PGCC MICROBIOLOGY



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PGCC Microbiology

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TABLE OF CONTENTS

Licensing

1: Introduction to Microbiology

- 1.1: Introduction to Microbiology
- 1.2: The Roles of Microbes
- 1.3: Classification, Phylogeny, and Nomenclature
- 1.4: Carl Woese and the Phylogenetic Tree
- 1.5: Types of Cells and Microorganisms
- 1.6: History of Microbiology
  - 1.6.1: Hooke, van Leeuwenhoek, and Cohn
  - 1.6.2: Pasteur and Spontaneous Generation
  - 1.6.3: What Causes Diseases?
  - 1.6.4: Koch and Pure Culture
  - 1.6.5: Koch's Postulate
  - 1.6.6: Improvement in Public Health
  - 1.6.7: The Discovery of Antimicrobial Drugs
  - 1.6.E: The History (Exercises)

2: Chemistry of Microbiology

- 2.1: Atomic Structure
  - 2.1.1: Overview of Atomic Structure
  - 2.1.2: Electronic Orbitals
- 2.2: Chemical Bonds
  - 2.2.1: Ions and Ionic Bonds
  - 2.2.2: Covalent Bonds and Other Bonds and Interaction
  - 2.2.3: Hydrogen Bonding
- 2.3: Chemical Reactions
  - 2.3.1: The Chemical Basis of Life
  - 2.3.2: Synthesis of Biological Macromolecules
  - 2.3.3: Energy and Biochemical Reactions
- 2.4: Water and pH
  - 2.4.1: Biochemical Properties of Water
  - 2.4.2: pH, Buffers, Acids, and Bases
- 2.5: Organic Compounds
  - 2.5.1: Organic Molecules
  - 2.5.2: Carbohydrate Molecules
  - 2.5.3: Lipid Molecules
  - 2.5.4: DNA and RNA
  - 2.5.5: Amino Acids
  - 2.5.6: ATP- Adenosine Triphosphate
  - 2.5.E: Organic Molecules (Exercises)

3: Cell Structure and Function

- 3.1: Characteristics of Life
- 3.2: Cells and Cell Theory
  - 3.2.1: Comparing Prokaryotic and Eukaryotic Cells
- 3.3: Prokaryotic External Structures
  - 3.3.1: Bacteria Surface Structures
  - 3.3.2: Flagella
  - 3.3.3: Fimbriae and Pili
- 3.4: Prokaryote Cell Wall
  - 3.4.1: Antibiotics and Selective Toxicity
  - 3.4.2: Gram-Negative Wall
  - 3.4.3: Gram-Positive Cell Wall
  - 3.4.4: Archaea Cell Wall
- 3.5: Prokaryote Plasma Membrane
  - 3.5.1: Components and Structure - Fluid Mosaic Model
- 3.6: Endospores
  - 3.6.1: Fungal Spores
- 3.7: Eukaryotic External Structures
- 3.8: Eukaryotic Plasma Membrane and The Cytoplasm
  - 3.8.1: The Plasma Membrane and Transport
    - 3.8.1.1: The Plasma Membrane
    - 3.8.1.2: Transport Across Membranes
    - 3.8.1.3: Passive Transport - Diffusion
    - 3.8.1.4: Passive Transport - Facilitated Transport
    - 3.8.1.5: Passive Transport - Osmosis
    - 3.8.1.6: Active Transport
  - 3.8.2: Endocytosis
- 3.9: Eukaryote Organelles
- 3.10: Serial Endosymbiosis Theory (SET)

4: Microscopy, Staining, and Classification

- 4.1: Units of Measure
- 4.2: Staining Microscopic Specimens
- 4.3: Classification and Identification
  - 4.3.1: Kingdom Classification According to Whittaker
  - 4.3.2: Domain Classification According to Woese
- 4.4: Microscopy
  - 4.4.1: The Properties of Light
  - 4.4.2: Types of Microscopes

5: Infectious Diseases and Epidemiology

- 5.1: Microbe and Human Symbiotic Relationships
- 5.2: Human Microbiota
  - 5.2.1: Opportunistic Pathogens
- 5.3: Infectious Diseases Characteristics and Classification

- 5.3.1: Koch's Postulates
- 5.4: Virulence Factors
  - 5.4.1: Virulence Factors that Promote Colonization
    - 5.4.1.1: The Ability to Use Motility and Other Means to Contact Host Cells
    - 5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal
    - 5.4.1.3: The Ability to Evade Adaptive Immune Defenses
  - 5.4.2: Virulence Factors that Damage the Host
- 5.5: Reservoirs of Infection and Disease Transmission
- 5.6: Introduction to Epidemiology
  - 5.6.1: Epidemiology
    - 5.6.1.1: Descriptive Epidemiology
    - 5.6.1.2: Analytical Epidemiology
    - 5.6.1.3: Experimental Epidemiology
  - 5.6.2: Nosocomial Infections
    - 5.6.2.1: Transmission of Nosocomial Infection
    - 5.6.2.2: Control of Nosocomial Infections
  - 5.6.3: Global Public Health
    - 5.6.3I: Current Epidemics

6: Immunology

- 6.1: Overview of the Immune System
  - 6.1.1: Cells and Organs of the Immune System
- 6.2: Non-Adaptive Immunity
  - 6.2.1: Pathogen Recognition
    - 6.2.1.1: Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)
    - 6.2.1.2: Pattern-Recognition Receptors (PRRs)
  - 6.2.2: The Complement System
  - 6.2.3: Interferons
  - 6.2.4: Natural Killer Cells
  - 6.2.5: Dendritic Cells
  - 6.2.6: Inflammation and Fever
- 6.3: Adaptive Immunity
  - 6.3.1: Antigen Epitopes
  - 6.3.2: Humoral Immune Response
    - 6.3.2.1: B Lymphocytes and Antibodies
    - 6.3.2.2: Antibody Structure
    - 6.3.2.3: Antibody Functions
  - 6.3.3: Cell-Mediated Immunity
    - 6.3.3.1: T Lymphocytes
  - 6.3.4: Immunological Memory, Primary, and Secondary Adaptive Response

7: Microbial Metabolism

- 7.1: Introduction to Metabolism and Enzymes
  - 7.1.1: Factors Affecting Enzyme Activity
- 7.2: Catabolism of Glucose (1)- Stages 1-3 of Cellular Respiration

- 7.2A: Alternative to Glycolysis- the Entner-Doudoroff Pathway
- 7.2B: Alternative to Glycolysis- the Pentose Phosphate Shunt
- 7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
- 7.4: Catabolism of Glucose (3)- In the Absence of Oxygen
  - 7.4.1: Electron Donors and Acceptors in Anaerobic Respiration
  - 7.4.2: Fermentation
- 7.5: Catabolism of Lipids and Proteins
- 7.6: Anabolism of Glucose- Photosynthesis
  - 7.6.1: The Two Parts of Photosynthesis
  - 7.6.2: The Process of Photosynthesis
- 7.7: Other Anabolism
  - 7.7.1: Amino Acid Synthesis
  - 7.7.2: Polysaccharide Biosynthesis
  - 7.7.3: Lipid Biosynthesis
- 7.E: Microbial Metabolism (Exercises)

8: Microbial Growth

- 8.1: How Bacteria Grow
- 8.2: Factors that Affect Bacterial Growth
  - 8.2.1: Temperature Effects on Bacterial Growth
  - 8.2.2: pH Effects on Bacterial Growth
  - 8.2.3: Nitrogen Effects on Bacterial Growth
  - 8.2.4: Oxygen Effects on Bacterial Growth
  - 8.2.5: Other Environmental Effects on Bacterial Growth
- 8.3: Bacterial Quorum Sensing
- 8.4: Culturing Bacteria
  - 8.4.1: Media Used for Bacterial Growth
  - 8.4.2: Culture Media
  - 8.4.3: Complex and Synthetic Media
  - 8.4.4: Selective and Differential Media
  - 8.4.5: Special Culture Techniques
- 8.5: Growth Characteristics
  - 8.5.1: Generation Time

9: Microbial Genetics

- 9.1: Genome, DNA, and Genes
  - 9.1.1: The Structure and Function of Cellular Genomes
  - 9.1.2: Plasmids
    - 9.1.2A: Introduction to Plasmids
    - 9.1.2B: Types of Plasmids and Their Biological Significance
  - 9.1.3: Structure and Function of DNA
  - 9.1.4: Structure and Function of RNA
  - 9.1.5: What Are Genes?
  - 9.1.E: Biochemistry of the Genome (Exercises)
- 9.2: DNA Replication
- 9.3: RNA Transcription

- 9.3A: mRNA Processing
- 9.4: Protein Synthesis (Translation)
- 9.5: Mutations
  - 9.5A: DNA Repair
- 9.6: Gene Regulation - Operon Theory
- 9.7: How Asexual Prokaryotes Achieve Genetic Diversity
- 9.E: Mechanisms of Microbial Genetics (Exercises)

10: Control of Microbial Growth

- 10.1: Controlling Microbial Growth
- 10.2: Testing the Effectiveness of Antiseptics and Disinfectants
- 10.3: Using Physical Methods to Control Microorganisms
- 10.4: Using Chemicals to Control Microorganisms
- 10.E: Control of Microbial Growth (Exercises)

11: Viruses and Other Agents

- 11.1: Viruses - General Characteristics
- 11.2: The Viral Life Cycle and Host Infection Specificity
  - 11.2.1: Medical Importance of Viruses
  - 11.2.2: The Baltimore Virus Classification
  - 11.2.3: Evolution of Viruses
- 11.3: Prevention and Treatment of Viral Infections
- 11.4: The Role of Viruses in Tumor Production
- 11.5: Culturing Viruses
  - 11.5.1: Isolation, Culture, and Identification of Viruses
  - 11.5.2: Tissue Culture of Animal Viruses
  - 11.5.3: Batch Culture of Bacteriophages
- 11.6: Viroids and Prions

12: Antimicrobial Drugs

- 12.1: Overview of Antimicrobial Therapy
  - 12.1A: Origins of Antimicrobial Drugs
  - 12.1B: Antibiotic Discovery
  - 12.1C: Antibiotics and Selective Toxicity
  - 12.1D: Spectrum of Antimicrobial Activity
  - 12.1E: Antibiotic Classifications
- 12.2: Functions of Antimicrobial Drugs
  - 12.2A: Inhibiting Cell Wall Synthesis
  - 12.2B: Injuring the Plasma Membrane
  - 12.2C: Inhibiting Nucleic Acid Synthesis
  - 12.2D: Inhibiting Protein Synthesis
  - 12.2E: Inhibiting Essential Metabolite Synthesis
- 12.3: Commonly Used Antimicrobial Drugs
  - 12.3A: Synthetic Antimicrobial Drugs
  - 12.3B: Naturally Occurring Antimicrobial Drugs- Antibiotics
  - 12.3C: Beta-Lactam Antibiotics- Penicillins and Cephalosporins

- 12.3D: Antibiotics from Prokaryotes
- 12.3E: Antimycobacterial Antibiotics
- 12.4: Interactions Between Drug and Host
  - 12.4A: Organ Toxicity
  - 12.4B: Allergic Responses to Drugs
  - 12.4C: Suppression and Alteration of Microbiota by Antimicrobials
  - 12.4D: Effects of Drug Combinations
- 12.5: Measuring Drug Susceptibility
  - 12.5A: Minimal Inhibitory Concentration (MIC)
  - 12.5B: Kirby-Bauer Disk Susceptibility Test
- 12.6: Drug Resistance
  - 12.6A: Mechanisms of Resistance
  - 12.6B: Antibiotic Misuse
  - 12.6C: Cost and Prevention of Resistance
  - 12.6D: Biofilms, Persisters, and Antibiotic Tolerance
  - 12.6E: Finding New Antimicrobial Drugs

Index

Glossary

Detailed Licensing

Licensing

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CHAPTER OVERVIEW

1: Introduction to Microbiology

1.1: Introduction to Microbiology
1.2: The Roles of Microbes
1.3: Classification, Phylogeny, and Nomenclature
1.4: Carl Woese and the Phylogenetic Tree
1.5: Types of Cells and Microorganisms
1.6: History of Microbiology
1.6.1: Hooke, van Leeuwenhoek, and Cohn
1.6.2: Pasteur and Spontaneous Generation
1.6.3: What Causes Diseases?
1.6.4: Koch and Pure Culture
1.6.5: Koch's Postulate
1.6.6: Improvement in Public Health
1.6.7: The Discovery of Antimicrobial Drugs
1.6.E: The History (Exercises)

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1.1: Introduction to Microbiology

Obviously microbes are small. The traditional definition describes microbes as organisms or agents that are invisible to the naked eye, indicating that one needs assistance in order to see them. That assistance is typically in the form of a microscope of some type. The only problem with that definition is that there are microbes that you can see without a microscope. Not well, but you can see them. It would be easy to dismiss these organisms as non-microbes, but in all other respects they look/act/perform like other well-studied microbes (who follow the size restriction).

So, the traditional definition is modified to describe microbes as fairly simple agents/organisms that are not highly **differentiated**, meaning even the multicellular microbes are composed of cells that can act independently– there is no set division of labor. If you take a giant fungus and chop half the cells off, the remaining cells will continue to function unimpeded. Versus if you chopped half my cells off, well, that would be a problem. Multicellular microbes, even if composed of billions of cells, are relatively simple in design, usually composed of branching filaments.

It is also acknowledged that research in the field of microbiology will require certain common techniques, largely related to the size of the quarry. Because microbes are so small and there are so many around, it is important to be able to isolate the one type that you are interested in. This involves methods of **sterilization**, to prevent unwanted contamination, and **observation**, to confirm that you have fully isolated the microbe that you want to study.

Microbe Size

Since size is a bit of theme in microbiology, let us talk about actual measurements. How small is small? The cellular microbes are typically measured in **micrometers** (**µm**). A typical bacterial cell (let us say *E. coli*) is about 1 µm wide by 4 µm long. A typical protozoal cell (let us say *Paramecium*) is about 25 µm wide by 100 µm long. There are 1000 µm in every millimeter, so that shows why it is difficult to see most microbes without assistance. (An exception would be a multicellular microbe, such as a fungus. If you get enough cells together in one place, you can definitely see them without a microscope!)

When we talk about the acellular microbes we have to use an entirely different scale. A typical virus (let us say influenza virus) has a diameter of about 100 **nanometers** (**nm**). There are 1000 nanometers in every micrometer, so that shows why you need a more powerful microscope to see a virus. If a typical bacterium (let us pick on *E.coli* again) were inflated to be the size of the Statue of Liberty, a typical virus (again, influenza virus works) would be the size of an adult human, if we keep the correct proportions.

http://learn.genetics.utah.edu/content/cells/scale/

The Discovery of Microbes

The small size of microbes definitely hindered their discovery. It is hard to get people to believe that their skin is covered with billions of small creatures, if you cannot show it to them. “Seeing is believing,” that is what I always say. Or someone says that.

In microbiology, there are two people that are given the credit for the discovery of microbes. Or at least providing the proof of their discovery, both around the same time period:

Robert Hooke (1635-1703)

Robert Hooke was a scientist who used a **compound microscope**, or microscope with two lenses in tandem, to observe many different objects. He made detailed drawings of his observations, publishing them in the scientific literature of the day, and is credited with publishing the first drawings of microorganisms. In 1665 he published a book by the name of *Micrographia*, with drawing of microbes such as fungi, as well as other organisms and cell structures. His microscopes were restricted in their resolution, or clarity, which appeared to limit what microbes he was able to observe.

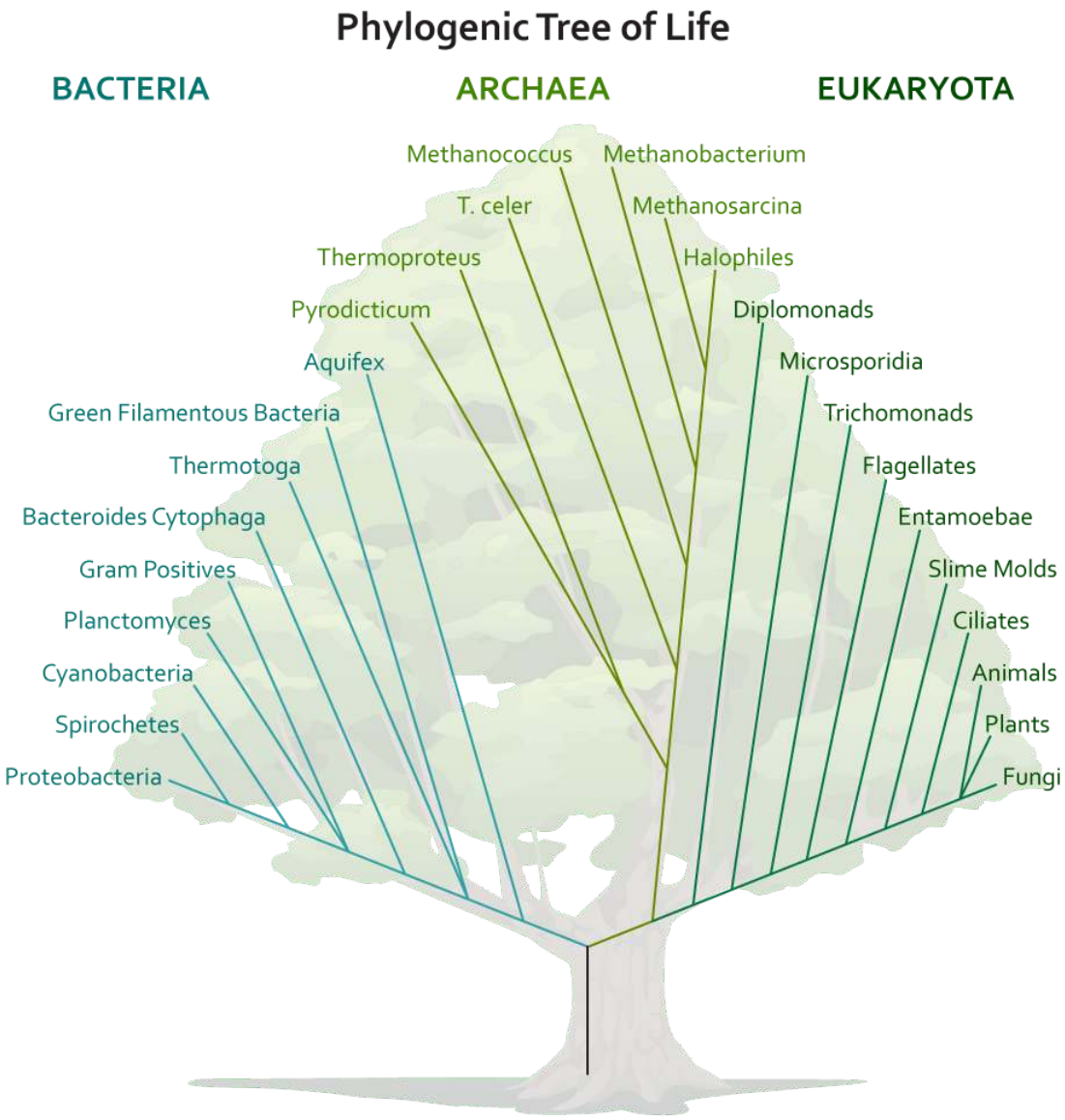
Antony van Leeuwenhoek (1632-1723)

Antony van Leeuwenhoek was a Dutch cloth merchant, who also happened to dabble in microscopes. He constructed a **simple microscope** (which has a single lens), where the lens was held between two silver plates. Apparently he relished viewing microbes from many different sample types – pond water, fecal material, teeth scrapings, etc. He made detailed drawings and notes about his observations and discoveries, sending them off to the **Royal Society of London**, the scientific organization of that time. This

invaluable record clearly indicates that he saw both bacteria and a wide variety of protists. Some microbiologists refer to van Leeuwenhoek as the "**Father of Microbiology**," because of his contributions to the field.

Microbial Groups

Classification of organisms, or the determination of how to group them, continually changes as we acquire new information and new tools of assessing the characteristics of an organism. Currently all organisms are grouped into one of three categories or domains: ***Bacteria***, ***Archaea***, and ***Eukarya***. The **Three Domain Classification**, first proposed by Carl Woese in the 1970s, is based on **ribosomal RNA** (**rRNA**) sequences and widely accepted by scientists today as the most accurate current portrayal of organism relatedness.



Tree of Life.

Bacteria

The *Bacteria* domain contains some of the best known microbial examples (*E. coli*, anyone?). Most of the members are unicellular, cells lack a **nucleus** or any other **organelle**, most members have a cell wall with a particular substance known as **peptidoglycan**

(not found anywhere else but in bacteria!), and humans are intimately familiar with many members, since they are common in soil, water, our foods, and our own bodies.

Archaea

Archaea is a relatively new domain, since these organisms used to be grouped with the bacteria. There are some obvious similarities, since they are mostly unicellular and cells lack a nucleus or any other organelle. But they have completely different cell walls that vary markedly in composition (but notably lack peptidoglycan) and their rRNA sequences have shown that they are not closely related to the *Bacteria* at all. In fact, they appear to be more closely related to the eukaryotes! These organisms are found in soil, water, even sometimes in the human body, but they are also found in some very extreme environments on Earth – very cold, very hot, very salty, very pressurized, very acidic, earning them the commonly used name "the extremophiles," or extreme-loving organisms.

Eukarya

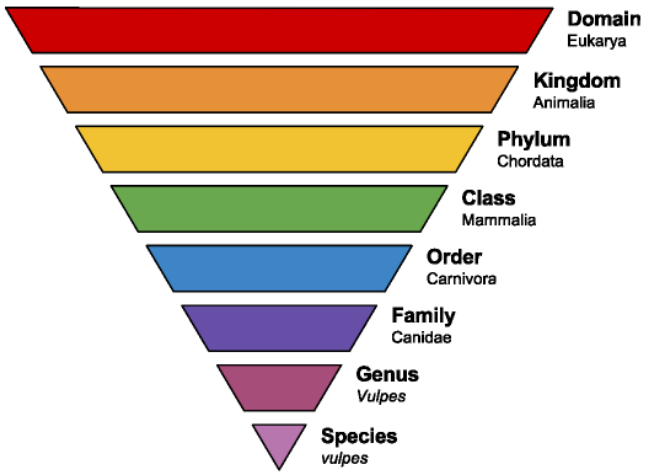
The *Eukarya* Domain includes many non-microbes, such as animals and plants, but there are numerous microbial examples as well, such as fungi, protists, slime molds, and water molds. The eukaryotic cell type has a nucleus, as well as many organelles, such as mitochondria or endoplasmic reticulum.

Viruses

Viruses are not part of the Three Domain Classification, since they lack ribosomes and therefore lack rRNA sequences for comparison. They are classified separately, using characteristics specific to viruses. Viruses are typically described as "**obligate intracellular parasites**," a reference to their strict requirement for a host cell in order to replicate or increase in number. These acellular entities are often agents of disease, a result of their cell invasion.

Taxonomic Ranks

Taxonomic ranks are a way for scientists to organize information about organisms, by determining relatedness. Domains are the largest grouping used, followed by numerous smaller groupings, where each smaller grouping consists of organisms that share specific features in common. Each level becomes more and more restrictive as to whom can be a member. Eventually we get down to **genus** and **species**, the groupings used for formation of a scientific name. This is the **binomial nomenclature** devised by Carl Linnaeus in the 1750s.



Taxonomic Ranks. By Annina Breen (Own work) [CC BY-SA 4.0], via Wikimedia Commons

Binomial Nomenclature

When referring to the actual scientific name assigned to an organism, it is important to follow convention, so it is clear to everyone that you are referring to the scientific name. There are rules in science (just like in English class, where you would never refer to "mr. robert louis stevenson," or at least not without expecting to get your paper back with red all over it).

A scientific name is composed of a genus and a species, where the genus is a generic name and the species is specific. The species name, once assigned, is permanent for the organism, while the genus can change if new information becomes available. For example, the bacterium previously known as *Streptococcus faecalis* is now *Enterococcus faecalis* because sequencing information indicates that it is more closely related to the members of the *Enterococcus* genus. It is important to note that it is inappropriate to refer to an organism by the species alone (i.e. you should never refer to *E. coli* as "*coli*" alone. Other bacteria can have the species "*coli*" as well.)

Now for the rules: The genus is always capitalized. The species is always lowercase. And both the genus and the species are italicized (common if typewritten) or underlined (common if handwritten). The genus may be shortened to its starting letter, but only if the name has been referred to in the text in its entirety at least once first (the exception to this is *E. coli*, due to its commonality, where hardly anyone spells out the *Escherichia* genus anymore).

Key Words

microbiology, microorganisms, microbes, unicellular, multicellular, differentiation, sterilization, observation, micrometers (µm), nanometers (nm), Robert Hooke, compound microscope, Antony van Leeuwenhoek, simple microscope, Royal Society of London, Father of Microbiology, Three Domain Classification, ribosomal RNA (rRNA), *Bacteria*, *Archaea*, *Eukarya*, obligate intracellular parasites, taxonomic ranks, genus, species, binomial nomenclature

Study Questions

1. Who are the members of the microbial world?
2. What is the complete definition of microbiology? What characteristics are relevant?
3. What size are different groups of microbes?
4. What were the contributions of Hooke and Van Leeuwenhoek to the field of microbiology? How did they make these contributions?
5. What is the basis for Woese's classification and what are the three domains?
6. What are the basic characteristics of members of the three domains? Where do microbes fit in?
7. What are the basic characteristics of viruses? Why are they not classified in one of the three domains?
8. What are taxonomic ranks? What is the system of binomial nomenclature? What are the basic rules? How are bacteria named? What is a genus and species? Be able to write a bacterial name correctly.

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1.2: The Roles of Microbes

Learning Objectives

1. State three harmful effects and four beneficial effects associated with the activities of microorganisms.
2. Define microbiota and microbiome.
3. Briefly describe two different beneficial things the human microbiome does for the normal function of our body.
4. State several diseases associated with a change in our "normal" microbiota.
5. List and recognize a description of the each of the 5 basic groups of microbes.

Microorganisms are the dominant life forms on earth, are found in almost every conceivable environment, and are essential to sustaining life on this planet. There are five basic groups of microorganisms:

- **Bacteria** are typically unicellular, microscopic, prokaryotic organisms that reproduce by binary fission.
- **Fungi (**yeasts and molds) are typically unicellular, microscopic, eukaryotic fungi that reproduce asexually by budding. Molds are typically filamentous, eukaryotic fungi that reproduce by producing asexual reproductive spores.
- **Viruses** are typically submicroscopic, acellular infectious particles that can only replicate inside a living host cell. The vast majority of viruses possess either DNA or RNA, but not both.
- **Protozoa** are typically unicellular, microscopic, eukaryotic organisms that lack a cell wall.
- **Algae** are typically eukaryotic microorganisms that carry out photosynthesis.

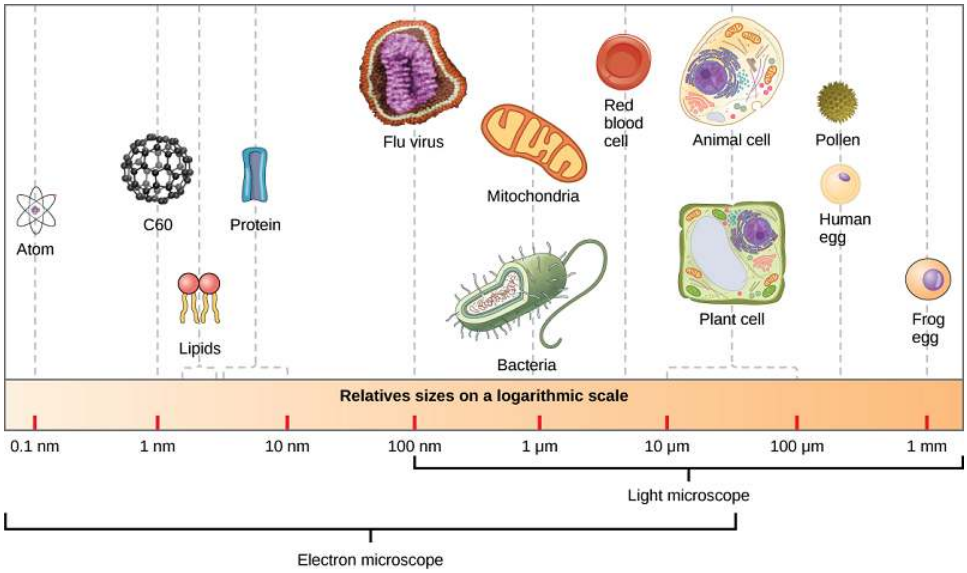
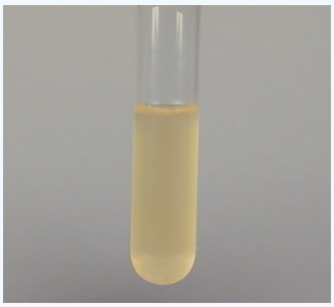


Figure **1.2.1**: The size of a virus is very small relative to the size of cells and organelles.

To get us started on our introduction of microorganisms we will go through the following Think-Pair-Share Questions.

Exercise 1.2.1: Think-Pair-Share Questions



This tube contains 7 milliliters of a culture of *Escherichia coli*. The total number of bacteria in this tube is equal to:

a. The number of people in Baltimore city.
b. The number of people in Maryland.
c. The number of people in North America.
d. The number of people in the world.

? Exercise 1.2.2: Think-Pair-Share Questions

Are microbes such as bacteria mostly beneficial or harmful? Briefly explain your answer.

? Exercise 1.2.3: Think-Pair-Share Questions

- In what ways might microbes such as bacteria be beneficial?
- In what ways might microbes such as bacteria be harmful?

In this course we will be looking at various fundamental concepts of microbiology, with particular emphasis on their relationships to human health. The overall goal is to better understand the total picture of infectious diseases in terms of host-infectious agent interaction. We will look at various groups of microbes and learn what they might do to establish infection and harm the body, we will look at the body to see the ways in which it defends itself against these microbes, and we will learn what can be done to help the body in its defense efforts.

The Big Picture of Infectious Diseases

One of the most important things in microbiology is learining the "Big Picture of Infectious Diseases," which is the biological basis of host parasite interaction. There are four interlocking parts to this big picture:

A. **The microbe's side of the story - why some microbes have more potential to be harmful:** The overwhelming majority of microbes are harmless to humans and, in fact, many are beneficial, being key players in the recycling of nutrients in nature. We will look at the major groups of microbes, learn what they are composed of chemically and structurally, and see how how they carry out their metabolism and reproduce. We will learn of a variety of factors some microbes may possess that play a role in increasing their ability to cause disease. Also we will learn how, through mutation, genetic recombination, and natural selection, microbes may adapt to resist our control attempts.
B. **The body's side of the story - ways in which the body is able to defend itself naturally against infectious disease agents:** Here will learn about the phenomenal defenses the body has available to defend itself against infectious disease agents, as well as altered body cells such as cancer cells and infected cells. The body is able to do this through the innate immune system and the adaptive immune system. Innate immune defenses are those you are born with and include anatomical barriers, mechanical removal, cytokines, pattern-recognition receptors, phagocytosis, inflammation, the complement pathways, and fever. The adaptive immune defenses are those you develop throughout your life and include antibody production and cell-mediated immunity.
C. **Ways in which we can artificially help the body defend itself by removing the microbes or enhancing body defenses:** We will learn how we can artificially help ourselves to avoid or reduce the risk of infection. Also we will learn ways in which we are able to artificially remove microbes from the body and its environment using agents such as antiseptics, disinfectants, physical agents such as heat and cold, antimicrobial chemotherapeutic chemicals, and antibiotics. Finally we will learn ways we are currently able to - or potentially in the future will be able to - improve or restore the body's immune responses through such techniques as immunization, adoptive immunotherapy, or immune modulation.
D. **Relationship between the Human Microbiome and Human Health:** The complex mutually beneficial symbiotic relationship between humans and their natural microbes is critical to good health. It is now recognized that the millions of genes associated with the normal flora or microbiota of the human body -especially in the intestinal tract - aid in the digestion of many foods, the regulation of multiple host metabolic pathways, and the regulation the body's immune defenses.

Benefits of Microbial Activity

Most people tend to think of microorganisms as harmful because of their roles in causing infectious diseases in humans and other animals, and agricultural loss as a result of infectious diseases of plants and the spoilage of food. The fact is, however, the vast majority of microorganisms are not harmful but rather beneficial. Without them there would be no life on earth. Therefore, we will start this course by looking at a few of the many benefits from microbial activity on this planet.

1. **Food production:** Many food products employ microorganisms in their production. These include the microbial fermentation processes used to produce yogurt, buttermilk, cheeses, alcoholic beverages, leavened breads, sauerkraut, pickles, and kimchi.
2. **Energy production and cleaning up the environment**: Methane, or natural gas, is a product of *methanogenic microorganisms*. Many aquatic microbes capture light energy and store it in molecules used as food then used by other organisms. Animal wastes, domestic refuse, biomass, and grain can be converted to biofuels such as ethanol and methane by microorganisms. In addition, through a process called bioremediation , some pollutants such pesticides, solvents, and oil spills can be cleaned up with the aid of microbes.
3. **Sustaining agriculture**: Through their roles in recycling nitrogen, carbon, and sulfur, microorganism are able to convert these essential elements into forms that can be used by plants in their growth. They are also essential in enabling ruminant animals such as cows and sheep to digest cellulose from the grasses they eat.
4. **Production of useful natural gene products or products from bioengineering**. Examples include specific enzymes, antibiotics, vaccines, and medications such as human insulin, interferons, and growth hormones.
5. **The human microbiota and microbiome**: Where we be without microorganisms? While the typical human body contains an estimated 37 trillion human cells, it also contains over 100 trillion bacteria and other microbes. The human body has 3 times as many bacterial cells as it does human cells! It is estimated the the mass of the human microbiota is 2.5 pounds.

The complex mutually beneficial symbiotic relationship between humans and their natural microbes is critical to good health. It is now recognized that the millions of genes associated with the microbiota of the human body -especially in the intestinal tract - aid in the digestion of many foods, the regulation of multiple host metabolic pathways, and the regulation the body's immune defenses. These collective microbes and their genes are referred to as the human microbiome. There are currently an estimated 5,000,000 - 10,000,000 genes from over 1000 species that constitute the human microbiome compared to the approximately 20,000 - 23,000 genes that make up the human genome. There are approximately 300 non-human genes in the human body for every human gene.

a. The mutually beneficial interaction between the human host and its resident microbiota is essential to human health. Microbial genes produce metabolites essential to the host while human genes contribute to development of the microbiota. The microbiome aids in the following:
    1. The digestion of many foods, especially plant polysaccharides that would normally be indigestible by humans.
    2. The regulation of many host metabolic pathways. The metabolism of many substrates in the human body is carried out by a combination of genes from both the microbiome and the human genome. Within the intestinal tract there is constant chemical communication not only between microbial species but also between microbial cells and human cells. Multiple factors, including diet, antibiotic use, disease, life style, and a person's environment can alter the composition of the microbiota within the gastrointestinal tract and, as a result, influence host biochemistry and the body's susceptibility to disease.
    3. Metabolic disorders such as diabetes, nonalcoholic fatty liver disease, hypertension, obesity, gastric ulcers, colon cancer, and possibly some mood and behavior changes through hormone signaling have been linked to alterations in the microbiota.

b.

Summary

1. Microorganisms are typically too small to be seen with the naked eye.
2. Bacteria, fungi, viruses, protozoa, and algae are the major groups of microorganisms.
3. The vast majority of microorganisms are not harmful but rather beneficial.
4. Microbiota refers to all of the microorganisms that live in a particular environment.
5. A microbiome is the entire collection of genes found in all of the microbes associated with a particular host.
6. The microbiome of the human body - especially in the intestinal tract - aid in the digestion of many foods, the regulation of multiple host metabolic pathways, and the regulation the body's immune defenses.

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1.3: Classification, Phylogeny, and Nomenclature

So Many Species!

The collage below shows six kingdoms into which all of Earth's living things are commonly classified. How many species are there in each kingdom? In a word, millions. A total of almost 2 million living species have already been identified, and new species are being discovered all the time. Scientists estimate that there may be as many as 30 million different species alive on Earth today! Clearly, there is a tremendous variety of life on Earth.

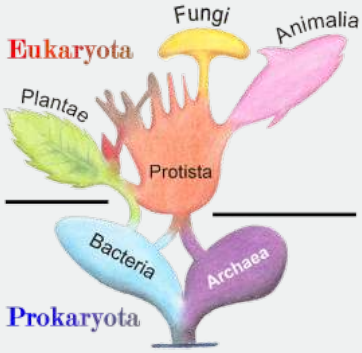


Figure **1.3.1**: Six kingdoms of life: Archaea, Bacteria, Protista, Fungi, Animalia, and Plantae

What Is Biodiversity?

Biological diversity, or **biodiversity,** refers to all of the variety of life that exists on Earth. Biodiversity can be described and measured at three different levels: species, genetic, and ecosystem diversity.

- Species diversity refers to the number of different species in an ecosystem or on Earth as a whole. This is the commonest way to measure biodiversity. Current estimates for Earth's total number of living species range from 5 to 30 million species.
- Genetic diversity refers to the variation in genes within all these species.
- Ecosystem diversity refers to the variety of ecosystems on Earth. An ecosystem is a system formed by populations of many different species interacting with each other and their environment.

Defining a Species

Biodiversity is most often measured by counting species, but what is a species? The answer to that question is not as straightforward as you might think. The formal biological definition of **species** is a group of actually or potentially interbreeding organisms. This means that members of the same species are similar enough to each other to produce fertile offspring together. By this definition of species, all human beings alive today belong to one species, *Homo sapiens*. All humans can potentially interbreed with each other but not with members of any other species.

In the real world, it isn't always possible to make the observations needed to determine whether different organisms can interbreed. For one thing, many species reproduce asexually, so individuals never interbreed even with members of their own species. When studying extinct species represented by fossils, it is usually impossible to know whether different organisms could interbreed. Therefore, in practice, many biologists and virtually all paleontologists generally define species on the basis of morphology, rather than breeding behavior. **Morphology** refers to the form and structure of organisms. For classification purposes, it generally refers to relatively obvious physical traits. Typically, the more similar to one another different organisms appear, the greater the chance that they will be classified in the same species.

Classifying Living Things

People have been trying to classify the tremendous diversity of life on Earth for more than two thousand years. The science of classifying organisms is called **taxonomy.** Classification is an important step in understanding the present diversity and past evolutionary history of life on Earth. It helps make sense of the overwhelming diversity of living things.

Linnaean Classification

All modern classification systems have their roots in the Linnaean classification system. It was developed by Swedish botanist Carolus Linnaeus in the 1700s. He tried to classify all living things that were known at his time. He grouped together organisms that shared obvious morphological traits, such as the number of legs or shape of leaves.

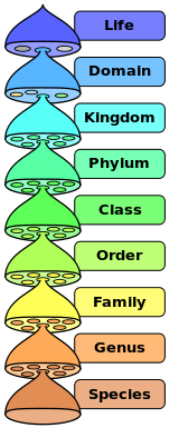


Figure **1.3.2**: Classification of life into smaller subcategories: Domain, Kingdom, phylum, class, order, family, genus, species.

The Linnaean system of classification consists of a hierarchy of groupings, called taxa (singular, taxon). Figure **1.3.2** shows an expanded version of Linnaeus's original classification system. In the original system, taxa range from the kingdom to the species. The kingdom is the largest and most inclusive grouping. It consists of organisms that share just a few basic similarities. Examples are the plant and animal kingdoms. The species is the smallest and most exclusive grouping. Ideally, it consists of organisms that are similar enough to interbreed, as discussed above. Similar species are classified together in the same genus (plural, genera), similar genera are classified together in the same family, and so on all the way up to the kingdom.

Binomial Nomenclature

Perhaps the single greatest contribution Linnaeus made to science was his method of naming species. This method, called **binomial nomenclature,** gives each species a unique, two-word Latin name consisting of the genus name followed by a specific species identifier. An example is *Homo sapiens*, the two-word Latin name for humans. It literally means “wise human.” This is a reference to our big brains.

Why is having two names so important? It is similar to people having a first and a last name. You may know several people with the first name Michael, but adding Michael’s last name usually pins down exactly who you mean. In the same way, having two names uniquely identifies a species.

Revisions in the Linnaean Classification

Linnaeus published his classification system in the 1700s. Since then, many new species have been discovered. Scientists can also now classify organisms on the basis of their biochemical and genetic similarities and differences rather than just their outward morphology. These changes have led to revisions in the original Linnaean system of classification.

A major change to the Linnaean system is the addition of a new taxon called the domain. The **domain** is a taxon that is larger and more inclusive than the kingdom, as shown in Figure **1.3.2**. Most biologists agree that there are three domains of life on Earth: Bacteria, Archaea, and Eukarya (Figure **1.3.3**). Both the Bacteria and the Archaea domains consist of single-celled organisms that lack a nucleus. This means that their genetic material is not enclosed within a membrane inside the cell. The Eukarya domain, in contrast, consists of all organisms whose cells have a nucleus. In other words, their genetic material is enclosed within a membrane inside the cell. The Eukarya domain is made up of both single-celled and multicellular organisms. This domain includes several kingdoms, including the animal, plant, fungus, and protist kingdoms.

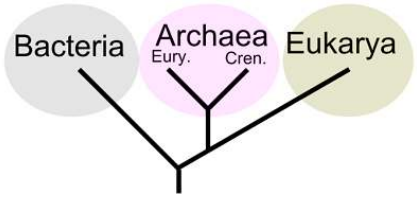


Figure **1.3.3**: Three domains of life: Bacteria, Archaea, and Eukarya

Phylogenetic Classification

Linnaeus classified organisms based on morphology. Basically, organisms were grouped together if they looked alike. After Darwin published his theory of evolution in the 1800s, scientists looked for a way to classify organisms that took into account phylogeny. **Phylogeny** is the evolutionary history of a group of related organisms. It is represented by a phylogenetic tree, or some other tree-like diagram, like the one in Figure **1.3.3** for the three domains. A **phylogenetic tree** shows how closely related different groups of organisms are to one another. Each branching point represents a common ancestor of the branching groups. Figure **1.3.3**, for example, shows that the Eukarya shared a more recent common ancestor with the Archaea than they did with the Bacteria. This is based on comparisons of important similarities and differences between the three domains.

Review

1. What is biodiversity? Identify three ways that biodiversity may be measured.
2. Define biological species. Why is this definition often difficult to apply?
3. Explain why it is important to classify living things and outline the Linnaean system of classification.
4. What is binomial nomenclature? Give an example.
5. Contrast Linnaean and phylogenetic systems of classification.
6. Describe the taxon called the domain, and compare the three widely recognized domains of living things.
7. *True or False.* Humans have identified all of the species on Earth.
8. *True or False.* In the binomial nomenclature for humans, *Homo* is the genus and *sapiens* refers to the specific species.
9. A kingdom is a:
    A. domain
    B. taxon
    C. genera
    D. phylogeny
10. In Linnaean classification, similar classes together make up a _____ .
11. Based on the phylogenetic tree for the three domains of life above, explain whether you think Bacteria are more closely related to Archaea or Eukarya.
12. A scientist discovers a new single-celled organism. Answer the following questions about this discovery.
    A. If this is all you know, can you place the organism into a particular domain? If so, what is the domain and if not, why not?
    B. What is one type of information that could help the scientist classify the organism?
13. Define morphology. Give an example of a morphological trait in humans.
14. Which type of biodiversity is represented by the differences between humans?
15. Why do you think it is important for the definition of a species that members of a species can produce *fertile* offspring?

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1.4: CARL WOESE AND THE PHYLOGENETIC TREE

LEARNING OBJECTIVES

- Recognize the three major domains used for classification

The fact that biology has such a broad scope as a science has to do with the tremendous diversity of life on Earth. The source of this diversity is evolution, the process of gradual change during which new species arise from older species. Evolutionary biologists study the evolution of living things in everything from the microscopic world to ecosystems.

The evolution of various life forms on Earth can be summarized in a phylogenetic tree using phylogeny. A phylogenetic tree is a diagram showing the evolutionary relationships among biological species based on similarities and differences in genetic or physical traits or both. A phylogenetic tree is composed of nodes and branches. The internal nodes represent ancestors and are points in evolution when, based on scientific evidence, an ancestor is thought to have diverged to form two new species. The length of each branch is proportional to the time elapsed since the split.

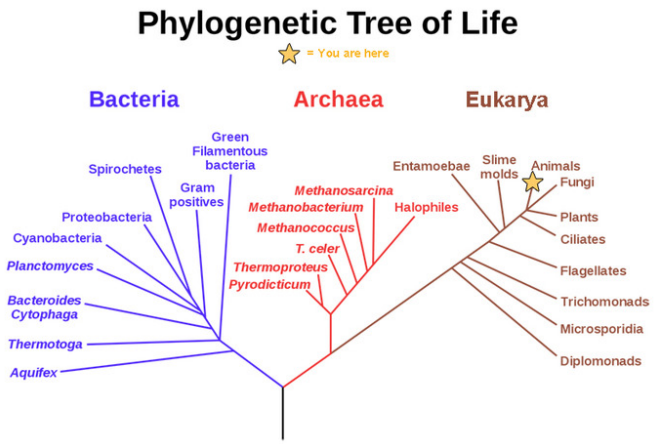


Figure **1.4.1**: Phylogenetic Tree of Life: This phylogenetic tree was constructed by microbiologist Carl Woese using data obtained from sequencing ribosomal RNA genes. The tree shows the separation of living organisms into three domains: Bacteria, Archaea, and Eukarya. Bacteria and Archaea are prokaryotes, single-celled organisms lacking intracellular organelles.

CARL WOESE AND THE PHYLOGENETIC TREE

In the past, biologists grouped living organisms into five kingdoms: animals, plants, fungi, protists, and bacteria. The organizational scheme was based mainly on physical features, as opposed to physiology, biochemistry, or molecular biology, all of which are used by modern systematics. The pioneering work of American microbiologist Carl Woese in the early 1970s has shown, however, that life on Earth has evolved along three lineages, now called domains—Bacteria, Archaea, and Eukarya. The first two are prokaryotic cells with microbes that lack membrane-enclosed nuclei and organelles. The third domain contains the eukaryotes and includes unicellular microorganisms together with the four original kingdoms (excluding bacteria). Woese defined Archaea as a new domain, and this resulted in a new taxonomic tree. Many organisms belonging to the Archaea domain live under extreme conditions and are called extremophiles. To construct his tree, Woese used genetic relationships rather than similarities based on morphology (shape).

Woese's tree was constructed from comparative sequencing of the genes that are universally distributed, present in every organism, and conserved (meaning that these genes have remained essentially unchanged throughout evolution). Woese's approach was revolutionary because comparisons of physical features are insufficient to differentiate between the prokaryotes that appear fairly similar in spite of their tremendous biochemical diversity and genetic variability. The comparison of homologous DNA and RNA sequences provided Woese with a sensitive device that revealed the extensive variability of prokaryotes, and which justified the separation of the prokaryotes into two domains: bacteria and archaea. DNA, the universal genetic material, contains the instructions for the structure and function of all living organisms and can be divided into genes whose expression varies between organisms. The RNA, which is transcribed from DNA, varies between organisms as well based on the expression of specific genes. Thus, to examine differences at this molecular level provides a more accurate depiction of the diversity which exists.

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KEY POINTS

- The three major Domains of Life include: Domain Bacteria, Domain Eukarya and Domain Archaea.
- Domain Bacteria and Domain Archaea include prokaryotic cells that lack membrane-enclosed nuclei and organelles.
- Domain Eukarya include eukaryotes and more complex organisms that contain membrane-bound nuclei and organelles.
- Carl Woese defined Archaea as a new domain and constructed the phylogentic tree of life which shows separation of all living organisms.
- The phylogenetic tree of life was constructed by Carl Woese using sequencing data of ribosomal RNA genes. Therefore, genetics classification surpassed morphological cataloguing, which was the traditional way of organizing living beings.

KEY TERMS

- **phylogeny**: the evolutionary history of an organism
- **extremophile**: an organism that lives under extreme conditions of temperature, salinity, etc; commercially important as a source of enzymes that operate under similar conditions
- **DNA**: a biopolymer of deoxyribonucleic acids (a type of nucleic acid) that has four different chemical groups, called bases: adenine, guanine, cytosine, and thymine

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1.5: Types of Cells and Microorganisms

Learning Objectives

- List the various types of microorganisms and describe their defining characteristics
- Give examples of different types of cellular and viral microorganisms and infectious agents
- Describe the similarities and differences between archaea and bacteria
- Provide an overview of the field of microbiology

Most microbes are unicellular and small enough that they require artificial magnification to be seen. However, there are some unicellular microbes that are visible to the naked eye, and some multicellular organisms that are microscopic. An object must measure about 100 micrometers (µm) to be visible without a microscope, but most microorganisms are many times smaller than that. For some perspective, consider that a typical animal cell measures roughly 10 µm across but is still microscopic. Bacterial cells are typically about 1 µm, and viruses can be 10 times smaller than bacteria (Figure 1.5.1). See Table 1.5.1 for units of length used in microbiology.

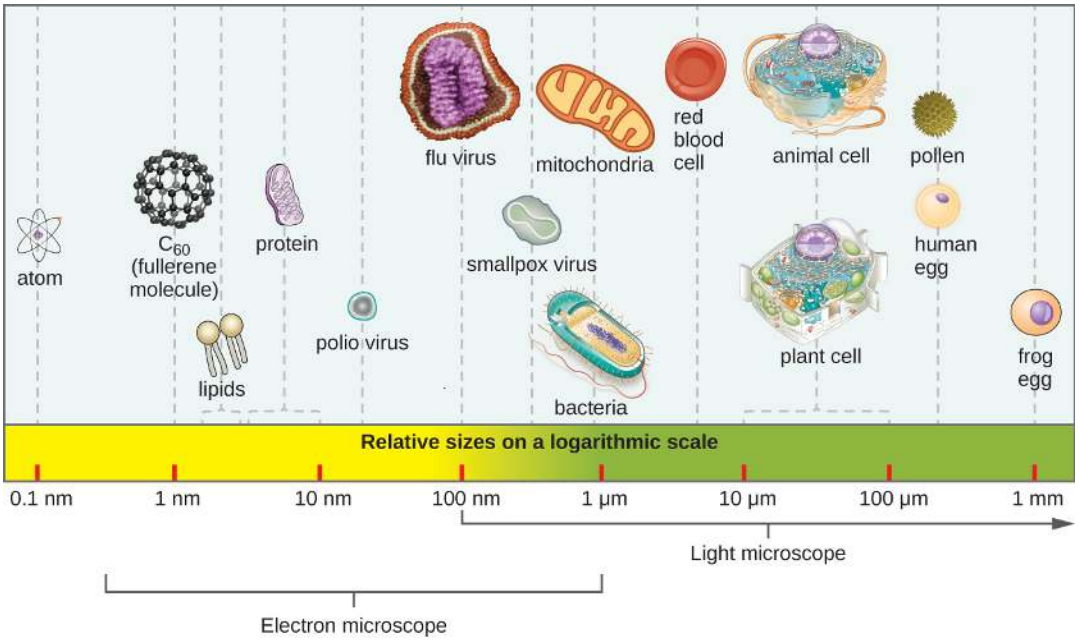


Figure 1.5.1: The relative sizes of various microscopic and nonmicroscopic objects. Note that a typical virus measures about 100 nm, 10 times smaller than a typical bacterium (~1 µm), which is at least 10 times smaller than a typical plant or animal cell (~10–100 µm). An object must measure about 100 µm to be visible without a microscope.

Table 1.5.1: Units of Length Commonly Used in Microbiology

Metric Unit	Meaning of Prefix	Metric Equivalent
meter (m)	—	1 m = 10^0 m
decimeter (dm)	1/10	1 dm = 0.1 m = 10^{-1} m
centimeter (cm)	1/100	1 cm = 0.01 m = 10^{-2} m
millimeter (mm)	1/1000	1 mm = 0.001 m = 10^{-3} m
micrometer (µm)	1/1,000,000	1 µm = 0.000001 m = 10^{-6} m
nanometer (nm)	1/1,000,000,000	1 nm = 0.000000001 m = 10^{-9} m

Microorganisms differ from each other not only in size, but also in structure, habitat, metabolism, and many other characteristics. While we typically think of microorganisms as being unicellular, there are also many multicellular organisms that are too small to

be seen without a microscope. Some microbes, such as viruses, are even acellular (not composed of cells).

Microorganisms are found in each of the three domains of life: Archaea, Bacteria, and Eukarya. Microbes within the domains Bacteria and Archaea are all prokaryotes (their cells lack a nucleus), whereas microbes in the domain Eukarya are eukaryotes (their cells have a nucleus). Some microorganisms, such as viruses, do not fall within any of the three domains of life. In this section, we will briefly introduce each of the broad groups of microbes. Later chapters will go into greater depth about the diverse species within each group.

Prokaryotic Microorganisms

Bacteria are found in nearly every habitat on earth, including within and on humans. Most bacteria are harmless or helpful, but some are pathogens, causing disease in humans and other animals. Bacteria are prokaryotic because their genetic material (DNA) is not housed within a true nucleus. Most bacteria have cell walls that contain peptidoglycan.

Bacteria are often described in terms of their general shape. Common shapes include spherical (coccus), rod-shaped (bacillus), or curved (spirillum, spirochete, or vibrio). Figure **1.5.2** shows examples of these shapes.

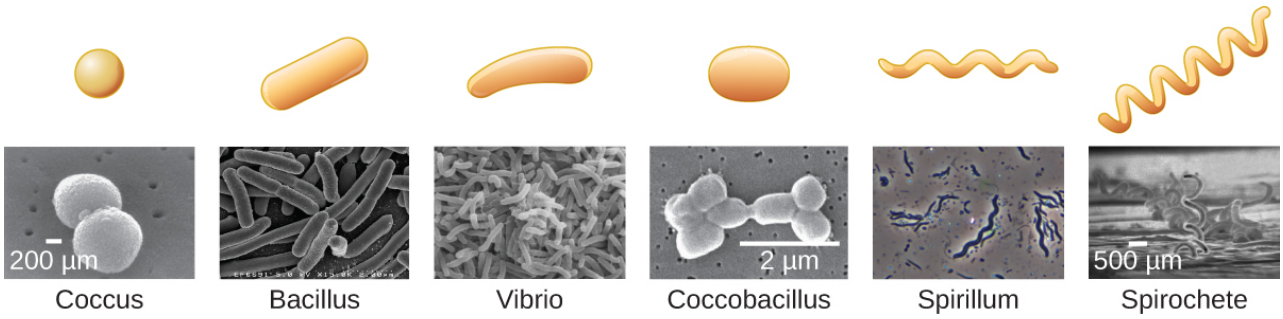


Figure **1.5.2**: Common bacterial shapes. Note how coccobacillus is a combination of spherical (coccus) and rod-shaped (bacillus). (credit “Coccus”: modification of work by Janice Haney Carr, Dr. Richard Facklam, Centers for Disease Control and Prevention; credit “Bacillus”: modification of work by “Elapied”/Wikimedia Commons)

They have a wide range of metabolic capabilities and can grow in a variety of environments, using different combinations of nutrients. Some bacteria are photosynthetic, such as oxygenic cyanobacteria and anoxygenic green sulfur and green nonsulfur bacteria; these bacteria use energy derived from sunlight, and fix carbon dioxide for growth. Other types of bacteria are nonphotosynthetic, obtaining their energy from organic or inorganic compounds in their environment.

Archaea are also unicellular prokaryotic organisms. Archaea and bacteria have different evolutionary histories, as well as significant differences in genetics, metabolic pathways, and the composition of their cell walls and membranes. Unlike most bacteria, archaeal cell walls do not contain peptidoglycan, but their cell walls are often composed of a similar substance called pseudopeptidoglycan. Like bacteria, archaea are found in nearly every habitat on earth, even extreme environments that are very cold, very hot, very basic, or very acidic (Figure **1.5.3**). Some archaea live in the human body, but none have been shown to be human pathogens.



Figure **1.5.3**: Some archaea live in extreme environments, such as the Morning Glory pool, a hot spring in Yellowstone National Park. The color differences in the pool result from the different communities of microbes that are able to thrive at various water temperatures.

? Exercise 1.5.1

1. What are the two main types of prokaryotic organisms?
2. Name some of the defining characteristics of each type.

Eukaryotic Microorganisms

The domain Eukarya contains all eukaryotes, including uni- or multicellular eukaryotes such as protists, fungi, plants, and animals. The major defining characteristic of eukaryotes is that their cells contain a nucleus.

Protists

Protists are unicellular eukaryotes that are not plants, animals, or fungi. Algae and protozoa are examples of protists.

Algae (singular: alga) are plant-like protists that can be either unicellular or multicellular (Figure **1.5.4**). Their cells are surrounded by cell walls made of cellulose, a type of carbohydrate. Algae are photosynthetic organisms that extract energy from the sun and release oxygen and carbohydrates into their environment. Because other organisms can use their waste products for energy, algae are important parts of many ecosystems. Many consumer products contain ingredients derived from algae, such as carrageenan or alginic acid, which are found in some brands of ice cream, salad dressing, beverages, lipstick, and toothpaste. A derivative of algae also plays a prominent role in the microbiology laboratory. Agar, a gel derived from algae, can be mixed with various nutrients and used to grow microorganisms in a Petri dish. Algae are also being developed as a possible source for biofuels.

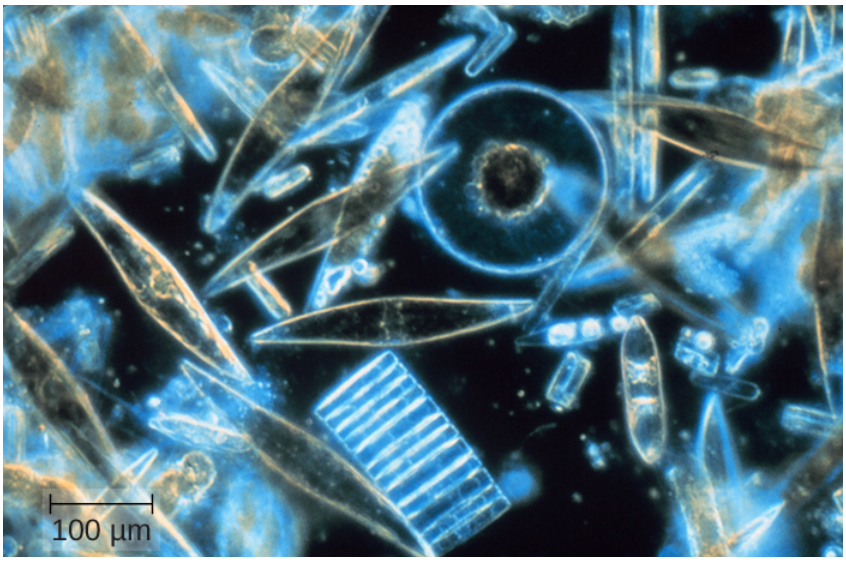


Figure 1.5.4: Assorted diatoms, a kind of algae, live in annual sea ice in McMurdo Sound, Antarctica. Diatoms range in size from 2 µm to 200 µm and are visualized here using light microscopy. (credit: National Oceanic and Atmospheric Administration)

Protozoa (singular: protozoan) are protists that make up the backbone of many food webs by providing nutrients for other organisms. Protozoa are very diverse. Some protozoa move with help from hair-like structures called cilia or whip-like structures called flagella. Others extend part of their cell membrane and cytoplasm to propel themselves forward. These cytoplasmic extensions are called pseudopods ("false feet"). Some protozoa are photosynthetic; others feed on organic material. Some are free-living, whereas others are parasitic, only able to survive by extracting nutrients from a host organism. Most protozoa are harmless, but some are pathogens that can cause disease in animals or humans (Figure 1.5.5).



Figure 1.5.5: Giardia lamblia, an intestinal protozoan parasite that infects humans and other mammals, causing severe diarrhea. (credit: modification of work by Centers for Disease Control and Prevention)

Fungi

Fungi (singular: fungus) are also eukaryotes. Some multicellular fungi, such as mushrooms, resemble plants, but they are actually quite different. Fungi are not photosynthetic, and their cell walls are usually made out of chitin rather than cellulose.

Unicellular fungi—yeasts—are included within the study of microbiology. There are more than 1000 known species. Yeasts are found in many different environments, from the deep sea to the human navel. Some yeasts have beneficial uses, such as causing bread to rise and beverages to ferment; but yeasts can also cause food to spoil. Some even cause diseases, such as vaginal yeast infections and oral thrush (Figure 1.5.6).

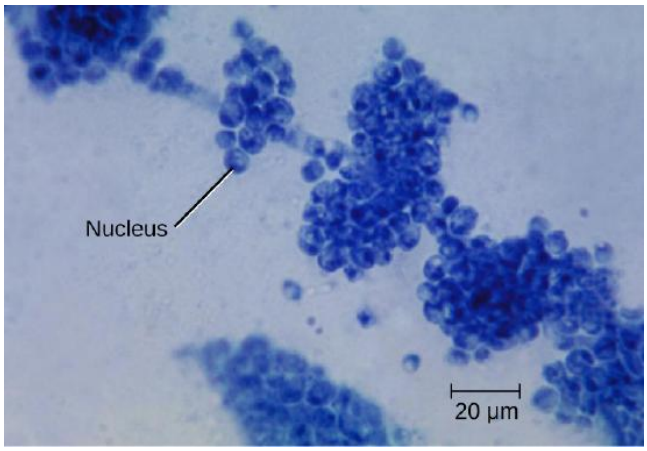


Figure 1.5.5: Candida albicans is a unicellular fungus, or yeast. It is the causative agent of vaginal yeast infections as well as oral thrush, a yeast infection of the mouth that commonly afflicts infants. *C. albicans* has a morphology similar to that of coccus bacteria; however, yeast is a eukaryotic organism (note the nuclei) and is much larger. (credit: modification of work by Centers for Disease Control and Prevention)

Other fungi of interest to microbiologists are multicellular organisms called molds. Molds are made up of long filaments that form visible colonies (Figure 1.5.6). Molds are found in many different environments, from soil to rotting food to dank bathroom corners. Molds play a critical role in the decomposition of dead plants and animals. Some molds can cause allergies, and others produce disease-causing metabolites called mycotoxins. Molds have been used to make pharmaceuticals, including penicillin, which is one of the most commonly prescribed antibiotics, and cyclosporine, used to prevent organ rejection following a transplant.



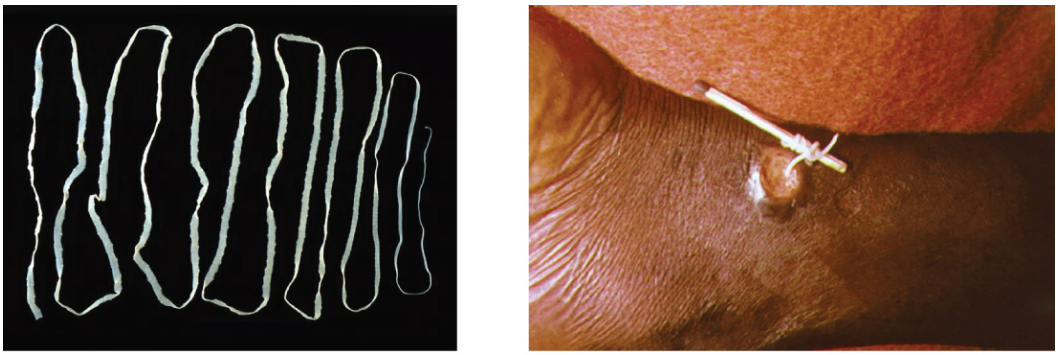
Figure 1.5.6: Large colonies of microscopic fungi can often be observed with the naked eye, as seen on the surface of these moldy oranges.

? Exercise 1.5.2

1. Name two types of protists and two types of fungi.
2. Name some of the defining characteristics of each type.

Helminths

Multicellular parasitic worms called helminths are not technically microorganisms, as most are large enough to see without a microscope. However, these worms fall within the field of microbiology because diseases caused by helminths involve microscopic eggs and larvae. One example of a helminth is the guinea worm, or *Dracunculus medinensis*, which causes dizziness, vomiting, diarrhea, and painful ulcers on the legs and feet when the worm works its way out of the skin (Figure 1.5.7). Infection typically occurs after a person drinks water containing water fleas infected by guinea-worm larvae. In the mid-1980s, there were an estimated 3.5 million cases of guinea-worm disease, but the disease has been largely eradicated. In 2014, there were only 126 cases reported, thanks to the coordinated efforts of the World Health Organization (WHO) and other groups committed to improvements in drinking water sanitation.[12]

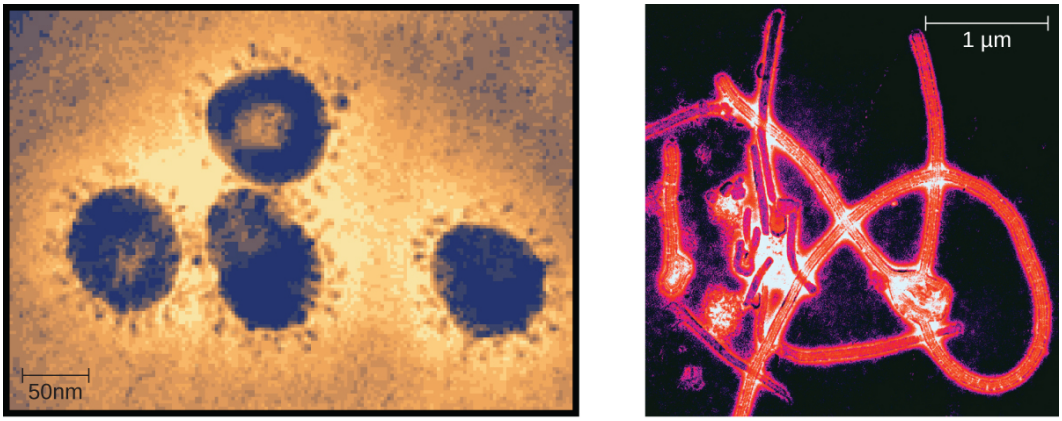


(a) (b)

Figure **1.5.7**: The beef tapeworm, *Taenia saginata*, infects both cattle and humans. *T. saginata* eggs are microscopic (around 50 µm), but adult worms like the one shown here can reach 4–10 m, taking up residence in the digestive system. (b) An adult guinea worm, *Dracunculus medinensis*, is removed through a lesion in the patient's skin by winding it around a matchstick. (credit b: modification of work by Centers for Disease Control and Prevention)

Viruses

Viruses are acellular microorganisms, which means they are not composed of cells. Essentially, a virus consists of proteins and genetic material—either DNA or RNA, but never both—that are inert outside of a host organism. However, by incorporating themselves into a host cell, viruses are able to co-opt the host's cellular mechanisms to multiply and infect other hosts. Viruses can infect all types of cells, from human cells to the cells of other microorganisms. In humans, viruses are responsible for numerous diseases, from the common cold to deadly Ebola (Figure **1.5.8**). However, many viruses do not cause disease.



(a) (b)

Figure **1.5.8**: (a) Members of the Coronavirus family can cause respiratory infections like the common cold, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS). Here they are viewed under a transmission electron microscope (TEM). (b) Ebolavirus, a member of the Filovirus family, as visualized using a TEM. (credit b: modification of work by Thomas W. Geisbert).

? Exercise 1.5.3

1. Are helminths microorganisms? Explain why or why not.
2. How are viruses different from other microorganisms?

Microbiology as a Field of Study

Microbiology is a broad term that encompasses the study of all different types of microorganisms. But in practice, microbiologists tend to specialize in one of several subfields. For example, bacteriology is the study of bacteria; mycology is the study of fungi;protozoology is the study of protozoa; parasitology is the study of helminths and other parasites; and virology is the study of viruses (Figure **1.5.9**). Immunology, the study of the immune system, is often included in the study of microbiology because host–

pathogen interactions are central to our understanding of infectious disease processes. Microbiologists can also specialize in certain areas of microbiology, such as clinical microbiology, environmental microbiology, applied microbiology, or food microbiology.

In this textbook, we are primarily concerned with clinical applications of microbiology, but since the various subfields of microbiology are highly interrelated, we will often discuss applications that are not strictly clinical.



Figure **1.5.9**: A virologist samples eggs from this nest to be tested for the influenza A virus, which causes avian flu in birds. (credit: Don Becker)

Bioethics in Microbiology

In the 1940s, the U.S. government was looking for a solution to a medical problem: the prevalence of sexually transmitted diseases (STDs) among soldiers. Several now-infamous government-funded studies used human subjects to research common STDs and treatments. In one such study, American researchers intentionally exposed more than 1300 human subjects in Guatemala to syphilis, gonorrhea, and chancroid to determine the ability of penicillin and other antibiotics to combat these diseases. Subjects of the study included Guatemalan soldiers, prisoners, prostitutes, and psychiatric patients—none of whom were informed that they were taking part in the study. Researchers exposed subjects to STDs by various methods, from facilitating intercourse with infected prostitutes to inoculating subjects with the bacteria known to cause the diseases. This latter method involved making a small wound on the subject's genitals or elsewhere on the body, and then putting bacteria directly into the wound.[3] In 2011, a U.S. government commission tasked with investigating the experiment revealed that only some of the subjects were treated with penicillin, and 83 subjects died by 1953, likely as a result of the study.[4]

Unfortunately, this is one of many horrific examples of microbiology experiments that have violated basic ethical standards. Even if this study had led to a life-saving medical breakthrough (it did not), few would argue that its methods were ethically sound or morally justifiable. But not every case is so clear cut. Professionals working in clinical settings are frequently confronted with ethical dilemmas, such as working with patients who decline a vaccine or life-saving blood transfusion. These are just two examples of life-and-death decisions that may intersect with the religious and philosophical beliefs of both the patient and the health-care professional.

No matter how noble the goal, microbiology studies and clinical practice must be guided by a certain set of ethical principles. Studies must be done with integrity. Patients and research subjects provide informed consent (not only agreeing to be treated or studied but demonstrating an understanding of the purpose of the study and any risks involved). Patients' rights must be

respected. Procedures must be approved by an institutional review board. When working with patients, accurate record-keeping, honest communication, and confidentiality are paramount. Animals used for research must be treated humanely, and all protocols must be approved by an institutional animal care and use committee. These are just a few of the ethical principles explored in the *Eye on Ethics* boxes throughout this book.

Clinical Focus: Resolution

Cora's CSF samples show no signs of inflammation or infection, as would be expected with a viral infection. However, there is a high concentration of a particular protein, 14-3-3 protein, in her CSF. An electroencephalogram (EEG) of her brain function is also abnormal. The EEG resembles that of a patient with a neurodegenerative disease like Alzheimer's or Huntington's, but Cora's rapid cognitive decline is not consistent with either of these. Instead, her doctor concludes that Cora hasCreutzfeldt-Jakob disease (CJD), a type of transmissible spongiform encephalopathy (TSE).

CJD is an extremely rare disease, with only about 300 cases in the United States each year. It is not caused by a bacterium, fungus, or virus, but rather by prions—which do not fit neatly into any particular category of microbe. Like viruses, prions are not found on the tree of life because they are acellular. Prions are extremely small, about one-tenth the size of a typical virus. They contain no genetic material and are composed solely of a type of abnormal protein.

CJD can have several different causes. It can be acquired through exposure to the brain or nervous-system tissue of an infected person or animal. Consuming meat from an infected animal is one way such exposure can occur. There have also been rare cases of exposure to CJD through contact with contaminated surgical equipment[5] and from cornea and growth-hormone donors who unknowingly had CJD.[6][7] In rare cases, the disease results from a specific genetic mutation that can sometimes be hereditary. However, in approximately 85% of patients with CJD, the cause of the disease is spontaneous (or sporadic) and has no identifiable cause.[8] Based on her symptoms and their rapid progression, Cora is diagnosed with sporadic CJD.

Unfortunately for Cora, CJD is a fatal disease for which there is no approved treatment. Approximately 90% of patients die within 1 year of diagnosis.[9] Her doctors focus on limiting her pain and cognitive symptoms as her disease progresses. Eight months later, Cora dies. Her CJD diagnosis is confirmed with a brain autopsy.

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Go back to the previous Clinical Focus box.

Summary

- Microorganisms are very diverse and are found in all three domains of life: Archaea, Bacteria, and Eukarya.
- Archaea and bacteria are classified as prokaryotes because they lack a cellular nucleus. Archaea differ from bacteria in evolutionary history, genetics, metabolic pathways, and cell wall and membrane composition.
- Archaea inhabit nearly every environment on earth, but no archaea have been identified as human pathogens.
- Eukaryotes studied in microbiology include algae, protozoa, fungi, and helminths.
- Algae are plant-like organisms that can be either unicellular or multicellular, and derive energy via photosynthesis.
- Protozoa are unicellular organisms with complex cell structures; most are motile.
- Microscopic fungi include molds and yeasts.
- Helminths are multicellular parasitic worms. They are included in the field of microbiology because their eggs and larvae are often microscopic.
- Viruses are acellular microorganisms that require a host to reproduce.
- The field of microbiology is extremely broad. Microbiologists typically specialize in one of many subfields, but all health professionals need a solid foundation in clinical microbiology.

Footnotes

1. 1 C. Greenaway "Dracunculiasis (Guinea Worm Disease)." *Canadian Medical Association Journal* 170 no. 4 (2004):495–500.
2. 2 World Health Organization. "Dracunculiasis (Guinea-Worm Disease)." *WHO*. 2015. http://www.who.int/mediacentre/factsheets/fs359/en/. Accessed October 2, 2015.

3. 3 Kara Rogers. “Guatemala Syphilis Experiment: American Medical Research Project”. *Encylopaedia Britannica*. www.britannica.com/event/Guat...lis-experiment. Accessed June 24, 2015.
4. 4 Susan Donaldson James. “Syphilis Experiments Shock, But So Do Third-World Drug Trials.” *ABC World News*. August 30, 2011. http://abcnews.go.com/Health/guatema...ry?id=14414902. Accessed June 24, 2015.
5. 5 Greg Botelho. “Case of Creutzfeldt-Jakob Disease Confirmed in New Hampshire.” *CNN*. 2013. http://www.cnn.com/2013/09/20/health...brain-disease/.
6. 6 P. Rudge et al. “Iatrogenic CJD Due to Pituitary-Derived Growth Hormone With Genetically Determined Incubation Times of Up to 40 Years.” *Brain* 138 no. 11 (2015): 3386–3399.
7. 7 J.G. Heckmann et al. “Transmission of Creutzfeldt-Jakob Disease via a Corneal Transplant.” *Journal of Neurology, Neurosurgery & Psychiatry* 63 no. 3 (1997): 388–390.
8. 8 National Institute of Neurological Disorders and Stroke. “Creutzfeldt-Jakob Disease Fact Sheet.” *NIH*. 2015. http://www.ninds.nih.gov/disorders/c....htm#288133058.
9. 9 National Institute of Neurological Disorders and Stroke. “Creutzfeldt-Jakob Disease Fact Sheet.” *NIH*. 2015. http://www.ninds.nih.gov/disorders/c....htm#288133058. Accessed June 22, 2015.

Glossary

acellular
not consisting of a cell or cells

algae
(singular: alga) any of various unicellular and multicellular photosynthetic eukaryotic organisms; distinguished from plants by their lack of vascular tissues and organs

archaea
any of various unicellular prokaryotic microorganisms, typically having cell walls containing pseudopeptidoglycan

bacteria
(singular: bacterium) any of various unicellular prokaryotic microorganisms typically (but not always) having cell wells that contain peptidoglycan

bacteriology
the study of bacteria

Eukarya
the domain of life that includes all unicellular and multicellular organisms with cells that contain membrane-bound nuclei and organelles

fungi
(singular: fungus) any of various unicellular or multicellular eukaryotic organisms, typically having cell walls made out of chitin and lacking photosynthetic pigments, vascular tissues, and organs

helminth
a multicellular parasitic worm

immunology
the study of the immune system

microbiology
the study of microorganisms

mold
a multicellular fungus, typically made up of long filaments

mycology
the study of fungi

parasitology
the study of parasites

pathogen
a disease-causing microorganism

protist
a unicellular eukaryotic microorganism, usually a type of algae or protozoa

protozoan
(plural: protozoa) a unicellular eukaryotic organism, usually motile

protozoology
the study of protozoa

virology
the study of viruses

virus
an acellular microorganism, consisting of proteins and genetic material (DNA or RNA), that can replicate itself by infecting a host cell

yeast
any unicellular fungus

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1.6: History of Microbiology

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1.6.1: Hooke, van Leeuwenhoek, and Cohn

Learning Objectives

- Explain how Van Leeuwenhoek, Spallanzani, Pasteur, Cohn and Koch contributed to the field of microbiology

Pre-microbiology, the possibility that microorganisms existed was discussed for many centuries before their actual discovery in the 17th century. The existence of unseen microbiological life was postulated by Jainism, which is based on Mahavira's teachings as early as 6th century BCE. In his first century book, *On Agriculture*, Roman scholar Marcus Terentius Varro was the first known to suggest the possibility of disease spreading by yet unseen organisms. In his book, he warns against locating a homestead near swamps because "there are bred certain minute creatures that cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose and there cause serious diseases. " In *The Canon of Medicine* (1020), Abū Alī ibn Sīnā (Avicenna) hypothesized that tuberculosis and other diseases might be contagious. In 1546, Girolamo Fracastoro proposed that epidemic diseases were caused by transferable seed-like entities that could transmit infection by direct or indirect contact, or even without contact over long distances. All these early claims about the existence of microorganisms were speculative and were not based on any data or science. Microorganisms were neither proven, observed, nor correctly and accurately described until the 17th century. The reason for this was that all these early studies lacked the microscope.

The Microscope and Discovery of Microorganisms

Antonie van Leeuwenhoek (1632–1723) was one of the first people to observe microorganisms, using a microscope of his own design, and made one of the most important contributions to biology. Robert Hooke was the first to use a microscope to observe living things. Hooke's 1665 book, *Micrographia*, contained descriptions of plant cells. Before Van Leeuwenhoek's discovery of microorganisms in 1675, it had been a mystery why grapes could be turned into wine, milk into cheese, or why food would spoil. Van Leeuwenhoek did not make the connection between these processes and microorganisms, but using a microscope, he did establish that there were forms of life that were not visible to the naked eye. Van Leeuwenhoek's discovery, along with subsequent observations by Spallanzani and Pasteur, ended the long-held belief that life spontaneously appeared from non-living substances during the process of spoilage.



Figure: ***Antoni van Leeuwenhoek****: A drawing of Antoni van Leeuwenhoek, one of the first scientists to use a microscope and identify microbes.*

Lazzaro Spallanzani (1729–1799) found that boiling broth would sterilize it and kill any microorganisms in it. He also found that new microorganisms could settle only in a broth if the broth was exposed to the air.

Louis Pasteur (1822–1895) expanded upon Spallanzani's findings by exposing boiled broths to the air in vessels that contained a filter to prevent all particles from passing through to the growth medium. He also did this in vessels with no filter at all, with air being admitted via a curved tube that prevented dust particles from coming in contact with the broth. By boiling the broth beforehand, Pasteur ensured that no microorganisms survived within the broths at the beginning of his experiment. Nothing grew in the broths in the course of Pasteur's experiment. This meant that the living organisms that grew in such broths came from outside,

as spores on dust, rather than spontaneously generated within the broth. Thus, Pasteur dealt the death blow to the theory of spontaneous generation and supported germ theory instead.



Figure: ***Louis Pasteur****: The famous scientist Louis Pasteur, one of the founders of microbiology.*

Ferdinand Julius Cohn (January 24, 1828 – June 25, 1898) was a German biologist. His classification of bacteria into four groups based on shape (sphericals, short rods, threads, and spirals) is still in use today. Among other things Cohn is remembered for being the first to show that *Bacillus* can change from a vegetative state to an endospore state when subjected to an environment deleterious to the vegetative state. His studies would lay the foundation for the classification of microbes and gave some of the first insights into the incredible complexity and diversity of microbial life.

In 1876, Robert Koch (1843–1910) established that microbes can cause disease. He found that the blood of cattle who were infected with anthrax always had large numbers of *Bacillus anthracis*. Koch found that he could transmit anthrax from one animal to another by taking a small sample of blood from the infected animal and injecting it into a healthy one, and this caused the healthy animal to become sick. He also found that he could grow the bacteria in a nutrient broth, then inject it into a healthy animal, and cause illness. Based on these experiments, he devised criteria for establishing a causal link between a microbe and a disease and these are now known as Koch's postulates. Although these postulates cannot be applied in all cases, they do retain historical importance to the development of scientific thought and are still being used today.

Key Points

- Van Leeuwenhoek is largely credited with the discovery of microbes, while Hooke is credited as the first scientist to describe live processes under a microscope.
- Spallanzani and Pasteur performed several experiments to demonstrate that microbial life does not arise spontaneously.
- Cohn laid the groundwork for discovering and cataloging microbes, while Koch conclusively showed that microbes can cause diseases.

Key Terms

- **classification**: the act of forming into a class or classes; a distribution into groups, as classes, orders, families, etc., according to some common relations or attributes.

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1.6.2: Pasteur and Spontaneous Generation

Learning Objectives

- Explain the theory of spontaneous generation and why people once accepted it as an explanation for the existence of certain types of organisms
- Explain how certain individuals (van Helmont, Redi, Needham, Spallanzani, and Pasteur) tried to prove or disprove spontaneous generation

Clinical Focus: Part 1

Barbara is a 19-year-old college student living in the dormitory. In January, she came down with a sore throat, headache, mild fever, chills, and a violent but unproductive (i.e., no mucus) cough. To treat these symptoms, Barbara began taking an over-the-counter cold medication, which did not seem to work. In fact, over the next few days, while some of Barbara's symptoms began to resolve, her cough and fever persisted, and she felt very tired and weak.

Exercise 1.6.2.1

What types of respiratory disease may be responsible?

Humans have been asking for millennia: Where does new life come from? Religion, philosophy, and science have all wrestled with this question. One of the oldest explanations was the theory of spontaneous generation, which can be traced back to the ancient Greeks and was widely accepted through the Middle Ages.

The Theory of Spontaneous Generation

The Greek philosopher Aristotle (384–322 BC) was one of the earliest recorded scholars to articulate the theory of spontaneous generation, the notion that life can arise from nonliving matter. Aristotle proposed that life arose from nonliving material if the material contained *pneuma* ("vital heat"). As evidence, he noted several instances of the appearance of animals from environments previously devoid of such animals, such as the seemingly sudden appearance of fish in a new puddle of water.[1]

This theory persisted into the 17th century, when scientists undertook additional experimentation to support or disprove it. By this time, the proponents of the theory cited how frogs simply seem to appear along the muddy banks of the Nile River in Egypt during the annual flooding. Others observed that mice simply appeared among grain stored in barns with thatched roofs. When the roof leaked and the grain molded, mice appeared. Jan Baptista van Helmont, a 17th century Flemish scientist, proposed that mice could arise from rags and wheat kernels left in an open container for 3 weeks. In reality, such habitats provided ideal food sources and shelter for mouse populations to flourish.

However, one of van Helmont's contemporaries, Italian physician Francesco Redi (1626–1697), performed an experiment in 1668 that was one of the first to refute the idea that maggots (the larvae of flies) spontaneously generate on meat left out in the open air. He predicted that preventing flies from having direct contact with the meat would also prevent the appearance of maggots. Redi left meat in each of six containers (Figure **1.6.2.1**). Two were open to the air, two were covered with gauze, and two were tightly sealed. His hypothesis was supported when maggots developed in the uncovered jars, but no maggots appeared in either the gauze-covered or the tightly sealed jars. He concluded that maggots could only form when flies were allowed to lay eggs in the meat, and that the maggots were the offspring of flies, not the product of spontaneous generation.

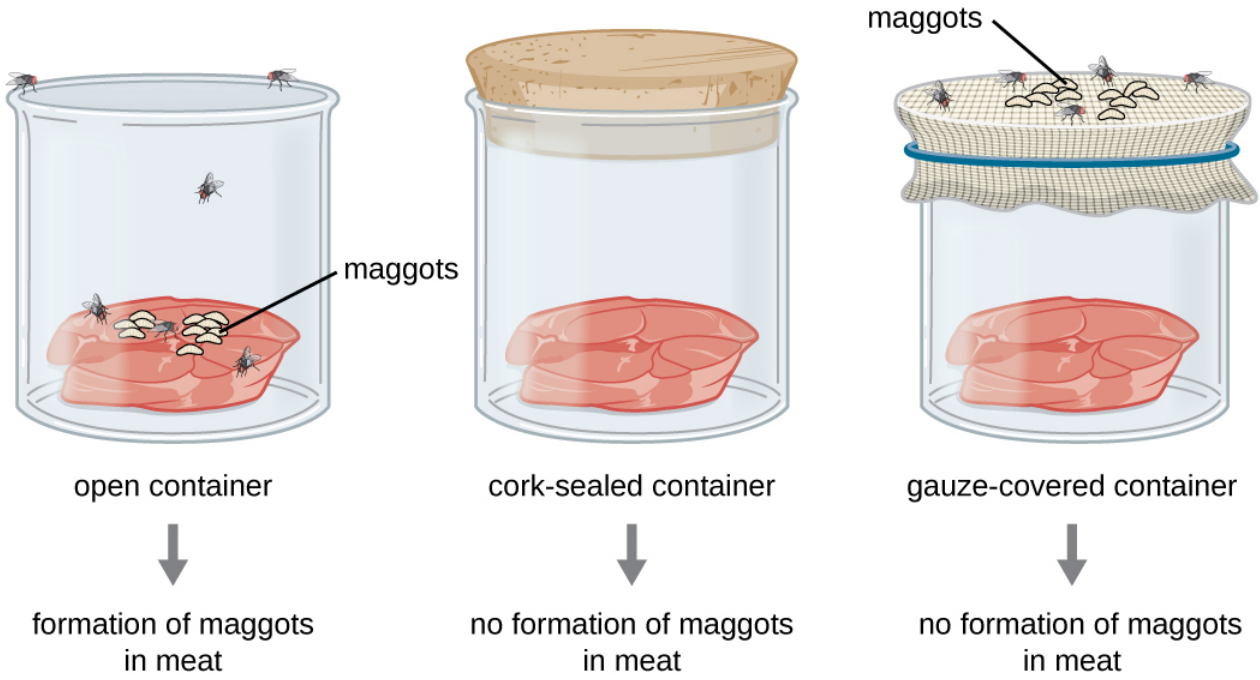


Figure **1.6.2.1**: Francesco Redi's experimental setup consisted of an open container, a container sealed with a cork top, and a container covered in mesh that let in air but not flies. Maggots only appeared on the meat in the open container. However, maggots were also found on the gauze of the gauze-covered container.

In 1745, John Needham (1713–1781) published a report of his own experiments, in which he briefly boiled broth infused with plant or animal matter, hoping to kill all preexisting microbes.[2] He then sealed the flasks. After a few days, Needham observed that the broth had become cloudy and a single drop contained numerous microscopic creatures. He argued that the new microbes must have arisen spontaneously. In reality, however, he likely did not boil the broth enough to kill all preexisting microbes.

Lazzaro Spallanzani (1729–1799) did not agree with Needham's conclusions, however, and performed hundreds of carefully executed experiments using heated broth.[3] As in Needham's experiment, broth in sealed jars and unsealed jars was infused with plant and animal matter. Spallanzani's results contradicted the findings of Needham: Heated but sealed flasks remained clear, without any signs of spontaneous growth, unless the flasks were subsequently opened to the air. This suggested that microbes were introduced into these flasks from the air. In response to Spallanzani's findings, Needham argued that life originates from a "life force" that was destroyed during Spallanzani's extended boiling. Any subsequent sealing of the flasks then prevented new life force from entering and causing spontaneous generation (Figure **1.6.2.2**).



Figure **1.6.2.2**: (a) Francesco Redi, who demonstrated that maggots were the offspring of flies, not products of spontaneous generation. (b) John Needham, who argued that microbes arose spontaneously in broth from a "life force." (c) Lazzaro Spallanzani, whose experiments with broth aimed to disprove those of Needham.

? Exercise 1.6.2.2

1. Describe the theory of spontaneous generation and some of the arguments used to support it.
2. Explain how the experiments of Redi and Spallanzani challenged the theory of spontaneous generation.

Disproving Spontaneous Generation

The debate over spontaneous generation continued well into the 19th century, with scientists serving as proponents of both sides. To settle the debate, the Paris Academy of Sciences offered a prize for resolution of the problem. Louis Pasteur, a prominent French chemist who had been studying microbial fermentation and the causes of wine spoilage, accepted the challenge. In 1858, Pasteur filtered air through a gun-cotton filter and, upon microscopic examination of the cotton, found it full of microorganisms, suggesting that the exposure of a broth to air was not introducing a "life force" to the broth but rather airborne microorganisms.

Later, Pasteur made a series of flasks with long, twisted necks ("swan-neck" flasks), in which he boiled broth to sterilize it (Figure 1.6.2.3). His design allowed air inside the flasks to be exchanged with air from the outside, but prevented the introduction of any airborne microorganisms, which would get caught in the twists and bends of the flasks' necks. If a life force besides the airborne microorganisms were responsible for microbial growth within the sterilized flasks, it would have access to the broth, whereas the microorganisms would not. He correctly predicted that sterilized broth in his swan-neck flasks would remain sterile as long as the swan necks remained intact. However, should the necks be broken, microorganisms would be introduced, contaminating the flasks and allowing microbial growth within the broth.

Pasteur's set of experiments irrefutably disproved the theory of spontaneous generation and earned him the prestigious Alhumbert Prize from the Paris Academy of Sciences in 1862. In a subsequent lecture in 1864, Pasteur articulated "*Omne vivum ex vivo*" ("Life only comes from life"). In this lecture, Pasteur recounted his famous swan-neck flask experiment, stating that "…life is a germ and a germ is life. Never will the doctrine of spontaneous generation recover from the mortal blow of this simple experiment."[4] To Pasteur's credit, it never has.

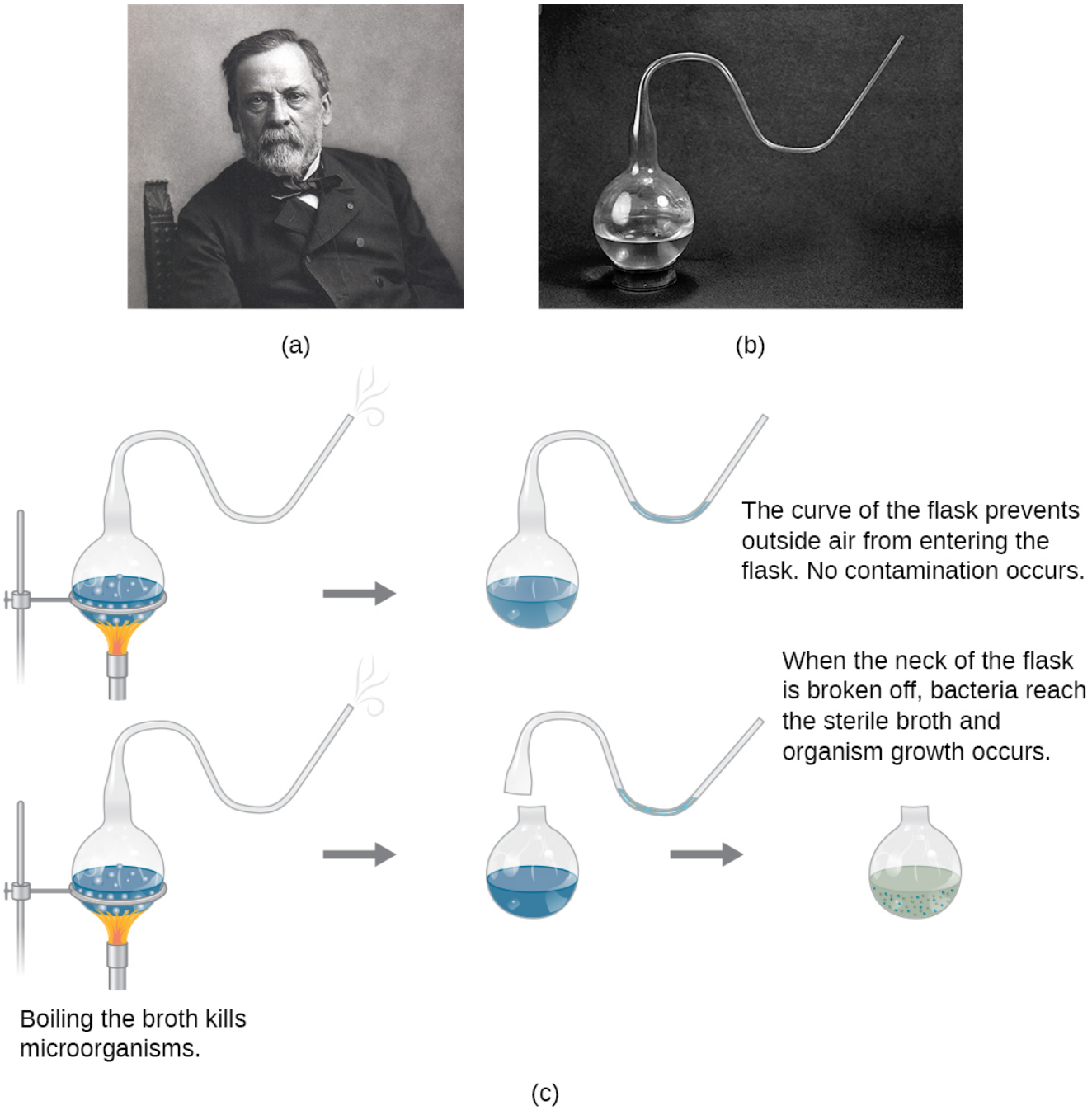


Figure **1.6.2.3**: (a) French scientist Louis Pasteur, who definitively refuted the long-disputed theory of spontaneous generation. (b) The unique swan-neck feature of the flasks used in Pasteur's experiment allowed air to enter the flask but prevented the entry of bacterial and fungal spores. (c) Pasteur's experiment consisted of two parts. In the first part, the broth in the flask was boiled to sterilize it. When this broth was cooled, it remained free of contamination. In the second part of the experiment, the flask was boiled and then the neck was broken off. The broth in this flask became contaminated. (credit b: modification of work by "Wellcome Images"/Wikimedia Commons)

? Exercise 1.6.2.3

1. How did Pasteur's experimental design allow air, but not microbes, to enter, and why was this important?
2. What was the control group in Pasteur's experiment and what did it show?

Summary

- The theory of **spontaneous generation** states that life arose from nonliving matter. It was a long-held belief dating back to Aristotle and the ancient Greeks.
- Experimentation by Francesco Redi in the 17th century presented the first significant evidence refuting spontaneous generation by showing that flies must have access to meat for maggots to develop on the meat. Prominent scientists designed experiments and argued both in support of (John Needham) and against (Lazzaro Spallanzani) spontaneous generation.
- Louis Pasteur is credited with conclusively disproving the theory of spontaneous generation with his famous swan-neck flask experiment. He subsequently proposed that “life only comes from life.”

Footnotes

1. 1 K. Zwier. “Aristotle on Spontaneous Generation.” www.sju.edu/int/academics/cas...R.%20Zwier.pdf
2. 2 E. Capanna. “Lazzaro Spallanzani: At the Roots of Modern Biology.” *Journal of Experimental Zoology* 285 no. 3 (1999):178–196.
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1.6.3: What Causes Diseases?

Learning Objectives

- Explain the key points of cell theory and the individual contributions of Hooke, Schleiden, Schwann, Remak, and Virchow
- Explain the key points of endosymbiotic theory and cite the evidence that supports this concept
- Explain the contributions of Semmelweis, Snow, Pasteur, Lister, and Koch to the development of germ theory

While some scientists were arguing over the theory of spontaneous generation, other scientists were making discoveries leading to a better understanding of what we now call the cell theory. Modern cell theory has two basic tenets:

- All cells only come from other cells (the principle of biogenesis).
- Cells are the fundamental units of organisms.

Today, these tenets are fundamental to our understanding of life on earth. However, modern cell theory grew out of the collective work of many scientists.

The Origins of Cell Theory

The English scientist Robert Hooke first used the term “cells” in 1665 to describe the small chambers within cork that he observed under a microscope of his own design. To Hooke, thin sections of cork resembled “Honey-comb,” or “small Boxes or Bladders of Air.” He noted that each “Cavern, Bubble, or Cell” was distinct from the others (Figure **1.6.3.1**). At the time, Hooke was not aware that the cork cells were long dead and, therefore, lacked the internal structures found within living cells.

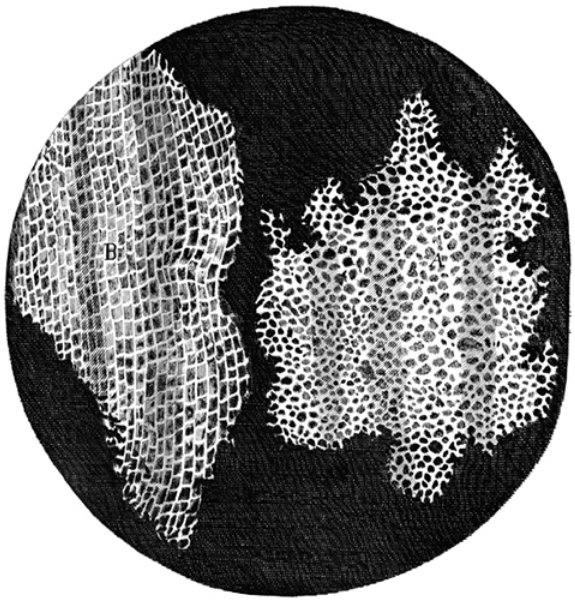


Figure **1.6.3.1**: Robert Hooke (1635–1703) was the first to describe cells based upon his microscopic observations of cork. This illustration was published in his work *Micrographia*.

Despite Hooke’s early description of cells, their significance as the fundamental unit of life was not yet recognized. Nearly 200 years later, in 1838, Matthias Schleiden (1804–1881), a German botanist who made extensive microscopic observations of plant tissues, described them as being composed of cells. Visualizing plant cells was relatively easy because plant cells are clearly separated by their thick cell walls. Schleiden believed that cells formed through crystallization, rather than cell division.

Theodor Schwann (1810–1882), a noted German physiologist, made similar microscopic observations of animal tissue. In 1839, after a conversation with Schleiden, Schwann realized that similarities existed between plant and animal tissues. This laid the foundation for the idea that cells are the fundamental components of plants and animals.

In the 1850s, two Polish scientists living in Germany pushed this idea further, culminating in what we recognize today as the modern cell theory. In 1852, Robert Remak (1815–1865), a prominent neurologist and embryologist, published convincing

evidence that cells are derived from other cells as a result of cell division. However, this idea was questioned by many in the scientific community. Three years later, Rudolf Virchow (1821–1902), a well-respected pathologist, published an editorial essay entitled "Cellular Pathology," which popularized the concept of cell theory using the Latin phrase *omnis cellula a cellula* ("all cells arise from cells"), which is essentially the second tenet of modern cell theory.[1]Given the similarity of Virchow's work to Remak's, there is some controversy as to which scientist should receive credit for articulating cell theory. See the following Eye on Ethics feature for more about this controversy.

Science and Plagiarism

Rudolf Virchow, a prominent, Polish-born, German scientist, is often remembered as the "Father of Pathology." Well known for innovative approaches, he was one of the first to determine the causes of various diseases by examining their effects on tissues and organs. He was also among the first to use animals in his research and, as a result of his work, he was the first to name numerous diseases and created many other medical terms. Over the course of his career, he published more than 2,000 papers and headed various important medical facilities, including the Charité – Universitätsmedizin Berlin, a prominent Berlin hospital and medical school. But he is, perhaps, best remembered for his 1855 editorial essay titled "Cellular Pathology," published in *Archiv für Pathologische Anatomie und Physiologie*, a journal that Virchow himself cofounded and still exists today.

Despite his significant scientific legacy, there is some controversy regarding this essay, in which Virchow proposed the central tenet of modern cell theory—that all cells arise from other cells. Robert Remak, a former colleague who worked in the same laboratory as Virchow at the University of Berlin, had published the same idea 3 years before. Though it appears Virchow was familiar with Remak's work, he neglected to credit Remak's ideas in his essay. When Remak wrote a letter to Virchow pointing out similarities between Virchow's ideas and his own, Virchow was dismissive. In 1858, in the preface to one of his books, Virchow wrote that his 1855 publication was just an editorial piece, not a scientific paper, and thus there was no need to cite Remak's work.



(a) (b)

Figure **1.6.3.2**: (a) Rudolf Virchow (1821–1902) popularized the cell theory in an 1855 essay entitled "Cellular Pathology." (b) The idea that all cells originate from other cells was first published in 1852 by his contemporary and former colleague Robert Remak (1815–1865).

By today's standards, Virchow's editorial piece would certainly be considered an act of plagiarism, since he presented Remak's ideas as his own. However, in the 19th century, standards for academic integrity were much less clear. Virchow's strong reputation, coupled with the fact that Remak was a Jew in a somewhat anti-Semitic political climate, shielded him from any significant repercussions. Today, the process of peer review and the ease of access to the scientific literature help discourage plagiarism. Although scientists are still motivated to publish original ideas that advance scientific knowledge, those who would consider plagiarizing are well aware of the serious consequences.

In academia, plagiarism represents the theft of both individual thought and research—an offense that can destroy reputations and end careers.[2 3 4 5]

? Exercise 1.6.3.1

1. What are the key points of the cell theory?
2. What contributions did Rudolf Virchow and Robert Remak make to the development of the cell theory?

The Germ Theory of Disease

Prior to the discovery of microbes during the 17th century, other theories circulated about the origins of disease. For example, the ancient Greeks proposed the miasma theory, which held that disease originated from particles emanating from decomposing matter, such as that in sewage or cesspits. Such particles infected humans in close proximity to the rotting material. Diseases including the Black Death, which ravaged Europe's population during the Middle Ages, were thought to have originated in this way.

In 1546, Italian physician Girolamo Fracastoro proposed, in his essay *De Contagione et Contagiosis Morbis*, that seed-like spores may be transferred between individuals through direct contact, exposure to contaminated clothing, or through the air. We now recognize Fracastoro as an early proponent of the germ theory of disease, which states that diseases may result from microbial infection. However, in the 16th century, Fracastoro's ideas were not widely accepted and would be largely forgotten until the 19th century.

In 1847, Hungarian obstetrician Ignaz Semmelweis (Figure **1.6.3.4**) observed that mothers who gave birth in hospital wards staffed by physicians and medical students were more likely to suffer and die from puerperal fever after childbirth (10%–20% mortality rate) than were mothers in wards staffed by midwives (1% mortality rate). Semmelweis observed medical students performing autopsies and then subsequently carrying out vaginal examinations on living patients without washing their hands in between. He suspected that the students carried disease from the autopsies to the patients they examined. His suspicions were supported by the untimely death of a friend, a physician who contracted a fatal wound infection after a postmortem examination of a woman who had died of a puerperal infection. The dead physician's wound had been caused by a scalpel used during the examination, and his subsequent illness and death closely paralleled that of the dead patient.

Although Semmelweis did not know the true cause of puerperal fever, he proposed that physicians were somehow transferring the causative agent to their patients. He suggested that the number of puerperal fever cases could be reduced if physicians and medical students simply washed their hands with chlorinated lime water before and after examining every patient. When this practice was implemented, the maternal mortality rate in mothers cared for by physicians dropped to the same 1% mortality rate observed among mothers cared for by midwives. This demonstrated that handwashing was a very effective method for preventing disease transmission. Despite this great success, many discounted Semmelweis's work at the time, and physicians were slow to adopt the simple procedure of handwashing to prevent infections in their patients because it contradicted established norms for that time period.

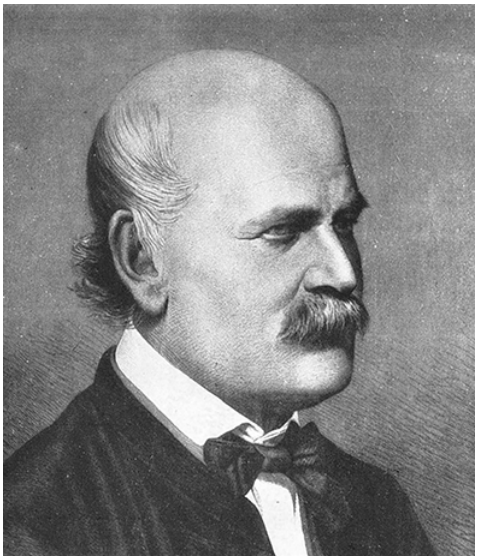


Figure **1.6.3.4**: Ignaz Semmelweis (1818–1865) was a proponent of the importance of handwashing to prevent transfer of disease between patients by physicians.

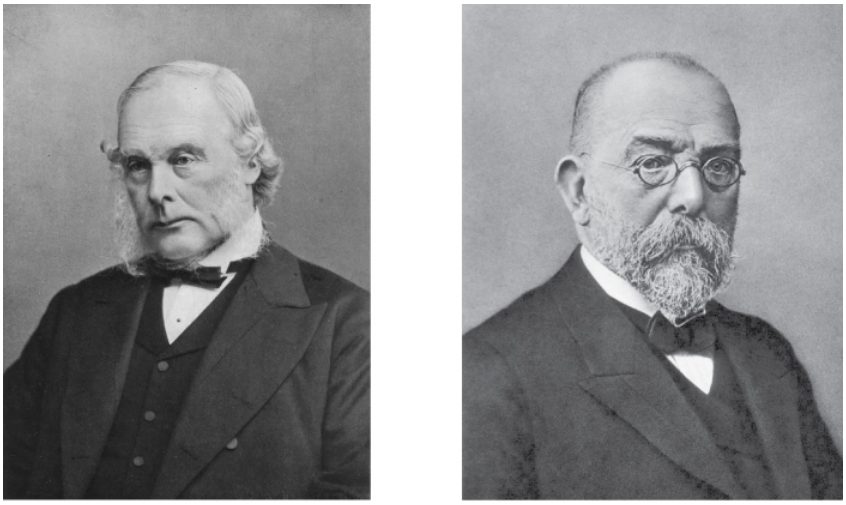
Around the same time Semmelweis was promoting handwashing, in 1848, British physician John Snow conducted studies to track the source of cholera outbreaks in London. By tracing the outbreaks to two specific water sources, both of which were contaminated by sewage, Snow ultimately demonstrated that cholera bacteria were transmitted via drinking water. Snow's work is influential in that it represents the first known epidemiological study, and it resulted in the first known public health response to an epidemic. The work of both Semmelweis and Snow clearly refuted the prevailing miasma theory of the day, showing that disease is not only transmitted through the air but also through contaminated items.

Although the work of Semmelweis and Snow successfully showed the role of sanitation in preventing infectious disease, the cause of disease was not fully understood. The subsequent work of Louis Pasteur, Robert Koch, and Joseph Listerwould further substantiate the germ theory of disease.

While studying the causes of beer and wine spoilage in 1856, Pasteur discovered properties of fermentation by microorganisms. He had demonstrated with his swan-neck flask experiments (link) that airborne microbes, not spontaneous generation, were the cause of food spoilage, and he suggested that if microbes were responsible for food spoilage and fermentation, they could also be responsible for causing infection. This was the foundation for the germ theory of disease.

Meanwhile, British surgeon Joseph Lister (Figure **1.6.3.5**) was trying to determine the causes of postsurgical infections. Many physicians did not give credence to the idea that microbes on their hands, on their clothes, or in the air could infect patients' surgical wounds, despite the fact that 50% of surgical patients, on average, were dying of postsurgical infections.[11] Lister, however, was familiar with the work of Semmelweis and Pasteur; therefore, he insisted on handwashing and extreme cleanliness during surgery. In 1867, to further decrease the incidence of postsurgical wound infections, Lister began using carbolic acid (phenol) spray disinfectant/antiseptic during surgery. His extremely successful efforts to reduce postsurgical infection caused his techniques to become a standard medical practice.

A few years later, Robert Koch (Figure **1.6.3.5**) proposed a series of postulates (Koch's postulates) based on the idea that the cause of a specific disease could be attributed to a specific microbe. Using these postulates, Koch and his colleagues were able to definitively identify the causative pathogens of specific diseases, including anthrax, tuberculosis, and cholera. Koch's "one microbe, one disease" concept was the culmination of the 19th century's paradigm shift away from miasma theory and toward the germ theory of disease. Koch's postulates are discussed more thoroughly in How Pathogens Cause Disease.



(a) (b)

Figure **1.6.3.5**: (a) Joseph Lister developed procedures for the proper care of surgical wounds and the sterilization of surgical equipment. (b) Robert Koch established a protocol to determine the cause of infectious disease. Both scientists contributed significantly to the acceptance of the germ theory of disease.

? Exercise 1.6.3.3

1. Compare and contrast the miasma theory of disease with the germ theory of disease.

2. How did Joseph Lister's work contribute to the debate between the miasma theory and germ theory and how did this increase the success of medical procedures?

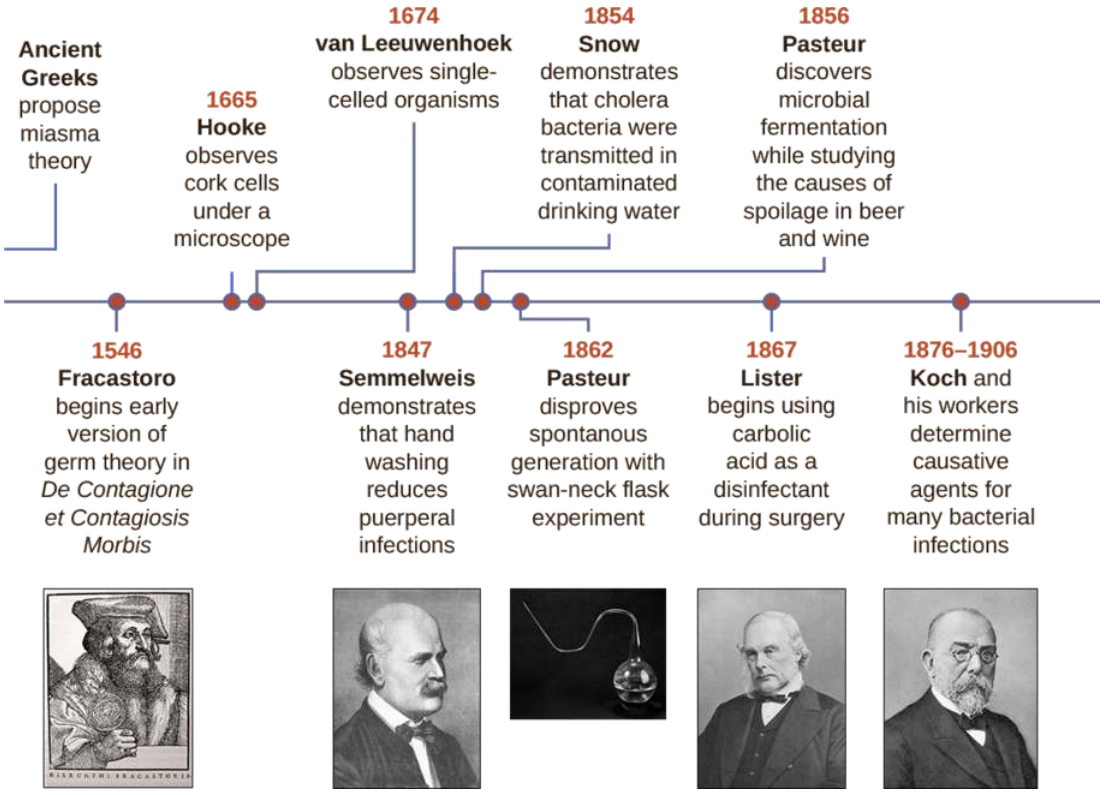


Figure 1.6.3.7: (credit "swan-neck flask": modification of work by Wellcome Images)

Key Concepts and Summary

- Although cells were first observed in the 1660s by Robert Hooke, **cell theory** was not well accepted for another 200 years. The work of scientists such as Schleiden, Schwann, Remak, and Virchow contributed to its acceptance.
- The **miasma theory of disease** was widely accepted until the 19th century, when it was replaced by the **germ theory of disease** thanks to the work of Semmelweis, Snow, Pasteur, Lister, and Koch, and others.

Footnotes

1. 1 M. Schultz. "Rudolph Virchow." *Emerging Infectious Diseases* 14 no. 9 (2008):1480–1481.
2. 2 B. Kisch. "Forgotten Leaders in Modern Medicine, Valentin, Gouby, Remak, Auerbach." *Transactions of the American Philosophical Society* 44 (1954):139–317.
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10. 10 J.M. Jaynes, L.P. Vernon. "The Cyanelle of *Cyanophora paradoxa*: Almost a Cyanobacterial Chloroplast." *Trends in Biochemical Sciences* 7 no. 1 (1982):22–24.
11. 11 Alexander, J. Wesley. "The Contributions of Infection Control to a Century of Progress" *Annals of Surgery* 201:423-428, 1985.

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1.6.4: Koch and Pure Culture

Learning Objectives

- Explain Robert Koch's postulates

Robert Koch was born in Clausthal in the Harz Mountains, then part of the Kingdom of Hanover, as the son of a mining official. He studied medicine at the University of Göttingen and graduated in 1866. He then served in the Franco-Prussian War and later became district medical officer in Wollstein (Wolsztyn), Prussian Poland. Working with very limited resources, he became one of the founders of bacteriology, the other major figure being Louis Pasteur.



Figure: ***Robert Koch****: An image of Robert Koch, a pioneering microbiologist. Koch's research and methods helped link the causal nature of microbes to certain diseases, including anthrax.*

After Casimir Davaine demonstrated the direct transmission of the anthrax bacillus between cows, Koch studied anthrax more closely. He invented methods to purify the bacillus from blood samples and grow pure cultures. He found that, while it could not survive outside a host for long, anthrax built persisting endospores that could last a long time. These endospores, embedded in soil, were the cause of unexplained "spontaneous" outbreaks of anthrax. Koch published his findings in 1876 and was rewarded with a job at the Imperial Health Office in Berlin in 1880. In 1881, he urged for the sterilization of surgical instruments using heat.

Probably as important as his work on tuberculosis, for which he was awarded a Nobel Prize in 1905, are Koch's postulates. These postulates stated that to establish that an organism is the cause of a disease, it must be found in all cases of the disease examined. Additionally, it must be absent in healthy organisms prepared and maintained in a pure culture capable of producing the original infection, even after several generations in culture retrievable from an inoculated animal and cultured again. By using his methods, Koch's pupils found the organisms responsible for diphtheria, typhoid, pneumonia, gonorrhoea, cerebrospinal meningitis, leprosy, bubonic plague, tetanus, and syphilis.

Perhaps the key method Koch developed was the ability to isolate pure cultures, explained in brief here. Pure cultures of multicellular organisms are often more easily isolated by simply picking out a single individual to initiate a culture. This is a useful technique for pure culture of fungi, multicellular algae, and small metazoa. Developing pure culture techniques is crucial to the observation of the specimen in question. The most common method to isolate individual microbes and produce a pure culture is to prepare a streak plate. The streak plate method is a way to physically separate the microbial population and is done by spreading the inoculate back and forth with an inoculating loop over the solid agar plate. Upon incubation, colonies will arise and single cells will have been isolated from the biomass.

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Key Points

- Koch’s research and methods helped link the causal nature of microbes to certain diseases, such as anthrax.
- As developed by Koch, pure cultures allow the pure isolation of a microbe, which is vital in understanding how an individual microbe may contribute to a disease.
- According to Koch’s postulates, for an organism to be the cause of a disease, it must be found in all cases of the disease and must be absent from healthy organisms, as well as maintained in pure culture capable of producing the original infection.

Key Terms

- **anthrax**: An infectious bacterial disease of herbivores than can also occur in humans through contact with infected animals, tissue from infected animals, or high concentrations of anthrax spores.
- **metazoa**: All those multicellular animals, of the subkingdom Metazoa, that have differentiated tissue.
- **tuberculosis**: An infectious disease of humans and animals caused by a species of mycobacterium mainly infecting the lungs where it causes tubercles characterized by the expectoration of mucus and sputum, fever, weight loss, and chest pain, and transmitted through inhalation or ingestion of bacteria.

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1.6.5: Koch's Postulate

Learning Objectives

- Summarize Koch's postulates and molecular Koch's postulates, respectively, and explain their significance and limitations
- Explain the concept of pathogenicity (virulence) in terms of infectious and lethal dose
- Distinguish between primary and opportunistic pathogens and identify specific examples of each
- Summarize the stages of pathogenesis
- Explain the roles of portals of entry and exit in the transmission of disease and identify specific examples of these portals

For most infectious diseases, the ability to accurately identify the causative pathogen is a critical step in finding or prescribing effective treatments. Today's physicians, patients, and researchers owe a sizable debt to the physician Robert Koch (1843–1910), who devised a systematic approach for confirming causative relationships between diseases and specific pathogens.

Koch's Postulates

In 1884, Koch published four postulates that summarized his method for determining whether a particular microorganism was the cause of a particular disease. Each of Koch's postulates represents a criterion that must be met before a disease can be positively linked with a pathogen. In order to determine whether the criteria are met, tests are performed on laboratory animals and cultures from healthy and diseased animals are compared (Figure **1.6.5.1**).

Koch's Postulates

1. The suspected pathogen must be found in every case of disease and not be found in healthy individuals.
2. The suspected pathogen can be isolated and grown in pure culture.
3. A healthy test subject infected with the suspected pathogen must develop the same signs and symptoms of disease as seen in postulate
4. The pathogen must be re-isolated from the new host and must be identical to the pathogen from postulate 2.

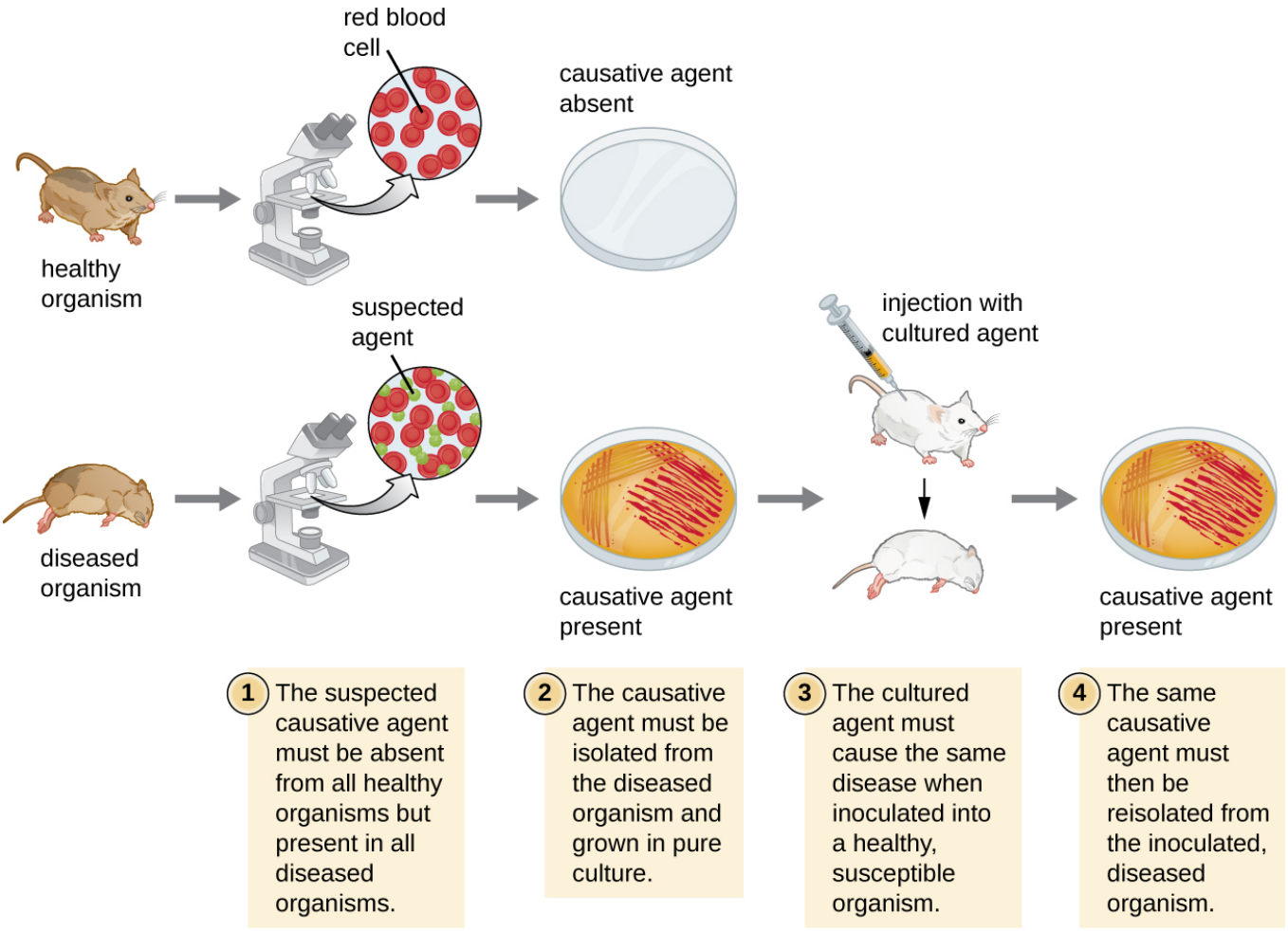


Figure **1.6.5.1**: The steps for confirming that a pathogen is the cause of a particular disease using Koch's postulates.

In many ways, Koch's postulates are still central to our current understanding of the causes of disease. However, advances in microbiology have revealed some important limitations in Koch's criteria. Koch made several assumptions that we now know are untrue in many cases. The first relates to postulate 1, which assumes that pathogens are only found in diseased, not healthy, individuals. This is not true for many pathogens. For example, *H. pylori*, described earlier in this chapter as a pathogen causing chronic gastritis, is also part of the normal microbiota of the stomach in many healthy humans who never develop gastritis. It is estimated that upwards of 50% of the human population acquires *H. pylori* early in life, with most maintaining it as part of the normal microbiota for the rest of their life without ever developing disease.

Koch's second faulty assumption was that all healthy test subjects are equally susceptible to disease. We now know that individuals are not equally susceptible to disease. Individuals are unique in terms of their microbiota and the state of their immune system at any given time. The makeup of the resident microbiota can influence an individual's susceptibility to an infection. Members of the normal microbiota play an important role in immunity by inhibiting the growth of transient pathogens. In some cases, the microbiota may prevent a pathogen from establishing an infection; in others, it may not prevent an infection altogether but may influence the severity or type of signs and symptoms. As a result, two individuals with the same disease may not always present with the same signs and symptoms. In addition, some individuals have stronger immune systems than others. Individuals with immune systems weakened by age or an unrelated illness are much more susceptible to certain infections than individuals with strong immune systems.

Koch also assumed that all pathogens are microorganisms that can be grown in pure culture (postulate 2) and that animals could serve as reliable models for human disease. However, we now know that not all pathogens can be grown in pure culture, and many human diseases cannot be reliably replicated in animal hosts. Viruses and certain bacteria, including *Rickettsia* and *Chlamydia*, are obligate intracellular pathogens that can grow only when inside a host cell. If a microbe cannot be cultured, a researcher cannot

move past postulate 2. Likewise, without a suitable nonhuman host, a researcher cannot evaluate postulate 2 without deliberately infecting humans, which presents obvious ethical concerns. AIDS is an example of such a disease because the human immunodeficiency virus (HIV) only causes disease in humans.

? Exercise 1.6.5.1

Briefly summarize the limitations of Koch's postulates.

Key Concepts and Summary

- **Koch's postulates** are used to determine whether a particular microorganism is a pathogen.

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1.6.6: Improvement in Public Health

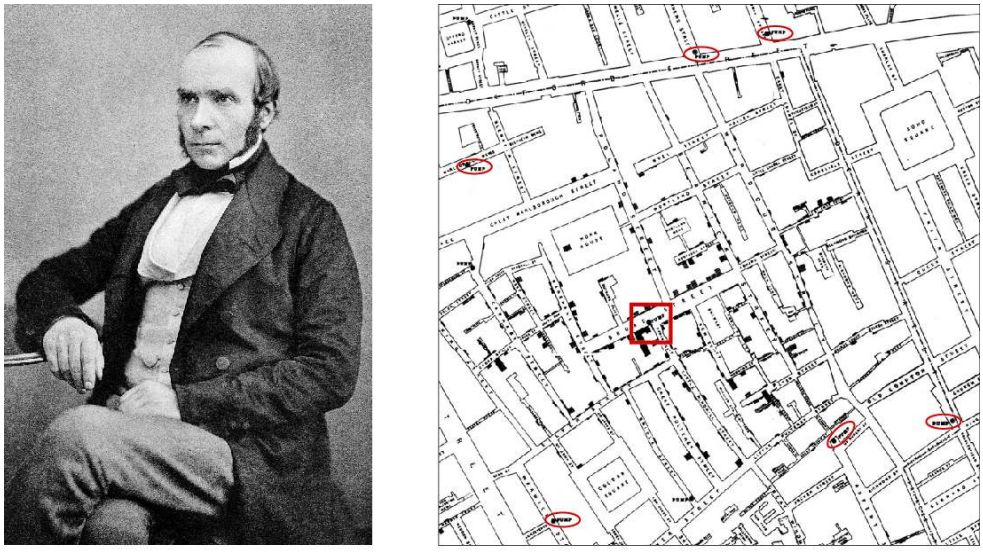
Learning Objectives

- Explain the research approaches used by the pioneers of epidemiology
- Explain how descriptive, analytical, and experimental epidemiological studies go about determining the cause of morbidity and mortality

Epidemiology has its roots in the work of physicians who looked for patterns in disease occurrence as a way to understand how to prevent it. The idea that disease could be transmitted was an important precursor to making sense of some of the patterns. In 1546, Girolamo Fracastoro first proposed the germ theory of disease in his essay *De Contagione et Contagiosis Morbis*, but this theory remained in competition with other theories, such as the miasma hypothesis, for many years (see What Our Ancestors Knew). Uncertainty about the cause of disease was not an absolute barrier to obtaining useful knowledge from patterns of disease. Some important researchers, such as Florence Nightingale, subscribed to the miasma hypothesis. The transition to acceptance of the germ theory during the 19th century provided a solid mechanistic grounding to the study of disease patterns. The studies of 19th century physicians and researchers such as John Snow, Florence Nightingale, Ignaz Semmelweis, Joseph Lister, Robert Koch, Louis Pasteur, and others sowed the seeds of modern epidemiology.

Pioneers of Epidemiology

John Snow (Figure **1.6.6.1**) was a British physician known as the father of epidemiology for determining the source of the 1854 Broad Street cholera epidemic in London. Based on observations he had made during an earlier cholera outbreak (1848–1849), Snow proposed that cholera was spread through a fecal-oral route of transmission and that a microbe was the infectious agent. He investigated the 1854 cholera epidemic in two ways. First, suspecting that contaminated water was the source of the epidemic, Snow identified the source of water for those infected. He found a high frequency of cholera cases among individuals who obtained their water from the River Thames downstream from London. This water contained the refuse and sewage from London and settlements upstream. He also noted that brewery workers did not contract cholera and on investigation found the owners provided the workers with beer to drink and stated that they likely did not drink water.[1] Second, he also painstakingly mapped the incidence of cholera and found a high frequency among those individuals using a particular water pump located on Broad Street. In response to Snow's advice, local officials removed the pump's handle,[2] resulting in the containment of the Broad Street cholera epidemic.



(a) (b)

Figure **1.6.6.1**: (a) John Snow (1813–1858), British physician and father of epidemiology. (b) Snow's detailed mapping of cholera incidence led to the discovery of the contaminated water pump on Broad street (red square) responsible for the 1854 cholera epidemic. (credit a: modification of work by "Rsabbatini"/Wikimedia Commons)

Snow's work represents an early epidemiological study and it resulted in the first known public health response to an epidemic. Snow's meticulous case-tracking methods are now common practice in studying disease outbreaks and in associating new diseases with their causes. His work further shed light on unsanitary sewage practices and the effects of waste dumping in the Thames. Additionally, his work supported the germ theory of disease, which argued disease could be transmitted through contaminated items, including water contaminated with fecal matter.

Snow's work illustrated what is referred to today as a common source spread of infectious disease, in which there is a single source for all of the individuals infected. In this case, the single source was the contaminated well below the Broad Street pump. Types of common source spread include point source spread, continuous common source spread, and intermittent common source spread. In point source spread of infectious disease, the common source operates for a short time period—less than the incubation period of the pathogen. An example of point source spread is a single contaminated potato salad at a group picnic. In continuous common source spread, the infection occurs for an extended period of time, longer than the incubation period. An example of continuous common source spread would be the source of London water taken downstream of the city, which was continuously contaminated with sewage from upstream. Finally, with intermittent common source spread, infections occur for a period, stop, and then begin again. This might be seen in infections from a well that was contaminated only after large rainfalls and that cleared itself of contamination after a short period.

In contrast to common source spread, propagated spread occurs through direct or indirect person-to-person contact. With propagated spread, there is no single source for infection; each infected individual becomes a source for one or more subsequent infections. With propagated spread, unless the spread is stopped immediately, infections occur for longer than the incubation period. Although point sources often lead to large-scale but localized outbreaks of short duration, propagated spread typically results in longer duration outbreaks that can vary from small to large, depending on the population and the disease (Figure **1.6.6.2**). In addition, because of person-to-person transmission, propagated spread cannot be easily stopped at a single source like point source spread.

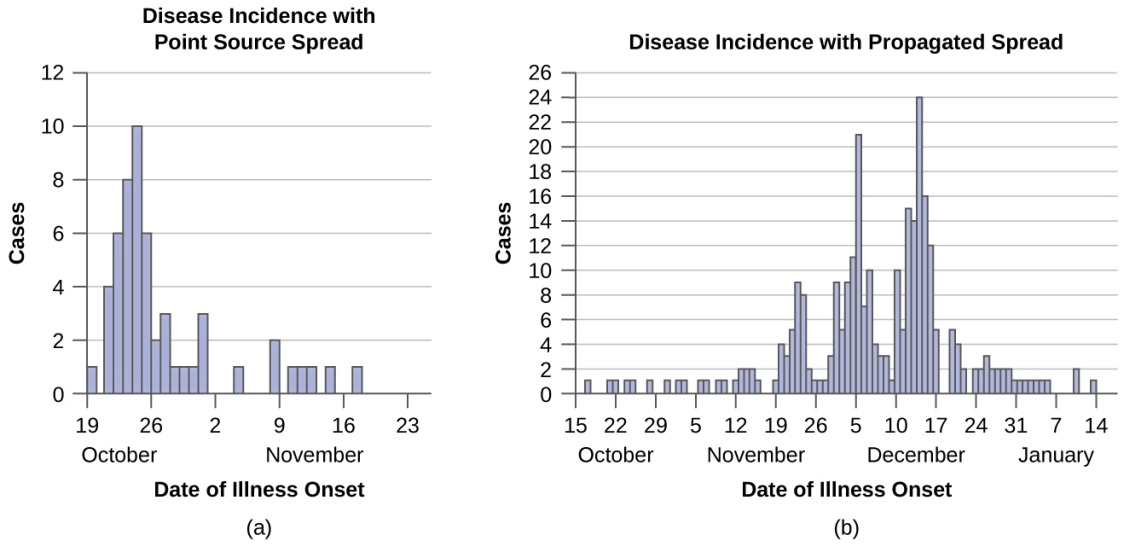
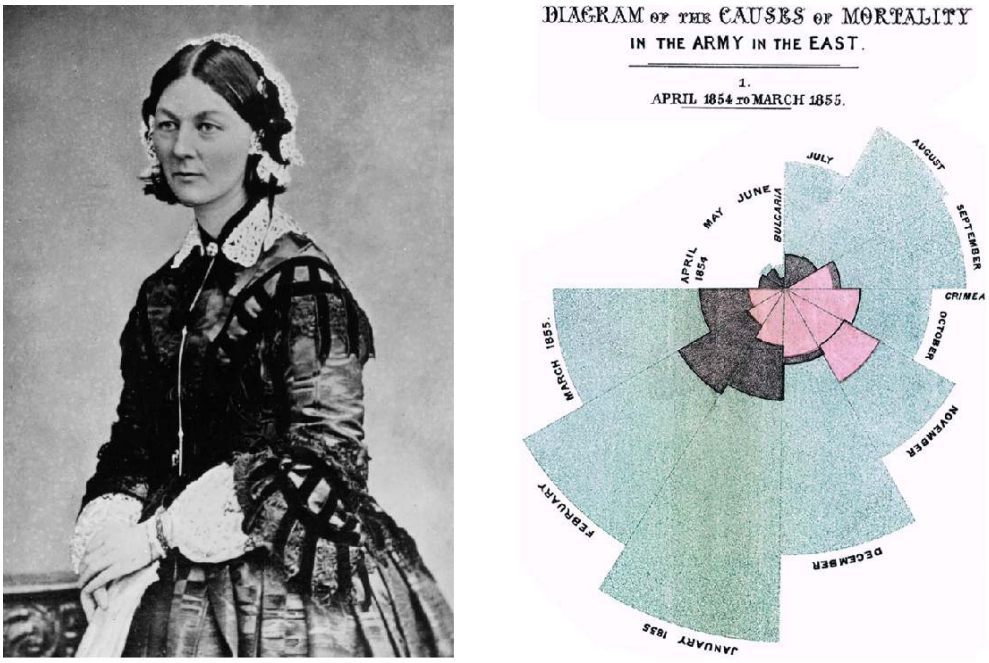


Figure **1.6.6.2**: (a) Outbreaks that can be attributed to point source spread often have a short duration. (b) Outbreaks attributed to propagated spread can have a more extended duration. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Florence Nightingale's work is another example of an early epidemiological study. In 1854, Nightingale was part of a contingent of nurses dispatched by the British military to care for wounded soldiers during the Crimean War. Nightingale kept meticulous records regarding the causes of illness and death during the war. Her recordkeeping was a fundamental task of what would later become the science of epidemiology. Her analysis of the data she collected was published in 1858. In this book, she presented monthly frequency data on causes of death in a wedge chart histogram (Figure **1.6.6.3**). This graphical presentation of data, unusual at the time, powerfully illustrated that the vast majority of casualties during the war occurred not due to wounds sustained in action but to what Nightingale deemed preventable infectious diseases. Often these diseases occurred because of poor sanitation and lack of access to hospital facilities. Nightingale's findings led to many reforms in the British military's system of medical care.

Joseph Lister provided early epidemiological evidence leading to good public health practices in clinics and hospitals. These settings were notorious in the mid-1800s for fatal infections of surgical wounds at a time when the germ theory of disease was not yet widely accepted (see Foundations of Modern Cell Theory). Most physicians did not wash their hands between patient visits or clean and sterilize their surgical tools. Lister, however, discovered the disinfecting properties of carbolic acid, also known as phenol (see Using Chemicals to Control Microorganisms). He introduced several disinfection protocols that dramatically lowered post-surgical infection rates.[3] He demanded that surgeons who worked for him use a 5% carbolic acid solution to clean their surgical tools between patients, and even went so far as to spray the solution onto bandages and over the surgical site during operations (Figure 1.6.6.4). He also took precautions not to introduce sources of infection from his skin or clothing by removing his coat, rolling up his sleeves, and washing his hands in a dilute solution of carbolic acid before and during the surgery.



(a) (b)

Figure 1.6.6.3: (a) Florence Nightingale reported on the data she collected as a nurse in the Crimean War. (b) Nightingale's diagram shows the number of fatalities in soldiers by month of the conflict from various causes. The total number dead in a particular month is equal to the area of the wedge for that month. The colored sections of the wedge represent different causes of death: wounds (pink), preventable infectious diseases (gray), and all other causes (brown).

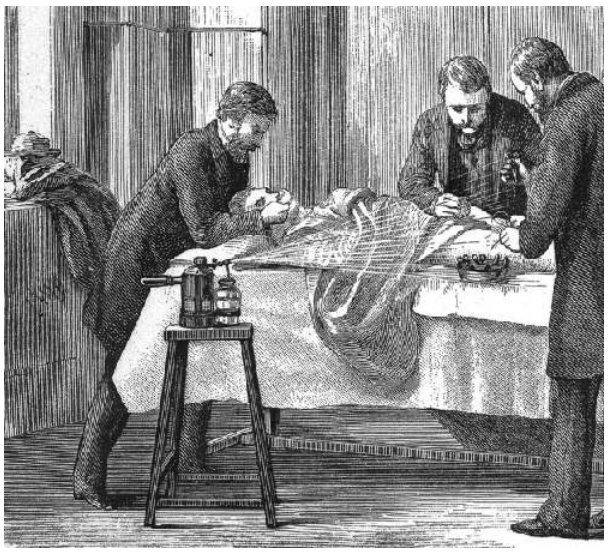


Figure 1.6.6.4: Joseph Lister initiated the use of a carbolic acid (phenol) during surgeries. This illustration of a surgery shows a pressurized canister of carbolic acid being sprayed over the surgical site.

Link to Learning

John Snow's own account of his work has additional links and information.

This CDC resource further breaks down the pattern expected from a point-source outbreak.

Learn more about Nightingale's wedge chart here.

Exercise 1.6.6.1

1. Explain the difference between common source spread and propagated spread of disease.
2. Describe how the observations of John Snow, Florence Nightingale, and Joseph Lister led to improvements in public health.

Key Concepts and Summary

- Early pioneers of epidemiology such as John Snow, Florence Nightingale, and Joseph Lister, studied disease at the population level and used data to disrupt disease transmission.
- **Descriptive epidemiology** studies rely on case analysis and patient histories to gain information about outbreaks, frequently while they are still occurring.
- **Retrospective epidemiology** studies use historical data to identify associations with the disease state of present cases. **Prospective epidemiology** studies gather data and follow cases to find associations with future disease states.
- **Analytical epidemiology** studies are observational studies that are carefully designed to compare groups and uncover associations between environmental or genetic factors and disease.
- **Experimental epidemiology** studies generate strong evidence of causation in disease or treatment by manipulating subjects and comparing them with control subjects.

Footnotes

1. John Snow. *On the Mode of Communication of Cholera. Second edition, Much Enlarged*. John Churchill, 1855.
2. John Snow. "The Cholera near Golden-Wquare, and at Deptford." *Medical Times and Gazette* 9 (1854): 321–322. http://www.ph.ucla.edu/epi/snow/chol...densquare.html.
3. O.M. Lidwell. "Joseph Lister and Infection from the Air." *Epidemiology and Infection* 99 (1987): 569–578. www.ncbi.nlm.nih.gov/pmc/arti...00006-0004.pdf.

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1.6.7: The Discovery of Antimicrobial Drugs

Learning Objectives

- Describe the concept of ‘antibiosis’ and the contributions of the different scientists who discovered it

Before the early 20th century, treatments for infections were based primarily on medicinal folklore. Mixtures with antimicrobial properties that were used in treatments of infections were described over 2000 years ago. Many ancient cultures, including the ancient Egyptians and ancient Greeks, used specially selected mold and plant materials and extracts to treat infections. More recent observations made in the laboratory of antibiosis between micro- organisms led to the discovery of natural antibacterials produced by microorganisms.

Louis Pasteur observed, “if we could intervene in the antagonism observed between some bacteria, it would offer perhaps the greatest hopes for therapeutics”. The term ‘antibiosis’, meaning “against life,” was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these early antibacterial drugs. Antibiosis was first described in 1877 in bacteria when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of Bacillus anthracis. These drugs were later renamed antibiotics by Selman Waksman, an American microbiologist, in 1942.

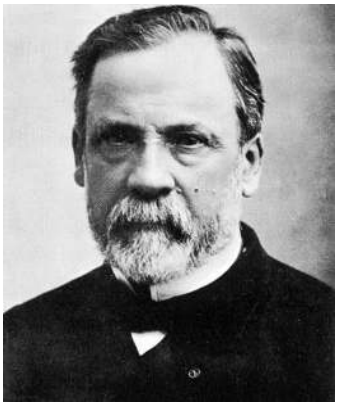


Figure: **Louis Pasteur**: Louis Pasteur was a French microbiologist and chemist best known for their experiments supporting the Germ theory of disease, and for his vaccinations, most notably the first vaccine against rabies.

John Tyndall first described antagonistic activities by fungi against bacteria in England in 1875. Synthetic antibiotic chemotherapy as a science and development of antibacterials began in Germany with Paul Ehrlich in the late 1880s. Ehrlich noted certain dyes would color human, animal, or bacterial cells, while others did not. He then proposed the idea that it might be possible to create chemicals that would act as a selective drug that would bind to and kill bacteria without harming the human host. After screening hundreds of dyes against various organisms, he discovered a medicinally useful drug, the synthetic antibacterial Salvarsan now called arsphenamine. In 1895, Vincenzo Tiberio, physician of the University of Naples discovered that a mold (Penicillium) in a water well has an antibacterial action. After this initial chemotherapeutic compound proved effective, others pursued similar lines of inquiry, but it was not until in 1928 that Alexander Fleming observed antibiosis against bacteria by a fungus of the genus Penicillium. Fleming postulated the effect was mediated by an antibacterial compound named penicillin, and that its antibacterial properties could be exploited for chemotherapy. He initially characterized some of its biological properties, but he did not pursue its further development.

Key Points

- Before the early 20th century, treatments for infections were based primarily on medicinal folklore.
- Louis Pasteur observed, “if we could intervene in the antagonism observed between some bacteria, it would offer perhaps the greatest hopes for therapeutics”.
- The term ‘antibiosis’, meaning “against life,” was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these early antibacterial drugs.

Key Terms

- **micro-organism**: A microorganism (from the Greek: μ, mikrós, “small” and ὀ, organismós, “organism”; also spelled micro-organism, micro organism or microörganism) or microbe is a microscopic organism that comprises either a single cell (unicellular), cell clusters, or multicellular relatively complex organisms.
- **chemotherapy**: Any chemical treatment intended to be therapeutic with respect to a disease state.
- **infection**: An uncontrolled growth of harmful microorganisms in a host.

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1.6.E: The History (Exercises)

3.1: Spontaneous Generation

The theory of spontaneous generation states that life arose from nonliving matter. It was a long-held belief dating back to Aristotle and the ancient Greeks. Experimentation by Francesco Redi in the 17th century presented the first significant evidence refuting spontaneous generation by showing that flies must have access to meat for maggots to develop on the meat. Louis Pasteur is credited with conclusively disproving the theory and proposed that “life only comes from life.”

Multiple Choice

Which of the following individuals argued in favor of the theory of spontaneous generation?

A. Francesco Redi
B. Louis Pasteur
C. John Needham
D. Lazzaro Spallanzani

Answer

C

Which of the following individuals is credited for definitively refuting the theory of spontaneous generation using broth in swan-neck flask?

A. Aristotle
B. Jan Baptista van Helmont
C. John Needham
D. Louis Pasteur

Answer

D

Which of the following experimented with raw meat, maggots, and flies in an attempt to disprove the theory of spontaneous generation.

A. Aristotle
B. Lazzaro Spallanzani
C. Antonie van Leeuwenhoek
D. Francesco Redi

Answer

D

Fill in the Blank

The assertion that “life only comes from life” was stated by Louis Pasteur in regard to his experiments that definitively refuted the theory of _____.

Answer

spontaneous generation

True/False

Exposure to air is necessary for microbial growth.

Answer

False

Short Answer

Explain in your own words Pasteur's swan-neck flask experiment.

Explain why the experiments of Needham and Spallanzani yielded in different results even though they used similar methodologies.

Critical Thinking

What would the results of Pasteur's swan-neck flask experiment have looked like if they supported the theory of spontaneous generation?

3.2: Foundations of Modern Cell Theory

Although cells were first observed in the 1660s by Robert Hooke, cell theory was not well accepted for another 200 years. The work of scientists such as Schleiden, Schwann, Remak, and Virchow contributed to its acceptance. Endosymbiotic theory states that mitochondria and chloroplasts, organelles found in many types of organisms, have their origins in bacteria. Significant structural and genetic information support this theory. The miasma theory was widely accepted until the 19th century.

Multiple Choice

Which of the following individuals did not contribute to the establishment of cell theory?

A. Girolamo Fracastoro
B. Matthias Schleiden
C. Robert Remak
D. Robert Hooke

Answer

A

Whose proposal of the endosymbiotic theory of mitochondrial and chloroplast origin was ultimately accepted by the greater scientific community?

A. Rudolf Virchow
B. Ignaz Semmelweis
C. Lynn Margulis
D. Theodor Schwann

Answer

C

Which of the following developed a set of postulates for determining whether a particular disease is caused by a particular pathogen?

A. John Snow
B. Robert Koch
C. Joseph Lister
D. Louis Pasteur

Answer

B

Fill in the Blank

John Snow is known as the Father of _____.

Answer

epidemiology

The _____ theory states that disease may originate from proximity to decomposing matter and is not due to person-to-person contact.

Answer

miasma

The scientist who first described cells was _____.

Answer

Robert Hooke

Short Answer

How did the explanation of Virchow and Remak for the origin of cells differ from that of Schleiden and Schwann?

What evidence exists that supports the endosymbiotic theory?

What were the differences in mortality rates due to puerperal fever that Ignaz Semmelweis observed? How did he propose to reduce the occurrence of puerperal fever? Did it work?

Critical Thinking

Why are mitochondria and chloroplasts unable to multiply outside of a host cell?

Why was the work of Snow so important in supporting the germ theory?

3.3: Unique Characteristics of Prokaryotic Cells

Prokaryotic cells differ from eukaryotic cells in that their genetic material is contained in a nucleoid rather than a membrane-bound nucleus. In addition, prokaryotic cells generally lack membrane-bound organelles. Prokaryotic cells of the same species typically share a similar cell morphology and cellular arrangement. Most prokaryotic cells have a cell wall that helps the organism maintain cellular morphology and protects it against changes in osmotic pressure.

Multiple Choice

Which of the following terms refers to a prokaryotic cell that is comma shaped?

A. coccus
B. coccobacilli
C. vibrio
D. spirillum

Answer

C

Which bacterial structures are important for adherence to surfaces? (Select all that apply.)

A. endospores
B. cell walls
C. fimbriae
D. capsules
E. flagella

Answer

C, D

Which of the following cell wall components is unique to gram-negative cells?

A. lipopolysaccharide
B. teichoic acid
C. mycolic acid
D. peptidoglycan

Answer

A

Which of the following terms refers to a bacterial cell having a single tuft of flagella at one end?

A. monotrichous
B. amphitrichous
C. peritrichous
D. lophotrichous

Answer

D

Bacterial cell walls are primarily composed of which of the following?

A. phospholipid
B. protein
C. carbohydrate
D. peptidoglycan

Answer

D

True/False

Bacteria have 80S ribosomes each composed of a 60S large subunit and a 40S small subunit.

Answer

False

Fill in the Blank

Prokaryotic cells that are rod-shaped are called _____.

Answer

bacilli

The type of inclusion containing polymerized inorganic phosphate is called _____.

Answer

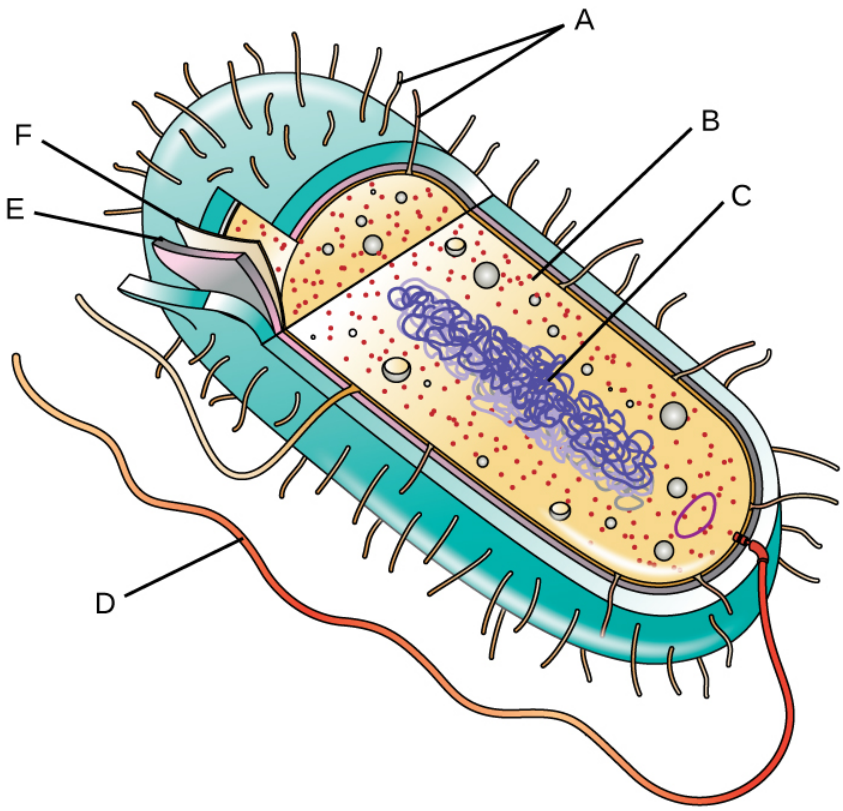
volutin (or metachromatic granule)

Short Answer

What is the direction of water flow for a bacterial cell living in a hypotonic environment? How do cell walls help bacteria living in such environments?

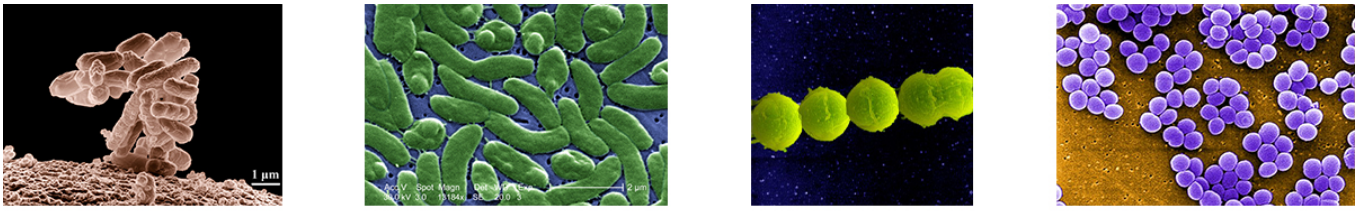
How do bacterial flagella respond to a chemical gradient of an attractant to move toward a higher concentration of the chemical?

Label the parts of the prokaryotic cell.



Critical Thinking

Which of the following slides is a good example of staphylococci?



(credit a: modification of work by U.S. Department of Agriculture; credit b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by NIAID)

Provide some examples of bacterial structures that might be used as antibiotic targets and explain why.

The causative agent of botulism, a deadly form of food poisoning, is an endospore-forming bacterium called *Clostridium botulinim*. Why might it be difficult to kill this bacterium in contaminated food?

3.4: Unique Characteristics of Eukaryotic Cells

Eukaryotic cells are defined by the presence of a nucleus containing the DNA genome and bound by a nuclear membrane (or nuclear envelope) composed of two lipid bilayers that regulate transport of materials into and out of the nucleus through nuclear pores. Eukaryotic cell morphologies vary greatly and may be maintained by various structures, including the cytoskeleton, the cell membrane, and/or the cell wall. The nucleolus in the nucleus of eukaryotic cells is the site of ribosomal synthesis.

Multiple Choice

Which of the following organelles is not part of the endomembrane system?

A. endoplasmic reticulum

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B. Golgi apparatus
C. lysosome
D. peroxisome

Answer

D

Which type of cytoskeletal fiber is important in the formation of the nuclear lamina?

A. microfilaments
B. intermediate filaments
C. microtubules
D. fibronectin

Answer

B

Sugar groups may be added to proteins in which of the following?

A. smooth endoplasmic reticulum
B. rough endoplasmic reticulum
C. Golgi apparatus
D. lysosome

Answer

C

Which of the following structures of a eukaryotic cell is not likely derived from endosymbiotic bacterium?

A. mitochondrial DNA
B. mitochondrial ribosomes
C. inner membrane
D. outer membrane

Answer

D

Which type of nutrient uptake involves the engulfment of small dissolved molecules into vesicles?

A. active transport
B. pinocytosis
C. receptor-mediated endocytosis
D. facilitated diffusion

Answer

B

Which of the following is not composed of microtubules?

A. desmosomes
B. centrioles
C. eukaryotic flagella
D. eukaryotic cilia

Answer

A

True/False

Mitochondria in eukaryotic cells contain ribosomes that are structurally similar to those found in prokaryotic cells.

Answer

True

Fill in the Blank

Peroxisomes typically produce _____, a harsh chemical that helps break down molecules.

Answer

hydrogen peroxide

Microfilaments are composed of _____ monomers.

Answer

actin

Short Answer

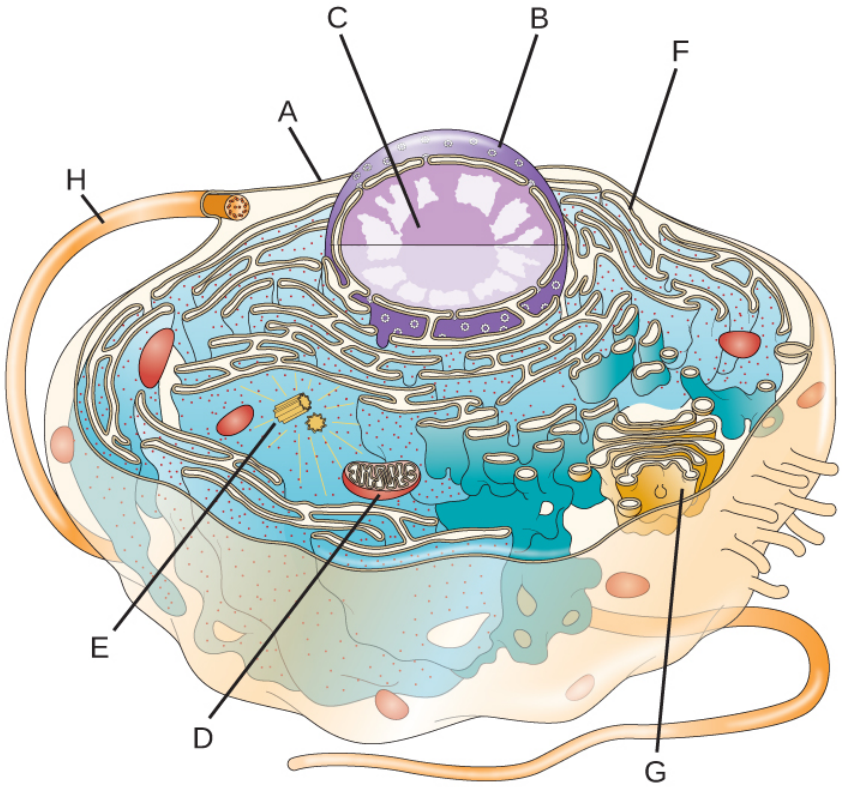
What existing evidence supports the theory that mitochondria are of prokaryotic origin?

Why do eukaryotic cells require an endomembrane system?

Name at least two ways that prokaryotic flagella are different from eukaryotic flagella.

Critical Thinking

Label the lettered parts of this eukaryotic cell.



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How are peroxisomes more like mitochondria than like the membrane-bound organelles of the endomembrane system? How do they differ from mitochondria?

Why must the functions of both lysosomes and peroxisomes be compartmentalized?

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CHAPTER OVERVIEW

2: Chemistry of Microbiology

2.1: Atomic Structure

2.1.1: Overview of Atomic Structure

2.1.2: Electronic Orbitals

2.2: Chemical Bonds

2.2.1: Ions and Ionic Bonds

2.2.2: Covalent Bonds and Other Bonds and Interaction

2.2.3: Hydrogen Bonding

2.3: Chemical Reactions

2.3.1: The Chemical Basis of Life

2.3.2: Synthesis of Biological Macromolecules

2.3.3: Energy and Biochemical Reactions

2.4: Water and pH

2.4.1: Biochemical Properties of Water

2.4.2: pH, Buffers, Acids, and Bases

2.5: Organic Compounds

2.5.1: Organic Molecules

2.5.2: Carbohydrate Molecules

2.5.3: Lipid Molecules

2.5.4: DNA and RNA

2.5.5: Amino Acids

2.5.6: ATP- Adenosine Triphosphate

2.5.E: Organic Molecules (Exercises)

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SECTION OVERVIEW

2.1: Atomic Structure

Topic hierarchy

2.1.1: Overview of Atomic Structure

2.1.2: Electronic Orbitals

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2.1.1: Overview of Atomic Structure

Learning Objectives

- Discuss the electronic and structural properties of an atom

An atom is the smallest unit of matter that retains all of the chemical properties of an element. Atoms combine to form molecules, which then interact to form solids, gases, or liquids. For example, water is composed of hydrogen and oxygen atoms that have combined to form water molecules. Many biological processes are devoted to breaking down molecules into their component atoms so they can be reassembled into a more useful molecule.

Atomic Particles

Atoms consist of three basic particles: protons, electrons, and neutrons. The nucleus (center) of the atom contains the protons (positively charged) and the neutrons (no charge). The outermost regions of the atom are called electron shells and contain the electrons (negatively charged). Atoms have different properties based on the arrangement and number of their basic particles.

The hydrogen atom (H) contains only one proton, one electron, and no neutrons. This can be determined using the atomic number and the mass number of the element (see the concept on atomic numbers and mass numbers).

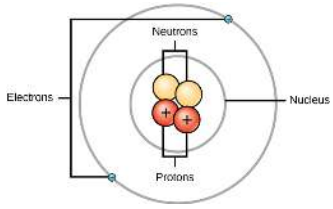


Figure: **Structure of an atom**: Elements, such as helium, depicted here, are made up of atoms. Atoms are made up of protons and neutrons located within the nucleus, with electrons in orbitals surrounding the nucleus.

Atomic Mass

Protons and neutrons have approximately the same mass, about 1.67×10^{-24} grams. Scientists define this amount of mass as one atomic mass unit (amu) or one Dalton. Although similar in mass, protons are positively charged, while neutrons have no charge. Therefore, the number of neutrons in an atom contributes significantly to its mass, but not to its charge.

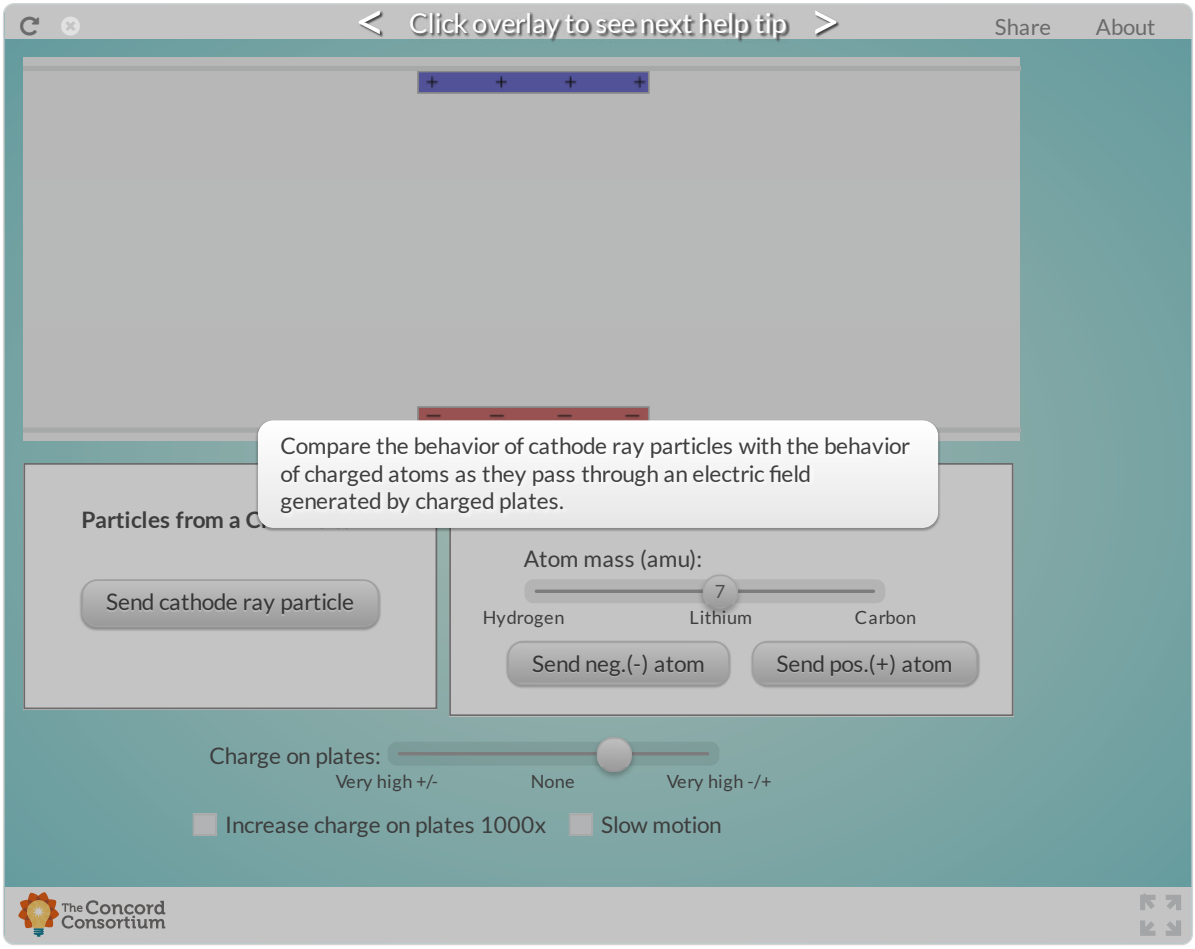
Electrons are much smaller in mass than protons, weighing only 9.11×10^{-28}grams, or about 1/1800 of an atomic mass unit. Therefore, they do not contribute much to an element's overall atomic mass. When considering atomic mass, it is customary to ignore the mass of any electrons and calculate the atom's mass based on the number of protons and neutrons alone.

Electrons contribute greatly to the atom's charge, as each electron has a negative charge equal to the positive charge of a proton. Scientists define these charges as "+1" and "-1. " In an uncharged, neutral atom, the number of electrons orbiting the nucleus is equal to the number of protons inside the nucleus. In these atoms, the positive and negative charges cancel each other out, leading to an atom with no net charge.

Protons, Neutrons, and Electrons			
	Charge	**Mass (amu)**	**Location**
Proton	+1	1	nucleus
Neutron	0	1	nucleus
Electron	–1	0	orbitals

Figure: **Protons, neutrons, and electrons**: Both protons and neutrons have a mass of 1 amu and are found in the nucleus. However, protons have a charge of +1, and neutrons are uncharged. Electrons have a mass of approximately 0 amu, orbit the nucleus, and have a charge of -1.

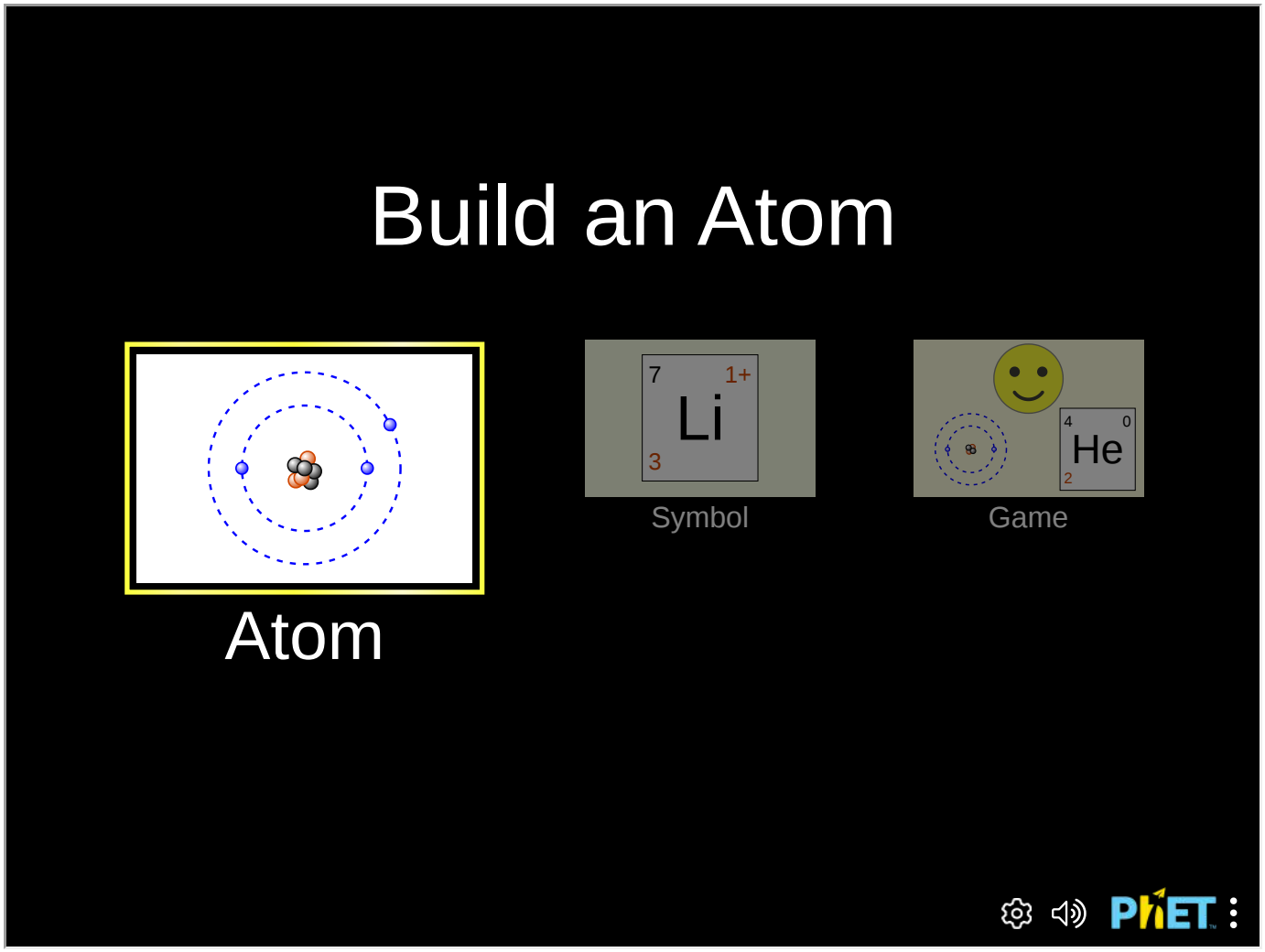
Exploring Electron Properties: Compare the behavior of electrons to that of other charged particles to discover properties of electrons such as charge and mass.



Volume of Atoms

Accounting for the sizes of protons, neutrons, and electrons, most of the volume of an atom—greater than 99 percent—is, in fact, empty space. Despite all this empty space, solid objects do not just pass through one another. The electrons that surround all atoms are negatively charged and cause atoms to repel one another, preventing atoms from occupying the same space. These intermolecular forces prevent you from falling through an object like your chair.

Interactive: Build an Atom: Build an atom out of protons, neutrons, and electrons, and see how the element, charge, and mass change. Then play a game to test your ideas!



Key Points

- An atom is composed of two regions: the nucleus, which is in the center of the atom and contains protons and neutrons, and the outer region of the atom, which holds its electrons in orbit around the nucleus.
- Protons and neutrons have approximately the same mass, about 1.67 × 10-24 grams, which scientists define as one atomic mass unit (amu) or one Dalton.
- Each electron has a negative charge (-1) equal to the positive charge of a proton (+1).
- Neutrons are uncharged particles found within the nucleus.

Key Terms

- **atom**: The smallest possible amount of matter which still retains its identity as a chemical element, consisting of a nucleus surrounded by electrons.
- **proton**: Positively charged subatomic particle forming part of the nucleus of an atom and determining the atomic number of an element. It weighs 1 amu.
- **neutron**: A subatomic particle forming part of the nucleus of an atom. It has no charge. It is equal in mass to a proton or it weighs 1 amu.

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2.1.2: Electronic Orbitals

Learning Objectives

- Distinguish between electron orbitals in the Bohr model versus the quantum mechanical orbitals

Although useful to explain the reactivity and chemical bonding of certain elements, the Bohr model of the atom does not accurately reflect how electrons are spatially distributed surrounding the nucleus. They do not circle the nucleus like the earth orbits the sun, but are rather found in electron orbitals. These relatively complex shapes result from the fact that electrons behave not just like particles, but also like waves. Mathematical equations from quantum mechanics known as wave functions can predict within a certain level of probability where an electron might be at any given time. The area where an electron is most likely to be found is called its orbital.

First Electron Shell

The closest orbital to the nucleus, called the 1s orbital, can hold up to two electrons. This orbital is equivalent to the innermost electron shell of the Bohr model of the atom. It is called the 1s orbital because it is spherical around the nucleus. The 1s orbital is always filled before any other orbital. Hydrogen has one electron; therefore, it has only one spot within the 1s orbital occupied. This is designated as $1s^1$, where the superscripted 1 refers to the one electron within the 1s orbital. Helium has two electrons; therefore, it can completely fill the 1s orbital with its two electrons. This is designated as $1s^2$, referring to the two electrons of helium in the 1s orbital. On the periodic table, hydrogen and helium are the only two elements in the first row (period); this is because they are the sole elements to have electrons only in their first shell, the 1s orbital.

Second Electron Shell

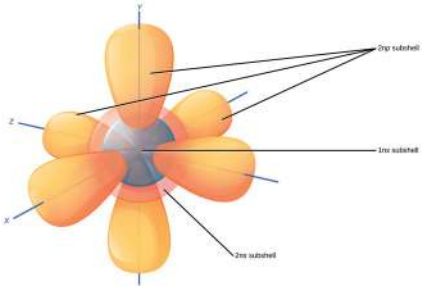


Figure: **Diagram of the S and P orbitals**: The s subshells are shaped like spheres. Both the 1n and 2n principal shells have an s orbital, but the size of the sphere is larger in the 2n orbital. Each sphere is a single orbital. p subshells are made up of three dumbbell-shaped orbitals. Principal shell 2n has a p subshell, but shell 1 does not.

The second electron shell may contain eight electrons. This shell contains another spherical s orbital and three “dumbbell” shaped p orbitals, each of which can hold two electrons. After the 1s orbital is filled, the second electron shell is filled, first filling its 2s orbital and then its three p orbitals. When filling the p orbitals, each takes a single electron; once each p orbital has an electron, a second may be added. Lithium (Li) contains three electrons that occupy the first and second shells. Two electrons fill the 1s orbital, and the third electron then fills the 2s orbital. Its electron configuration is $1s^2 2s^1$. Neon (Ne), on the other hand, has a total of ten electrons: two are in its innermost 1s orbital, and eight fill its second shell (two each in the 2s and three p orbitals). Thus, it is an inert gas and energetically stable: it rarely forms a chemical bond with other atoms.

Third Electron Shell

Larger elements have additional orbitals, making up the third electron shell. Subshells d and f have more complex shapes and contain five and seven orbitals, respectively. Principal shell 3n has s, p, and d subshells and can hold 18 electrons. Principal shell 4n has s, p, d, and f orbitals and can hold 32 electrons. Moving away from the nucleus, the number of electrons and orbitals found in the energy levels increases. Progressing from one atom to the next in the periodic table, the electron structure can be worked out by fitting an extra electron into the next available orbital. While the concepts of electron shells and orbitals are closely related, orbitals provide a more accurate depiction of the electron configuration of an atom because the orbital model specifies the different shapes and special orientations of all the places that electrons may occupy.

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Key Points

- The Bohr model of the atom does not accurately reflect how electrons are spatially distributed around the nucleus as they do not circle the nucleus like the earth orbits the sun.
- The electron orbitals are the result of mathematical equations from quantum mechanics known as wave functions and can predict within a certain level of probability where an electron might be at any given time.
- The number and type of orbitals increases with increasing atomic number, filling in various electron shells.
- The area where an electron is most likely to be found is called its orbital.

Key Terms

- **electron shell**: The collective states of all electrons in an atom having the same principal quantum number (visualized as an orbit in which the electrons move).
- **orbital**: A specification of the energy and probability density of an electron at any point in an atom or molecule.

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SECTION OVERVIEW

2.2: Chemical Bonds

Topic hierarchy

2.2.1: Ions and Ionic Bonds

2.2.2: Covalent Bonds and Other Bonds and Interaction

2.2.3: Hydrogen Bonding

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2.2.1: Ions and Ionic Bonds

Learning Objectives

- Predict whether a given element will more likely form a cation or an anion

Ions and Ionic Bonds

Some atoms are more stable when they gain or lose an electron (or possibly two) and form ions. This results in a full outermost electron shell and makes them energetically more stable. Now, because the number of electrons does not equal the number of protons, each ion has a net charge. Cations are positive ions that are formed by losing electrons (as the number of protons is now greater than the number of electrons). Negative ions are formed by gaining electrons and are called anions (wherein there are more electrons than protons in a molecule). Anions are designated by their elemental name being altered to end in "-ide". For example, the anion of chlorine is called chloride, and the anion of sulfur is called sulfide.

This movement of electrons from one element to another is referred to as electron transfer. As illustrated, sodium (Na) only has one electron in its outer electron shell. It takes less energy for sodium to donate that one electron than it does to accept seven more electrons to fill the outer shell. When sodium loses an electron, it will have 11 protons, 11 neutrons, and only 10 electrons. This leaves it with an overall charge of +1 since there are now more protons than electrons. It is now referred to as a sodium ion. Chlorine (Cl) in its lowest energy state (called the ground state) has seven electrons in its outer shell. Again, it is more energy efficient for chlorine to gain one electron than to lose seven. Therefore, it tends to gain an electron to create an ion with 17 protons, 17 neutrons, and 18 electrons. This gives it a net charge of -1 since there are now more electrons than protons. It is now referred to as a chloride ion. In this example, sodium will donate its one electron to empty its shell, and chlorine will accept that electron to fill its shell. Both ions now satisfy the octet rule and have complete outer shells. These transactions can normally only take place simultaneously; in order for a sodium atom to lose an electron, it must be in the presence of a suitable recipient like a chlorine atom.

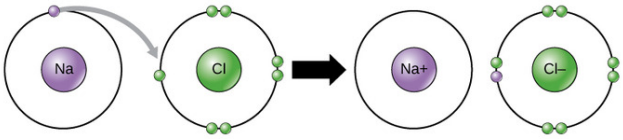


Figure: **Electron Transfer Between Na and Cl**: In the formation of an ionic compound, metals lose electrons and nonmetals gain electrons to achieve an octet. In this example, sodium loses one electron to empty its shell and chlorine accepts that electron to fill its shell.

Ionic bonds are formed between ions with opposite charges. For instance, positively charged sodium ions and negatively charged chloride ions bond together to form sodium chloride, or table salt, a crystalline molecule with zero net charge. The attractive force holding the two atoms together is called the electromagnetic force and is responsible for the attraction between oppositely charged ions.

Certain salts are referred to in physiology as electrolytes (including sodium, potassium, and calcium). Electrolytes are ions necessary for nerve impulse conduction, muscle contractions, and water balance. Many sports drinks and dietary supplements provide these ions to replace those lost from the body via sweating during exercise.

Key Points

- Ions form from elements when they gain or lose an electron causing the number of protons to be unequal to the number of electrons, resulting in a net charge.
- If there are more electrons than protons (from an element gaining one or more electrons), the ion is negatively charged and called an anion.
- If there are more protons than electrons (via loss of electrons), the ion is positively charged and is called a cation.
- Ionic bonds result from the interaction between a positively charged cation and a negatively charged anion.

Key Terms

- **ion**: An atom, or group of atoms, bearing an electrical charge, such as the sodium and chlorine atoms in a salt solution.
- **ionic bond**: A strong chemical bond caused by the electrostatic attraction between two oppositely charged ions.

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2.2.2: Covalent Bonds and Other Bonds and Interaction

Learning Objectives

- Compare the relative strength of different types of bonding interactions

Covalent Bonds and Other Bonds and Interactions

The octet rule can be satisfied by the sharing of electrons between atoms to form covalent bonds. These bonds are stronger and much more common than are ionic bonds in the molecules of living organisms. Covalent bonds are commonly found in carbon-based organic molecules, such as DNA and proteins. Covalent bonds are also found in inorganic molecules such as H_2O, CO_2, and O_2. One, two, or three pairs of electrons may be shared between two atoms, making single, double, and triple bonds, respectively. The more covalent bonds between two atoms, the stronger their connection. Thus, triple bonds are the strongest.

The strength of different levels of covalent bonding is one of the main reasons living organisms have a difficult time in acquiring nitrogen for use in constructing nitrogenous molecules, even though molecular nitrogen, N_2, is the most abundant gas in the atmosphere. Molecular nitrogen consists of two nitrogen atoms triple bonded to each other. The resulting strong triple bond makes it difficult for living systems to break apart this nitrogen in order to use it as constituents of biomolecules, such as proteins, DNA, and RNA.

The formation of water molecules is an example of covalent bonding. The hydrogen and oxygen atoms that combine to form water molecules are bound together by covalent bonds. The electron from the hydrogen splits its time between the incomplete outer shell of the hydrogen atom and the incomplete outer shell of the oxygen atom. In return, the oxygen atom shares one of its electrons with the hydrogen atom, creating a two-electron single covalent bond. To completely fill the outer shell of oxygen, which has six electrons in its outer shell, two electrons (one from each hydrogen atom) are needed. Each hydrogen atom needs only a single electron to fill its outer shell, hence the well-known formula H_2O. The electrons that are shared between the two elements fill the outer shell of each, making both elements more stable.

Polar Covalent Bonds

There are two types of covalent bonds: polar and nonpolar. In a polar covalent bond, the electrons are unequally shared by the atoms because they are more attracted to one nucleus than the other. The relative attraction of an atom to an electron is known as its electronegativity: atoms that are more attracted to an electron are considered to be more electronegative. Because of the unequal distribution of electrons between the atoms of different elements, a slightly positive (δ+) or slightly negative (δ-) charge develops. This partial charge is known as a dipole; this is an important property of water and accounts for many of its characteristics. The dipole in water occurs because oxygen has a higher electronegativity than hydrogen, which means that the shared electrons spend more time in the vicinity of the oxygen nucleus than they do near the nucleus of the hydrogen atoms.

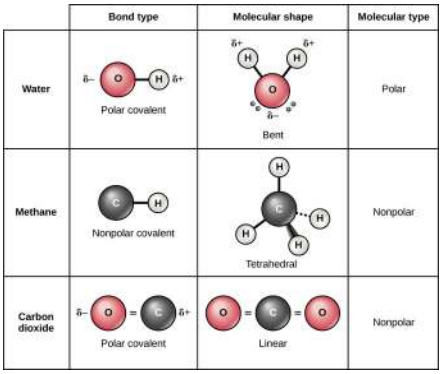


Figure: **Polar and Nonpolar Covalent Bonds**: Whether a molecule is polar or nonpolar depends both on bond type and molecular shape. Both water and carbon dioxide have polar covalent bonds, but carbon dioxide is linear, so the partial charges on the molecule cancel each other out.

Nonpolar Covalent Bonds

Nonpolar covalent bonds form between two atoms of the same element or between different elements that share electrons equally. For example, molecular oxygen (O_2) is nonpolar because the electrons will be equally distributed between the two oxygen atoms. The four bonds of methane are also considered to be nonpolar because the electronegativities of carbon and hydrogen are nearly identical.

Hydrogen Bonds and Van Der Waals Interactions

Not all bonds are ionic or covalent; weaker bonds can also form between molecules. Two types of weak bonds that frequently occur are hydrogen bonds and van der Waals interactions. Without these two types of bonds, life as we know it would not exist.

Hydrogen bonds provide many of the critical, life-sustaining properties of water and also stabilize the structures of proteins and DNA, the building block of cells. When polar covalent bonds containing hydrogen are formed, the hydrogen atom in that bond has a slightly positive charge (δ+) because the shared electrons are pulled more strongly toward the other element and away from the hydrogen atom. Because the hydrogen has a slightly positive

charge, it's attracted to neighboring negative charges. The weak interaction between the δ+ charge of a hydrogen atom from one molecule and the δ- charge of a more electronegative atom is called a hydrogen bond. Individual hydrogen bonds are weak and easily broken; however, they occur in very large numbers in water and in organic polymers, and the additive force can be very strong. For example, hydrogen bonds are responsible for zipping together the DNA double helix.

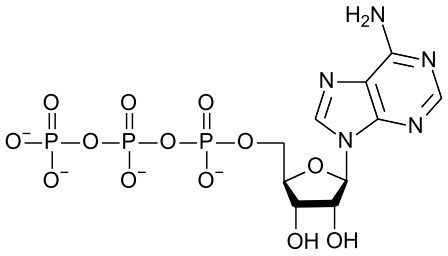
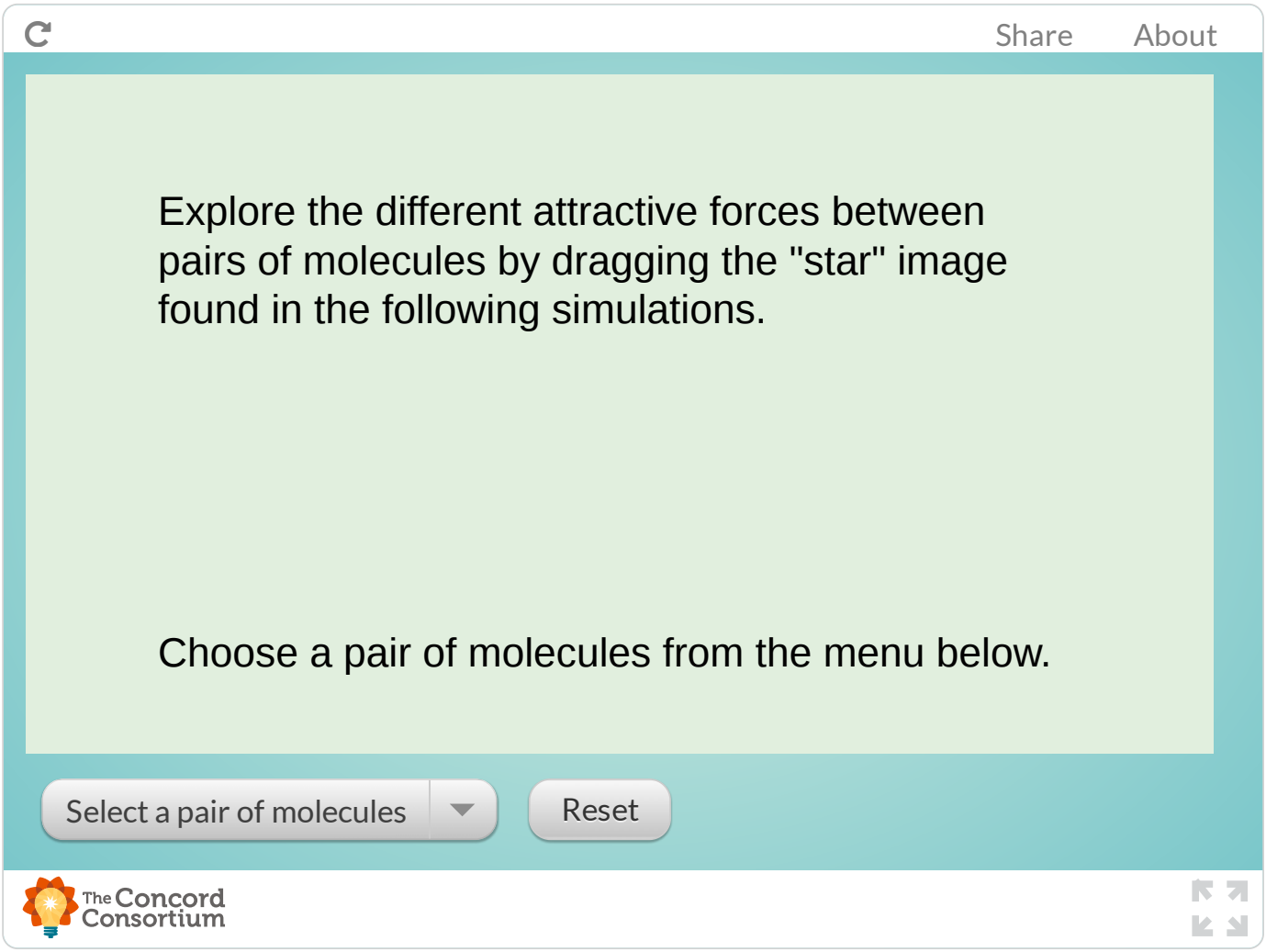


Figure: **Adenosine Triphosphate, ATP**: Adenosine Triphosphate, or ATP, is the most commonly used cofactor in nature. Its biosynthesis involves the fixation of nitrogen to provide feedstocks that eventually produce the carbon-nitrogen bonds it contains.

Like hydrogen bonds, van der Waals interactions are weak interactions between molecules. Van der Waals attractions can occur between any two or more molecules and are dependent on slight fluctuations of the electron densities, which can lead to slight temporary dipoles around a molecule. For these attractions to happen, the molecules need to be very close to one another. These bonds, along with hydrogen bonds, help form the three-dimensional structures of the proteins in our cells that are required for their proper function.

Interactions between different types of molecules: In this interactive, you can explore how different types of molecules interact with each other based on their bonds.



Key Points

- A polar covalent bond arises when two atoms of different electronegativity share two electrons unequally.
- A non-polar covalent bond is one in which the electrons are shared equally between two atoms.
- Hydrogen bonds and Van Der Waals are responsible for the folding of proteins, the binding of ligands to proteins, and many other processes between molecules.

Key Terms

- **hydrogen bond**: A weak bond in which a hydrogen atom in one molecule is attracted to an electronegative atom (usually nitrogen or oxygen) in the same or different molecule.
- **covalent bond**: A type of chemical bond where two atoms are connected to each other by the sharing of two or more electrons.
- **dipole**: Any object (such as a magnet, polar molecule or antenna), that is oppositely charged at two points (or poles).

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2.2.3: Hydrogen Bonding

Learning Objectives

- Describe the properties of hydrogen bonding.

Forming a Hydrogen Bond

A hydrogen bond is the electromagnetic attraction created between a partially positively charged hydrogen atom attached to a highly electronegative atom and another nearby electronegative atom. A hydrogen bond is a type of dipole-dipole interaction; it is not a true chemical bond. These attractions can occur between molecules (*inter*molecularly) or within different parts of a single molecule (*intra*molecularly).

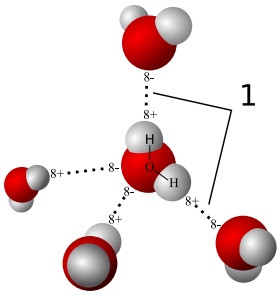


Figure: **Hydrogen bonding in water**: This is a space-filling ball diagram of the interactions between separate water molecules.

Hydrogen Bond Donor

A hydrogen atom attached to a relatively electronegative atom is a hydrogen bond donor. This electronegative atom is usually fluorine, oxygen, or nitrogen. The electronegative atom attracts the electron cloud from around the hydrogen nucleus and, by decentralizing the cloud, leaves the hydrogen atom with a positive partial charge. Because of the small size of hydrogen relative to other atoms and molecules, the resulting charge, though only partial, is stronger. In the molecule ethanol, there is one hydrogen atom bonded to an oxygen atom, which is very electronegative. This hydrogen atom is a hydrogen bond donor.

Hydrogen Bond Acceptor

A hydrogen bond results when this strong partial positive charge attracts a lone pair of electrons on another atom, which becomes the hydrogen bond acceptor. An electronegative atom such as fluorine, oxygen, or nitrogen is a hydrogen bond acceptor, regardless of whether it is bonded to a hydrogen atom or not. Greater electronegativity of the hydrogen bond acceptor will create a stronger hydrogen bond. The diethyl ether molecule contains an oxygen atom that is not bonded to a hydrogen atom, making it a hydrogen bond acceptor.

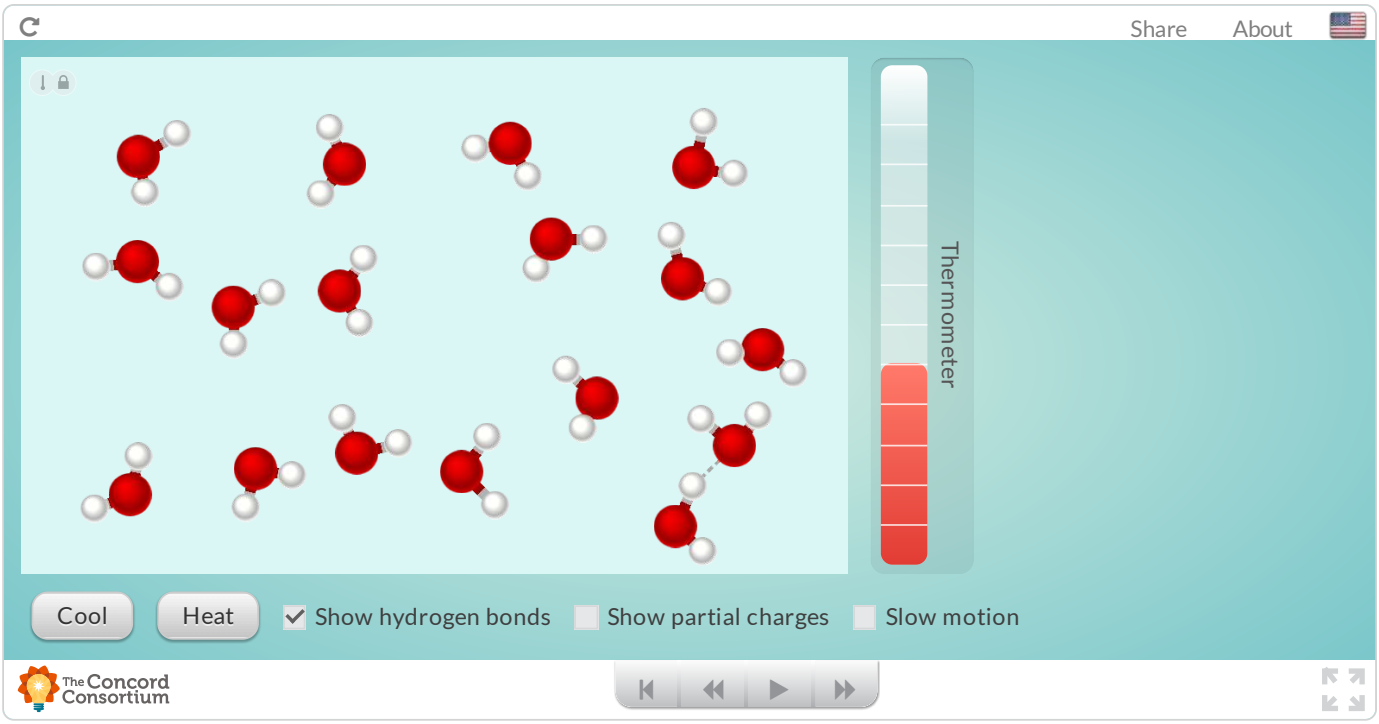
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Hydrogen bond donor and hydrogen bond acceptor: Ethanol contains a hydrogen atom that is a hydrogen bond donor because it is bonded to an electronegative oxygen atom, which is very electronegative, so the hydrogen atom is slightly positive. Diethyl ether contains an oxygen atom that is a hydrogen bond acceptor because it is not bonded to a hydrogen atom and so is slightly negative.

A hydrogen attached to carbon can also participate in hydrogen bonding when the carbon atom is bound to electronegative atoms, as is the case in chloroform ($CHCl_3$). As in a molecule where a hydrogen is attached to nitrogen, oxygen, or fluorine, the electronegative atom attracts the electron cloud from around the hydrogen nucleus and, by decentralizing the cloud, leaves the hydrogen atom with a positive partial charge.

Interactive: Hydrogen Bonding: Explore hydrogen bonds forming between polar molecules, such as water. Hydrogen bonds are shown with dotted lines. Show partial charges and run the model. Where do hydrogen bonds form? Try changing the temperature of the model. How does the pattern of

hydrogen bonding explain the lattice that makes up ice crystals?



Applications for Hydrogen Bonds

Hydrogen bonds occur in inorganic molecules, such as water, and organic molecules, such as DNA and proteins. The two complementary strands of DNA are held together by hydrogen bonds between complementary nucleotides (A&T, C&G). Hydrogen bonding in water contributes to its unique properties, including its high boiling point (100 °C) and surface tension.



Figure: **Water droplets on a leaf**: The hydrogen bonds formed between water molecules in water droplets are stronger than the other intermolecular forces between the water molecules and the leaf, contributing to high surface tension and distinct water droplets.

In biology, intramolecular hydrogen bonding is partly responsible for the secondary, tertiary, and quaternary structures of proteins and nucleic acids. The hydrogen bonds help the proteins and nucleic acids form and maintain specific shapes.

Key Points

- Hydrogen bonds are strong intermolecular forces created when a hydrogen atom bonded to an electronegative atom approaches a nearby electronegative atom.
- Greater electronegativity of the hydrogen bond acceptor will lead to an increase in hydrogen-bond strength.
- The hydrogen bond is one of the strongest intermolecular attractions, but weaker than a covalent or an ionic bond.
- Hydrogen bonds are responsible for holding together DNA, proteins, and other macromolecules.

Key Terms

- **electronegativity**: The tendency of an atom or molecule to draw electrons towards itself, form dipoles, and thus form bonds.
- **hydrogen bond**: The attraction between a partially positively charged hydrogen atom attached to a highly electronegative atom (such as nitrogen, oxygen, or fluorine) and another nearby electronegative atom.
- **intermolecular**: A type of interaction between two different molecules.

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SECTION OVERVIEW

2.3: Chemical Reactions

Topic hierarchy

2.3.1: The Chemical Basis of Life

2.3.2: Synthesis of Biological Macromolecules

2.3.3: Energy and Biochemical Reactions

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2.3.1: The Chemical Basis of Life

Learning Objectives

- Explain the properties of carbon that allow it to serve as a building block for biomolecules

Carbon is the fourth most abundant element in the universe and is the building block of life on earth. On earth, carbon circulates through the land, ocean, and atmosphere, creating what is known as the Carbon Cycle. This global carbon cycle can be divided further into two separate cycles: the geological carbon cycles takes place over millions of years, whereas the biological or physical carbon cycle takes place from days to thousands of years. In a nonliving environment, carbon can exist as carbon dioxide (CO_2), carbonate rocks, coal, petroleum, natural gas, and dead organic matter. Plants and algae convert carbon dioxide to organic matter through the process of photosynthesis, the energy of light.



Figure: **Carbon is present in all life**: All living things contain carbon in some form, and carbon is the primary component of macromolecules, including proteins, lipids, nucleic acids, and carbohydrates. Carbon exists in many forms in this leaf, including in the cellulose to form the leaf's structure and in chlorophyll, the pigment which makes the leaf green.

Carbon is Important to Life

In its metabolism of food and respiration, an animal consumes glucose ($C_6H_{12}O_6$), which combines with oxygen (O_2) to produce carbon dioxide (CO_2), water (H_2O), and energy, which is given off as heat. The animal has no need for the carbon dioxide and releases it into the atmosphere. A plant, on the other hand, uses the opposite reaction of an animal through photosynthesis. It intakes carbon dioxide, water, and energy from sunlight to make its own glucose and oxygen gas. The glucose is used for chemical energy, which the plant metabolizes in a similar way to an animal. The plant then emits the remaining oxygen into the environment.

Cells are made of many complex molecules called macromolecules, which include proteins, nucleic acids (RNA and DNA), carbohydrates, and lipids. The macromolecules are a subset of organic molecules (any carbon-containing liquid, solid, or gas) that are especially important for life. The fundamental component for all of these macromolecules is carbon. The carbon atom has unique properties that allow it to form covalent bonds to as many as four different atoms, making this versatile element ideal to serve as the basic structural component, or "backbone," of the macromolecules.

Structure of Carbon

Individual carbon atoms have an incomplete outermost electron shell. With an atomic number of 6 (six electrons and six protons), the first two electrons fill the inner shell, leaving four in the second shell. Therefore, carbon atoms can form four covalent bonds with other atoms to satisfy the octet rule. The methane molecule provides an example: it has the chemical formula CH_4. Each of its four hydrogen atoms forms a single covalent bond with the carbon atom by sharing a pair of electrons. This results in a filled outermost shell.

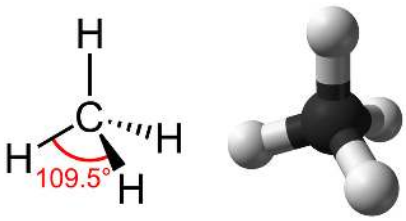


Figure: **Structure of Methane**: Methane has a tetrahedral geometry, with each of the four hydrogen atoms spaced 109.5° apart.

Key Points

- All living things contain carbon in some form.
- Carbon is the primary component of macromolecules, including proteins, lipids, nucleic acids, and carbohydrates.
- Carbon's molecular structure allows it to bond in many different ways and with many different elements.
- The carbon cycle shows how carbon moves through the living and non-living parts of the environment.

Key Terms

- **octet rule**: A rule stating that atoms lose, gain, or share electrons in order to have a full valence shell of 8 electrons (has some exceptions).
- **carbon cycle**: the physical cycle of carbon through the earth's biosphere, geosphere, hydrosphere, and atmosphere; includes such processes as photosynthesis, decomposition, respiration and carbonification
- **macromolecule**: a very large molecule, especially used in reference to large biological polymers (e.g., nucleic acids and proteins)

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2.3.2: Synthesis of Biological Macromolecules

Skills to Develop

- Understand the synthesis of macromolecules
- Explain dehydration (or condensation) and hydrolysis reactions

As you've learned, biological macromolecules are large molecules, necessary for life, that are built from smaller organic molecules. There are four major classes of biological macromolecules (carbohydrates, lipids, proteins, and nucleic acids); each is an important cell component and performs a wide array of functions. Combined, these molecules make up the majority of a cell's dry mass (recall that water makes up the majority of its complete mass). Biological macromolecules are organic, meaning they contain carbon. In addition, they may contain hydrogen, oxygen, nitrogen, and additional minor elements.

Dehydration Synthesis

Most macromolecules are made from single subunits, or building blocks, called monomers. The monomers combine with each other using covalent bonds to form larger molecules known as polymers. In doing so, monomers release water molecules as byproducts. This type of reaction is known as dehydration synthesis, which means "to put together while losing water."

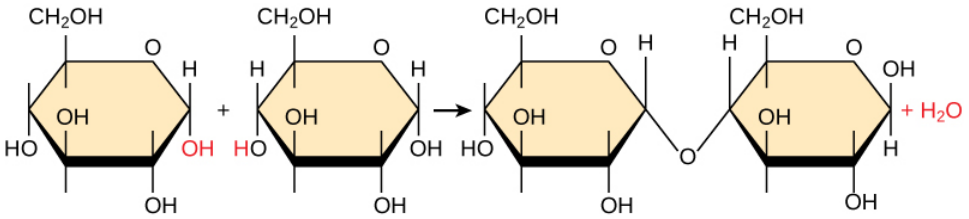


Figure **2.3.2.1**: In the dehydration synthesis reaction depicted above, two molecules of glucose are linked together to form the disaccharide maltose. In the process, a water molecule is formed.

In a dehydration synthesis reaction (Figure **2.3.2.1**), the hydrogen of one monomer combines with the hydroxyl group of another monomer, releasing a molecule of water. At the same time, the monomers share electrons and form covalent bonds. As additional monomers join, this chain of repeating monomers forms a polymer. Different types of monomers can combine in many configurations, giving rise to a diverse group of macromolecules. Even one kind of monomer can combine in a variety of ways to form several different polymers: for example, glucose monomers are the constituents of starch, glycogen, and cellulose.

Hydrolysis

Polymers are broken down into monomers in a process known as hydrolysis, which means "to split water," a reaction in which a water molecule is used during the breakdown (Figure **2.3.2.2**). During these reactions, the polymer is broken into two components: one part gains a hydrogen atom (H+) and the other gains a hydroxyl molecule (OH–) from a split water molecule.

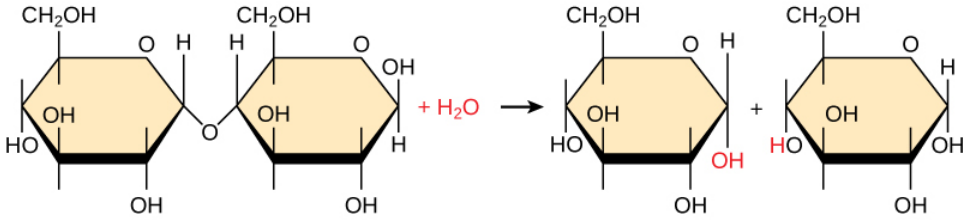


Figure **2.3.2.2**: In the hydrolysis reaction shown here, the disaccharide maltose is broken down to form two glucose monomers with the addition of a water molecule. Note that this reaction is the reverse of the synthesis reaction shown in Figure **2.3.2.1**.

Dehydration and hydrolysis reactions are catalyzed, or "sped up," by specific enzymes; dehydration reactions involve the formation of new bonds, requiring energy, while hydrolysis reactions break bonds and release energy. These reactions are similar for most macromolecules, but each monomer and polymer reaction is specific for its class. For example, in our bodies, food is hydrolyzed, or broken down, into smaller molecules by catalytic enzymes in the digestive system. This allows for easy absorption of nutrients by cells in the intestine. Each macromolecule is broken down by a specific enzyme. For instance, carbohydrates are broken down

by amylase, sucrase, lactase, or maltase. Proteins are broken down by the enzymes pepsin and peptidase, and by hydrochloric acid. Lipids are broken down by lipases. Breakdown of these macromolecules provides energy for cellular activities.

Link to Learning



Visit this site to see visual representations of dehydration synthesis and hydrolysis.

Summary

Proteins, carbohydrates, nucleic acids, and lipids are the four major classes of biological macromolecules—large molecules necessary for life that are built from smaller organic molecules. Macromolecules are made up of single units known as monomers that are joined by covalent bonds to form larger polymers. The polymer is more than the sum of its parts: it acquires new characteristics, and leads to an osmotic pressure that is much lower than that formed by its ingredients; this is an important advantage in the maintenance of cellular osmotic conditions. A monomer joins with another monomer with the release of a water molecule, leading to the formation of a covalent bond. These types of reactions are known as dehydration or condensation reactions. When polymers are broken down into smaller units (monomers), a molecule of water is used for each bond broken by these reactions; such reactions are known as hydrolysis reactions. Dehydration and hydrolysis reactions are similar for all macromolecules, but each monomer and polymer reaction is specific to its class. Dehydration reactions typically require an investment of energy for new bond formation, while hydrolysis reactions typically release energy by breaking bonds.

Glossary

biological macromolecule
large molecule necessary for life that is built from smaller organic molecules

dehydration synthesis
(also, condensation) reaction that links monomer molecules together, releasing a molecule of water for each bond formed

hydrolysis
reaction causes breakdown of larger molecules into smaller molecules with the utilization of water

monomer
smallest unit of larger molecules called polymers

polymer
chain of monomer residues that is linked by covalent bonds; polymerization is the process of polymer formation from monomers by condensation

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2.3.3: Energy and Biochemical Reactions



What is energy? Where does your energy come from? Can energy be recycled?

This team of ants is breaking down a dead tree. A classic example of teamwork. And all that work takes energy. In fact, each chemical reaction - the chemical reactions that allow the cellsin those ants to do the *work* - needs energy to get started. And all that energy comes from the food the ants eat. Whatever eats the ants gets their energy from the ants. Energy passes through an ecosystem in one direction only.

Chemical Reactions and Energy

Chemical reactions always involve energy. **Energy** is a property of matter that is defined as the ability to do work. When methane burns, for example, it releases energy in the form ofheat and light. Other chemical reactions absorb energy rather than release it.

Exothermic Reactions

A chemical reaction that releases energy (as heat) is called an **exothermic reaction**. This type of reaction can be represented by a general chemical equation:

Reactants → Products + Heat

In addition to methane burning, another example of an exothermic reaction is chlorine combining with sodium to form table salt. This reaction also releases energy.

Endothermic Reaction

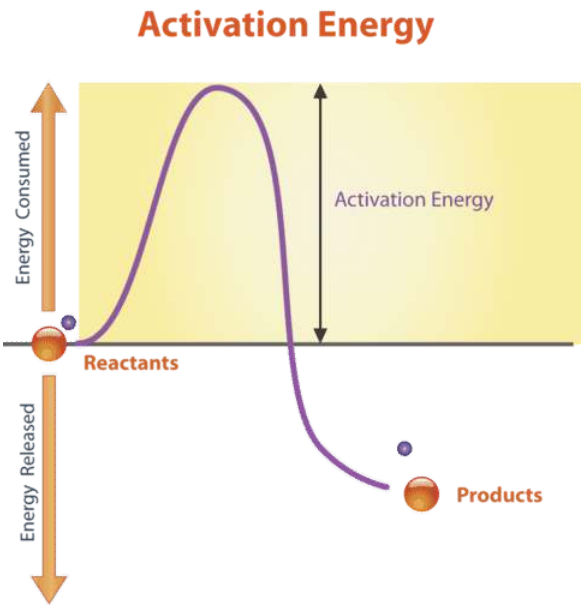
A chemical reaction that absorbs energy is called an **endothermic reaction**. This type of reaction can also be represented by a general chemical equation:

Reactants + Heat → Products

Did you ever use a chemical cold pack? The pack cools down because of an endothermic reaction. When a tube inside the pack is broken, it releases a chemical that reacts with waterinside the pack. This reaction absorbs heat energy and quickly cools down the pack.

Activation Energy

All chemical reactions need energy to get started. Even reactions that release energy need a boost of energy in order to begin. The energy needed to start a chemical reaction is called**activation energy**. Activation energy is like the push a child needs to start going down a playground slide. The push gives the child enough energy to start moving, but once she starts, she keeps moving without being pushed again. Activation energy is illustrated in**Figure** below.



Activation Energy. Activation energy provides the “push” needed to start a chemical reaction. Is the chemical reaction in this figure an exothermic or endothermic reaction?

Why do all chemical reactions need energy to get started? In order for reactions to begin, reactant molecules must bump into each other, so they must be moving, and movement requires energy. When reactant molecules bump together, they may repel each other because of intermolecular forces pushing them apart. Overcoming these forces so the molecules can come together and react also takes energy.

An overview of activation energy can be viewed at http://www.youtube.com/watch?v=VbIaK6PLrRM (1:16).



> *As you view Activation energy, focus on these concepts:*
>
> 1. *the role of activation energy,*
> 2. *what an energy diagram demonstrates.*

Summary

- Chemical reactions always involve energy. A chemical reaction that releases energy is an exothermic reaction, and a chemical reaction that absorbs energy is an endothermic reaction. The energy needed to start a chemical reaction is the activation energy.

Explore More

Use this resource to answer the questions that follow.

- http://www.hippocampus.org/Biology → Non-Majors Biology → Search: **Energy**

1. What is energy?
2. Why do living organisms need energy?
3. What is the main difference between potential and kinetic energy?
4. What is the original source of most energy used by living organisms on Earth?

Review

1. What is an exothermic reaction?
2. What is the general chemical equation for an endothermic reaction?
3. What is the activation energy?
4. Why do all chemical reactions require activation energy?

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SECTION OVERVIEW

2.4: Water and pH

Topic hierarchy

2.4.1: Biochemical Properties of Water

2.4.2: pH, Buffers, Acids, and Bases

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2.4.1: Biochemical Properties of Water

The Blue Marble

It's often called the "water planet," and it's been given the nickname "the blue marble." You probably just call it "home." Almost three-quarters of our home planet is covered by water, and without it, life as we know it could not exist on Earth. Water, like carbon, has a special role in living things. It is needed by all known forms of life. Although water consists of simple molecules, each containing just three atoms, its structure gives it unique properties that help explain why it is vital to all living organisms.



Figure **2.4.1.1**: Planet Earth

Chemical Structure and Properties of Water

You are probably already familiar with many of the water's properties. For example, you no doubt know that water is tasteless, odorless, and transparent. In small quantities, it is also colorless. However, when a large amount of water is observed, as in a lake or the ocean, it is actually light blue in color. The blue hue of the water is an intrinsic property and is caused by selective absorption and scattering of white light. These and other properties of water depend on its chemical structure.

The transparency of water is important for organisms that live in water. Because water is transparent, sunlight can pass through it. Sunlight is needed by water plants and other water organisms for photosynthesis.

Chemical Structure of Water

Each molecule of water consists of one atom of oxygen and two atoms of hydrogen, so it has the chemical formula H_2O. The arrangement of atoms in a water molecule, shown in Figure **2.4.1.2**, explains many of the water's chemical properties. In each water molecule, the nucleus of the oxygen atom (with 8 positively charged protons) attracts electrons much more strongly than the hydrogen nuclei (with only one positively charged proton). This results in a negative electrical charge near the oxygen atom (due to the "pull" of the negatively charged electrons toward the oxygen nucleus) and a positive electrical charge near the hydrogen atoms. A difference in electrical charge between different parts of a molecule is called **polarity**. A **polar molecule** is a molecule in which part of the molecule is positively charged, and part of the molecule is negatively charged.

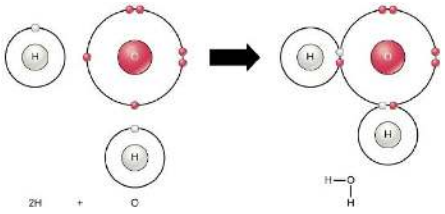


Figure **2.4.1.2**: This model is an atomic diagram of water, showing the two hydrogen atoms and an oxygen atom in the center.

Water is a good solvent

Water is considered a very good solvent in biochemical reactions. Figure **2.4.1.3** illustrates how water dissolves salts. Table salt (NaCl) consists of a positively charged sodium ion and a negatively charged chloride ion. The oxygen of water is attracted to the positive Na ion. The hydrogens of water are attracted to the negative Cl ion.

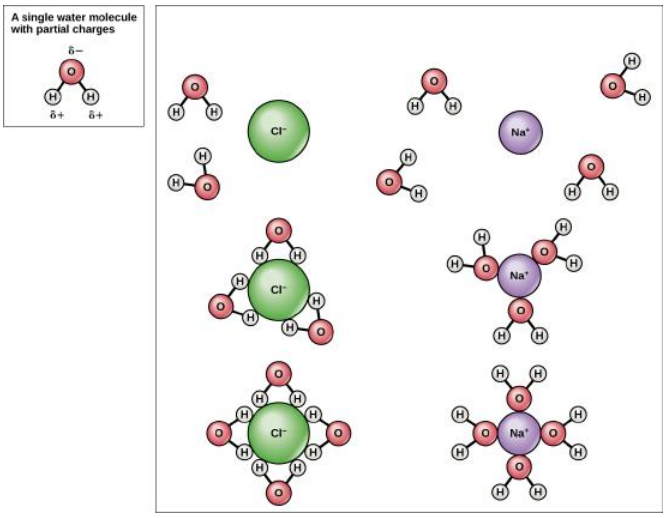


Figure **2.4.1.3**: This diagram shows the positive and negative parts of a water molecule. It also depicts how a charge, such as on an ion (Na or Cl, for example) can interact with a water molecule.

Hydrogen Bonding

Opposite electrical charges attract one another. Therefore, the positive part of one water molecule is attracted to the negative parts of other water molecules. Because of this attraction, bonds form between hydrogen and oxygen atoms of adjacent water molecules, as demonstrated in Figure **2.4.1.4**. This type of bond always involves a hydrogen atom, so it is called a **hydrogen bond**.

Hydrogen bonds can also form within a single large organic molecule. For example, hydrogen bonds that form between different parts of a protein molecule bend the molecule into a distinctive shape, which is important for the protein's functions. Hydrogen bonds also hold together the two nucleotide chains of a DNA molecule.

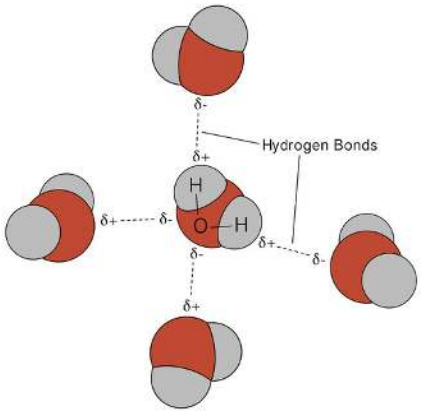


Figure **2.4.1.4**: Hydrogen bonds form between positively and negatively charged parts of water molecules. The bonds hold the water molecules together. How do you think this might affect water's properties?

Sticky, Wet Water

Water has some unusual properties due to its hydrogen bonds. One property is **cohesion**, the tendency for water molecules to stick together. The cohesive forces between water molecules are responsible for the phenomenon known as **surface tension**. The molecules at the surface do not have other like molecules on all sides of them, and consequently, they cohere more strongly with those directly associated with them on the surface. For example, if you drop a tiny amount of water onto a very smooth surface, the water molecules will stick together and form a droplet rather than spread out over the surface. The same thing happens when water slowly drips from a leaky faucet. The water doesn't fall from the faucet as individual water molecules but as droplets of water. The tendency of water to stick together in droplets is also illustrated by the dew drops in Figure **2.4.1.5**.



Figure 2.4.1.5: Droplets of dew cling to a spider web, demonstrating cohesion, the tendency of water molecules to stick together because of hydrogen bonds.

Another important physical property of water is **adhesion**. In terms of water, adhesion is the bonding of a water molecule to another substance, such as the sides of a leaf's veins. This process happens because hydrogen bonds are special in that they break and reform with great frequency. This constant rearranging of hydrogen bonds allows a percentage of all the molecules in a given sample to bond to another substance. This grip-like characteristic that water molecules form causes **capillary action**, the ability of a liquid to flow against gravity in a narrow space. An example of capillary action is when you place a straw into a glass of water. The water seems to climb up the straw before you even place your mouth on the straw. The water has created hydrogen bonds with the surface of the straw, causing the water to adhere to the sides of the straw. As the hydrogen bonds keep interchanging with the straw's surface, the water molecules interchange positions, and some begin to ascend the straw.

Adhesion and capillary action are necessary to the survival of most organisms. It is the mechanism that is responsible for water transport in plants through roots and stems, and in animals through small blood vessels.

Hydrogen bonds also explain why water's boiling point (100°C) is higher than the boiling points of similar substances without hydrogen bonds. Because of water's relatively high boiling point, most water exists in a liquid state on Earth. Liquid water is needed by all living organisms. Therefore, the availability of liquid water enables life to survive over much of the planet.

Furthermore, water has a high specific heat because it takes a lot of energy to raise or lower the temperature of the water. As a result, water plays a very important role in temperature regulation. Since cells are made up of water, this property helps to maintain homeostasis.

The Density of Ice and Water

The **melting point** of water is 0°C. Below this temperature, water is a solid (ice). Unlike most chemical substances, water in a solid state has a lower density than water in a liquid state. This is because water expands when it freezes. Again, hydrogen bonding is the reason. Hydrogen bonds cause water molecules to line up less efficiently in ice than in liquid water. As a result, water molecules are spaced farther apart in ice, giving ice a lower density than liquid water. A substance with lower density floats on a substance with higher density. This explains why ice floats on liquid water, whereas many other solids sink to the bottom of liquid water.

In a large body of water, such as a lake or the ocean, the water with the greatest density always sinks to the bottom. Water is most dense at about 4°C. As a result, the water at the bottom of a lake or the ocean usually has a temperature of about 4°C. In climates with cold winters, this layer of 4°C water insulates the bottom of a lake from freezing temperatures. Lake organisms such as fish can survive the winter by staying in this cold but unfrozen water at the bottom of the lake.

Review

1. Describe the structure of a water molecule. What is polarity, and why is water polar?
2. Explain how the internal polarity of the water molecule makes it a good solvent.
3. Explain how hydrogen bonds cause molecules of liquid water to stick together.
4. What is capillary action? Give an example.
5. What property of water helps to maintain homeostasis and how?

Explore More

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2.4.2: pH, Buffers, Acids, and Bases

Learning Objectives

- Explain the composition of buffer solutions and how they maintain a steady pH

Self-Ionization of Water

Hydrogen ions are spontaneously generated in pure water by the dissociation (ionization) of a small percentage of water molecules into equal numbers of hydrogen (H^+) ions and hydroxide (OH^-) ions. The hydroxide ions remain in solution because of their hydrogen bonds with other water molecules; the hydrogen ions, consisting of naked protons, are immediately attracted to un-ionized water molecules and form hydronium ions (H_30^+). By convention, scientists refer to hydrogen ions and their concentration as if they were free in this state in liquid water.

2H2O⇋H3O++OH−2H2O⇋H3O++OH−

The concentration of hydrogen ions dissociating from pure water is 1×10^{-7}moles H^+ ions per liter of water. The pH is calculated as the negative of the base 10 logarithm of this concentration:

$pH = -\log[H^+]$

The negative log of 1×10^{-7} is equal to 7.0, which is also known as neutral pH. Human cells and blood each maintain near-neutral pH.

pH Scale

The pH of a solution indicates its acidity or basicity (alkalinity). The pH scale is an inverse logarithm that ranges from 0 to 14: anything below 7.0 (ranging from 0.0 to 6.9) is acidic, and anything above 7.0 (from 7.1 to 14.0) is basic (or alkaline). Extremes in pH in either direction from 7.0 are usually considered inhospitable to life. The pH in cells (6.8) and the blood (7.4) are both very close to neutral, whereas the environment in the stomach is highly acidic, with a pH of 1 to 2.

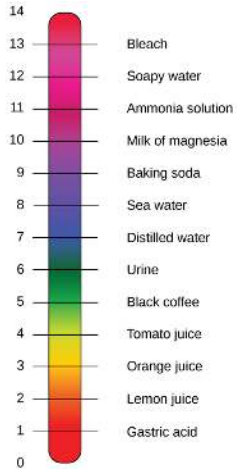


Figure: **The pH scale**: The pH scale measures the concentration of hydrogen ions (H^+) in a solution.

Non-neutral pH readings result from dissolving acids or bases in water. Using the negative logarithm to generate positive integers, high concentrations of hydrogen ions yield a low pH, and low concentrations a high pH.

An acid is a substance that increases the concentration of hydrogen ions (H^+) in a solution, usually by dissociating one of its hydrogen atoms. A base provides either hydroxide ions (OH^-) or other negatively-charged ions that react with hydrogen ions in solution, thereby reducing the concentration of H^+ and raising the pH.

Strong Acids and Strong Bases

The stronger the acid, the more readily it donates H^+. For example, hydrochloric acid (HCl) is highly acidic and completely dissociates into hydrogen and chloride ions, whereas the acids in tomato juice or vinegar do not completely dissociate and are

considered weak acids; conversely, strong bases readily donate OH^- and/or react with hydrogen ions. Sodium hydroxide (NaOH) and many household cleaners are highly basic and give up OH^- rapidly when placed in water; the OH^- ions react with H^+ in solution, creating new water molecules and lowering the amount of free H^+ in the system, thereby raising the overall pH. An example of a weak basic solution is seawater, which has a pH near 8.0, close enough to neutral that well-adapted marine organisms thrive in this alkaline environment.

Buffers

How can organisms whose bodies require a near-neutral pH ingest acidic and basic substances (a human drinking orange juice, for example) and survive? Buffers are the key. Buffers usually consist of a weak acid and its conjugate base; this enables them to readily absorb excess H^+ or OH^-, keeping the system's pH within a narrow range.

Maintaining a constant blood pH is critical to a person's well-being. The buffer that maintains the pH of human blood involves carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-), and carbon dioxide (CO_2). When bicarbonate ions combine with free hydrogen ions and become carbonic acid, hydrogen ions are removed, moderating pH changes. Similarly, excess carbonic acid can be converted into carbon dioxide gas and exhaled through the lungs; this prevents too many free hydrogen ions from building up in the blood and dangerously reducing its pH; likewise, if too much OH^- is introduced into the system, carbonic acid will combine with it to create bicarbonate, lowering the pH. Without this buffer system, the body's pH would fluctuate enough to jeopardize survival.

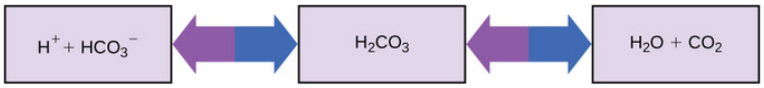


Figure: **Buffers in the body**: This diagram shows the body's buffering of blood pH levels: the blue arrows show the process of raising pH as more CO2 is made; the purple arrows indicate the reverse process, lowering pH as more bicarbonate is created.

Antacids, which combat excess stomach acid, are another example of buffers. Many over-the-counter medications work similarly to blood buffers, often with at least one ion (usually carbonate) capable of absorbing hydrogen and moderating pH, bringing relief to those that suffer "heartburn" from stomach acid after eating.

Key Points

- A basic solution will have a pH above 7.0, while an acidic solution will have a pH below 7.0.
- Buffers are solutions that contain a weak acid and its a conjugate base; as such, they can absorb excess H^+ ions or OH^- ions, thereby maintaining an overall steady pH in the solution.
- pH is equal to the negative logarithm of the concentration of H^+ ions in solution: $pH = -\log[H^+]$.

Key Terms

- **alkaline**: having a pH greater than 7; basic
- **acidic**: having a pH less than 7
- **buffer**: a solution composed of a weak acid and its conjugate base that can be used to stabilize the pH of a solution

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SECTION OVERVIEW

2.5: Organic Compounds

Topic hierarchy

2.5.1: Organic Molecules

2.5.2: Carbohydrate Molecules

2.5.3: Lipid Molecules

2.5.4: DNA and RNA

2.5.5: Amino Acids

2.5.6: ATP- Adenosine Triphosphate

2.5.E: Organic Molecules (Exercises)

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2.5.1: Organic Molecules

Learning Objectives

- Identify common elements and structures found in organic molecules
- Explain the concept of isomerism
- Identify examples of functional groups
- Describe the role of functional groups in synthesizing polymers

Clinical Focus: Part 1

Penny is a 16-year-old student who visited her doctor, complaining about an itchy skin rash. She had a history of allergic episodes. The doctor looked at her sun-tanned skin and asked her if she switched to a different sunscreen. She said she had, so the doctor diagnosed an allergic eczema. The symptoms were mild so the doctor told Penny to avoid using the sunscreen that caused the reaction and prescribed an over-the-counter moisturizing cream to keep her skin hydrated and to help with itching.

Exercise 2.5.1.1

1. What kinds of substances would you expect to find in a moisturizing cream?
2. What physical or chemical properties of these substances would help alleviate itching and inflammation of the skin?

Biochemistry is the discipline that studies the chemistry of life, and its objective is to explain form and function based on chemical principles. Organic chemistry is the discipline devoted to the study of carbon-based chemistry, which is the foundation for the study of biomolecules and the discipline of biochemistry. Both biochemistry and organic chemistry are based on the concepts of general chemistry, some of which are presented in Appendix A.

Elements in Living Cells

The most abundant element in cells is hydrogen (H), followed by carbon (C), oxygen (O), nitrogen (N), phosphorous (P), and sulfur (S). We call these elements macronutrients, and they account for about 99% of the dry weight of cells. Some elements, such as sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), calcium (Ca), molybdenum (Mo), copper (Cu), cobalt (Co), manganese (Mn), or vanadium (Va), are required by some cells in very small amounts and are called micronutrients or trace elements. All of these elements are essential to the function of many biochemical reactions, and, therefore, are essential to life.

The four most abundant elements in living matter (C, N, O, and H) have low atomic numbers and are thus light elements capable of forming strong bonds with other atoms to produce molecules (Figure **2.5.1.1**). Carbon forms four chemical bonds, whereas nitrogen forms three, oxygen forms two, and hydrogen forms one. When bonded together within molecules, oxygen, sulfur, and nitrogen often have one or more “lone pairs” of electrons that play important roles in determining many of the molecules’ physical and chemical properties (see Appendix A). These traits in combination permit the formation of a vast number of diverse molecular species necessary to form the structures and enable the functions of living organisms.

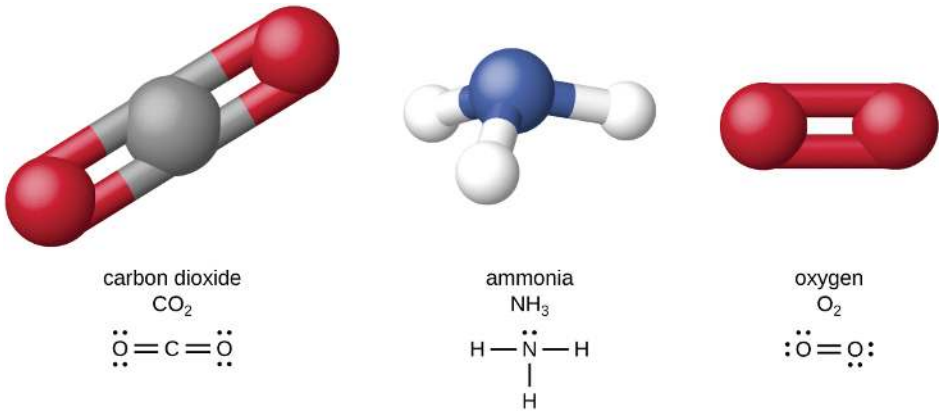


Figure **2.5.1.1**: Some common molecules include carbon dioxide, ammonia, and oxygen, which consist of combinations of oxygen atoms (red spheres), carbon atoms (gray spheres), hydrogen atoms (white spheres), or nitrogen atoms (blue spheres).

Living organisms contain inorganic compounds (mainly water and salts; see Appendix A) and organic molecules. Organic molecules contain carbon; inorganic compounds do not. Carbon oxides and carbonates are exceptions; they contain carbon but are considered inorganic because they do not contain hydrogen. The atoms of an organic molecule are typically organized around chains of carbon atoms.

Inorganic compounds make up 1%–1.5% of a living cell's mass. They are small, simple compounds that play important roles in the cell, although they do not form cell structures. Most of the carbon found in organic molecules originates from inorganic carbon sources such as carbon dioxide captured via carbon fixation by microorganisms.

? Exercise 2.5.1.2

1. Describe the most abundant elements in nature.
2. What are the differences between organic and inorganic molecules?

Organic Molecules and Isomerism

Organic molecules in organisms are generally larger and more complex than inorganic molecules. Their carbon skeletons are held together by covalent bonds. They form the cells of an organism and perform the chemical reactions that facilitate life. All of these molecules, called biomolecules because they are part of living matter, contain carbon, which is the building block of life. Carbon is a very unique element in that it has four valence electrons in its outer orbitals and can form four single covalent bonds with up to four other atoms at the same time (see Appendix A). These atoms are usually oxygen, hydrogen, nitrogen, sulfur, phosphorous, and carbon itself; the simplest organic compound is methane, in which carbon binds only to hydrogen (Figure **2.5.1.2**).

As a result of carbon's unique combination of size and bonding properties, carbon atoms can bind together in large numbers, thus producing a chain or carbon skeleton. The carbon skeleton of organic molecules can be straight, branched, or ring shaped (cyclic). Organic molecules are built on chains of carbon atoms of varying lengths; most are typically very long, which allows for a huge number and variety of compounds. No other element has the ability to form so many different molecules of so many different sizes and shapes.

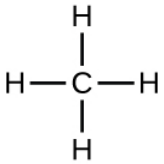


Figure **2.5.1.2**: A carbon atom can bond with up to four other atoms. The simplest organic molecule is methane (CH_4), depicted here.

Molecules with the same atomic makeup but different structural arrangement of atoms are called isomers. The concept of isomerism is very important in chemistry because the structure of a molecule is always directly related to its function. Slight changes in the structural arrangements of atoms in a molecule may lead to very different properties. Chemists represent molecules by their structural formula, which is a graphic representation of the molecular structure, showing how the atoms are arranged.

Compounds that have identical molecular formulas but differ in the bonding sequence of the atoms are called structural isomers. The monosaccharides glucose, galactose, and fructose all have the same molecular formula, $C_6H_{12}O_6$, but we can see from Figure **2.5.1.3** that the atoms are bonded together differently.

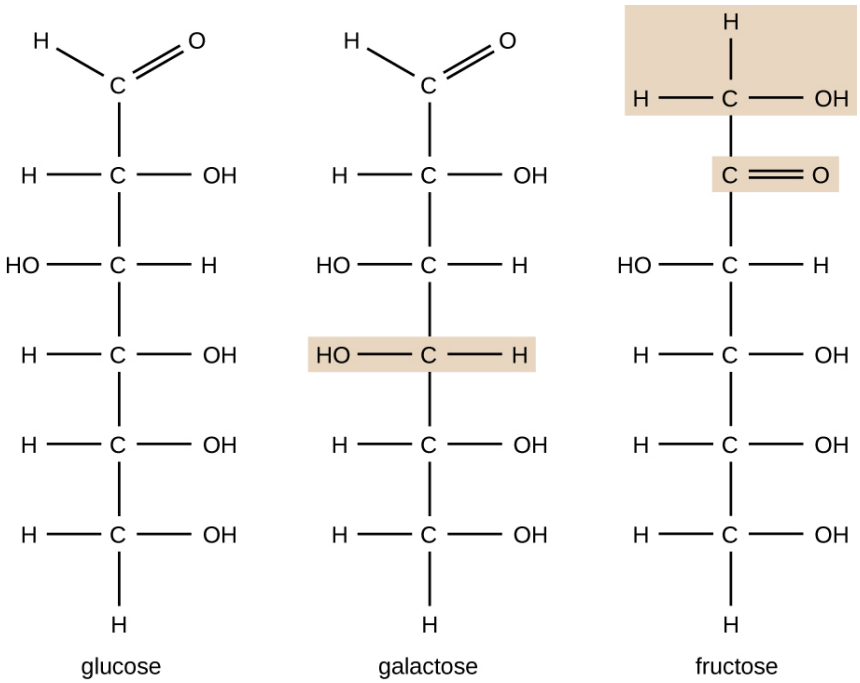


Figure **2.5.1.3**: Glucose, galactose, and fructose have the same chemical formula ($C_6H_{12}O_6$), but these structural isomers differ in their physical and chemical properties.

Isomers that differ in the spatial arrangements of atoms are called stereoisomers; one unique type is enantiomers. The properties of enantiomers were originally discovered by Louis Pasteur in 1848 while using a microscope to analyze crystallized fermentation products of wine. Enantiomers are molecules that have the characteristic of chirality, in which their structures are nonsuperimposable mirror images of each other. Chirality is an important characteristic in many biologically important molecules, as illustrated by the examples of structural differences in the enantiomeric forms of the monosaccharide glucose or the amino acid alanine (Figure **2.5.1.4**).

Many organisms are only able to use one enantiomeric form of certain types of molecules as nutrients and as building blocks to make structures within a cell. Some enantiomeric forms of amino acids have distinctly different tastes and smells when consumed as food. For example, L-aspartame, commonly called aspartame, tastes sweet, whereas D-aspartame is tasteless. Drug enantiomers can have very different pharmacologic affects. For example, the compound methorphan exists as two enantiomers, one of which acts as an antitussive (*dextro*methorphan, a cough suppressant), whereas the other acts as an analgesic (*levo*methorphan, a drug similar in effect to codeine).

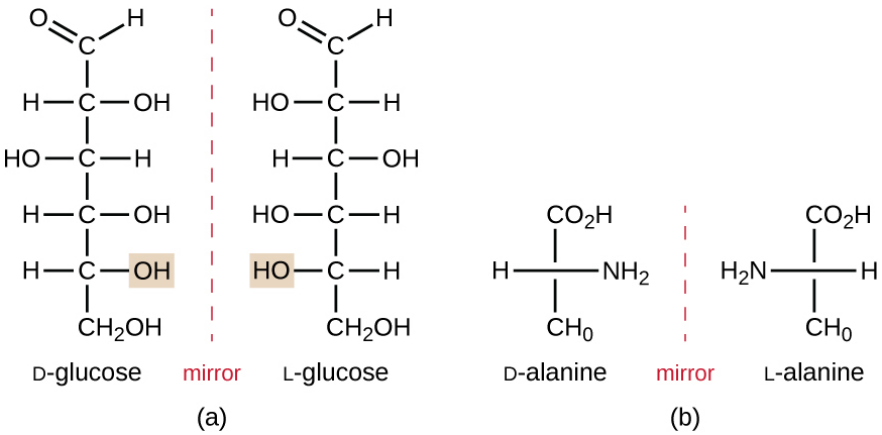


Figure **2.5.1.4**: Enantiomers are stereoisomers that exhibit chirality. Their chemical structures are nonsuperimposable mirror images of each other. (a) D-glucose and L-glucose are monosaccharides that are enantiomers. (b) The enantiomers D-alanine and L-alanine are enantiomers found in bacterial cell walls and human cells, respectively.

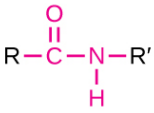
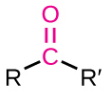
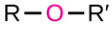
Enantiomers are also called optical isomers because they can rotate the plane of polarized light. Some of the crystals Pasteur observed from wine fermentation rotated light clockwise whereas others rotated the light counterclockwise. Today, we denote enantiomers that rotate polarized light clockwise (+) as *d* forms, and the mirror image of the same molecule that rotates polarized light counterclockwise (−) as the *l* form. The *d* and *l* labels are derived from the Latin words *dexter* (on the right) and *laevus* (on the left), respectively. These two different optical isomers often have very different biological properties and activities. Certain species of molds, yeast, and bacteria, such as *Rhizopus*, *Yarrowia*, and *Lactobacillus* spp., respectively, can only metabolize one type of optical isomer; the opposite isomer is not suitable as a source of nutrients. Another important reason to be aware of optical isomers is the therapeutic use of these types of chemicals for drug treatment, because some microorganisms can only be affected by one specific optical isomer.

? Exercise 2.5.1.3

We say that life is carbon based. What makes carbon so suitable to be part of all the macromolecules of living organisms?

Biologically Significant Functional Groups

In addition to containing carbon atoms, biomolecules also contain functional groups—groups of atoms within molecules that are categorized by their specific chemical composition and the chemical reactions they perform, regardless of the molecule in which the group is found. Some of the most common functional groups are listed in Figure **2.5.1.5**. In the formulas, the symbol R stands for “residue” and represents the remainder of the molecule. R might symbolize just a single hydrogen atom or it may represent a group of many atoms. Notice that some functional groups are relatively simple, consisting of just one or two atoms, while some comprise two of these simpler functional groups. For example, a carbonyl group is a functional group composed of a carbon atom double bonded to an oxygen atom: C=O. It is present in several classes of organic compounds as part of larger functional groups such as ketones, aldehydes, carboxylic acids, and amides. In ketones, the carbonyl is present as an internal group, whereas in aldehydes it is a terminal group.

Common Functional Groups Found in Biomolecules		
Name	**Functional Group**	**Compounds**
Aldehyde		Carbohydrates
Amide		Proteins
Amino		Amino acids, proteins
Carbonyl		Ketones, aldehydes, carboxylic acids, amides
Carboxylic acid		Amino acids, proteins, fatty acids
Ester		Lipids, nucleic acids
Ether		Disaccharides, polysaccharides, lipids
Hydroxyl		Alcohols, monosaccharides, amino acids, nucleic acids
Ketone		Carbohydrates
Methyl		Methylated compounds such as methyl alcohols and methyl esters
Phosphate		Nucleic acids, phospholipids, ATP
Sulfhydryl		Amino acids, proteins

*Functional groups are represented in pink. Ketone and aldehyde both contain a carbonyl group, highlighted in blue.

Figure **2.5.1.5**: Functional groups.

Macromolecules

Carbon chains form the skeletons of most organic molecules. Functional groups combine with the chain to form biomolecules. Because these biomolecules are typically large, we call them macromolecules. Many biologically relevant macromolecules are formed by linking together a great number of identical, or very similar, smaller organic molecules. The smaller molecules act as building blocks and are called monomers, and the macromolecules that result from their linkage are called polymers. Cells and cell

structures include four main groups of carbon-containing macromolecules: polysaccharides, proteins, lipids, and nucleic acids. The first three groups of molecules will be studied throughout this chapter. The biochemistry of nucleic acids will be discussed in Biochemistry of the Genome.

Of the many possible ways that monomers may be combined to yield polymers, one common approach encountered in the formation of biological macromolecules is dehydration synthesis. In this chemical reaction, monomer molecules bind end to end in a process that results in the formation of water molecules as a byproduct:

$$\begin{aligned} &\text{H—monomer—OH} + \text{H—monomer—OH} \\ &\longrightarrow \text{H—monomer—monomer—OH} + \text{H}_2\text{O} \end{aligned} \tag{2.5.1.1}$$

Figure 2.5.1.6 shows dehydration synthesis of glucose binding together to form maltose and a water molecule. Table 2.5.1.1 summarizes macromolecules and some of their functions.

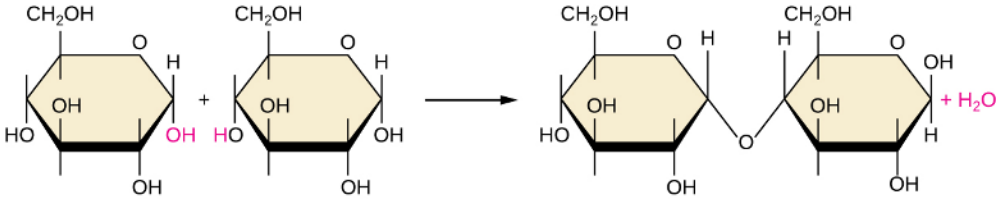


Figure 2.5.1.6: In this dehydration synthesis reaction, two molecules of glucose are linked together to form maltose. In the process, a water molecule is formed.

Table 2.5.1.1: Functions of Macromolecules

Macromolecule	Functions
Carbohydrates	Energy storage, receptors, food, structural role in plants, fungal cell walls, exoskeletons of insects
Lipids	Energy storage, membrane structure, insulation, hormones, pigments
Nucleic acids	Storage and transfer of genetic information
Proteins	Enzymes, structure, receptors, transport, structural role in the cytoskeleton of a cell and the extracellular matrix

? Exercise 2.5.1.4

What is the byproduct of a dehydration synthesis reaction?

Key Concepts and Summary

- The most abundant elements in cells are hydrogen, carbon, oxygen, nitrogen, phosphorus, and sulfur.
- Life is carbon based. Each carbon atom can bind to another one producing a **carbon skeleton** that can be straight, branched, or ring shaped.
- The same numbers and types of atoms may bond together in different ways to yield different molecules called **isomers**. Isomers may differ in the bonding sequence of their atoms (**structural isomers**) or in the spatial arrangement of atoms whose bonding sequences are the same (**stereoisomers**), and their physical and chemical properties may vary slightly or drastically.
- **Functional groups** confer specific chemical properties to molecules bearing them. Common functional groups in biomolecules are hydroxyl, methyl, carbonyl, carboxyl, amino, phosphate, and sulfhydryl.
- **Macromolecules** are **polymers** assembled from individual units, the **monomers**, which bind together like building blocks. Many biologically significant macromolecules are formed by **dehydration synthesis**, a process in which monomers bind together by combining their functional groups and generating water molecules as byproducts.

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2.5.2: Carbohydrate Molecules

Learning Objectives

- Describe the structure of mono-, di-, and poly-saccharides

Carbohydrates can be represented by the stoichiometric formula $(CH_2O)_n$, where n is the number of carbons in the molecule. Therefore, the ratio of carbon to hydrogen to oxygen is 1:2:1 in carbohydrate molecules. The origin of the term "carbohydrate" is based on its components: carbon ("carbo") and water ("hydrate"). Carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides

Monosaccharides (mono- = "one"; sacchar- = "sweet") are simple sugars. In monosaccharides, the number of carbons usually ranges from three to seven. If the sugar has an aldehyde group (the functional group with the structure R-CHO), it is known as an aldose, and if it has a ketone group (the functional group with the structure RC(=O)R'), it is known as a ketose. Depending on the number of carbons in the sugar, they also may be known as trioses (three carbons), pentoses (five carbons), and or hexoses (six carbons). Monosaccharides can exist as a linear chain or as ring-shaped molecules; in aqueous solutions they are usually found in ring forms.

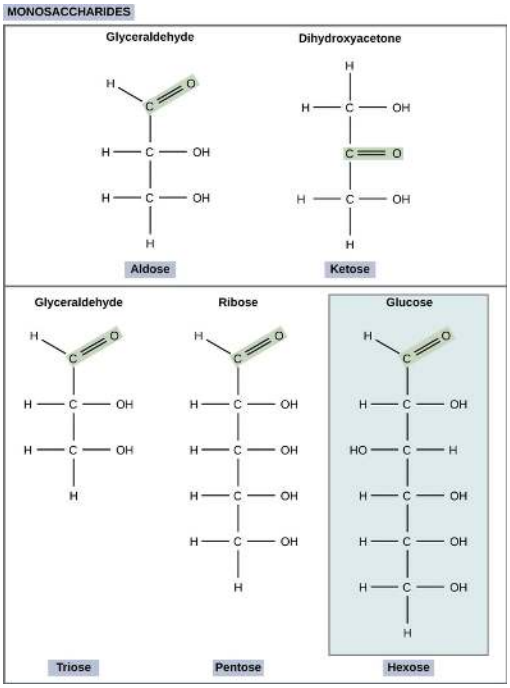


Figure: **Monosaccharides**: Monosaccharides are classified based on the position of their carbonyl group and the number of carbons in the backbone. Aldoses have a carbonyl group (indicated in green) at the end of the carbon chain, and ketoses have a carbonyl group in the middle of the carbon chain. Trioses, pentoses, and hexoses have three, five, and six carbon backbones, respectively.

Common Monosaccharides

Glucose ($C_6H_{12}O_6$) is a common monosaccharide and an important source of energy. During cellular respiration, energy is released from glucose and that energy is used to help make adenosine triphosphate (ATP). Plants synthesize glucose using carbon dioxide and water, and glucose, in turn, is used for energy requirements for the plant.

Galactose (a milk sugar) and fructose (found in fruit) are other common monosaccharides. Although glucose, galactose, and fructose all have the same chemical formula ($C_6H_{12}O_6$), they differ structurally and stereochemically. This makes them different molecules despite sharing the same atoms in the same proportions, and they are all isomers of one another, or isomeric monosaccharides. Glucose and galactose are aldoses, and fructose is a ketose.

Disaccharides

Disaccharides (di- = “two”) form when two monosaccharides undergo a dehydration reaction (also known as a condensation reaction or dehydration synthesis). During this process, the hydroxyl group of one monosaccharide combines with the hydrogen of another monosaccharide, releasing a molecule of water and forming a covalent bond. A covalent bond formed between a carbohydrate molecule and another molecule (in this case, between two monosaccharides) is known as a glycosidic bond. Glycosidic bonds (also called glycosidic linkages) can be of the alpha or the beta type.

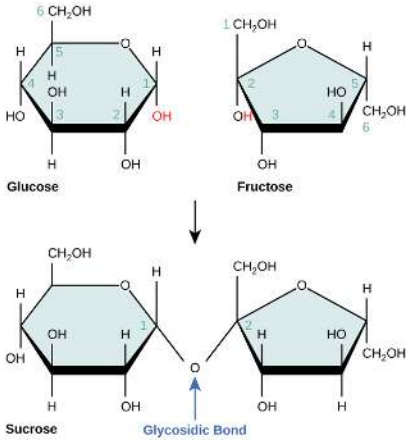


Figure: **Disaccharides**: Sucrose is formed when a monomer of glucose and a monomer of fructose are joined in a dehydration reaction to form a glycosidic bond. In the process, a water molecule is lost. By convention, the carbon atoms in a monosaccharide are numbered from the terminal carbon closest to the carbonyl group. In sucrose, a glycosidic linkage is formed between carbon 1 in glucose and carbon 2 in fructose.

Common Disaccharides

Common disaccharides include lactose, maltose, and sucrose. Lactose is a disaccharide consisting of the monomers glucose and galactose. It is found naturally in milk. Maltose, or malt sugar, is a disaccharide formed by a dehydration reaction between two glucose molecules. The most common disaccharide is sucrose, or table sugar, which is composed of the monomers glucose and fructose.

Polysaccharides

A long chain of monosaccharides linked by glycosidic bonds is known as a polysaccharide (poly- = “many”). The chain may be branched or unbranched, and it may contain different types of monosaccharides. Starch, glycogen, cellulose, and chitin are primary examples of polysaccharides.

Plants are able to synthesize glucose, and the excess glucose is stored as starch in different plant parts, including roots and seeds. Starch is the stored form of sugars in plants and is made up of glucose monomers that are joined by α1-4 or 1-6 glycosidic bonds. The starch in the seeds provides food for the embryo as it germinates while the starch that is consumed by humans is broken down by enzymes into smaller molecules, such as maltose and glucose. The cells can then absorb the glucose.

Common Polysaccharides

Glycogen is the storage form of glucose in humans and other vertebrates. It is made up of monomers of glucose. Glycogen is the animal equivalent of starch and is a highly branched molecule usually stored in liver and muscle cells. Whenever blood glucose levels decrease, glycogen is broken down to release glucose in a process known as glycogenolysis.

Cellulose is the most abundant natural biopolymer. The cell wall of plants is mostly made of cellulose and provides structural support to the cell. Cellulose is made up of glucose monomers that are linked by β 1-4 glycosidic bonds. Every other glucose monomer in cellulose is flipped over, and the monomers are packed tightly as extended long chains. This gives cellulose its rigidity and high tensile strength—which is so important to plant cells.

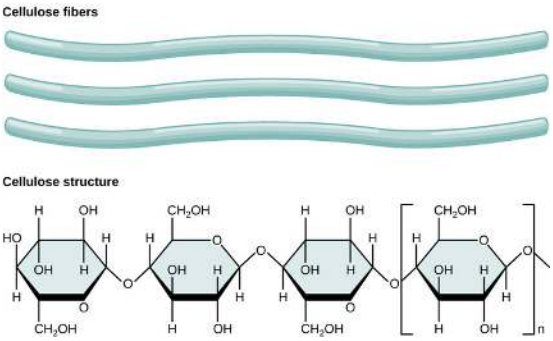


Figure: **Polysaccharides**: In cellulose, glucose monomers are linked in unbranched chains by β 1-4 glycosidic linkages. Because of the way the glucose subunits are joined, every glucose monomer is flipped relative to the next one resulting in a linear, fibrous structure.

Carbohydrate Function

Carbohydrates serve various functions in different animals. Arthropods have an outer skeleton, the exoskeleton, which protects their internal body parts. This exoskeleton is made of chitin, which is a polysaccharide-containing nitrogen. It is made of repeating units of N-acetyl-β-d-glucosamine, a modified sugar. Chitin is also a major component of fungal cell walls

Key Points

- Monosaccharides are simple sugars made up of three to seven carbons, and they can exist as a linear chain or as ring-shaped molecules.
- Glucose, galactose, and fructose are monosaccharide isomers, which means they all have the same chemical formula but differ structurally and chemically.
- Disaccharides form when two monosaccharides undergo a dehydration reaction (a condensation reaction); they are held together by a covalent bond.
- Sucrose (table sugar) is the most common disaccharide, which is composed of the monomers glucose and fructose.
- A polysaccharide is a long chain of monosaccharides linked by glycosidic bonds; the chain may be branched or unbranched and can contain many types of monosaccharides.

Key Terms

- **isomer**: Any of two or more compounds with the same molecular formula but with different structure.
- **dehydration reaction**: A chemical reaction in which two molecules are covalently linked in a reaction that generates H2O as a second product.
- **biopolymer**: Any macromolecule of a living organism that is formed from the polymerization of smaller entities; a polymer that occurs in a living organism or results from life.

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2.5.3: Lipid Molecules

Learning Objectives

- Differentiate between saturated and unsaturated fatty acids

Fats have important functions, and many vitamins are fat soluble. Fats serve as a long-term storage form of fatty acids and act as a source of energy. They also provide insulation for the body.

Glycerol and Fatty Acids

A fat molecule consists of two main components: glycerol and fatty acids. Glycerol is an alcohol with three carbons, five hydrogens, and three hydroxyl (OH) groups. Fatty acids have a long chain of hydrocarbons with a carboxyl group attached and may have 4-36 carbons; however, most of them have 12-18. In a fat molecule, the fatty acids are attached to each of the three carbons of the glycerol molecule with an ester bond through the oxygen atom. During the ester bond formation, three molecules are released. Since fats consist of three fatty acids and a glycerol, they are also called triacylglycerols or triglycerides.

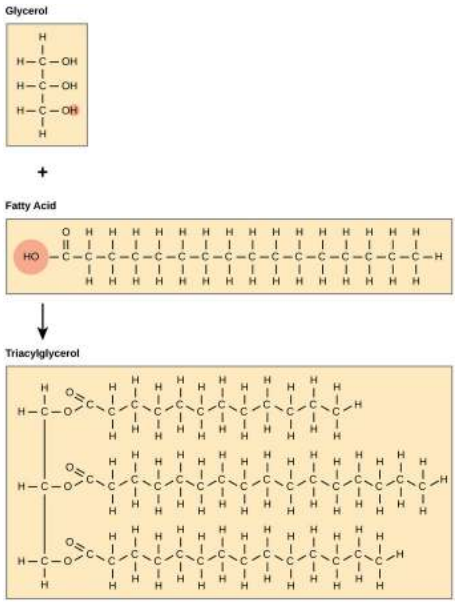


Figure: **Triacylglycerols**: Triacylglycerol is formed by the joining of three fatty acids to a glycerol backbone in a dehydration reaction. Three molecules of water are released in the process.

Saturated vs. Unsaturated Fatty Acids

Fatty acids may be saturated or unsaturated. In a fatty acid chain, if there are only single bonds between neighboring carbons in the hydrocarbon chain, the fatty acid is said to be saturated. Saturated fatty acids are saturated with hydrogen since single bonds increase the number of hydrogens on each carbon. Stearic acid and palmitic acid, which are commonly found in meat, are examples of saturated fats.

When the hydrocarbon chain contains a double bond, the fatty acid is said to be unsaturated. Oleic acid is an example of an unsaturated fatty acid. Most unsaturated fats are liquid at room temperature and are called oils. If there is only one double bond in the molecule, then it is known as a monounsaturated fat; e.g. olive oil. If there is more than one double bond, then it is known as a polyunsaturated fat; e.g. canola oil. Unsaturated fats help to lower blood cholesterol levels whereas saturated fats contribute to plaque formation in the arteries.

Unsaturated fats or oils are usually of plant origin and contain cis unsaturated fatty acids. Cis and trans indicate the configuration of the molecule around the double bond. If hydrogens are present in the same plane, it is referred to as a cis fat; if the hydrogen atoms are on two different planes, it is referred to as a trans fat. The cis double bond causes a bend or a “kink” that prevents the fatty acids from packing tightly, keeping them liquid at room temperature.

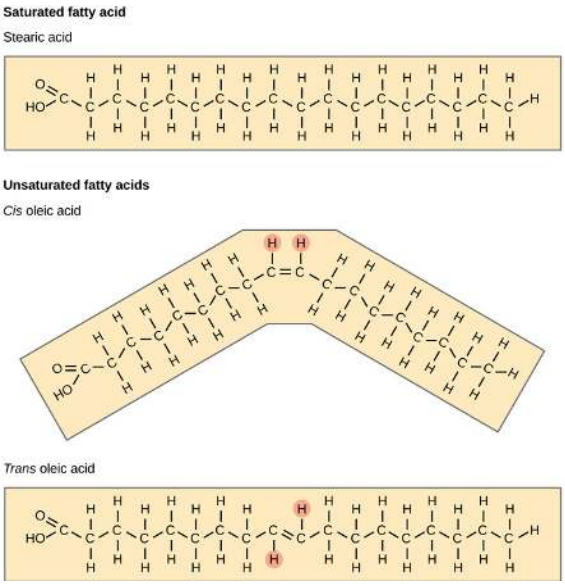


Figure: **Fatty Acids**: Saturated fatty acids have hydrocarbon chains connected by single bonds only. Unsaturated fatty acids have one or more double bonds. Each double bond may be in a cis or trans configuration. In the cis configuration, both hydrogens are on the same side of the hydrocarbon chain. In the trans configuration, the hydrogens are on opposite sides. A cis double bond causes a kink in the chain.

Trans Fats

In the food industry, oils are artificially hydrogenated to make them semi-solid and of a consistency desirable for many processed food products. During this hydrogenation process, gas is bubbled through oils to solidify them, and the double bonds of the cis-conformation in the hydrocarbon chain may be converted to double bonds in the trans-conformation.

Margarine, some types of peanut butter, and shortening are examples of artificially-hydrogenated trans fats. Recent studies have shown that an increase in trans fats in the human diet may lead to an increase in levels of low-density lipoproteins (LDL), or “bad” cholesterol, which in turn may lead to plaque deposition in the arteries, resulting in heart disease. Many fast food restaurants have recently banned the use of trans fats, and food labels are required to display the trans fat content.

Essential Fatty Acids

Essential fatty acids are fatty acids required for biological processes, but not synthesized by the human body. Consequently, they have to be supplemented through ingestion via the diet and are nutritionally very important. Omega-3 fatty acid, or alpha-linoleic acid (ALA), falls into this category and is one of only two fatty acids known to be essential for humans (the other being omega-6 fatty acid, or linoleic acid). These polyunsaturated fatty acids are called omega-3 because the third carbon from the end of the hydrocarbon chain is connected to its neighboring carbon by a double bond. Salmon, trout, and tuna are good sources of omega-3 fatty acids.

Research indicates that omega-3 fatty acids reduce the risk of sudden death from heart attacks, reduce triglycerides in the blood, lower blood pressure, and prevent thrombosis by inhibiting blood clotting. They also reduce inflammation and may help reduce the risk of some cancers in animals.

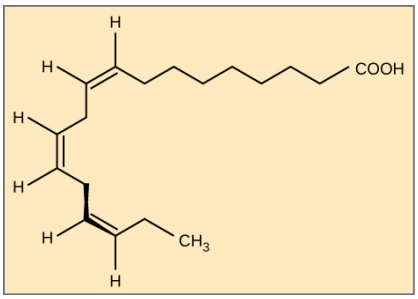


Figure: **Omega Fatty Acids**: Alpha-linolenic acid is an example of an omega-3 fatty acid. It has three cis double bonds and, as a result, a curved shape. For clarity, the carbons are not shown. Each singly bonded carbon has two hydrogens associated with it, also not shown.

Key Points

- Fats provide energy, insulation, and storage of fatty acids for many organisms.
- Fats may be saturated (having single bonds) or unsaturated (having double bonds).
- Unsaturated fats may be cis (hydrogens in same plane) or trans (hydrogens in two different planes).
- Olive oil, a monounsaturated fat, has a single double bond whereas canola oil, a polyunsaturated fat, has more than one double bond.
- Omega-3 fatty acid and omega-6 fatty acid are essential for human biological processes, but they must be ingested in the diet because they cannot be synthesized.

Key Terms

- **hydrogenation**: The chemical reaction of hydrogen with another substance, especially with an unsaturated organic compound, and usually under the influence of temperature, pressure and catalysts.
- **ester**: Compound most often formed by the condensation of an alcohol and an acid, by removing water. It contains the functional group carbon-oxygen double bond joined via carbon to another oxygen atom.
- **carboxyl**: A univalent functional group consisting of a carbonyl and a hydroxyl functional group (-CO.OH); characteristic of carboxylic acids.

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2.5.4: DNA and RNA

Learning Objectives

- Describe the structure of nucleic acids and the types of molecules that contain them

Types of Nucleic Acids

The two main types of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the genetic material found in all living organisms, ranging from single-celled bacteria to multicellular mammals. It is found in the nucleus of eukaryotes and in the chloroplasts and mitochondria. In prokaryotes, the DNA is not enclosed in a membranous envelope, but rather free-floating within the cytoplasm.

The entire genetic content of a cell is known as its genome and the study of genomes is genomics. In eukaryotic cells, but not in prokaryotes, DNA forms a complex with histone proteins to form chromatin, the substance of eukaryotic chromosomes. A chromosome may contain tens of thousands of genes. Many genes contain the information to make protein products; other genes code for RNA products. DNA controls all of the cellular activities by turning the genes “on” or “off. ”

The other type of nucleic acid, RNA, is mostly involved in protein synthesis. In eukaryotes, the DNA molecules never leave the nucleus but instead use an intermediary to communicate with the rest of the cell. This intermediary is the messenger RNA (mRNA). Other types of RNA—like rRNA, tRNA, and microRNA—are involved in protein synthesis and its regulation.

Nucleotides

DNA and RNA are made up of monomers known as nucleotides. The nucleotides combine with each other to form a polynucleotide: DNA or RNA. Each nucleotide is made up of three components:

1. a nitrogenous base
2. a pentose (five-carbon) sugar
3. a phosphate group

Each nitrogenous base in a nucleotide is attached to a sugar molecule, which is attached to one or more phosphate groups.

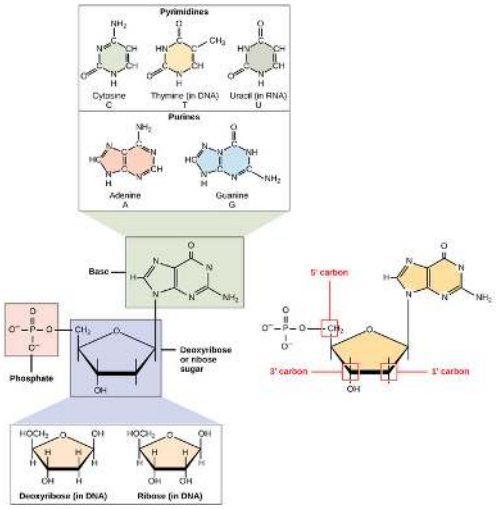


Figure: **DNA and RNA**: A nucleotide is made up of three components: a nitrogenous base, a pentose sugar, and one or more phosphate groups. Carbon residues in the pentose are numbered 1′ through 5′ (the prime distinguishes these residues from those in the base, which are numbered without using a prime notation). The base is attached to the 1′ position of the ribose, and the phosphate is attached to the 5′ position. When a polynucleotide is formed, the 5′ phosphate of the incoming nucleotide attaches to the 3′ hydroxyl group at the end of the growing chain. Two types of pentose are found in nucleotides, deoxyribose (found in DNA) and ribose (found in RNA). Deoxyribose is similar in structure to ribose, but it has an H instead of an OH at the 2′ position. Bases can be divided into two categories: purines and pyrimidines. Purines have a double ring structure, and pyrimidines have a single ring.

Nitrogenous Base

The nitrogenous bases are organic molecules and are so named because they contain carbon and nitrogen. They are bases because they contain an amino group that has the potential of binding an extra hydrogen, and thus, decreasing the hydrogen ion concentration in its environment, making it more basic. Each nucleotide in DNA contains one of four possible nitrogenous bases: adenine (A), guanine (G) cytosine (C), and thymine (T).

Adenine and guanine are classified as purines. The primary structure of a purine consists of two carbon-nitrogen rings. Cytosine, thymine, and uracil are classified as pyrimidines which have a single carbon-nitrogen ring as their primary structure. Each of these basic carbon-nitrogen rings has different functional groups attached to it. In molecular biology shorthand, the nitrogenous bases are simply known by their symbols A, T, G, C, and U. DNA contains A, T, G, and C whereas RNA contains A, U, G, and C.

Five-Carbon Sugar

The pentose sugar in DNA is deoxyribose and in RNA it is ribose. The difference between the sugars is the presence of the hydroxyl group on the second carbon of the ribose and hydrogen on the second carbon of the deoxyribose. The carbon atoms of the sugar molecule are numbered as 1′, 2′, 3′, 4′, and 5′ (1′ is read as “one prime”).

Phosphate Group

The phosphate residue is attached to the hydroxyl group of the 5′ carbon of one sugar and the hydroxyl group of the 3′ carbon of the sugar of the next nucleotide, which forms a 5′3′ phosphodiester linkage. The phosphodiester linkage is not formed by simple dehydration reaction like the other linkages connecting monomers in macromolecules: its formation involves the removal of two phosphate groups. A polynucleotide may have thousands of such phosphodiester linkages.

Key Points

- The two main types of nucleic acids are DNA and RNA.
- Both DNA and RNA are made from nucleotides, each containing a five-carbon sugar backbone, a phosphate group, and a nitrogen base.
- DNA provides the code for the cell ‘s activities, while RNA converts that code into proteins to carry out cellular functions.
- The sequence of nitrogen bases (A, T, C, G) in DNA is what forms an organism’s traits.
- The nitrogen bases A and T (or U in RNA) always go together and C and G always go together, forming the 5′-3′ phosphodiester linkage found in the nucleic acid molecules.

Key Terms

- **nucleotide**: the monomer comprising DNA or RNA molecules; consists of a nitrogenous heterocyclic base that can be a purine or pyrimidine, a five-carbon pentose sugar, and a phosphate group
- **genome**: the cell’s complete genetic information packaged as a double-stranded DNA molecule
- **monomer**: A relatively small molecule which can be covalently bonded to other monomers to form a polymer.

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2.5.5: Amino Acids

Learning Objectives

- Describe the structure of an amino acid and the features that confer its specific properties

Structure of an Amino Acid

Amino acids are the monomers that make up proteins. Each amino acid has the same fundamental structure, which consists of a central carbon atom, also known as the alpha (α) carbon, bonded to an amino group (NH_2), a carboxyl group (COOH), and to a hydrogen atom. In the aqueous environment of the cell, the both the amino group and the carboxyl group are ionized under physiological conditions, and so have the structures $-NH_3^+$ and $-COO^-$, respectively. Every amino acid also has another atom or group of atoms bonded to the central atom known as the R group. This R group, or side chain, gives each amino acid proteins specific characteristics, including size, polarity, and pH.

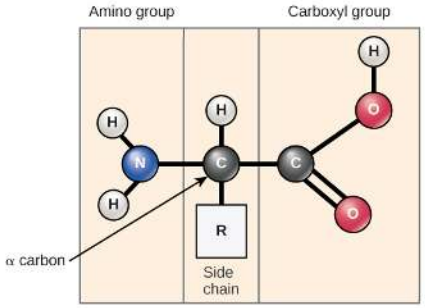


Figure: **Amino acid structure**: Amino acids have a central asymmetric carbon to which an amino group, a carboxyl group, a hydrogen atom, and a side chain (R group) are attached. This amino acid is unionized, but if it were placed in water at pH 7, its amino group would pick up another hydrogen and a positive charge, and the hydroxyl in its carboxyl group would lose and a hydrogen and gain a negative charge.

Types of Amino Acids

The name “amino acid” is derived from the amino group and carboxyl-acid-group in their basic structure. There are 21 amino acids present in proteins, each with a specific R group or side chain. Ten of these are considered essential amino acids in humans because the human body cannot produce them and they must be obtained from the diet. All organisms have different essential amino acids based on their physiology.

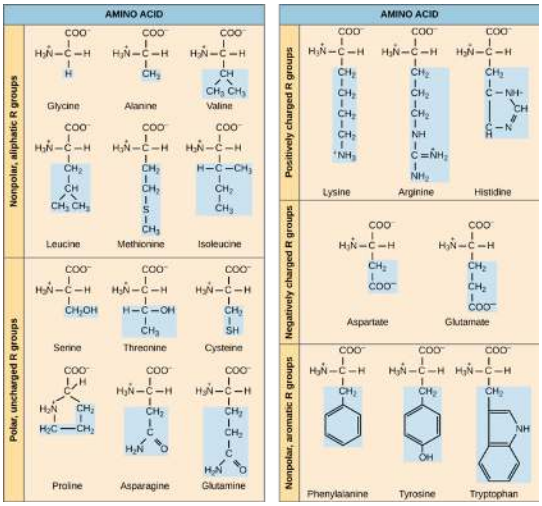


Figure: **Types of amino acids**: There are 21 common amino acids commonly found in proteins, each with a different R group (variant group) that determines its chemical nature. The 21st amino acid, not shown here, is selenocysteine, with an R group of $-CH_2$-SeH.

Characteristics of Amino Acids

Which categories of amino acid would you expect to find on the surface of a soluble protein, and which would you expect to find in the interior? What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?

The chemical composition of the side chain determines the characteristics of the amino acid. Amino acids such as valine, methionine, and alanine are nonpolar (hydrophobic), while amino acids such as serine, threonine, and cysteine are polar (hydrophilic). The side chains of lysine and arginine are positively charged so these amino acids are also known as basic (high pH) amino acids. Proline is an exception to the standard structure of an amino acid because its R group is linked to the amino group, forming a ring-like structure.

Amino acids are represented by a single upper case letter or a three-letter abbreviation. For example, valine is known by the letter V or the three-letter symbol val.

Peptide Bonds

The sequence and the number of amino acids ultimately determine the protein's shape, size, and function. Each amino acid is attached to another amino acid by a covalent bond, known as a peptide bond. When two amino acids are covalently attached by a peptide bond, the carboxyl group of one amino acid and the amino group of the incoming amino acid combine and release a molecule of water. Any reaction that combines two monomers in a reaction that generates H_2O as one of the products is known as a dehydration reaction, so peptide bond formation is an example of a dehydration reaction.

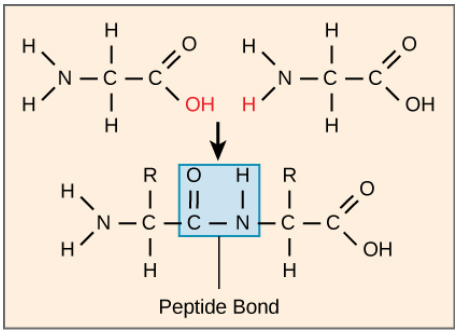


Figure: **Peptide bond formation**: Peptide bond formation is a dehydration synthesis reaction. The carboxyl group of one amino acid is linked to the amino group of the incoming amino acid. In the process, a molecule of water is released.

Polypeptide Chains

The resulting chain of amino acids is called a polypeptide chain. Each polypeptide has a free amino group at one end. This end is called the N terminal, or the amino terminal, and the other end has a free carboxyl group, also known as the C or carboxyl terminal. When reading or reporting the amino acid sequence of a protein or polypeptide, the convention is to use the N-to-C direction. That is, the first amino acid in the sequence is assumed to the be one at the N terminal and the last amino acid is assumed to be the one at the C terminal.

Although the terms polypeptide and protein are sometimes used interchangeably, a polypeptide is technically any polymer of amino acids, whereas the term protein is used for a polypeptide or polypeptides that have folded properly, combined with any additional components needed for proper functioning, and is now functional.

Key Points

- Each amino acid contains a central C atom, an amino group (NH2), a carboxyl group (COOH), and a specific R group.
- The R group determines the characteristics (size, polarity, and pH) for each type of amino acid.
- Peptide bonds form between the carboxyl group of one amino acid and the amino group of another through dehydration synthesis.
- A chain of amino acids is a polypeptide.

Key Terms

- **amino acid**: Any of 20 naturally occurring α-amino acids (having the amino, and carboxylic acid groups on the same carbon atom), and a variety of side chains, that combine, via peptide bonds, to form proteins.
- **R group**: The R group is a side chain specific to each amino acid that confers particular chemical properties to that amino acid.
- **polypeptide**: Any polymer of (same or different) amino acids joined via peptide bonds.

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2.5.6: ATP- Adenosine Triphosphate

Learning Objectives

- Explain the role of ATP as the currency of cellular energy

ATP: Adenosine Triphosphate

Adenosine triphosphate (ATP) is the energy currency for cellular processes. ATP provides the energy for both energy-consuming endergonic reactions and energy-releasing exergonic reactions, which require a small input of activation energy. When the chemical bonds within ATP are broken, energy is released and can be harnessed for cellular work. The more bonds in a molecule, the more potential energy it contains. Because the bond in ATP is so easily broken and reformed, ATP is like a rechargeable battery that powers cellular process ranging from DNA replication to protein synthesis.

Molecular Structure

Adenosine triphosphate (ATP) is comprised of the molecule adenosine bound to three phosphate groups. Adenosine is a nucleoside consisting of the nitrogenous base adenine and the five-carbon sugar ribose. The three phosphate groups, in order of closest to furthest from the ribose sugar, are labeled alpha, beta, and gamma. Together, these chemical groups constitute an energy powerhouse. The two bonds between the phosphates are equal high-energy bonds (phosphoanhydride bonds) that, when broken, release sufficient energy to power a variety of cellular reactions and processes. The bond between the beta and gamma phosphate is considered “high-energy” because when the bond breaks, the products [adenosine diphosphate (ADP) and one inorganic phosphate group (P_i)] have a lower free energy than the reactants (ATP and a water molecule). ATP breakdown into ADP and P_i is called hydrolysis because it consumes a water molecule (hydro-, meaning “water”, and lysis, meaning “separation”).

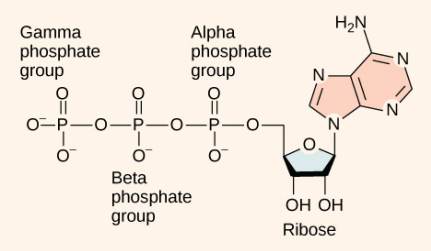


Figure: **Adenosine Triphosphate (ATP)**: ATP is the primary energy currency of the cell. It has an adenosine backbone with three phosphate groups attached.

ATP Hydrolysis and Synthesis

ATP is hydrolyzed into ADP in the following reaction:

$ATP + H_2O \rightarrow ADP + P_i + \text{free energy}$

Like most chemical reactions, the hydrolysis of ATP to ADP is reversible. The reverse reaction combines ADP + P_i to regenerate ATP from ADP. Since ATP hydrolysis releases energy, ATP synthesis must require an input of free energy.

ADP is combined with a phosphate to form ATP in the following reaction:

$ADP + P_i + \text{free energy} \rightarrow ATP + H_2O$

ATP and Energy Coupling

Exactly how much free energy (ΔG) is released with the hydrolysis of ATP, and how is that free energy used to do cellular work? The calculated ΔG for the hydrolysis of one mole of ATP into ADP and P_i is −7.3 kcal/mole (−30.5 kJ/mol). However, this is only true under standard conditions, and the ΔG for the hydrolysis of one mole of ATP in a living cell is almost double the value at standard conditions: 14 kcal/mol (−57 kJ/mol).

ATP is a highly unstable molecule. Unless quickly used to perform work, ATP spontaneously dissociates into ADP + P_i, and the free energy released during this process is lost as heat. To harness the energy within the bonds of ATP, cells use a strategy called

energy coupling.

Energy Coupling in Sodium-Potassium Pumps

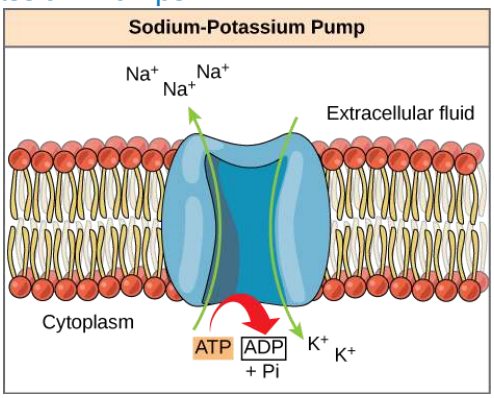


Figure: **Energy Coupling**: Sodium-potassium pumps use the energy derived from exergonic ATP hydrolysis to pump sodium and potassium ions across the cell membrane.

Cells couple the exergonic reaction of ATP hydrolysis with the endergonic reactions of cellular processes. For example, transmembrane ion pumps in nerve cells use the energy from ATP to pump ions across the cell membrane and generate an action potential. The sodium-potassium pump (Na^+/K^+ pump) drives sodium out of the cell and potassium into the cell. When ATP is hydrolyzed, it transfers its gamma phosphate to the pump protein in a process called phosphorylation. The Na^+/K^+ pump gains the free energy and undergoes a conformational change, allowing it to release three Na^+ to the outside of the cell. Two extracellular K^+ ions bind to the protein, causing the protein to change shape again and discharge the phosphate. By donating free energy to the Na^+/K^+ pump, phosphorylation drives the endergonic reaction.

Energy Coupling in Metabolism

During cellular metabolic reactions, or the synthesis and breakdown of nutrients, certain molecules must be altered slightly in their conformation to become substrates for the next step in the reaction series. In the very first steps of cellular respiration, glucose is broken down through the process of glycolysis. ATP is required for the phosphorylation of glucose, creating a high-energy but unstable intermediate. This phosphorylation reaction causes a conformational change that allows enzymes to convert the phosphorylated glucose molecule to the phosphorylated sugar fructose. Fructose is a necessary intermediate for glycolysis to move forward. In this example, the exergonic reaction of ATP hydrolysis is coupled with the endergonic reaction of converting glucose for use in the metabolic pathway.

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Key Points

- Adenosine triphosphate is composed of the nitrogenous base adenine, the five-carbon sugar ribose, and three phosphate groups.
- ATP is hydrolyzed to ADP in the reaction $ATP + H_2O \rightarrow ADP + P_i$ + free energy; the calculated ΔG for the hydrolysis of 1 mole of ATP is -57 kJ/mol.

- ADP is combined with a phosphate to form ATP in the reaction ADP+Pi+free energy → ATP+H2O.
- The energy released from the hydrolysis of ATP into ADP is used to perform cellular work, usually by coupling the exergonic reaction of ATP hydrolysis with endergonic reactions.
- Sodium-potassium pumps use the energy derived from exergonic ATP hydrolysis to pump sodium and potassium ions across the cell membrane while phosphorylation drives the endergonic reaction.

Key Terms

- **energy coupling**: Energy coupling occurs when the energy produced by one reaction or system is used to drive another reaction or system.
- **endergonic**: Describing a reaction that absorbs (heat) energy from its environment.
- **exergonic**: Describing a reaction that releases energy (heat) into its environment.
- **free energy**: Gibbs free energy is a thermodynamic potential that measures the useful or process-initiating work obtainable from a thermodynamic system at a constant temperature and pressure (isothermal, isobaric).
- **hydrolysis**: A chemical process of decomposition involving the splitting of a bond by the addition of water.

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2.5.E: Organic Molecules (Exercises)

7.1: Organic Molecules

Biochemistry is the discipline that studies the chemistry of life, and its objective is to explain form and function based on chemical principles. Organic chemistry is the discipline devoted to the study of carbon-based chemistry, which is the foundation for the study of biomolecules and the discipline of biochemistry. Both biochemistry and organic chemistry are based on the concepts of general chemistry.

Multiple Choice

Which of these elements is *not* a micronutrient?

A. C
B. Ca
C. Co
D. Cu

Answer

A

Which of the following is the name for molecules whose structures are nonsuperimposable mirror images?

A. structural isomers
B. monomers
C. polymers
D. enantiomers

Answer

D

True/False

Aldehydes, amides, carboxylic acids, esters, and ketones all contain carbonyl groups.

Answer

True

Two molecules containing the same types and numbers of atoms but different bonding sequences are called enantiomers.

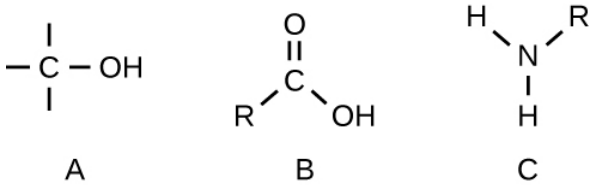
Answer

False

Short Answer

Why are carbon, nitrogen, oxygen, and hydrogen the most abundant elements in living matter and, therefore, considered macronutrients?

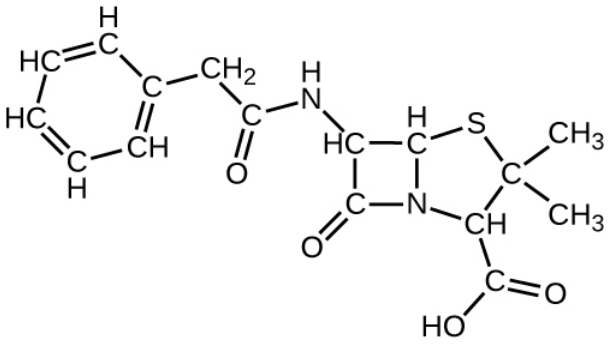
Identify the functional group in each of the depicted structural formulas.



A B C

Critical Thinking

The structural formula shown corresponds to penicillin G, a narrow-spectrum antibiotic that is given intravenously or intramuscularly as a treatment for several bacterial diseases. The antibiotic is produced by fungi of the genus *Penicillium*. (a) Identify three major functional groups in this molecule that each comprise two simpler functional groups. (b) Name the two simpler functional groups composing each of the major functional groups identified in (a).



7.2: Carbohydrates

The most abundant biomolecules on earth are carbohydrates. From a chemical viewpoint, carbohydrates are primarily a combination of carbon and water, and many of them have the empirical formula $(CH_2O)_n$, where *n* is the number of repeated units. This view represents these molecules simply as “hydrated” carbon atom chains in which water molecules attach to each carbon atom, leading to the term “carbohydrates.”

Multiple Choice

By definition, carbohydrates contain which elements?

A. carbon and hydrogen
B. carbon, hydrogen, and nitrogen
C. carbon, hydrogen, and oxygen
D. carbon and oxygen

Answer

C

Monosaccharides may link together to form polysaccharides by forming which type of bond?

A. hydrogen
B. peptide
C. ionic
D. glycosidic

Answer

D

Matching

Match each polysaccharide with its description.

___chitin	A. energy storage polymer in plants
___glycogen	B. structural polymer found in plants
___starch	C. structural polymer found in cell walls of fungi and exoskeletons of some animals
___cellulose	D. energy storage polymer found in animal cells and bacteria

Answer

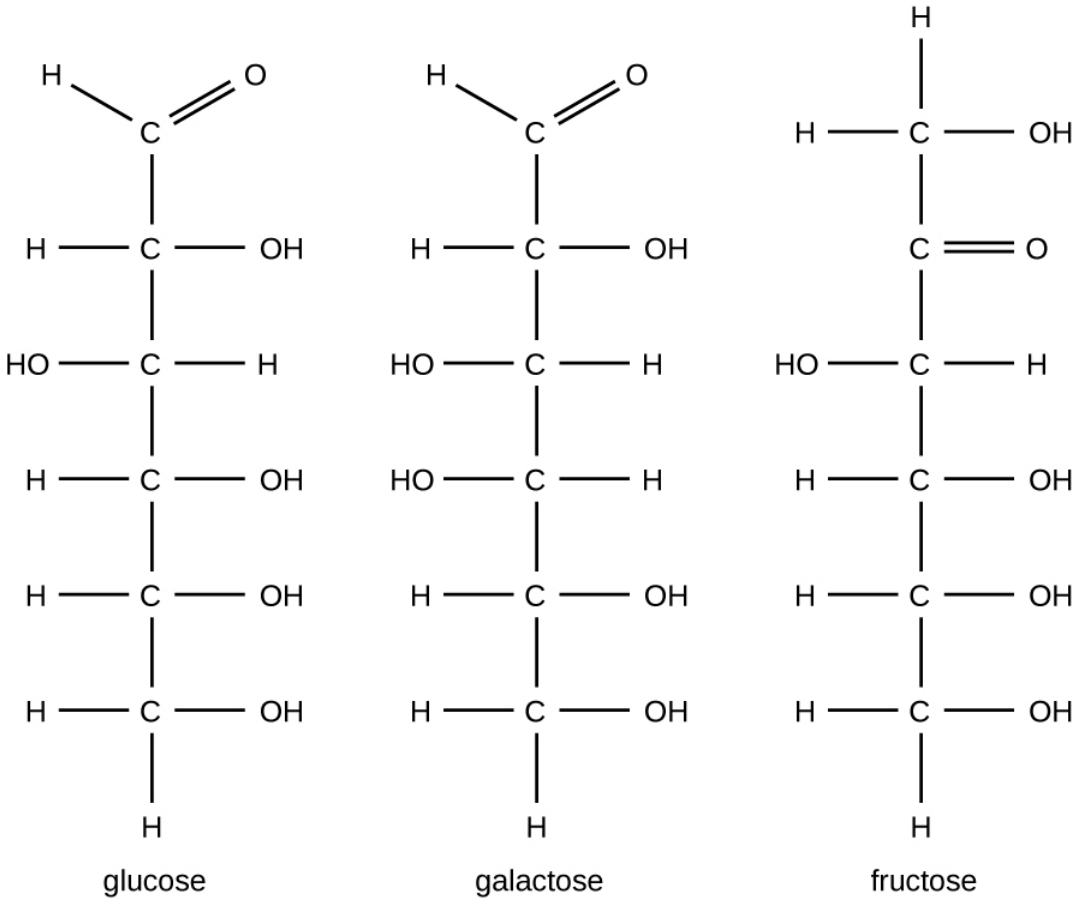
C, D, A, B

Short Answer

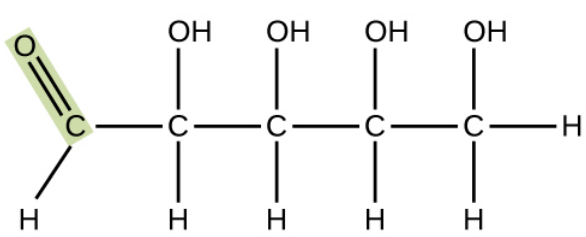
What are monosaccharides, disaccharides, and polysaccharides?

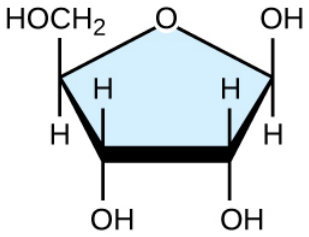
Critical Thinking

The figure depicts the structural formulas of glucose, galactose, and fructose. (a) Circle the functional groups that classify the sugars either an aldose or a ketose, and identify each sugar as one or the other. (b) The chemical formula of these compounds is the same, although the structural formula is different. What are such compounds called?



Structural diagrams for the linear and cyclic forms of a monosaccharide are shown. (a) What is the molecular formula for this monosaccharide? (Count the C, H and O atoms in each to confirm that these two molecules have the same formula, and report this formula.) (b) Identify which hydroxyl group in the linear structure undergoes the ring-forming reaction with the carbonyl group.





The term “dextrose” is commonly used in medical settings when referring to the biologically relevant isomer of the monosaccharide glucose. Explain the logic of this alternative name.

7.3: Lipids

Although they are composed primarily of carbon and hydrogen, lipid molecules may also contain oxygen, nitrogen, sulfur, and phosphorous. Lipids serve numerous and diverse purposes in the structure and functions of organisms. They can be a source of nutrients, a storage form for carbon, energy-storage molecules, or structural components of membranes and hormones. Lipids comprise a broad class of many chemically distinct compounds, the most common of which are discussed in this section.

Multiple Choice

Which of the following describes lipids?

A. a source of nutrients for organisms
B. energy-storage molecules
C. molecules having structural role in membranes
D. molecules that are part of hormones and pigments
E. all of the above

Answer

E

Molecules bearing both polar and nonpolar groups are said to be which of the following?

A. hydrophilic
B. amphipathic
C. hydrophobic
D. polyfunctional

Answer

B

True/False

Lipids are a naturally occurring group of substances that are not soluble in water but are freely soluble in organic solvents.

Answer

False

Fatty acids having no double bonds are called “unsaturated.”

Answer

False

A triglyceride is formed by joining three glycerol molecules to a fatty acid backbone in a dehydration reaction.

Answer

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False

Fill in the Blank

Waxes contain esters formed from long-chain _____ and saturated _____, and they may also contain substituted hydrocarbons.

Answer

alcohols; fatty acids

Cholesterol is the most common member of the _____ group, found in animal tissues; it has a tetracyclic carbon ring system with a _____ bond in one of the rings and one free _____group.

Answer

steroid; double; hydroxyl

Critical Thinking

Microorganisms can thrive under many different conditions, including high-temperature environments such as hot springs. To function properly, cell membranes have to be in a fluid state. How do you expect the fatty acid content (saturated versus unsaturated) of bacteria living in high-temperature environments might compare with that of bacteria living in more moderate temperatures?

Short Answer

Describe the structure of a typical phospholipid. Are these molecules polar or nonpolar?

7.4: Proteins

Amino acids are capable of bonding together in essentially any number, yielding molecules of essentially any size that possess a wide array of physical and chemical properties and perform numerous functions vital to all organisms. The molecules derived from amino acids can function as structural components of cells and subcellular entities, as sources of nutrients, as atom- and energy-storage reservoirs, and as functional species such as hormones, enzymes, receptors, and transport molecules.

Multiple Choice

Which of the following groups varies among different amino acids?

A. hydrogen atom
B. carboxyl group
C. *R* group
D. amino group

Answer

C

The amino acids present in proteins differ in which of the following?

A. size
B. shape
C. side groups
D. all of the above

Answer

D

Which of the following bonds are not involved in tertiary structure?

A. peptide bonds
B. ionic bonds
C. hydrophobic interactions
D. hydrogen bonds

Answer

A

Fill in the Blank

The sequence of amino acids in a protein is called its _____.

Answer

Primary structure

Denaturation implies the loss of the _____ and _____ structures without the loss of the _____ structure.

Answer

secondary, tertiary, primary

True/False

A change in one amino acid in a protein sequence always results in a loss of function.

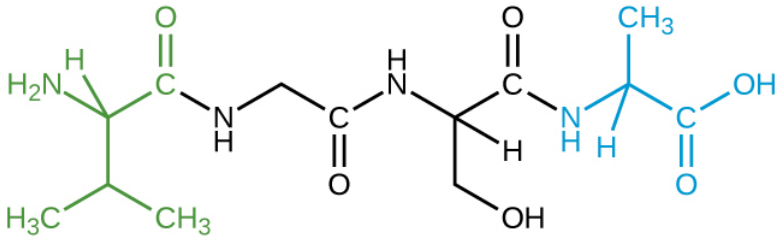
Answer

False

Critical Thinking

Heating a protein sufficiently may cause it to denature. Considering the definition of denaturation, what does this statement say about the strengths of peptide bonds in comparison to hydrogen bonds?

The image shown represents a tetrapeptide. (a) How many peptide bonds are in this molecule? (b) Identify the side groups of the four amino acids composing this peptide.



7.5: Using Biochemistry to Identify Microorganisms

Accurate identification of bacteria is essential in a clinical laboratory for diagnostic and management of epidemics, pandemics, and food poisoning caused by bacterial outbreaks. In this section, we discuss a few methods that use biochemical characteristics to identify microorganisms.

Multiple Choice

Which of the following characteristics/compounds is not considered to be a phenotypic biochemical characteristic used of microbial identification?

A. poly-β-hydroxybutyrate
B. small-subunit (16S) rRNA gene
C. carbon utilization
D. lipid composition

Answer

B

Proteomic analysis is a methodology that deals with which of the following?

A. the analysis of proteins functioning as enzymes within the cell
B. analysis of transport proteins in the cell
C. the analysis of integral proteins of the cell membrane
D. the study of all accumulated proteins of an organism

Answer

D

Which method involves the generation of gas phase ions from intact microorganisms?

A. FAME
B. PLFA
C. MALDI-TOF
D. Lancefield group testing

Answer

C

Which method involves the analysis of membrane-bound carbohydrates?

A. FAME
B. PLFA
C. MALDI-TOF
D. Lancefield group testing

Answer

D

Which method involves conversion of a microbe's lipids to volatile compounds for analysis by gas chromatography?

A. FAME
B. proteomic analysis
C. MALDI-TOF
D. Lancefield group testing

Answer

A

Fill in the Blank

A FAME analysis involves the conversion of _____ to more volatile _____ for analysis using _____.

Answer

fatty acids, methyl esters, gas chromatography

True/False

MALDI-TOF relies on obtaining a unique mass spectrum for the bacteria tested and then checking the acquired mass spectrum against the spectrum databases registered in the analysis software to identify the microorganism.

Answer

True

Lancefield group tests can identify microbes using antibodies that specifically bind cell-surface proteins.

Answer

False

Short Answer

Compare MALDI-TOF, FAME, and PLFA, and explain how each technique would be used to identify pathogens.

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CHAPTER OVERVIEW

3: Cell Structure and Function

- 3.1: Characteristics of Life
- 3.2: Cells and Cell Theory
  - 3.2.1: Comparing Prokaryotic and Eukaryotic Cells
- 3.3: Prokaryotic External Structures
  - 3.3.1: Bacteria Surface Structures
  - 3.3.2: Flagella
  - 3.3.3: Fimbriae and Pili
- 3.4: Prokaryote Cell Wall
  - 3.4.1: Antibiotics and Selective Toxicity
  - 3.4.2: Gram-Negative Wall
  - 3.4.3: Gram-Positive Cell Wall
  - 3.4.4: Archaea Cell Wall
- 3.5: Prokaryote Plasma Membrane
  - 3.5.1: Components and Structure - Fluid Mosaic Model
- 3.6: Endospores
  - 3.6.1: Fungal Spores
- 3.7: Eukaryotic External Structures
- 3.8: Eukaryotic Plasma Membrane and The Cytoplasm
  - 3.8.1: The Plasma Membrane and Transport
    - 3.8.1.1: The Plasma Membrane
    - 3.8.1.2: Transport Across Membranes
    - 3.8.1.3: Passive Transport - Diffusion
    - 3.8.1.4: Passive Transport - Facilitated Transport
    - 3.8.1.5: Passive Transport - Osmosis
    - 3.8.1.6: Active Transport
  - 3.8.2: Endocytosis
- 3.9: Eukaryote Organelles
- 3.10: Serial Endosymbiosis Theory (SET)

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3.1: CHARACTERISTICS OF LIFE

LEARNING OBJECTIVES

- Describe the properties of life

All living organisms share several key characteristics or functions: order, sensitivity or response to the environment, reproduction, growth and development, regulation, homeostasis, and energy processing. When viewed together, these eight characteristics serve to define life.



Figure **3.1.1**: Multicellular Organisms: A toad represents a highly organized structure consisting of cells, tissues, organs, and organ systems.

ORDER

Organisms are highly organized, coordinated structures that consist of one or more cells. Even very simple, single-celled organisms are remarkably complex: inside each cell, atoms make up molecules; these in turn make up cell organelles and other cellular inclusions. In multicellular organisms, similar cells form tissues. Tissues, in turn, collaborate to create organs (body structures with a distinct function). Organs work together to form organ systems.



Figure **3.1.2**: Response to Stimuli: The leaves of this sensitive plant (Mimosa pudica) will instantly droop and fold when touched. After a few minutes, the plant returns to normal.

SENSITIVITY OR RESPONSE TO STIMULI

Organisms can respond to diverse stimuli. For example, plants can grow toward a source of light, climb on fences and walls, or respond to touch. Even tiny bacteria can move toward or away from chemicals (a process called chemotaxis) or light (phototaxis). Movement toward a stimulus is considered a positive response, while movement away from a stimulus is considered a negative response.

REPRODUCTION

Single-celled organisms reproduce by first duplicating their DNA. They then divide it equally as the cell prepares to divide to form two new cells. Multicellular organisms often produce specialized reproductive germline cells that will form new individuals. When reproduction occurs, genes containing DNA are passed along to an organism's offspring. These genes ensure that the offspring will belong to the same species and will have similar characteristics, such as size and shape.



Figure **3.1.3**: Reproduction: Although no two look alike, these kittens have inherited genes from both parents and share many of the same characteristics.

GROWTH AND DEVELOPMENT

All organisms grow and develop following specific instructions coded for by their genes. These genes provide instructions that will direct cellular growth and development, ensuring that a species' young will grow up to exhibit many of the same characteristics as its parents.

REGULATION

Even the smallest organisms are complex and require multiple regulatory mechanisms to coordinate internal functions, respond to stimuli, and cope with environmental stresses. Two examples of internal functions regulated in an organism are nutrient transport and blood flow. Organs (groups of tissues working together) perform specific functions, such as carrying oxygen throughout the body, removing wastes, delivering nutrients to every cell, and cooling the body.



Figure **3.1.4**: Homeostasis: Polar bears (Ursus maritimus) and other mammals living in ice-covered regions maintain their body temperature by generating heat and reducing heat loss through thick fur and a dense layer of fat under their skin.

HOMEOSTASIS

In order to function properly, cells need to have appropriate conditions such as proper temperature, pH, and appropriate concentration of diverse chemicals. These conditions may, however, change from one moment to the next. Organisms are able to maintain internal conditions within a narrow range almost constantly, despite environmental changes, through homeostasis (literally, "steady state")—the ability of an organism to maintain constant internal conditions. For example, an organism needs to regulate body temperature through a process known as thermoregulation. Organisms that live in cold climates, such as the polar bear, have body structures that help them withstand low temperatures and conserve body heat. Structures that aid in this type of insulation include fur, feathers, blubber, and fat. In hot climates, organisms have methods (such as perspiration in humans or panting in dogs) that help them to shed excess body heat.



Figure **3.1.5**: Energy Processing: The California condor (Gymnogyps californianus) uses chemical energy derived from food to power flight.

ENERGY PROCESSING

All organisms use a source of energy for their metabolic activities. Some organisms capture energy from the sun and convert it into chemical energy in food; others use chemical energy in molecules they take in as food.



Figure **3.1.6**: Adaptation in the flat-tailed horned lizard: This lizard exhibits a flattened body and coloring that helps camouflage it, both of which are adaptive traits that help it avoid predators.

EVOLUTION

As a population of organisms interacts with the environment, individuals with traits that contribute to reproduction and survival in that particular environment will leave more offspring. Over time those advantageous traits (called adaptations) will become more common in the population. This process, change over time, is called evolution, and it is one of the processes that explain the diverse species seen in biology. Adaptations help organisms survive in their ecological niches, and adaptive traits may be structural, behavioral, or physiological; as such, adaptations frequently involve other properties of organisms such as homeostasis, reproduction, and growth and development.

KEY POINTS

- Order can include highly organized structures such as cells, tissues, organs, and organ systems.
- Interaction with the environment is shown by response to stimuli.
- The ability to reproduce, grow and develop are defining features of life.
- The concepts of biological regulation and maintenance of homeostasis are key to survival and define major properties of life.
- Organisms use energy to maintain their metabolic processes.
- Populations of organisms evolve to produce individuals that are adapted to their specific environment.

KEY TERMS

- **phototaxis**: The movement of an organism either towards or away from a source of light
- **gene**: a unit of heredity; the functional units of chromosomes that determine specific characteristics by coding for specific proteins
- **chemotaxis**: the movement of a cell or an organism in response to a chemical stimulant

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3.2: CELLS AND CELL THEORY

LEARNING OBJECTIVES

- Identify the components of cell theory

CELL THEORY

The microscopes we use today are far more complex than those used in the 1600s by Antony van Leeuwenhoek, a Dutch shopkeeper who had great skill in crafting lenses. Despite the limitations of his now-ancient lenses, van Leeuwenhoek observed the movements of protista (a type of single-celled organism) and sperm, which he collectively termed “animalcules. ”

In a 1665 publication called *Micrographia*, experimental scientist Robert Hooke coined the term “cell” for the box-like structures he observed when viewing cork tissue through a lens. In the 1670s, van Leeuwenhoek discovered bacteria and protozoa. Later advances in lenses, microscope construction, and staining techniques enabled other scientists to see some components inside cells.

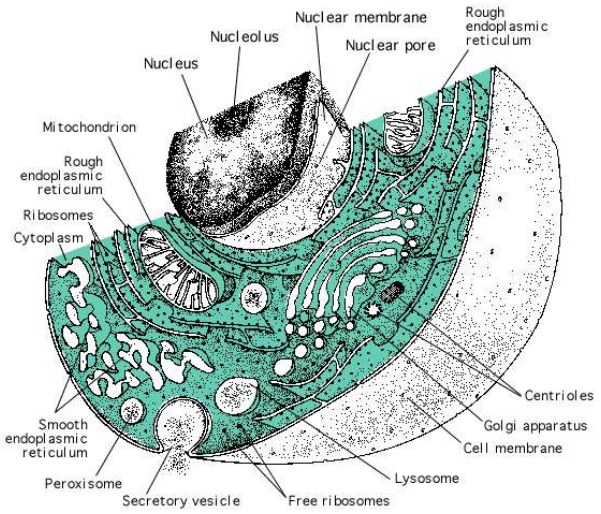


Figure **3.2.1**: Structure of an Animal Cell: The cell is the basic unit of life and the study of the cell led to the development of the cell theory.

By the late 1830s, botanist Matthias Schleiden and zoologist Theodor Schwann were studying tissues and proposed the unified cell theory. The unified cell theory states that: all living things are composed of one or more cells; the cell is the basic unit of life; and new cells arise from existing cells. Rudolf Virchow later made important contributions to this theory.

Schleiden and Schwann proposed spontaneous generation as the method for cell origination, but spontaneous generation (also called abiogenesis) was later disproven. Rudolf Virchow famously stated “Omnis cellula e cellula”… “All cells only arise from pre-existing cells. “The parts of the theory that did not have to do with the origin of cells, however, held up to scientific scrutiny and are widely agreed upon by the scientific community today. The generally accepted portions of the modern Cell Theory are as follows:

1. The cell is the fundamental unit of structure and function in living things.
2. All organisms are made up of one or more cells.
3. Cells arise from other cells through cellular division.

The expanded version of the cell theory can also include:

- Cells carry genetic material passed to daughter cells during cellular division
- All cells are essentially the same in chemical composition
- Energy flow (metabolism and biochemistry) occurs within cells

KEY POINTS

- The cell theory describes the basic properties of all cells.
- The three scientists that contributed to the development of cell theory are Matthias Schleiden, Theodor Schwann, and Rudolf Virchow.
- A component of the cell theory is that all living things are composed of one or more cells.
- A component of the cell theory is that the cell is the basic unit of life.
- A component of the cell theory is that all new cells arise from existing cells.

KEY TERMS

- **cell theory**: The scientific theory that all living organisms are made of cells as the smallest functional unit.

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3.2.1: Comparing Prokaryotic and Eukaryotic Cells

Cells fall into one of two broad categories: prokaryotic and eukaryotic. The predominantly single-celled organisms of the domains Bacteria and Archaea are classified as prokaryotes (*pro-* = before; *-karyon-* = nucleus). Animal cells, plant cells, fungi, and protists are eukaryotes (*eu-* = true).

Components of Prokaryotic Cells

All cells share four common components: 1) a plasma membrane, an outer covering that separates the cell's interior from its surrounding environment; 2) cytoplasm, consisting of a jelly-like region within the cell in which other cellular components are found; 3) DNA, the genetic material of the cell; and 4) ribosomes, particles that synthesize proteins. However, prokaryotes differ from eukaryotic cells in several ways.

A prokaryotic cell is a simple, single-celled (unicellular) organism that lacks a nucleus, or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is found in the central part of the cell: a darkened region called the nucleoid (Figure **3.2.1.1**).

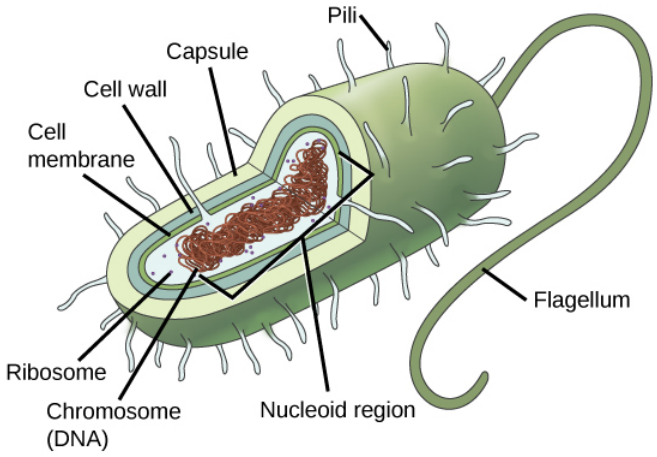


Figure 3.2.1.1: This figure shows the generalized structure of a prokaryotic cell.

Unlike Archaea and eukaryotes, bacteria have a cell wall made of peptidoglycan, comprised of sugars and amino acids, and many have a polysaccharide capsule (Figure **3.2.1.1**). The cell wall acts as an extra layer of protection, helps the cell maintain its shape, and prevents dehydration. The capsule enables the cell to attach to surfaces in its environment. Some prokaryotes have flagella, pili, or fimbriae. Flagella are used for locomotion, while most pili are used to exchange genetic material during a type of reproduction called conjugation.

Eukaryotic Cells

In nature, the relationship between form and function is apparent at all levels, including the level of the cell, and this will become clear as we explore eukaryotic cells. The principle "form follows function" is found in many contexts. For example, birds and fish have streamlined bodies that allow them to move quickly through the medium in which they live, be it air or water. It means that, in general, one can deduce the function of a structure by looking at its form, because the two are matched.

A eukaryotic cell is a cell that has a membrane-bound nucleus and other membrane-bound compartments or sacs, called organelles, which have specialized functions. The word eukaryotic means "true kernel" or "true nucleus," alluding to the presence of the membrane-bound nucleus in these cells. The word "organelle" means "little organ," and, as already mentioned, organelles have specialized cellular functions, just as the organs of your body have specialized functions.

Cell Size

At 0.1–5.0 µm in diameter, prokaryotic cells are significantly smaller than eukaryotic cells, which have diameters ranging from 10–100 µm (Figure **3.2.1.2**). The small size of prokaryotes allows ions and organic molecules that enter them to quickly spread to other parts of the cell. Similarly, any wastes produced within a prokaryotic cell can quickly move out. However, larger eukaryotic cells

have evolved different structural adaptations to enhance cellular transport. Indeed, the large size of these cells would not be possible without these adaptations. In general, cell size is limited because volume increases much more quickly than does cell surface area. As a cell becomes larger, it becomes more and more difficult for the cell to acquire sufficient materials to support the processes inside the cell, because the relative size of the surface area across which materials must be transported declines.

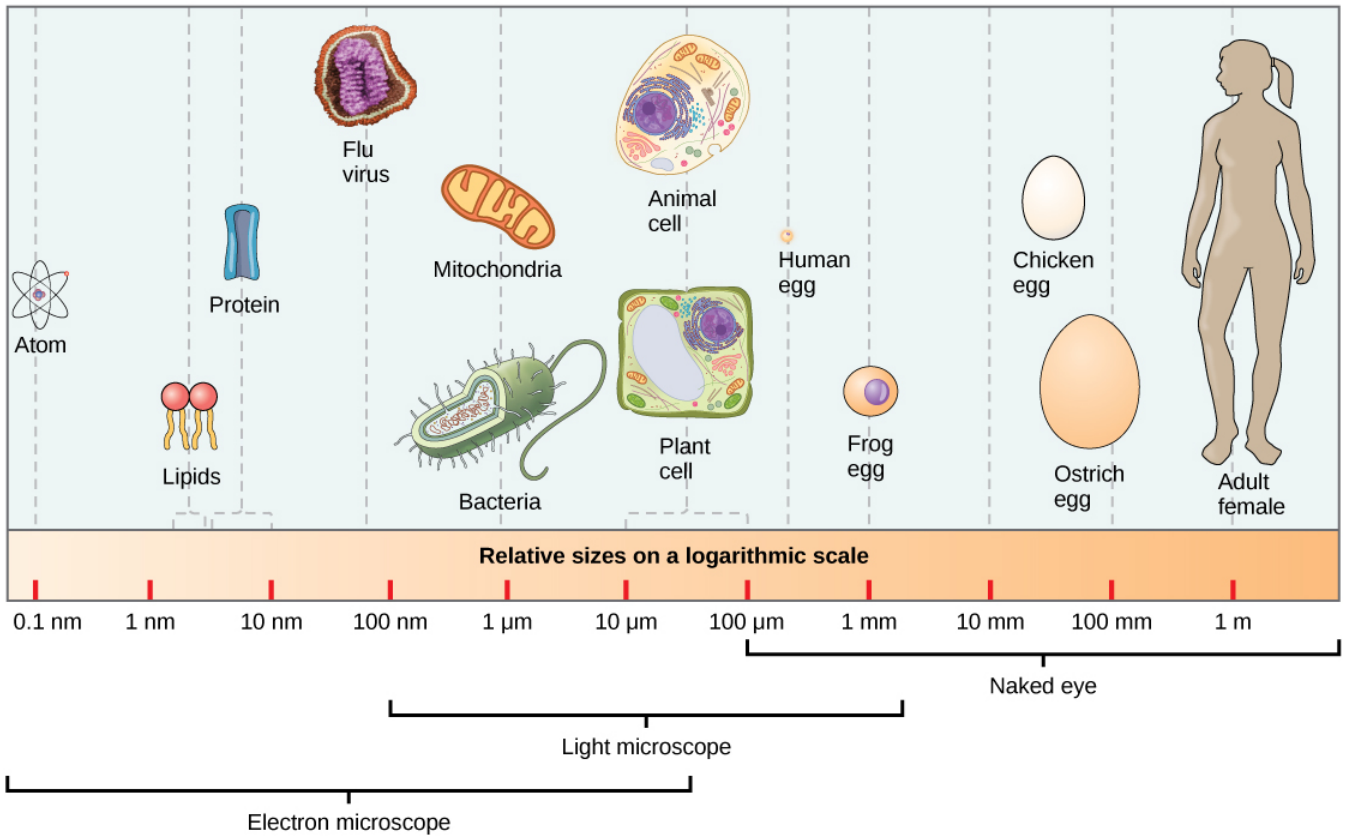


Figure 3.2.1.2: This figure shows the relative sizes of different kinds of cells and cellular components. An adult human is shown for comparison.

Section Summary

Prokaryotes are predominantly single-celled organisms of the domains Bacteria and Archaea. All prokaryotes have plasma membranes, cytoplasm, ribosomes, a cell wall, DNA, and lack membrane-bound organelles. Many also have polysaccharide capsules. Prokaryotic cells range in diameter from 0.1–5.0 µm.

Like a prokaryotic cell, a eukaryotic cell has a plasma membrane, cytoplasm, and ribosomes, but a eukaryotic cell is typically larger than a prokaryotic cell, has a true nucleus (meaning its DNA is surrounded by a membrane), and has other membrane-bound organelles that allow for compartmentalization of functions. Eukaryotic cells tend to be 10 to 100 times the size of prokaryotic cells.

Glossary

eukaryotic cell

a cell that has a membrane-bound nucleus and several other membrane-bound compartments or sacs

organelle

a membrane-bound compartment or sac within a cell

prokaryotic cell

a unicellular organism that lacks a nucleus or any other membrane-bound organelle

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3.3: PROKARYOTIC EXTERNAL STRUCTURES

LEARNING OBJECTIVES

- Describe the structure of prokaryotic cells

COMPONENTS OF PROKARYOTIC CELLS

All cells share four common components:

1. a plasma membrane: an outer covering that separates the cell's interior from its surrounding environment.
2. cytoplasm: a jelly-like cytosol within the cell in which other cellular components are found
3. DNA: the genetic material of the cell
4. ribosomes: where protein synthesis occurs

However, prokaryotes differ from eukaryotic cells in several ways.

A prokaryote is a simple, single-celled (unicellular) organism that lacks an organized nucleus or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is found in a central part of the cell: the nucleoid.

Most prokaryotes have a peptidoglycan cell wall and many have a polysaccharide capsule. The cell wall acts as an extra layer of protection, helps the cell maintain its shape, and prevents dehydration. The capsule enables the cell to attach to surfaces in its environment. Some prokaryotes have flagella, pili, or fimbriae. Flagella are used for locomotion. Pili are used to exchange genetic material during a type of reproduction called conjugation. Fimbriae are used by bacteria to attach to a host cell.

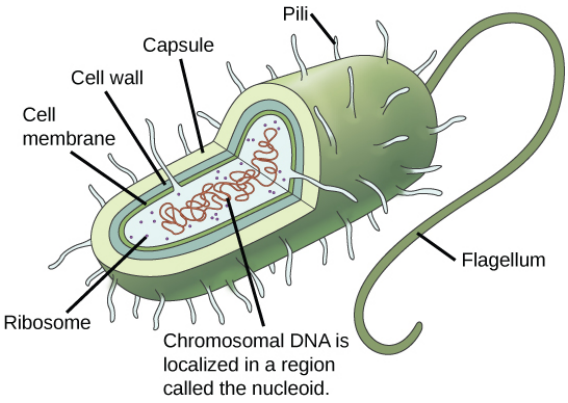


Figure **3.3.1**: General Structure of a Prokaryotic Cell: This figure shows the generalized structure of a prokaryotic cell.All prokaryotes have chromosomal DNA localized in a nucleoid, ribosomes, a cell membrane, and a cell wall.The other structures shown are present in some, but not all, bacteria.

CELL SIZE

At 0.1 to 5.0 µm in diameter, prokaryotic cells are significantly smaller than eukaryotic cells, which have diameters ranging from 10 to 100 µm. The small size of prokaryotes allows ions and organic molecules that enter them to quickly diffuse to other parts of the cell. Similarly, any wastes produced within a prokaryotic cell can quickly diffuse out. This is not the case in eukaryotic cells, which have developed different structural adaptations to enhance intracellular transport.

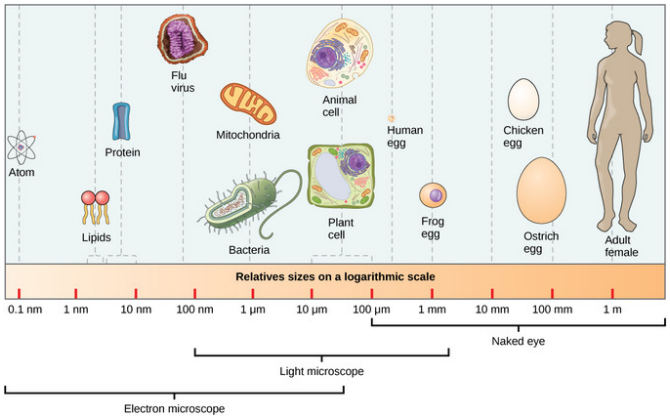


Figure **3.3.1**: Microbial Size: This figure shows relative sizes of microbes on a logarithmic scale (recall that each unit of increase in a logarithmic scale represents a 10-fold increase in the quantity being measured).

Small size, in general, is necessary for all cells, whether prokaryotic or eukaryotic. Let's examine why that is so. First, we'll consider the area and volume of a typical cell. Not all cells are spherical in shape, but most tend to approximate a sphere. You may remember from your high school geometry course that the formula for the surface area of a sphere is $4\pi r^2$, while the formula for its volume is $4/3\pi r^3$. Thus, as the radius of a cell increases, its surface area increases as the square of its radius, but its volume increases as the cube of its radius (much more rapidly). Therefore, as a cell increases in size, its surface area-to-volume ratio decreases. This same principle would apply if the cell had the shape of a cube. If the cell grows too large, the plasma membrane will not have sufficient surface area to support the rate of diffusion required for the increased volume. In other words, as a cell grows, it becomes less efficient. One way to become more efficient is to divide; another way is to develop organelles that perform specific tasks. These adaptations led to the development of more sophisticated cells called eukaryotic cells.

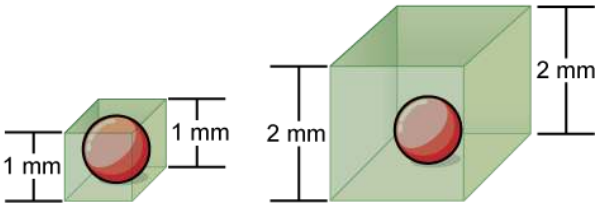


Figure **3.3.1**: Cell Surface Size: Notice that as a cell increases in size, its surface area-to-volume ratio decreases.When there is insufficient surface area to support a cell's increasing volume, a cell will either divide or die.The cell on the left has a volume of 1 mm3 and a surface area of 6 mm2, with a surface area-to-volume ratio of 6 to 1, whereas the cell on the right has a volume of 8 mm3 and a surface area of 24 mm2, with a surface area-to-volume ratio of 3 to 1.

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KEY POINTS

- Prokaryotes lack an organized nucleus and other membrane-bound organelles.
- Prokaryotic DNA is found in a central part of the cell called the nucleoid.
- The cell wall of a prokaryote acts as an extra layer of protection, helps maintain cell shape, and prevents dehydration.
- Prokaryotic cell size ranges from 0.1 to 5.0 μm in diameter.
- The small size of prokaryotes allows quick entry and diffusion of ions and molecules to other parts of the cell while also allowing fast removal of waste products out of the cell.

KEY TERMS

- **eukaryotic**: Having complex cells in which the genetic material is organized into membrane-bound nuclei.
- **prokaryotic**: Of cells, lacking a nucleus.
- **nucleoid**: the irregularly-shaped region within a prokaryote cell where the genetic material is localized

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3.3.1: Bacteria Surface Structures

Layers Outside the Cell Wall

What have we learned so far, in terms of cell layers? All cells have a cell membrane. Most bacteria have a cell wall. But there are a couple of additional layers that bacteria may, or may not, have. These would be found outside of both the cell membrane and the cell wall, if present.

Capsule

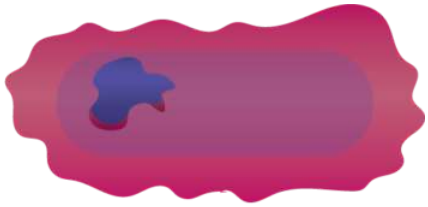
A bacterial **capsule** is a polysaccharide layer that completely envelopes the cell. It is well organized and tightly packed, which explains its resistance to staining under the microscope. The capsule offers protection from a variety of different threats to the cell, such as desiccation, hydrophobic toxic materials (i.e. detergents), and bacterial viruses. The capsule can enhance the ability of bacterial pathogens to cause disease and can provide protection from phagocytosis (engulfment by white blood cells known as phagocytes). Lastly, it can help in attachment to surfaces.

Slime Layer

A bacterial **slime layer** is similar to the capsule in that it is typically composed of polysaccharides and it completely surrounds the cell. It also offers protection from various threats, such as desiccation and antibiotics. It can also allow for adherence to surfaces. So, how does it differ from the capsule? A slime layer is a loose, unorganized layer that is easily stripped from the cell that made it, as opposed to a capsule which integrates firmly around the bacterial cell wall.



Capsule



Slime Layer

S-Layer

Some bacteria have a highly organized layer made of secreted proteins or glycoproteins that self-assemble into a matrix on the outer part of the cell wall. This regularly structured **S-layer** is anchored into the cell wall, although it is not considered to be officially part of the cell wall in bacteria. S-layers have very important roles for the bacteria that have them, particularly in the areas of growth and survival, and cell integrity.

S layers help maintain overall rigidity of the cell wall and surface layers, as well as cell shape, which are important for reproduction. S layers protect the cell from ion/pH changes, osmotic stress, detrimental enzymes, bacterial viruses, and predator bacteria. They can provide cell adhesion to other cells or surfaces. For pathogenic bacteria they can provide protection from phagocytosis.

Structures Outside the Cell Wall

Bacteria can also have structures outside of the cell wall, often bound to the cell wall and/or cell membrane. The building blocks for these structures are typically made within the cell and then secreted past the cell membrane and cell wall, to be assembled on the outside of the cell.

Fimbriae (sing. fimbria)

Fimbriae are thin filamentous appendages that extend from the cell, often in the tens or hundreds. They are composed of **pilin** proteins and are used by the cell to attach to surfaces. They can be particularly important for pathogenic bacteria, which use them to attach to host tissues.

Pili (sing. pilin)

Pili are very similar to fimbriae (some textbooks use the terms interchangeably) in that they are thin filamentous appendages that extend from the cell and are made of pilin proteins. Pili can be used for attachment as well, to both surfaces and host cells, such as the *Neisseria gonorrhea* cells that use their pili to grab onto sperm cells, for passage to the next human host. So, why would some researchers bother differentiating between fimbriae and pili?

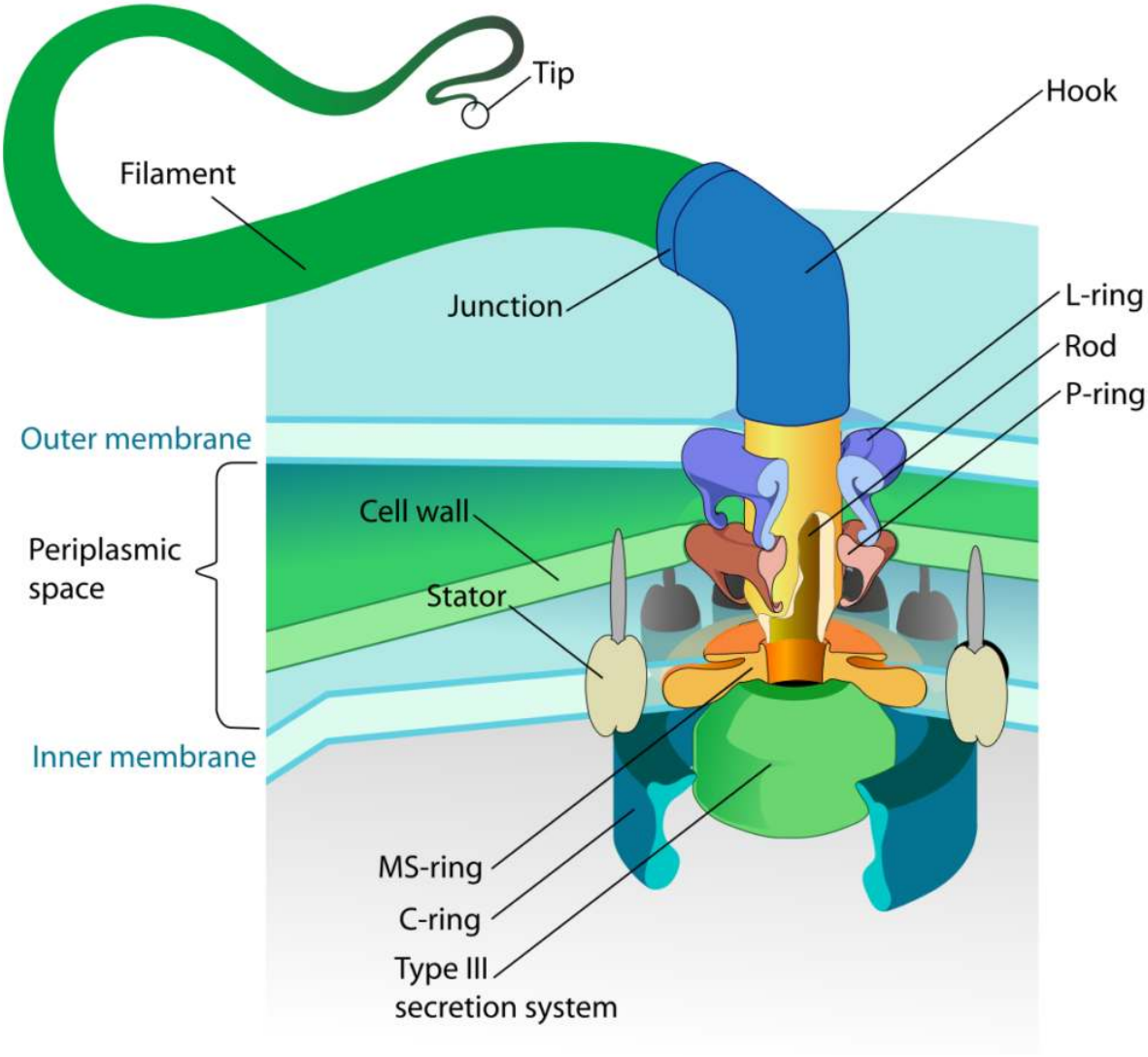
Pili are typically longer than fimbriae, with only 1-2 present on each cell, but that hardly seems enough to set the two structures apart. It really boils down to the fact that a few specific pili participate in functions beyond attachment. The **conjugative pili** participate in the process known as **conjugation**, which allows for the transfer of a small piece of DNA from a donor cell to a recipient cell. The **type IV pili** play a role in an unusual type of motility known as **twitching motility**, where a pilus attaches to a solid surface and then contracts, pulling the bacterium forward in a jerky motion.

Flagella (sing. flagellum)

Bacterial motility is typically provided by structures known as **flagella**. The bacterial flagellum differs in composition, structure, and function from the eukaryotic flagellum, which operates as a flexible whip-like tail utilizing microtubules. The bacterial flagellum is rigid in nature and operates more like the propeller on a boat.

There are three main components to the bacterial flagellum:

1. the **filament** – a long thin appendage that extends from the cell surface. The filament is composed of the protein **flagellin** and is hollow. Flagellin proteins are transcribed in the cell cytoplasm and then transported across the cell membrane and cell wall. A bacterial flagellar filament grows from its tip (unlike the hair on your head), adding more and more flagellin units to extend the length until the correct size is reached. The flagellin units are guided into place by a protein cap.
2. the **hook** – this is a curved coupler that attaches the filament to the flagellar motor.
3. the **motor** – a rotary motor that spans both the cell membrane and the cell wall, with additional components for the gram negative outer membrane. The motor has two components: the **basal body**, which provides the rotation, and the **stator**, which provides the torque necessary for rotation to occur. The basal body consists of a central shaft surrounded by protein rings, two in the gram positive bacteria and four in the gram negative bacteria. The stator consists of **Mot proteins** that surround the ring(s) embedded within the cell membrane.



Flagellum base diagram. By LadyofHats (Own work) [Public Domain], Via Wikipedia Commons

Bacterial Movement

Bacterial movement typically involves the use of flagella, although there are a few other possibilities as well (such as the use of type IV pili for twitching motility). But certainly the most common type of bacterial movement is **swimming**, which is accomplished with the use of a flagellum or flagella.

Swimming

Rotation of the flagellar basal body occurs due to the proton motive force, where protons that accumulate on the outside of the cell membrane are driven through pores in the Mot proteins, interacting with charges in the ring proteins as they pass across the membrane. The interaction causes the basal body to rotate and turns the filament extending from the cell. Rotation can occur at 200-1000 rpm and result in speeds of 60 cell lengths/second (for comparison, a cheetah moves at a maximum rate of 25 body lengths/second).

Rotation can occur in a **clockwise (CW)** or a **counterclockwise (CCW)**direction, with different results to the cell. A bacterium will move forward, called a "**run**," when there is a CCW rotation, and reorient randomly, called a "**tumble**," when there is a CW rotation.

Corkscrew Motility

Some spiral-shaped bacteria, known as the **Spirochetes**, utilize a **corkscrew-motility** due to their unusual morphology and flagellar conformation. These gram negative bacteria have specialized flagella that attach to one end of the cell, extend back through the periplasm and then attach to the other end of the cell. When these **endoflagella** rotate they put torsion on the entire cell, resulting in a flexing motion that is particularly effective for burrowing through viscous liquids.

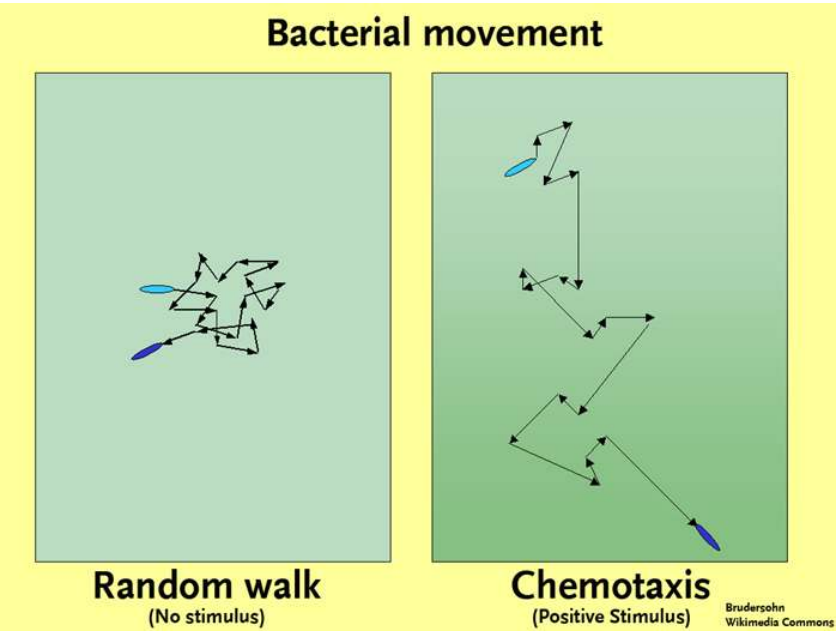
Gliding Motility

Gliding motility is just like it sounds, a slower and more graceful movement than the other forms covered so far. Gliding motility is exhibited by certain filamentous or bacillus bacteria and does not require the use of flagella. It does require that the cells be in contact with a solid surface, although more than one mechanism has been identified. Some cells rely on slime propulsion, where secreted slime propels the cell forward, where other cells rely on surface layer proteins to pull the cell forward.

Chemotaxis

Now that we have covered the basics of the bacterial flagellar motor and mechanics of bacterial swimming, let us combine the two topics to talk about **chemotaxis** or any other type of taxes (just not my taxes). Chemotaxis refers to the movement of an organism towards or away from a chemical. You can also have **phototaxis**, where an organism is responding to light. In chemotaxis, a favorable substance (such as a nutrient) is referred to as an **attractant**, while a substance with an adverse effect on the cell (such as a toxin) is referred to as a **repellant**. In the absence of either an attractant or a repellant a cell will engage in a "**random walk**," where it alternates between tumbles and runs, in the end getting nowhere in particular. In the presence of a gradient of some type, the movements of the cell will become biased, resulting over time in the movement of the bacterium towards an attractant and away from any repellants. How does this happen?

First, let us cover how a bacterium knows which direction to go. Bacteria rely on protein receptors embedded within their membrane, called **chemoreceptors**, which bind specific molecules. Binding typically results in methylation or phosphorylation of the chemoreceptor, which triggers an elaborate protein pathway that eventually impacts the rotation of the flagellar motor. The bacteria engage in **temporal sensing**, where they compare the concentration of a substance with the concentration obtained just a few seconds (or microseconds) earlier. In this way they gather information about the orientation of the concentration gradient of the substance. As a bacterium moves closer to the higher concentrations of an attractant, runs (dictated by CCW flagellar rotation) become longer, while tumbling (dictated by CW flagellar rotation) decreases. There will still be times that the bacterium will head off in the wrong direction away from an attractant since tumbling results in a random reorientation of the cell, but it won't head in the wrong direction for very long. The resulting "**biased random walk**" allows the cell to quickly move up the gradient of an attractant (or move down the gradient of a repellant).



Bacterial Movement. By Brudersohn (Own work (Original text: selbst erstellt)) [CC BY-SA 2.0 de], via Wikimedia Commons

Key Words

capsule, slime layer, S-layer, fimbriae/fimbria, pilin, pili/pilus, conjugative pili, conjugation, type IV pili, twitching motility, flagella/flagellum, filament, flagellin, hook, motor, basal body, stator, Mot proteins, swimming, clockwise (CW), counterclockwise (CCW), run, tumble, Spirochetes, corkscrew-motility, endoflagella, gliding motility, chemotaxis, phototaxis, attractant, repellant, random walk, chemoreceptors, temporal sensing, biased random walk.

Essential Questions/Objectives

1. What are the compositions and functions of capsules and slime layers? When are they produced? How do capsules or slime layers increase the survival chances of bacteria in different environments?
2. What are fimbriae and pili; what are their compositions and functions?What is the size of bacterial flagella and how can they be arranged on a bacterial cell? How common are flagella in bacteria?
3. What is the basic composition of a bacterial flagellum and how does this differ from flagella found in eukaryotes? How do bacterial flagella grow and how are proteins transported across the membrane? How do they cause movement? How is the movement different from Eukaryotic flagella?
4. How are bacterial flagella attached to the body? How do the 2 inner rings work to cause movement and what powers the movement? What is the purpose of the 2 outer rings found in the basal body of gram-bacteria? What do gram + have instead?
5. How do endoflagella differ from flagella and in what type of bacteria are they found? Where do they work better than flagella?
6. What is chemotaxis? How does the direction of rotation of the flagella affect the way a bacteria moves? What do we know about the mechanism of chemotaxis in terms of membrane binding-proteins and chemotactic mediator? How long do stimuli last in chemotaxis and why is this important to the phenomenon?

Exploratory Questions (OPTIONAL)

1. How could chemotaxis in microbes be used to address environmental pollution problems?

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3.3.2: Flagella

Describe the basic structure of a bacterial flagellum and state its function.
- State what provides the energy for bacterial flagellar rotation.
- Define the following flagellar arrangements:

a. monotrichous
b. lophotrichous
c. amphitrichous
d. peritrichous
e. axial filaments

- Define taxis.
- Compare and contrast how bacteria with peritrichous flagella and bacteria with polar flagella carry out taxis.
- State how bacterial flagella may play a role in the initiation of innate immune defenses.
- Briefly describe how bacterial flagella and chemotaxis may play a role in the pathogenocity of some bacteri

Highlighted Bacterium 1. Read the description of *Treponema pallidum* and match the bacterium with the description of the organism and the infection it causes.

Why might having flagella better enable those bacteria to cause disease?
- Our defense cells have a surface PRR called TLR-5 that recognizes bacterial flagellin. In terms of preventing infection, why is this an advantage?
- Most pathogenic spirochetes such as Treponema pallidum and Borrelia burgdorferi disseminate from the original infection site. How are they able to do this?

Structure and Composition of Flagella

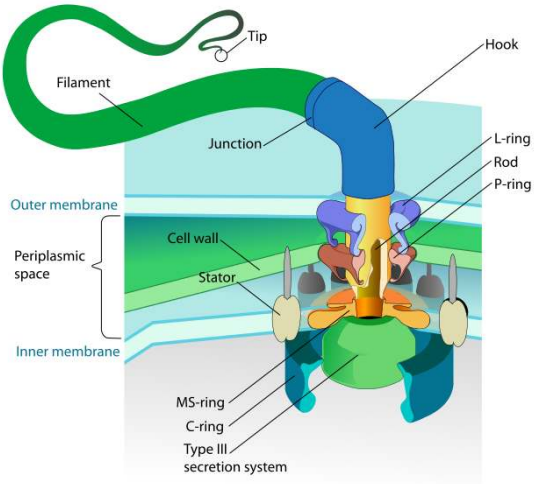


Figure **3.3.2.4**B.1: A flagellum (plural: flagella) is a long, slender projection from the cell body, whose function is to propel a unicellular or small multicellular organism. The depicted type of flagellum is found in bacteria such as E. coli and Salmonella, and rotates like a propeller when the bacterium swims. The bacterial movement can be divided into 2 kinds: run, resulting from a counterclockwise rotation of the flagellum, and tumbling, from a clockwise rotation of the flagellum. from Wikipedia (LadyofHats)

1. The filament is the rigid, helical structure that extends from the cell surface. It is composed of the protein flagellin arranged in helical chains so as to form a hollow core. During synthesis of the flagellar filament, flagellin molecules coming off of the ribosomes are transported through the hollow core of the filament where they attach to the growing tip of the filament causing it to lengthen. With the exception of a few bacteria, such as *Bdellovibrio* and *Vibrio cholerae*, the flagellar filament is not surrounded by a sheath (see Figure **3.3.2.1**).
2. The hook is a flexible coupling between the filament and the basal body (see Figure **3.3.2.1**).
3. The basal body consists of a rod and a series of rings that anchor the flagellum to the cell wall and the cytoplasmic membrane (see Figure **3.3.2.1**). Unlike eukaryotic flagella, the bacterial flagellum has no internal fibrils and does not flex. Instead, the basal body acts as a rotary molecular motor, enabling the flagellum to rotate and propel the bacterium through the surrounding fluid. In fact, the flagellar motor rotates very rapidly. (Some flagella can rotate up to 300 revolutions per second!)

The MotA and MotB proteins form the stator of the flagellar motor and function to generate torque for rotation of the flagellum. The MS and C rings function as the rotor. (See Figure **3.3.2.1**). Energy for rotation comes from the proton motive force provided by protons moving through the Mot proteins along a concentration gradient from the peptidoglycan and periplasm towards the cytoplasm.

For More Information: Review of Proton Motive Force from Unit 7

Electron micrograph and illustration of the basal body of bacterial flagella; *Cover photo of Molecular Biology of the Cell*, May 1, 2000

- Electron micrograph and illustration of the basal body of bacterial flagella; Cover photo of *Molecular Biology of the Cell*, May 1, 2000.
- Animation of a rotating bacterial flagellum from the ARN Molecular Museum
- YouTube movie of the assembly and rotation of a bacterial flagellum

Bacteria flagella (see Figure 3.3.2.2 and Figure 3.3.2.3) are 10-20 µm long and between 0.01 and 0.02 µm in diameter.

Flagellar Arrangements (see Figure 3.3.2.4)

1. monotrichous: a single flagellum, usually at one pole

- Scanning electron micrograph showing monotrichous flagellum of *Vibrio*; courtesy of CDC.

2. amphitrichous: a single flagellum at both ends of the organism

3. lophotrichous: two or more flagella at one or both poles

- Scanning electron micrograph of *Helicobacter pylori* showing lophotrichous arrangement of flagella ; from Science Photolab.com

4. peritrichous: flagella over the entire surface

- Scanning electron micrograph of *Proteus vulgaris* showing peritrichous arrangement of flagella and pili; from fineartamerica.com

5. axial filaments: internal flagella found only in the spirochetes. Axial filaments are composed of from two to over a hundred axial fibrils (or endoflagella) that extend from both ends of the bacterium between the outer membrane and the cell wall, often overlapping in the center of the cell. (see Figure 3.3.2.5 and Figure 3.3.2.6). A popular theory as to the mechanism behind spirochete motility presumes that as the endoflagella rotate in the periplasmic space between the outer membrane and the cell wall, this could cause the corkscrew-shaped outer membrane of the spirochete to rotate and propel the bacterium through the surrounding fluid.

- Axial filaments of the spirochete Leptospira; Midlands Technical College, Bio 255 course site

Concept map for Bacterial Flagella

Functions

Flagella are the organelles of locomotion for most of the bacteria that are capable of motility. Two proteins in the flagellar motor, called MotA and MotB, form a proton channel through the cytoplasmic membrane and rotation of the flagellum is driven by a proton gradient. This driving proton motive force occurs as protons accumulating in the space between the cytoplasmic membrane and the cell wall as a result of the electron transport system travel through the channel back into the bacterium's cytoplasm. Most bacterial flagella can rotate both counterclockwise and clockwise and this rotation contributes to the bacterium's ability to change direction as it swims. A protein switch in the molecular motor of the basal body controls the direction of rotation.

1. A bacterium with peritrichous flagella:

If a bacterium has a peritrichous arrangement of flagella, counterclockwise rotation of the flagella causes them to form a single bundle that propels the bacterium in long, straight or curved runs without a change in direction. Counterclockwise rotation causes the flagellum to exhibit a left-handed helix. During a run, that lasts about one second, the bacterium moves 10 - 20 times its length before it stops. This occurs when some of the the flagella rotate clockwise, disengage from the bundle, and trigger a tumbling motion. Clockwise rotation causes the flagellum to assume a right-handed helix. A tumble only lasts about one-tenth of a second and no real forward progress is made. After a “tumble”, the direction of the next bacterial run is random because every time the bacterium stops swimming, Brownian motion and fluid currents cause the bacterium to reorient in a new direction.

Movie of swimming *Escherichia coli* as seen with phase contrast microscopy.
Flagella are not visible with under phase contrast microscopy. Note runs and tumbles.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Movie of motile *Escherichia coli* with fluorescent labelled-flagella #1.
This technique allows the the flagella to be seen as the bacteria swim. Note some flagella leaving the flagellar bundle to initiate tumbling.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Movie of motile *Escherichia coli* with fluorescent labelled-flagella #2.
This technique allows the the flagella to be seen as the bacteria swim. Note some flagella leaving the flagellar bundle to initiate tumbling.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

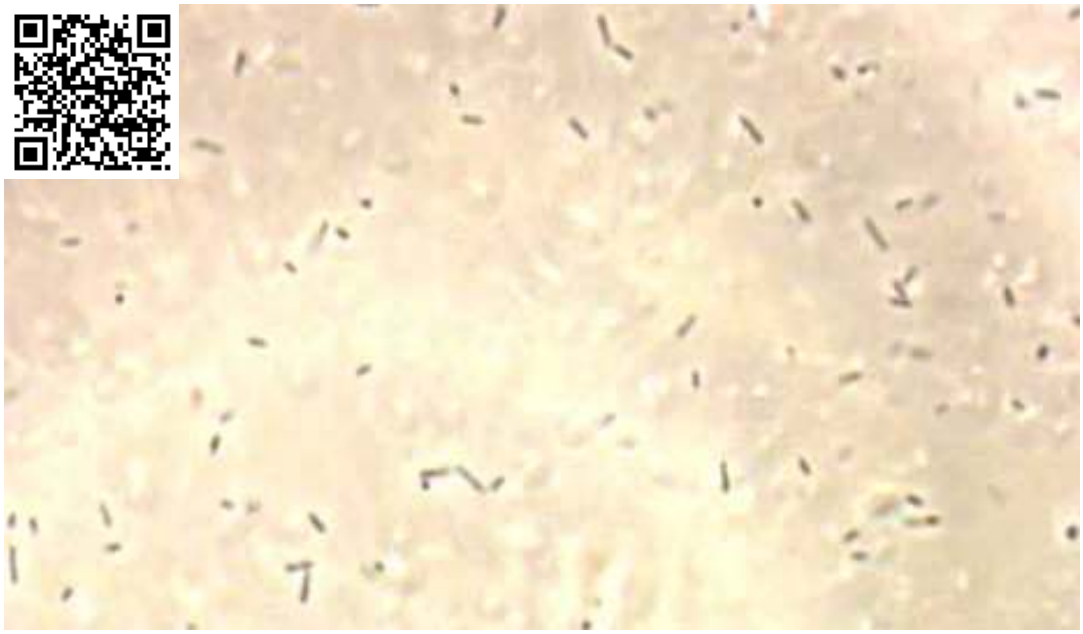
Movie of tethered *Escherichia coli* showing that the bacterial flagella rotate.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

When bacteria with a peritrichous arrangement grow on a nutrient-rich solid surface, they can exhibit a swarming motility wherein the bacteria elongate, synthesize additional flagella, secrete wetting agents, and move across the surface in coordinated manner.

Movie of swarming motility of *Escherichia coli*.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

2. A bacterium with polar flagella:

Most bacteria with polar flagella, like the peritrichous above, can rotate their flagella both clockwise and counterclockwise. If the flagellum is rotating counterclockwise, it pushes the bacterium forward. When it rotates clockwise, it pulls the bacterium backward. These bacteria change direction by changing the rotation of their flagella.



Video 3.3.2.4*B.1: Phase contrast movie of motile Pseudomonas. Pseudomonas has a single polar flagellum that can rotate both counterclockwise and clockwise but is not visible under phase contrast microscopy (http://www.youtube.com/embed/EWj2TGsTQEI).*

Movie of *Spirillum volutans*, a spiral-shaped bacterium with a bundle of flagella at either end.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Some bacteria with polar flagella can only rotate their flagellum clockwise. In this case, clockwise rotation pushes the bacterium forward. Every time the bacterium stops, Brownian motion and fluid currents cause the bacterium to reorient in a new direction.

Movie of *Rhodobacter spheroides* with fluorescent-labelled flagella.
The flagellum can only rotate clockwise.
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Concept map for Bacterial Flagella

Taxis

Around half of all known bacteria are motile. Motility serves to keep bacteria in an optimum environment via taxis. Taxis is a motile response to an environmental stimulus. Bacteria can respond to chemicals (chemotaxis), light (phototaxis), osmotic pressure (osmotaxis), oxygen (aerotaxis), and temperature (thermotaxis). Chemotaxis is a response to a chemical gradient of attractant or repellent molecules in the bacterium's environment.

- In an environment that lacks a gradient of attractant or repellent, the bacterium moves randomly. In this way the bacterium keeps searching for a gradient.

- In an environment that has a gradient of attractant or repellent, the net movement of the bacterium is towards the attractant or away from the repellent.

For More Information: Chemotaxis in *Escherichia coli*

Chemotaxis is regulated by chemoreceptors located in the cytoplasmic membrane or periplasm of the bacterium bind chemical attractants or repellents. In most cases, this leads to either the methylation or demethylation of methyl-accepting chemotaxis proteins (MCPs) that in turn, eventually trigger either a counterclockwise or clockwise rotation of the flagellum. An increasing concentration of attractant or decreasing concentration of repellent (both conditions beneficial) causes less tumbling and longer runs; a decreasing concentration of attractant or increasing concentration of repellent (both conditions harmful) causes normal tumbling and a greater chance of reorienting in a "better" direction. As a result, the organism's net movement is toward the optimum environment..

Significance of Flagella in the Initiation of Body Defense

Initiation of Innate Immunity

To protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells. These unique molecules are called pathogen-associated molecular patterns or PAMPs. (Because all microbes, not just pathogenic microbes, possess PAMPs, pathogen-associated molecular patterns are sometimes referred to as microbe-associated molecular patterns or MAMPs.)

The protein flagellin in bacterial flagella is a PAMP that binds to pattern-recognition receptors or PRRs on a variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

For More Information: Pathogen-Associated Molecular Patterns (PAMPs) from Unit 5

For More Information: Pattern-Recognition Receptors from Unit 5

Initiation of Adaptive Immunity

Proteins associated with bacterial flagella function as antigens and initiate adaptive immunity. An antigen is defined as a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they fit specific antigen receptors on our B-lymphocytes and T-lymphocytes, the cells that carry out adaptive immunity.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes. An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen, or 3-4 sugar residues branching off of a polysaccharide antigen. A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against.

The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. Humoral immunity: Humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against flagellar antigens can stick bacteria to phagocytes, a process called opsonization. They can also interfere with bacterial motility.
2. Cell-mediated immunity: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.

Adaptive immunity will be discussed in greater detail in Unit 6.

For More Information: Review of antigens and epitopes from Unit 6

Significance of Motility to Bacterial Pathogenicity

Motility and chemotaxis probably help some intestinal pathogens to move through the mucous layer so they can attach to the epithelial cells of the mucous membranes. In fact, many bacteria that can colonize the mucous membranes of the bladder and the intestines are motile. Motility probably

helps these bacteria move through the mucus in places where it is less viscous.

Flash animation showing a motile bacterium contacting a host cell by swimming through the mucus.

html5 version of animation for iPad showing a motile bacterium contacting a host cell by swimming through the mucus.

Motility and chemotaxis also enable spirochetes to move through viscous environments and penetrate cell membranes. Examples include *Treponema pallidum (inf)*, *Leptospira (inf)*, and *Borrelia burgdorferi*) *(inf)*. Because of their thinness, their internal flagella (axial filaments), and their motility, spirochetes are more readily able to penetrate host mucous membranes, skin abrasions, etc., and enter the body. Motility and invasins may also enable the spirochetes to penetrate deeper in tissue and enter the lymphatics and bloodstream and disseminate to other body sites.

Flash animation showing spirochetes using motility to enter a blood vessel.

html5 version of animation for iPad showing spirochetes using motility to enter a blood vessel.

Movie of motile *Borrelia bergdorferi*, the spirochete that causes Lyme disease. Note corkscrewing motility.
From You Tube, courtesy of CytoVivo.

Electron micrograph of *Treponema pallidum* invading a host cell.

This will be discussed in more detail under Bacterial Pathogenesis in Unit 3.

For More Information: The Ability to Contact Host Cells from Unit 3

For More Information: The Ability to Invade Host Cells from Unit 3

Concept map for Bacterial Flagella

Summary

1. Many bacteria are motile and use flagella to swim through liquid environments.
2. The basal body of a bacterial flagellum functions as a rotary molecular motor, enabling the flagellum to rotate and propel the bacterium through the surrounding fluid.
3. Bacterial flagella appear in several arrangements, each unique to a particular organism.
4. Motility serves to keep bacteria in an optimum environment via taxis.
5. Taxis refers to a motile response to an environmental stimulus enabling the net movement of bacteria towards some beneficial attractant or away from some harmful repellent.
6. Most bacterial flagella can rotate both clockwise and counterclockwise enabling to stop and change direction.
7. The protein flagellin that forms the filament of bacterial flagella functions as a pathogen-associated molecular pattern or PAMP that binds to pattern-recognition receptors or PRRs on a variety of defense cells of the body to trigger innate immune defenses.
8. Motility and chemotaxis probably help some intestinal pathogens to move through the mucous layer so they can attach to the epithelial cells of the mucous membranes and colonize the intestines.
9. Motility enables some spirochetes to penetrate deeper in tissue and enter the lymphatics and bloodstream and disseminate to other body sites.

Questions

Study the material in this section and then write out the answers to these questions. Do not just click on the answers and write them out. This will not test your understanding of this tutorial.

1. _____ a single flagellum at both ends (ans)
   A. monotrichous
   B. amphitrichous
   C. lophotrichous
   D. peritrichous
   E. axial filaments
2. State how bacterial flagella may play a role in the initiation of innate immune defenses. *(ans)*
3. Briefly describe how bacterial flagella and chemotaxis may play a role in the pathogenocity of some bacteria. *(ans)*
4. Multiple Choice *(ans)*

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3.3.3: Fimbriae and Pili

Learning Objectives

1. State the chemical composition, structure, and function of the short adhesion pili of bacteria.
2. State the function of a bacterial conjugation (sex) pilus.
3. Define bacterial conjugation.
4. State how the ability to change the shape of the adhesive tip of its pili could be an advantage to a bacterium.
5. Briefly describe twitching motility induced by type IV pili.

Highlighted Bacterium

1. Read the description of *Neisseria gonorrhoeae* and match the bacterium with the description of the organism and the infection it causes.

Structure and Composition

Fimbriae and pili are thin, protein tubes originating from the cytoplasmic membrane of many bacteria. Both are able to stick bacteria to surfaces, but pili are typically longer and fewer in number than fimbriae. They are found in virtually all Gram-negative bacteria but not in many Gram-positive bacteria. The fimbriae and pili have a shaft composed of a protein called pilin. At the end of the shaft is the adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell (Figure **3.3.3.1**). There are two basic types of pili: **short attachment pili** and l**ong conjugation pili**.

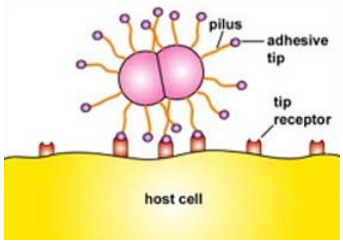


Figure **3.3.3.1**: Adhesive Tip of Bacterial Pili Binding to Host Cell Receptors

Short attachment pili, also known as *fimbriae*, are usually short and quite numerous (Figure **3.3.3.1**) and enable bacteria to colonize environmental surfaces or cells and resist flushing.

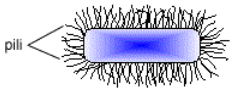


Figure **3.3.3.2**: Bacterial Pili.

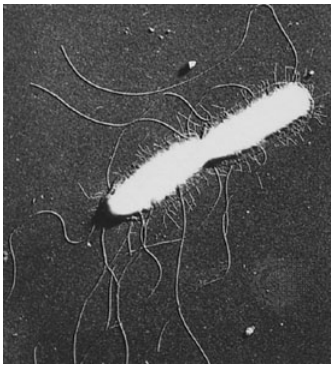


Figure **3.3.3.3**: Electron micrograph of *Salmonella* showing both flagella and pili from the Wiki Biodiversityserene.

Long conjugation pili, also called "F" or sex pili (Figure **3.3.3.4**), that are longer and very few in number. The conjugation pilus enables conjugation. As will be seen later in this unit, conjugation is the transfer of DNA from one bacterium to another by cell-to-cell contact. In gram-negative bacteria it is typically the transfer of DNA from a donor or "male bacterium" with a sex pilus to a recipient or "female bacterium" to enable genetic recombination.

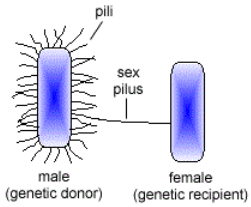


Figure **3.3.3.4**: Conjugation (Sex) Pilus

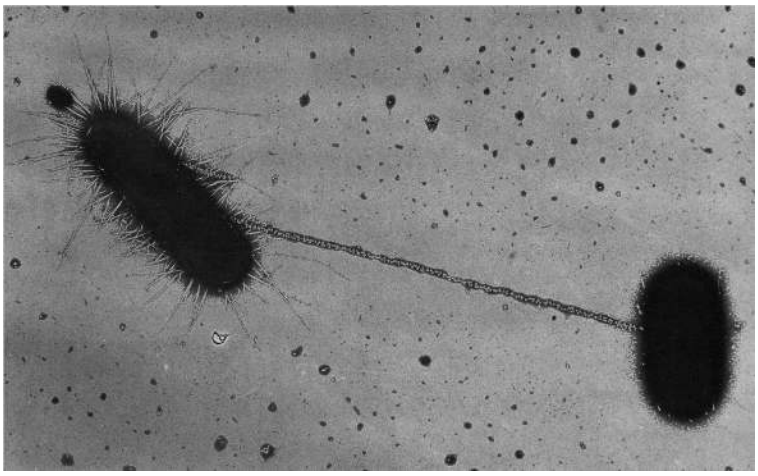


Figure **3.3.3.5**: Scanning electron micrograph of E.coli bacteria exchanging genes. Courtesy of Charles C. Brinton Jr. (NIH)

Significance of Pili to Bacterial Pathogenicity

The short attachment pili or fimbriae are organelles of adhesion allowing bacteria to colonize environmental surfaces or cells and resist flushing. The pilus has a shaft composed of a protein called *pilin*. At the end of the shaft is the adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell (Figure **3.3.3.1**). Because both the bacteria and the host cells have a negative charge, pili may enable the bacteria to bind to host cells without initially having to get close enough to be pushed away by electrostatic repulsion. Once attached to the host cell, the pili can depolymerize and enable adhesions in the bacterial cell wall to make more intimate contact.

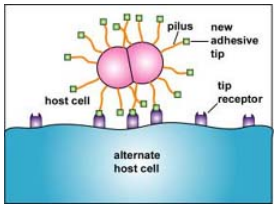


Figure **3.3.3.6**: Bacteria Altering the Adhesive Tips of Their Pili. By genetically altering the adhesive tips of their pili, certain bacteria are able to: 1) adhere to and colonize different cell types with different receptors, and 2) evade antibodies made against the previous pili.

Bacteria are constantly losing and reforming pili as they grow in the body and the same bacterium may switch the adhesive tips of the pili in order to adhere to different types of cells and evade immune defenses (Figure **3.3.3.6**). This will be discussed in detail

later in Unit 3 under Bacterial Pathogenesis. Bacteria that use pili to initially colonize host cells include *Neisseria gonorrhoeae*, *Neisseria meningitidis (inf)*, uropathogenic strains of *Escherichia coli*, and *Pseudomonas aeruginosa (inf)*.

Highlighted Bacterium: *Neisseria gonorrhoeae*
Click on this link, read the description of *Neisseria gonorrhoeae*, and be able to match the bacterium with its description on an exam.

Flash animation showing bacteria lacking pili being flushed out of the urethra.

Flash animation showing how bacteria with pili may resist being flushed out of the urethra.

html5 version of animation for iPad showing bacteria lacking pili being flushed out of the urethra.

html5 version of animation for iPad showing how bacteria with pili may resist being flushed out of the urethra.

One class of pili, known as type IV pili , not only allow for attachment but also enable a twitching motility. They are located at the poles of bacilli and allow for a gliding motility along a solid surface such as a host cell. Extension and retraction of these pili allows the bacterium to drag itself along the solid surface (see Figure 3.3.3.5). In addition, bacteria can use their type IV pili to "slingshot" the bacterium over a cellular surface. In this case, as the pili contract they are thought to become taut like a stretched rubber band. When an anchoring pilus detaches, the taut pili "slingshot" the bacterium in the opposite direction (see Figure 3.3.3.6). This motion typically alternates with the twitching motility and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm.

This enables bacteria with these types of pili within a biofilm to move around a cellular surface and find an optimum area on that cell for attachment and growth once they have initially bound. Bacteria with type IV pili include *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Vibrio cholerae*.

- Electron micrograph of type IV pili of *Neisseria gonorrhoeae* from Magdalene So, University of Arizona

Flash animation showing a bacterium using type IV pili to drag itself (twitching motility) along a surface.

html5 version of animation for iPad showing a bacterium using type IV pili to drag itself (twitching motility) along a surface.

Flash animation showing a bacterium using type IV pili to "slingshot" itself along a surface.

html5 version of animation for iPad showing a bacterium using type IV pili to "slingshot" itself along a surface.

You Tube movie showing twitching motility in *Pseudomonas* due to type IV pili
Courtesy of Dr. Lori Burrows You Tube videos

You Tube movie showing *Pseudomonas* using type IV pili to "walk" on end following binary fission.
Courtesy of Gerard Wong, UCLA Bioengineering, CNSI

Movie of twitching motility of *Pseudomonas*
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

Retraction of pili of *Pseudomonas* used in twitching motility
Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.

You Tube movie showing a *Pseudomonas* using pili to hop or slingshot itself over a surface.

Exercise: Think-Pair-Share Questions

Neisseria gonorrhoeae is a gram-negative diplococcus that has multiple alleles coding for different and distinct pili adhesive tips as well as different and distinct cell wall adhesins called Opa proteins.

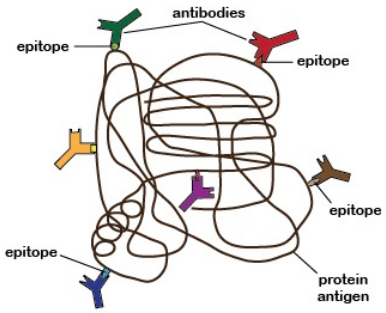
The gonococcus is able to colonize and infect a numerous sites in the body, including the urethra, the rectum, the throat, the conjunctiva of the eye, and the fallopian tubes. It can also colonize sperm.

1. Considering the locations in the body where it colonizes, why doesn't the body simply flush the bacterium out of the body?
2. Why is *N. gonorrhoea*e able to colonize so many different sites in the body?
3. We recognize pili adhesive tips and cell wall adhesins as foreign and, during adaptive immunity, make antibodies that bind to these microbial molecules. State how this might help to protect the body.

Significance of Fimbriae and Pili in the Initiation of Body Defense

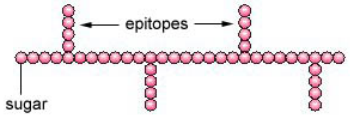
Initiation of Adaptive Immunity

Proteins associated with bacterial fimbriae and pili function as antigens and initiate adaptive immunity. An antigen is defined as a molecular shape that reacts with antibody molecules and with antigen receptors on lymphocytes. We recognize those molecular shapes as foreign or different from our body's molecular shapes because they fit specific antigen receptors on our B-lymphocytes and T-lymphocytes, the cells that carry out adaptive immunity.



Epitopes of an Antigen (Polysaccharide). Proteins have many epitopes of different specificities. During humoral immunity, antibodies are made to fit each epitope of each antigen.

The actual portions or fragments of an antigen that react with antibodies and with receptors on B-lymphocytes and T-lymphocytes are called epitopes . An epitope is typically a group of 5-15 amino acids with a unique shape that makes up a portion of a protein antigen, or 3-4 sugar residues branching off of a polysaccharide antigen. A single microorganism has many hundreds of different shaped epitopes that our lymphocytes can recognize as foreign and mount an adaptive immune response against.



Epitopes of an Antigen (Polysaccharide)

The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR).

There are two major branches of the adaptive immune responses: humoral immunity and cell-mediated immunity.

1. Humoral immunity: Humoral immunity involves the production of antibody molecules in response to an antigen and is mediated by B-lymphocytes. Through a variety of mechanisms, these antibodies are able to remove or neutralize microorganisms and their toxins after binding to their epitopes. For example, antibodies made against pili antigens can stick bacteria to phagocytes, a process called *opsonization*. Antibodies made against the adhesive tips of pili can prevent bacteria from adhering to and colonizing host cells.

2. Cell-mediated immunity: Cell-mediated immunity involves the production of cytotoxic T-lymphocytes, activated macrophages, activated NK cells, and cytokines in response to an antigen and is mediated by T-lymphocytes. These defense cells help to remove infected cells and cancer cells displaying foreign epitopes.

Adaptive immunity will be discussed in greater detail in Unit 6.

For More Information: Review of antigens and epitopes from Unit 6

Medscape article on infections associated with organisms mentioned in this Learning Object. Registration to access this website is free.

- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Vibrio cholerae*

Summary

1. Fimbriae and pili are thin, protein tubes originating from the cytoplasmic membrane found in virtually all Gram-negative bacteria but not in many Gram-positive bacteria. Pili are typically longer and fewer in number than fimbriae.
2. The short attachment pili or fimbriae are organelles of adhesion allowing bacteria to colonize environmental surfaces or cells and resist flushing.
3. The long conjugation pilus enables conjugation in Gram-negative bacteria.
4. The pilus has a shaft composed of a protein called pilin with an adhesive tip structure at the end having a shape corresponding to that of specific receptors on a host cell.
5. The same bacterium may switch the adhesive tips of the pili in order to adhere to different types of cells and evade immune defenses.
6. Type IV pili not only allow for attachment but also enable a twitching motility that enables bacteria to “crawl” or “walk” over the surfaces to which they have attached by extending and retracting their type IV pili.

Questions

Study the material in this section and then write out the answers to these questions. Do not just click on the answers and write them out. This will not test your understanding of this tutorial.

1. State the function of the short adhesion pili of bacteria. *(ans)*
2. Define bacterial conjugation. *(ans)*
3. State how the ability to change the shape of the adhesive tip of its pili could be an advantage to a bacterium. *(ans)*
4. Multiple Choice *(ans)*

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3.4: Prokaryote Cell Wall

It is important to note that not all bacteria have a **cell wall**. Having said that though, it is also important to note that **most** bacteria (about 90%) have a cell wall and they typically have one of two types: a **gram positive** cell wall or a **gram negative** cell wall. The two different cell wall types can be identified in the lab by a differential stain known as the **Gram stain**. Developed in 1884, it's been in use ever since. Originally, it was not known why the Gram stain allowed for such reliable separation of bacterial into two groups. Once the electron microscope was invented in the 1940s, it was found that the staining difference correlated with differences in the cell walls. Here is a website that shows the actual steps of the Gram stain. After this stain technique is applied the gram positive bacteria will stain purple, while the gram negative bacteria will stain pink.



Overview of Bacterial Cell Walls

A cell wall, not just of bacteria but for all organisms, is found outside of the cell membrane. It's an additional layer that typically provides some strength that the cell membrane lacks, by having a semi-rigid structure.

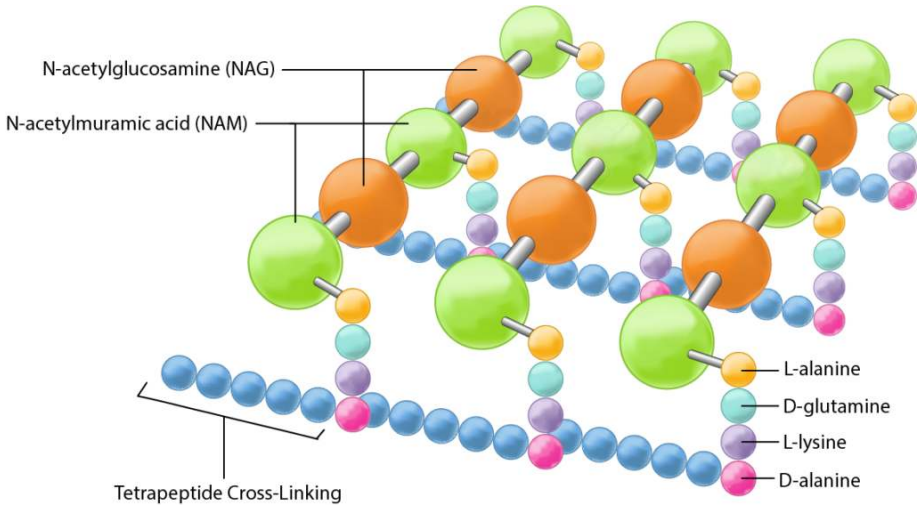
Both gram positive and gram negative cell walls contain an ingredient known as **peptidoglycan** (also known as **murein**). This particular substance hasn't been found anywhere else on Earth, other than the cell walls of bacteria. But both bacterial cell wall types contain additional ingredients as well, making the bacterial cell wall a complex structure overall, particularly when compared with the cell walls of eukaryotic microbes. The cell walls of eukaryotic microbes are typically composed of a single ingredient, like the cellulose found in algal cell walls or the chitin in fungal cell walls.

The bacterial cell wall performs several functions as well, in addition to providing overall strength to the cell. It also helps maintain the cell shape, which is important for how the cell will grow, reproduce, obtain nutrients, and move. It protects the cell from **osmotic lysis**, as the cell moves from one environment to another or transports in nutrients from its surroundings. Since water can freely move across both the cell membrane and the cell wall, the cell is at risk for an osmotic imbalance, which could put pressure on the relatively weak plasma membrane. Studies have actually shown that the internal pressure of a cell is similar to the pressure found inside a fully inflated car tire. That is a lot of pressure for the plasma membrane to withstand! The cell wall can keep out certain molecules, such as toxins, particularly for gram negative bacteria. And lastly, the bacterial cell wall can contribute to the pathogenicity or disease –causing ability of the cell for certain bacterial pathogens.

Structure of Peptidoglycan

Let us start with peptidoglycan, since it is an ingredient that both bacterial cell walls have in common. Peptidoglycan is a polysaccharide made of two glucose derivatives, **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**, alternated in long chains. The chains are cross-linked to one another by a **tetrapeptide** that extends off the NAM sugar unit, allowing a lattice-like structure to form. The four amino acids that compose the tetrapeptide are: **L-alanine, D-glutamine, L-lysine or *meso*-diaminopimelic acid (DPA),** and **D-alanine**. Typically only the L-isomeric form of amino acids are utilized by cells but the use of the mirror image D-amino acids provides protection from proteases that might compromise the integrity of the cell wall by attacking the peptidoglycan. The tetrapeptides can be **directly cross-linked** to one another, with the D-alanine on one tetrapeptide binding to the L-lysine/ DPA on another tetrapeptide. In many gram positive bacteria there is a cross-bridge of five amino acids such as glycine (**peptide interbridge**) that serves to connect one tetrapeptide to another. In either case the cross-linking serves to increase the strength of the overall structure, with more strength derived from **complete cross-linking**, where every tetrapeptide is bound in some way to a tetrapeptide on another NAG-NAM chain.

While much is still unknown about peptidoglycan, research in the past ten years suggests that peptidoglycan is synthesized as a cylinder with a coiled substructure, where each coil is cross-linked to the coil next to it, creating an even stronger structure overall.

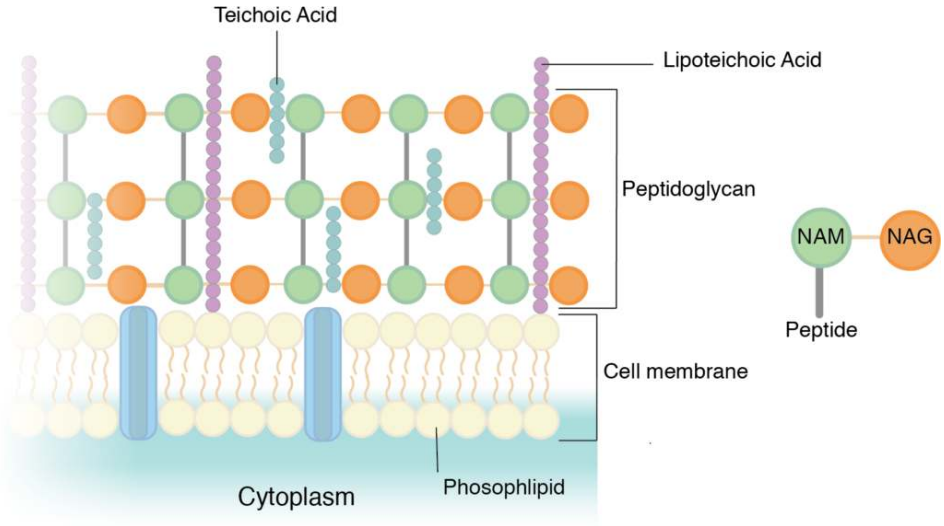


Peptidoglycan Structure.

Gram Positive Cell walls

The cell walls of gram positive bacteria are composed predominantly of peptidoglycan. In fact, peptidoglycan can represent up to 90% of the cell wall, with layer after layer forming around the cell membrane. The NAM tetrapeptides are typically cross-linked with a peptide interbridge and complete cross-linking is common. All of this combines together to create an incredibly strong cell wall.

The additional component in a gram positive cell wall is **teichoic acid**, a glycopolymer, which is embedded within the peptidoglycan layers. Teichoic acid is believed to play several important roles for the cell, such as generation of the net negative charge of the cell, which is essential for development of a proton motive force. Teichoic acid contributes to the overall rigidity of the cell wall, which is important for the maintenance of the cell shape, particularly in rod-shaped organisms. There is also evidence that teichoic acids participate in cell division, by interacting with the peptidoglycan biosynthesis machinery. Lastly, teichoic acids appear to play a role in resistance to adverse conditions such as high temperatures and high salt concentrations, as well as to β-lactam antibiotics. Teichoic acids can either be covalently linked to peptidoglycan (**wall teichoic acids or WTA**) or connected to the cell membrane via a lipid anchor, in which case it is referred to as **lipoteichoic acid**.



Gram Positive Bacteria Cell Wall

Since peptidoglycan is relatively porous, most substances can pass through the gram positive cell wall with little difficulty. But some nutrients are too large, requiring the cell to rely on the use of **exoenzymes**. These extracellular enzymes are made within the cell's cytoplasm and then secreted past the cell membrane, through the cell wall, where they function outside of the cell to break down large macromolecules into smaller components.

Gram Negative Cell Walls

The cell walls of gram negative bacteria are more complex than that of gram positive bacteria, with more ingredients overall. They do contain peptidoglycan as well, although only a couple of layers, representing 5-10% of the total cell wall. What is most notable about the gram negative cell wall is the presence of a plasma membrane located outside of the peptidoglycan layers, known as the **outer membrane**. This makes up the bulk of the gram negative cell wall. The outer membrane is composed of a lipid bilayer, very similar in composition to the cell membrane with polar heads, fatty acid tails, and integral proteins. It differs from the cell membrane by the presence of large molecules known as **lipopolysaccharide (LPS)**, which are anchored into the outer membrane and project from the cell into the environment. LPS is made up of three different components: 1) the **O-antigen or O-polysaccharide**, which represents the outermost part of the structure , 2) the **core polysaccharide**, and 3) **lipid A**, which anchors the LPS into the outer membrane. LPS is known to serve many different functions for the cell, such as contributing to the net negative charge for the cell, helping to stabilize the outer membrane, and providing protection from certain chemical substances by physically blocking access to other parts of the cell wall. In addition, LPS plays a role in the host response to pathogenic gram negative bacteria. The O-antigen triggers an immune response in an infected host, causing the generation of antibiotics specific to that part of LPS (think of *E. coli* **O**157). Lipid A acts as a toxin, specifically an **endotoxin**, causing general symptoms of illness such as fever and diarrhea. A large amount of lipid A released into the bloodstream can trigger endotoxic shock, a body-wide inflammatory response which can be life-threatening.

The outer membrane does present an obstacle for the cell. While there are certain molecules it would like to keep out, such as antibiotics and toxic chemicals, there are nutrients that it would like to let in and the additional lipid bilayer presents a formidable barrier. Large molecules are broken down by enzymes, in order to allow them to get past the LPS. Instead of exoenzymes (like the gram positive bacteria), the gram negative bacteria utilize **periplasmic enzymes** that are stored in the **periplasm**. Where is the periplasm, you ask? It is the space located between the outer surface of the cell membrane and the inner surface of the outer membrane, and it contains the gram negative peptidoglycan. Once the periplasmic enzymes have broken nutrients down to smaller molecules that can get past the LPS, they still need to be transported across the outer membrane, specifically the lipid bilayer. Gram negative cells utilize **porins**, which are transmembrane proteins composed of a trimer of three subunits, which form a pore across the membrane. Some porins are non-specific and transport any molecule that fits, while some porins are specific and only transport substances that they recognize by use of a binding site. Once across the outer membrane and in the periplasm, molecules work their way through the porous peptidoglycan layers before being transported by integral proteins across the cell membrane.

The peptidoglycan layers are linked to the outer membrane by the use of a lipoprotein known as **Braun's lipoprotein** (good ol' Dr. Braun). At one end the lipoprotein is covalently bound to the peptidoglycan while the other end is embedded into the outer membrane via its polar head. This linkage between the two layers provides additional structural integrity and strength.

Unusual and Wall-less Bacteria

Having emphasized the important of a cell wall and the ingredient peptidoglycan to both the gram positive and the gram negative bacteria, it does seem important to point out a few exceptions as well. Bacteria belonging to the phylum **Chlamydiae** appear to lack peptidoglycan, although their cell walls have a gram negative structure in all other regards (i.e. outer membrane, LPS, porin, etc). It has been suggested that they might be using a protein layer that functions in much the same way as peptidoglycan. This has an advantage to the cell in providing resistance to β-lactam antibiotics (such as penicillin), which attack peptidoglycan.

Bacteria belonging to the phylum **Tenericutes** lack a cell wall altogether, which makes them extremely susceptible to osmotic changes. They often strengthen their cell membrane somewhat by the addition of **sterols**, a substance usually associated with eukaryotic cell membranes. Many members of this phylum are pathogens, choosing to hide out within the protective environment of a host.

Key Words

cell wall, gram positive bacteria, gram negative bacteria, Gram stain, peptidoglycan, murein, osmotic lysis, N-acetylglucosamine (NAG), N-acetylmuramic acid (NAM), tetrapeptide, L-alanine, D-glutamine, L-lysine, *meso*-diaminopimelic acid (DPA), D-alanine, direct cross-link, peptide interbridge, complete cross-linking, teichoic acid, wall teichoic acid (WTA), lipoteichoic acid, exoenzymes, outer membrane, lipopolysaccharide (LPS), O-antigen or O-polysaccharide, core polysaccharide, lipid A, endotoxin, periplasmic enzymes, periplasm, porins, Braun's lipoprotein, Chlamydiae, Tenericutes, sterols.

Essential Questions/Objectives

1. What are the basic characteristics and functions of the cell wall in *Bacteria*?
2. What is the Gram stain and how does it relate to the different cell wall types of *Bacteria*?
3. What is the basic unit structure of peptidoglycan? What components are present and how do they interact? Be able to diagram peptidoglycan and its' components.
4. What is cross linking and why does this play such an important role in the cell wall? What different types of cross-linking are there?
5. Why are D-amino acids unusual and how does having D-amino acids in the peptidoglycan keep this macromolecule stable?
6. What are the differences between gram positive and negative organisms in terms of thickness of peptidoglycan, different constituents of PG and variations in cross linkage and strength, and other molecules associated with cell wall?
7. What is teichoic acid and what are its' proposed roles and functions? What are lipteichoic acids?
8. What is the periplasm of gram negative bacteria? What purpose can it serve? What alternatives are available for cells?
9. What is the general composition of the outer membrane of gram-negative microorganisms, its function and toxic properties? How is it linked to the cell? What is a porin and what are their functions?
10. What group of bacteria lack peptidoglycan in their cell wall? What advantage does this confer?
11. What group of bacteria normally does not have cell walls and how do they maintain themselves?

Exploratory Questions (OPTIONAL)

1. How does the mechanism of the Gram stain relate to specific components of the bacterial cell wall?

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3.4.1: Antibiotics and Selective Toxicity

Learning Objectives

- Describe selective toxicity

Selective Toxicity in Antibiotics

Synthetic antibiotic chemotherapy as a science and development of antibacterials began in Germany with Paul Ehrlich in the late 1880s. Ehrlich noted that certain dyes would color human, animal, or bacterial cells, while others did not. He then proposed the idea that it might be possible to create chemicals that would act as a selective drug that would bind to and kill bacteria without harming the human host. After screening hundreds of dyes against various organisms, he discovered a medicinally useful drug, the synthetic antibacterial Salvarsan now called arsphenamine.

Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. More specifically, narrow spectrum antibiotics target specific types of bacteria, such as Gram-negative or Gram-positive bacteria, whereas broad spectrum antibiotics affect a wide range of bacteria. Following a 40-year hiatus in discovering new classes of antibacterial compounds, three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).



Figure: **Bacterial Cultures**: In antibacterial production, microorganisms must be isolated, cultured, and tested for growth inhibition of target organisms and for their selective toxicity.

Some antibacterials have been associated with a range of adverse effects. Side-effects range from mild to very serious depending on the antibiotics used, the microbial organisms targeted, and the individual patient. Safety profiles of newer drugs are often not as well established as for those that have a long history of use. Adverse effects range from fever and nausea to major allergic reactions, including photodermatitis and anaphylaxis. Common side-effects include diarrhea, resulting from disruption of the species composition in the intestinal flora, resulting, for example, in overgrowth of pathogenic bacteria, such as *Clostridium difficile*. Antibacterials can also affect the vaginal flora, and may lead to overgrowth of yeast species of the genus *Candida* in the vulvo-vaginal area. Additional side-effects can result from interaction with other drugs, such as elevated risk of tendon damage from administration of a quinolone antibiotic with a systemic corticosteroid.

Antibacterial Production

Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics. Other antibiotics simply lack advantage over those already in use, or have no other practical applications. Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and

therapeutic activities, and the best candidates can be examined and possibly modified. A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

Key Points

- Their mechanism of action, chemical structure, or spectrum of activity are ways in which antibiotics are classified.
- Broad spectrum antibiotics affect a wide range of bacteria, while narrow spectrum antibiotics are able to target specific types.
- Antibiotics must go through a screening process, where they are isolated, cultured, and then tested for production of diffusible products that inhibit the growth of specific test organisms.
- Due to potential adverse side effects, antibiotics must also be tested for their selective toxicities.

Key Terms

- **antibacterial**: A drug having the effect of killing or inhibiting bacteria.
- **bactericidal**: An agent that kills bacteria.
- **bacteriostatic**: A drug that prevents bacterial growth and reproduction but does not necessarily kill them. When it is removed from the environment the bacteria start growing again.

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3.4.2: Gram-Negative Wall

Learning Objectives

- Recognize the characteristics of a gram-negative bacteria

The Gram-negative cell wall is composed of an outer membrane, a *peptidoglygan* layer, and a *periplasm*.

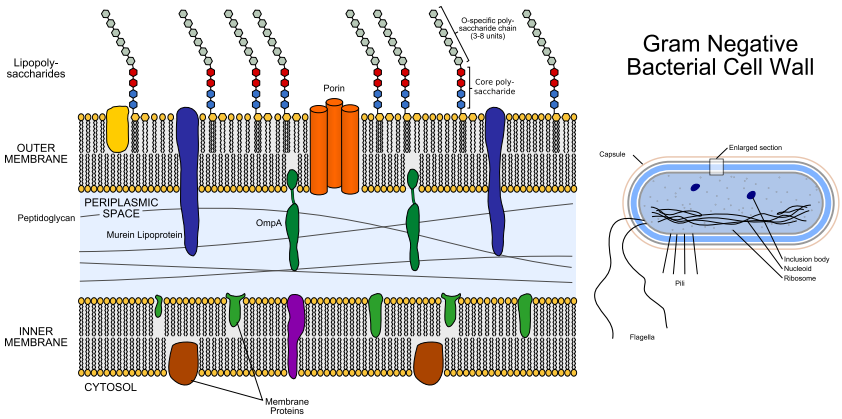


Figure: **Structure of Gram-negative cell wall**: Gram-negative outer membrane composed of lipopolysaccharides.

In the Gram-negative Bacteria the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The gram-negative bacteria do not retain crystal violet but are able to retain a counterstain, commonly safranin, which is added after the crystal violet. The safranin is responsible for the red or pink color seen with a gram-negative bacteria. The Gram-negative's cell wall is thinner (10 nanometers thick) and less compact than that of Gram-positive bacteria, but remains strong, tough, and elastic to give them shape and protect them against extreme environmental conditions. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS) in addition to proteins and phospholipids. The LPS molecule is toxic and is classified as an endotoxin that elicits a strong immune response when the bacteria infect animals.

In Gram-negative bacteria the outer membrane is usually thought of as part of the outer leaflet of the membrane structure and is relatively permeable. It contains structures that help bacteria adhere to animal cells and cause disease. The peptidoglycan layer is non-covalently anchored to lipoprotein molecules called Braun's lipoproteins through their hydrophobic head. Sandwiched between the outer membrane and the plasma membrane, a concentrated gel-like matrix (the periplasm) is found in the periplasmic space. It is in fact an integral compartment of the gram-negative cell wall and contains binding proteins for amino acids, sugars, vitamins, iron, and enzymes essential for bacterial nutrition. The periplasm space can act as reservoir for virulence factors and a dynamic flux of macromolecules representing the cell's metabolic status and its response to environmental factors. Together, the plasma membrane and the cell wall (outer membrane, peptidoglycan layer, and periplasm) constitute the gram-negative envelope.

Key Points

- The outer membrane of Gram-negative bacteria contains lipopolysaccharides, proteins, and phospholipids.
- The lipopolysaccharide component acts as a virulence factor and causes disease in animals.
- More virulence factors are harbored in the periplasmic space between the outer membrane and the plasma membrane.

Key Terms

- **lipopolysaccharide**: any of a large class of lipids conjugated with polysaccharides
- **endotoxin**: Any toxin secreted by a microorganism and released into the surrounding environment only when it dies.

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3.4.3: Gram-Positive Cell Wall

Learning Objectives

- Compare and contrast a gram-positive and negative stain

Gram-positive bacteria are stained dark blue or violet by Gram staining. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram-variable and Gram-indeterminate groups as well.

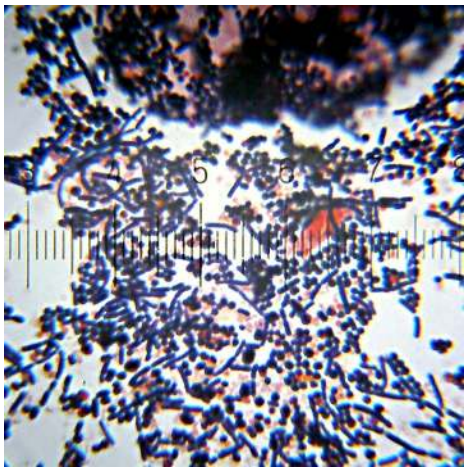


Figure: **Gram-positive bacteria**: These bacteria stain violet by Gram staining.

It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram-positive bacteria. A Gram-positive results in a purple/blue color while a Gram-negative results in a pink/red color. The Gram stain is almost always the first step in the identification of a bacterial organism, and is the default stain performed by laboratories over a sample when no specific culture is referred.

In Gram-positive bacteria, the cell wall is thick (15-80 nanometers), and consists of several layers of peptidoglycan. They lack the outer membrane envelope found in Gram-negative bacteria. Running perpendicular to the peptidoglycan sheets is a group of molecules called teichoic acids, which are unique to the Gram-positive cell wall. Teichoic acids are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars.

The teichoic acid polymers are occasionally anchored to the plasma membrane (called lipoteichoic acid, LTA), and apparently directed outward at right angles to the layers of peptidoglycan. Teichoic acids give the Gram-positive cell wall an overall negative charge due to the presence of phosphodiester bonds between teichoic acid monomers. The functions of teichoic acid are not fully known but it is believed to serve as a chelating agent and means of adherence for the bacteria. These are essential to the viability of Gram-positive bacteria in the environment and provide chemical and physical protection.

One idea is that they provide a channel of regularly-oriented, negative charges for threading positively-charged substances through the complicated peptidoglycan network. Another theory is that teichoic acids are in some way involved in the regulation and assembly of muramic acid sub-units on the outside of the plasma membrane.

There are instances, particularly in the streptococci, wherein teichoic acids have been implicated in the adherence of the bacteria to tissue surfaces and are thought to contribute to the pathogenicity of Gram-positive bacteria.

Key Points

- Gram-positive bacteria stain violet by Gram staining due the presence of peptidoglycan in their cell wall.
- Peptidoglycans are attached to negatively-charged lipoteichoic acid monomers important for cell direction and adherence.
- Lipoteichoic acids are covalently linked to lipids within the cytoplasmic membrane, thus connecting the peptidoglycans to the cell cytoplasm.

Key Terms

- **Gram stain**: A method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative).

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3.4.4: Archaea Cell Wall

Learning Objectives

- State the similarities between the cell walls of archaea and bacteria

As with other living organisms, archaeal cells have an outer cell membrane that serves as a protective barrier between the cell and its environment. Within the membrane is the cytoplasm, where the living functions of the archeon take place and where the DNA is located. Around the outside of nearly all archaeal cells is a cell wall, a semi-rigid layer that helps the cell maintain its shape and chemical equilibrium. All three of these regions may be distinguished in the cells of bacteria and most other living organisms.

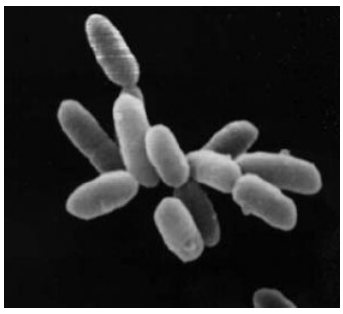


Figure: **Archaea**: Cluster of halobacterium (archaea)

A closer look at each region reveals structural similarities but major differences in chemical composition between bacterial and archaeal cell wall. Archaea builds the same structures as other organisms, but they build them from different chemical components. For instance, the cell walls of all bacteria contain the chemical peptidoglycan. Archaeal cell walls do not contain this compound, though some species contain a similar one. It is assembled from surface-layer proteins called S-layers. Likewise, archaea do not produce walls of cellulose (as do plants) or chitin (as do fungi). The cell wall of archaeans is chemically distinct. Methanogens are the only exception and possess pseudopeptidoglycan chains in their cell wall that lacks amino acids and N-acetylmuramic acid in their chemical composition. The most striking chemical differences between Archaea and other living things lie in their cell membrane. There are four fundamental differences between the archaeal membrane and those of all other cells: (1) chirality of glycerol, (2) ether linkage, (3) isoprenoid chains, and (4) branching of side chains.

Key Points

- Archaea are single-celled microorganisms that lack a cell nucleus and membrane -bound organelles.
- Like other living organisms, archaea have a semi-rigid cell wall that protects them from the environment.
- The cell wall of archaea is composed of S-layers and lack peptidoglycan molecules with the exception of methanobacteria who have pseudopeptidoglycan in their cell wall.

Key Terms

- **cellulose**: A complex carbohydrate that forms the main constituent of the cell wall in most plants and is important in the manufacture of numerous products, such as paper, textiles, pharmaceuticals, and explosives.
- **chitin**: A complex polysaccharide, a polymer of N-acetylglucosamine, found in the exoskeletons of arthropods and in the cell walls of fungi; thought to be responsible for some forms of asthma in humans.
- **cytoplasm**: The contents of a cell except for the nucleus. It includes cytosol, organelles, vesicles, and the cytoskeleton.

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3.5: Prokaryote Plasma Membrane

Learning Objectives

- Describe the basic structure of a typical prokaryote
- Describe important differences in structure between Archaea and Bacteria

There are many differences between prokaryotic and eukaryotic cells. However, all cells have four common structures: the plasma membrane, which functions as a barrier for the cell and separates the cell from its environment; the cytoplasm, a jelly-like substance inside the cell; nucleic acids, the genetic material of the cell; and ribosomes, where protein synthesis takes place. Prokaryotes come in various shapes, but many fall into three categories: cocci (spherical), bacilli (rod-shaped), and spirilli (spiral-shaped) (Figure **3.5.1**).

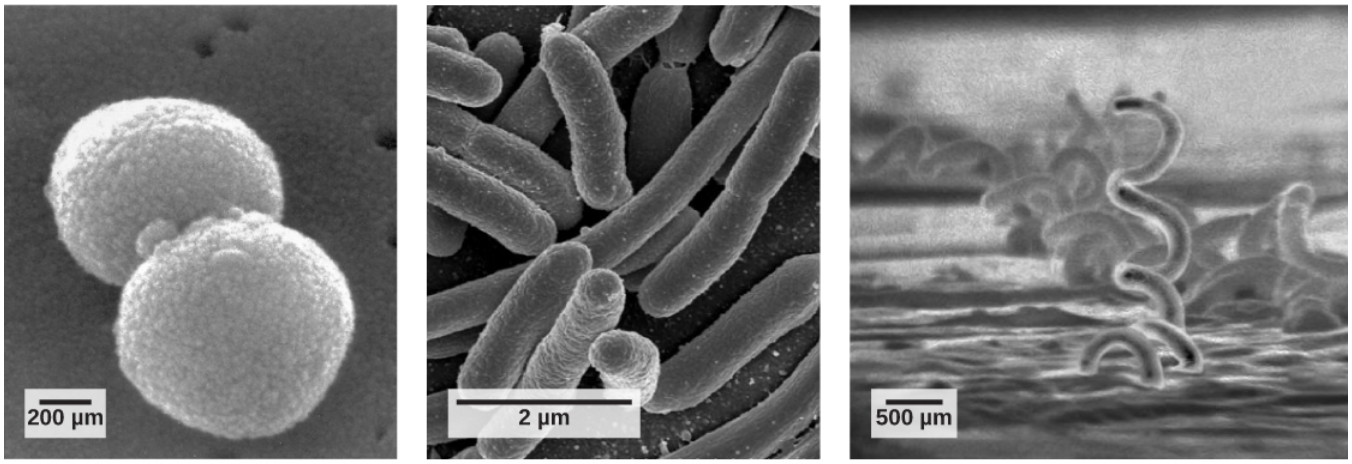


Figure **3.5.1**: Prokaryotes fall into three basic categories based on their shape, visualized here using scanning electron microscopy: (a) cocci, or spherical (a pair is shown); (b) bacilli, or rod-shaped; and (c) spirilli, or spiral-shaped. (credit a: modification of work by Janice Haney Carr, Dr. Richard Facklam, CDC; credit c: modification of work by Dr. David Cox; scale-bar data from Matt Russell)

The Prokaryotic Cell

Recall that prokaryotes (Figure **3.5.2**) are unicellular organisms that lack organelles or other internal membrane-bound structures. Therefore, they do not have a nucleus but instead generally have a single chromosome—a piece of circular, double-stranded DNA located in an area of the cell called the nucleoid. Most prokaryotes have a cell wall outside the plasma membrane.

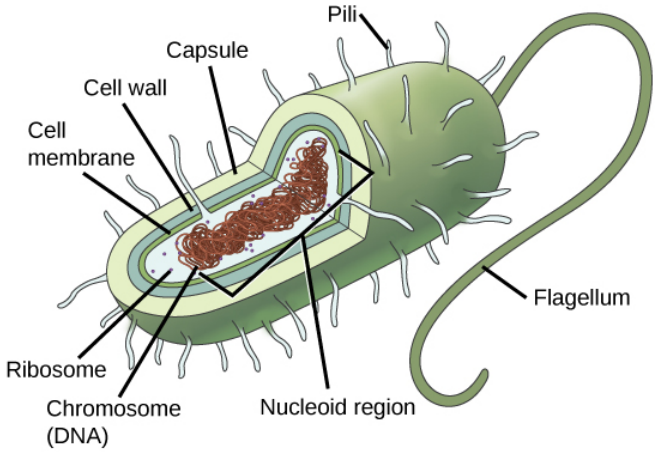


Figure **3.5.2**: The features of a typical prokaryotic cell are shown.

Recall that prokaryotes are divided into two different domains, Bacteria and Archaea, which together with Eukarya, comprise the three domains of life (Figure **3.5.3**).

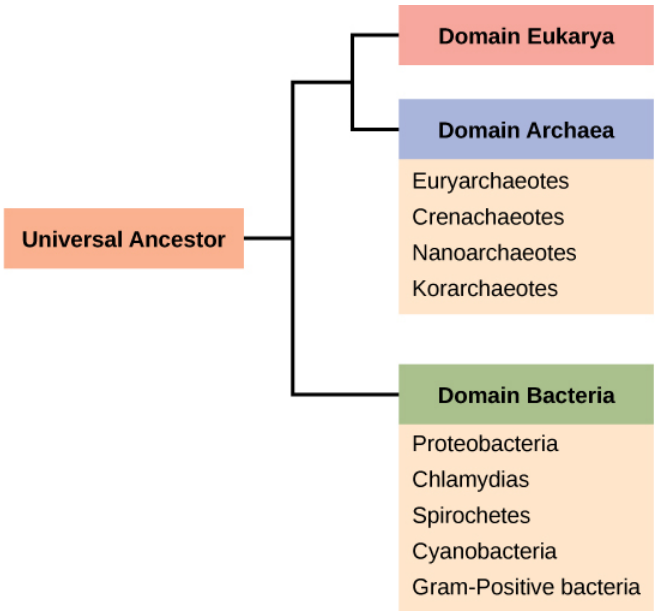
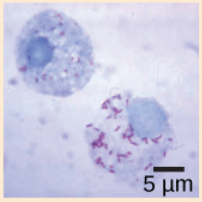
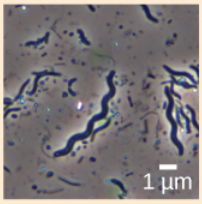
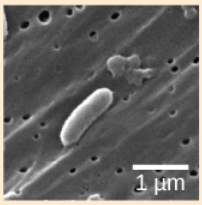
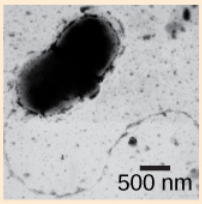
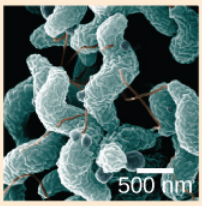


Figure **3.5.3**: Bacteria and Archaea are both prokaryotes but differ enough to be placed in separate domains. An ancestor of modern Archaea is believed to have given rise to Eukarya, the third domain of life. Archaeal and bacterial phyla are shown; the evolutionary relationship between these phyla is still open to debate.

The composition of the cell wall differs significantly between the domains Bacteria and Archaea. The composition of their cell walls also differs from the eukaryotic cell walls found in plants (cellulose) or fungi and insects (chitin). The cell wall functions as a protective layer, and it is responsible for the organism's shape. Some bacteria have an outer capsule outside the cell wall. Other structures are present in some prokaryotic species, but not in others (Table **3.5.1**). For example, the capsule found in some species enables the organism to attach to surfaces, protects it from dehydration and attack by phagocytic cells, and makes pathogens more resistant to our immune responses. Some species also have flagella (singular, flagellum) used for locomotion, and pili (singular, pilus) used for attachment to surfaces. Plasmids, which consist of extra-chromosomal DNA, are also present in many species of bacteria and archaea.

Characteristics of phyla of Bacteria are described in Figure **3.5.4** and Figure **3.5.5**; Archaea are described in Figure **3.5.6**.

Bacteria of Phylum Proteobacteria		
Class	**Representative organisms**	**Representative micrograph**
Alpha Proteobacteria Some species are photoautotrophic but some are symbionts of plants and animals and others are pathogens. Eukaryotic mitochondria are thought be derived from bacteria in this group.	*Rhizobium* Nitrogen-fixing endosymbiont associated with the roots of legumes *Rickettsia* Obligate intracellular parasite that causes typhus and Rocky Mountain Spotted Fever (but not rickets, which is caused by Vitamin C deficiency)	 *Rickettsia rickettsia,* stained red, grow inside a host cell.
Beta Proteobacteria This group of bacteria is diverse. Some species play an important role in the nitrogen cycle.	*Nitrosomas* Species from this group oxidize ammonia into nitrite. *Spirillum minus* Causes rat-bite fever	 *Spirillum minus*
Gamma Proteobacteria Many are beneficial symbionts that populate the human gut, but others are familiar human pathogens. Some species from this subgroup oxidize sulfur compounds.	*Escherichia coli* Normally beneficial microbe of the human gut, but some strains cause disease *Salmonella* Certain strains cause food poisoning or typhoid fever *Yersinia pestis* Causative agent of Bubonic plague *Psuedomonas aeruginosa* Causes lung infections *Vibrio cholera* Causative agent of cholera *Chromatium* Sulfur-producing bacteria that oxidize sulfur, producing H_2S	 *Vibrio cholera*
Delta Proteobacteria Some species generate a spore-forming fruiting body in adverse conditions. Others reduce sulfate and sulfur.	*Myxobacteria* Generate spore-forming fruiting bodies in adverse conditions *Desulfovibrio vulgaris* Aneorobic, sulfate-reducing bacterium	 *Desulfovibrio vulgaris*
Epsilon Proteobacteria Many species inhabit the digestive tract of animals as symbionts or pathogens. Bacteria from this group have been found in deep-sea hydrothermal vents and cold seep habitats.	*Campylobacter* Causes blood poisoning and intestinal inflammation *Heliobacter pylori* Causes stomach ulcers	 *Campylobacter*

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Figure 3.5.4: Phylum Proteobacteria is one of up to 52 bacteria phyla. Proteobacteria is further subdivided into five classes, Alpha through Epsilon. (credit "Rickettsia rickettsia": modification of work by CDC; credit "Spirillum minus": modification of work by Wolframm Adlassnig; credit "Vibrio cholera": modification of work by Janice Haney Carr, CDC; credit "Desulfovibrio vulgaris": modification of work by Graham Bradley; credit "Campylobacter": modification of work by De Wood, Pooley, USDA, ARS, EMU; scale-bar data from Matt Russell)

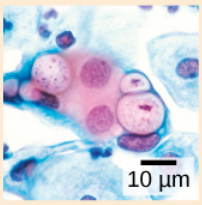
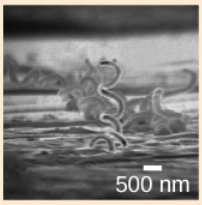
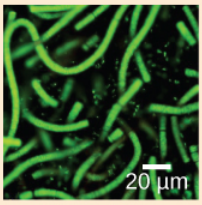
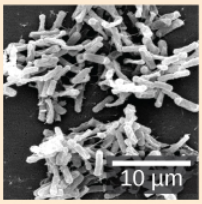
Bacteria: Chlamydia, Spirochaetae, Cyanobacteria, and Gram-positive		
Phylum	**Representative organisms**	**Representative micrograph**
Chlamydias All members of this group are obligate intracellular parasites of animal cells. Cells walls lack peptidoglycan.	*Chlamydia trachomatis* Common sexually transmitted disease that can lead to blindness	 In this pap smear, *Chlamydia trachomatis* appear as pink inclusions inside cells.
Spirochetes Most members of this species, which has spiral-shaped cells, are free-living aneaerobes, but some are pathogenic. Flagella run lengthwise in the periplasmic space between the inner and outer membrane.	*Treponema pallidum* Causative agent of syphilis *Borrelia burgdorferi* Causative agent of Lyme disease	 *Treponema pallidum*
Cyanobacteria Also known as blue-green algae, these bacteria obtain their energy through photosynthesis. They are ubiquitous, found in terrestrial, marine, and freshwater environments. Eukaryotic chloroplasts are thought be derived from bacteria in this group.	*Prochlorococcus* Believed to be the most abundant photosynthetic organism on earth; responsible for generating half the world's oxygen	 *Phormidium*
Gram-positive Bacteria Soil-dwelling members of this subgroup decompose organic matter. Some species cause disease. They have a thick cell wall and lack an outer membrane.	*Bacillus anthracis* Causes anthrax *Clostridium botulinum* Causes Botulism *Clostridium difficile* Causes diarrhea during antibiotic therapy *Streptomyces* Many antibiotics, including streptomycin, are derived from these bacteria. *Mycoplasmas* These tiny bacteria, the smallest known, lack a cell wall. Some are free-living, and some are pathogenic.	 *Clostridium difficile*

Figure 3.5.5: Chlamydia, Spirochetes, Cyanobacteria, and Gram-positive bacteria are described in this table. Note that bacterial shape is not phylum-dependent; bacteria within a phylum may be cocci, rod-shaped, or spiral. (credit "Chlamydia trachomatis": modification of work by Dr. Lance Liotta Laboratory, NCI; credit "Treponema pallidum": modification of work by Dr. David Cox, CDC; credit "Phormidium": modification of work by USGS; credit "Clostridium difficile": modification of work by Lois S. Wiggs, CDC; scale-bar data from Matt Russell)

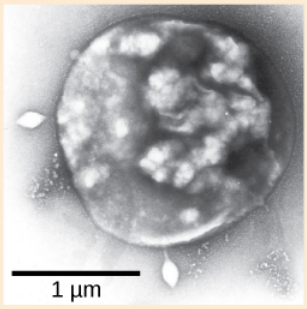
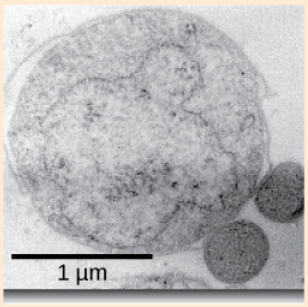
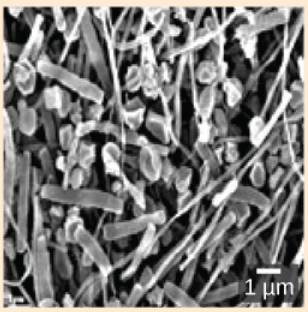
Archaea		
Phylum	**Representative organisms**	**Representative micrograph**
Euryarchaeota This phylum includes methanogens, which produce methane as a metabolic waste product, and halobacteria, which live in an extreme saline environment.	*Methanogens* Methane production causes flatulence in humans and other animals. *Halobacteria* Large blooms of this salt-loving archaea appear reddish due to the presence of bacterirhodopsin in the membrane. Bacteriorhodopsin is related to the retinal pigment rhodopsin.	 *Halobacterium* strain NRC-1
Crenarchaeota Members of the ubiquitous phylum play an important role in the fixation of carbon. Many members of this group are sulfur-dependent extremophiles. Some are thermophilic or hyperthermophilic.	*Sulfolobus* Members of this genus grow in volcanic springs at temperatures between 75° and 80°C and at a pH between 2 and 3.	 *Sulfolobus* being infected by bacteriophage
Nanoarchaeota This group currently contains only one species, *Nanoarchaeum equitans*.	*Nanoarchaeum equitans* This species was isolated from the bottom of the Atlantic Ocean and from a hydrothermal vent at Yellowstone National Park. It is an obligate symbiont with *Ignicoccus*, another species of archaea.	 *Nanoarchaeum equitans* (small dark spheres) are in contact with their larger host, *Ignicoccus*.
Korarchaeota Members of this phylum, considered to be one of the most primitive forms of life, have only been found in the Obsidian Pool, a hot spring at Yellowstone National Park.	No members of this species have been cultivated.	 This image shows a variety of korarchaeota species from the Obsidian Pool at Yellowstone National Park.

Figure **3.5.6**: Archaea are separated into four phyla: the Korarchaeota, Euryarchaeota, Crenarchaeota, and Nanoarchaeota. (credit "Halobacterium": modification of work by NASA; credit "Nanoarchaeotum equitans": modification of work by Karl O. Stetter; credit "korarchaeota": modification of work by Office of Science of the U.S. Dept. of Energy; scale-bar data from Matt Russell)

The Plasma Membrane

The plasma membrane is a thin lipid bilayer (6 to 8 nanometers) that completely surrounds the cell and separates the inside from the outside. Its selectively permeable nature keeps ions, proteins, and other molecules within the cell and prevents them from diffusing into the extracellular environment, while other molecules may move through the membrane. Recall that the general structure of a cell membrane is a phospholipid bilayer composed of two layers of lipid molecules. In archaeal cell membranes, isoprene (phytanyl) chains linked to glycerol replace the fatty acids linked to glycerol in bacterial membranes. Some archaeal membranes are lipid monolayers instead of bilayers (Figure **3.5.7**).

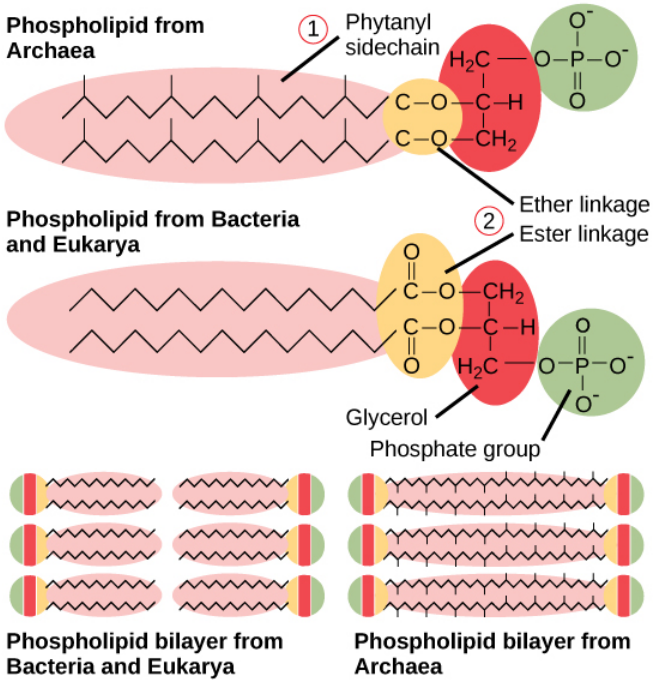


Figure **3.5.7**: Archaeal phospholipids differ from those found in Bacteria and Eukarya in two ways. First, they have branched phytanyl sidechains instead of linear ones. Second, an ether bond instead of an ester bond connects the lipid to the glycerol.

The Cell Wall

The cytoplasm of prokaryotic cells has a high concentration of dissolved solutes. Therefore, the osmotic pressure within the cell is relatively high. The cell wall is a protective layer that surrounds some cells and gives them shape and rigidity. It is located outside the cell membrane and prevents osmotic lysis (bursting due to increasing volume). The chemical composition of the cell walls varies between archaea and bacteria, and also varies between bacterial species.

Bacterial cell walls contain peptidoglycan, composed of polysaccharide chains that are cross-linked by unusual peptides containing both L- and D-amino acids including D-glutamic acid and D-alanine. Proteins normally have only L-amino acids; as a consequence, many of our antibiotics work by mimicking D-amino acids and therefore have specific effects on bacterial cell wall development. There are more than 100 different forms of peptidoglycan. S-layer (surface layer) proteins are also present on the outside of cell walls of both archaea and bacteria.

Bacteria are divided into two major groups: Gram positive and Gram negative, based on their reaction to Gram staining. Note that all Gram-positive bacteria belong to one phylum; bacteria in the other phyla (Proteobacteria, Chlamydias, Spirochetes, Cyanobacteria, and others) are Gram-negative. The Gram staining method is named after its inventor, Danish scientist Hans Christian Gram (1853–1938). The different bacterial responses to the staining procedure are ultimately due to cell wall structure. Gram-positive organisms typically lack the outer membrane found in Gram-negative organisms (Figure **3.5.8**). Up to 90 percent of the cell wall in Gram-positive bacteria is composed of peptidoglycan, and most of the rest is composed of acidic substances called teichoic acids. Teichoic acids may be covalently linked to lipids in the plasma membrane to form lipoteichoic acids. Lipoteichoic acids anchor the cell wall to the cell membrane. Gram-negative bacteria have a relatively thin cell wall composed of a few layers of peptidoglycan (only 10 percent of the total cell wall), surrounded by an outer envelope containing lipopolysaccharides (LPS) and

lipoproteins. This outer envelope is sometimes referred to as a second lipid bilayer. The chemistry of this outer envelope is very different, however, from that of the typical lipid bilayer that forms plasma membranes.

Art Connection

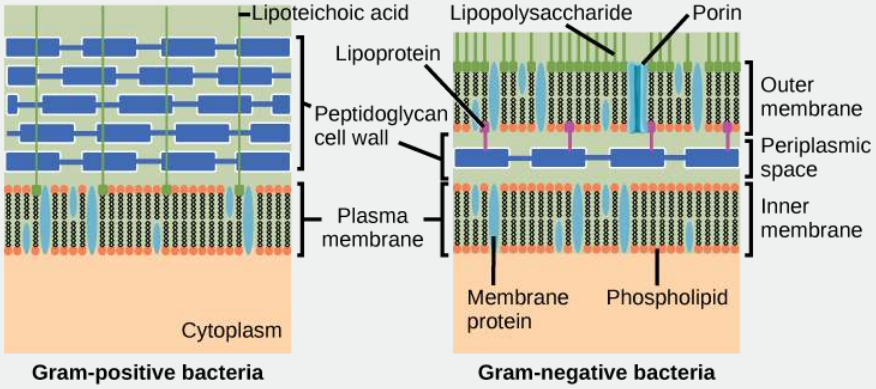


Figure **3.5.8**: Bacteria are divided into two major groups: Gram positive and Gram negative. Both groups have a cell wall composed of peptidoglycan: in Gram-positive bacteria, the wall is thick, whereas in Gram-negative bacteria, the wall is thin. In Gram-negative bacteria, the cell wall is surrounded by an outer membrane that contains lipopolysaccharides and lipoproteins. Porins are proteins in this cell membrane that allow substances to pass through the outer membrane of Gram-negative bacteria. In Gram-positive bacteria, lipoteichoic acid anchors the cell wall to the cell membrane. (credit: modification of work by "Franciscosp2"/Wikimedia Commons)

Which of the following statements is true?

A. Gram-positive bacteria have a single cell wall anchored to the cell membrane by lipoteichoic acid.
B. Porins allow entry of substances into both Gram-positive and Gram-negative bacteria.
C. The cell wall of Gram-negative bacteria is thick, and the cell wall of Gram-positive bacteria is thin.
D. Gram-negative bacteria have a cell wall made of peptidoglycan, whereas Gram-positive bacteria have a cell wall made of lipoteichoic acid.

Archaean cell walls do not have peptidoglycan. There are four different types of Archaean cell walls. One type is composed of pseudopeptidoglycan, which is similar to peptidoglycan in morphology but contains different sugars in the polysaccharide chain. The other three types of cell walls are composed of polysaccharides, glycoproteins, or pure protein.

Table **3.5.1**: Structural Differences and Similarities between Bacteria and Archaea

Structural Characteristic	Bacteria	Archaea
Cell type	Prokaryotic	Prokaryotic
Cell morphology	Variable	Variable
Cell wall	Contains peptidoglycan	Does not contain peptidoglycan
Cell membrane type	Lipid bilayer	Lipid bilayer or lipid monolayer
Plasma membrane lipids	Fatty acids	Phytanyl groups

Reproduction

Reproduction in prokaryotes is asexual and usually takes place by binary fission. Recall that the DNA of a prokaryote exists as a single, circular chromosome. Prokaryotes do not undergo mitosis. Rather the chromosome is replicated and the two resulting copies separate from one another, due to the growth of the cell. The prokaryote, now enlarged, is pinched inward at its equator and the two resulting cells, which are clones, separate. Binary fission does not provide an opportunity for genetic recombination or genetic diversity, but prokaryotes can share genes by three other mechanisms.

In transformation, the prokaryote takes in DNA found in its environment that is shed by other prokaryotes. If a nonpathogenic bacterium takes up DNA for a toxin gene from a pathogen and incorporates the new DNA into its own chromosome, it too may

become pathogenic. In transduction, bacteriophages, the viruses that infect bacteria, sometimes also move short pieces of chromosomal DNA from one bacterium to another. Transduction results in a recombinant organism. Archaea are not affected by bacteriophages but instead have their own viruses that translocate genetic material from one individual to another. In conjugation, DNA is transferred from one prokaryote to another by means of a pilus, which brings the organisms into contact with one another. The DNA transferred can be in the form of a plasmid or as a hybrid, containing both plasmid and chromosomal DNA. These three processes of DNA exchange are shown in Figure **3.5.9**.

Reproduction can be very rapid: a few minutes for some species. This short generation time coupled with mechanisms of genetic recombination and high rates of mutation result in the rapid evolution of prokaryotes, allowing them to respond to environmental changes (such as the introduction of an antibiotic) very quickly.

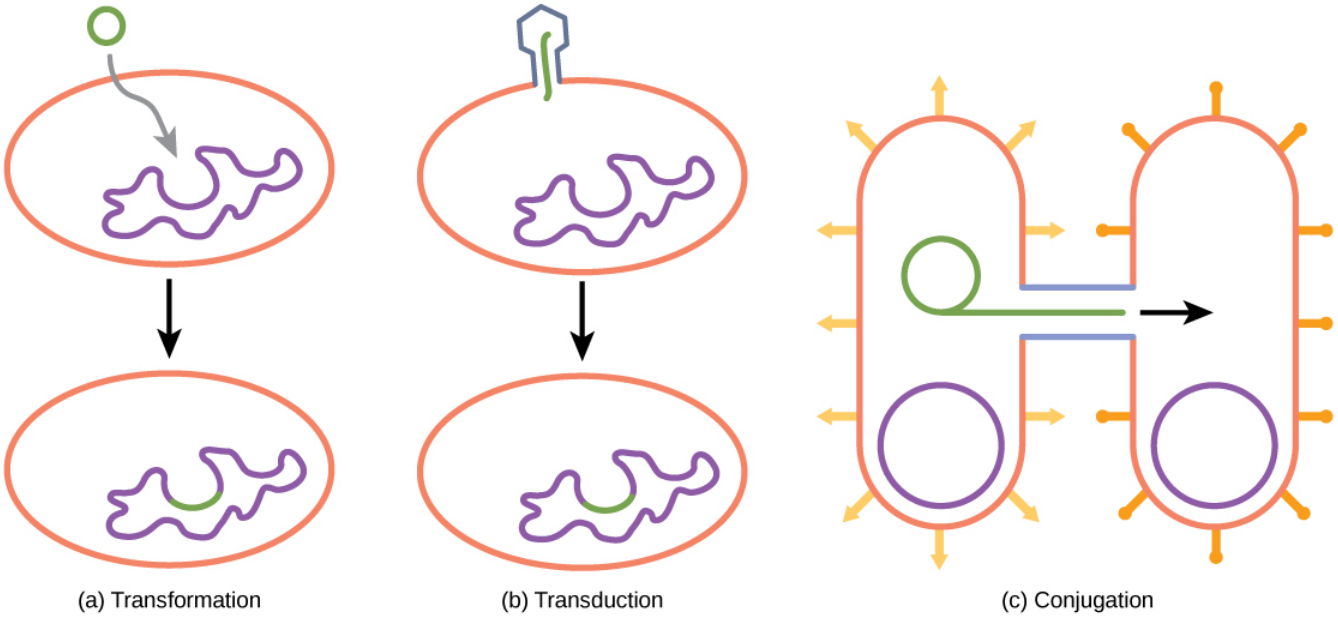


Figure **3.5.9**: Besides binary fission, there are three other mechanisms by which prokaryotes can exchange DNA. In (a) transformation, the cell takes up prokaryotic DNA directly from the environment. The DNA may remain separate as plasmid DNA or be incorporated into the host genome. In (b) transduction, a bacteriophage injects DNA into the cell that contains a small fragment of DNA from a different prokaryote. In (c) conjugation, DNA is transferred from one cell to another via a mating bridge that connects the two cells after the sex pilus draws the two bacteria close enough to form the bridge.

Evolution Connection: The Evolution of Prokaryotes

How do scientists answer questions about the evolution of prokaryotes? Unlike with animals, artifacts in the fossil record of prokaryotes offer very little information. Fossils of ancient prokaryotes look like tiny bubbles in rock. Some scientists turn to genetics and to the principle of the molecular clock, which holds that the more recently two species have diverged, the more similar their genes (and thus proteins) will be. Conversely, species that diverged long ago will have more genes that are dissimilar.

Scientists at the NASA Astrobiology Institute and at the European Molecular Biology Laboratory collaborated to analyze the molecular evolution of 32 specific proteins common to 72 species of prokaryotes.[1] The model they derived from their data indicates that three important groups of bacteria—Actinobacteria, *Deinococcus*, and Cyanobacteria (which the authors call *Terrabacteria*)—were the first to colonize land. (Recall that *Deinococcus* is a genus of prokaryote—a bacterium—that is highly resistant to ionizing radiation.) Cyanobacteria are photosynthesizers, while Actinobacteria are a group of very common bacteria that include species important in decomposition of organic wastes.

The timelines of divergence suggest that bacteria (members of the domain Bacteria) diverged from common ancestral species between 2.5 and 3.2 billion years ago, whereas archaea diverged earlier: between 3.1 and 4.1 billion years ago. Eukarya later diverged off the Archaean line. The work further suggests that stromatolites that formed prior to the advent of cyanobacteria (about 2.6 billion years ago) photosynthesized in an anoxic environment and that because of the modifications of the

Terrabacteria for land (resistance to drying and the possession of compounds that protect the organism from excess light), photosynthesis using oxygen may be closely linked to adaptations to survive on land.

Summary

Prokaryotes (domains Archaea and Bacteria) are single-celled organisms lacking a nucleus. They have a single piece of circular DNA in the nucleoid area of the cell. Most prokaryotes have a cell wall that lies outside the boundary of the plasma membrane. Some prokaryotes may have additional structures such as a capsule, flagella, and pili. Bacteria and Archaea differ in the lipid composition of their cell membranes and the characteristics of the cell wall. In archaeal membranes, phytanyl units, rather than fatty acids, are linked to glycerol. Some archaeal membranes are lipid monolayers instead of bilayers.

The cell wall is located outside the cell membrane and prevents osmotic lysis. The chemical composition of cell walls varies between species. Bacterial cell walls contain peptidoglycan. Archaean cell walls do not have peptidoglycan, but they may have pseudopeptidoglycan, polysaccharides, glycoproteins, or protein-based cell walls. Bacteria can be divided into two major groups: Gram positive and Gram negative, based on the Gram stain reaction. Gram-positive organisms have a thick cell wall, together with teichoic acids. Gram-negative organisms have a thin cell wall and an outer envelope containing lipopolysaccharides and lipoproteins.

Art Connections

Figure 3.5.8: Which of the following statements is true?

A. Gram-positive bacteria have a single cell wall anchored to the cell membrane by lipoteichoic acid.
B. Porins allow entry of substances into both Gram-positive and Gram-negative bacteria.
C. The cell wall of Gram-negative bacteria is thick, and the cell wall of Gram-positive bacteria is thin.
D. Gram-negative bacteria have a cell wall made of peptidoglycan, whereas Gram-positive bacteria have a cell wall made of lipoteichoic acid.

Answer

A

Footnotes

1. 1 Battistuzzi, FU, Feijao, A, and Hedges, SB. A genomic timescale of prokaryote evolution: Insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BioMed Central: Evolutionary Biology* 4 (2004): 44, doi:10.1186/1471-2148-4-44.

Glossary

capsule
external structure that enables a prokaryote to attach to surfaces and protects it from dehydration

conjugation
process by which prokaryotes move DNA from one individual to another using a pilus

Gram negative
bacterium whose cell wall contains little peptidoglycan but has an outer membrane

Gram positive
bacterium that contains mainly peptidoglycan in its cell walls

peptidoglycan
material composed of polysaccharide chains cross-linked to unusual peptides

pilus

surface appendage of some prokaryotes used for attachment to surfaces including other prokaryotes

pseudopeptidoglycan
component of archaea cell walls that is similar to peptidoglycan in morphology but contains different sugars

S-layer
surface-layer protein present on the outside of cell walls of archaea and bacteria

teichoic acid
polymer associated with the cell wall of Gram-positive bacteria

transduction
process by which a bacteriophage moves DNA from one prokaryote to another

transformation
process by which a prokaryote takes in DNA found in its environment that is shed by other prokaryotes

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3.5.1: COMPONENTS AND STRUCTURE - FLUID MOSAIC MODEL

LEARNING OBJECTIVES

- Describe the fluid mosaic model of cell membranes

The fluid mosaic model was first proposed by S.J. Singer and Garth L. Nicolson in 1972 to explain the structure of the plasma membrane. The model has evolved somewhat over time, but it still best accounts for the structure and functions of the plasma membrane as we now understand them. The fluid mosaic model describes the structure of the plasma membrane as a mosaic of components —including phospholipids, cholesterol, proteins, and carbohydrates—that gives the membrane a fluid character. Plasma membranes range from 5 to 10 nm in thickness. For comparison, human red blood cells, visible via light microscopy, are approximately 8 µm wide, or approximately 1,000 times wider than a plasma membrane. The proportions of proteins, lipids, and carbohydrates in the plasma membrane vary with cell type. For example, myelin contains 18% protein and 76% lipid. The mitochondrial inner membrane contains 76% protein and 24% lipid.

The Components and Functions of the Plasma Membrane	
Component	**Location**
Phospholipid	Main fabric of the membrane
Cholesterol	Attached between phospholipids and between the two phospholipid layers
Integral proteins (for example, integrins)	Embedded within the phospholipid layer(s). May or may not penetrate through both layers
Peripheral proteins	On the inner or outer surface of the phospholipid bilayer; not embedded within the phospholipids
Carbohydrates (components of glycoproteins and glycolipids)	Generally attached to proteins on the outside membrane layer

Figure **3.5.1.1**: The Components and functions of the Plasma Membrane: The principal components of a plasma membrane are lipids (phospholipids and cholesterol), proteins, and carbohydrates attached to some of the lipids and some of the proteins.

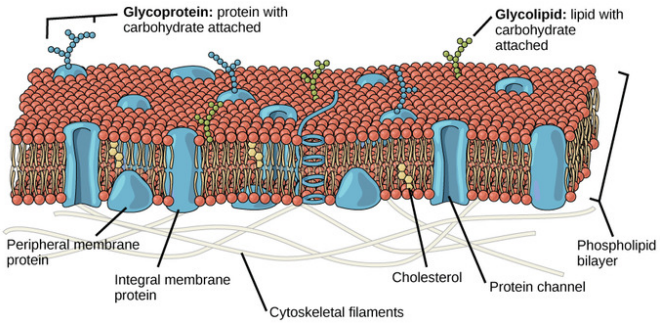


Figure **3.5.1.1**: The fluid mosaic model of the plasma membrane: The fluid mosaic model of the plasma membrane describes the plasma membrane as a fluid combination of phospholipids, cholesterol, and proteins. Carbohydrates attached to lipids (glycolipids) and to proteins (glycoproteins) extend from the outward-facing surface of the membrane.

The main fabric of the membrane is composed of amphiphilic or dual-loving, phospholipid molecules. The hydrophilic or water-loving areas of these molecules are in contact with the aqueous fluid both inside and outside the cell. Hydrophobic, or water-hating molecules, tend to be non- polar. A phospholipid molecule consists of a three-carbon glycerol backbone with two fatty acid molecules attached to carbons 1 and 2, and a phosphate-containing group attached to the third carbon. This arrangement gives the overall molecule an area described as its head (the phosphate-containing group), which has a polar character or negative charge, and an area called the tail (the fatty acids), which has no charge. They interact with other non-polar molecules in chemical reactions, but generally do not interact with polar molecules. When placed in water, hydrophobic molecules tend to form a ball or cluster. The hydrophilic regions of the phospholipids tend to form hydrogen bonds with water and other polar molecules on both the exterior and interior of the cell. Thus, the membrane surfaces that face the interior and exterior of the cell are hydrophilic. In contrast, the middle of the cell membrane is hydrophobic and will not interact with water. Therefore, phospholipids form an excellent lipid bilayer cell membrane that separates fluid within the cell from the fluid outside of the cell.

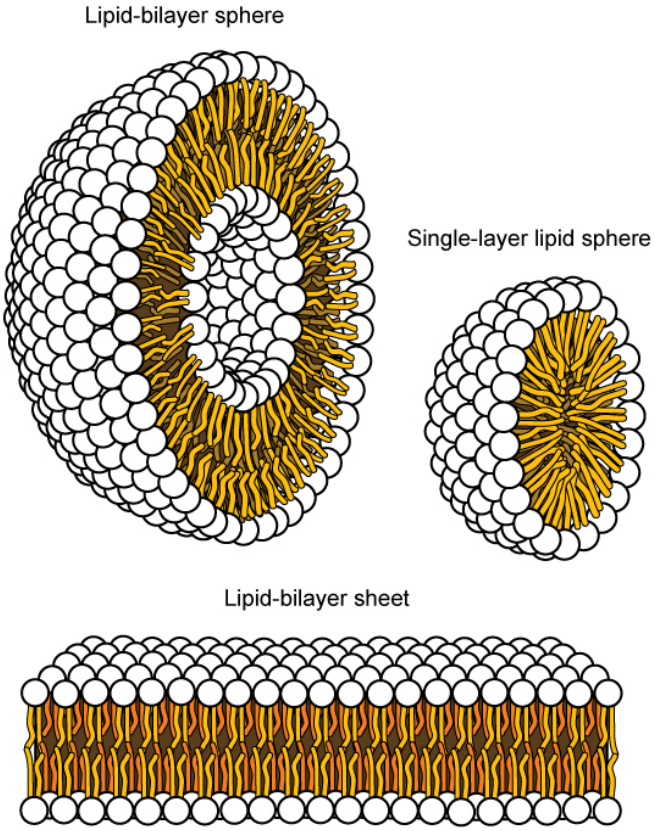


Figure **3.5.1.1**: Phospholipid aggregation: In an aqueous solution, phospholipids tend to arrange themselves with their polar heads facing outward and their hydrophobic tails facing inward.

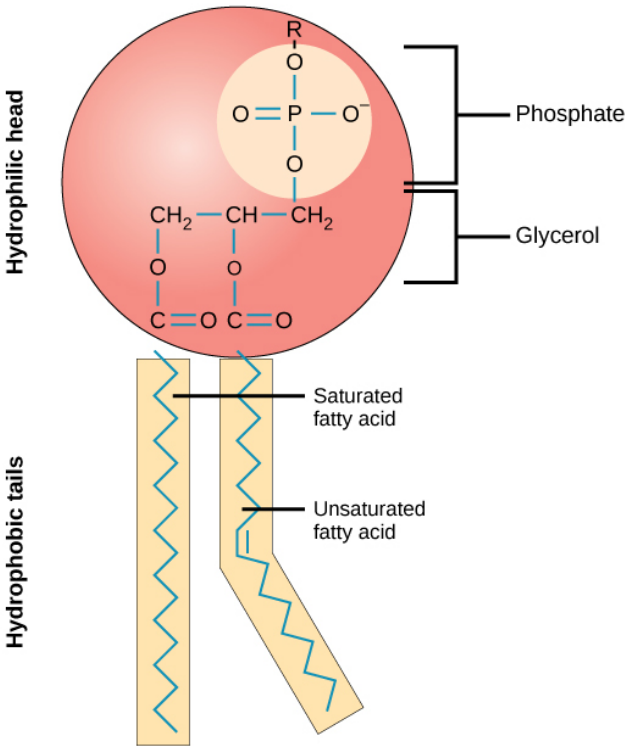


Figure **3.5.1.1**: The structure of a phospholipid molecule: This phospholipid molecule is composed of a hydrophilic head and two hydrophobic tails. The hydrophilic head group consists of a phosphate-containing group attached to a glycerol molecule. The hydrophobic tails, each containing either a saturated or an unsaturated fatty acid, are long hydrocarbon chains.

Proteins make up the second major component of plasma membranes. Integral proteins (some specialized types are called integrins) are, as their name suggests, integrated completely into the membrane structure, and their hydrophobic membrane-spanning regions interact with the hydrophobic region of the the phospholipid bilayer. Single-pass integral membrane proteins usually have a hydrophobic transmembrane segment that consists of 20–25 amino acids. Some span only part of the membrane—associating with a single layer—while others stretch from one side of the membrane to the other, and are exposed on either side. Some complex proteins are composed of up to 12 segments of a single protein, which are extensively folded and embedded in the membrane. This type of protein has a hydrophilic region or regions, and one or several mildly hydrophobic regions. This arrangement of regions of the protein tends to orient the protein alongside the phospholipids, with the hydrophobic region of the protein adjacent to the tails of the phospholipids and the hydrophilic region or regions of the protein protruding from the membrane and in contact with the cytosol or extracellular fluid.

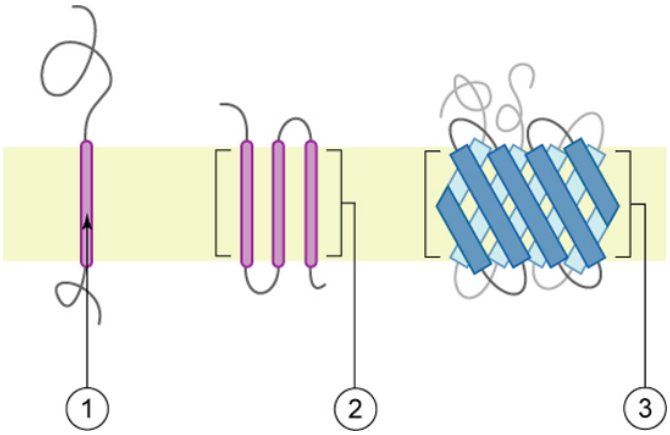


Figure **3.5.1.1**: Structure of integral membrane proteins: Integral membrane proteins may have one or more alpha-helices that span the membrane (examples 1 and 2), or they may have beta-sheets that span the membrane (example 3).

Carbohydrates are the third major component of plasma membranes. They are always found on the exterior surface of cells and are bound either to proteins (forming glycoproteins) or to lipids (forming glycolipids). These carbohydrate chains may consist of 2–60 monosaccharide units and can be either straight or branched. Along with peripheral proteins, carbohydrates form specialized sites on the cell surface that allow cells to recognize each other. This recognition function is very important to cells, as it allows the immune system to differentiate between body cells (called “self”) and foreign cells or tissues (called “non-self”). Similar types of glycoproteins and glycolipids are found on the surfaces of viruses and may change frequently, preventing immune cells from recognizing and attacking them. These carbohydrates on the exterior surface of the cell—the carbohydrate components of both glycoproteins and glycolipids—are collectively referred to as the glycocalyx (meaning “sugar coating”). The glycocalyx is highly hydrophilic and attracts large amounts of water to the surface of the cell. This aids in the interaction of the cell with its watery environment and in the cell’s ability to obtain substances dissolved in the water.

KEY POINTS

- The main fabric of the membrane is composed of amphiphilic or dual-loving, phospholipid molecules.
- Integral proteins, the second major component of plasma membranes, are integrated completely into the membrane structure with their hydrophobic membrane-spanning regions interacting with the hydrophobic region of the phospholipid bilayer.
- Carbohydrates, the third major component of plasma membranes, are always found on the exterior surface of cells where they are bound either to proteins (forming glycoproteins) or to lipids (forming glycolipids).

KEY TERMS

- **amphiphilic**: Having one surface consisting of hydrophilic amino acids and the opposite surface consisting of hydrophobic (or lipophilic) ones.

- **hydrophilic**: Having an affinity for water; able to absorb, or be wetted by water, “water-loving.”
- **hydrophobic**: Lacking an affinity for water; unable to absorb, or be wetted by water, “water-fearing.”

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3.6: Endospores

Learning Objectives

- Describe the function and advantage of endospore formation, as well as the methods for viewing it.

An endospore is a dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum. Endospore formation is usually triggered by lack of nutrients, and usually occurs in Gram-positive bacteria. In endospore formation, the bacterium divides within its cell wall. One side then engulfs the other. Endospores enable bacteria to lie dormant for extended periods, even centuries. When the environment becomes more favorable, the endospore can reactivate itself to the vegetative state. Examples of bacteria that can form endospores include Bacillus and Clostridium. The endospore consists of the bacterium's DNA and part of its cytoplasm, surrounded by a very tough outer coating. Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants. They are commonly found in soil and water, where they may survive for long periods of time. Bacteria produce a single endospore internally.

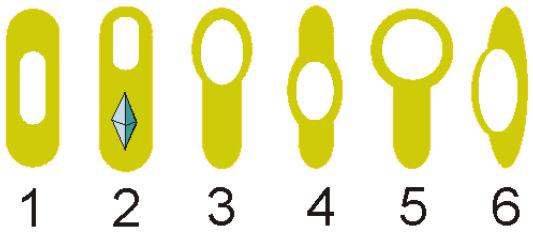


Figure: ***Endospore morphology****: Variations in endospore morphology: (1, 4) central endospore; (2, 3, 5) terminal endospore; (6) lateral endospore.*

Viewing endospores under the light microscope can be difficult due to the impermeability of the endospore wall to dyes and stains. While the rest of a bacterial cell may stain, the endospore is left colorless. To combat this, a special stain technique called a Moeller stain is used. That allows the endospore to show up as red, while the rest of the cell stains blue. Another staining technique for endospores is the Schaeffer-Fulton stain, which stains endospores green and bacterial bodies red. There are variations in endospore morphology. Examples of bacteria having terminal endospores include Clostridium tetani, the pathogen that causes the disease tetanus. Bacteria having a centrally placed endospore include Bacillus cereus, and those having a subterminal endospore include Bacillus subtilis. Sometimes the endospore can be so large that the cell can be distended around the endospore. This is typical of Clostridium tetani.

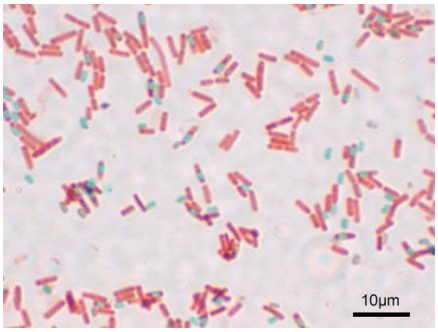


Figure: **Bacillus subtilis stained with the Schaeffer-Fulton stain.**: A stained preparation of Bacillus subtilis showing endospores as green and the vegetative cell as red.

When a bacterium detects environmental conditions are becoming unfavorable it may start the process of endosporulation, which takes about eight hours. The DNA is replicated and a membrane wall known as a spore septum begins to form between it and the rest of the cell. The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around the DNA, and the developing structure is now known as a *forespore*. Calcium dipicolinate is incorporated into the forespore during this time. Next the peptidoglycan cortex forms between the two layers and the bacterium adds a spore coat to the outside of the forespore. Sporulation is now complete, and the mature endospore will be released when the surrounding vegetative cell is degraded.

While resistant to extreme heat and radiation, endospores can be destroyed by burning or by autoclaving. Endospores are able to survive boiling at 100°C for hours, although the longer the number of hours the fewer that will survive. An indirect way to destroy them is to place them in an environment that reactivates them to their vegetative state. They will germinate within a day or two with the right environmental conditions, and then the vegetative cells can be straightforwardly destroyed. This indirect method is called Tyndallization. It was the usual method for a while in the late 19th century before the advent of inexpensive autoclaves. Prolonged exposure to ionising radiation, such as x-rays and gamma rays, will also kill most endospores.

Reactivation of the endospore occurs when conditions are more favourable and involves activation, germination, and outgrowth. Even if an endospore is located in plentiful nutrients, it may fail to germinate unless activation has taken place. This may be triggered by heating the endospore. Germination involves the dormant endospore starting metabolic activity and thus breaking hibernation. It is commonly characterised by rupture or absorption of the spore coat, swelling of the endospore, an increase in metabolic activity, and loss of resistance to environmental stress.

As a simplified model for cellular differentiation, the molecular details of endospore formation have been extensively studied, specifically in the model organism Bacillus subtilis. These studies have contributed much to our understanding of the regulation of gene expression, transcription factors, and the sigma factor subunits of RNA polymerase.

Endospores of the bacterium Bacillus anthracis were used in the 2001 anthrax attacks. The powder found in contaminated postal letters was composed of extracellular anthrax endospores. Inhalation, ingestion or skin contamination of these endospores led to a number of deaths.

Geobacillus stearothermophilus endospores are used as biological indicators when an autoclave is used in sterilization procedures. Bacillus subtilis spores are useful for the expression of recombinant proteins and in particular for the surface display of peptides and proteins as a tool for fundamental and applied research in the fields of microbiology, biotechnology and vaccination.

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Key Points

- Examples of bacteria that can form endospores include Bacillus and Clostridium.
- Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants.
- While resistant to extreme heat and radiation, endospores can be destroyed by burning or by autoclaving.

Key Terms

- **endospore**: A dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum.

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3.6.1: Fungal Spores



Figure 1 Although many fungi reproduce without sex, the sexual organ, if present, is the most visible structure of the organism and, until the advent of molecular techniques, it was the structure used to identify fungi. In many fungi the sexual organ is too small to be seen but in some, like this pale chanterelle, the 'fruiting body' is not only large, its also edible!

The 'vegetative' (i.e., not associated with sex and reproduction) structure of most fungi are generally very consistent: they are composed of branched filaments that explore the volume of material that they feed on(be it living or dead) and this structure provides for acquisition of materials that they need to grow and survive. The consistency of vegetative form makes it difficult to classify fungibecause they mostly look alike, although some vary on the basis of whether or not the filaments on coenocytic or septate (with cross walls and therefore cellular). However, fungi do show variation, and can be grouped, based upon structures that are associated with sexual reproduction. The exceptions to this are the two groups where sex is either completely absent (Glomeromycota—the endomycorhizal forming fungi) or rare (Chytridomycota—the chytrids). The remaining three groups (bread molds = Zygomycota, cup fungi = Ascomycota, and club fungi = Basidiomycota usually exhibit specific features associated with sexual reproduction that are used define thesegroups (generally considered phyla). Fungi that show no sexual features used to be put into a group called the 'Fungi Imperfecti' (Deuteromycota), but molecular techniques now allow these fungi to be placed in one of the groups mentioned above.

Fungi have the following features associated with sex and reproduction that are different from most familiar sexual organisms:

- R eproduction often involves both sexual aspects and asexual aspects. Cells called **spores** — defined as cells with both dispersal capabilities (i.e., mobile cells) and reproductive capabilities (i.e., cells that can grow into new fungal organisms) are often important in both asexual and sexual reproduction.
- **Gametes**(cells that can fuse with other gametes) are quite different from what most wouldconsider 'typical' ; they are usually hyphal cells (part of the fungal filaments) that have the ability to fuse with other hyphae. Depending upon the group, these special hyphae may or may not have structural features that would distinguish themfrom the normal hyphae.
- **Syngamy**often involves two steps separated in time and often in space. This is a consequence of the fact that the fusion of the hyphae serving as gametes is generally not followed immediately by the fusion of nuclei. Cellular fusion is called plasmogamy and nuclear fusion is called karyogamy. Plasmogamy followed by a delayed karyogamy allows fungi to have a novel condition, the dikaryon state, wherea cell hastwo nuclei ('dikaryon' means 'two nuclei'), one from each parent. This condition is often perpetuated: the dikaryon cell divides while both nuclei divide, thereby forming a new cell that is also dikaryon. This process can continue, producing multiple dikaryon cells and dikaryon hyphae. Note that although a dikaryon cell has two copies of each chromosome, it is not considered diploid because each nucleus is haploid, with only one copy of each chromosome.
- At some point, some of the dikaryon cells become diploid as a result of the fusion of the two nuclei(karyogamy).The diploid cells are **NOT**perpetuated; they undergo meiosis to form haploid nucleiwhich develop walls to become haploid cells. Thus, the

diploid state is very brief in extent(i.e., size and number of cells) and often brief in time as well—the diploid cells neverdivide mitotically to form more diploid cells, they only divide meiotically to form haploid cells. These haploid cells are, or soon become, spores and aredispersed from the parent fungus to a new location where they germinate and form haploid hyphae.

- 'Mating strains' are a common mechanism thatensures that fungi do not mate with themselves. Within a particular species there are two to many mating types. A particular mating type needs to find a different mating type in order to interact sexually (i.e., fuse hyphae). The simplest situation has two mating types: “+” and “-” and they need to find each other to mate, but there may bemore than just two types, e.g., types A, B, C, D, E, and A could mate with any of the othersbut not with another A. Generally, the interaction, or lack of it, involves chemical signals (pheromones)that are emitted by one mating type and sensed only by fungi of a different mating type. In response to the pheromone, hyphae grow and find each other, bringing about plasmogamy.

TOPICS

- Zygomycetes (bread molds)
- Ascomycetes (cup fungi)
- Basidiomycetes (club fungi)

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3.7: Eukaryotic External Structures

Skills to Develop

- Describe the structure of eukaryotic cells
- Compare animal cells with plant cells
- State the role of the plasma membrane
- Summarize the functions of the major cell organelles

Have you ever heard the phrase “form follows function?” It’s a philosophy practiced in many industries. In architecture, this means that buildings should be constructed to support the activities that will be carried out inside them. For example, a skyscraper should be built with several elevator banks; a hospital should be built so that its emergency room is easily accessible.

Our natural world also utilizes the principle of form following function, especially in cell biology, and this will become clear as we explore eukaryotic cells (Figure **3.7.1**). Unlike prokaryotic cells, eukaryotic cells have: 1) a membrane-bound nucleus; 2) numerous membrane-bound organelles such as the endoplasmic reticulum, Golgi apparatus, chloroplasts, mitochondria, and others; and 3) several, rod-shaped chromosomes. Because a eukaryotic cell’s nucleus is surrounded by a membrane, it is often said to have a “true nucleus.” The word “organelle” means “little organ,” and, as already mentioned, organelles have specialized cellular functions, just as the organs of your body have specialized functions.

At this point, it should be clear to you that eukaryotic cells have a more complex structure than prokaryotic cells. Organelles allow different functions to be compartmentalized in different areas of the cell. Before turning to organelles, let’s first examine two important components of the cell: the plasma membrane and the cytoplasm.

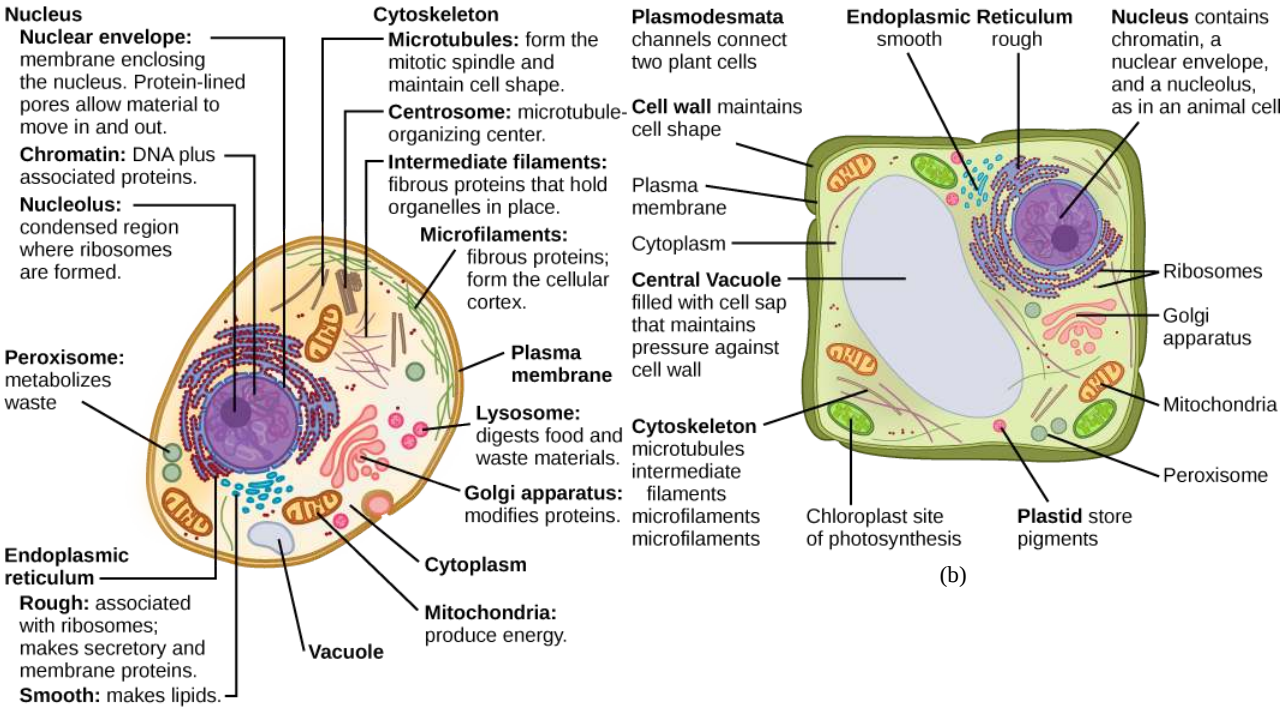


Figure **3.7.1**: These figures show the major organelles and other cell components of (a) a typical animal cell and (b) a typical eukaryotic plant cell. The plant cell has a cell wall, chloroplasts, plastids, and a central vacuole—structures not found in animal cells. Plant cells do not have lysosomes or centrosomes.

Exercise 3.7.1

If the nucleolus were not able to carry out its function, what other cellular organelles would be affected?

The Plasma Membrane

Like prokaryotes, eukaryotic cells have a plasma membrane (Figure 3.7.2), a phospholipid bilayer with embedded proteins that separates the internal contents of the cell from its surrounding environment. A phospholipid is a lipid molecule with two fatty acid chains and a phosphate-containing group. The plasma membrane controls the passage of organic molecules, ions, water, and oxygen into and out of the cell. Wastes (such as carbon dioxide and ammonia) also leave the cell by passing through the plasma membrane.

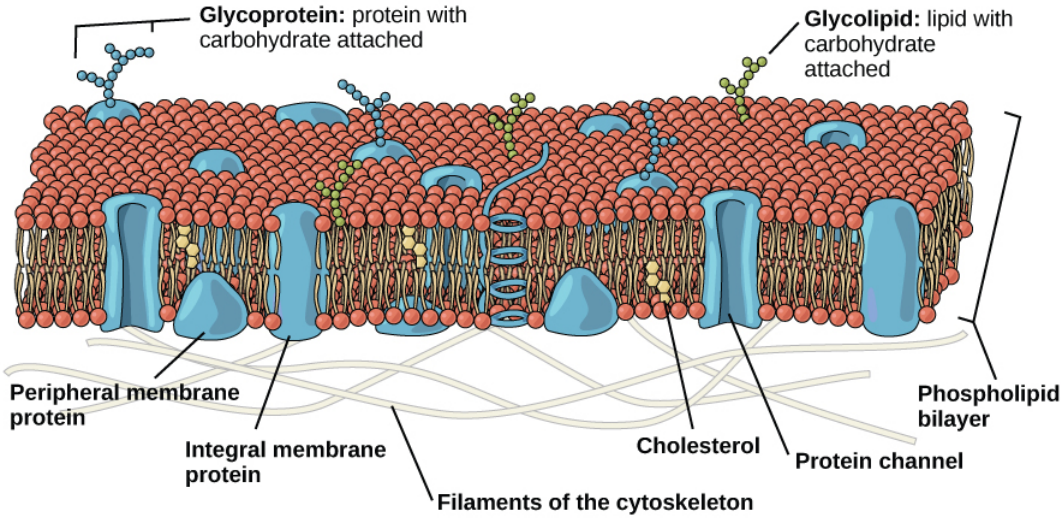


Figure 3.7.2: The eukaryotic plasma membrane is a phospholipid bilayer with proteins and cholesterol embedded in it.

The plasma membranes of cells that specialize in absorption are folded into fingerlike projections called microvilli (singular = microvillus); (Figure 3.7.3). Such cells are typically found lining the small intestine, the organ that absorbs nutrients from digested food. This is an excellent example of form following function. People with celiac disease have an immune response to gluten, which is a protein found in wheat, barley, and rye. The immune response damages microvilli, and thus, afflicted individuals cannot absorb nutrients. This leads to malnutrition, cramping, and diarrhea. Patients suffering from celiac disease must follow a gluten-free diet.

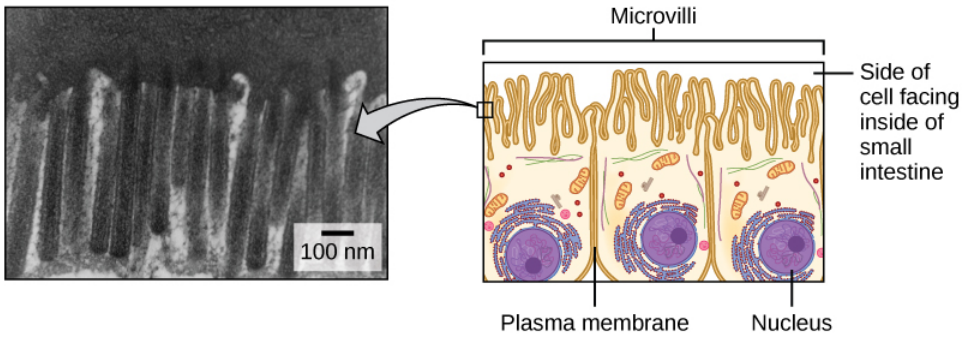


Figure 3.7.3: Microvilli, shown here as they appear on cells lining the small intestine, increase the surface area available for absorption. These microvilli are only found on the area of the plasma membrane that faces the cavity from which substances will be absorbed. (credit "micrograph": modification of work by Louisa Howard)

The Cytoplasm

The cytoplasm is the entire region of a cell between the plasma membrane and the nuclear envelope (a structure to be discussed shortly). It is made up of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals (Figure 3.7.1). Even though the cytoplasm consists of 70 to 80 percent water, it has a semi-solid consistency, which comes from the proteins within it.

However, proteins are not the only organic molecules found in the cytoplasm. Glucose and other simple sugars, polysaccharides, amino acids, nucleic acids, fatty acids, and derivatives of glycerol are found there, too. Ions of sodium, potassium, calcium, and many other elements are also dissolved in the cytoplasm. Many metabolic reactions, including protein synthesis, take place in the cytoplasm.

The Nucleus

Typically, the nucleus is the most prominent organelle in a cell (Figure **3.7.1**). The nucleus (plural = nuclei) houses the cell's DNA and directs the synthesis of ribosomes and proteins. Let's look at it in more detail (Figure **3.7.4**).

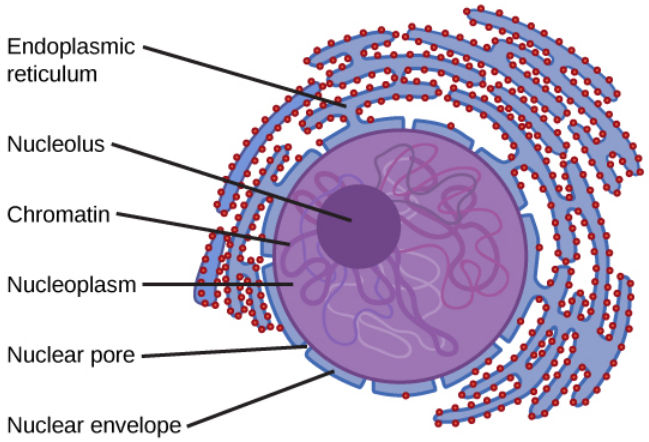


Figure **3.7.4**: The nucleus stores chromatin (DNA plus proteins) in a gel-like substance called the nucleoplasm. The nucleolus is a condensed region of chromatin where ribosome synthesis occurs. The boundary of the nucleus is called the nuclear envelope. It consists of two phospholipid bilayers: an outer membrane and an inner membrane. The nuclear membrane is continuous with the endoplasmic reticulum. Nuclear pores allow substances to enter and exit the nucleus.

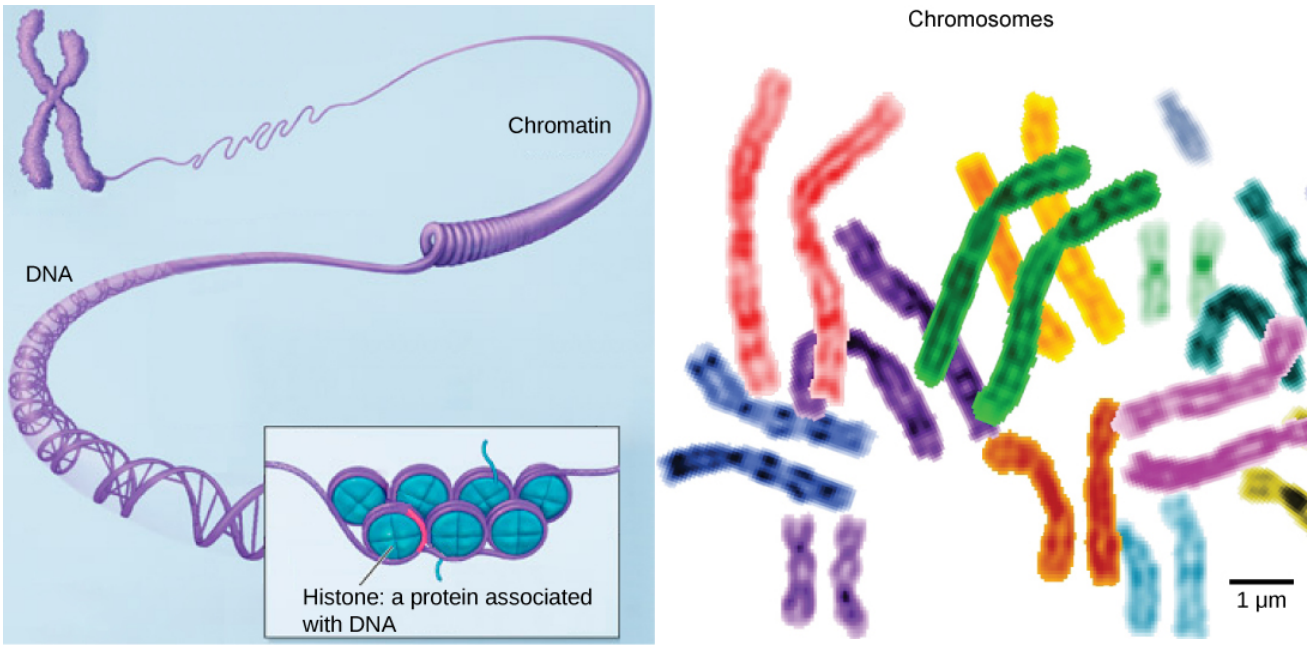
The Nuclear Envelope

The nuclear envelope is a double-membrane structure that constitutes the outermost portion of the nucleus (Figure **3.7.4**). Both the inner and outer membranes of the nuclear envelope are phospholipid bilayers.

The nuclear envelope is punctuated with pores that control the passage of ions, molecules, and RNA between the nucleoplasm and cytoplasm. The nucleoplasm is the semi-solid fluid inside the nucleus, where we find the chromatin and the nucleolus.

Chromatin and Chromosomes

To understand chromatin, it is helpful to first consider chromosomes. Chromosomes are structures within the nucleus that are made up of DNA, the hereditary material. You may remember that in prokaryotes, DNA is organized into a single circular chromosome. In eukaryotes, chromosomes are linear structures. Every eukaryotic species has a specific number of chromosomes in the nuclei of its body's cells. For example, in humans, the chromosome number is 46, while in fruit flies, it is eight. Chromosomes are only visible and distinguishable from one another when the cell is getting ready to divide. When the cell is in the growth and maintenance phases of its life cycle, proteins are attached to chromosomes, and they resemble an unwound, jumbled bunch of threads. These unwound protein-chromosome complexes are called chromatin (Figure **3.7.5**); chromatin describes the material that makes up the chromosomes both when condensed and decondensed.



(a) (b)

Figure **3.7.5**: (a) This image shows various levels of the organization of chromatin (DNA and protein). (b) This image shows paired chromosomes. (credit b: modification of work by NIH; scale-bar data from Matt Russell)

The Nucleolus

We already know that the nucleus directs the synthesis of ribosomes, but how does it do this? Some chromosomes have sections of DNA that encode ribosomal RNA. A darkly staining area within the nucleus called the nucleolus (plural = nucleoli) aggregates the ribosomal RNA with associated proteins to assemble the ribosomal subunits that are then transported out through the pores in the nuclear envelope to the cytoplasm.

Ribosomes

Ribosomes are the cellular structures responsible for protein synthesis. When viewed through an electron microscope, ribosomes appear either as clusters (polyribosomes) or single, tiny dots that float freely in the cytoplasm. They may be attached to the cytoplasmic side of the plasma membrane or the cytoplasmic side of the endoplasmic reticulum and the outer membrane of the nuclear envelope (Figure **3.7.1**). Electron microscopy has shown us that ribosomes, which are large complexes of protein and RNA, consist of two subunits, aptly called large and small (Figure **3.7.6**). Ribosomes receive their “orders” for protein synthesis from the nucleus where the DNA is transcribed into messenger RNA (mRNA). The mRNA travels to the ribosomes, which translate the code provided by the sequence of the nitrogenous bases in the mRNA into a specific order of amino acids in a protein. Amino acids are the building blocks of proteins.

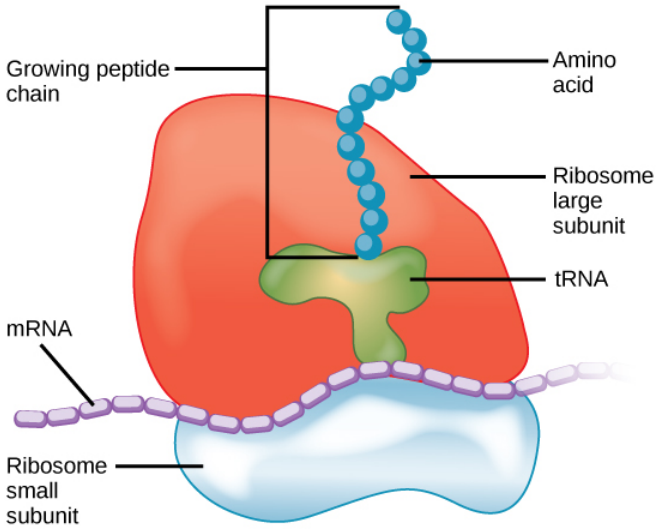


Figure 3.7.6: Ribosomes are made up of a large subunit (top) and a small subunit (bottom). During protein synthesis, ribosomes assemble amino acids into proteins.

Because proteins synthesis is an essential function of all cells (including enzymes, hormones, antibodies, pigments, structural components, and surface receptors), ribosomes are found in practically every cell. Ribosomes are particularly abundant in cells that synthesize large amounts of protein. For example, the pancreas is responsible for creating several digestive enzymes and the cells that produce these enzymes contain many ribosomes. Thus, we see another example of form following function.

Mitochondria

Mitochondria (singular = mitochondrion) are often called the “powerhouses” or “energy factories” of a cell because they are responsible for making adenosine triphosphate (ATP), the cell’s main energy-carrying molecule. ATP represents the short-term stored energy of the cell. Cellular respiration is the process of making ATP using the chemical energy found in glucose and other nutrients. In mitochondria, this process uses oxygen and produces carbon dioxide as a waste product. In fact, the carbon dioxide that you exhale with every breath comes from the cellular reactions that produce carbon dioxide as a byproduct.

In keeping with our theme of form following function, it is important to point out that muscle cells have a very high concentration of mitochondria that produce ATP. Your muscle cells need a lot of energy to keep your body moving. When your cells don’t get enough oxygen, they do not make a lot of ATP. Instead, the small amount of ATP they make in the absence of oxygen is accompanied by the production of lactic acid.

Mitochondria are oval-shaped, double membrane organelles (Figure 3.7.7) that have their own ribosomes and DNA. Each membrane is a phospholipid bilayer embedded with proteins. The inner layer has folds called cristae. The area surrounded by the folds is called the mitochondrial matrix. The cristae and the matrix have different roles in cellular respiration.

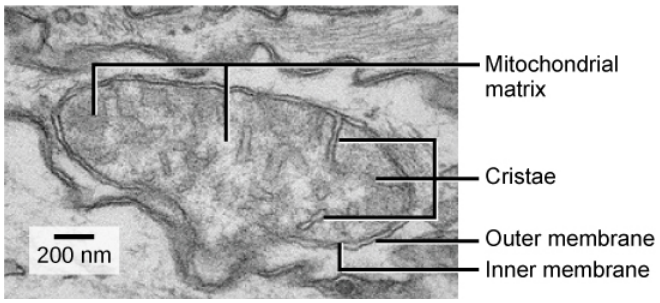


Figure 3.7.7: This electron micrograph shows a mitochondrion as viewed with a transmission electron microscope. This organelle has an outer membrane and an inner membrane. The inner membrane contains folds, called cristae, which increase its surface area. The space between the two membranes is called the intermembrane space, and the space inside the inner membrane is called the mitochondrial matrix. ATP synthesis takes place on the inner membrane. (credit: modification of work by Matthew Britton; scale-bar data from Matt Russell)

Peroxisomes

Peroxisomes are small, round organelles enclosed by single membranes. They carry out oxidation reactions that break down fatty acids and amino acids. They also detoxify many poisons that may enter the body. (Many of these oxidation reactions release hydrogen peroxide, H_2O_2, which would be damaging to cells; however, when these reactions are confined to peroxisomes, enzymes safely break down the H_2O_2 into oxygen and water.) For example, alcohol is detoxified by peroxisomes in liver cells. Glyoxysomes, which are specialized peroxisomes in plants, are responsible for converting stored fats into sugars.

Vesicles and Vacuoles

Vesicles and vacuoles are membrane-bound sacs that function in storage and transport. Other than the fact that vacuoles are somewhat larger than vesicles, there is a very subtle distinction between them: The membranes of vesicles can fuse with either the plasma membrane or other membrane systems within the cell. Additionally, some agents such as enzymes within plant vacuoles break down macromolecules. The membrane of a vacuole does not fuse with the membranes of other cellular components.

Animal Cells versus Plant Cells

At this point, you know that each eukaryotic cell has a plasma membrane, cytoplasm, a nucleus, ribosomes, mitochondria, peroxisomes, and in some, vacuoles, but there are some striking differences between animal and plant cells. While both animal and plant cells have microtubule organizing centers (MTOCs), animal cells also have centrioles associated with the MTOC: a complex called the centrosome. Animal cells each have a centrosome and lysosomes, whereas plant cells do not. Plant cells have a cell wall, chloroplasts and other specialized plastids, and a large central vacuole, whereas animal cells do not.

The Centrosome

The centrosome is a microtubule-organizing center found near the nuclei of animal cells. It contains a pair of centrioles, two structures that lie perpendicular to each other (Figure **3.7.8**). Each centriole is a cylinder of nine triplets of microtubules.

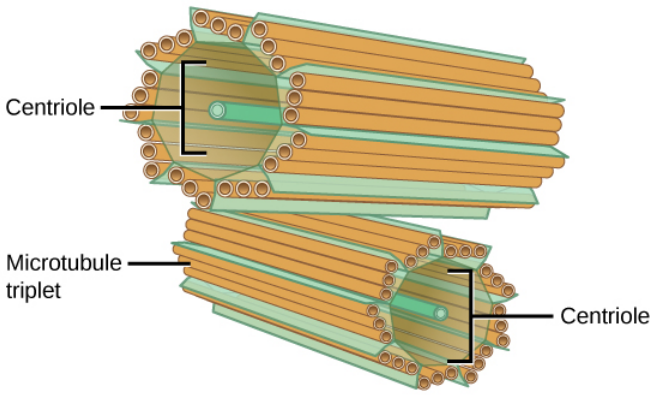


Figure **3.7.8**: The centrosome consists of two centrioles that lie at right angles to each other. Each centriole is a cylinder made up of nine triplets of microtubules. Nontubulin proteins (indicated by the green lines) hold the microtubule triplets together.

The centrosome (the organelle where all microtubules originate) replicates itself before a cell divides, and the centrioles appear to have some role in pulling the duplicated chromosomes to opposite ends of the dividing cell. However, the exact function of the centrioles in cell division isn't clear, because cells that have had the centrosome removed can still divide, and plant cells, which lack centrosomes, are capable of cell division.

Lysosomes

Animal cells have another set of organelles not found in plant cells: lysosomes. The lysosomes are the cell's "garbage disposal." In plant cells, the digestive processes take place in vacuoles. Enzymes within the lysosomes aid the breakdown of proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles. These enzymes are active at a much lower pH than that of the cytoplasm. Therefore, the pH within lysosomes is more acidic than the pH of the cytoplasm. Many reactions that take place in the cytoplasm could not occur at a low pH, so again, the advantage of compartmentalizing the eukaryotic cell into organelles is apparent.

The Cell Wall

If you examine Figure 3.7.1b, the diagram of a plant cell, you will see a structure external to the plasma membrane called the cell wall. The cell wall is a rigid covering that protects the cell, provides structural support, and gives shape to the cell. Fungal and protistan cells also have cell walls. While the chief component of prokaryotic cell walls is peptidoglycan, the major organic molecule in the plant cell wall is cellulose (Figure 3.7.9), a polysaccharide made up of glucose units. Have you ever noticed that when you bite into a raw vegetable, like celery, it crunches? That's because you are tearing the rigid cell walls of the celery cells with your teeth.

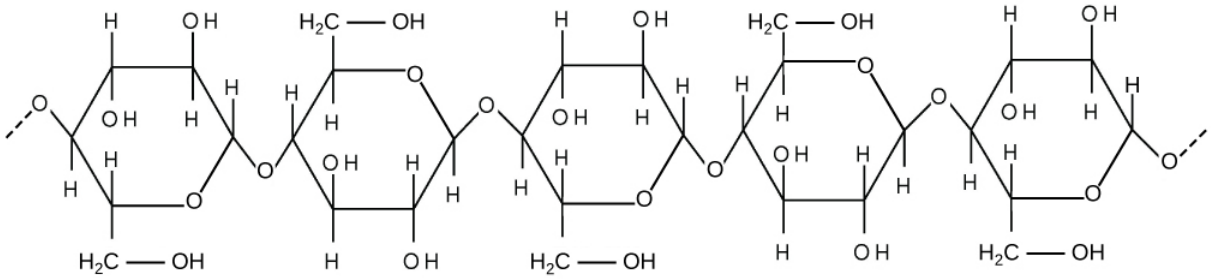


Figure 3.7.9: Cellulose is a long chain of β-glucose molecules connected by a 1-4 linkage. The dashed lines at each end of the figure indicate a series of many more glucose units. The size of the page makes it impossible to portray an entire cellulose molecule.

Chloroplasts

Like the mitochondria, chloroplasts have their own DNA and ribosomes, but chloroplasts have an entirely different function. Chloroplasts are plant cell organelles that carry out photosynthesis. Photosynthesis is the series of reactions that use carbon dioxide, water, and light energy to make glucose and oxygen. This is a major difference between plants and animals; plants (autotrophs) are able to make their own food, like sugars, while animals (heterotrophs) must ingest their food.

Like mitochondria, chloroplasts have outer and inner membranes, but within the space enclosed by a chloroplast's inner membrane is a set of interconnected and stacked fluid-filled membrane sacs called thylakoids (Figure 3.7.10). Each stack of thylakoids is called a granum (plural = grana). The fluid enclosed by the inner membrane that surrounds the grana is called the stroma.

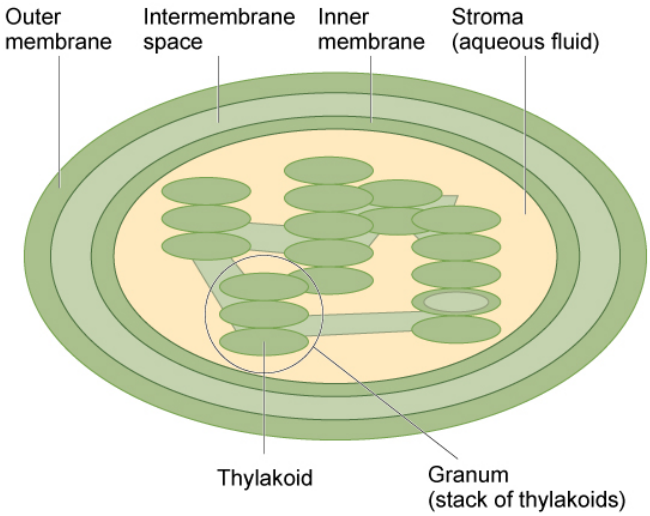


Figure 3.7.10: The chloroplast has an outer membrane, an inner membrane, and membrane structures called thylakoids that are stacked into grana. The space inside the thylakoid membranes is called the thylakoid space. The light harvesting reactions take place in the thylakoid membranes, and the synthesis of sugar takes place in the fluid inside the inner membrane, which is called the stroma. Chloroplasts also have their own genome, which is contained on a single circular chromosome.

The chloroplasts contain a green pigment called chlorophyll, which captures the light energy that drives the reactions of photosynthesis. Like plant cells, photosynthetic protists also have chloroplasts. Some bacteria perform photosynthesis, but their chlorophyll is not relegated to an organelle.

Evolution Connection: Endosymbiosis

We have mentioned that both mitochondria and chloroplasts contain DNA and ribosomes. Have you wondered why? Strong evidence points to endosymbiosis as the explanation.

Symbiosis is a relationship in which organisms from two separate species depend on each other for their survival. Endosymbiosis (endo- = "within") is a mutually beneficial relationship in which one organism lives inside the other. Endosymbiotic relationships abound in nature. We have already mentioned that microbes that produce vitamin K live inside the human gut. This relationship is beneficial for us because we are unable to synthesize vitamin K. It is also beneficial for the microbes because they are protected from other organisms and from drying out, and they receive abundant food from the environment of the large intestine.

Scientists have long noticed that bacteria, mitochondria, and chloroplasts are similar in size. We also know that bacteria have DNA and ribosomes, just as mitochondria and chloroplasts do. Scientists believe that host cells and bacteria formed an endosymbiotic relationship when the host cells ingested both aerobic and autotrophic bacteria (cyanobacteria) but did not destroy them. Through many millions of years of evolution, these ingested bacteria became more specialized in their functions, with the aerobic bacteria becoming mitochondria and the autotrophic bacteria becoming chloroplasts.

The Central Vacuole

Previously, we mentioned vacuoles as essential components of plant cells. If you look at Figure **3.7.1**b, you will see that plant cells each have a large central vacuole that occupies most of the area of the cell. The central vacuole plays a key role in regulating the cell's concentration of water in changing environmental conditions. Have you ever noticed that if you forget to water a plant for a few days, it wilts? That's because as the water concentration in the soil becomes lower than the water concentration in the plant, water moves out of the central vacuoles and cytoplasm. As the central vacuole shrinks, it leaves the cell wall unsupported. This loss of support to the cell walls of plant cells results in the wilted appearance of the plant.

The central vacuole also supports the expansion of the cell. When the central vacuole holds more water, the cell gets larger without having to invest a lot of energy in synthesizing new cytoplasm.

Summary

Like a prokaryotic cell, a eukaryotic cell has a plasma membrane, cytoplasm, and ribosomes, but a eukaryotic cell is typically larger than a prokaryotic cell, has a true nucleus (meaning its DNA is surrounded by a membrane), and has other membrane-bound organelles that allow for compartmentalization of functions. The plasma membrane is a phospholipid bilayer embedded with proteins. The nucleus's nucleolus is the site of ribosome assembly. Ribosomes are either found in the cytoplasm or attached to the cytoplasmic side of the plasma membrane or endoplasmic reticulum. They perform protein synthesis. Mitochondria participate in cellular respiration; they are responsible for the majority of ATP produced in the cell. Peroxisomes hydrolyze fatty acids, amino acids, and some toxins. Vesicles and vacuoles are storage and transport compartments. In plant cells, vacuoles also help break down macromolecules.

Animal cells also have a centrosome and lysosomes. The centrosome has two bodies perpendicular to each other, the centrioles, and has an unknown purpose in cell division. Lysosomes are the digestive organelles of animal cells.

Plant cells and plant-like cells each have a cell wall, chloroplasts, and a central vacuole. The plant cell wall, whose primary component is cellulose, protects the cell, provides structural support, and gives shape to the cell. Photosynthesis takes place in chloroplasts. The central vacuole can expand without having to produce more cytoplasm.

Art Connections

Figure **3.7.1**: If the nucleolus were not able to carry out its function, what other cellular organelles would be affected?

Answer

Free ribosomes and rough endoplasmic reticulum (which contains ribosomes) would not be able to form.

cell wall
rigid cell covering made of cellulose that protects the cell, provides structural support, and gives shape to the cell

central vacuole
large plant cell organelle that regulates the cell's storage compartment, holds water, and plays a significant role in cell growth as the site of macromolecule degradation

centrosome
region in animal cells made of two centrioles

chlorophyll
green pigment that captures the light energy that drives the light reactions of photosynthesis

chloroplast
plant cell organelle that carries out photosynthesis

chromatin
protein-DNA complex that serves as the building material of chromosomes

chromosome
structure within the nucleus that is made up of chromatin that contains DNA, the hereditary material

cytoplasm
entire region between the plasma membrane and the nuclear envelope, consisting of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals

cytosol
gel-like material of the cytoplasm in which cell structures are suspended

eukaryotic cell
cell that has a membrane-bound nucleus and several other membrane-bound compartments or sacs

lysosome
organelle in an animal cell that functions as the cell's digestive component; it breaks down proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles

mitochondria
(singular = mitochondrion) cellular organelles responsible for carrying out cellular respiration, resulting in the production of ATP, the cell's main energy-carrying molecule

nuclear envelope
double-membrane structure that constitutes the outermost portion of the nucleus

nucleolus
darkly staining body within the nucleus that is responsible for assembling the subunits of the ribosomes

nucleoplasm
semi-solid fluid inside the nucleus that contains the chromatin and nucleolus

nucleus

cell organelle that houses the cell’s DNA and directs the synthesis of ribosomes and proteins

organelle
compartment or sac within a cell

peroxisome
small, round organelle that contains hydrogen peroxide, oxidizes fatty acids and amino acids, and detoxifies many poisons

plasma membrane
phospholipid bilayer with embedded (integral) or attached (peripheral) proteins, and separates the internal content of the cell from its surrounding environment

ribosome
cellular structure that carries out protein synthesis

vacuole
membrane-bound sac, somewhat larger than a vesicle, which functions in cellular storage and transport

vesicle
small, membrane-bound sac that functions in cellular storage and transport; its membrane is capable of fusing with the plasma membrane and the membranes of the endoplasmic reticulum and Golgi apparatus

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3.8: Eukaryotic Plasma Membrane and The Cytoplasm

At this point, it should be clear that eukaryotic cells have a more complex structure than do prokaryotic cells. Organelles allow for various functions to occur in the cell at the same time. Before discussing the functions of organelles within a eukaryotic cell, let us first examine two important components of all cells (prokaryotic and eukaryotic): the plasma membrane and the cytoplasm.

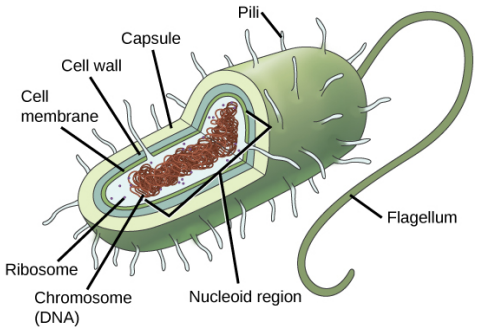


Figure 3.8.1: A prokaryotic cell. The cytoplasm is not labeled, but is the light blue area inside the cell membrane. The ribosome label is pointing to one of the small brown dots representing the ribosome.

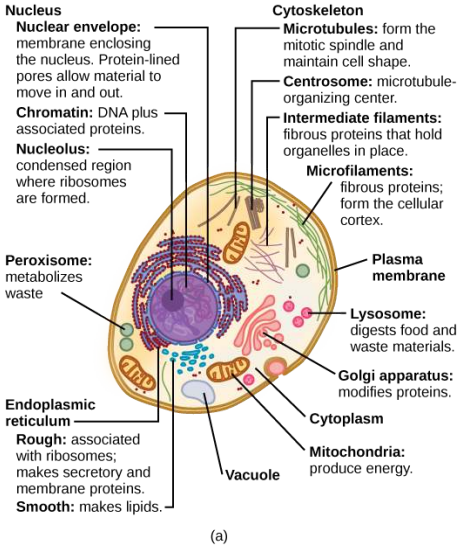


Figure 3.8.2: This figure shows a typical animal cell.

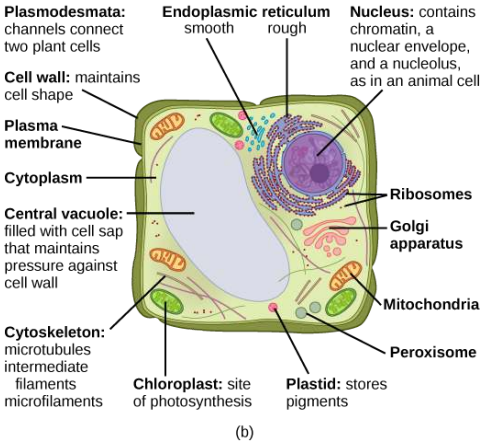


Figure 3.8.3: This figure shows a typical plant cell.

The Plasma Membrane

Like prokaryotes, eukaryotic cells have a **plasma membrane** (Find it in **Figures 3.8.1-3.8.3**, then look at the detailed structure in **Figure 3.8.4**) made up of a phospholipid bilayer with embedded proteins that separates the internal contents of the cell from its surrounding environment. A phospholipid is a lipid molecule composed of two fatty acid chains, a glycerol backbone, and a phosphate group. The plasma membrane regulates the passage of some substances, such as organic molecules, ions, and water, preventing the passage of some to maintain internal conditions, while actively bringing in or removing others. Other compounds move passively across the membrane.

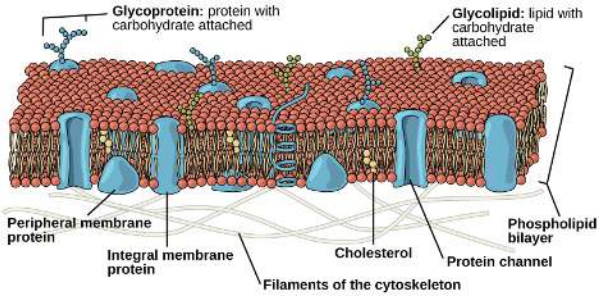


Figure 3.8.4: The plasma membrane is a phospholipid bilayer with embedded proteins. There are other components, such as cholesterol and carbohydrates, which can be found in the membrane in addition to phospholipids and protein.

The plasma membranes of cells that specialize in absorption are folded into fingerlike projections called **microvilli** (singular = microvillus). This folding increases the surface area of the plasma membrane. Such cells are typically found lining the small intestine, the organ that absorbs nutrients from digested food (Figure **3.8.5**). This is an excellent example of form matching the function of a structure.

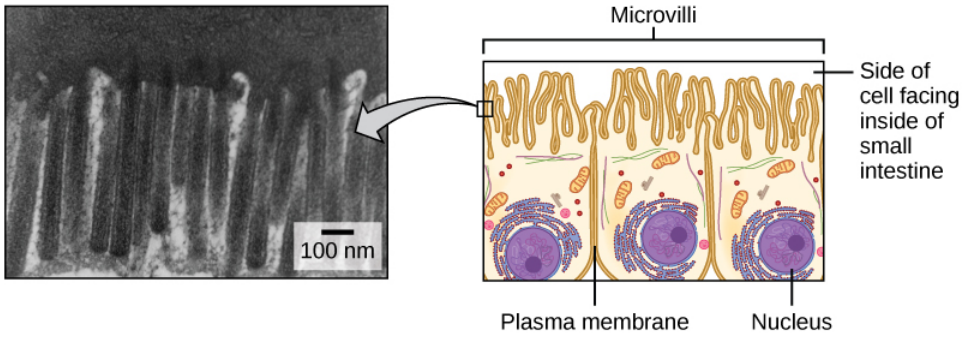


Figure 3.8.5: Microvilli, shown here as they appear on cells lining the small intestine, increase the surface area available for absorption. These microvilli are only found on the area of the plasma membrane that faces the cavity from which substances will be absorbed. (credit "micrograph": modification of work by Louisa Howard)

The Cytoplasm

The **cytoplasm** comprises the contents of a cell between the plasma membrane and the nuclear envelope (a structure to be discussed shortly). It is made up of organelles suspended in the gel-like **cytosol**, the cytoskeleton, and various chemicals (Find it in **Figures 3.8.1-3.8.3**). Even though the cytoplasm consists of 70 to 80 percent water, it has a semi-solid consistency, which comes from the proteins within it. However, proteins are not the only organic molecules found in the cytoplasm. Glucose and other simple sugars, polysaccharides, amino acids, nucleic acids, fatty acids, and derivatives of glycerol are found there too. Ions of sodium, potassium, calcium, and many other elements are also dissolved in the cytoplasm. Many metabolic reactions, including protein synthesis, take place in the cytoplasm. Take note that the cytoplasm is not "empty" or "filler" – it is a vitally important component of cells that allows chemical reactions to take place!

References

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SECTION OVERVIEW

3.8.1: The Plasma Membrane and Transport

Learning Objectives

By the end of this section, you will be able to:

- Explain how the structure of cell membranes leads to its various functions including selective permeability and transport, and cell signaling.

The plasma membrane, which is also called the cell membrane, has many functions, but the most basic one is to define the borders of the cell and keep the cell functional. The plasma membrane is selectively permeable. This means that the membrane allows some materials to freely enter or leave the cell, while other materials cannot move freely, but require the use of a specialized structure, and occasionally, even energy investment for crossing.

3.8.1.1: The Plasma Membrane

3.8.1.2: Transport Across Membranes

3.8.1.3: Passive Transport - Diffusion

3.8.1.4: Passive Transport - Facilitated Transport

3.8.1.5: Passive Transport - Osmosis

3.8.1.6: Active Transport

References

Text adapted from: OpenStax, Concepts of Biology. OpenStax CNX. May 18, 2016 https://cnx.org/contents/GFy_h8cu@10...2/Introduction

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3.8.1.1: The Plasma Membrane

Cells closely control the exchange of substances in and out of the cell. Some substances are excluded, others are taken in, and still others are excreted – all in controlled quantities. Although the **plasma membrane** encloses the cell's borders, it is far from being a static barrier; it is dynamic and constantly in flux. The plasma membrane must be sufficiently flexible to allow certain cells, such as red blood cells and white blood cells, to change shape as they pass through narrow capillaries. In addition to these more obvious functions, the surface of the plasma membrane carries markers which allow cells to recognize one another. This is vital as these markers play a role in the "self" versus "non-self" distinction of the immune response.

Fluid Mosaic Model

In 1972, S. J. Singer and Garth L. Nicolson proposed a new model of the plasma membrane. This theory, compared to earlier theories, best explains both microscopic observations and the function of the plasma membrane. This theory is called the **fluid mosaic model**. The model has evolved somewhat over time, but still best accounts for the structure and functions of the plasma membrane as we now understand them. The fluid mosaic model describes the structure of the plasma membrane as comprised of diverse components—including phospholipids, cholesterol, proteins, and carbohydrates—that are able to flow and change position, while maintaining the basic integrity of the membrane. Both phospholipid molecules and embedded proteins are able to move laterally in the membrane. The fluidity of the plasma membrane is necessary for the activities of certain enzymes and transport molecules within the membrane.

Plasma membranes range from 5–10 nm thick. As a comparison, human red blood cells, visible via light microscopy, are approximately 8 μm thick, or approximately 1,000 times thicker than a plasma membrane.

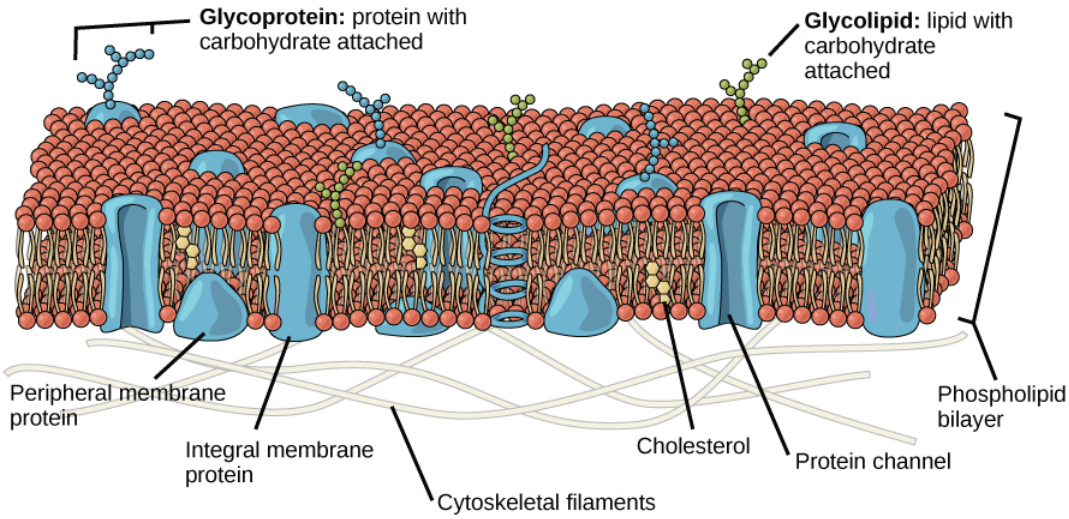


Figure 3.8.1.1.1: The fluid mosaic model of the plasma membrane structure describes the plasma membrane as a fluid combination of phospholipids, cholesterol, proteins, and carbohydrates.

Components of the Plasma Membrane

The plasma membrane is made up primarily of a bilayer of phospholipids with embedded proteins, carbohydrates, glycolipids, and glycoproteins, and, in animal cells, cholesterol (**Figure 3.8.1.1.1**).

Phospholipids

The main fabric of the membrane is composed of two layers of phospholipid molecules, and the polar ends of these molecules (which look like a collection of balls in an artist's rendition of the model) (**Figure 3.8.1.1.2**) are in contact with aqueous fluid both inside and outside the cell. Thus, both surfaces of the plasma membrane are **hydrophilic** ("water loving"). In contrast, the interior of the membrane, between its two surfaces, is a **hydrophobic** ("water fearing") or nonpolar region because of the fatty acid tails. This region has no attraction for water or other polar molecules.

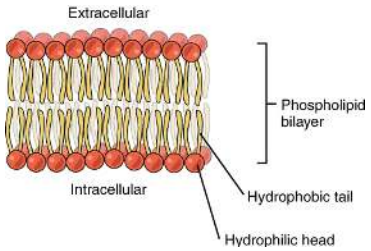


Figure 3.8.1.1.2: Phospholipid bilayer. “Extracellular” = outside the cell; “Intracellular” = inside the cell. Photo credit: OpenStax Anatomy and Physiology.

A phospholipid molecule (**Figure 3.8.1.1.3**) consists of a three-carbon glycerol backbone with two fatty acid molecules attached to carbons 1 and 2, and a phosphate-containing group attached to the third carbon. This arrangement gives the overall molecule an area described as its head (the phosphate-containing group), which has a polar character or negative charge, and an area called the tail (the fatty acids), which has no charge. The head can form hydrogen bonds, but the tail cannot.

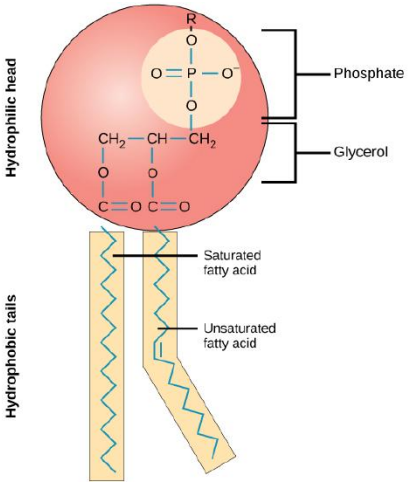


Figure 3.8.1.1.3: This phospholipid molecule is composed of a hydrophilic head and two hydrophobic tails. The hydrophilic head group consists of a phosphate-containing group attached to a glycerol molecule. The hydrophobic tails, each containing either a saturated or an unsaturated fatty acid, are long hydrocarbon chains.

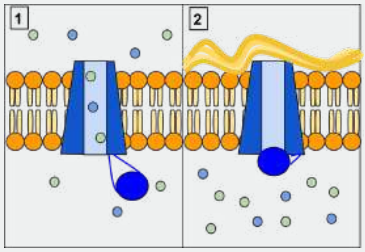
Proteins

Proteins make up the second major chemical component of plasma membranes (see **Figure 3.8.1.1.1**). **Integral proteins** are embedded in the plasma membrane and may span all or part of the membrane (**Figure 3.8.1.1.1**). Integral proteins may serve as channels or pumps to move materials into or out of the cell. **Peripheral proteins** are found on the exterior or interior surfaces of membranes, attached either to integral proteins or to phospholipid molecules (**Figure 3.8.1.1.1**). Both integral and peripheral proteins may serve as enzymes, as structural attachments for the fibers of the cytoskeleton, or as part of the cell’s recognition sites.

The recognition sites on the plasma membrane are called **receptors,** which are attachment sites for substances that interact with the cell. Each receptor is structured to bind with a specific substance. The binding of a specific substance to its receptor on the plasma membrane can activate processes within the interior of the cell – such as activating enzymes involved in metabolic pathways. These metabolic pathways might be vital for providing the cell with energy, making substances for the cell, or breaking down cellular waste or toxins for disposal. Likewise, extracellular hormones and neurotransmitters bind to plasma membrane receptors which transmit a signal into the cell to intracellular molecules. Some recognition sites are used by viruses as attachment points. Although they are highly specific, pathogens like viruses may evolve to exploit receptors to gain entry to a cell by mimicking the specific substance that the receptor is meant to bind. This specificity helps to explain why human immunodeficiency virus (HIV) or any of the five types of hepatitis viruses invade only specific cells.

Cystic Fibrosis

Cystic Fibrosis is caused by a defect in an integral protein in the cell membrane which acts as a channel. The CFTR protein moves ions from one side of the membrane to another. When it is not functioning correctly, this causes very thick mucus to build up in the lungs and digestive tract.



When the CFTR channel protein is functioning correctly (1), ions (small balls) are able to pass through the membrane. When it is not functioning correctly (2), ions are unable to cross the membrane. Photo credit: LBudd14, May, 2013. Wikimedia.

Carbohydrates

Carbohydrates are the third major component of plasma membranes. They are always found on the exterior surface of cells and are bound either to proteins (forming **glycoproteins**) or to lipids (forming **glycolipids**). These carbohydrate chains may consist of 2–60 monosaccharide units and may be either straight or branched. Along with peripheral proteins, carbohydrates form specialized sites on the cell surface that allow cells to recognize each other. These sites have unique patterns that allow the cell to be recognized, much the way that the facial features unique to each person allow him or her to be recognized. This recognition function is very important to cells, as it allows the immune system to differentiate between body cells (called "self") and foreign cells or tissues (called "non-self"). Similar types of glycoproteins and glycolipids are found on the surfaces of viruses and may change frequently, preventing immune cells from recognizing and attacking them.

Blood Types

The carbohydrates that make up glycoproteins are responsible for determining human A, B, O blood types. These glycoproteins are recognized by the immune system, which leads to incompatibility in blood types.

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

ABO Blood types. In this figure, the membrane carbohydrate is represented by the "lollipops". They are termed "antigens". Photo credit: InvictaHOG, 2006. Wikimedia.

Membrane Fluidity

The mosaic characteristic of the membrane, described in the fluid mosaic model, helps to illustrate its nature. The proteins and other components that exist in the membrane can move with respect to each other, rather like boats floating on a lake. The membrane is not like a balloon, however, that can expand and contract; rather, it is fairly rigid and can burst if penetrated or if a cell takes in too much water. However, because of its mosaic nature, a very fine needle can easily penetrate a plasma membrane without causing it to burst, and the membrane will flow and self-seal when the needle is extracted.

The mosaic characteristics of the membrane explain some but not all of its fluidity. There are two other factors that help maintain this fluid characteristic. One factor is the nature of the phospholipids themselves. The structure of the fatty acid tails in each phospholipid can make the membrane more dense and rigid, or less dense and flexible. The relative fluidity of the membrane is particularly important in a cold environment. A cold environment tends to make membranes less fluid and more susceptible to rupturing. Many organisms (fish are one example) are capable of adapting to cold environments by changing the proportion of different types of fatty acids in their membranes in response to the lowering of the temperature.

Animals have an additional membrane constituent that assists in maintaining fluidity. **Cholesterol,** which lies alongside the phospholipids in the membrane, tends to dampen the effects of temperature on the membrane. Thus, this lipid functions as a buffer, preventing lower temperatures from inhibiting fluidity and preventing increased temperatures from increasing fluidity too much. Thus, cholesterol extends, in both directions, the range of temperature in which the membrane is appropriately fluid and consequently functional. Cholesterol also serves other functions, such as organizing clusters of transmembrane proteins into lipid rafts.

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3.8.1.2: Transport Across Membranes

Plasma membranes act not only as a barrier, but also as a gatekeeper. It must allow needed substances to enter and cell products to leave the cell, while preventing entrance of harmful material and exit of essential material. In other words, plasma membranes are **selectively permeable**—they allow some substances through but not others. If the membrane were to lose this selectivity, the cell would no longer be able to maintain homeostasis, or to sustain itself, and it would be destroyed. Some cells require larger amounts of specific substances than other cells; they must have a way of obtaining these materials from the extracellular fluids.

This may happen passively, as certain materials move back and forth, or the cell may have special mechanisms that ensure transport. Most cells expend most of their energy, in the form of adenosine triphosphate (ATP), to create and maintain an uneven distribution of ions on the opposite sides of their membranes. The structure of the plasma membrane contributes to these functions.

Selective Permeability

Plasma membranes are asymmetric, meaning that despite the mirror image formed by the phospholipids, the interior of the membrane is not identical to the exterior of the membrane. Integral proteins that act as channels or pumps work in one direction. Carbohydrates, attached to lipids or proteins, are also found on the exterior surface of the plasma membrane.

These carbohydrate complexes help the cell bind substances in the extracellular fluid that the cell needs. This adds considerably to the selective nature of plasma membranes.

Recall that plasma membranes have hydrophilic and hydrophobic regions. This characteristic helps the movement of certain materials through the membrane and hinders the movement of others. Lipid-soluble material can easily slip through the hydrophobic lipid core of the membrane. Substances such as the fat-soluble vitamins A, D, E, and K readily pass through the plasma membranes in the digestive tract and other tissues. Fat-soluble drugs also gain easy entry into cells and are readily transported into the body's tissues and organs. Molecules of oxygen and carbon dioxide have no charge and pass through by simple diffusion.

Polar substances, with the exception of water, present problems for the membrane. While some polar molecules connect easily with the outside of a cell, they cannot readily pass through the lipid core of the plasma membrane. Additionally, whereas small ions could easily slip through the spaces in the mosaic of the membrane, their charge prevents them from doing so. Ions such as sodium, potassium, calcium, and chloride must have a special means of penetrating plasma membranes. Simple sugars and amino acids also need help with transport across plasma membranes.

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3.8.1.3: Passive Transport - Diffusion

The most direct forms of membrane transport are passive. **Passive transport** is a naturally occurring phenomenon and does not require the cell to expend energy to accomplish the movement. In passive transport, substances move from an area of higher concentration to an area of lower concentration in a process called **diffusion**. A physical space in which there is a different concentration of a single substance is said to have a **concentration gradient**.

Diffusion

Diffusion is a passive process of transport. A single substance tends to move from an area of high concentration to an area of low concentration until the concentration is equal across the space. You are familiar with diffusion of substances through the air. For example, think about someone opening a bottle of perfume in a room filled with people. The perfume is at its highest concentration in the bottle and is at its lowest at the edges of the room. The perfume vapor will diffuse, or spread away, from the bottle, and gradually, more and more people will smell the perfume as it spreads. Materials move within the cell's cytosol by diffusion, and certain materials move through the plasma membrane by diffusion (**Figure 3.8.1.3.1**). Diffusion expends no energy. Rather the different concentrations of materials in different areas are a form of potential energy, and diffusion is the dissipation of that potential energy as materials move down their concentration gradients, from high to low.

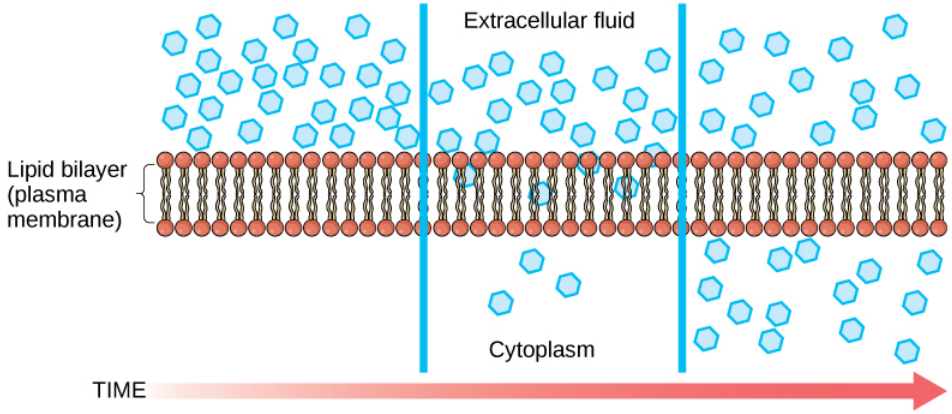


Figure 3.8.1.3.1: Diffusion through a permeable membrane follows the concentration gradient of a substance, moving the substance from an area of high concentration to one of low concentration. (credit: modification of work by Mariana Ruiz Villarreal)

Each separate substance in a medium, such as the extracellular fluid, has its own concentration gradient, independent of the concentration gradients of other materials. Additionally, each substance will diffuse according to that gradient.

Several factors affect the rate of diffusion:

- Extent of the concentration gradient: The greater the difference in concentration, the more rapid the diffusion. The closer the distribution of the material gets to equilibrium, the slower the rate of diffusion becomes.
- Mass of the molecules diffusing: More massive molecules move more slowly, because it is more difficult for them to move between the molecules of the substance they are moving through; therefore, they diffuse more slowly.
- Temperature: Higher temperatures increase the energy and therefore the movement of the molecules, increasing the rate of diffusion.
- Solvent density: As the density of the solvent increases, the rate of diffusion decreases. The molecules slow down because they have a more difficult time getting through the denser medium.

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3.8.1.4: Passive Transport - Facilitated Transport

In **facilitated transport**, also called facilitated diffusion, material moves across the plasma membrane with the assistance of transmembrane proteins down a concentration gradient (from high to low concentration) *without* the expenditure of cellular energy. However, the substances that undergo facilitated transport would otherwise not diffuse easily or quickly across the plasma membrane. The solution to moving polar substances and other substances across the plasma membrane rests in the proteins that span its surface. The material being transported is first attached to protein or glycoprotein receptors on the exterior surface of the plasma membrane. This allows the material that is needed by the cell to be removed from the extracellular fluid. The substances are then passed to specific integral proteins that facilitate their passage, because they form channels or pores that allow certain substances to pass through the membrane. The integral proteins involved in facilitated transport are collectively referred to as transport proteins, and they function as either channels for the material or carriers.

Channels

The integral proteins involved in facilitated transport are collectively referred to as transport proteins, and they function as either channels for the material or carriers. In both cases, they are transmembrane proteins (they span across the membrane). Channels are specific for the substance that is being transported. Channel proteins have hydrophilic domains exposed to the intracellular and extracellular fluids; they additionally have a hydrophilic channel through their core that provides a hydrated opening through the membrane layers (**Figure 3.8.1.4.1**). Passage through the channel allows polar compounds to avoid the nonpolar central layer of the plasma membrane that would otherwise slow or prevent their entry into the cell. Aquaporins are channel proteins that allow water to pass through the membrane at a very high rate.

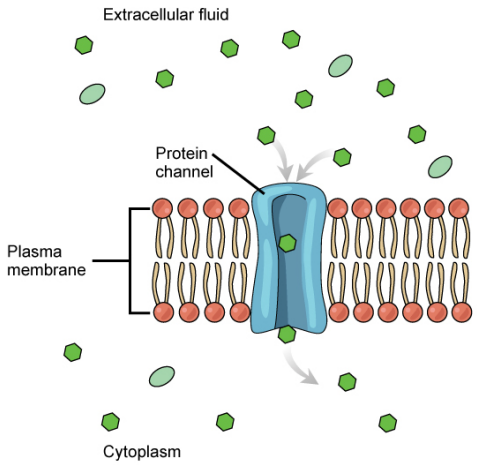


Figure 3.8.1.4.1: Facilitated transport moves substances down their concentration gradients. They may cross the plasma membrane with the aid of channel proteins. (credit: modification of work by Mariana Ruiz Villareal)

Carrier Proteins

Another type of protein embedded in the plasma membrane is a carrier protein. This aptly named protein binds a substance and, in doing so, triggers a change of its own shape, moving the bound molecule from the outside of the cell to its interior (**Figure 3.8.1.4.2**); depending on the gradient, the material may move in the opposite direction. Carrier proteins are typically specific for a single substance. This selectivity adds to the overall selectivity of the plasma membrane. The exact mechanism for the change of shape is poorly understood. Proteins can change shape when their hydrogen bonds are affected, but this may not fully explain this mechanism. Each carrier protein is specific to one substance, and there are a finite number of these proteins in any membrane. This can cause problems in transporting enough of the material for the cell to function properly. When all of the proteins are bound to their ligands, they are saturated and the rate of transport is at its maximum. Increasing the concentration gradient at this point will not result in an increased rate of transport.

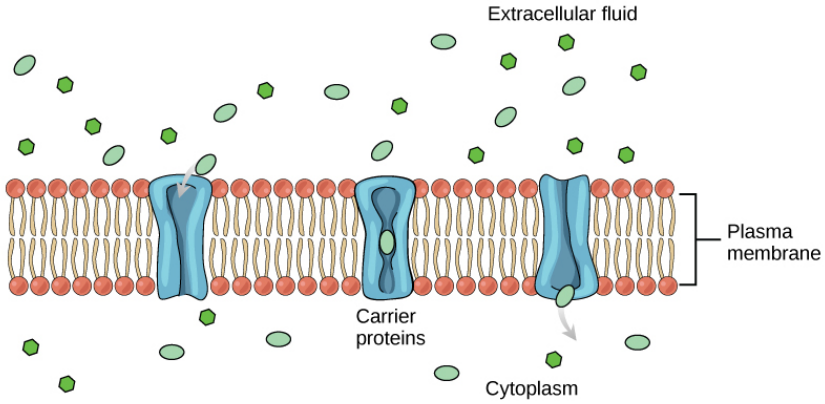


Figure 3.8.1.4.2: (credit: modification of work by Mariana Ruiz Villareal)

An example of this process occurs in the kidney. Glucose, water, salts, ions, and amino acids needed by the body are filtered in one part of the kidney. This filtrate, which includes glucose, is then reabsorbed in another part of the kidney. Because there are only a finite number of carrier proteins for glucose, if more glucose is present than the proteins can handle, the excess is not transported and it is excreted from the body in the urine. In a diabetic individual, this is described as “spilling glucose into the urine.” A different group of carrier proteins called glucose transport proteins, or GLUTs, are involved in transporting glucose and other hexose sugars through plasma membranes within the body.

Channel and carrier proteins transport material at different rates. Channel proteins transport much more quickly than do carrier proteins. Channel proteins facilitate diffusion at a rate of tens of millions of molecules per second, whereas carrier proteins work at a rate of a thousand to a million molecules per second.

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3.8.1.5: Passive Transport - Osmosis

Osmosis is the diffusion of water through a semipermeable membrane according to the concentration gradient of water across the membrane. Whereas diffusion transports material across membranes and within cells, osmosis transports *only water* across a membrane and the membrane limits the diffusion of solutes in the water. Osmosis is a special case of diffusion. Water, like other substances, moves from an area of higher concentration to one of lower concentration. Imagine a beaker with a semipermeable membrane, separating the two sides or halves (**Figure 3.8.1.5.1**). On both sides of the membrane, the water level is the same, but there are different concentrations on each side of a dissolved substance, or **solute**, that cannot cross the membrane. If the volume of the water is the same, but the concentrations of solute are different, then there are also different concentrations of water, the **solvent,** on either side of the membrane.

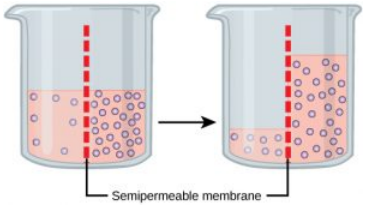


Figure 3.8.1.5.1: In osmosis, water always moves from an area of higher concentration (of water) to one of lower concentration (of water). In this system, the solute cannot pass through the selectively permeable membrane.

A principle of diffusion is that the molecules move around and will spread evenly throughout the medium if they can. However, only the material capable of getting through the membrane will diffuse through it. In this example, the solute cannot diffuse through the membrane, but the water can. Water has a concentration gradient in this system. Therefore, water will diffuse down its concentration gradient, crossing the membrane to the side where it is less concentrated. This diffusion of water through the membrane— **osmosis** —will continue until the concentration gradient of water goes to zero. Osmosis proceeds constantly in living systems.

Tonicity

Tonicity describes the amount of solute in a solution. The measure of the tonicity of a solution, or the total amount of solutes dissolved in a specific amount of solution, is called its **osmolarity**. Three terms—hypotonic, isotonic, and hypertonic—are used to relate the osmolarity of a cell to the osmolarity of the extracellular fluid that contains the cells. All three of these terms are a *comparison* between two different solutions (for example, inside a cell compared to outside the cell).

In a **hypotonic** solution, such as tap water, the extracellular fluid has a lower concentration of solutes than the fluid inside the cell, and water enters the cell. (In living systems, the point of reference is always the cytoplasm, so the prefix *hypo–* means that the extracellular fluid has a lower concentration of solutes, or a lower osmolarity, than the cell cytoplasm.) It also means that the extracellular fluid has a higher concentration of water than does the cell. In this situation, water will follow its concentration gradient and enter the cell. This may cause an animal cell to burst, or **lyse.**

In a **hypertonic** solution (the prefix *hyper–* refers to the extracellular fluid having a higher concentration of solutes than the cell's cytoplasm), the fluid contains less water than the cell does, such as seawater. Because the cell has a lower concentration of solutes, the water will leave the cell. In effect, the solute is drawing the water out of the cell. This may cause an animal cell to shrivel, or **crenate.**

In an **isotonic** solution, the extracellular fluid has the same osmolarity as the cell. If the concentration of solutes of the cell matches that of the extracellular fluid, there will be no net movement of water into or out of the cell. The cell will retain its "normal" appearance. Blood cells in hypertonic, isotonic, and hypotonic solutions take on characteristic appearances (**Figure 3.8.1.5.2**).

Remember that all three of these terms are *comparisons* between two solutions (i.e. inside and outside the cell). A solution can't be hypotonic, that would be like saying that Bob is taller. That doesn't make sense – you need to say that Bob is taller than Mike. You can say that the solution inside the cell is hypotonic to the solution outside the cell. That also means that the solution outside is hypertonic to the solution inside (just like Mike would be shorter than Bob).

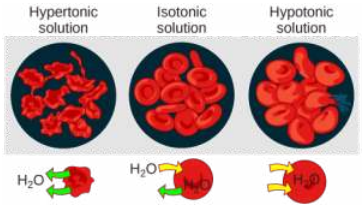


Figure 3.8.1.5.2: Osmotic pressure changes the shape of red blood cells in hypertonic, isotonic, and hypotonic solutions. (credit: modification of work by Mariana Ruiz Villarreal)

Some organisms, such as plants, fungi, bacteria, and some protists, have **cell walls** that surround the plasma membrane and prevent cell lysis. The plasma membrane can only expand to the limit of the cell wall, so the cell will not lyse. In fact, the cytoplasm in plants is always slightly hypertonic compared to the cellular environment, and water will always enter the plant cell if water is available. This influx of water produces **turgor pressure**, which stiffens the cell walls of the plant (**Figure 3.8.1.5.3**). In nonwoody plants, turgor pressure supports the plant. If the plant cells become hypertonic, as occurs in drought or if a plant is not watered adequately, water will leave the cell. Plants lose turgor pressure in this condition and wilt.

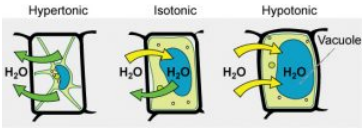


Figure 3.8.1.5.3: The turgor pressure within a plant cell depends on the tonicity of the solution that it is bathed in. (credit: modification of work by Mariana Ruiz Villarreal)

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3.8.1.6: Active Transport

Active transport mechanisms require the use of the cell's energy, usually in the form of adenosine triphosphate (ATP). If a substance must move into the cell against its concentration gradient, that is, if the concentration of the substance inside the cell must be greater than its concentration in the extracellular fluid, the cell must use energy to move the substance. Some active transport mechanisms move small-molecular weight material, such as ions, through the membrane.

In addition to moving small ions and molecules through the membrane, cells also need to remove and take in larger molecules and particles. Some cells are even capable of engulfing entire unicellular microorganisms. You might have correctly hypothesized that the uptake and release of large particles by the cell requires energy. A large particle, however, cannot pass through the membrane, even with energy supplied by the cell.

Electrochemical Gradient

We have discussed simple concentration gradients—differential concentrations of a substance across a space or a membrane. However, in living systems gradients are more complex. Cells contain many proteins, most of which are negatively charged. Due to these negatively charged proteins, coupled with the movement of ions into and out of cells, there is an electrical gradient (a difference of charge) across the plasma membrane. The interior of living cells is electrically negative as compared to the extracellular fluid in which cells are bathed; at the same time, cells contain higher concentrations of potassium (K^+) and lower concentrations of sodium (Na^+) than does the extracellular fluid. Thus, in a living cell, the concentration gradient and electrical gradient of Na^+ promotes diffusion of the ion into the cell, and the electrical gradient of Na^+ (a positive ion) tends to drive it inward to the negatively charged interior. The situation is more complex, however, for other elements such as potassium. The electrical gradient of K^+ promotes diffusion of the ion *into* the cell, but the concentration gradient of K^+ promotes diffusion *out* of the cell (**Figure 3.8.1.6.1**). The combined gradient that affects an ion is called its **electrochemical gradient**, and it is especially important to muscle and nerve cells.

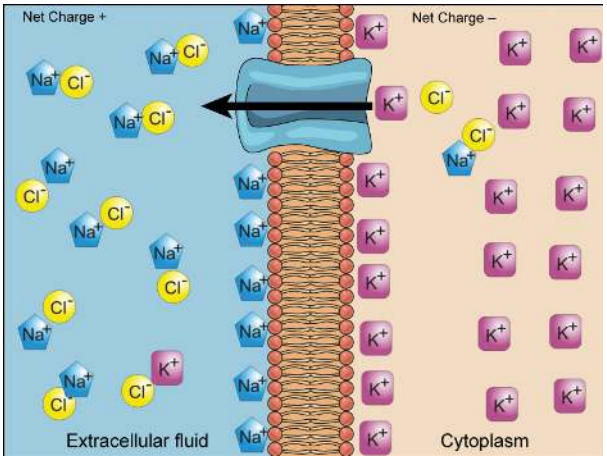


Figure 3.8.1.6.1: Electrochemical gradients arise from the combined effects of concentration gradients and electrical gradients. (credit: modification of work by "Synaptitude"/Wikimedia Commons)

Moving Against a Gradient

To move substances against a concentration or an electrochemical gradient, the cell must use energy. This energy is harvested from ATP that is generated through cellular metabolism. Active transport mechanisms, collectively called pumps or carrier proteins, work against electrochemical gradients. With the exception of ions, small substances constantly pass through plasma membranes. Active transport maintains concentrations of ions and other substances needed by living cells in the face of these passive changes. Much of a cell's supply of metabolic energy may be spent maintaining these processes. As active transport mechanisms depend on cellular metabolism for energy, they are sensitive to many metabolic poisons that interfere with the supply of ATP.

Two mechanisms exist for the transport of small-molecular weight material and macromolecules. **Primary active transport** moves ions across a membrane and creates a difference in charge across that membrane. The primary active transport system uses ATP to move a substance, such as an ion, into the cell, and often at the same time, a second substance is moved out of the cell. The

sodium-potassium pump, an important pump in animal cells, expends energy to move potassium ions into the cell and a different number of sodium ions out of the cell. The action of this pump results in a concentration and charge difference across the membrane.

Secondary active transport describes the movement of material using the energy of the electrochemical gradient established by primary active transport. Using the energy of the electrochemical gradient created by the primary active transport system, other substances such as amino acids and glucose can be brought into the cell through membrane channels. ATP itself is formed through secondary active transport using a hydrogen ion gradient in the mitochondrion.

Endocytosis

Endocytosis is a type of active transport that moves particles, such as large molecules, parts of cells, and even whole cells, into a cell. There are different variations of endocytosis, but all share a common characteristic: The plasma membrane of the cell invaginates, forming a pocket around the target particle. The pocket pinches off, resulting in the particle being contained in a newly created vacuole that is formed from the plasma membrane.

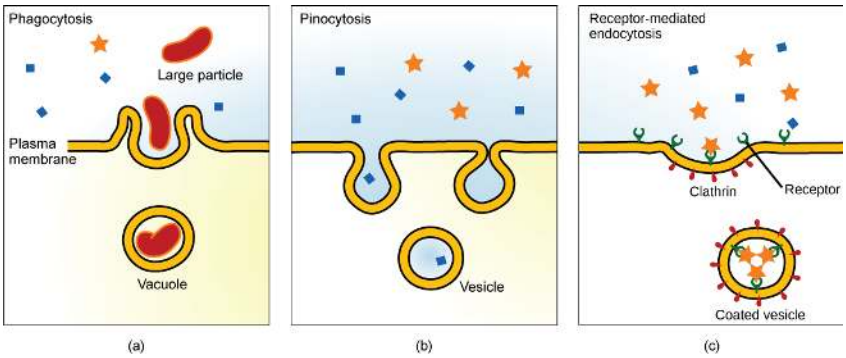


Figure 3.8.1.6.2: Three variations of endocytosis are shown. (a) In one form of endocytosis, phagocytosis, the cell membrane surrounds the particle and pinches off to form an intracellular vacuole. (b) In another type of endocytosis, pinocytosis, the cell membrane surrounds a small volume of fluid and pinches off, forming a vesicle. (c) In receptor-mediated endocytosis, uptake of substances by the cell is targeted to a single type of substance that binds at the receptor on the external cell membrane. (credit: modification of work by Mariana Ruiz Villarreal)

Phagocytosis is the process by which large particles, such as cells, are taken in by a cell. For example, when microorganisms invade the human body, a type of white blood cell called a neutrophil removes the invader through this process, surrounding and engulfing the microorganism, which is then destroyed by the neutrophil (**Figure 3.8.1.6.2**a).

A variation of endocytosis is called **pinocytosis**. This literally means “cell drinking” and was named at a time when the assumption was that the cell was purposefully taking in extracellular fluid. In reality, this process takes in solutes that the cell needs from the extracellular fluid (**Figure 3.8.1.6.2**b).

A targeted variation of endocytosis employs binding proteins in the plasma membrane that are specific for certain substances (**Figure 3.8.1.6.2**c). The particles bind to the proteins and the plasma membrane invaginates, bringing the substance and the proteins into the cell. If passage across the membrane of the target of **receptor-mediated endocytosis** is ineffective, it will not be removed from the tissue fluids or blood. Instead, it will stay in those fluids and increase in concentration.

Some human diseases are caused by a failure of receptor-mediated endocytosis. For example, the form of cholesterol termed low-density lipoprotein or LDL (also referred to as “bad” cholesterol) is removed from the blood by receptor mediated endocytosis. In the human genetic disease familial hypercholesterolemia, the LDL receptors are defective or missing entirely. People with this condition have life-threatening levels of cholesterol in their blood, because their cells cannot clear the chemical from their blood.

Exocytosis

In contrast to these methods of moving material into a cell is the process of exocytosis. **Exocytosis** is the opposite of the processes discussed above in that its purpose is to expel material from the cell into the extracellular fluid. A particle enveloped in membrane fuses with the interior of the plasma membrane. This fusion opens the membranous envelope to the exterior of the cell, and the particle is expelled into the extracellular space (**Figure 3.8.1.6.3**).

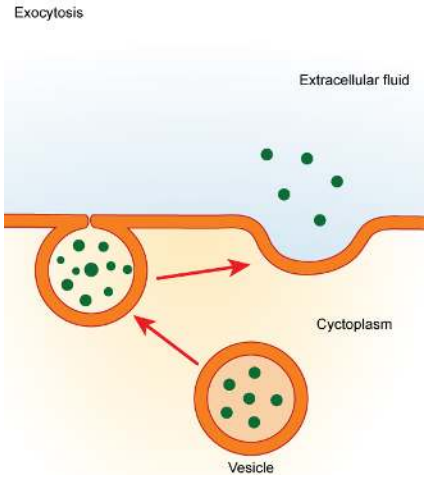


Figure 3.8.1.6.3: In exocytosis, a vesicle migrates to the plasma membrane, binds, and releases its contents to the outside of the cell. (credit: modification of work by Mariana Ruiz Villarreal)

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3.8.2: Endocytosis

Learning Objectives

- Describe endocytosis, including phagocytosis, pinocytosis, and receptor-mediated endocytosis.

Endocytosis is a type of active transport that moves particles, such as large molecules, parts of cells, and even whole cells, into a cell. There are different variations of endocytosis, but all share a common characteristic: the plasma membrane of the cell invaginates, forming a pocket around the target particle. The pocket pinches off, resulting in the particle being contained in a newly-created intracellular vesicle formed from the plasma membrane.

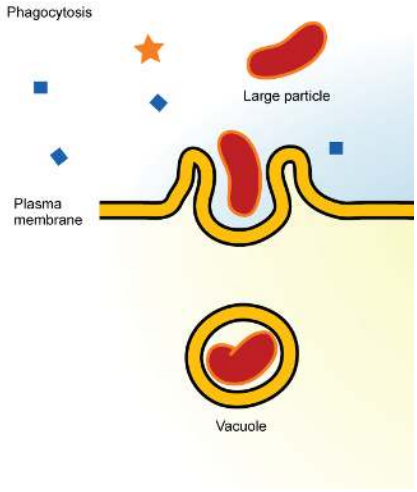


Figure: **Phagocytosis**: In phagocytosis, the cell membrane surrounds the particle and engulfs it.

Phagocytosis

Phagocytosis (the condition of “cell eating”) is the process by which large particles, such as cells or relatively large particles, are taken in by a cell. For example, when microorganisms invade the human body, a type of white blood cell called a neutrophil will remove the invaders through this process, surrounding and engulfing the microorganism, which is then destroyed by the neutrophil.

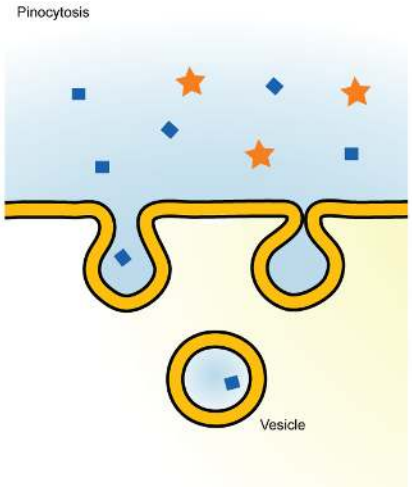


Figure: **Pinocytosis**: In pinocytosis, the cell membrane invaginates, surrounds a small volume of fluid, and pinches off.

In preparation for phagocytosis, a portion of the inward-facing surface of the plasma membrane becomes coated with a protein called clathrin, which stabilizes this section of the membrane. The coated portion of the membrane then extends from the body of the cell and surrounds the particle, eventually enclosing it. Once the vesicle containing the particle is enclosed within the cell, the clathrin disengages from the membrane and the vesicle merges with a lysosome for the breakdown of the material in the newly-

formed compartment (endosome). When accessible nutrients from the degradation of the vesicular contents have been extracted, the newly-formed endosome merges with the plasma membrane and releases its contents into the extracellular fluid. The endosomal membrane again becomes part of the plasma membrane.

Pinocytosis

A variation of endocytosis is called pinocytosis. This literally means “cell drinking” and was named at a time when the assumption was that the cell was purposefully taking in extracellular fluid. In reality, this is a process that takes in molecules, including water, which the cell needs from the extracellular fluid. Pinocytosis results in a much smaller vesicle than does phagocytosis, and the vesicle does not need to merge with a lysosome.

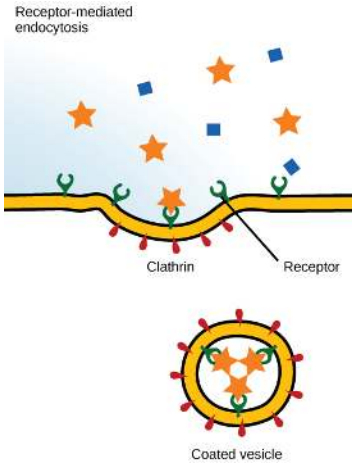


Figure: **Receptor-Mediated Endocytosis**: In receptor-mediated endocytosis, uptake of substances by the cell is targeted to a single type of substance that binds to the receptor on the external surface of the cell membrane.

Potocytosis, a variant of pinocytosis, is a process that uses a coating protein, called caveolin, on the cytoplasmic side of the plasma membrane, which performs a similar function to clathrin. The cavities in the plasma membrane that form the vacuoles have membrane receptors and lipid rafts in addition to caveolin. The vacuoles or vesicles formed in caveolae (singular caveola) are smaller than those in pinocytosis. Potocytosis is used to bring small molecules into the cell and to transport these molecules through the cell for their release on the other side of the cell, a process called transcytosis.

Receptor-mediated Endocytosis

A targeted variation of endocytosis, known as receptor-mediated endocytosis, employs receptor proteins in the plasma membrane that have a specific binding affinity for certain substances. In receptor-mediated endocytosis, as in phagocytosis, clathrin is attached to the cytoplasmic side of the plasma membrane. If uptake of a compound is dependent on receptor-mediated endocytosis and the process is ineffective, the material will not be removed from the tissue fluids or blood. Instead, it will stay in those fluids and increase in concentration. Some human diseases are caused by the failure of receptor-mediated endocytosis. For example, the form of cholesterol termed low-density lipoprotein or LDL (also referred to as “bad” cholesterol) is removed from the blood by receptor-mediated endocytosis. In the human genetic disease familial hypercholesterolemia, the LDL receptors are defective or missing entirely. People with this condition have life-threatening levels of cholesterol in their blood, because their cells cannot clear LDL particles from their blood.

Although receptor-mediated endocytosis is designed to bring specific substances that are normally found in the extracellular fluid into the cell, other substances may gain entry into the cell at the same site. Flu viruses, diphtheria, and cholera toxin all have sites that cross-react with normal receptor-binding sites and gain entry into cells.

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Key Points

- Endocytosis consists of phagocytosis, pinocytosis, and receptor -mediated endocytosis.
- Endocytosis takes particles into the cell that are too large to passively cross the cell membrane.
- Phagocytosis is the taking in of large food particles, while pinocytosis takes in liquid particles.
- Receptor-mediated endocytosis uses special receptor proteins to help carry large particles across the cell membrane.

Key Terms

- **endosome**: An endocytic vacuole through which molecules internalized during endocytosis pass en route to lysosomes
- **neutrophil**: A cell, especially a white blood cell that consumes foreign invaders in the blood.

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3.9: EUKARYOTE ORGANELLES

RIBOSOME REVIEW

Figure **3.9.1** represents an important structure in living cells. It is a component of a ribosome, the cell structure where proteins are synthesized. Large ribosomal subunit (50S) of *Haloarcula marismortui*, facing the 30S subunit. The ribosomal proteins are shown in blue, the rRNA in ochre (a shade of brown and yellow), the active site in red. All living cells contain ribosomes, whether they are prokaryotic or eukaryotic cells. However, only eukaryotic cells also contain a nucleus and several other types of organelles.

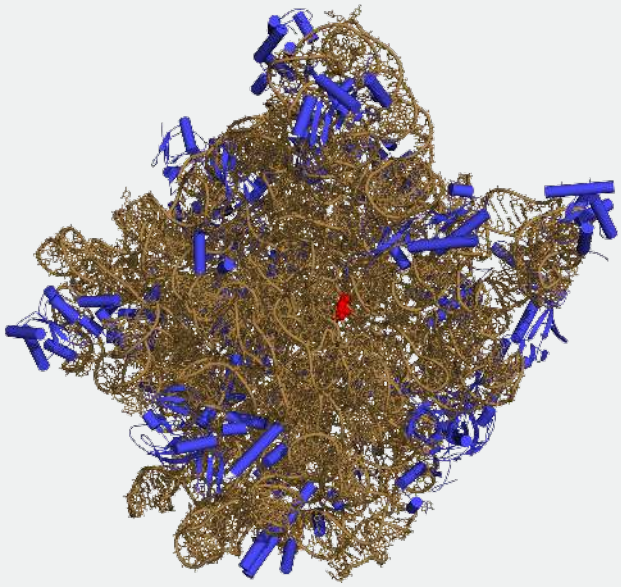


Figure **3.9.1**: Ribosomal subunit

An organelle is a structure within the cytoplasm of a eukaryotic cell that is enclosed within a membrane and performs a specific job. Organelles are involved in many vital cell functions. Organelles in animal cells include the nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, vesicles, and vacuoles. Ribosomes are not enclosed within a membrane but are still commonly referred to as organelles in eukaryotic cells.

THE NUCLEUS

The nucleus is the largest organelle in a eukaryotic cell and is considered to be the cell's control center. It contains most of the cell's DNA, which makes up chromosomes and is encoded with the genetic instructions for making proteins. The function of the nucleus is to regulate gene expression, including controlling which proteins the cell makes. In addition to DNA, the nucleus contains a thick liquid called nucleoplasm that is similar in composition to the cytosol found in the cytoplasm outside the nucleus (Figure **3.9.2**). Most eukaryotic cells contain just a single nucleus, but some types of cells, such as red blood cells, contain no nucleus. A few other types of cells, such as muscle cells, contain multiple nuclei.

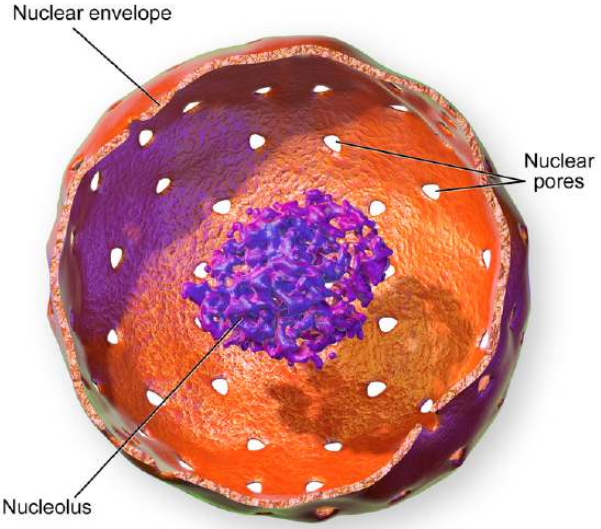


Figure **3.9.2**: This closeup of a cell nucleus shows that it is surrounded by a structure called the nuclear envelope, which contains tiny perforations, or pores. The nucleus also contains a dense center called the nucleolus.

As you can see from the model in Figure **3.9.2**, the membrane enclosing the nucleus is called the **nuclear envelope**. This is actually a double membrane that encloses the entire organelle and isolates its contents from the cellular cytoplasm. Tiny holes, called **nuclear pores**, allow large molecules to pass through the nuclear envelope with the help of special proteins. Large proteins and RNA molecules must be able to pass through the nuclear envelope so proteins can be synthesized in the cytoplasm and the genetic material can be maintained inside the nucleus. The **nucleolus** shown in the model below is mainly involved in the assembly of ribosomes. After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they are involved in the synthesis of proteins.

MITOCHONDRIA

The **mitochondrion** (plural, **mitochondria)** is an organelle that makes energy available to the cell (Figure **3.9.3**). This is why mitochondria are sometimes referred to as the power plants of the cell. They use energy from organic compounds such as glucose to make molecules of **ATP (adenosine triphosphate)**, an energy-carrying molecule that is used almost universally inside cells for energy.

Scientists think that mitochondria were once free-living organisms because they contain their own DNA. They theorize that ancient prokaryotes infected (or were engulfed by) larger prokaryotic cells, and the two organisms evolved a symbiotic relationship that benefited both of them. The larger cells provided the smaller prokaryotes with a place to live. In return, the larger cells got extra energy from the smaller prokaryotes. Eventually, the smaller prokaryotes became permanent guests of the larger cells, as organelles inside them. This theory is called the **endosymbiotic theory**, and it is widely accepted by biologists today

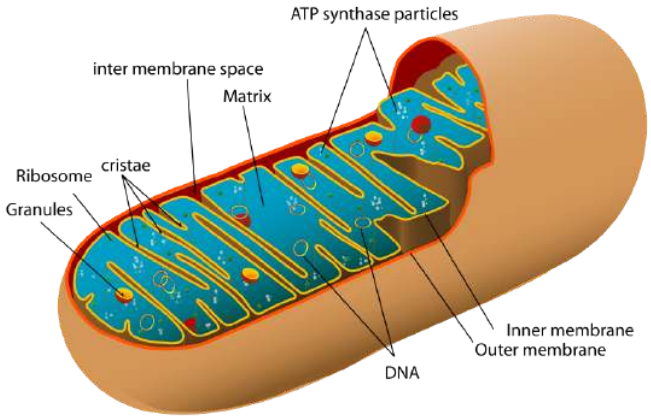


Figure **3.9.3**: Mitochondria, organelles specialized to carry out aerobic respiration, contain an inner membrane folded into cristae, which form two separate compartments: the inner membrane space and the matrix. The Krebs Cycle takes place in the matrix. The electron transport chain is embedded in the inner membrane and uses both compartments to make ATP by chemiosmosis. Mitochondria have their own DNA and ribosomes, resembling those of prokaryotic organisms.

MITOCHONDRIAL COMPARTMENTS

The double membrane nature of the mitochondria results in five distinct compartments, each with an important role in cellular respiration. These compartments are:

1. the outer mitochondrial membrane,
2. the intermembrane space (the space between the outer and inner membranes),
3. the inner mitochondrial membrane,
4. the cristae (formed by infoldings of the inner membrane), and
5. the matrix (space within the inner membrane).

ENDOPLASMIC RETICULUM

The *endoplasmic reticulum* **(ER)** (plural, reticuli) is a network of phospholipid membranes that form hollow tubes, flattened sheets, and round sacs. These flattened, hollow folds and sacs are called *cisternae*. The ER has two major functions:

- Transport: Molecules, such as proteins, can move from place to place inside the ER, much like on an intracellular highway.
- Synthesis: Ribosomes that are attached to the ER, similar to unattached ribosomes, make proteins. Lipids are also produced in the ER.

There are two types of endoplasmic reticulum, rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER):

- **Rough endoplasmic reticulum** is studded with ribosomes, which gives it a “rough” appearance. These ribosomes make proteins that are then transported from the ER in small sacs called transport vesicles. The transport vesicles pinch off the ends of the ER. The rough endoplasmic reticulum works with the Golgi apparatus to move new proteins to their proper destinations in the cell. The membrane of the RER is continuous with the outer layer of the nuclear envelope.
- **Smooth endoplasmic reticulum** does not have any ribosomes attached to it, and so it has a smooth appearance. SER has many different functions, some of which include lipid synthesis, calcium ion storage, and drug detoxification. The smooth endoplasmic reticulum is found in both animal and plant cells and it serves different functions in each. The SER is made up of tubules and vesicles that branch out to form a network. In some cells, there are dilated areas like the sacs of RER. Smooth endoplasmic reticulum and RER form an interconnected network.

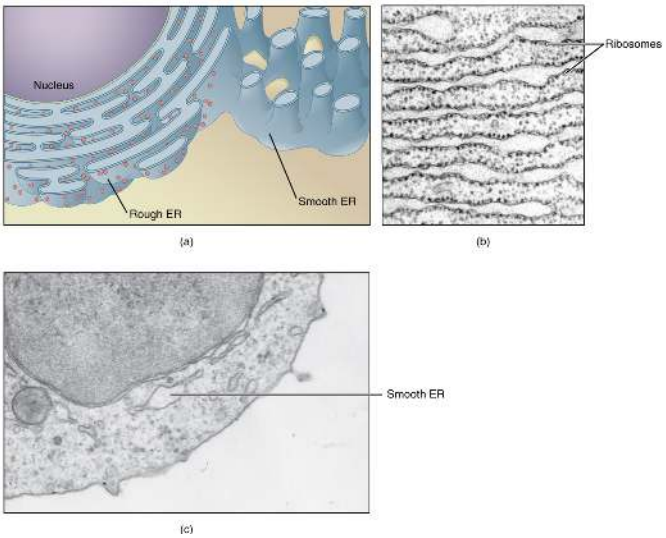


Figure **3.9.4**: The ER is a winding network of thin membranous sacs found in close association with the cell nucleus. The smooth and rough endoplasmic reticula are very different in appearance and function (source: mouse tissue). (b) Rough ER is studded with numerous ribosomes, which are sites of protein synthesis (source: mouse tissue). EM × 110,000. (c) Smooth ER synthesizes phospholipids, steroid hormones, regulates the concentration of cellular Ca^{2+}, metabolizes some carbohydrates, and breaks down certain toxins.

GOLGI APPARATUS

The **Golgi apparatus** (Figure **3.9.5**) is a large organelle that processes proteins and prepares them for use both inside and outside the cell. It was identified in 1898 by the Italian physician Camillo Golgi. The Golgi apparatus modifies, sorts, and packages different substances for secretion out of the cell, or for use within the cell. The Golgi apparatus is found close to the nucleus of the cell where it modifies proteins that have been delivered in transport vesicles from the Rough Endoplasmic Reticulum. It is also involved in the transport of lipids around the cell. Pieces of the Golgi membrane pinch off to form vesicles that transport molecules around the cell. The Golgi apparatus can be thought of as similar to a post office; it packages and labels "items" and then sends them to different parts of the cell. The Golgi apparatus tends to be larger and more numerous in cells that synthesize and secrete large quantities of materials; for example, the plasma B cells and the antibody-secreting cells of the immune system have prominent Golgi complexes.

The Golgi apparatus manipulates products from the Rough Endoplasmic Reticulum (ER) and also produces new organelles called lysosomes. Proteins and other products of the ER are sent to the Golgi apparatus, which organizes, modifies, packages, and tags them. Some of these products are transported to other areas of the cell and some are exported from the cell through exocytosis. Enzymatic proteins are packaged as new lysosomes.

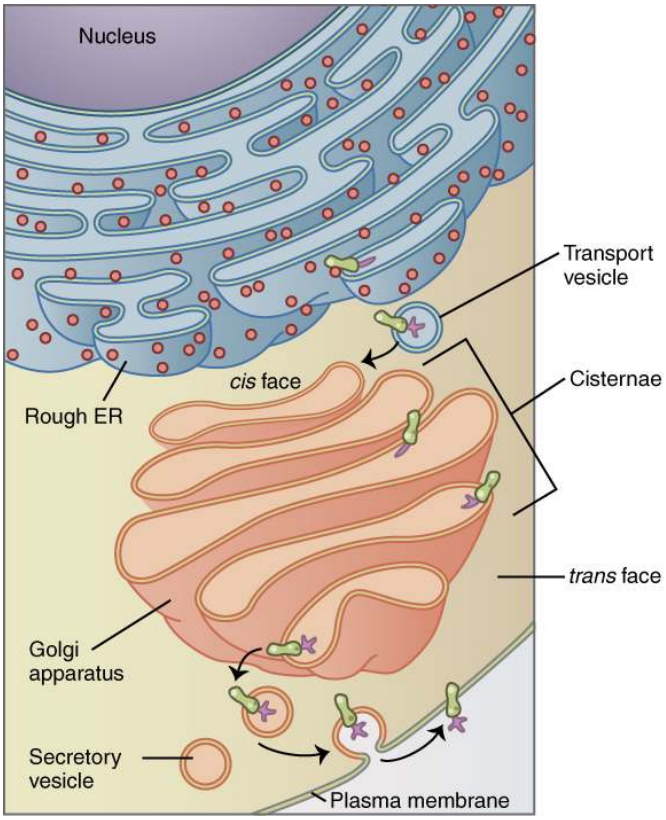


Figure 3.9.5: The rough ER is continuous with the nuclear envelope and has ribosomes on it's surface. The ribosomes produce proteins such as the one shown which remains bound to the membrane of the rough ER. The membrane of the rough ER pinches off to form a transport vesicle containing the protein. The vesicle fuses with the cis face of the Golgi apparatus. The protein is now found on the membrane of the Golgi apparatus and travels along the cisternae. Once it reaches the trans face of the Golgi apparatus, it gets packaged into a secretory vesicle that sends the protein to the plasma membrane.

The stack of **cisternae** has four functional regions: the **cis-Golgi network**, medial-Golgi, endo-Golgi, and trans-Golgi network. Vesicles from the ER fuse with the network and subsequently progress through the stack from the cis- to the **trans-Golgi network**, where they are packaged and sent to their destination. Each cisterna includes special Golgi enzymes which modify or help to modify proteins that travel through it. Proteins may be modified by the addition of a carbohydrate group (glycosylation) or phosphate group (phosphorylation). These modifications may form a signal sequence on the protein, which determines the final destination of the protein. For example, the addition of mannose-6-phosphate signals the protein for lysosomes.

VESICLES AND VACUOLES

Both **vesicles** and **vacuoles** are sac-like organelles that store and transport materials in the cell. Vesicles are much smaller than vacuoles and have a variety of functions. The vesicles that pinch off from the membranes of the ER and Golgi apparatus store and transport protein and lipid molecules. You can see an example of this type of transport vesicle in the figure above. Some vesicles are used as chambers for biochemical reactions. Other vesicles include:

- Lysosomes, which use enzymes to break down foreign matter and dead cells.
- Peroxisomes, which use oxygen to break down poisons.
- Transport vesicles, transport contents between organelle as well as between cell exterior and interior.

CENTRIOLES

Centrioles are organelles involved in cell division. The function of centrioles is to help organize the chromosomes before cell division occurs so that each daughter cell has the correct number of chromosomes after the cell divides. Centrioles are found only in animal cells and are located near the nucleus. Each centriole is made mainly of a protein named **tubulin**. The centriole is cylindrical in shape and consists of many microtubules, as shown in the model pictured below.

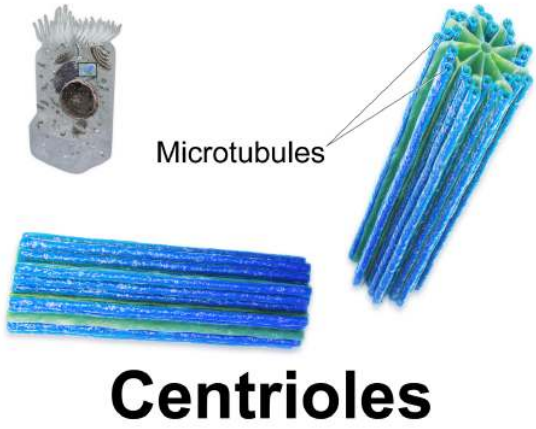


Figure 3.9.6: Centrioles are tiny cylinders near the nucleus, enlarged here to show their tubular structure.

RIBOSOMES

Ribosomes are small structures where proteins are made. Although they are not enclosed within a membrane, they are frequently considered organelles. Each ribosome is formed of two subunits, like the one pictured at the top of this section. Both subunits consist of proteins and RNA. RNA from the nucleus carries the genetic code, copied from DNA, which remains in the nucleus. At the ribosome, the genetic code in RNA is used to assemble and join together amino acids to make proteins. Ribosomes can be found alone or in groups within the cytoplasm as well as on the RER.

REVIEW

1. Define organelle.
2. Describe the structure and function of the nucleus.
3. Explain how the nucleus, ribosomes, rough endoplasmic reticulum, and Golgi apparatus work together to make and transport proteins.
4. Why are mitochondria referred to as the power plants of the cell?
5. What roles are played by vesicles and vacuoles?
6. Why do all cells need ribosomes, even prokaryotic cells that lack a nucleus and other cell organelles?
7. Explain endosymbiotic theory as it relates to mitochondria. What is one piece of evidence that supports this theory?
8. Lysosomes and peroxisomes are types of:
   A. A. Organelles
   B. B. Vesicles
   C. C. Vacuoles
   D. D. Both A and B
9. Which of the following organelles fits best with each description of function? Choose only one organelle for each answer: Golgi apparatus, centrioles, nucleolus, nucleus, rough endoplasmic reticulum
   A. a. Contains the genetic instructions for the production of proteins
   B. b. Organizes chromosomes before cell division
   C. c. Provides a framework for ribosomes
   D. d. Packages and labels proteins
   E. e. Assembles ribosomes
10. *True or False.* All eukaryotic cells have a nucleus.
11. *True or False.* The outer surface of the nucleus of a eukaryotic cell is not completely solid.

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3.10: Serial Endosymbiosis Theory (SET)

Skills to Develop

- List the unifying characteristics of eukaryotes
- Describe what scientists know about the origins of eukaryotes based on the last common ancestor
- Explain endosymbiotic theory

Living things fall into three large groups: Archaea, Bacteria, and Eukarya. The first two have prokaryotic cells, and the third contains all eukaryotes. A relatively sparse fossil record is available to help discern what the first members of each of these lineages looked like, so it is possible that all the events that led to the last common ancestor of extant eukaryotes will remain unknown. However, comparative biology of extant organisms and the limited fossil record provide some insight into the history of Eukarya.

The earliest fossils found appear to be Bacteria, most likely cyanobacteria. They are about 3.5 billion years old and are recognizable because of their relatively complex structure and, for prokaryotes, relatively large cells. Most other prokaryotes have small cells, 1 or 2 µm in size, and would be difficult to pick out as fossils. Most living eukaryotes have cells measuring 10 µm or greater. Structures this size, which might be fossils, appear in the geological record about 2.1 billion years ago.

Characteristics of Eukaryotes

Data from these fossils have led comparative biologists to the conclusion that living eukaryotes are all descendants of a single common ancestor. Mapping the characteristics found in all major groups of eukaryotes reveals that the following characteristics must have been present in the last common ancestor, because these characteristics are present in at least some of the members of each major lineage.

1. Cells with nuclei surrounded by a nuclear envelope with nuclear pores. This is the single characteristic that is both necessary and sufficient to define an organism as a eukaryote. All extant eukaryotes have cells with nuclei.
2. Mitochondria. Some extant eukaryotes have very reduced remnants of mitochondria in their cells, whereas other members of their lineages have “typical” mitochondria.
3. A cytoskeleton containing the structural and motility components called actin microfilaments and microtubules. All extant eukaryotes have these cytoskeletal elements.
4. Flagella and cilia, organelles associated with cell motility. Some extant eukaryotes lack flagella and/or cilia, but they are descended from ancestors that possessed them.
5. Chromosomes, each consisting of a linear DNA molecule coiled around basic (alkaline) proteins called histones. The few eukaryotes with chromosomes lacking histones clearly evolved from ancestors that had them.
6. Mitosis, a process of nuclear division wherein replicated chromosomes are divided and separated using elements of the cytoskeleton. Mitosis is universally present in eukaryotes.
7. Sex, a process of genetic recombination unique to eukaryotes in which diploid nuclei at one stage of the life cycle undergo meiosis to yield haploid nuclei and subsequent karyogamy, a stage where two haploid nuclei fuse together to create a diploid zygote nucleus.
8. Members of all major lineages have cell walls, and it might be reasonable to conclude that the last common ancestor could make cell walls during some stage of its life cycle. However, not enough is known about eukaryotes’ cell walls and their development to know how much homology exists among them. If the last common ancestor could make cell walls, it is clear that this ability must have been lost in many groups.

Endosymbiosis and the Evolution of Eukaryotes

In order to understand eukaryotic organisms fully, it is necessary to understand that all extant eukaryotes are descendants of a chimeric organism that was a composite of a host cell and the cell(s) of an alpha-proteobacterium that “took up residence” inside it. This major theme in the origin of eukaryotes is known as endosymbiosis, one cell engulfing another such that the engulfed cell survives and both cells benefit. Over many generations, a symbiotic relationship can result in two organisms that depend on each other so completely that neither could survive on its own. Endosymbiotic events likely contributed to the origin of the last common

ancestor of today's eukaryotes and to later diversification in certain lineages of eukaryotes (Figure **3.10.4**). Before explaining this further, it is necessary to consider metabolism in prokaryotes.

Prokaryotic Metabolism

Many important metabolic processes arose in prokaryotes, and some of these, such as nitrogen fixation, are never found in eukaryotes. The process of aerobic respiration is found in all major lineages of eukaryotes, and it is localized in the mitochondria. Aerobic respiration is also found in many lineages of prokaryotes, but it is not present in all of them, and many forms of evidence suggest that such anaerobic prokaryotes never carried out aerobic respiration nor did their ancestors.

While today's atmosphere is about one-fifth molecular oxygen (O_2), geological evidence shows that it originally lacked O_2. Without oxygen, aerobic respiration would not be expected, and living things would have relied on fermentation instead. At some point before, about 3.5 billion years ago, some prokaryotes began using energy from sunlight to power anabolic processes that reduce carbon dioxide to form organic compounds. That is, they evolved the ability to photosynthesize. Hydrogen, derived from various sources, was captured using light-powered reactions to reduce fixed carbon dioxide in the Calvin cycle. The group of Gram-negative bacteria that gave rise to cyanobacteria used water as the hydrogen source and released O_2 as a waste product.

Eventually, the amount of photosynthetic oxygen built up in some environments to levels that posed a risk to living organisms, since it can damage many organic compounds. Various metabolic processes evolved that protected organisms from oxygen, one of which, aerobic respiration, also generated high levels of ATP. It became widely present among prokaryotes, including in a group we now call alpha-proteobacteria. Organisms that did not acquire aerobic respiration had to remain in oxygen-free environments. Originally, oxygen-rich environments were likely localized around places where cyanobacteria were active, but by about 2 billion years ago, geological evidence shows that oxygen was building up to higher concentrations in the atmosphere. Oxygen levels similar to today's levels only arose within the last 700 million years.

Recall that the first fossils that we believe to be eukaryotes date to about 2 billion years old, so they appeared as oxygen levels were increasing. Also, recall that all extant eukaryotes descended from an ancestor with mitochondria. These organelles were first observed by light microscopists in the late 1800s, where they appeared to be somewhat worm-shaped structures that seemed to be moving around in the cell. Some early observers suggested that they might be bacteria living inside host cells, but these hypotheses remained unknown or rejected in most scientific communities.

Endosymbiotic Theory

As cell biology developed in the twentieth century, it became clear that mitochondria were the organelles responsible for producing ATP using aerobic respiration. In the 1960s, American biologist Lynn Margulis developed endosymbiotic theory, which states that eukaryotes may have been a product of one cell engulfing another, one living within another, and evolving over time until the separate cells were no longer recognizable as such. In 1967, Margulis introduced new work on the theory and substantiated her findings through microbiological evidence. Although Margulis' work initially was met with resistance, this once-revolutionary hypothesis is now widely (but not completely) accepted, with work progressing on uncovering the steps involved in this evolutionary process and the key players involved. Much still remains to be discovered about the origins of the cells that now make up the cells in all living eukaryotes.

Broadly, it has become clear that many of our nuclear genes and the molecular machinery responsible for replication and expression appear closely related to those in Archaea. On the other hand, the metabolic organelles and genes responsible for many energy-harvesting processes had their origins in bacteria. Much remains to be clarified about how this relationship occurred; this continues to be an exciting field of discovery in biology. For instance, it is not known whether the endosymbiotic event that led to mitochondria occurred before or after the host cell had a nucleus. Such organisms would be among the extinct precursors of the last common ancestor of eukaryotes.

Mitochondria

One of the major features distinguishing prokaryotes from eukaryotes is the presence of mitochondria. Eukaryotic cells may contain anywhere from one to several thousand mitochondria, depending on the cell's level of energy consumption. Each mitochondrion measures 1 to 10 or greater micrometers in length and exists in the cell as an organelle that can be ovoid to worm-shaped to intricately branched (Figure **3.10.1**). Mitochondria arise from the division of existing mitochondria; they may fuse

together; and they may be moved around inside the cell by interactions with the cytoskeleton. However, mitochondria cannot survive outside the cell. As the atmosphere was oxygenated by photosynthesis, and as successful aerobic prokaryotes evolved, evidence suggests that an ancestral cell with some membrane compartmentalization engulfed a free-living aerobic prokaryote, specifically an alpha-proteobacterium, thereby giving the host cell the ability to use oxygen to release energy stored in nutrients. Alpha-proteobacteria are a large group of bacteria that includes species symbiotic with plants, disease organisms that can infect humans via ticks, and many free-living species that use light for energy. Several lines of evidence support that mitochondria are derived from this endosymbiotic event. Most mitochondria are shaped like alpha-proteobacteria and are surrounded by two membranes, which would result when one membrane-bound organism was engulfed into a vacuole by another membrane-bound organism. The mitochondrial inner membrane is extensive and involves substantial infoldings called cristae that resemble the textured, outer surface of alpha-proteobacteria. The matrix and inner membrane are rich with the enzymes necessary for aerobic respiration.

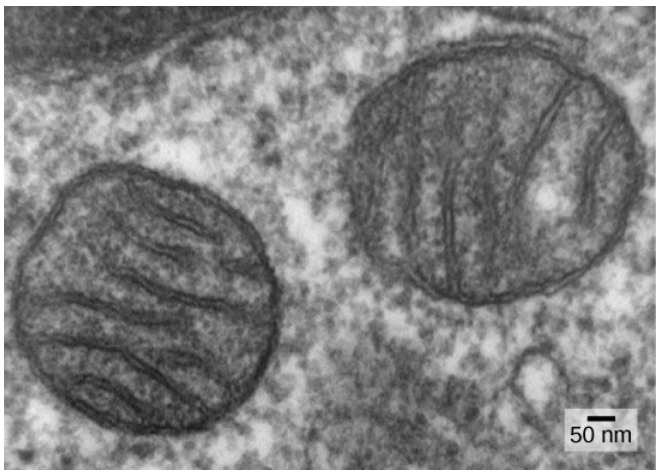


Figure 3.10.1: In this transmission electron micrograph of mitochondria in a mammalian lung cell, the cristae, infoldings of the mitochondrial inner membrane, can be seen in cross-section. (credit: Louise Howard)

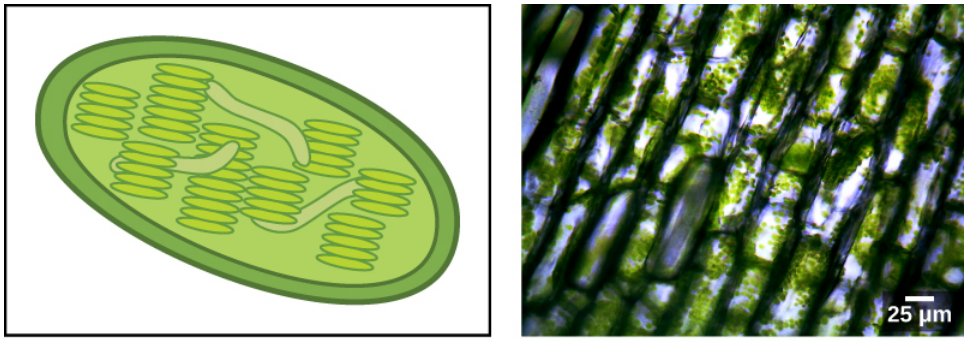
Mitochondria divide independently by a process that resembles binary fission in prokaryotes. Specifically, mitochondria are not formed from scratch (de novo) by the eukaryotic cell; they reproduce within it and are distributed with the cytoplasm when a cell divides or two cells fuse. Therefore, although these organelles are highly integrated into the eukaryotic cell, they still reproduce as if they are independent organisms within the cell. However, their reproduction is synchronized with the activity and division of the cell. Mitochondria have their own (usually) circular DNA chromosome that is stabilized by attachments to the inner membrane and carries genes similar to genes expressed by alpha-proteobacteria. Mitochondria also have special ribosomes and transfer RNAs that resemble these components in prokaryotes. These features all support that mitochondria were once free-living prokaryotes.

Mitochondria that carry out aerobic respiration have their own genomes, with genes similar to those in alpha-proteobacteria. However, many of the genes for respiratory proteins are located in the nucleus. When these genes are compared to those of other organisms, they appear to be of alpha-proteobacterial origin. Additionally, in some eukaryotic groups, such genes are found in the mitochondria, whereas in other groups, they are found in the nucleus. This has been interpreted as evidence that genes have been transferred from the endosymbiont chromosome to the host genome. This loss of genes by the endosymbiont is probably one explanation why mitochondria cannot live without a host.

Some living eukaryotes are anaerobic and cannot survive in the presence of too much oxygen. Some appear to lack organelles that could be recognized as mitochondria. In the 1970s to the early 1990s, many biologists suggested that some of these eukaryotes were descended from ancestors whose lineages had diverged from the lineage of mitochondrion-containing eukaryotes before endosymbiosis occurred. However, later findings suggest that reduced organelles are found in most, if not all, anaerobic eukaryotes, and that all eukaryotes appear to carry some genes in their nuclei that are of mitochondrial origin. In addition to the aerobic generation of ATP, mitochondria have several other metabolic functions. One of these functions is to generate clusters of iron and sulfur that are important cofactors of many enzymes. Such functions are often associated with the reduced mitochondrion-derived organelles of anaerobic eukaryotes. Therefore, most biologists accept that the last common ancestor of eukaryotes had mitochondria.

Plastids

Some groups of eukaryotes are photosynthetic. Their cells contain, in addition to the standard eukaryotic organelles, another kind of organelle called a plastid. When such cells are carrying out photosynthesis, their plastids are rich in the pigment chlorophyll *a* and a range of other pigments, called accessory pigments, which are involved in harvesting energy from light. Photosynthetic plastids are called chloroplasts (Figure **3.10.2**).



(a) (b)

Figure **3.10.2**: (a) This chloroplast cross-section illustrates its elaborate inner membrane organization. Stacks of thylakoid membranes compartmentalize photosynthetic enzymes and provide scaffolding for chloroplast DNA. (b) In this micrograph of *Elodea* sp., the chloroplasts can be seen as small green spheres. (credit b: modification of work by Brandon Zierer; scale-bar data from Matt Russell)

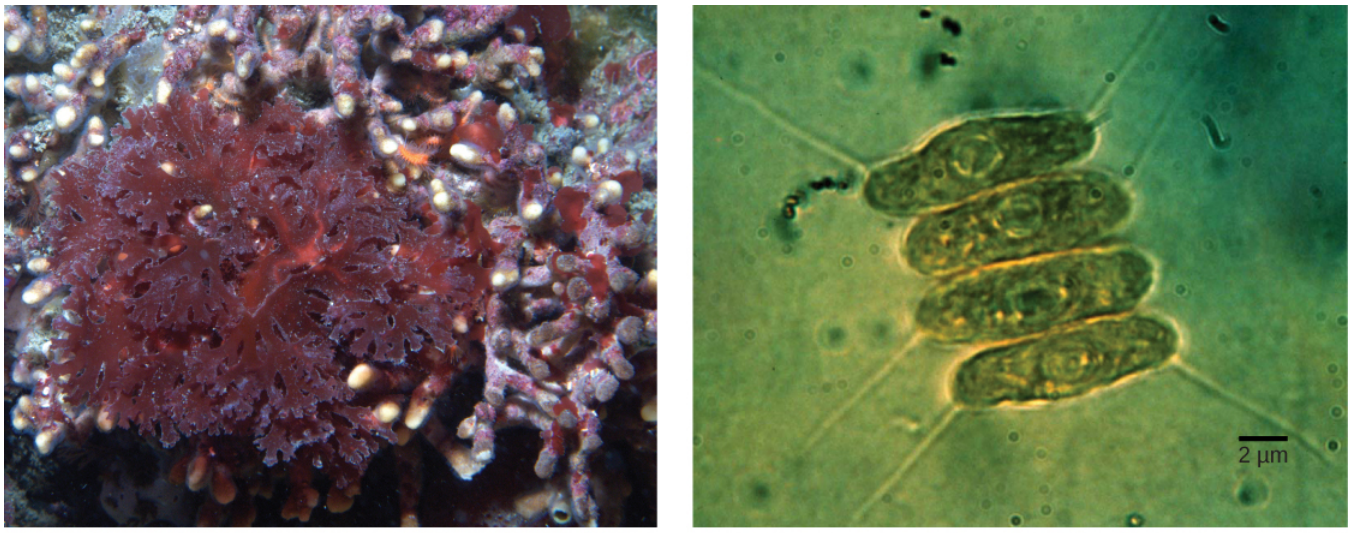
Like mitochondria, plastids appear to have an endosymbiotic origin. This hypothesis was also championed by Lynn Margulis. Plastids are derived from cyanobacteria that lived inside the cells of an ancestral, aerobic, heterotrophic eukaryote. This is called primary endosymbiosis, and plastids of primary origin are surrounded by two membranes. The best evidence is that this has happened twice in the history of eukaryotes. In one case, the common ancestor of the major lineage/supergroup Archaeplastida took on a cyanobacterial endosymbiont; in the other, the ancestor of the small amoeboid rhizarian taxon, *Paulinella*, took on a different cyanobacterial endosymbiont. Almost all photosynthetic eukaryotes are descended from the first event, and only a couple of species are derived from the other.

Cyanobacteria are a group of Gram-negative bacteria with all the conventional structures of the group. However, unlike most prokaryotes, they have extensive, internal membrane-bound sacs called thylakoids. Chlorophyll is a component of these membranes, as are many of the proteins of the light reactions of photosynthesis. Cyanobacteria also have the peptidoglycan wall and lipopolysaccharide layer associated with Gram-negative bacteria.

Chloroplasts of primary origin have thylakoids, a circular DNA chromosome, and ribosomes similar to those of cyanobacteria. Each chloroplast is surrounded by two membranes. In the group of Archaeplastida called the glaucophytes and in *Paulinella*, a thin peptidoglycan layer is present between the outer and inner plastid membranes. All other plastids lack this relictual cyanobacterial wall. The outer membrane surrounding the plastid is thought to be derived from the vacuole in the host, and the inner membrane is thought to be derived from the plasma membrane of the symbiont.

There is also, as with the case of mitochondria, strong evidence that many of the genes of the endosymbiont were transferred to the nucleus. Plastids, like mitochondria, cannot live independently outside the host. In addition, like mitochondria, plastids are derived from the division of other plastids and never built from scratch. Researchers have suggested that the endosymbiotic event that led to Archaeplastida occurred 1 to 1.5 billion years ago, at least 5 hundred million years after the fossil record suggests that eukaryotes were present.

Not all plastids in eukaryotes are derived directly from primary endosymbiosis. Some of the major groups of algae became photosynthetic by secondary endosymbiosis, that is, by taking in either green algae or red algae (both from Archaeplastida) as endosymbionts (Figure **3.10.3**). Numerous microscopic and genetic studies have supported this conclusion. Secondary plastids are surrounded by three or more membranes, and some secondary plastids even have clear remnants of the nucleus of endosymbiotic alga. Others have not “kept” any remnants. There are cases where tertiary or higher-order endosymbiotic events are the best explanations for plastids in some eukaryotes.



(a) (b)

Figure **3.10.3**: (a) Red algae and (b) green algae (visualized by light microscopy) share similar DNA sequences with photosynthetic cyanobacteria. Scientists speculate that, in a process called endosymbiosis, an ancestral prokaryote engulfed a photosynthetic cyanobacterium that evolved into modern-day chloroplasts. (credit a: modification of work by Ed Bierman; credit b: modification of work by G. Fahnenstiel, NOAA; scale-bar data from Matt Russell)

Art Connection

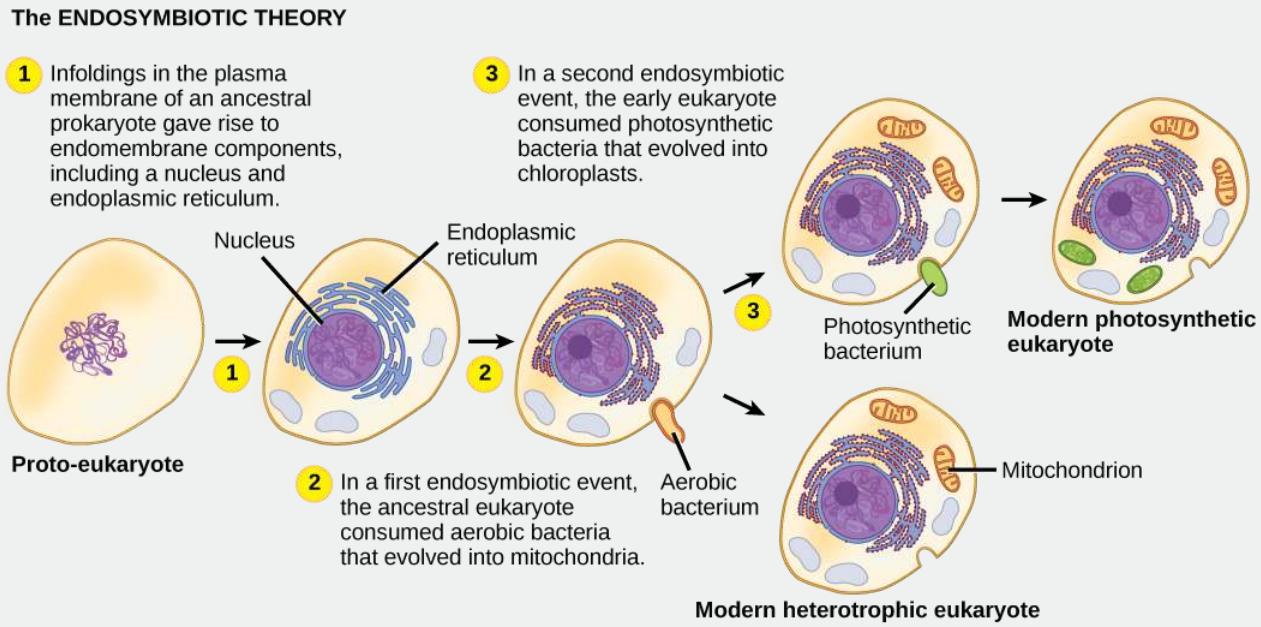


Figure **3.10.4**: The first eukaryote may have originated from an ancestral prokaryote that had undergone membrane proliferation, compartmentalization of cellular function (into a nucleus, lysosomes, and an endoplasmic reticulum), and the establishment of endosymbiotic relationships with an aerobic prokaryote, and, in some cases, a photosynthetic prokaryote, to form mitochondria and chloroplasts, respectively.

What evidence is there that mitochondria were incorporated into the ancestral eukaryotic cell before chloroplasts?

Evolution Connection: Secondary Endosymbiosis in Chlorarachniophytes

Endosymbiosis involves one cell engulfing another to produce, over time, a coevolved relationship in which neither cell could survive alone. The chloroplasts of red and green algae, for instance, are derived from the engulfment of a photosynthetic cyanobacterium by an early prokaryote.

This leads to the question of the possibility of a cell containing an endosymbiont to itself become engulfed, resulting in a secondary endosymbiosis. Molecular and morphological evidence suggest that the chlorarachniophyte protists are derived from a secondary endosymbiotic event. Chlorarachniophytes are rare algae indigenous to tropical seas and sand that can be classified into the rhizarian supergroup. Chlorarachniophytes extend thin cytoplasmic strands, interconnecting themselves with other chlorarachniophytes, in a cytoplasmic network. These protists are thought to have originated when a eukaryote engulfed a green alga, the latter of which had already established an endosymbiotic relationship with a photosynthetic cyanobacterium (Figure **3.10.5**).

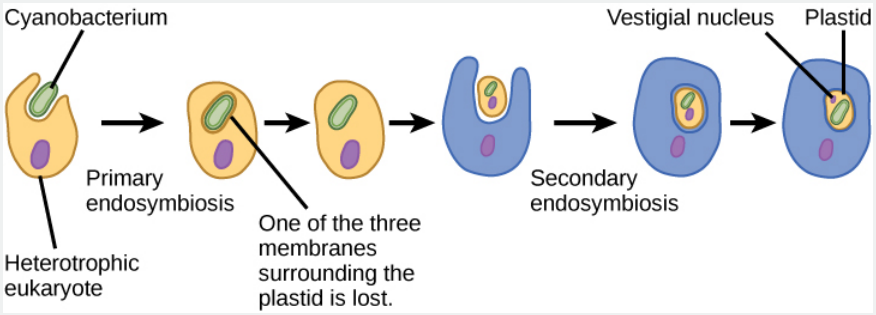


Figure **3.10.5**: The hypothesized process of endosymbiotic events leading to the evolution of chlorarachniophytes is shown. In a primary endosymbiotic event, a heterotrophic eukaryote consumed a cyanobacterium. In a secondary endosymbiotic event, the cell resulting from primary endosymbiosis was consumed by a second cell. The resulting organelle became a plastid in modern chlorarachniophytes.

Several lines of evidence support that chlorarachniophytes evolved from secondary endosymbiosis. The chloroplasts contained within the green algal endosymbionts still are capable of photosynthesis, making chlorarachniophytes photosynthetic. The green algal endosymbiont also exhibits a stunted vestigial nucleus. In fact, it appears that chlorarachniophytes are the products of an evolutionarily recent secondary endosymbiotic event. The plastids of chlorarachniophytes are surrounded by four membranes: The first two correspond to the inner and outer membranes of the photosynthetic cyanobacterium, the third corresponds to the green alga, and the fourth corresponds to the vacuole that surrounded the green alga when it was engulfed by the chlorarachniophyte ancestor. In other lineages that involved secondary endosymbiosis, only three membranes can be identified around plastids. This is currently rectified as a sequential loss of a membrane during the course of evolution.

The process of secondary endosymbiosis is not unique to chlorarachniophytes. In fact, secondary endosymbiosis of green algae also led to euglenid protists, whereas secondary endosymbiosis of red algae led to the evolution of dinoflagellates, apicomplexans, and stramenopiles.

Summary

The oldest fossil evidence of eukaryotes is about 2 billion years old. Fossils older than this all appear to be prokaryotes. It is probable that today's eukaryotes are descended from an ancestor that had a prokaryotic organization. The last common ancestor of today's Eukarya had several characteristics, including cells with nuclei that divided mitotically and contained linear chromosomes where the DNA was associated with histones, a cytoskeleton and endomembrane system, and the ability to make cilia/flagella during at least part of its life cycle. It was aerobic because it had mitochondria that were the result of an aerobic alpha-proteobacterium that lived inside a host cell. Whether this host had a nucleus at the time of the initial symbiosis remains unknown. The last common ancestor may have had a cell wall for at least part of its life cycle, but more data are needed to confirm this hypothesis. Today's eukaryotes are very diverse in their shapes, organization, life cycles, and number of cells per individual.

Art Connections

Figure **3.10.4**: What evidence is there that mitochondria were incorporated into the ancestral eukaryotic cell before chloroplasts?

Answer

All eukaryotic cells have mitochondria, but not all eukaryotic cells have chloroplasts.

Glossary

endosymbiosis
engulfment of one cell within another such that the engulfed cell survives, and both cells benefit; the process responsible for the evolution of mitochondria and chloroplasts in eukaryotes

endosymbiotic theory
theory that states that eukaryotes may have been a product of one cell engulfing another, one living within another, and evolving over time until the separate cells were no longer recognizable as such

plastid
one of a group of related organelles in plant cells that are involved in the storage of starches, fats, proteins, and pigments

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CHAPTER OVERVIEW

4: Microscopy, Staining, and Classification

4.1: Units of Measure

4.2: Staining Microscopic Specimens

4.3: Classification and Identification

4.3.1: Kingdom Classification According to Whittaker

4.3.2: Domain Classification According to Woese

4.4: Microscopy

4.4.1: The Properties of Light

4.4.2: Types of Microscopes

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4.1: Units of Measure

The Metric System

The **metric system** is an internationally agreed-upon measurement system based on decimals or powers of 10. Scientists use a refined version called the **International System of Units** (abbreviated **SI**). In biology, you will often find a need to describe measurements of length, volume, mass, time, temperature or amount of substance.

International System of Units

BASIC SI UNITS		
MEASURE	**SI UNIT**	**SYMBOL**
length	meter	**m**
mass	kilogram	**kg**
time	second	**s**
temperature	Kelvin *(Celsius is used in Biology)*	**K** *(°C)*
quantity	mole	**mol**
current	Ampere	**A**
luminosity	candela	**cd**

Metric Units

- length: meter (**m**)
- volume: liter (**L**)
- mass: gram (**g**)
- time: second (**s**)
- temperature: Celsius (**°C**)
  - Kelvin (**K**) is a unit of thermodynamic temperature and is the SI unit. The Kelvin scale in the same as the Celsius or centigrade scale but offset by 273.16
  - Biology uses Celsius predominantly because of the range in which organisms live.
- amount of substance: mole (**mol**)
  - A mole is a number representing 6.022×10^{23} of something
  - Just as a pair of shoes equals 2 shoes, a mole of shoes is 6.022×10^{23} shoes
  - Just as a dozen eggs equals 12 eggs, a mole of eggs is 6.022×10^{23} eggs

METRIC PREFIXES IN EVERYDAY USE			
PREFIX	**SYMBOL**	**SCIENTIFIC NOTATION**	**FACTOR**
tera	T	10^{12}	1 000 000 000 000
giga	G	10^{9}	1 000 000 000
mega	M	10^{6}	1 000 000
kilo	k	10^{3}	1 000
hecto	h	10^{2}	100
deca	da	10^{1}	10
BASE UNIT	(none)	10^{0}	1
deci	d	10^{-1}	0.1
centi	c	10^{-2}	0.01
milli	m	10^{-3}	0.001
micro	μ	10^{-6}	0.000 001
nano	n	10^{-9}	0.000 000 001
pico	p	10^{-12}	0.000 000 000 001

Strategy for Conversions

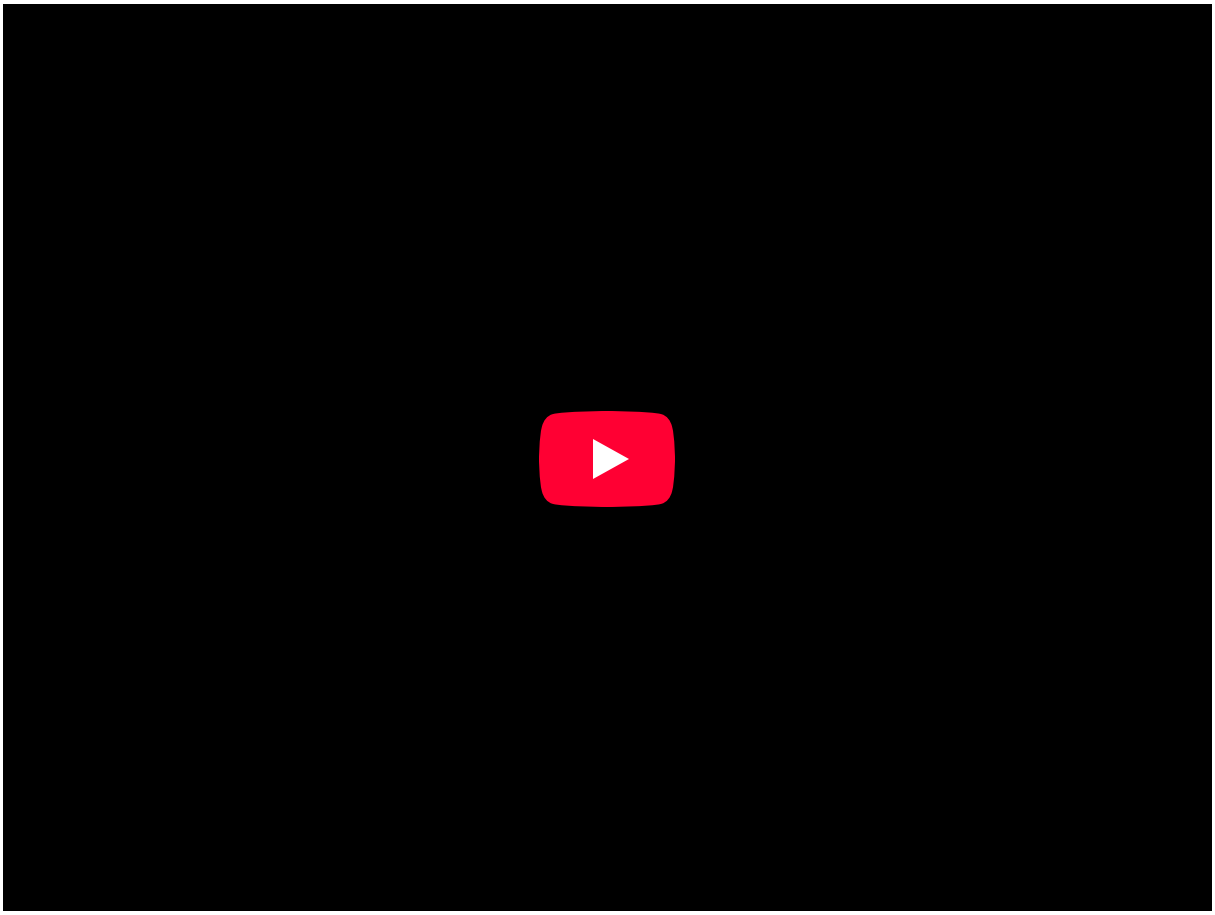
1. What unit is being asked for?
   - 500ml = ____L → liters
2. What unit are you starting from?
   - 500ml = ____L → milliliters
3. Which unit is larger? By how much is that unit larger?
   - Liters are the larger unit. Liters are 1,000X (10^3) greater than milliliters.
4. Which direction are we moving?
   - Since we are moving to a larger unit, our value will be smaller. In this case, the value is smaller by 1,000X
   - In other words, the value is 1/1000 or 0.001 the value.
   - So what is the answer?

Factoring Out

Using the idea of factors of ten, you can assess the difference between the two units and cancel out the original unit algebraically to reach the desired final unit.

- 500ml=_____L
  - $1ml = \frac{1}{1000}L$ or, $\frac{1L}{1000ml}$
    - which states 1000 milliliter in every 1 liter
  - $500ml \times \frac{1L}{1000ml} = \frac{500L}{1000} = 0.5L$
    - pay attention to the units and how we've canceled out the ml in the numerator of 500ml and in the denominator in the conversion of 1L in 1000ml

Additional Resources





Accuracy and Precision

Accuracy refers to how closely a measured value agrees with the correct or target value.

Precision refers to how closely individual measurements agree with each other and reflects the repeatability in those measurements.



This illustrates accuracy. Measurements are on target.



This illustrates precision. Measurements are very close to each other and repeatable.



This illustrates Accuracy AND Precision. Each measurement is on target and also highly repeatable.

Instruments have a finite amount of accuracy and it is important to report measurements within that level of accuracy. **Significant figures**, report the number of digits that are known to some degree of confidence with the measuring device. With the increased sensitivity of the equipment, the number of significant figures increases.

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4.2: Staining Microscopic Specimens

Learning Objectives

- Differentiate between simple and differential stains
- Describe the unique features of commonly used stains
- Explain the procedures and name clinical applications for Gram, endospore, acid-fast, negative capsule, and flagella staining

In their natural state, most of the cells and microorganisms that we observe under the microscope lack color and contrast. This makes it difficult, if not impossible, to detect important cellular structures and their distinguishing characteristics without artificially treating specimens. We have already alluded to certain techniques involving stains and fluorescent dyes, and in this section we will discuss specific techniques for sample preparation in greater detail. Indeed, numerous methods have been developed to identify specific microbes, cellular structures, DNA sequences, or indicators of infection in tissue samples, under the microscope. Here, we will focus on the most clinically relevant techniques.

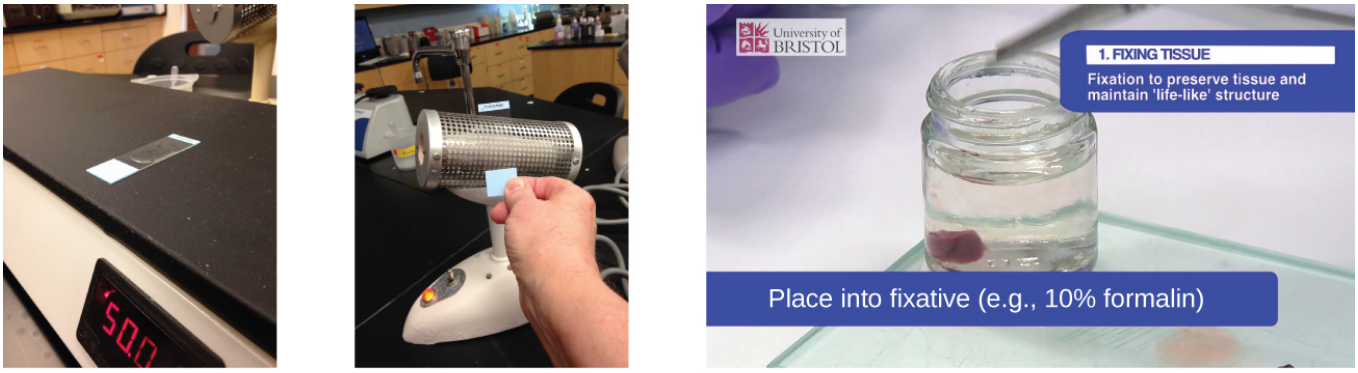
Preparing Specimens for Light Microscopy

In clinical settings, light microscopes are the most commonly used microscopes. There are two basic types of preparation used to view specimens with a light microscope: wet mounts and fixed specimens.

The simplest type of preparation is the wet mount, in which the specimen is placed on the slide in a drop of liquid. Some specimens, such as a drop of urine, are already in a liquid form and can be deposited on the slide using a dropper. Solid specimens, such as a skin scraping, can be placed on the slide before adding a drop of liquid to prepare the wet mount. Sometimes the liquid used is simply water, but often stains are added to enhance contrast. Once the liquid has been added to the slide, a coverslip is placed on top and the specimen is ready for examination under the microscope.

The second method of preparing specimens for light microscopy is fixation. The “fixing” of a sample refers to the process of attaching cells to a slide. Fixation is often achieved either by heating (heat fixing) or chemically treating the specimen. In addition to attaching the specimen to the slide, fixation also kills microorganisms in the specimen, stopping their movement and metabolism while preserving the integrity of their cellular components for observation.

To heat-fix a sample, a thin layer of the specimen is spread on the slide (called a smear), and the slide is then briefly heated over a heat source (Figure 4.2.1). Chemical fixatives are often preferable to heat for tissue specimens. Chemical agents such as acetic acid, ethanol, methanol, formaldehyde (formalin), and glutaraldehyde can denature proteins, stop biochemical reactions, and stabilize cell structures in tissue samples (Figure 4.2.1).

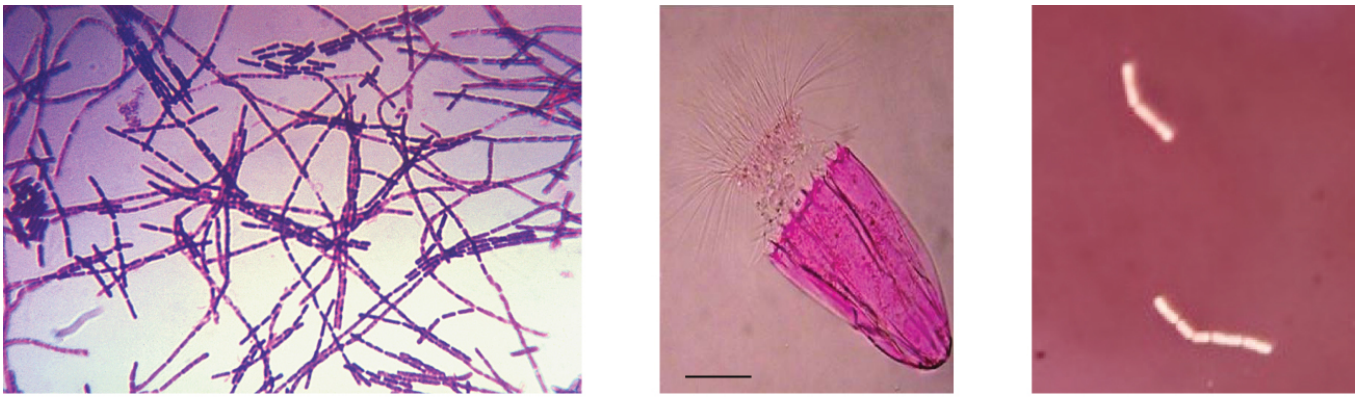


(a) (b) (c)

Figure 4.2.1: (a) A specimen can be heat-fixed by using a slide warmer like this one. (b) Another method for heat-fixing a specimen is to hold a slide with a smear over a microincinerator. (c) This tissue sample is being fixed in a solution of formalin (also known as formaldehyde). Chemical fixation kills microorganisms in the specimen, stopping degradation of the tissues and preserving their structure so that they can be examined later under the microscope. (credit a: modification of work by Nina Parker; credit b: modification of work by Nina Parker; credit c: modification of work by “University of Bristol”/YouTube)

In addition to fixation, staining is almost always applied to color certain features of a specimen before examining it under a light microscope. Stains, or dyes, contain salts made up of a positive ion and a negative ion. Depending on the type of dye, the positive or the negative ion may be the chromophore (the colored ion); the other, uncolored ion is called the counterion. If the chromophore is the positively charged ion, the stain is classified as a basic dye; if the negative ion is the chromophore, the stain is considered an acidic dye.

Dyes are selected for staining based on the chemical properties of the dye and the specimen being observed, which determine how the dye will interact with the specimen. In most cases, it is preferable to use a positive stain, a dye that will be absorbed by the cells or organisms being observed, adding color to objects of interest to make them stand out against the background. However, there are scenarios in which it is advantageous to use a negative stain, which is absorbed by the background but not by the cells or organisms in the specimen. Negative staining produces an outline or silhouette of the organisms against a colorful background (Figure **4.2.2**).



(a) (b) (c)

Figure **4.2.2**: (a) These *Bacillus anthracis* cells have absorbed crystal violet, a basic positive stain. (b) This specimen of *Spinoloricus*, a microscopic marine organism, has been stained with rose bengal, a positive acidic stain. (c) These *B. megaterium* appear to be white because they have not absorbed the negative red stain applied to the slide. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Roberto Danovaro, Antonio Pusceddu, Cristina Gambi, Iben Heiner, Reinhardt Mobjerg Kristensen; credit c: modification of work by Anh-Hue Tu)

Because cells typically have negatively charged cell walls, the positive chromophores in basic dyes tend to stick to the cell walls, making them positive stains. Thus, commonly used basic dyes such as basic fuchsin, crystal violet, malachite green, methylene blue, and safranin typically serve as positive stains. On the other hand, the negatively charged chromophores in acidic dyes are repelled by negatively charged cell walls, making them negative stains. Commonly used acidic dyes include acid fuchsin, eosin, and rose bengal. Figure **4.2.10** provides more detail.

Some staining techniques involve the application of only one dye to the sample; others require more than one dye. In simple staining, a single dye is used to emphasize particular structures in the specimen. A simple stain will generally make all of the organisms in a sample appear to be the same color, even if the sample contains more than one type of organism. In contrast, differential stainingdistinguishes organisms based on their interactions with multiple stains. In other words, two organisms in a differentially stained sample may appear to be different colors. Differential staining techniques commonly used in clinical settings include Gram staining, acid-fast staining, endospore staining, flagella staining, and capsule staining. Figure **4.2.11** provides more detail on these differential staining techniques.

? Exercise 4.2.1

1. Explain why it is important to fix a specimen before viewing it under a light microscope.
2. What types of specimens should be chemically fixed as opposed to heat-fixed?
3. Why might an acidic dye react differently with a given specimen than a basic dye?
4. Explain the difference between a positive stain and a negative stain.
5. Explain the difference between simple and differential staining.

Gram Staining

The Gram stain procedure is a differential staining procedure that involves multiple steps. It was developed by Danish microbiologist Hans Christian Gram in 1884 as an effective method to distinguish between bacteria with different types of cell walls, and even today it remains one of the most frequently used staining techniques. The steps of the Gram stain procedure are listed below and illustrated in Figure **4.2.3**.

1. First, crystal violet, a primary stain, is applied to a heat-fixed smear, giving all of the cells a purple color.
2. Next, Gram's iodine, a mordant, is added. A mordant is a substance used to set or stabilize stains or dyes; in this case, Gram's iodine acts like a trapping agent that complexes with the crystal violet, making the crystal violet–iodine complex clump and stay contained in thick layers of peptidoglycan in the cell walls.
3. Next, a decolorizing agent is added, usually ethanol or an acetone/ethanol solution. Cells that have thick peptidoglycan layers in their cell walls are much less affected by the decolorizing agent; they generally retain the crystal violet dye and remain purple. However, the decolorizing agent more easily washes the dye out of cells with thinner peptidoglycan layers, making them again colorless.
4. Finally, a secondary counterstain, usually safranin, is added. This stains the decolorized cells pink and is less noticeable in the cells that still contain the crystal violet dye.

Gram stain process

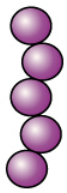
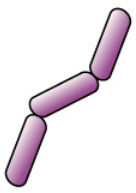
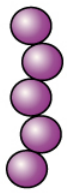
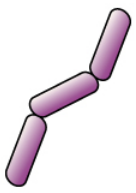
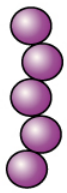
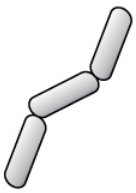
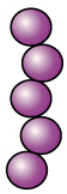
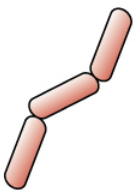
Gram staining steps	Cell effects	Gram-positive	Gram-negative
Step 1 **Crystal violet** *primary stain added to specimen smear.*	Stains cells purple or blue.		
Step 2 **Iodine** *mordant makes dye less soluble so it adheres to cell walls.*	Cells remain purple or blue.		
Step 3 **Alcohol** *decolorizer washes away stain from gram-negative cell walls.*	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 **Safranin** *counterstain allows dye adherance to gram-negative cells.*	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

Figure **4.2.1**: Gram-staining is a differential staining technique that uses a primary stain and a secondary counterstain to distinguish between gram-positive and gram-negative bacteria.

The purple, crystal-violet stained cells are referred to as gram-positive cells, while the red, safranin-dyed cells are gram-negative (Figure **4.2.4**). However, there are several important considerations in interpreting the results of a Gram stain. First, older bacterial cells may have damage to their cell walls that causes them to appear gram-negative even if the species is gram-positive. Thus, it is

best to use fresh bacterial cultures for Gram staining. Second, errors such as leaving on decolorizer too long can affect the results. In some cases, most cells will appear gram-positive while a few appear gram-negative (as in Figure **4.2.4**). This suggests damage to the individual cells or that decolorizer was left on for too long; the cells should still be classified as gram-positive if they are all the same species rather than a mixed culture.

Besides their differing interactions with dyes and decolorizing agents, the chemical differences between gram-positive and gram-negative cells have other implications with clinical relevance. For example, Gram staining can help clinicians classify bacterial pathogens in a sample into categories associated with specific properties. Gram-negative bacteria tend to be more resistant to certain antibiotics than gram-positive bacteria. We will discuss this and other applications of Gram staining in more detail in later chapters.

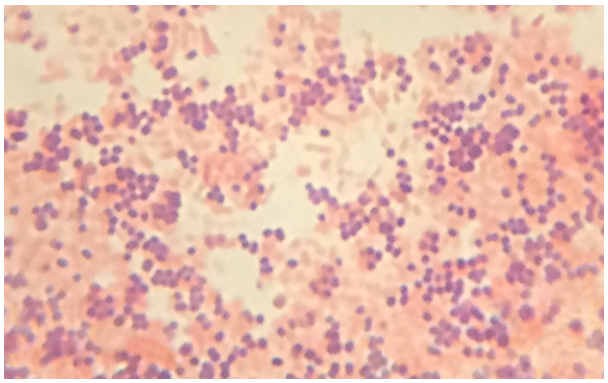


Figure **4.2.4**: In this specimen, the gram-positive bacterium *Staphylococcus aureus* retains crystal violet dye even after the decolorizing agent is added. Gram-negative *Escherichia coli*, the most common Gram stain quality-control bacterium, is decolorized, and is only visible after the addition of the pink counterstain safranin. (credit: modification of work by Nina Parker)

? Exercise 4.2.2

1. Explain the role of Gram's iodine in the Gram stain procedure.
2. Explain the role of alcohol in the Gram stain procedure.
3. What color are gram-positive and gram-negative cells, respectively, after the Gram stain procedure?

Clinical Focus: Part 3

Viewing Cindy's specimen under the darkfield microscope has provided the technician with some important clues about the identity of the microbe causing her infection. However, more information is needed to make a conclusive diagnosis. The technician decides to make a Gram stain of the specimen. This technique is commonly used as an early step in identifying pathogenic bacteria. After completing the Gram stain procedure, the technician views the slide under the brightfield microscope and sees purple, grape-like clusters of spherical cells (Figure **4.2.5**).

Exercise 4.2.3

1. Are these bacteria gram-positive or gram-negative?
2. What does this reveal about their cell walls?

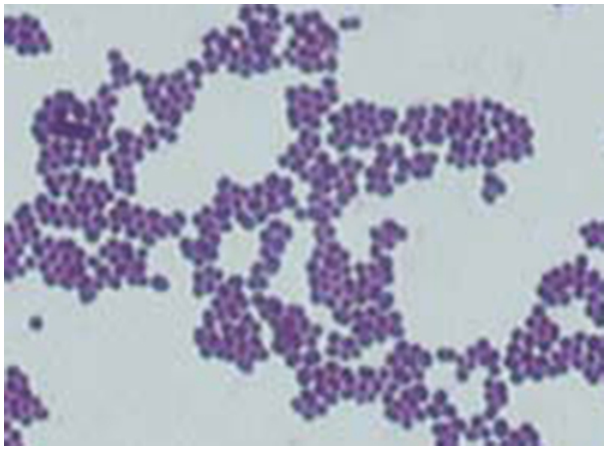


Figure **4.2.5**: Cindy's specimen (credit: modification of work by American Society for Microbiology)

Acid-Fast Stains

Acid-fast staining is another commonly used, differential staining technique that can be an important diagnostic tool. An acid-fast stain is able to differentiate two types of gram-positive cells: those that have waxy mycolic acids in their cell walls, and those that do not. Two different methods for acid-fast staining are the Ziehl-Neelsen technique and the Kinyoun technique. Both use carbolfuchsin as the primary stain. The waxy, acid-fast cells retain the carbolfuchsin even after a decolorizing agent (an acid-alcohol solution) is applied. A secondary counterstain, methylene blue, is then applied, which renders non–acid-fast cells blue.

The fundamental difference between the two carbolfuchsin-based methods is whether heat is used during the primary staining process. The Ziehl-Neelsen method uses heat to infuse the carbolfuchsin into the acid-fast cells, whereas the Kinyoun method does not use heat. Both techniques are important diagnostic tools because a number of specific diseases are caused by acid-fast bacteria(AFB). If AFB are present in a tissue sample, their red or pink color can be seen clearly against the blue background of the surrounding tissue cells (Figure **4.2.6**).

? Exercise 4.2.4

Why are acid-fast stains useful?

Using Microscopy to Diagnose Tuberculosis

Mycobacterium tuberculosis, the bacterium that causes tuberculosis, can be detected in specimens based on the presence of acid-fast bacilli. Often, a smear is prepared from a sample of the patient's sputum and then stained using the Ziehl-Neelsen technique (Figure **4.2.6**). If acid-fast bacteria are confirmed, they are generally cultured to make a positive identification. Variations of this approach can be used as a first step in determining whether *M. tuberculosis* or other acid-fast bacteria are present, though samples from elsewhere in the body (such as urine) may contain other *Mycobacterium* species.

An alternative approach for determining the presence of *M. tuberculosis* is immunofluorescence. In this technique, fluorochrome-labeled antibodies bind to *M. tuberculosis*, if present. Antibody-specific fluorescent dyes can be used to view the mycobacteria with a fluorescence microscope.

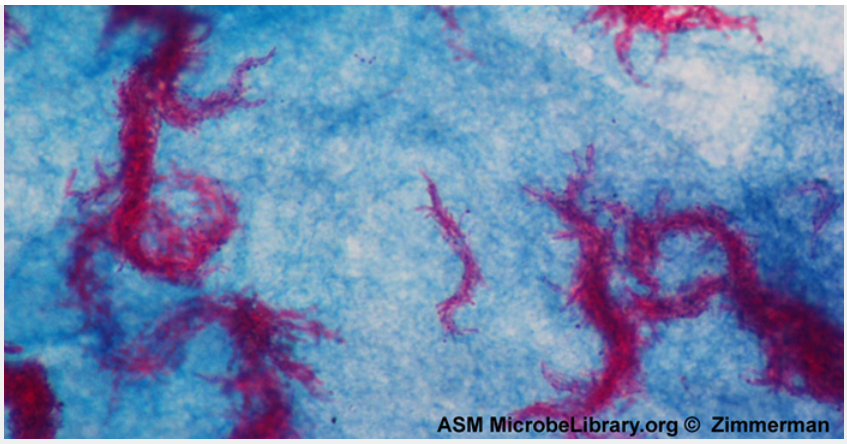
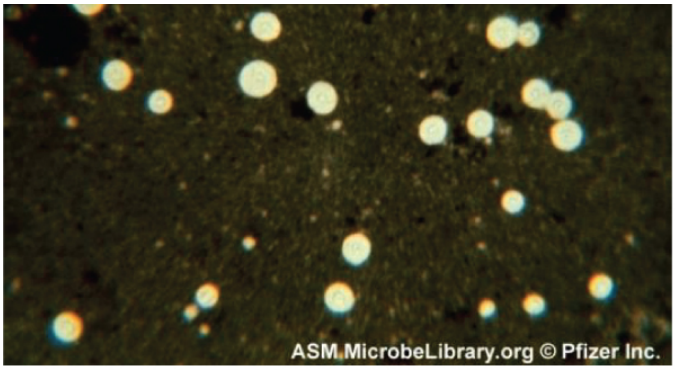


Figure 4.2.6: Ziehl-Neelsen staining has rendered these *Mycobacterium tuberculosis* cells red and the surrounding growth indicator medium blue. (credit: modification of work by American Society for Microbiology)

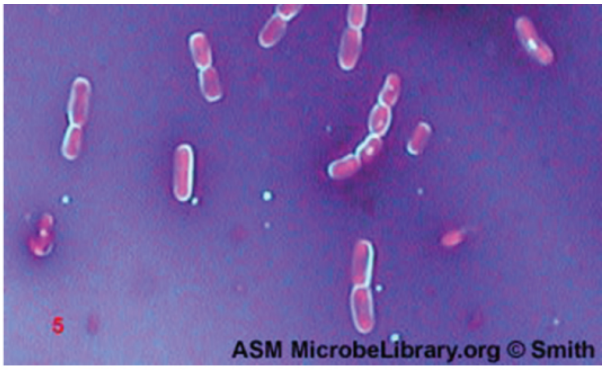
Capsule Staining

Certain bacteria and yeasts have a protective outer structure called a capsule. Since the presence of a capsule is directly related to a microbe's virulence (its ability to cause disease), the ability to determine whether cells in a sample have capsules is an important diagnostic tool. Capsules do not absorb most basic dyes; therefore, a negative staining technique (staining around the cells) is typically used for capsule staining. The dye stains the background but does not penetrate the capsules, which appear like halos around the borders of the cell. The specimen does not need to be heat-fixed prior to negative staining.

One common negative staining technique for identifying encapsulated yeast and bacteria is to add a few drops of India ink or nigrosin to a specimen. Other capsular stains can also be used to negatively stain encapsulated cells (Figure 4.2.7). Alternatively, positive and negative staining techniques can be combined to visualize capsules: The positive stain colors the body of the cell, and the negative stain colors the background but not the capsule, leaving halo around each cell.



(a)



(b)

Figure 4.2.7: (a) India-ink was used to stain the background around these cells of the yeast *Cryptococcus neoformans*. The halos surrounding the cells are the polysaccharide capsules. (b) Crystal violet and copper sulfate dyes cannot penetrate the encapsulated *Bacillus* cells in this negatively stained sample. Encapsulated cells appear to have a light-blue halo. (credit a: modification of work by American Society for Microbiology; credit b: modification of work by American Society for Microbiology)

? Exercise 4.2.5

How does negative staining help us visualize capsules?

Endospore Staining

Endospores are structures produced within certain bacterial cells that allow them to survive harsh conditions. Gram staining alone cannot be used to visualize endospores, which appear clear when Gram-stained cells are viewed. Endospore staining uses two stains to differentiate endospores from the rest of the cell. The Schaeffer-Fulton method (the most commonly used endospore-

staining technique) uses heat to push the primary stain (malachite green) into the endospore. Washing with water decolorizes the cell, but the endospore retains the green stain. The cell is then counterstained pink with safranin. The resulting image reveals the shape and location of endospores, if they are present. The green endospores will appear either within the pink vegetative cells or as separate from the pink cells altogether. If no endospores are present, then only the pink vegetative cells will be visible (Figure **4.2.8**).

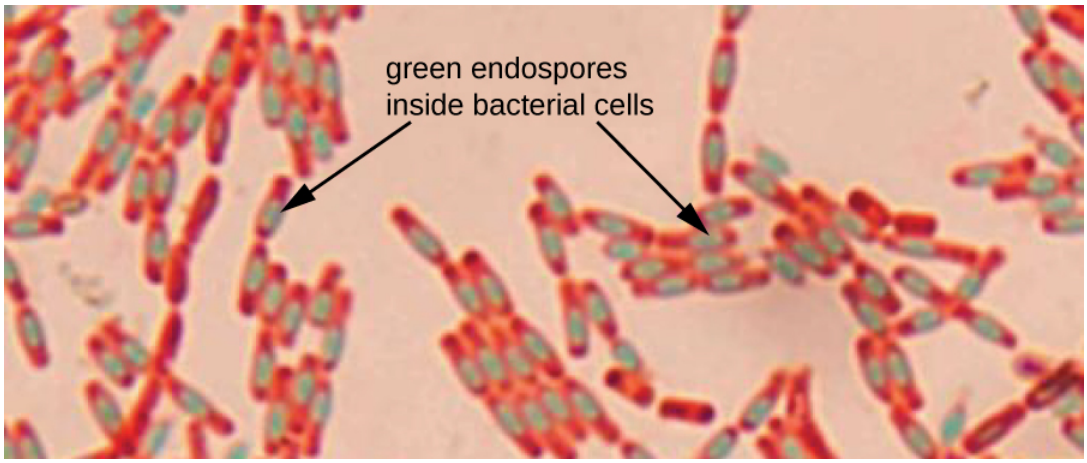


Figure **4.2.8**: A stained preparation of *Bacillus subtilis* showing endospores as green and the vegetative cells as pink. (credit: modification of work by American Society for Microbiology)

Endospore-staining techniques are important for identifying *Bacillus* and *Clostridium*, two genera of endospore-producing bacteria that contain clinically significant species. Among others, *B. anthracis*(which causes anthrax) has been of particular interest because of concern that its spores could be used as a bioterrorism agent. *C. difficile* is a particularly important species responsible for the typically hospital-acquired infection known as “C. diff.”

? Exercise 4.2.6

Is endospore staining an example of positive, negative, or differential staining?

Flagella Staining

Flagella (singular: flagellum) are tail-like cellular structures used for locomotion by some bacteria, archaea, and eukaryotes. Because they are so thin, flagella typically cannot be seen under a light microscope without a specialized flagella staining technique. Flagella staining thickens the flagella by first applying mordant (generally tannic acid, but sometimes potassium alum), which coats the flagella; then the specimen is stained with pararosaniline (most commonly) or basic fuchsin (Figure **4.2.9**).

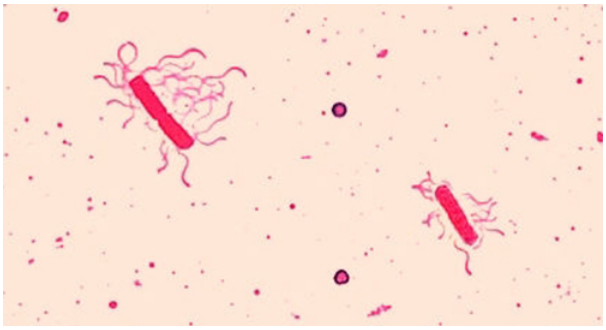


Figure **4.2.9**: A flagella stain of *Bacillus cereus*, a common cause of foodborne illness, reveals that the cells have numerous flagella, used for locomotion. (credit: modification of work by Centers for Disease Control and Prevention)

Though flagella staining is uncommon in clinical settings, the technique is commonly used by microbiologists, since the location and number of flagella can be useful in classifying and identifying bacteria in a sample. When using this technique, it is important to handle the specimen with great care; flagella are delicate structures that can easily be damaged or pulled off, compromising attempts to accurately locate and count the number of flagella.

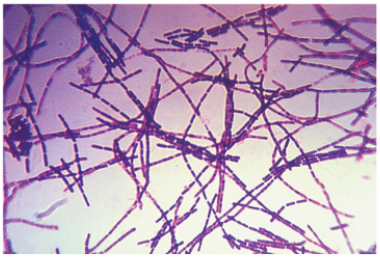
SIMPLE STAINS				
Stain Type	**Specific Dyes**	**Purpose**	**Outcome**	**Sample Images**
Basic stains	Methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, safranin	Stain negatively charged molecules and structures, such as nucleic acids and proteins	Positive stain	
Acidic stains	Eosin, acid fuchsin, rose bengal, Congo red	Stain positively charged molecules and structures, such as proteins	Can be either a positive or negative stain, depending on the cell's chemistry.	
Negative stains	India ink, nigrosin	Stains background, not specimen	Dark background with light specimen	

Figure **4.2.10**: (credit "basic stains": modification of work by Centers for Disease Control and Prevention; credit "Acidic stains": modification of work by Roberto Danovaro, Antonio Dell'Anno, Antonio Pusceddu, Cristina Gambi, Iben Heiner, Reinhardt Mobjerg Kristensen; credit "Negative stains": modification of work by Anh-Hue Tu)

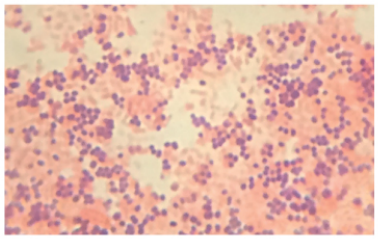
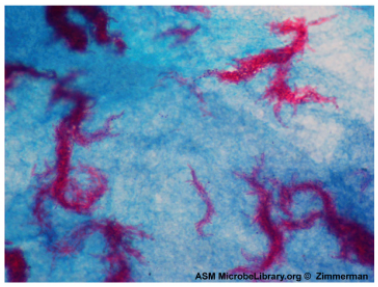
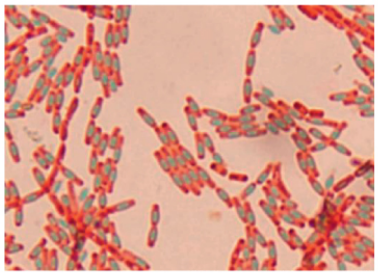
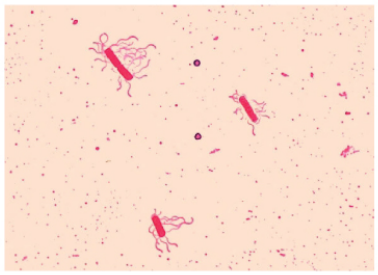
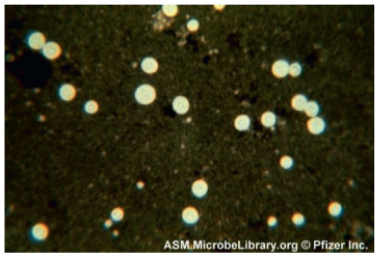
DIFFERENTIAL STAINS				
Stain Type	**Specific Dyes**	**Purpose**	**Outcome**	**Sample Images**
Gram stain	Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin	Used to distinguish cells by cell-wall type (gram-positive, gram-negative)	Gram-positive cells stain purple/violet. Gram-negative cells stain pink.	
Acid-fast stain	After staining with basic fuchsin, acid-fast bacteria resist decolorization by acid-alcohol. Non acid-fast bacteria are counterstained with methylene blue.	Used to distinguish acid-fast bacteria such as *M. tuberculosis*, from non–acid-fast cells	Acid-fast bacteria are red; non–acid-fast cells are blue.	
Endospore stain	Uses heat to stain endospores with malachite green (Schaeffer-Fulton procedure), then cell is washed and counterstained with safranin.	Used to distinguish organisms with endospores from those without; used to study the endospore.	Endospores appear bluish-green; other structures appear pink to red.	
Flagella stain	Flagella are coated with a tannic acid or potassium alum mordant, then stained using either pararosaline or basic fuchsin.	Used to view and study flagella in bacteria that have them.	Flagella are visible if present.	
Capsule stain	Negative staining with India ink or nigrosin is used to stain the background, leaving a clear area of the cell and the capsule. Counterstaining can be used to stain the cell while leaving the capsule clear.	Used to distinguish cells with capsules from those without.	Capsules appear clear or as halos if present.	

Figure **4.2.11**: (credit "Gram stain": modification of work by Nina Parker; credit "Acid-fast stain": modification of work by American Society for Microbiology; credit "Endospore stain": modification of work by American Society for Microbiology; credit "Capsule stain" : modification of work by American Society for Microbiology; credit "Flagella stain": modification of work by Centers for Disease Control and Prevention)

Preparing Specimens for Electron Microscopy

Samples to be analyzed using a TEM must have very thin sections. But cells are too soft to cut thinly, even with diamond knives. To cut cells without damage, the cells must be embedded in plastic resin and then dehydrated through a series of soaks in ethanol solutions (50%, 60%, 70%, and so on). The ethanol replaces the water in the cells, and the resin dissolves in ethanol and enters the cell, where it solidifies. Next, thin sections are cut using a specialized device called an ultramicrotome (Figure **4.2.12**). Finally, samples are fixed to fine copper wire or carbon-fiber grids and stained—not with colored dyes, but with substances like uranyl acetate or osmium tetroxide, which contain electron-dense heavy metal atoms.

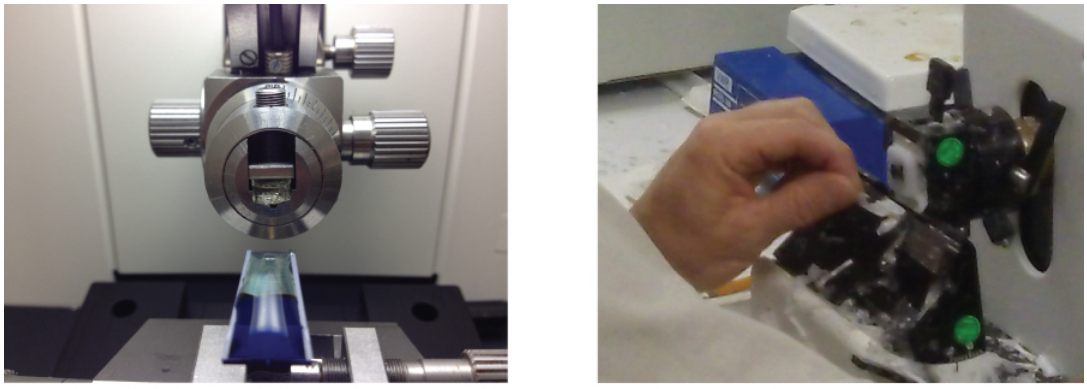


Figure **4.2.12**: (a) An ultramicrotome used to prepare specimens for a TEM. (b) A technician uses an ultramicrotome to slice a specimen into thin sections. (credit a: modification of work by "Frost Museum"/Flickr; credit b: modification of work by U.S. Fish and Wildlife Service Northeast Region)

When samples are prepared for viewing using an SEM, they must also be dehydrated using an ethanol series. However, they must be even drier than is necessary for a TEM. Critical point drying with inert liquid carbon dioxide under pressure is used to displace the water from the specimen. After drying, the specimens are sputter-coated with metal by knocking atoms off of a palladium target, with energetic particles. Sputter-coating prevents specimens from becoming charged by the SEM's electron beam.

? Exercise 4.2.7

1. Why is it important to dehydrate cells before examining them under an electron microscope?
2. Name the device that is used to create thin sections of specimens for electron microscopy.

Using Microscopy to Diagnose Syphilis

The causative agent of syphilis is *Treponema pallidum*, a flexible, spiral cell (spirochete) that can be very thin (<0.15 µm) and match the refractive index of the medium, making it difficult to view using brightfield microscopy. Additionally, this species has not been successfully cultured in the laboratory on an artificial medium; therefore, diagnosis depends upon successful identification using microscopic techniques and serology (analysis of body fluids, often looking for antibodies to a pathogen). Since fixation and staining would kill the cells, darkfield microscopy is typically used for observing live specimens and viewing their movements. However, other approaches can also be used. For example, the cells can be thickened with silver particles (in tissue sections) and observed using a light microscope. It is also possible to use fluorescence or electron microscopy to view *Treponema* (Figure **4.2.13**).

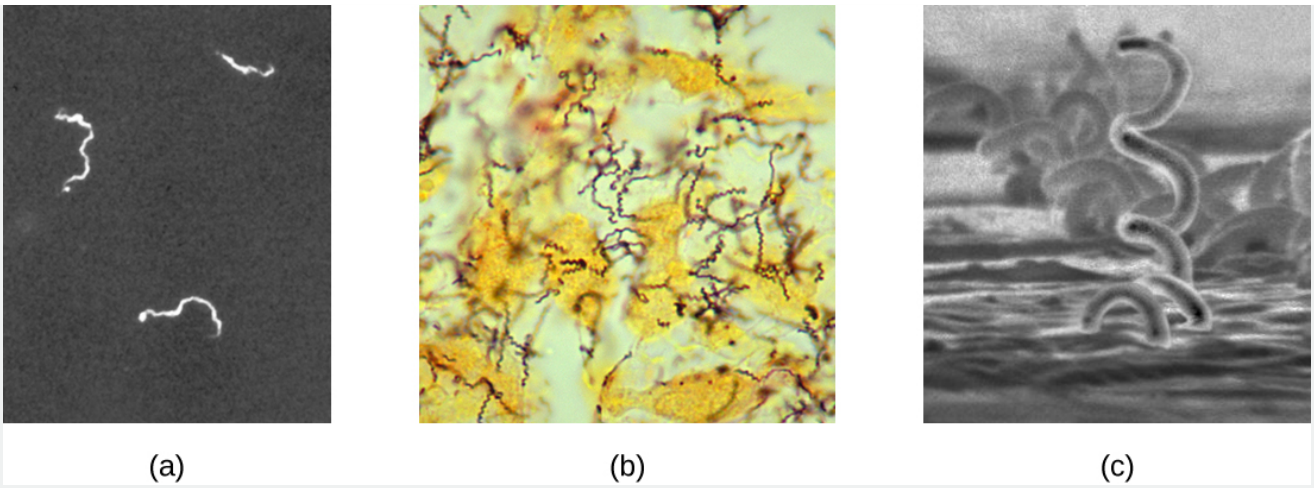


Figure 4.2.13: (a) Living, unstained *Treponema pallidum* spirochetes can be viewed under a darkfield microscope. (b) In this brightfield image, a modified Steiner silver stain is used to visualized *T. pallidum* spirochetes. Though the stain kills the cells, it increases the contrast to make them more visible. (c) While not used for standard diagnostic testing, *T. pallidum* can also be examined using scanning electron microscopy. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by Centers for Disease Control and Prevention)

In clinical settings, indirect immunofluorescence is often used to identify *Treponema*. A primary, unstained antibody attaches directly to the pathogen surface, and secondary antibodies "tagged" with a fluorescent stain attach to the primary antibody. Multiple secondary antibodies can attach to each primary antibody, amplifying the amount of stain attached to each *Treponema* cell, making them easier to spot (Figure 4.2.14).

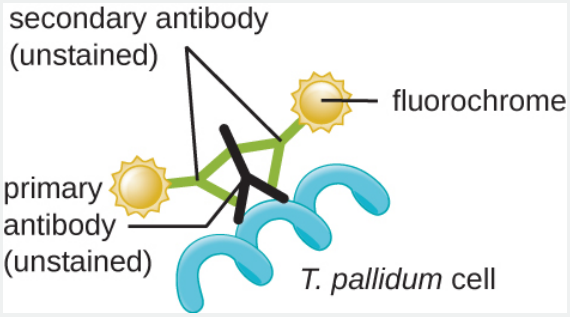


Figure 4.2.14: Indirect immunofluorescence can be used to identify *T. pallidum*, the causative agent of syphilis, in a specimen.

Preparation and Staining for Other Microscopes

Samples for fluorescence and confocal microscopy are prepared similarly to samples for light microscopy, except that the dyes are fluorochromes. Stains are often diluted in liquid before applying to the slide. Some dyes attach to an antibody to stain specific proteins on specific types of cells (immunofluorescence); others may attach to DNA molecules in a process called fluorescence in situ hybridization (FISH), causing cells to be stained based on whether they have a specific DNA sequence.

Sample preparation for two-photon microscopy is similar to fluorescence microscopy, except for the use of infrared dyes. Specimens for STM need to be on a very clean and atomically smooth surface. They are often mica coated with Au(111). Toluene vapor is a common fixative.

? Exercise 4.2.8

What is the main difference between preparing a sample for fluorescence microscopy versus light microscopy?

Link to Learning

Cornell University's Case Studies in Microscopy offers a series of clinical problems based on real-life events. Each case study walks you through a clinical problem using appropriate techniques in microscopy at each step.

Clinical Focus: Resolution

From the results of the Gram stain, the technician now knows that Cindy's infection is caused by spherical, gram-positive bacteria that form grape-like clusters, which is typical of staphylococcal bacteria. After some additional testing, the technician determines that these bacteria are the medically important species known as *Staphylococcus aureus*, a common culprit in wound infections. Because some strains of *S. aureus* are resistant to many antibiotics, skin infections may spread to other areas of the body and become serious, sometimes even resulting in amputations or death if the correct antibiotics are not used.

After testing several antibiotics, the lab is able to identify one that is effective against this particular strain of *S. aureus*. Cindy's doctor quickly prescribes the medication and emphasizes the importance of taking the entire course of antibiotics, even if the infection appears to clear up before the last scheduled dose. This reduces the risk that any especially resistant bacteria could survive, causing a second infection or spreading to another person.

Microscopy and Antibiotic Resistance

As the use of antibiotics has proliferated in medicine, as well as agriculture, microbes have evolved to become more resistant. Strains of bacteria such as methicillin-resistant *S. aureus* (MRSA), which has developed a high level of resistance to many antibiotics, are an increasingly worrying problem, so much so that research is underway to develop new and more diversified antibiotics.

Fluorescence microscopy can be useful in testing the effectiveness of new antibiotics against resistant strains like MRSA. In a test of one new antibiotic derived from a marine bacterium, MC21-A (bromophene), researchers used the fluorescent dye SYTOX Green to stain samples of MRSA. SYTOX Green is often used to distinguish dead cells from living cells, with fluorescence microscopy. Live cells will not absorb the dye, but cells killed by an antibiotic will absorb the dye, since the antibiotic has damaged the bacterial cell membrane. In this particular case, MRSA bacteria that had been exposed to MC21-A did, indeed, appear green under the fluorescence microscope, leading researchers to conclude that it is an effective antibiotic against MRSA.

Of course, some argue that developing new antibiotics will only lead to even more antibiotic-resistant microbes, so-called superbugs that could spawn epidemics before new treatments can be developed. For this reason, many health professionals are beginning to exercise more discretion in prescribing antibiotics. Whereas antibiotics were once routinely prescribed for common illnesses without a definite diagnosis, doctors and hospitals are much more likely to conduct additional testing to determine whether an antibiotic is necessary and appropriate before prescribing.

A sick patient might reasonably object to this stingy approach to prescribing antibiotics. To the patient who simply wants to feel better as quickly as possible, the potential benefits of taking an antibiotic may seem to outweigh any immediate health risks that might occur if the antibiotic is ineffective. But at what point do the risks of widespread antibiotic use supersede the desire to use them in individual cases?

Key Concepts and Summary

- Samples must be properly prepared for microscopy. This may involve **staining**, **fixation**, and/or cutting **thin sections**.
- A variety of staining techniques can be used with light microscopy, including **Gram staining, acid-fast staining**, **capsule staining**, **endospore staining,** and **flagella staining**.
- Samples for TEM require very thin sections, whereas samples for SEM require sputter-coating.
- Preparation for fluorescence microscopy is similar to that for light microscopy, except that fluorochromes are used.

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4.3: Classification and Identification

Learning Objecctives

- Describe how microorganisms are classified and distinguished as unique species
- Compare historical and current systems of taxonomy used to classify microorganisms

Once microbes became visible to humans with the help of microscopes, scientists began to realize their enormous diversity. Microorganisms vary in all sorts of ways, including their size, their appearance, and their rates of reproduction. To study this incredibly diverse new array of organisms, researchers needed a way to systematically organize them.

The Science of Taxonomy

Taxonomy is the classification, description, identification, and naming of living organisms. Classification is the practice of organizing organisms into different groups based on their shared characteristics. The most famous early taxonomist was a Swedish botanist, zoologist, and physician named Carolus Linnaeus (1701–1778). In 1735, Linnaeus published *Systema Naturae*, an 11-page booklet in which he proposed the Linnaean taxonomy, a system of categorizing and naming organisms using a standard format so scientists could discuss organisms using consistent terminology. He continued to revise and add to the book, which grew into multiple volumes (Figure **4.3.1**).



Figure **4.3.1**: Swedish botanist, zoologist, and physician Carolus Linnaeus developed a new system for categorizing plants and animals. In this 1853 portrait by Hendrik Hollander, Linnaeus is holding a twinflower, named *Linnaea borealis* in his honor.

In his taxonomy, Linnaeus divided the natural world into three kingdoms: animal, plant, and mineral (the mineral kingdom was later abandoned). Within the animal and plant kingdoms, he grouped organisms using a hierarchy of increasingly specific levels and sublevels based on their similarities. The names of the levels in Linnaeus's original taxonomy were kingdom, class, order, family, genus (plural: genera), and species. Species was, and continues to be, the most specific and basic taxonomic unit.

Evolving Trees of Life (Phylogenies)

With advances in technology, other scientists gradually made refinements to the Linnaean system and eventually created new systems for classifying organisms. In the 1800s, there was a growing interest in developing taxonomies that took into account the evolutionary relationships, or phylogenies, of all different species of organisms on earth. One way to depict these relationships is via a diagram called a phylogenetic tree (or tree of life). In these diagrams, groups of organisms are arranged by how closely related they are thought to be. In early phylogenetic trees, the relatedness of organisms was inferred by their visible similarities, such as the presence or absence of hair or the number of limbs. Now, the analysis is more complicated. Today, phylogenic analyses include genetic, biochemical, and embryological comparisons, as will be discussed later in this chapter.

Linnaeus's tree of life contained just two main branches for all living things: the animal and plant kingdoms. In 1866, Ernst Haeckel, a German biologist, philosopher, and physician, proposed another kingdom, Protista, for unicellular organisms (Figure **4.3.2**). He later proposed a fourth kingdom, Monera, for unicellular organisms whose cells lack nuclei, like bacteria.

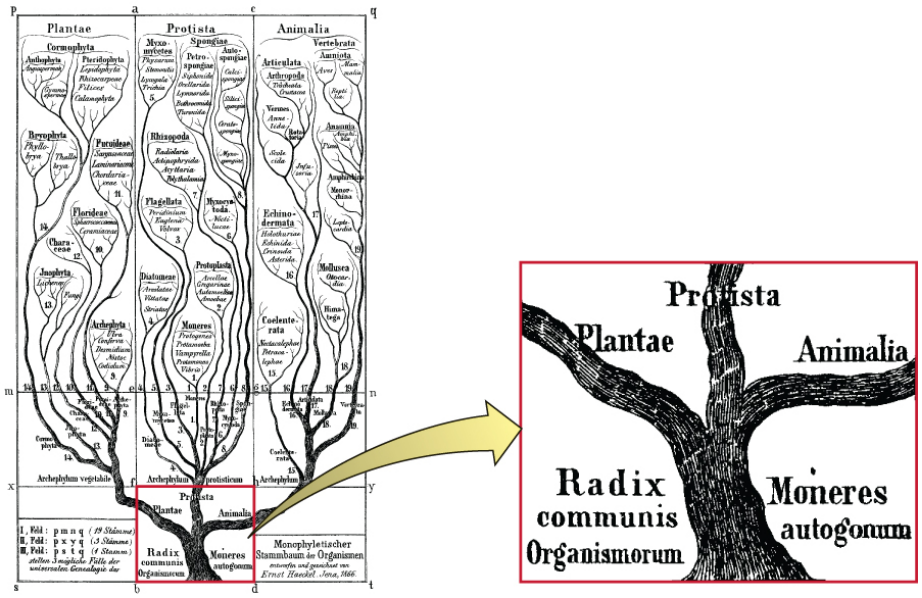


Figure **4.3.2**: Ernst Haeckel's rendering of the tree of life, from his 1866 book *General Morphology of Organisms*, contained three kingdoms: Plantae, Protista, and Animalia. He later added a fourth kingdom, Monera, for unicellular organisms lacking a nucleus.

Nearly 100 years later, in 1969, American ecologist Robert Whittaker (1920–1980) proposed adding another kingdom—Fungi—in his tree of life. Whittaker's tree also contained a level of categorization above the kingdom level—the empire or superkingdom level—to distinguish between organisms that have membrane-bound nuclei in their cells (eukaryotes) and those that do not (prokaryotes). Empire Prokaryota contained just the Kingdom Monera. The Empire Eukaryota contained the other four kingdoms: Fungi, Protista, Plantae, and Animalia. Whittaker's five-kingdom tree was considered the standard phylogeny for many years.

Figure **4.3.3** shows how the tree of life has changed over time. Note that viruses are not found in any of these trees. That is because they are not made up of cells and thus it is difficult to determine where they would fit into a tree of life.

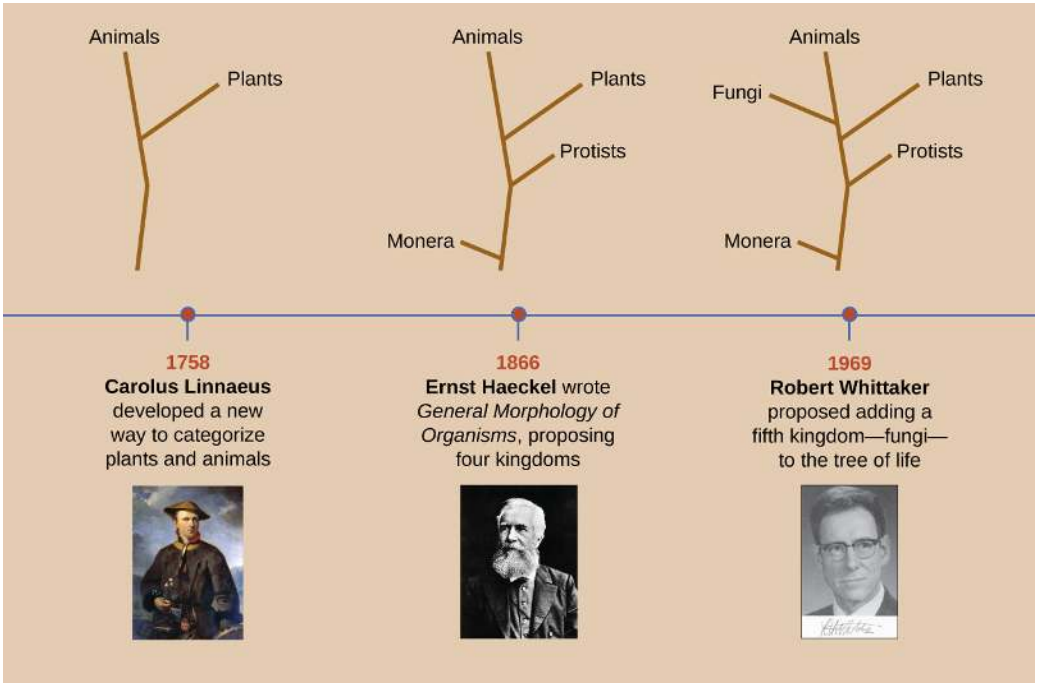


Figure **4.3.3**: This timeline shows how the shape of the tree of life has changed over the centuries. Even today, the taxonomy of living organisms is continually being reevaluated and refined with advances in technology.

Exercise 4.3.1

Briefly summarize how our evolving understanding of microorganisms has contributed to changes in the way that organisms are classified.

Clinical Focus: Part 2

Antibiotic drugs are specifically designed to kill or inhibit the growth of bacteria. But after a couple of days on antibiotics, Cora shows no signs of improvement. Also, her CSF cultures came back from the lab negative. Since bacteria or fungi were not isolated from Cora's CSF sample, her doctor rules out bacterial and fungal meningitis. Viral meningitis is still a possibility.

However, Cora now reports some troubling new symptoms. She is starting to have difficulty walking. Her muscle stiffness has spread from her neck to the rest of her body, and her limbs sometimes jerk involuntarily. In addition, Cora's cognitive symptoms are worsening. At this point, Cora's doctor becomes very concerned and orders more tests on the CSF samples.

Exercise 4.3.2

What types of microorganisms could be causing Cora's symptoms?

The Role of Genetics in Modern Taxonomy

Haeckel's and Whittaker's trees presented hypotheses about the phylogeny of different organisms based on readily observable characteristics. But the advent of molecular genetics in the late 20th century revealed other ways to organize phylogenetic trees. Genetic methods allow for a standardized way to compare all living organisms without relying on observable characteristics that can often be subjective. Modern taxonomy relies heavily on comparing the nucleic acids (deoxyribonucleic acid [DNA] or ribonucleic acid [RNA]) or proteins from different organisms. The more similar the nucleic acids and proteins are between two organisms, the more closely related they are considered to be.

In the 1970s, American microbiologist Carl Woese discovered what appeared to be a "living record" of the evolution of organisms. He and his collaborator George Fox created a genetics-based tree of life based on similarities and differences they observed in the small subunit ribosomal RNA (rRNA) of different organisms. In the process, they discovered that a certain type of bacteria, called archaebacteria (now known simply as archaea), were significantly different from other bacteria and eukaryotes in terms of the sequence of small subunit rRNA. To accommodate this difference, they created a tree with three Domains above the level of Kingdom: Archaea, Bacteria, and Eukarya (Figure **4.3.4**). Genetic analysis of the small subunit rRNA suggests archaea, bacteria, and eukaryotes all evolved from a common ancestral cell type. The tree is skewed to show a closer evolutionary relationship between Archaea and Eukarya than they have to Bacteria.

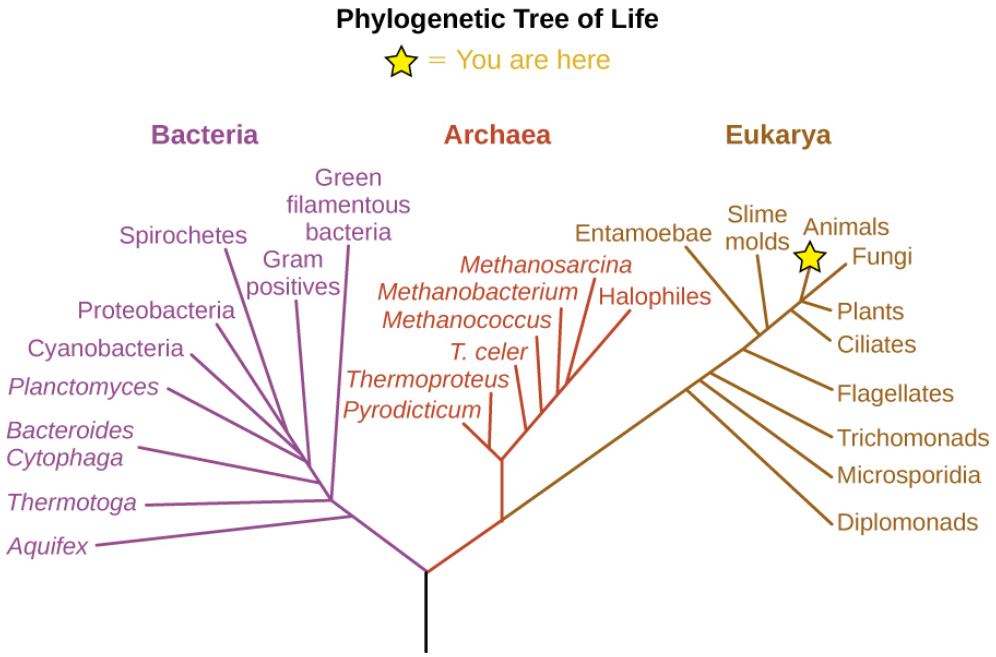


Figure **4.3.4**: Scientists continue to use analysis of RNA, DNA, and proteins to determine how organisms are related. One interesting, and complicating, discovery is that of horizontal gene transfer—when a gene of one species is absorbed into another organism's genome. Horizontal gene transfer is especially common in microorganisms and can make it difficult to determine how organisms are evolutionarily related. Consequently, some scientists now think in terms of "webs of life" rather than "trees of life."

? Exercise 4.3.3

1. In modern taxonomy, how do scientists determine how closely two organisms are related?
2. Explain why the branches on the "tree of life" all originate from a single "trunk."

Naming Microbes

In developing his taxonomy, Linnaeus used a system of binomial nomenclature, a two-word naming system for identifying organisms by genus and species. For example, modern humans are in the genus *Homo* and have the species name *sapiens*, so their scientific name in binomial nomenclature is *Homo sapiens*. In binomial nomenclature, the genus part of the name is always capitalized; it is followed by the species name, which is not capitalized. Both names are italicized.

Taxonomic names in the 18th through 20th centuries were typically derived from Latin, since that was the common language used by scientists when taxonomic systems were first created. Today, newly discovered organisms can be given names derived from Latin, Greek, or English. Sometimes these names reflect some distinctive trait of the organism; in other cases, microorganisms are named after the scientists who discovered them. The archaeon *Haloquadratum walsbyi* is an example of both of these naming schemes. The genus, *Haloquadratum*, describes the microorganism's saltwater habitat (*halo* is derived from the Greek word for "salt") as well as the arrangement of its square cells, which are arranged in square clusters of four cells (*quadratum* is Latin for "foursquare"). The species, *walsbyi*, is named after Anthony Edward Walsby, the microbiologist who discovered *Haloquadratum walsbyi* in in 1980. While it might seem easier to give an organism a common descriptive name—like a red-headed woodpecker—we can imagine how that could become problematic. What happens when another species of woodpecker with red head coloring is discovered? The systematic nomenclature scientists use eliminates this potential problem by assigning each organism a single, unique two-word name that is recognized by scientists all over the world.

In this text, we will typically abbreviate an organism's genus and species after its first mention. The abbreviated form is simply the first initial of the genus, followed by a period and the full name of the species. For example, the bacterium *Escherichia coli* is shortened to *E. coli* in its abbreviated form. You will encounter this same convention in other scientific texts as well.

Bergey's Manuals

Whether in a tree or a web, microbes can be difficult to identify and classify. Without easily observable macroscopic features like feathers, feet, or fur, scientists must capture, grow, and devise ways to study their biochemical properties to differentiate and classify microbes. Despite these hurdles, a group of microbiologists created and updated a set of manuals for identifying and classifying microorganisms. First published in 1923 and since updated many times, *Bergey's Manual of Determinative Bacteriology* and *Bergey's Manual of Systematic Bacteriology* are the standard references for identifying and classifying different prokaryotes. (Appendix D of this textbook is partly based on Bergey's manuals; it shows how the organisms that appear in this textbook are classified.) Because so many bacteria look identical, methods based on nonvisual characteristics must be used to identify them. For example, biochemical tests can be used to identify chemicals unique to certain species. Likewise, serological tests can be used to identify specific antibodies that will react against the proteins found in certain species. Ultimately, DNA and rRNA sequencing can be used both for identifying a particular bacterial species and for classifying newly discovered species.

? Exercise 4.3.4

- What is binomial nomenclature and why is it a useful tool for naming organisms?
- Explain why a resource like one of Bergey's manuals would be helpful in identifying a microorganism in a sample.

Same Name, Different Strain

Within one species of microorganism, there can be several subtypes called strains. While different strains may be nearly identical genetically, they can have very different attributes. The bacterium*Escherichia coli* is infamous for causing food poisoning and traveler's diarrhea. However, there are actually many different strains of *E. coli*, and they vary in their ability to cause disease.

One pathogenic (disease-causing) *E. coli* strain that you may have heard of is *E. coli* O157:H7. In humans, infection from *E. coli* O157:H7 can cause abdominal cramps and diarrhea. Infection usually originates from contaminated water or food, particularly raw vegetables and undercooked meat. In the 1990s, there were several large outbreaks of *E. coli* O157:H7 thought to have originated in undercooked hamburgers.

While *E. coli* O157:H7 and some other strains have given *E. coli* a bad name, most *E. coli* strains do not cause disease. In fact, some can be helpful. Different strains of *E. coli* found naturally in our gut help us digest our food, provide us with some needed chemicals, and fight against pathogenic microbes.

Summary

- Carolus Linnaeus developed a taxonomic system for categorizing organisms into related groups.
- Binomial nomenclature assigns organisms Latinized scientific names with a genus and species designation.
- A phylogenetic tree is a way of showing how different organisms are thought to be related to one another from an evolutionary standpoint.
- The first phylogenetic tree contained kingdoms for plants and animals; Ernst Haeckel proposed adding a kingdom for protists.
- Robert Whittaker's tree contained five kingdoms: Animalia, Plantae, Protista, Fungi, and Monera.
- Carl Woese used small subunit ribosomal RNA to create a phylogenetic tree that groups organisms into three domains based on their genetic similarity.
- Bergey's manuals of determinative and systemic bacteriology are the standard references for identifying and classifying bacteria, respectively.
- Bacteria can be identified through biochemical tests, DNA/RNA analysis, and serological testing methods.

Glossary

binomial nomenclature

a universal convention for the scientific naming of organisms using Latinized names for genus and species

eukaryote

an organism made up of one or more cells that contain a membrane-bound nucleus and organelles

phylogeny

the evolutionary history of a group of organisms

prokaryote

an organism whose cell structure does not include a membrane-bound nucleus

taxonomy

the classification, description, identification, and naming of living organisms

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4.3.1: Kingdom Classification According to Whittaker

Learning Objecctives

- Describe how microorganisms are classified and distinguished as unique species
- Compare historical and current systems of taxonomy used to classify microorganisms

Once microbes became visible to humans with the help of microscopes, scientists began to realize their enormous diversity. Microorganisms vary in all sorts of ways, including their size, their appearance, and their rates of reproduction. To study this incredibly diverse new array of organisms, researchers needed a way to systematically organize them.

The Science of Taxonomy

Taxonomy is the classification, description, identification, and naming of living organisms. Classification is the practice of organizing organisms into different groups based on their shared characteristics. The most famous early taxonomist was a Swedish botanist, zoologist, and physician named Carolus Linnaeus (1701–1778). In 1735, Linnaeus published *Systema Naturae*, an 11-page booklet in which he proposed the Linnaean taxonomy, a system of categorizing and naming organisms using a standard format so scientists could discuss organisms using consistent terminology. He continued to revise and add to the book, which grew into multiple volumes (Figure **4.3.1.1**).



Figure **4.3.1.1**: Swedish botanist, zoologist, and physician Carolus Linnaeus developed a new system for categorizing plants and animals. In this 1853 portrait by Hendrik Hollander, Linnaeus is holding a twinflower, named *Linnaea borealis* in his honor.

In his taxonomy, Linnaeus divided the natural world into three kingdoms: animal, plant, and mineral (the mineral kingdom was later abandoned). Within the animal and plant kingdoms, he grouped organisms using a hierarchy of increasingly specific levels and sublevels based on their similarities. The names of the levels in Linnaeus's original taxonomy were kingdom, class, order, family, genus (plural: genera), and species. Species was, and continues to be, the most specific and basic taxonomic unit.

Evolving Trees of Life (Phylogenies)

With advances in technology, other scientists gradually made refinements to the Linnaean system and eventually created new systems for classifying organisms. In the 1800s, there was a growing interest in developing taxonomies that took into account the evolutionary relationships, or phylogenies, of all different species of organisms on earth. One way to depict these relationships is via a diagram called a phylogenetic tree (or tree of life). In these diagrams, groups of organisms are arranged by how closely related they are thought to be. In early phylogenetic trees, the relatedness of organisms was inferred by their visible similarities, such as the presence or absence of hair or the number of limbs. Now, the analysis is more complicated. Today, phylogenic analyses include genetic, biochemical, and embryological comparisons, as will be discussed later in this chapter.

Linnaeus's tree of life contained just two main branches for all living things: the animal and plant kingdoms. In 1866, Ernst Haeckel, a German biologist, philosopher, and physician, proposed another kingdom, Protista, for unicellular organisms (Figure **4.3.1.2**). He later proposed a fourth kingdom, Monera, for unicellular organisms whose cells lack nuclei, like bacteria.

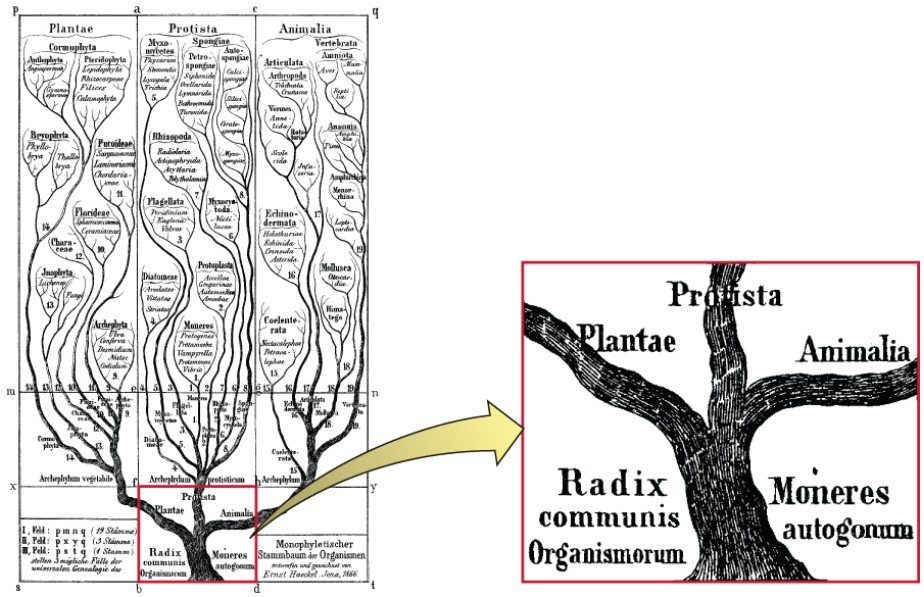


Figure 4.3.1.2: Ernst Haeckel's rendering of the tree of life, from his 1866 book *General Morphology of Organisms*, contained three kingdoms: Plantae, Protista, and Animalia. He later added a fourth kingdom, Monera, for unicellular organisms lacking a nucleus.

Nearly 100 years later, in 1969, American ecologist Robert Whittaker (1920–1980) proposed adding another kingdom—Fungi—in his tree of life. Whittaker's tree also contained a level of categorization above the kingdom level—the empire or superkingdom level—to distinguish between organisms that have membrane-bound nuclei in their cells (eukaryotes) and those that do not (prokaryotes). Empire Prokaryota contained just the Kingdom Monera. The Empire Eukaryota contained the other four kingdoms: Fungi, Protista, Plantae, and Animalia. Whittaker's five-kingdom tree was considered the standard phylogeny for many years.

Figure 4.3.1.3 shows how the tree of life has changed over time. Note that viruses are not found in any of these trees. That is because they are not made up of cells and thus it is difficult to determine where they would fit into a tree of life.

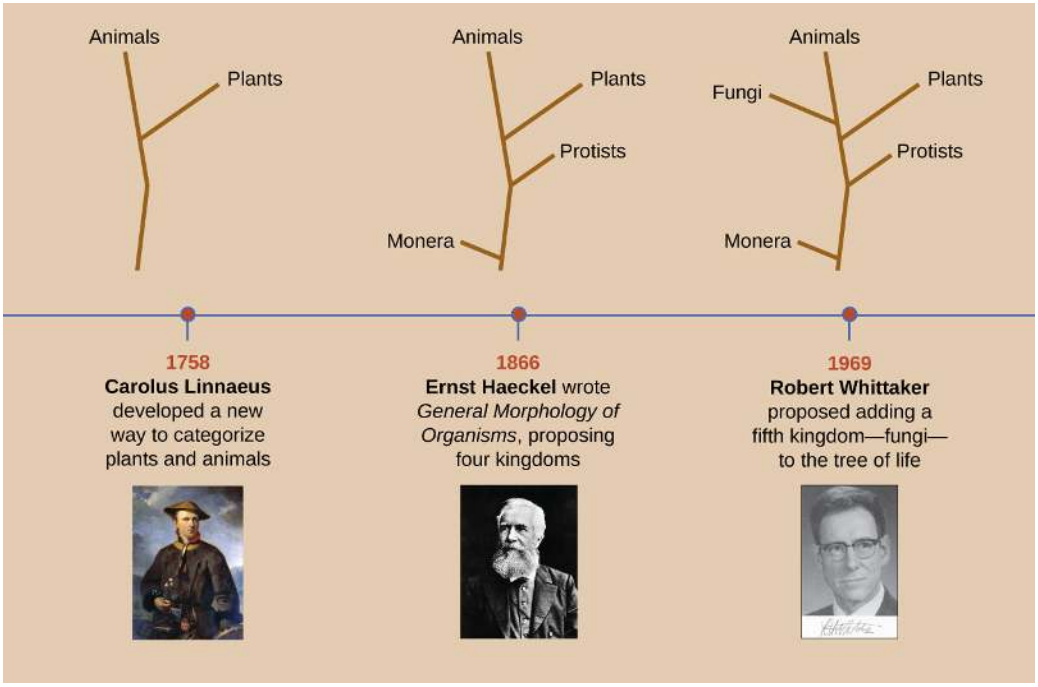


Figure 4.3.1.3: This timeline shows how the shape of the tree of life has changed over the centuries. Even today, the taxonomy of living organisms is continually being reevaluated and refined with advances in technology.

Exercise 4.3.1.1

Briefly summarize how our evolving understanding of microorganisms has contributed to changes in the way that organisms are classified.

Clinical Focus: Part 2

Antibiotic drugs are specifically designed to kill or inhibit the growth of bacteria. But after a couple of days on antibiotics, Cora shows no signs of improvement. Also, her CSF cultures came back from the lab negative. Since bacteria or fungi were not isolated from Cora's CSF sample, her doctor rules out bacterial and fungal meningitis. Viral meningitis is still a possibility.

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Exercise 4.3.1.2

What types of microorganisms could be causing Cora's symptoms?

The Role of Genetics in Modern Taxonomy

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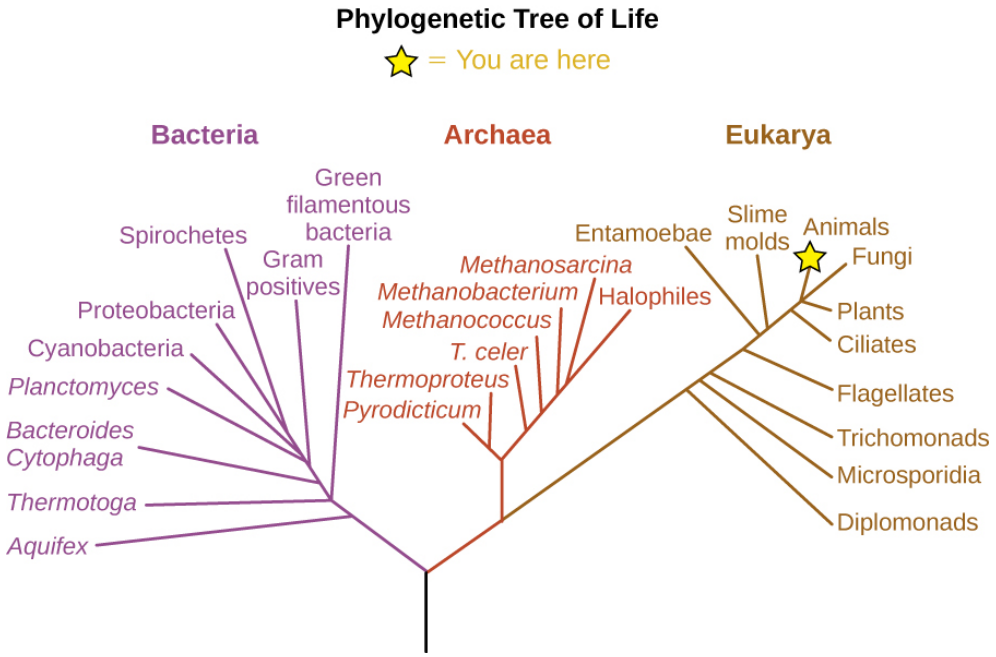


Figure **4.3.1.4**: Scientists continue to use analysis of RNA, DNA, and proteins to determine how organisms are related. One interesting, and complicating, discovery is that of horizontal gene transfer—when a gene of one species is absorbed into another organism's genome. Horizontal gene transfer is especially common in microorganisms and can make it difficult to determine how organisms are evolutionarily related. Consequently, some scientists now think in terms of "webs of life" rather than "trees of life."

? Exercise 4.3.1.3

1. In modern taxonomy, how do scientists determine how closely two organisms are related?
2. Explain why the branches on the "tree of life" all originate from a single "trunk."

Naming Microbes

In developing his taxonomy, Linnaeus used a system of binomial nomenclature, a two-word naming system for identifying organisms by genus and species. For example, modern humans are in the genus *Homo* and have the species name *sapiens*, so their scientific name in binomial nomenclature is *Homo sapiens*. In binomial nomenclature, the genus part of the name is always capitalized; it is followed by the species name, which is not capitalized. Both names are italicized.

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? Exercise 4.3.1.4

- What is binomial nomenclature and why is it a useful tool for naming organisms?
- Explain why a resource like one of Bergey's manuals would be helpful in identifying a microorganism in a sample.

Same Name, Different Strain

Within one species of microorganism, there can be several subtypes called strains. While different strains may be nearly identical genetically, they can have very different attributes. The bacterium*Escherichia coli* is infamous for causing food poisoning and traveler's diarrhea. However, there are actually many different strains of *E. coli*, and they vary in their ability to cause disease.

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Glossary

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eukaryote

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phylogeny
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prokaryote
an organism whose cell structure does not include a membrane-bound nucleus

taxonomy
the classification, description, identification, and naming of living organisms

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4.3.2: DOMAIN CLASSIFICATION ACCORDING TO WOESE

LEARNING OBJECTIVES

- Recognize the three major domains used for classification

The fact that biology has such a broad scope as a science has to do with the tremendous diversity of life on Earth. The source of this diversity is evolution, the process of gradual change during which new species arise from older species. Evolutionary biologists study the evolution of living things in everything from the microscopic world to ecosystems.

The evolution of various life forms on Earth can be summarized in a phylogenetic tree using phylogeny. A phylogenetic tree is a diagram showing the evolutionary relationships among biological species based on similarities and differences in genetic or physical traits or both. A phylogenetic tree is composed of nodes and branches. The internal nodes represent ancestors and are points in evolution when, based on scientific evidence, an ancestor is thought to have diverged to form two new species. The length of each branch is proportional to the time elapsed since the split.

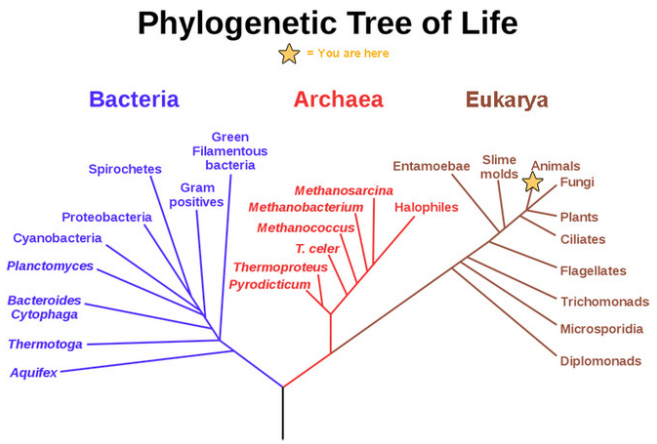


Figure **4.3.2.1**: Phylogenetic Tree of Life: This phylogenetic tree was constructed by microbiologist Carl Woese using data obtained from sequencing ribosomal RNA genes. The tree shows the separation of living organisms into three domains: Bacteria, Archaea, and Eukarya. Bacteria and Archaea are prokaryotes, single-celled organisms lacking intracellular organelles.

CARL WOESE AND THE PHYLOGENETIC TREE

In the past, biologists grouped living organisms into five kingdoms: animals, plants, fungi, protists, and bacteria. The organizational scheme was based mainly on physical features, as opposed to physiology, biochemistry, or molecular biology, all of which are used by modern systematics. The pioneering work of American microbiologist Carl Woese in the early 1970s has shown, however, that life on Earth has evolved along three lineages, now called domains—Bacteria, Archaea, and Eukarya. The first two are prokaryotic cells with microbes that lack membrane-enclosed nuclei and organelles. The third domain contains the eukaryotes and includes unicellular microorganisms together with the four original kingdoms (excluding bacteria). Woese defined Archaea as a new domain, and this resulted in a new taxonomic tree. Many organisms belonging to the Archaea domain live under extreme conditions and are called extremophiles. To construct his tree, Woese used genetic relationships rather than similarities based on morphology (shape).

Woese's tree was constructed from comparative sequencing of the genes that are universally distributed, present in every organism, and conserved (meaning that these genes have remained essentially unchanged throughout evolution). Woese's approach was revolutionary because comparisons of physical features are insufficient to differentiate between the prokaryotes that appear fairly similar in spite of their tremendous biochemical diversity and genetic variability. The comparison of homologous DNA and RNA sequences provided Woese with a sensitive device that revealed the extensive variability of prokaryotes, and which justified the separation of the prokaryotes into two domains: bacteria and archaea. DNA, the universal genetic material, contains the instructions for the structure and function of all living organisms and can be divided into genes whose expression varies between organisms. The RNA, which is transcribed from DNA, varies between organisms as well based on the expression of specific genes. Thus, to examine differences at this molecular level provides a more accurate depiction of the diversity which exists.

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KEY POINTS

- The three major Domains of Life include: Domain Bacteria, Domain Eukarya and Domain Archaea.
- Domain Bacteria and Domain Archaea include prokaryotic cells that lack membrane-enclosed nuclei and organelles.
- Domain Eukarya include eukaryotes and more complex organisms that contain membrane-bound nuclei and organelles.
- Carl Woese defined Archaea as a new domain and constructed the phylogentic tree of life which shows separation of all living organisms.
- The phylogenetic tree of life was constructed by Carl Woese using sequencing data of ribosomal RNA genes. Therefore, genetics classification surpassed morphological cataloguing, which was the traditional way of organizing living beings.

KEY TERMS

- **phylogeny**: the evolutionary history of an organism
- **extremophile**: an organism that lives under extreme conditions of temperature, salinity, etc; commercially important as a source of enzymes that operate under similar conditions
- **DNA**: a biopolymer of deoxyribonucleic acids (a type of nucleic acid) that has four different chemical groups, called bases: adenine, guanine, cytosine, and thymine

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4.4: Microscopy

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4.4.1: The Properties of Light

Learning Objectives

- Identify and define the characteristics of electromagnetic radiation (EMR) used in microscopy
- Explain how lenses are used in microscopy to manipulate visible and ultraviolet (UV) light

Clinical Focus: Part I

Cindy, a 17-year-old counselor at a summer sports camp, scraped her knee playing basketball 2 weeks ago. At the time, she thought it was only a minor abrasion that would heal, like many others before it. Instead, the wound began to look like an insect bite and has continued to become increasingly painful and swollen.

The camp nurse examines the lesion and observes a large amount of pus oozing from the surface. Concerned that Cindy may have developed a potentially aggressive infection, she swabs the wound to collect a sample from the infection site. Then she cleans out the pus and dresses the wound, instructing Cindy to keep the area clean and to come back the next day. When Cindy leaves, the nurse sends the sample to the closest medical lab to be analyzed under a microscope.

Exercise 4.4.1.1

What are some things we can learn about these bacteria by looking at them under a microscope?

Visible light consists of electromagnetic waves that behave like other waves. Hence, many of the properties of light that are relevant to microscopy can be understood in terms of light's behavior as a wave. An important property of light waves is the wavelength, or the distance between one peak of a wave and the next peak. The height of each peak (or depth of each trough) is called the amplitude. In contrast, the frequency of the wave is the rate of vibration of the wave, or the number of wavelengths within a specified time period (Figure **4.4.1.1**).

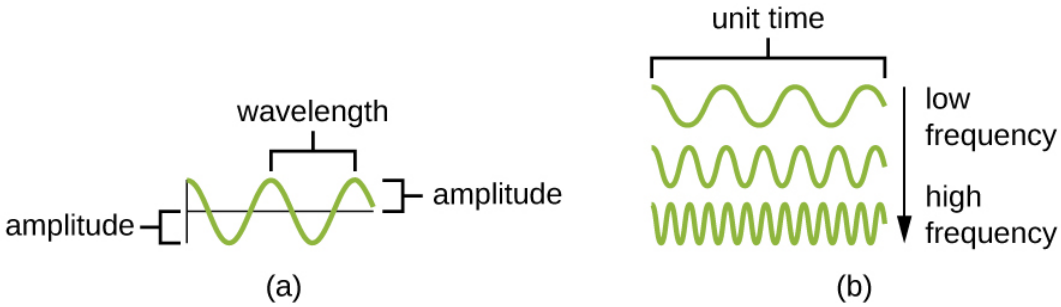
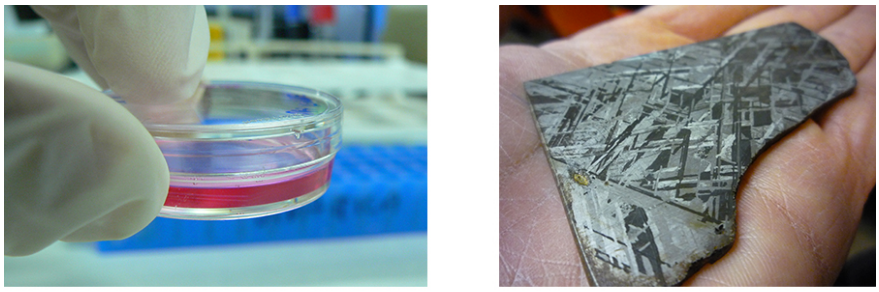


Figure **4.4.1.1**: (a) The amplitude is the height of a wave, whereas the wavelength is the distance between one peak and the next. (b) These waves have different frequencies, or rates of vibration. The wave at the top has the lowest frequency, since it has the fewest peaks per unit time. The wave at the bottom has the highest frequency.

Interactions of Light

Light waves interact with materials by being reflected, absorbed, or transmitted. Reflection occurs when a wave bounces off of a material. For example, a red piece of cloth may reflect red light to our eyes while absorbing other colors of light. Absorbanceoccurs when a material captures the energy of a light wave. In the case of glow-in-the-dark plastics, the energy from light can be absorbed and then later re-emitted as another form of phosphorescence. Transmission occurs when a wave travels through a material, like light through glass (the process of transmission is called transmittance). When a material allows a large proportion of light to be transmitted, it may do so because it is thinner, or more transparent (having more transparency and less opacity). Figure **4.4.1.2** illustrates the difference between transparency and opacity.



(a) (b)

Figure 4.4.1.2: (a) A Petri dish is made of transparent plastic or glass, which allows transmission of a high proportion of light. This transparency allows us to see through the sides of the dish to view the contents. (b) This slice of an iron meteorite is opaque (i.e., it has opacity). Light is not transmitted through the material, making it impossible to see the part of the hand covered by the object. (credit a: modification of work by Umberto Salvagnin; credit b: modification of work by "Waifer X"/Flickr)

Light waves can also interact with each other by interference, creating complex patterns of motion. Dropping two pebbles into a puddle causes the waves on the puddle's surface to interact, creating complex interference patterns. Light waves can interact in the same way.

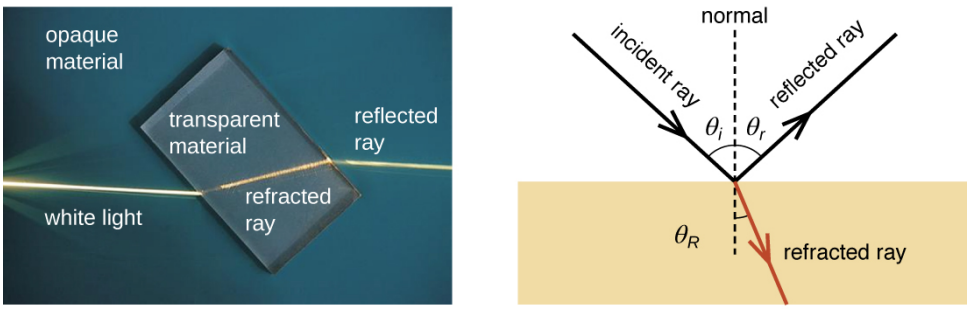
In addition to interfering with each other, light waves can also interact with small objects or openings by bending or scattering. This is called diffraction. Diffraction is larger when the object is smaller relative to the wavelength of the light (the distance between two consecutive peaks of a light wave). Often, when waves diffract in different directions around an obstacle or opening, they will interfere with each other.

? Exercise 4.4.1.2

1. If a light wave has a long wavelength, is it likely to have a low or high frequency?
2. If an object is transparent, does it reflect, absorb, or transmit light?

Lenses and Refraction

In the context of microscopy, refraction is perhaps the most important behavior exhibited by light waves. Refraction occurs when light waves change direction as they enter a new medium (Figure 4.4.1.3). Different transparent materials transmit light at different speeds; thus, light can change speed when passing from one material to another. This change in speed usually also causes a change in direction (refraction), with the degree of change dependent on the angle of the incoming light.



(a) (b)

Figure 4.4.1.3: (a) Refraction occurs when light passes from one medium, such as air, to another, such as glass, changing the direction of the light rays. (b) As shown in this diagram, light rays passing from one medium to another may be either refracted or reflected. (credit a: modification of work by "ajizai"/Wikimedia Commons).

The extent to which a material slows transmission speed relative to empty space is called the refractive index of that material. Large differences between the refractive indices of two materials will result in a large amount of refraction when light passes from one material to the other. For example, light moves much more slowly through water than through air, so light entering water from air can change direction greatly. We say that the water has a higher refractive index than air (Figure 4.4.1.4).



Figure 4.4.1.4: This straight pole appears to bend at an angle as it enters the water. This optical illusion is due to the large difference between the refractive indices of air and water.

When light crosses a boundary into a material with a higher refractive index, its direction turns to be closer to perpendicular to the boundary (i.e., more toward a normal to that boundary; Figure 4.4.1.5). This is the principle behind lenses. We can think of a lens as an object with a curved boundary (or a collection of prisms) that collects all of the light that strikes it and refracts it so that it all meets at a single point called the image point (focus). A convex lens can be used to magnify because it can focus at closer range than the human eye, producing a larger image. Concave lenses and mirrors can also be used in microscopes to redirect the light path. Figure 4.4.1.5 shows the focal point (the image point when light entering the lens is parallel) and the focal length (the distance to the focal point) for convex and concave lenses.

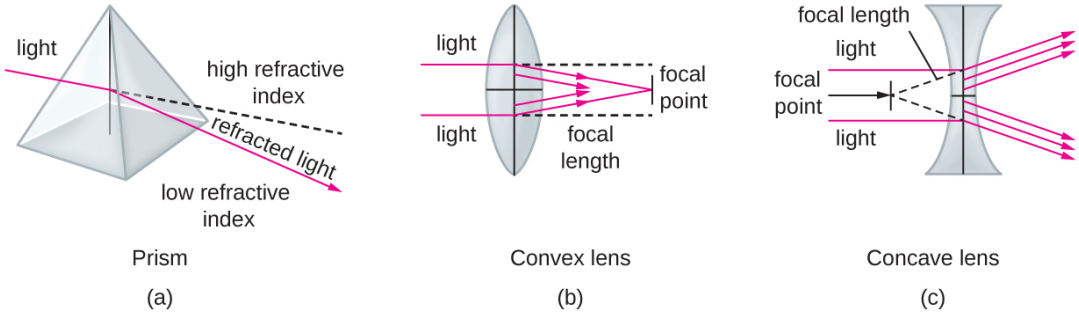


Figure 4.4.1.5: (a) A lens is like a collection of prisms, such as the one shown here. (b) When light passes through a convex lens, it is refracted toward a focal point on the other side of the lens. The focal length is the distance to the focal point. (c) Light passing through a concave lens is refracted away from a focal point in front of the lens.

The human eye contains a lens that enables us to see images. This lens focuses the light reflecting off of objects in front of the eye onto the surface of the retina, which is like a screen in the back of the eye. Artificial lenses placed in front of the eye (contact lenses, glasses, or microscopic lenses) focus light before it is focused (again) by the lens of the eye, manipulating the image that ends up on the retina (e.g., by making it appear larger).

Images are commonly manipulated by controlling the distances between the object, the lens, and the screen, as well as the curvature of the lens. For example, for a given amount of curvature, when an object is closer to the lens, the focal points are farther from the lens. As a result, it is often necessary to manipulate these distances to create a focused image on a screen. Similarly, more curvature creates image points closer to the lens and a larger image when the image is in focus. This property is often described in terms of the focal distance, or distance to the focal point.

? Exercise 4.4.1.3

1. Explain how a lens focuses light at the image point.
2. Name some factors that affect the focal length of a lens.

Electromagnetic Spectrum and Color

Visible light is just one form of electromagnetic radiation (EMR), a type of energy that is all around us. Other forms of EMR include microwaves, X-rays, and radio waves, among others. The different types of EMR fall on the electromagnetic spectrum, which is defined in terms of wavelength and frequency. The spectrum of visible light occupies a relatively small range of frequencies between infrared and ultraviolet light (Figure 4.4.1.6).

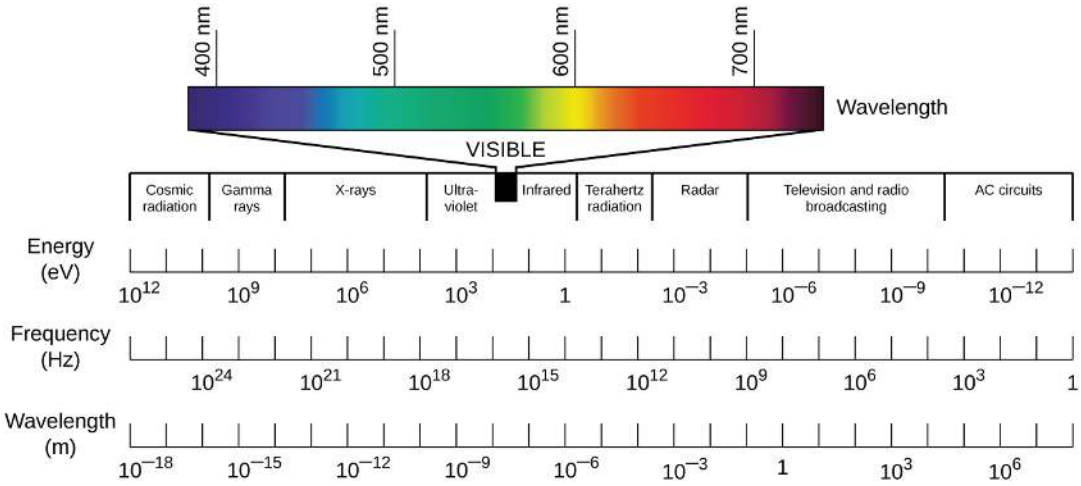


Figure **4.4.1.6**: The electromagnetic spectrum ranges from high-frequency gamma rays to low-frequency radio waves. Visible light is the relatively small range of electromagnetic frequencies that can be sensed by the human eye. On the electromagnetic spectrum, visible light falls between ultraviolet and infrared light. (credit: modification of work by Johannes Ahlmann).

Whereas wavelength represents the distance between adjacent peaks of a light wave, frequency, in a simplified definition, represents the rate of oscillation. Waves with higher frequencies have shorter wavelengths and, therefore, have more oscillations per unit time than lower-frequency waves. Higher-frequency waves also contain more energy than lower-frequency waves. This energy is delivered as elementary particles called photons. Higher-frequency waves deliver more energetic photons than lower-frequency waves.

Photons with different energies interact differently with the retina. In the spectrum of visible light, each color corresponds to a particular frequency and wavelength (Figure **4.4.1.6**).The lowest frequency of visible light appears as the color red, whereas the highest appears as the color violet. When the retina receives visible light of many different frequencies, we perceive this as white light. However, white light can be separated into its component colors using refraction. If we pass white light through a prism, different colors will be refracted in different directions, creating a rainbow-like spectrum on a screen behind the prism. This separation of colors is called dispersion, and it occurs because, for a given material, the refractive index is different for different frequencies of light.

Certain materials can refract nonvisible forms of EMR and, in effect, transform them into visible light. Certain fluorescent dyes, for instance, absorb ultraviolet or blue light and then use the energy to emit photons of a different color, giving off light rather than simply vibrating. This occurs because the energy absorption causes electrons to jump to higher energy states, after which they then almost immediately fall back down to their ground states, emitting specific amounts of energy as photons. Not all of the energy is emitted in a given photon, so the emitted photons will be of lower energy and, thus, of lower frequency than the absorbed ones. Thus, a dye such as Texas red may be excited by blue light, but emit red light; or a dye such as fluorescein isothiocyanate (FITC) may absorb (invisible) high-energy ultraviolet light and emit green light (Figure **4.4.1.7**). In some materials, the photons may be emitted following a delay after absorption; in this case, the process is called phosphorescence. Glow-in-the-dark plastic works by using phosphorescent material.

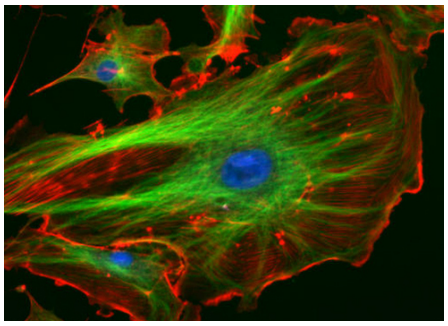


Figure **4.4.1.7**: The fluorescent dyes absorbed by these bovine pulmonary artery endothelial cells emit brilliant colors when excited by ultraviolet light under a fluorescence microscope. Various cell structures absorb different dyes. The nuclei are stained blue with 4’,6-diamidino-2-phenylindole (DAPI); microtubles are marked green by an antibody bound to FITC; and actin filaments are labeled red with phalloidin bound to tetramethylrhodamine (TRITC).

? Exercise 4.4.1.4

1. Which has a higher frequency: red light or green light?
2. Explain why dispersion occurs when white light passes through a prism.
3. Why do fluorescent dyes emit a different color of light than they absorb?

Magnification, Resolution, and Contrast

Microscopes magnify images and use the properties of light to create useful images of small objects. Magnification is defined as the ability of a lens to enlarge the image of an object when compared to the real object. For example, a magnification of 10× means that the image appears 10 times the size of the object as viewed with the naked eye.

Greater magnification typically improves our ability to see details of small objects, but magnification alone is not sufficient to make the most useful images. It is often useful to enhance the resolution of objects: the ability to tell that two separate points or objects are separate. A low-resolution image appears fuzzy, whereas a high-resolution image appears sharp. Two factors affect resolution. The first is wavelength. Shorter wavelengths are able to resolve smaller objects; thus, an electron microscope has a much higher resolution than a light microscope, since it uses an electron beam with a very short wavelength, as opposed to the long-wavelength visible light used by a light microscope. The second factor that affects resolution is numerical aperture, which is a measure of a lens’s ability to gather light. The higher the numerical aperture, the better the resolution.

Even when a microscope has high resolution, it can be difficult to distinguish small structures in many specimens because microorganisms are relatively transparent. It is often necessary to increase contrast to detect different structures in a specimen. Various types of microscopes use different features of light or electrons to increase contrast—visible differences between the parts of a specimen (see Instruments of Microscopy). Additionally, dyes that bind to some structures but not others can be used to improve the contrast between images of relatively transparent objects (see Staining Microscopic Specimens).

? Exercise 4.4.1.5

1. Explain the difference between magnification and resolution.
2. Explain the difference between resolution and contrast.
3. Name two factors that affect resolution.

Key Concepts and Summary

- Light waves interacting with materials may be reflected, absorbed, or transmitted, depending on the properties of the material.
- Light waves can interact with each other (interference) or be distorted by interactions with small objects or openings (diffraction).
- Refraction occurs when light waves change speed and direction as they pass from one medium to another. Differences in the refraction indices of two materials determine the magnitude of directional changes when light passes from one to the other.
- A lens is a medium with a curved surface that refracts and focuses light to produce an image.

- Visible light is part of the electromagnetic spectrum; light waves of different frequencies and wavelengths are distinguished as colors by the human eye.
- A prism can separate the colors of white light (dispersion) because different frequencies of light have different refractive indices for a given material.
- Fluorescent dyes and phosphorescent materials can effectively transform nonvisible electromagnetic radiation into visible light.
- The power of a microscope can be described in terms of its magnification and resolution.
- Resolution can be increased by shortening wavelength, increasing the numerical aperture of the lens, or using stains that enhance contrast.

Glossary

absorbance
when a molecule captures energy from a photon and vibrates or stretches, using the energy

amplitude
the height of a wave

contrast
visible differences between parts of a microscopic specimen

diffraction
the changing of direction (bending or spreading) that occurs when a light wave interacts with an opening or barrier

dispersion
the separation of light of different frequencies due to different degrees of refraction

fluorescent
the ability of certain materials to absorb energy and then immediately release that energy in the form of light

focal length
the distance from the lens to the image point when the object is at a definite distance from the lens (this is also the distance to the focal point)

focal point
a property of the lens; the image point when light entering the lens is parallel (i.e., the object is an infinite distance from the lens)

frequency
the rate of vibration for a light wave or other electromagnetic wave

image point (focus)
a property of the lens and the distance of the object to the lens; the point at which an image is in focus (the image point is often called the focus)

interference
distortion of a light wave due to interaction with another wave

magnification
the power of a microscope (or lens) to produce an image that appears larger than the actual specimen, expressed as a factor of the actual size

numerical aperture
a measure of a lens's ability to gather light

opacity
the property of absorbing or blocking light

phosphorescence
: the ability of certain materials to absorb energy and then release that energy as light after a delay

reflection
: when light bounces back from a surface

refraction
: bending of light waves, which occurs when a light wave passes from one medium to another

refractive index
: a measure of the magnitude of slowing of light waves by a particular medium

resolution
: the ability to distinguish between two points in an image

transmittance
: the amount of light that passes through a medium

transparency
: the property of allowing light to pass through

wavelength
: the distance between one peak of a wave and the next peak

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4.4.2: Types of Microscopes

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CHAPTER OVERVIEW

5: Infectious Diseases and Epidemiology

5.1: Microbe and Human Symbiotic Relationships
5.2: Human Microbiota
5.2.1: Opportunistic Pathogens
5.3: Infectious Diseases Characteristics and Classification
5.3.1: Koch’s Postulates
5.4: Virulence Factors
5.4.1: Virulence Factors that Promote Colonization
5.4.1.1: The Ability to Use Motility and Other Means to Contact Host Cells
5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal
5.4.1.3: The Ability to Evade Adaptive Immune Defenses
5.4.2: Virulence Factors that Damage the Host
5.5: Reservoirs of Infection and Disease Transmission
5.6: Introduction to Epidemiology
5.6.1: Epidemiology
5.6.1.1: Descriptive Epidemiology
5.6.1.2: Analytical Epidemiology
5.6.1.3: Experimental Epidemiology
5.6.2: Nosocomial Infections
5.6.2.1: Transmission of Nosocomial Infection
5.6.2.2: Control of Nosocomial Infections
5.6.3: Global Public Health
5.6.3I: Current Epidemics

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5.1: MICROBE AND HUMAN SYMBIOTIC RELATIONSHIPS

LEARNING OBJECTIVES

- Differentiate among the types of symbiosis: commensalism, mutualism, and parasitism

Symbiotic relationships, or symbioses (plural), are close interactions between individuals of different species over an extended period of time which impact the abundance and distribution of the associating populations. Most scientists accept this definition, but some restrict the term to only those species that are mutualistic, where both individuals benefit from the interaction.

COMMENSALISM

A commensalistic relationship occurs when one species benefits from the close, prolonged interaction, while the other neither benefits nor is harmed. Birds nesting in trees provide an example of a commensal relationship. The tree is not harmed by the presence of the nest among its branches. The nests are light and produce little strain on the structural integrity of the branch. Most of the leaves, which the tree uses to obtain energy by photosynthesis, are above the nest, so they are unaffected. The bird, on the other hand, benefits greatly. If the bird had to nest in the open, its eggs and young would be vulnerable to predators.



Figure **5.1.1**: Commensalistic relationship: The southern masked-weaver bird is starting to make a nest in a tree in Zambezi Valley, Zambia. This is an example of a commensal relationship, in which one species (the bird) benefits, while the other (the tree) neither benefits nor is harmed.

MUTUALISM

A second type of symbiotic relationship, mutualism, is where two species both benefit from their interaction. Some scientists believe that these are the only true examples of symbiosis. For example, termites have a mutualistic relationship with protozoa that live in the insect's gut. The termite benefits from the ability of bacterial symbionts within the protozoa to digest cellulose. The termite itself cannot do this; without the protozoa, it would not be able to obtain energy from its food (cellulose from the wood it chews and eats). The protozoa and the bacterial symbionts benefit by having a protective environment and a constant supply of food from the wood-chewing actions of the termite.

PARASITISM

A parasite is an organism that lives in or on another living organism, deriving nutrients from it. In this relationship the parasite benefits, but the organism being fed upon, the host, is harmed. The host is usually weakened by the parasite as it siphons resources the host would normally use to maintain itself. The parasite, however, is unlikely to kill the host. This is because the parasite needs the host to complete its reproductive cycle by spreading to another host.

The reproductive cycles of parasites are often very complex, sometimes requiring more than one host species. A tapeworm is a parasite that causes disease in humans when contaminated, undercooked meat such as pork, fish, or beef is consumed. The tapeworm can live inside the intestine of the host for several years, benefiting from the food the host is bringing into its gut by eating; it may grow to be over 50 ft long by adding segments. The parasite moves from species to species as it requires two hosts to complete its life cycle.

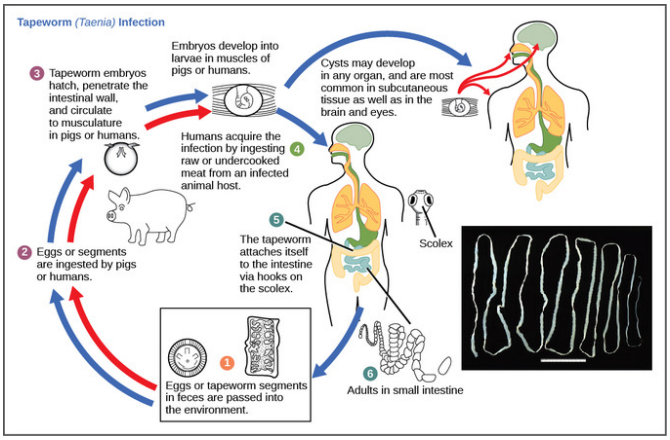


Figure **5.1.1**: Lifecycle of a parasitic tapeworm: This diagram shows the life cycle of a pork tapeworm (*Taenia solium*), a human worm parasite. The eggs of the tapeworm are ingested by the host. When they hatch, the worms travel through the wall of the intestine and begin to grow. Here, the parasite will absorb the nutrition from the host and continue to grow.

KEY POINTS

- Commensalism is when two organisms share the same environment, where one benefits and the other is unharmed.
- Trees and birds have a commensalistic relationship; the birds benefit from having a place to build their nests, while the trees are unharmed and not impacted by the bird's presence.

- Mutualism is when two species sharing the same environment both benefit from their interactions.
- The protozoans living within the intestines of termites create a mutualistic relationship with them; the protozoans get a safe place to live while the termites get help digesting the cellulose in their diet.
- Parasitism occurs when two organisms interact, but while one benefits, the other experiences harm.
- Parasites harm their hosts, as with the tapeworm attaching itself to the intestine of a cow; the tapeworm absorbs the nutrients from the cow's diet, preventing them from being absorbed by the cow.

KEY TERMS

- **mutualism**: Any interaction between two species that benefits both.
- **commensalism**: A sharing of the same environment by two organisms where one species benefits and the other is unaffected; e.g., barnacles on whales.
- **parasitism**: Interaction between two organisms, in which one organism (the parasite) benefits and the other (the host) is harmed.

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- **45.5C: Symbiosis** by Boundless is licensed CC BY-SA 4.0.

5.2: HUMAN MICROBIOTA

BENEFICIAL MICROBES: THE PHARMACY IN THE GUT

While some bacteria can cause disease, others play beneficial roles in human health. We have co-evolved with microbes in and on our body, with everyone having a unique set of microorganisms. The most abundant and well-studied microbiome is found in the gut. It has been estimated that the number of bacteria in the human gut may outnumber the cells in the body by an order of magnitude. Thus, one may consider the gut microbiome as a multicellular organ similar in size to the liver. Indeed, it is sometimes referred to as our "forgotten organ".

Generally, the microbiome within a given body habitat can be defined as the diversity and abundance distribution of distinct types of microorganisms. This microbial composition is highly influenced by individual factors such as diet, age, lifestyle, ethnicity, and host health, among others. Although no taxa are observed to be universally present among all individuals, some microbial patterns demonstrate broad prevalence. Most bacteria belong to the genera *Bacteroides*, *Clostridium*, *Fusobacterium*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, and *Bifidobacterium.*

THE EVOLUTION OF MICROBIOME DURING LIFE

Recent research suggests early in-utero microbial exposure during pregnancy. Following birth, the newborn's digestive tract is quickly colonized by microorganisms from the mother (vaginal, fecal, skin, breast milk, etc.) and the environment in which the delivery takes place. Following birth, the microbiome that enters and evolves in the infant's gut is dependent upon a number of factors, with delivery mode and feeding regime (breastfeeding vs infant formula feeding) of prime importance in the early days and weeks of life. By the age of 2 to 3 years, the microbiome becomes essentially established, having reached a steady state, and remains relatively stable throughout life. However, the gut microbiome continuously changes in response to daily variations in diet, lifestyle, age, and host physiological and immunological health.

HEALTH BENEFITS OF THE MICROBIOME

On the basis of the currently available literature, the gut microbiome is known to contribute to a number of important functions in the host, from protective, immunomodulatory, metabolic to trophic roles. These are promoted via a number of mechanisms. For example, members of the gut microbiome can produce anti-inflammatory factors, pain relieving compounds, antioxidants, and vitamins to protect and nurture the body. Additionally, they may prevent attachment and action of harmful bacteria that can produce toxins causing chronic disease. This close and specific contact with human cells, exchanging nutrients and metabolic wastes, makes symbiotic bacteria essentially a human organ.

GASTROINTESTINAL INFECTION PREVENTION

The indigenous intestinal microbiome serves as a line of resistance to colonization by exogenous microbes such as *Clostridium difficile* and *Helicobacter pylori*, and thus assists in competitive exclusion of pathogens preventing the potential invasion, termed colonization resistance. Indeed, antibiotic-associated diarrhea occurs when antibiotic treatment disturbs the natural balance of the gut microbiome causing harmful bacteria (i.e., *Clostridium difficile*) to proliferate and multiply. Oral probiotics may reduce antibiotic-associated diarrhea significantly.

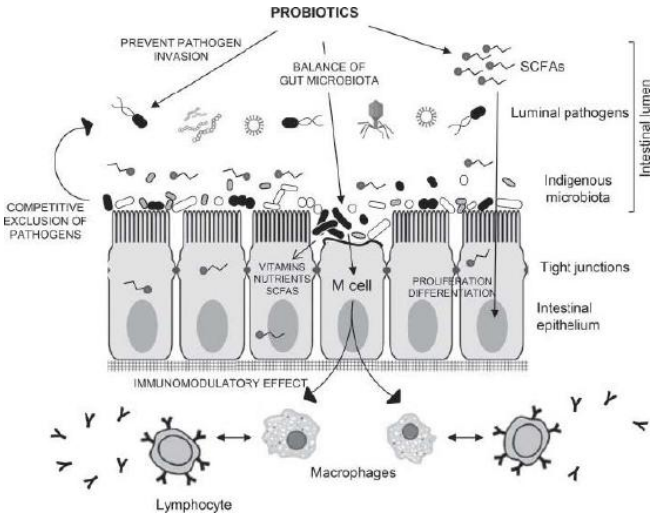


Figure **5.2.1**: Schematic representation of the cross-talk interaction of indigenous microbiome and oral probiotics with the intestinal epithelium. The intestinal microbiome protects the mucosa from invasion by pathogens. These probiotic bacteria may also allow beneficial effects through the release of nutrients (vitamins, SCFAs sugars) which are absorbed in the small intestine. Human microbes and probiotics would also interact with MALT macrophages and naive lymphocyte cells, allowing a mucosal anti-inflammatory response.

IMMUNOMODULATORY EFFECTS

Commensal bacteria can interact with the host immune system in ways that control the host's immune response and counteracts the development of the disease. The complex interactions that may occur between ingested probiotic bacteria, commensals, and the mucosal surface are possible because of the mucosa-associated immune system, typically organized into MALT (Mucosal Associated Lymphoid Tissue, such as Peyer's patches). This cross-talk interaction enhances cellular immune response characterized by activation of macrophages, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines. Furthermore, some probiotics may be effective in the prevention and/or alleviation of allergies and auto-immune diseases like irritable bowel syndrome and inflammatory bowel diseases (Crohn's disease and ulcerative colitis).

NUTRITIONAL BENEFITS

The metabolic activity of the gut microbiome makes an important contribution to the host's nutritional status via its ability to synthesize certain vitamins and various bioactive metabolites, such as short-chain fatty acids (SCFA), that then become bioavailable to the host. It has been reported that consumption of yogurt containing *Lactobacillus bulgaricus* or *acidophilus* could alleviate lactose intolerance during gastric passage through their enzyme lactase. However, the major metabolic function of the colonic microflora is the fermentation of nondigestible carbohydrates, which are key sources of energy in the colon. These carbohydrates also include large polysaccharides (i.e., resistant starches, pectins, and cellulose) and some oligosaccharides that escape digestion, as well as unabsorbed sugars and alcohols. Other benefits of the gut microbiome on human health, such as a role in supporting the health of the reproductive tract, oral cavity, lungs, skin, and gut-brain axis is currently under investigation.

PROBIOTIC IMBALANCE

When the normal composition of the microbiome is thrown off balance, there is a potential risk of disease. A decrease in microbiome diversity has been linked to cancer, asthma, Parkinson's, obesity, Alzheimer's, type-2 diabetes, cardiovascular disease, and possibly even autism in comparison to healthy subjects. Over-the-counter probiotics can help. In order to arrive alive at their workplace (i.e. the gastrointestinal tract), orally administered probiotics must be able to resist stomach acid, bile, and the effects of digestive enzymes. Certain mechanisms of action (such as the delivery of certain enzymes to the intestine) may not require live cells to play a physiologic benefit. Hence, a probiotic must contain as many live bacteria as claimed on the label. In addition, to survive the stomach and arrive at the intestine in optimal numbers, probiotic strains must be able to adhere to the intestinal epithelium and/or mucus, persist and multiply in the gut to maintain its metabolic activity and confer their probiotic properties in the human body.

RESOURCES

- BIOENGINEERED, 2016, VOL. 7, NO. 1, 11–20: http://dx.doi.org/10.1080/21655979.2015.1126015
- REVIEW: Beneficial Microbes: The pharmacy in the gut, Daniel M. Linares, Paul Ross, and Catherine Stanton, Food Biosciences Department, Teagasc Food Research Center, Moorepark, Fermoy, Cork, Ireland; APC, Microbiome Institute, University College Cork, Cork, Ireland; Biosciences Institute, University College Cork, Cork, Ireland

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5.2.1: Opportunistic Pathogens

Learning Objectives

- Differentiate between primary and opportunistic pathogens in regards to host involvement

Host-pathogen interactions are the interactions that take place between a pathogen (e.g. virus, bacteria) and their host (e.g. humans, plants). By definition, all pathogens damage their host to some extent. Infectious diseases result from the interplay between the pathogens and the defenses of the hosts they infect. The appearance and severity of disease resulting from the presence of any pathogen depends upon the ability of that pathogen to damage the host as well as the ability of the host to resist the pathogen.

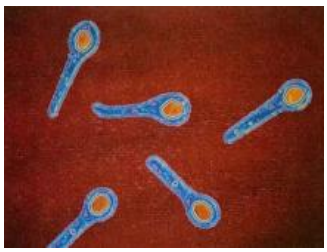


Figure: **Clostridium tetani**: Clostridium tetani are pathogenic bacteria.

Clinicians therefore classify infectious microorganisms or microbes according to the status of host defenses – either as primary pathogens or as opportunistic pathogens.

Primary pathogens cause disease as a result of their presence or activity within the normal, healthy host, and their intrinsic virulence is, in part, a necessary consequence of their need to reproduce and spread. Many of the most common primary pathogens of humans only infect humans; however many serious diseases are caused by organisms acquired from the environment or which infect non-human hosts.

Organisms which cause an infectious disease in a host with depressed resistance are classified as opportunistic pathogens. Opportunistic diseases may be caused by microbes that are ordinarily in contact with the host, such as pathogenic bacteria or fungi in the gastrointestinal or the upper respiratory tract, and they may also result from (otherwise innocuous) microbes acquired from other hosts or from the environment as a result of traumatic introduction. An opportunistic disease requires impairment of host defenses, which may occur as a result of several factors such as genetic defects, exposure to antimicrobial drugs or immunosuppressive chemicals, exposure to ionizing radiation, or as a result of an infectious disease with immunosuppressive activity. Primary pathogens may also cause more severe disease in a host with depressed resistance than would normally occur in an immunosufficient host.

Key Points

- All pathogens damage their host to some extent, usually resulting in an infectious disease from the interplay between the pathogens and the defenses of the hosts they infect.
- Clinicians classify infectious microorganisms or microbes according to the status of host defenses – either as primary pathogens or as opportunistic pathogens.
- Primary pathogens cause disease as a result of their presence or activity within the normal, healthy host, and their intrinsic virulence is, in part, a necessary consequence of their need to reproduce and spread.
- Organisms which cause an infectious disease in a host with depressed resistance are classified as opportunistic pathogens.

Key Terms

- **host**: A cell or organism which harbors another organism or biological entity, usually a parasite.
- **pathogen**: Any organism or substance, especially a microorganism, capable of causing disease, such as bacteria, viruses, protozoa, or fungi. Microorganisms are not considered to be pathogenic until they have reached a population size that is large enough to cause disease.

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- **10.2C: Extent of Host Involvement** by Boundless is licensed CC BY-SA 4.0. Original source: https://courses.lumenlearning.com/boundless-microbiology/.

5.3: Infectious Diseases Characteristics and Classification

Learning Objectives

- Distinguish between signs and symptoms of disease
- Explain the difference between a communicable disease and a noncommunicable disease
- Compare different types of infectious diseases, including iatrogenic, nosocomial, and zoonotic diseases
- Identify and describe the stages of an acute infectious disease in terms of number of pathogens present and severity of signs and symptoms

A disease is any condition in which the normal structure or functions of the body are damaged or impaired. Physical injuries or disabilities are not classified as disease, but there can be several causes for disease, including infection by a pathogen, genetics (as in many cancers or deficiencies), noninfectious environmental causes, or inappropriate immune responses. Our focus in this chapter will be on infectious diseases, although when diagnosing infectious diseases, it is always important to consider possible noninfectious causes.

Clinical Focus: Part 1

Michael, a 10-year-old boy in generally good health, went to a birthday party on Sunday with his family. He ate many different foods but was the only one in the family to eat the undercooked hot dogs served by the hosts. Monday morning, he woke up feeling achy and nauseous, and he was running a fever of 38 °C (100.4 °F). His parents, assuming Michael had caught the flu, made him stay home from school and limited his activities. But after 4 days, Michael began to experience severe headaches, and his fever spiked to 40 °C (104 °F). Growing worried, his parents finally decide to take Michael to a nearby clinic.

Exercise 5.3.1

1. What signs and symptoms is Michael experiencing?
2. What do these signs and symptoms tell us about the stage of Michael's disease?

Signs and Symptoms of Disease

An infection is the successful colonization of a host by a microorganism. Infections can lead to disease, which causes signs and symptoms resulting in a deviation from the normal structure or functioning of the host. Microorganisms that can cause disease are known as pathogens.

The signs of disease are objective and measurable, and can be directly observed by a clinician. Vital signs, which are used to measure the body's basic functions, include body temperature (normally 37 °C [98.6 °F]), heart rate (normally 60–100 beats per minute), breathing rate (normally 12–18 breaths per minute), and blood pressure (normally between 90/60 and 120/80 mm Hg). Changes in any of the body's vital signs may be indicative of disease. For example, having a fever (a body temperature significantly higher than 37 °C or 98.6 °F) is a sign of disease because it can be measured.

In addition to changes in vital signs, other observable conditions may be considered signs of disease. For example, the presence of antibodies in a patient's serum (the liquid portion of blood that lacks clotting factors) can be observed and measured through blood tests and, therefore, can be considered a sign. However, it is important to note that the presence of antibodies is not always a sign of an active disease. Antibodies can remain in the body long after an infection has resolved; also, they may develop in response to a pathogen that is in the body but not currently causing disease.

Unlike signs, symptoms of disease are subjective. Symptoms are felt or experienced by the patient, but they cannot be clinically confirmed or objectively measured. Examples of symptoms include nausea, loss of appetite, and pain. Such symptoms are important to consider when diagnosing disease, but they are subject to memory bias and are difficult to measure precisely. Some clinicians attempt to quantify symptoms by asking patients to assign a numerical value to their symptoms. For example, the Wong-Baker Faces pain-rating scale asks patients to rate their pain on a scale of 0–10. An alternative method of quantifying pain is

measuring skin conductance fluctuations. These fluctuations reflect sweating due to skin sympathetic nerve activity resulting from the stressor of pain.[1]

A specific group of signs and symptoms characteristic of a particular disease is called a syndrome. Many syndromes are named using a nomenclature based on signs and symptoms or the location of the disease. Table **5.3.1** lists some of the prefixes and suffixes commonly used in naming syndromes.

Table **5.3.1**: Nomenclature of Symptoms

Affix	Meaning	Example
cyto-	cell	cytopenia: reduction in the number of blood cells
hepat-	of the liver	hepatitis: inflammation of the liver
-pathy	disease	neuropathy: a disease affecting nerves
-emia	of the blood	bacteremia: presence of bacteria in blood
-itis	inflammation	colitis: inflammation of the colon
-lysis	destruction	hemolysis: destruction of red blood cells
-oma	tumor	lymphoma: cancer of the lymphatic system
-osis	diseased or abnormal condition	leukocytosis: abnormally high number of white blood cells
-derma	of the skin	keratoderma: a thickening of the skin

Clinicians must rely on signs and on asking questions about symptoms, medical history, and the patient's recent activities to identify a particular disease and the potential causative agent. Diagnosis is complicated by the fact that different microorganisms can cause similar signs and symptoms in a patient. For example, an individual presenting with symptoms of diarrhea may have been infected by one of a wide variety of pathogenic microorganisms. Bacterial pathogens associated with diarrheal disease include *Vibrio cholerae*, *Listeria monocytogenes*, *Campylobacter jejuni*, and enteropathogenic *Escherichia coli* (EPEC). Viral pathogens associated with diarrheal disease include norovirus and rotavirus. Parasitic pathogens associated with diarrhea include *Giardia lamblia* and *Cryptosporidium parvum*. Likewise, fever is indicative of many types of infection, from the common cold to the deadly Ebola hemorrhagic fever.

Finally, some diseases may be asymptomatic or subclinical, meaning they do not present any noticeable signs or symptoms. For example, most individual infected with herpes simplex virus remain asymptomatic and are unaware that they have been infected.

? Exercise 5.3.2

Explain the difference between signs and symptoms.

Classifications of Disease

The World Health Organization's (WHO) International Classification of Diseases (ICD) is used in clinical fields to classify diseases and monitor morbidity (the number of cases of a disease) and mortality (the number of deaths due to a disease). In this section, we will introduce terminology used by the ICD (and in health-care professions in general) to describe and categorize various types of disease.

An **infectious disease** is any disease caused by the direct effect of a pathogen. A pathogen may be cellular (bacteria, parasites, and fungi) or acellular (viruses, viroids, and prions). Some infectious diseases are also communicable, meaning they are capable of being spread from person to person through either direct or indirect mechanisms. Some infectious communicable diseases are also considered contagious diseases, meaning they are easily spread from person to person. Not all contagious diseases are equally so; the degree to which a disease is contagious usually depends on how the pathogen is transmitted. For example, measles is a highly

contagious viral disease that can be transmitted when an infected person coughs or sneezes and an uninfected person breathes in droplets containing the virus. Gonorrhea is not as contagious as measles because transmission of the pathogen (*Neisseria gonorrhoeae*) requires close intimate contact (usually sexual) between an infected person and an uninfected person.

Diseases that are contracted as the result of a medical procedure are known as **iatrogenic diseases**. Iatrogenic diseases can occur after procedures involving wound treatments, catheterization, or surgery if the wound or surgical site becomes contaminated. For example, an individual treated for a skin wound might acquire necrotizing fasciitis (an aggressive, "flesh-eating" disease) if bandages or other dressings became contaminated by *Clostridium perfringens* or one of several other bacteria that can cause this condition.

Diseases acquired in hospital settings are known as **nosocomial diseases**. Several factors contribute to the prevalence and severity of nosocomial diseases. First, sick patients bring numerous pathogens into hospitals, and some of these pathogens can be transmitted easily via improperly sterilized medical equipment, bed sheets, call buttons, door handles, or by clinicians, nurses, or therapists who do not wash their hands before touching a patient. Second, many hospital patients have weakened immune systems, making them more susceptible to infections. Compounding this, the prevalence of antibiotics in hospital settings can select for drug-resistant bacteria that can cause very serious infections that are difficult to treat.

Certain infectious diseases are not transmitted between humans directly but can be transmitted from animals to humans. Such a disease is called zoonotic disease (or zoonosis). According to WHO, a zoonosis is a disease that occurs when a pathogen is transferred from a vertebrate animal to a human; however, sometimes the term is defined more broadly to include diseases transmitted by all animals (including invertebrates). For example, rabies is a viral zoonotic disease spread from animals to humans through bites and contact with infected saliva. Many other zoonotic diseases rely on insects or other arthropods for transmission. Examples include yellow fever (transmitted through the bite of mosquitoes infected with yellow fever virus) and Rocky Mountain spotted fever (transmitted through the bite of ticks infected with *Rickettsia rickettsii*).

In contrast to communicable infectious diseases, a noncommunicable infectious disease is not spread from one person to another. One example is tetanus, caused by *Clostridium tetani*, a bacterium that produces endospores that can survive in the soil for many years. This disease is typically only transmitted through contact with a skin wound; it cannot be passed from an infected person to another person. Similarly, Legionnaires disease is caused by *Legionella pneumophila*, a bacterium that lives within amoebae in moist locations like water-cooling towers. An individual may contract Legionnaires disease via contact with the contaminated water, but once infected, the individual cannot pass the pathogen to other individuals.

In addition to the wide variety of noncommunicable infectious diseases, noninfectious diseases (those not caused by pathogens) are an important cause of morbidity and mortality worldwide. Noninfectious diseases can be caused by a wide variety factors, including genetics, the environment, or immune system dysfunction, to name a few. For example, sickle cell anemia is an inherited disease caused by a genetic mutation that can be passed from parent to offspring (Figure **5.3.1**). Other types of noninfectious diseases are listed in Table **5.3.2**.

Table **5.3.2**: Types of Noninfectious Diseases

Type	Definition	Example
Inherited	A genetic disease	Sickle cell anemia
Congenital	Disease that is present at or before birth	Down syndrome
Degenerative	Progressive, irreversible loss of function	Parkinson disease (affecting central nervous system)
Nutritional deficiency	Impaired body function due to lack of nutrients	Scurvy (vitamin C deficiency)
Endocrine	Disease involving malfunction of glands that release hormones to regulate body functions	Hypothyroidism – thyroid does not produce enough thyroid hormone, which is important for metabolism
Neoplastic	Abnormal growth (benign or malignant)	Some forms of cancer
Idiopathic	Disease for which the cause is unknown	Idiopathic juxtafoveal retinal telangiectasia (dilated, twisted blood vessels in the retina of the eye)

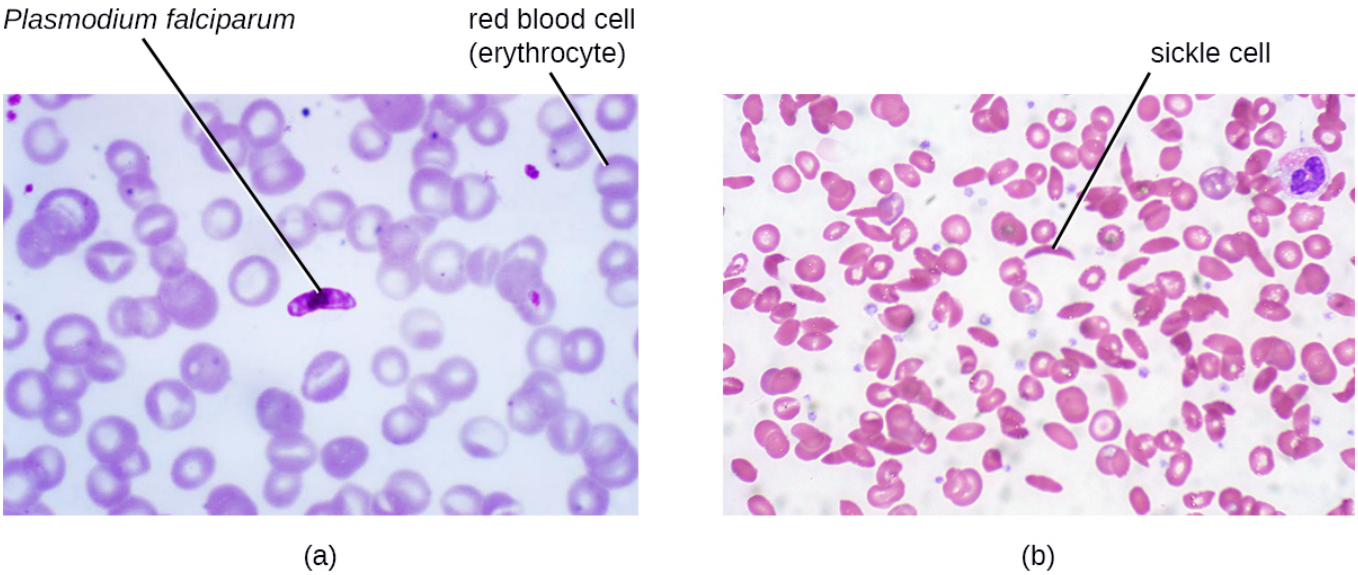


Figure **5.3.1**: Blood smears showing two diseases of the blood. (a) Malaria is an infectious, zoonotic disease caused by the protozoan pathogen *Plasmodium falciparum* (shown here) and several other species of the genus *Plasmodium*. It is transmitted by mosquitoes to humans. (b) Sickle cell disease is a noninfectious genetic disorder that results in abnormally shaped red blood cells, which can stick together and obstruct the flow of blood through the circulatory system. It is not caused by a pathogen, but rather a genetic mutation. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Ed Uthman)

Link to Learning

Lists of common infectious diseases can be found at the following Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), and International Classification of Diseases websites.

Exercise 5.3.3

1. Describe how a disease can be infectious but not contagious.
2. Explain the difference between iatrogenic disease and nosocomial disease.

Periods of Disease

The five periods of disease (sometimes referred to as stages or phases) include the incubation, prodromal, illness, decline, and convalescence periods (Figure 5.3.2). The incubation period occurs in an acute disease after the initial entry of the pathogen into the host (patient). It is during this time the pathogen begins multiplying in the host. However, there are insufficient numbers of pathogen particles (cells or viruses) present to cause signs and symptoms of disease. Incubation periods can vary from a day or two in acute disease to months or years in chronic disease, depending upon the pathogen. Factors involved in determining the length of the incubation period are diverse, and can include strength of the pathogen, strength of the host immune defenses, site of infection, type of infection, and the size infectious dose received. During this incubation period, the patient is unaware that a disease is beginning to develop.

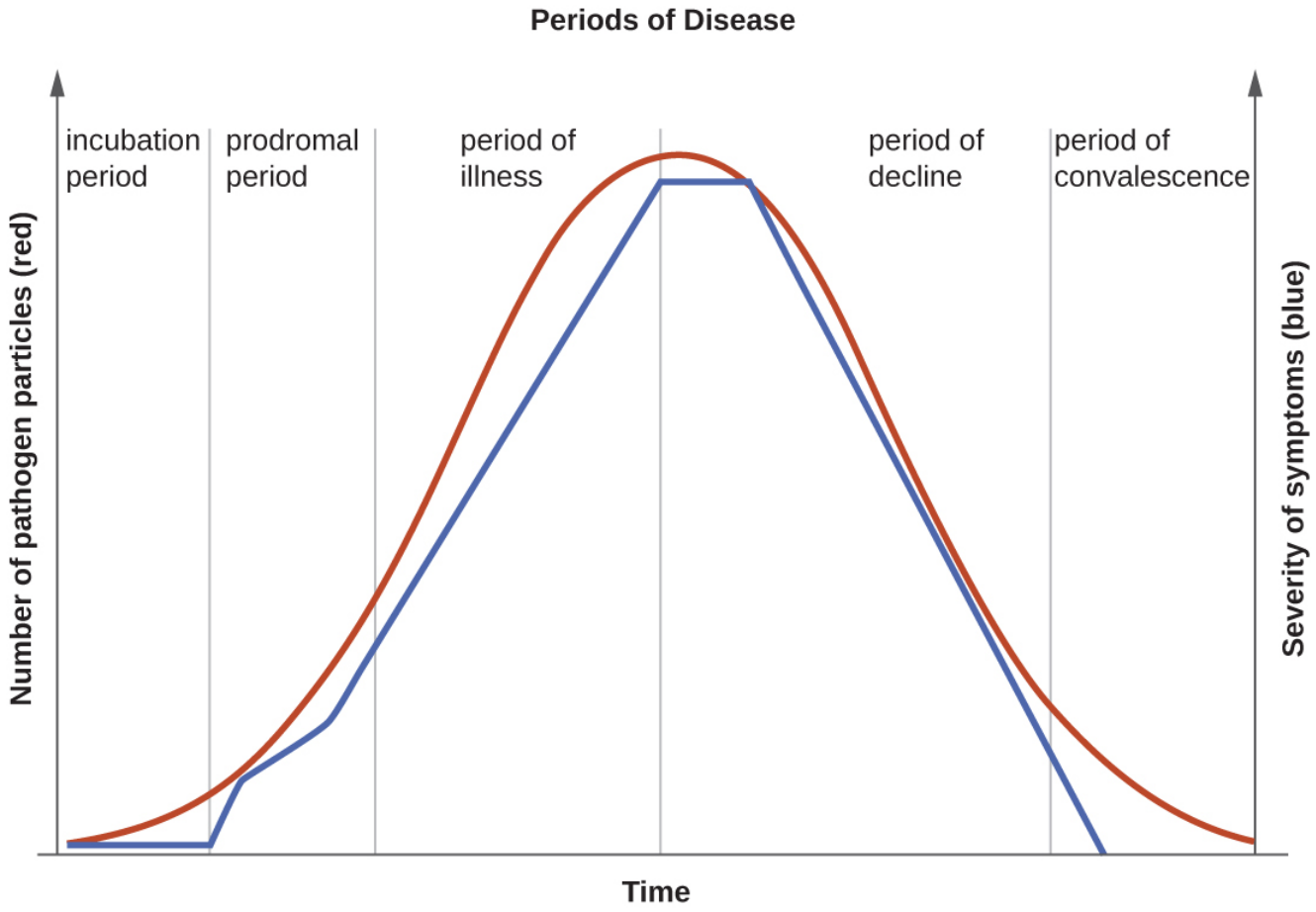


Figure 5.3.2: The progression of an infectious disease can be divided into five periods, which are related to the number of pathogen particles (red) and the severity of signs and symptoms (blue).

The prodromal period occurs after the incubation period. During this phase, the pathogen continues to multiply and the host begins to experience general signs and symptoms of illness, which typically result from activation of the immune system, such as fever, pain, soreness, swelling, or inflammation. Usually, such signs and symptoms are too general to indicate a particular disease. Following the prodromal period is the period of illness, during which the signs and symptoms of disease are most obvious and severe.

The period of illness is followed by the period of decline, during which the number of pathogen particles begins to decrease, and the signs and symptoms of illness begin to decline. However, during the decline period, patients may become susceptible to developing secondary infections because their immune systems have been weakened by the primary infection. The final period is known as the period of convalescence. During this stage, the patient generally returns to normal functions, although some diseases may inflict permanent damage that the body cannot fully repair.

Infectious diseases can be contagious during all five of the periods of disease. Which periods of disease are more likely to associated with transmissibility of an infection depends upon the disease, the pathogen, and the mechanisms by which the disease

develops and progresses. For example, with meningitis (infection of the lining of brain), the periods of infectivity depend on the type of pathogen causing the infection. Patients with bacterial meningitis are contagious during the incubation period for up to a week before the onset of the prodromal period, whereas patients with viral meningitis become contagious when the first signs and symptoms of the prodromal period appear. With many viral diseases associated with rashes (e.g., chickenpox, measles, rubella, roseola), patients are contagious during the incubation period up to a week before the rash develops. In contrast, with many respiratory infections (e.g., colds, influenza, diphtheria, strep throat, and pertussis) the patient becomes contagious with the onset of the prodromal period. Depending upon the pathogen, the disease, and the individual infected, transmission can still occur during the periods of decline, convalescence, and even long after signs and symptoms of the disease disappear. For example, an individual recovering from a diarrheal disease may continue to carry and shed the pathogen in feces for some time, posing a risk of transmission to others through direct contact or indirect contact (e.g., through contaminated objects or food).

? Exercise 5.3.4

Name some of the factors that can affect the length of the incubation period of a particular disease.

Acute and Chronic Diseases

The duration of the period of illness can vary greatly, depending on the pathogen, effectiveness of the immune response in the host, and any medical treatment received. For an acute disease, pathologic changes occur over a relatively short time (e.g., hours, days, or a few weeks) and involve a rapid onset of disease conditions. For example, influenza (caused by Influenzavirus) is considered an acute disease because the incubation period is approximately 1–2 days. Infected individuals can spread influenza to others for approximately 5 days after becoming ill. After approximately 1 week, individuals enter the period of decline.

For a chronic disease, pathologic changes can occur over longer time spans (e.g., months, years, or a lifetime). For example, chronic gastritis (inflammation of the lining of the stomach) is caused by the gram-negative bacterium *Helicobacter pylori*. *H. pylori* is able to colonize the stomach and persist in its highly acidic environment by producing the enzyme urease, which modifies the local acidity, allowing the bacteria to survive indefinitely.[2] Consequently, *H. pylori* infections can recur indefinitely unless the infection is cleared using antibiotics.[3] Hepatitis B virus can cause a chronic infection in some patients who do not eliminate the virus after the acute illness. A chronic infection with hepatitis B virus is characterized by the continued production of infectious virus for 6 months or longer after the acute infection, as measured by the presence of viral antigen in blood samples.

In latent diseases, as opposed to chronic infections, the causal pathogen goes dormant for extended periods of time with no active replication. Examples of diseases that go into a latent state after the acute infection include herpes(herpes simplex viruses [HSV-1 and HSV-2]), chickenpox (varicella-zoster virus [VZV]), and mononucleosis (Epstein-Barr virus [EBV]). HSV-1, HSV-2, and VZV evade the host immune system by residing in a latent form within cells of the nervous system for long periods of time, but they can reactivate to become active infections during times of stress and immunosuppression. For example, an initial infection by VZV may result in a case of childhood chickenpox, followed by a long period of latency. The virus may reactivate decades later, causing episodes of shingles in adulthood. EBV goes into latency in B cells of the immune system and possibly epithelial cells; it can reactivate years later to produce B-cell lymphoma.

? Exercise 5.3.5

Explain the difference between latent disease and chronic disease.

Key Concepts and Summary

- In an **infection**, a microorganism enters a host and begins to multiply. Some infections cause **disease**, which is any deviation from the normal function or structure of the host.
- **Signs** of a disease are objective and are measured. **Symptoms** of a disease are subjective and are reported by the patient.
- Diseases can either be **noninfectious** (due to genetics and environment) or **infectious** (due to pathogens). Some infectious diseases are **communicable** (transmissible between individuals) or **contagious** (easily transmissible between individuals); others are **noncommunicable**, but may be contracted via contact with environmental reservoirs or animals (**zoonoses**)
- **Nosocomial diseases** are contracted in hospital settings, whereas **iatrogenic disease** are the direct result of a medical procedure

- An **acute disease** is short in duration, whereas a **chronic disease** lasts for months or years. **Latent diseases** last for years, but are distinguished from chronic diseases by the lack of active replication during extended dormant periods.
- The periods of disease include the **incubation period**, the **prodromal period**, the **period of illness**, the **period of decline**, and the **period of convalescence**. These periods are marked by changes in the number of infectious agents and the severity of signs and symptoms.

Footnotes

1. 1 F. Savino et al. “Pain Assessment in Children Undergoing Venipuncture: The Wong–Baker Faces Scale Versus Skin Conductance Fluctuations.” *PeerJ* 1 (2013):e37; https://peerj.com/articles/37/
2. 2 J.G. Kusters et al. Pathogenesis of *Helicobacter pylori* Infection. *Clinical Microbiology Reviews* 19 no. 3 (2006):449–490.
3. 3 N.R. Salama et al. “Life in the Human Stomach: Persistence Strategies of the Bacterial Pathogen *Helicobacter pylori*.” *Nature Reviews Microbiology* 11 (2013):385–399.

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5.3.1: Koch's Postulates

Learning Objectives

- List Koch's postulates

Koch's postulates are four criteria designed to establish a causal relationship between a causative microbe and a disease. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 and refined and published by Koch in 1890. Koch applied the postulates to establish the etiology of anthrax and tuberculosis, but they have been generalized to other diseases.



Figure: ***Robert Koch****: Robert Koch circa 1900. Koch's postulates are four criteria designed in the 1880's to establish a causal relationship between a causative microbe and a disease.*

Koch's postulates were developed in the 19th century as general guidelines to identify pathogens that could be isolated with the techniques of the day. Even in Koch's time, it was recognized that some infectious agents were clearly responsible for disease even though they did not fulfill all of the postulates. Attempts to rigidly apply Koch's postulates to the diagnosis of viral diseases in the late 19th century, at a time when viruses could not be seen or isolated in culture, may have impeded the early development of the field of virology. Currently, a number of infectious agents are accepted as the cause of disease despite their not fulfilling all of Koch's postulates. Therefore, while Koch's postulates retain historical importance and continue to inform the approach to microbiologic diagnosis, fulfillment of all four postulates is not required to demonstrate causality.

Koch's postulates

Koch's postulates are the following:

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Koch's postulates have also influenced scientists who examine microbial pathogenesis from a molecular point of view. In the 1980s, a molecular version of Koch's postulates was developed to guide the identification of microbial genes encoding virulence factors.

Key Points

- The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 and refined and published by Koch in 1890.
- Postulate 1: The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
- Postulate 2: The microorganism must be isolated from a diseased organism and grown in pure culture.
- Postulate 3: The cultured microorganism should cause disease when introduced into a healthy organism.
- Postulate 4: The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Key Terms

- **Koch's postulates**: four criteria designed to establish a causal relationship between a causative microbe and a disease
- **postulate**: A fundamental element; a basic principle.

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5.4: Virulence Factors

Learning Objectives

- Explain how virulence factors contribute to signs and symptoms of infectious disease
- Differentiate between endotoxins and exotoxins
- Describe and differentiate between various types of exotoxins
- Describe the mechanisms viruses use for adhesion and antigenic variation

In the previous section, we explained that some pathogens are more virulent than others. This is due to the unique virulence factors produced by individual pathogens, which determine the extent and severity of disease they may cause. A pathogen's virulence factors are encoded by genes that can be identified using molecular Koch's postulates. When genes encoding virulence factors are inactivated, virulence in the pathogen is diminished. In this section, we examine various types and specific examples of virulence factors and how they contribute to each step of pathogenesis.

Virulence Factors for Adhesion

As discussed in the previous section, the first two steps in pathogenesis are exposure and adhesion. Recall that an adhesin is a protein or glycoprotein found on the surface of a pathogen that attaches to receptors on the host cell. Adhesins are found on bacterial, viral, fungal, and protozoan pathogens. One example of a bacterial adhesin is type 1 fimbrial adhesin, a molecule found on the tips of fimbriae of enterotoxigenic *E. coli* (ETEC). Recall that fimbriae are hairlike protein bristles on the cell surface. Type 1 fimbrial adhesin allows the fimbriae of ETEC cells to attach to the mannose glycans expressed on intestinal epithelial cells. Table **5.4.1** lists common adhesins found in some of the pathogens we have discussed or will be seeing later in this chapter.

Table **5.4.1**: Some Bacterial Adhesins and Their Host Attachment Sites

Pathogen	Disease	Adhesin	Attachment Site
Streptococcus pyogenes	Strep throat	Protein F	Respiratory epithelial cells
Streptococcus mutans	Dental caries	Adhesin P1	Teeth
Neisseria gonorrhoeae	Gonorrhea	Type IV pili	Urethral epithelial cells
Enterotoxigenic *E. coli* (ETEC)	Traveler's diarrhea	Type 1 fimbriae	Intestinal epithelial cells
Vibrio cholerae	Cholera	N-methylphenylalanine pili	Intestinal epithelial cells

Clinical Focus: Part 3

The presence of bacteria in Michael's blood is a sign of infection, since blood is normally sterile. There is no indication that the bacteria entered the blood through an injury. Instead, it appears the portal of entry was the gastrointestinal route. Based on Michael's symptoms, the results of his blood test, and the fact that Michael was the only one in the family to partake of the hot dogs, the physician suspects that Michael is suffering from a case of listeriosis.

Listeria monocytogenes, the facultative intracellular pathogen that causes listeriosis, is a common contaminant in ready-to-eat foods such as lunch meats and dairy products. Once ingested, these bacteria invade intestinal epithelial cells and translocate to the liver, where they grow inside hepatic cells. Listeriosis is fatal in about one in five normal healthy people, and mortality rates are slightly higher in patients with pre-existing conditions that weaken the immune response. A cluster of virulence genes encoded on a pathogenicity island is responsible for the pathogenicity of *L. monocytogenes*. These genes are regulated by a transcriptional factor known as peptide chain release factor 1 (PrfA). One of the genes regulated by PrfA is *hyl*, which encodes a toxin known as listeriolysin O (LLO), which allows the bacterium to escape vacuoles upon entry into a host cell. A second gene regulated by PrfA is *actA*, which encodes for a surface protein known as actin assembly-inducing protein (ActA). ActA is expressed on the surface of *Listeria* and polymerizes host actin. This enables the bacterium to produce actin tails, move around the cell's cytoplasm, and spread from cell to cell without exiting into the extracellular compartment.

Michael's condition has begun to worsen. He is now experiencing a stiff neck and hemiparesis (weakness of one side of the body). Concerned that the infection is spreading, the physician decides to conduct additional tests to determine what is causing these new symptoms.

Exercise 5.4.1

1. What kind of pathogen causes listeriosis, and what virulence factors contribute to the signs and symptoms Michael is experiencing?
2. Is it likely that the infection will spread from Michael's blood? If so, how might this explain his new symptoms?

Bacterial Exoenzymes and Toxins as Virulence Factors

After exposure and adhesion, the next step in pathogenesis is invasion, which can involve enzymes and toxins. Many pathogens achieve invasion by entering the bloodstream, an effective means of dissemination because blood vessels pass close to every cell in the body. The downside of this mechanism of dispersal is that the blood also includes numerous elements of the immune system. Various terms ending in –emia are used to describe the presence of pathogens in the bloodstream. The presence of bacteria in blood is called bacteremia. Bacteremia involving pyogens(pus-forming bacteria) is called pyemia. When viruses are found in the blood, it is called viremia. The term toxemiadescribes the condition when toxins are found in the blood. If bacteria are both present and multiplying in the blood, this condition is called septicemia.

Patients with septicemia are described as septic, which can lead to shock, a life-threatening decrease in blood pressure (systolic pressure <90 mm Hg) that prevents cells and organs from receiving enough oxygen and nutrients. Some bacteria can cause shock through the release of toxins (virulence factors that can cause tissue damage) and lead to low blood pressure. Gram-negative bacteria are engulfed by immune system phagocytes, which then release tumor necrosis factor, a molecule involved in inflammation and fever. Tumor necrosis factor binds to blood capillaries to increase their permeability, allowing fluids to pass out of blood vessels and into tissues, causing swelling, or edema(Figure 5.4.1). With high concentrations of tumor necrosis factor, the inflammatory reaction is severe and enough fluid is lost from the circulatory system that blood pressure decreases to dangerously low levels. This can have dire consequences because the heart, lungs, and kidneys rely on normal blood pressure for proper function; thus, multi-organ failure, shock, and death can occur.



Figure 5.4.1: This patient has edema in the tissue of the right hand. Such swelling can occur when bacteria cause the release of pro-inflammatory molecules from immune cells and these molecules cause an increased permeability of blood vessels, allowing fluid to escape the bloodstream and enter tissue.

Exoenzymes

Some pathogens produce extracellular enzymes, or exoenzymes, that enable them to invade host cells and deeper tissues. Exoenzymes have a wide variety of targets. Some general classes of exoenzymes and associated pathogens are listed in Table **5.4.2**. Each of these exoenzymes functions in the context of a particular tissue structure to facilitate invasion or support its own growth and defend against the immune system. For example, hyaluronidase S, an enzyme produced by pathogens like *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Clostridium perfringens*, degrades the glycoside hylauronan (hyaluronic acid), which acts as an intercellular cement between adjacent cells in connective tissue (Figure **5.4.2**). This allows the pathogen to pass through the tissue layers at the portal of entry and disseminate elsewhere in the body (Figure **5.4.2**).

Table **5.4.2**: Some Classes of Exoenzymes and Their Targets

Class	Example	Function
Glycohydrolases	Hyaluronidase S in *Staphylococcus aureus*	Degrades hyaluronic acid that cements cells together to promote spreading through tissues
Nucleases	DNAse produced by *S. aureus*	Degrades DNA released by dying cells (bacteria and host cells) that can trap the bacteria, thus promoting spread
Phospholipases	Phospholipase C of *Bacillus anthracis*	Degrades phospholipid bilayer of host cells, causing cellular lysis, and degrade membrane of phagosomes to enable escape into the cytoplasm
Proteases	Collagenase in *Clostridium perfringens*	Degrades collagen in connective tissue to promote spread

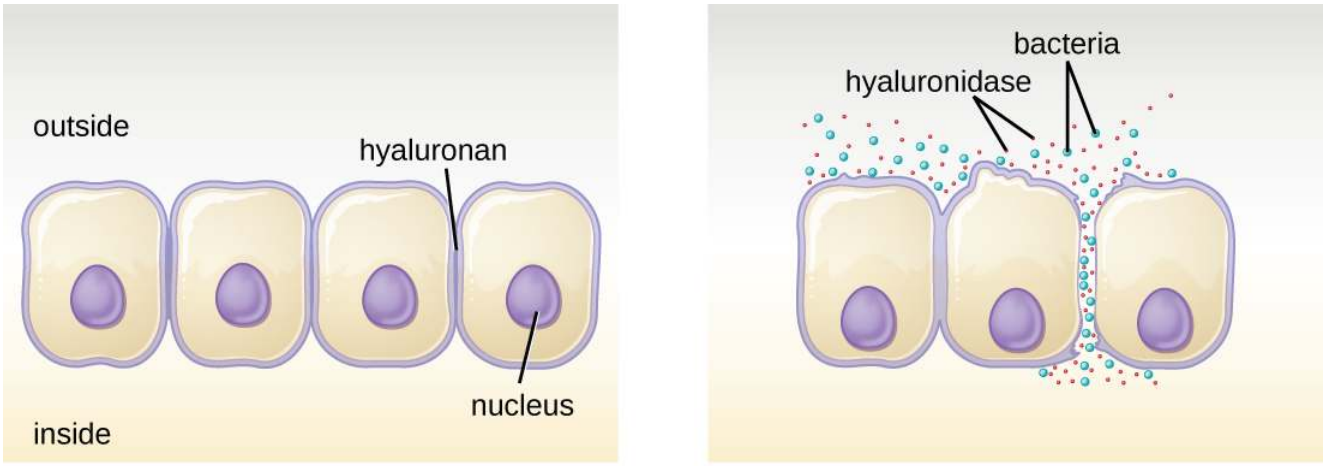


Figure **5.4.2**: (a) Hyaluronan is a polymer found in the layers of epidermis that connect adjacent cells. (b) Hyaluronidase produced by bacteria degrades this adhesive polymer in the extracellular matrix, allowing passage between cells that would otherwise be blocked.

Pathogen-produced nucleases, such as DNAse produced by *S. aureus*, degrade extracellular DNA as a means of escape and spreading through tissue. As bacterial and host cells die at the site of infection, they lyse and release their intracellular contents. The DNA chromosome is the largest of the intracellular molecules, and masses of extracellular DNA can trap bacteria and prevent their spread. *S. aureus* produces a DNAse to degrade the mesh of extracellular DNA so it can escape and spread to adjacent tissues. This strategy is also used by *S. aureus* and other pathogens to degrade and escape webs of extracellular DNA produced by immune system phagocytes to trap the bacteria.

Enzymes that degrade the phospholipids of cell membranes are called phospholipases. Their actions are specific in regard to the type of phospholipids they act upon and where they enzymatically cleave the molecules. The pathogen responsible for anthrax, *B. anthracis*, produces phospholipase C. When *B. anthracis* is ingested by phagocytic cells of the immune system, phospholipase C degrades the membrane of the phagosome before it can fuse with the lysosome, allowing the pathogen to escape into the cytoplasm and multiply. Phospholipases can also target the membrane that encloses the phagosome within phagocytic cells. As described earlier in this chapter, this is the mechanism used by intracellular pathogens such as *L. monocytogenes* and *Rickettsia* to escape the phagosome and multiply within the cytoplasm of phagocytic cells. The role of phospholipases in bacterial virulence is not restricted to phagosomal escape. Many pathogens produce phospholipases that act to degrade cell membranes and cause lysis of target cells. These phospholipases are involved in lysis of red blood cells, white blood cells, and tissue cells.

Bacterial pathogens also produce various protein-digesting enzymes, or proteases. Proteases can be classified according to their substrate target (e.g., serine proteases target proteins with the amino acid serine) or if they contain metals in their active site (e.g., zinc metalloproteases contain a zinc ion, which is necessary for enzymatic activity).

One example of a protease that contains a metal ion is the exoenzyme collagenase. Collagenase digests collagen, the dominant protein in connective tissue. Collagen can be found in the extracellular matrix, especially near mucosal membranes, blood vessels, nerves, and in the layers of the skin. Similar to hyaluronidase, collagenase allows the pathogen to penetrate and spread through the host tissue by digesting this connective tissue protein. The collagenase produced by the gram-positive bacterium *Clostridium perfringens*, for example, allows the bacterium to make its way through the tissue layers and subsequently enter and multiply in the blood (septicemia). *C. perfringens* then uses toxins and a phospholipase to cause cellular lysis and necrosis. Once the host cells have died, the bacterium produces gas by fermenting the muscle carbohydrates. The widespread necrosis of tissue and accompanying gas are characteristic of the condition known as gas gangrene (Figure **5.4.3**).

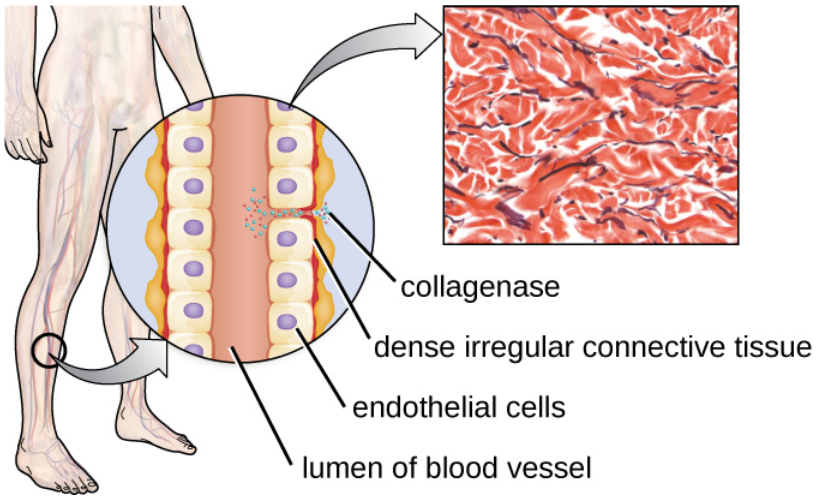


Figure **5.4.3**: The illustration depicts a blood vessel with a single layer of endothelial cells surrounding the lumen and dense connective tissue (shown in red) surrounding the endothelial cell layer. Collagenase produced by *C. perfringens* degrades the collagen between the endothelial cells, allowing the bacteria to enter the bloodstream. (credit illustration: modification of work by Bruce Blaus; credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Toxins

In addition to exoenzymes, certain pathogens are able to produce toxins, biological poisons that assist in their ability to invade and cause damage to tissues. The ability of a pathogen to produce toxins to cause damage to host cells is called toxigenicity.

Toxins can be categorized as endotoxins or exotoxins. The lipopolysaccharide (LPS) found on the outer membrane of gram-negative bacteria is called endotoxin (Figure **5.4.4**). During infection and disease, gram-negative bacterial pathogens release endotoxin either when the cell dies, resulting in the disintegration of the membrane, or when the bacterium undergoes binary fission. The lipid component of endotoxin, lipid A, is responsible for the toxic properties of the LPS molecule. Lipid A is relatively conserved across different genera of gram-negative bacteria; therefore, the toxic properties of lipid A are similar regardless of the gram-negative pathogen. In a manner similar to that of tumor necrosis factor, lipid A triggers the immune system's inflammatory response (see Inflammation and Fever). If the concentration of endotoxin in the body is low, the inflammatory response may

provide the host an effective defense against infection; on the other hand, high concentrations of endotoxin in the blood can cause an excessive inflammatory response, leading to a severe drop in blood pressure, multi-organ failure, and death.

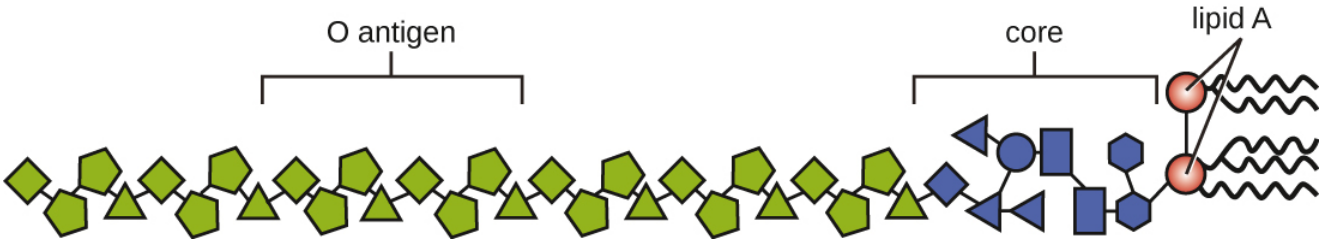


Figure **5.4.4**: Lipopolysaccharide is composed of lipid A, a core glycolipid, and an O-specific polysaccharide side chain. Lipid A is the toxic component that promotes inflammation and fever.

A classic method of detecting endotoxin is by using the *Limulus* amebocyte lysate (LAL) test. In this procedure, the blood cells (amebocytes) of the horseshoe crab (*Limulus polyphemus*) is mixed with a patient's serum. The amebocytes will react to the presence of any endotoxin. This reaction can be observed either chromogenically (color) or by looking for coagulation (clotting reaction) to occur within the serum. An alternative method that has been used is an enzyme-linked immunosorbent assay (ELISA) that uses antibodies to detect the presence of endotoxin.

Unlike the toxic lipid A of endotoxin, exotoxins are protein molecules that are produced by a wide variety of living pathogenic bacteria. Although some gram-negative pathogens produce exotoxins, the majority are produced by gram-positive pathogens. Exotoxins differ from endotoxin in several other key characteristics, summarized in Table **5.4.3**. In contrast to endotoxin, which stimulates a general systemic inflammatory response when released, exotoxins are much more specific in their action and the cells they interact with. Each exotoxin targets specific receptors on specific cells and damages those cells through unique molecular mechanisms. Endotoxin remains stable at high temperatures, and requires heating at 121 °C (250 °F) for 45 minutes to inactivate. By contrast, most exotoxins are heat labile because of their protein structure, and many are denatured (inactivated) at temperatures above 41 °C (106 °F). As discussed earlier, endotoxin can stimulate a lethal inflammatory response at very high concentrations and has a measured LD_{50} of 0.24 mg/kg. By contrast, very small concentrations of exotoxins can be lethal. For example, botulinum toxin, which causes botulism, has an LD_{50} of 0.000001 mg/kg (240,000 times more lethal than endotoxin).

Table **5.4.3**: Comparison of Endotoxin and Exotoxins Produced by Bacteria

Characteristic	Endotoxin	Exotoxin
Source	Gram-negative bacteria	Gram-positive (primarily) and gram-negative bacteria
Composition	Lipid A component of lipopolysaccharide	Protein
Effect on host	General systemic symptoms of inflammation and fever	Specific damage to cells dependent upon receptor-mediated targeting of cells and specific mechanisms of action
Heat stability	Heat stable	Most are heat labile, but some are heat stable
LD_{50}	High	Low

The exotoxins can be grouped into three categories based on their target: intracellular targeting, membrane disrupting, and superantigens. Table **5.4.4** provides examples of well-characterized toxins within each of these three categories.

Table **5.4.4**: Some Common Exotoxins and Associated Bacterial Pathogens

Category	Example	Pathogen	Mechanism and Disease
Intracellular-targeting toxins	Cholera toxin	*Vibrio cholerae*	Activation of adenylate cyclase in intestinal cells, causing increased levels of cyclic adenosine monophosphate (cAMP) and secretion of fluids and electrolytes out of cell, causing diarrhea
	Tetanus toxin	*Clostridium tetani*	Inhibits the release of inhibitory neurotransmitters in the central nervous system, causing spastic paralysis
	Botulinum toxin	*Clostridium botulinum*	Inhibits release of the neurotransmitter acetylcholine from neurons, resulting in flaccid paralysis
	Diphtheria toxin	*Corynebacterium diphtheriae*	Inhibition of protein synthesis, causing cellular death
Membrane-disrupting toxins	Streptolysin	*Streptococcus pyogenes*	Proteins that assemble into pores in cell membranes, disrupting their function and killing the cell
	Pneumolysin	*Streptococcus pneumoniae*	
	Alpha-toxin	*Staphylococcus aureus*	
	Alpha-toxin	*Clostridium perfringens*	Phospholipases that degrade cell membrane phospholipids, disrupting membrane function and killing the cell
	Phospholipase C	*Pseudomonas aeruginosa*	
	Beta-toxin	*Staphylococcus aureus*	
Superantigens	Toxic shock syndrome toxin	*Staphylococcus aureus*	Stimulates excessive activation of immune system cells and release of cytokines (chemical mediators) from immune system cells. Life-threatening fever, inflammation, and shock are the result.
	Streptococcal mitogenic exotoxin	*Streptococcus pyogenes*	
	Streptococcal pyrogenic toxins	*Streptococcus pyogenes*	

The intracellular targeting toxins comprise two components: A for activity and B for binding. Thus, these types of toxins are known as A-B exotoxins (Figure **5.4.5**). The B component is responsible for the cellular specificity of the toxin and mediates the initial attachment of the toxin to specific cell surface receptors. Once the A-B toxin binds to the host cell, it is brought into the cell by endocytosis and entrapped in a vacuole. The A and B subunits separate as the vacuole acidifies. The A subunit then enters the cell cytoplasm and interferes with the specific internal cellular function that it targets.

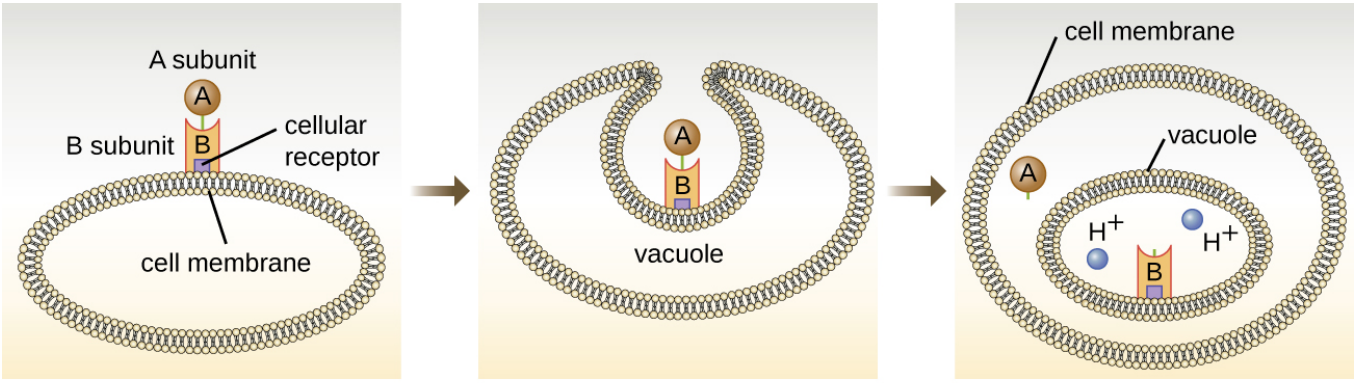


Figure 5.4.5: (a) In A-B toxins, the B component binds to the host cell through its interaction with specific cell surface receptors. (b) The toxin is brought in through endocytosis. (c) Once inside the vacuole, the A component (active component) separates from the B component and the A component gains access to the cytoplasm. (credit: modification of work by "Biology Discussion Forum"/YouTube)

Four unique examples of A-B toxins are the diphtheria, cholera, botulinum, and tetanus toxins. The diphtheria toxin is produced by the gram-positive bacterium *Corynebacterium diphtheriae*, the causative agent of nasopharyngeal and cutaneous diphtheria. After the A subunit of the diphtheria toxin separates and gains access to the cytoplasm, it facilitates the transfer of adenosine diphosphate (ADP)-ribose onto an elongation-factor protein (EF-2) that is needed for protein synthesis. Hence, diphtheria toxin inhibits protein synthesis in the host cell, ultimately killing the cell (Figure 5.4.6).

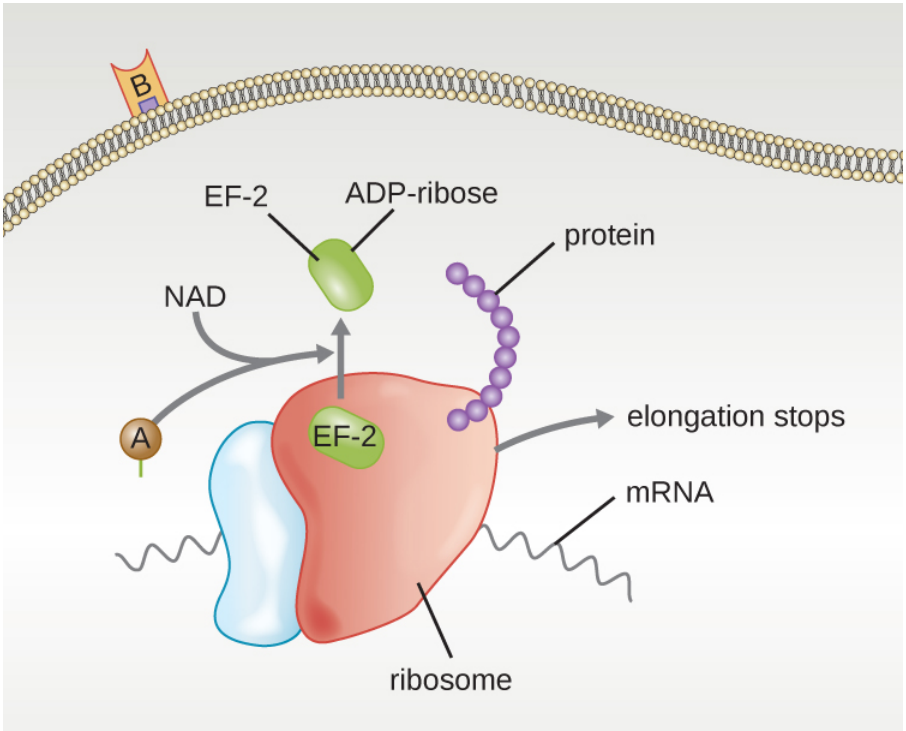


Figure 5.4.6: The mechanism of the diphtheria toxin inhibiting protein synthesis. The A subunit inactivates elongation factor 2 by transferring an ADP-ribose. This stops protein elongation, inhibiting protein synthesis and killing the cell.

Cholera toxin is an enterotoxin produced by the gram-negative bacterium *Vibrio cholerae* and is composed of one A subunit and five B subunits. The mechanism of action of the cholera toxin is complex. The B subunits bind to receptors on the intestinal epithelial cell of the small intestine. After gaining entry into the cytoplasm of the epithelial cell, the A subunit activates an intracellular G protein. The activated G protein, in turn, leads to the activation of the enzyme adenyl cyclase, which begins to produce an increase in the concentration of cyclic AMP (a secondary messenger molecule). The increased cAMP disrupts the

normal physiology of the intestinal epithelial cells and causes them to secrete excessive amounts of fluid and electrolytes into the lumen of the intestinal tract, resulting in severe “rice-water stool” diarrhea characteristic of cholera.

Botulinum toxin (also known as botox) is a neurotoxin produced by the gram-positive bacterium *Clostridium botulinum*. It is the most acutely toxic substance known to date. The toxin is composed of a light A subunit and heavy protein chain B subunit. The B subunit binds to neurons to allow botulinum toxin to enter the neurons at the neuromuscular junction. The A subunit acts as a protease, cleaving proteins involved in the neuron’s release of acetylcholine, a neurotransmitter molecule. Normally, neurons release acetylcholine to induce muscle fiber contractions. The toxin’s ability to block acetylcholine release results in the inhibition of muscle contractions, leading to muscle relaxation. This has the potential to stop breathing and cause death. Because of its action, low concentrations of botox are used for cosmetic and medical procedures, including the removal of wrinkles and treatment of overactive bladder.

Link to Learning

Click this link to see an animation of how the cholera toxin functions.

Click this link to see an animation of how the botulinum toxin functions.

Another neurotoxin is tetanus toxin, which is produced by the gram-positive bacterium *Clostridium tetani*. This toxin also has a light A subunit and heavy protein chain B subunit. Unlike botulinum toxin, tetanus toxin binds to inhibitory interneurons, which are responsible for release of the inhibitory neurotransmitters glycine and gamma-aminobutyric acid (GABA). Normally, these neurotransmitters bind to neurons at the neuromuscular junction, resulting in the inhibition of acetylcholine release. Tetanus toxin inhibits the release of glycine and GABA from the interneuron, resulting in permanent muscle contraction. The first symptom is typically stiffness of the jaw (lockjaw). Violent muscle spasms in other parts of the body follow, typically culminating with respiratory failure and death. Figure **5.4.7** shows the actions of both botulinum and tetanus toxins.

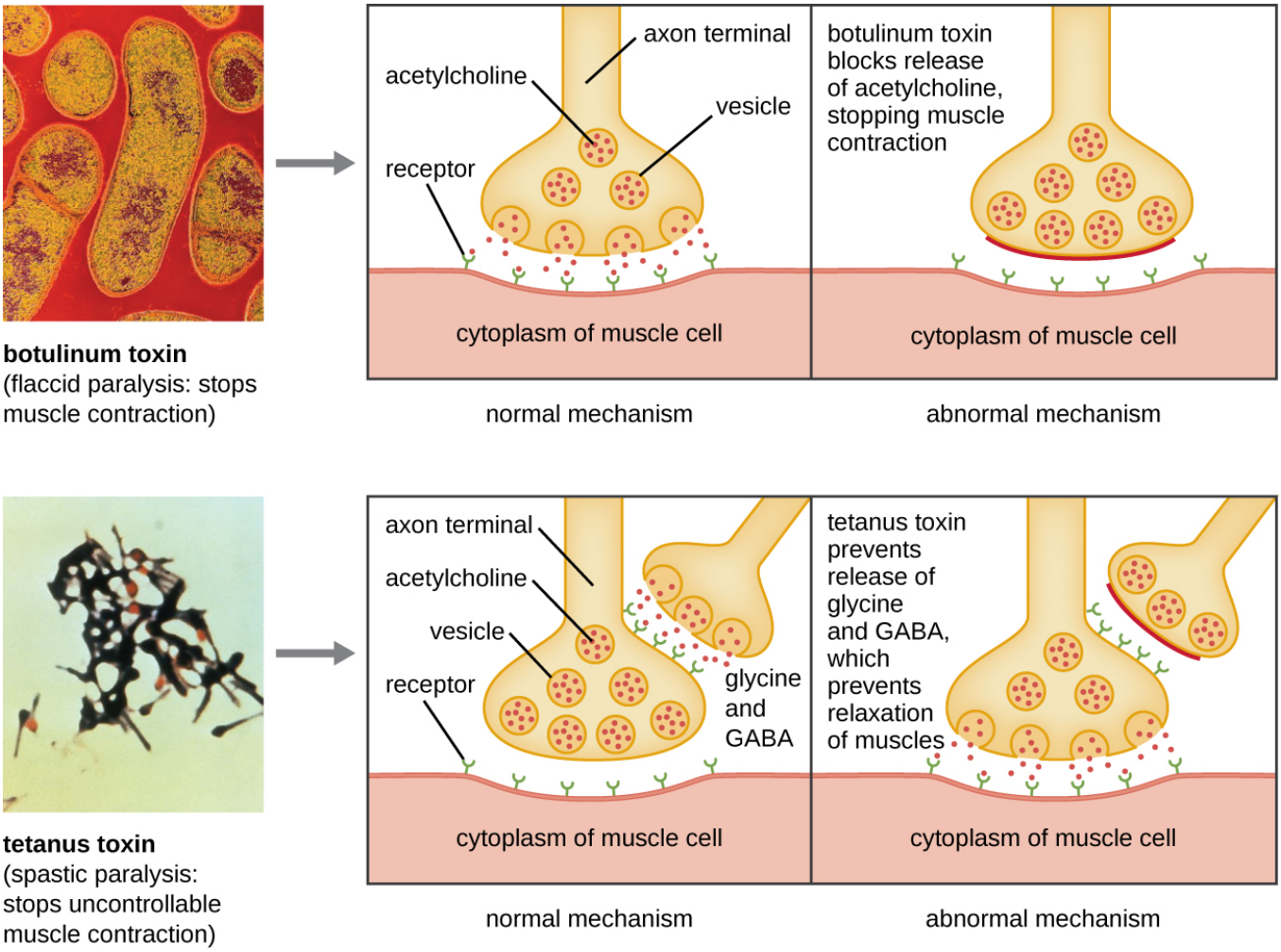


Figure **5.4.7**: Mechanisms of botulinum and tetanus toxins. (credit micrographs: modification of work by Centers for Disease Control and Prevention)

Membrane-disrupting toxins affect cell membrane function either by forming pores or by disrupting the phospholipid bilayer in host cell membranes. Two types of membrane-disrupting exotoxins are hemolysins and leukocidins, which form pores in cell membranes, causing leakage of the cytoplasmic contents and cell lysis. These toxins were originally thought to target red blood cells (erythrocytes) and white blood cells (leukocytes), respectively, but we now know they can affect other cells as well. The gram-positive bacterium *Streptococcus pyogenes* produces streptolysins, water-soluble hemolysins that bind to the cholesterol moieties in the host cell membrane to form a pore. The two types of streptolysins, O and S, are categorized by their ability to cause hemolysis in erythrocytes in the absence or presence of oxygen. Streptolysin O is not active in the presence of oxygen, whereas streptolysin S is active in the presence of oxygen. Other important pore-forming membrane-disrupting toxins include alpha toxin of *Staphylococcus aureus* and pneumolysin of *Streptococcus pneumoniae*.

Bacterial phospholipases are membrane-disrupting toxins that degrade the phospholipid bilayer of cell membranes rather than forming pores. We have already discussed the phospholipases associated with *B. anthracis*, *L. pneumophila*, and *Rickettsia* species that enable these bacteria to effect the lysis of phagosomes. These same phospholipases are also hemolysins. Other phospholipases that function as hemolysins include the alpha toxin of *Clostridium perfringens*, phospholipase C of *P. aeruginosa*, and beta toxin of *Staphylococcus aureus*.

Some strains of *S. aureus* also produce a leukocidin called Panton-Valentine leukocidin (PVL). PVL consists of two subunits, S and F. The S component acts like the B subunit of an A-B exotoxin in that it binds to glycolipids on the outer plasma membrane of animal cells. The F-component acts like the A subunit of an A-B exotoxin and carries the enzymatic activity. The toxin inserts and assembles into a pore in the membrane. Genes that encode PVL are more frequently present in *S. aureus* strains that cause skin infections and pneumonia.[1] PVL promotes skin infections by causing edema, erythema (reddening of the skin due to blood vessel

dilation), and skin necrosis. PVL has also been shown to cause necrotizing pneumonia. PVL promotes pro-inflammatory and cytotoxic effects on alveolar leukocytes. This results in the release of enzymes from the leukocytes, which, in turn, cause damage to lung tissue.

The third class of exotoxins is the superantigens. These are exotoxins that trigger an excessive, nonspecific stimulation of immune cells to secrete cytokines (chemical messengers). The excessive production of cytokines, often called a cytokine storm, elicits a strong immune and inflammatory response that can cause life-threatening high fevers, low blood pressure, multi-organ failure, shock, and death. The prototype superantigen is the toxic shock syndrome toxin of *S. aureus*. Most toxic shock syndrome cases are associated with vaginal colonization by toxin-producing *S. aureus* in menstruating women; however, colonization of other body sites can also occur. Some strains of *Streptococcus pyogenes* also produce superantigens; they are referred to as the streptococcal mitogenic exotoxins and the streptococcal pyrogenic toxins.

? Exercise 5.4.2

1. Describe how exoenzymes contribute to bacterial invasion.
2. Explain the difference between exotoxins and endotoxin.
3. Name the three classes of exotoxins.

Virulence Factors for Survival in the Host and Immune Evasion

Evading the immune system is also important to invasiveness. Bacteria use a variety of virulence factors to evade phagocytosis by cells of the immune system. For example, many bacteria produce capsules, which are used in adhesion but also aid in immune evasion by preventing ingestion by phagocytes. The composition of the capsule prevents immune cells from being able to adhere and then phagocytose the cell. In addition, the capsule makes the bacterial cell much larger, making it harder for immune cells to engulf the pathogen (Figure 5.4.8). A notable capsule-producing bacterium is the gram-positive pathogen *Streptococcus pneumoniae*, which causes pneumococcal pneumonia, meningitis, septicemia, and other respiratory tract infections. Encapsulated strains of *S. pneumoniae* are more virulent than nonencapsulated strains and are more likely to invade the bloodstream and cause septicemia and meningitis.

Some pathogens can also produce proteases to protect themselves against phagocytosis. As described in Adaptive Specific Host Defenses, the human immune system produces antibodies that bind to surface molecules found on specific bacteria (e.g., capsules, fimbriae, flagella, LPS). This binding initiates phagocytosis and other mechanisms of antibacterial killing and clearance. Proteases combat antibody-mediated killing and clearance by attacking and digesting the antibody molecules (Figure 5.4.8).

In addition to capsules and proteases, some bacterial pathogens produce other virulence factors that allow them to evade the immune system. The fimbriae of certain species of *Streptococcus* contain M protein, which alters the surface of *Streptococcus* and inhibits phagocytosis by blocking the binding of the complement molecules that assist phagocytes in ingesting bacterial pathogens. The acid-fast bacterium *Mycobacterium tuberculosis* (the causative agent of tuberculosis) produces a waxy substance known as mycolic acid in its cell envelope. When it is engulfed by phagocytes in the lung, the protective mycolic acid coat enables the bacterium to resist some of the killing mechanisms within the phagolysosome.

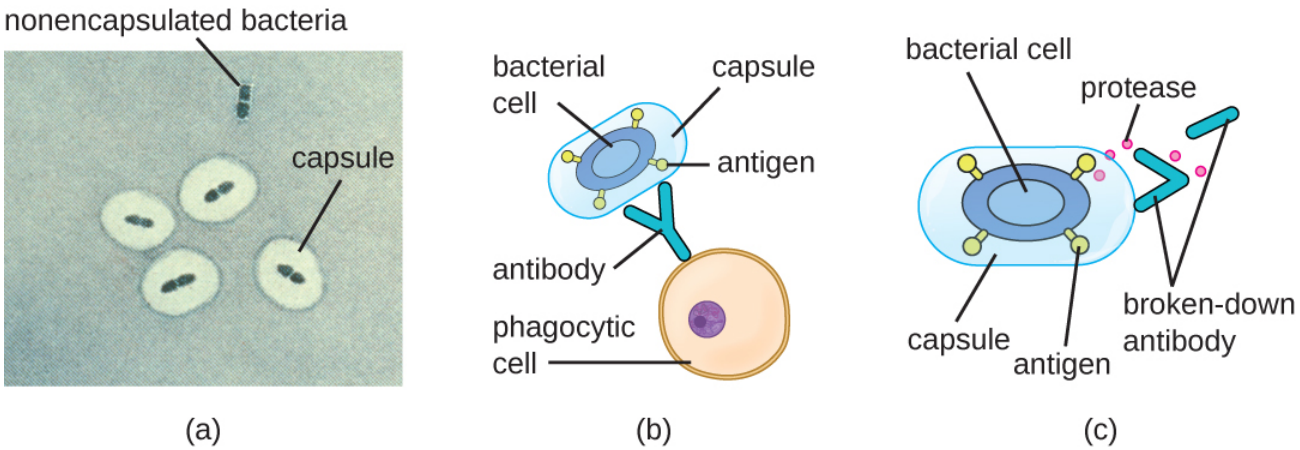


Figure 5.4.8: (a) A micrograph of capsules around bacterial cells. (b) Antibodies normally function by binding to antigens, molecules on the surface of pathogenic bacteria. Phagocytes then bind to the antibody, initiating phagocytosis. (c) Some bacteria also produce proteases, virulence factors that break down host antibodies to evade phagocytosis. (credit a: modification of work by Centers for Disease Control and Prevention)

Some bacteria produce virulence factors that promote infection by exploiting molecules naturally produced by the host. For example, most strains of *Staphylococcus aureus* produce the exoenzyme coagulase, which exploits the natural mechanism of blood clotting to evade the immune system. Normally, blood clotting is triggered in response to blood vessel damage; platelets begin to plug the clot, and a cascade of reactions occurs in which fibrinogen, a soluble protein made by the liver, is cleaved into fibrin. Fibrin is an insoluble, thread-like protein that binds to blood platelets, cross-links, and contracts to form a mesh of clumped platelets and red blood cells. The resulting clot prevents further loss of blood from the damaged blood vessels. However, if bacteria release coagulase into the bloodstream, the fibrinogen-to-fibrin cascade is triggered in the absence of blood vessel damage. The resulting clot coats the bacteria in fibrin, protecting the bacteria from exposure to phagocytic immune cells circulating in the bloodstream.

Whereas coagulase causes blood to clot, kinases have the opposite effect by triggering the conversion of plasminogen to plasmin, which is involved in the digestion of fibrin clots. By digesting a clot, kinases allow pathogens trapped in the clot to escape and spread, similar to the way that collagenase, hyaluronidase, and DNAse facilitate the spread of infection. Examples of kinases include staphylokinases and streptokinases, produced by *Staphylococcus aureus*and *Streptococcus pyogenes*, respectively. It is intriguing that *S. aureus* can produce both coagulase to promote clotting and staphylokinase to stimulate the digestion of clots. The action of the coagulase provides an important protective barrier from the immune system, but when nutrient supplies are diminished or other conditions signal a need for the pathogen to escape and spread, the production of staphylokinase can initiate this process.

A final mechanism that pathogens can use to protect themselves against the immune system is called antigenic variation, which is the alteration of surface proteins so that a pathogen is no longer recognized by the host's immune system. For example, the bacterium *Borrelia burgdorferi*, the causative agent of Lyme disease, contains a surface lipoprotein known as VlsE. Because of genetic recombination during DNA replication and repair, this bacterial protein undergoes antigenic variation. Each time fever occurs, the VlsE protein in *B. burgdorferi* can differ so much that antibodies against previous VlsE sequences are not effective. It is believed that this variation in the VlsE contributes to the ability *B. burgdorferi* to cause chronic disease. Another important human bacterial pathogen that uses antigenic variation to avoid the immune system is *Neisseria gonorrhoeae*, which causes the sexually transmitted disease gonorrhea. This bacterium is well known for its ability to undergo antigenic variation of its type IV pili to avoid immune defenses.

? Exercise 5.4.3

1. Name at least two ways that a capsule provides protection from the immune system.
2. Besides capsules, name two other virulence factors used by bacteria to evade the immune system.

Clinical Focus: Resolution

Based on Michael's reported symptoms of stiff neck and hemiparesis, the physician suspects that the infection may have spread to his nervous system. The physician decides to order a spinal tap to look for any bacteria that may have invaded the meninges and cerebrospinal fluid (CSF), which would normally be sterile. To perform the spinal tap, Michael's lower back is swabbed with an iodine antiseptic and then covered with a sterile sheet. The needle is aseptically removed from the manufacturer's sealed plastic packaging by the clinician's gloved hands. The needle is inserted and a small volume of fluid is drawn into an attached sample tube. The tube is removed, capped and a prepared label with Michael's data is affixed to it. This STAT (urgent or immediate analysis required) specimen is divided into three separate sterile tubes, each with 1 mL of CSF. These tubes are immediately taken to the hospital's lab, where they are analyzed in the clinical chemistry, hematology, and microbiology departments. The preliminary results from all three departments indicate there is a cerebrospinal infection occurring, with the microbiology department reporting the presence of a gram-positive rod in Michael's CSF.

These results confirm what his physician had suspected: Michael's new symptoms are the result of meningitis, acute inflammation of the membranes that protect the brain and spinal cord. Because meningitis can be life threatening and because the first antibiotic therapy was not effective in preventing the spread of infection, Michael is prescribed an aggressive course of two antibiotics, ampicillin and gentamicin, to be delivered intravenously. Michael remains in the hospital for several days for supportive care and for observation. After a week, he is allowed to return home for bed rest and oral antibiotics. After 3 weeks of this treatment, he makes a full recovery.

Viral Virulence

Although viral pathogens are not similar to bacterial pathogens in terms of structure, some of the properties that contribute to their virulence are similar. Viruses use adhesins to facilitate adhesion to host cells, and certain enveloped viruses rely on antigenic variation to avoid the host immune defenses. These virulence factors are discussed in more detail in the following sections.

Viral Adhesins

One of the first steps in any viral infection is adhesion of the virus to specific receptors on the surface of cells. This process is mediated by adhesins that are part of the viral capsid or membrane envelope. The interaction of viral adhesins with specific cell receptors defines the tropism (preferential targeting) of viruses for specific cells, tissues, and organs in the body. The spike protein hemagglutinin found on Influenzavirus is an example of a viral adhesin; it allows the virus to bind to the sialic acid on the membrane of host respiratory and intestinal cells. Another viral adhesin is the glycoprotein gp20, found on HIV. For HIV to infect cells of the immune system, it must interact with two receptors on the surface of cells. The first interaction involves binding between gp120 and the CD4 cellular marker that is found on some essential immune system cells. However, before viral entry into the cell can occur, a second interaction between gp120 and one of two chemokine receptors (CCR5 and CXCR4) must occur. Table **5.4.5** lists the adhesins for some common viral pathogens and the specific sites to which these adhesins allow viruses to attach.

Table **5.4.5**: Some Viral Adhesins and Their Host Attachment Sites

Pathogen	Disease	Adhesin	Attachment Site
Influenzavirus	Influenza	Hemagglutinin	Sialic acid of respiratory and intestinal cells
Herpes simplex virus I or II	Oral herpes, genital herpes	Glycoproteins gB, gC, gD	Heparan sulfate on mucosal surfaces of the mouth and genitals
Human immunodeficiency virus	HIV/AIDS	Glycoprotein gp120	CD4 and CCR5 or CXCR4 of immune system cells

Antigenic Variation in Viruses

Antigenic variation also occurs in certain types of enveloped viruses, including influenza viruses, which exhibit two forms of antigenic variation: antigenic drift and antigenic shift (Figure **5.4.9**). Antigenic drift is the result of point mutations causing slight changes in the spike proteins hemagglutinin (H) and neuraminidase (N). On the other hand, antigenic shift is a major change in

spike proteins due to gene reassortment. This reassortment for antigenic shift occurs typically when two different influenza viruses infect the same host.

The rate of antigenic variation in influenza viruses is very high, making it difficult for the immune system to recognize the many different strains of Influenzavirus. Although the body may develop immunity to one strain through natural exposure or vaccination, antigenic variation results in the continual emergence of new strains that the immune system will not recognize. This is the main reason that vaccines against Influenzavirus must be given annually. Each year's influenza vaccine provides protection against the most prevalent strains for that year, but new or different strains may be more prevalent the following year.

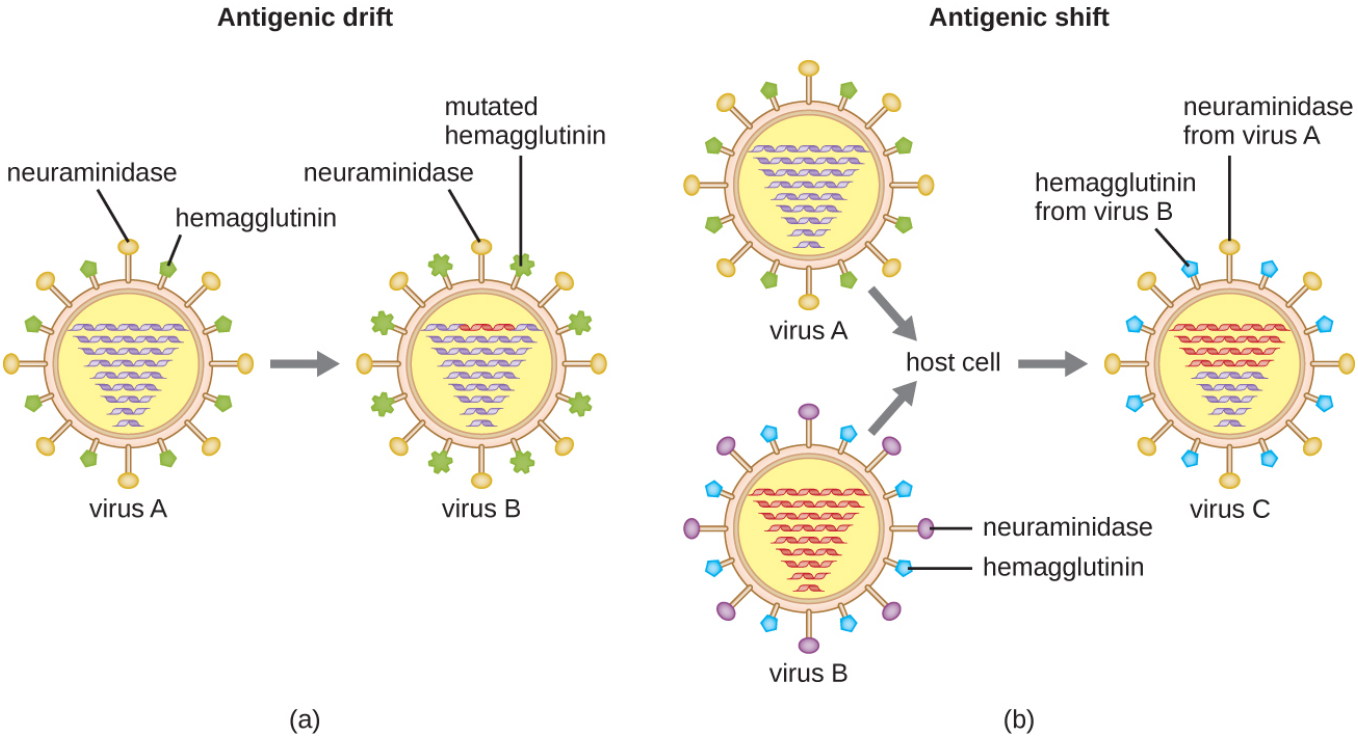


Figure **5.4.9**: Antigenic drift and antigenic shift in influenza viruses. (a) In antigenic drift, mutations in the genes for the surface proteins neuraminidase and/or hemagglutinin result in small antigenic changes over time. (b) In antigenic shift, simultaneous infection of a cell with two different influenza viruses results in mixing of the genes. The resultant virus possesses a mixture of the proteins of the original viruses. Influenza pandemics can often be traced to antigenic shifts.

Link to Learning

For another explanation of how antigenic shift and drift occur, watch this video.

Exercise 5.4.4

1. Describe the role of adhesins in viral tropism.
2. Explain the difference between antigenic drift and antigenic shift.

Key Concepts and Summary

Virulence factors contribute to a pathogen's ability to cause disease. **Exoenzymes** and **toxins** allow pathogens to invade host tissue and cause tissue damage. Exoenzymes are classified according to the macromolecule they target and exotoxins are classified based on their mechanism of action. Bacterial toxins include **endotoxin** and **exotoxins**. Endotoxin is the lipid A component of the LPS of the gram-negative cell envelope. Exotoxins are proteins secreted mainly by gram-positive bacteria, but also are secreted by gram-negative bacteria. Bacterial pathogens may evade the host immune response by producing **capsules** to avoid phagocytosis, surviving the intracellular environment of phagocytes, degrading antibodies, or through **antigenic variation**. Viral pathogens use adhesins for initiating infections and antigenic variation to avoid immune defenses. Influenza viruses use both **antigenic drift** and **antigenic shift** to avoid being recognized by the immune system.

Footnotes

1. 1 V. Meka. “Panton-Valentine Leukocidin.” http://www.antimicrobe.org/h04c.file...L-S-aureus.asp

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SECTION OVERVIEW

5.4.1: Virulence Factors that Promote Colonization

Virulence factors are molecules expressed and secreted by that enable them to colonize the host, evade or inhibit the immune responses of the host, enter into or out of a host cell, and/or obtain nutrition from the host. The following are virulence factors that promote bacterial colonization of the host .

1. The ability to use motility and other means to contact host cells and disseminate within a host.
2. The ability to adhere to host cells and resist physical removal.
3. The ability to invade host cells.
4. The ability to compete for iron and other nutrients.
5. The ability to resist innate immune defenses such as phagocytosis and complement.
6. The ability to evade adaptive immune defenses.

5.4.1.1: The Ability to Use Motility and Other Means to Contact Host Cells

5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal

5.4.1.3: The Ability to Evade Adaptive Immune Defenses

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5.4.1.1: The Ability to Use Motility and Other Means to Contact Host Cells

Learning Objectives

1. State why it might be of an advantage for a bacterium trying to colonize the bladder or the intestines to be motile.
2. **Describe** specifically how certain bacteria are able to use motility to contact host cells and state how this can promote colonization.
3. Briefly describe why being extremely thin and being motile by means of axial filaments may be an advantage to pathogenic spirochetes.
4. Give one example of how a nonmotile bacterium may be able to better disseminate within a host.
5. Give a brief description of how a bacterium may use toxins to better disseminate from one host to another.

Highlighted Bacterium

1. Read the description of *Helicobacter pylori* and match the bacterium with the description of the organism and the infection it causes.

The mucosal surfaces of the respiratory tract, the intestinal tract, and the genitourinary tract constantly flush bacteria away in order to prevent colonization of host mucous membranes. Motile bacteria can use their motility and chemotaxis to swim through mucus towards mucosal epithelial cells. Many bacteria that can colonize the mucous membranes of the bladder and the intestines, in fact, are motile. Motility probably helps these bacteria move through the mucus between the mucin strands or in places where the mucus is less viscous. Examples of motile opportunists and pathogens include *Helicobacter pylori, Salmonella* species, *Escherichia coli, Pseudomonas aeruginosa*, and *Vibrio cholerae*. Once bacteria contact host cells they can subsequently attach, and colonize. (Attachment will be discussed in the next section.)

- Movie of motile *Escherichia coli* with fluorescent labelled-flagella #1 Courtesy of Dr. Howard C. Berg from the Roland Institute at Harvard.
- Movie of motile *Pseudomonas* from YouTube.

For example, *Helicobacter pylori* , the bacterium that causes most gastric and duodenal ulcers, produces urease, an enzyme that breaks down urea into ammonia and carbon dioxide. The ammonia neutralizes the hydrochloric acid in the stomach. In addition, the urease is thought to alter the proteins in the mucus changing it from a solid gel to a thinner fluid that the bacteria are able to swim through by way of their flagella, and subsequently use adhesins to adhere to the epithelial cells of the mucous membranes. To further help protect the bacterium from the acid, *H. pylori* produces an acid-inhibitory protein that blocks acid secretion by surrounding parietal cells in the stomach. Bacterial toxins then lead to excessive production of cytokines and chemokines , as well as mucinase and phospholipase that damage the gastric mucosa. The cytokines and chemokines, in turn, result in a massive inflammatory response. Neutrophils leave the capillaries, accumulate at the area of infection, and discharge their lysosomes for extracellular killing. This not only kills the bacteria, it also destroys the mucus-secreting mucous membranes of the stomach. Without this protective layer, gastric acid causes ulceration of the stomach. This, in turn, leads to either gastritis or gastric and duodenal ulcers.



YouTube movie of a video endoscopy exam showing duodenal ulcers caused by Helicobacter pylori.

Click on this link, read the description of *Helicobacter pylori*, and be able to match the bacterium with its description on an exam.

Planktonic *Pseudomonas aeruginosa* uses its polar flagellum to move through water or mucus and make contact with a solid surface such as the body's mucous membranes (Figure 5.4.1.1.5.1.1). It then can use pili and cell wall adhesins to attach to the epithelial cells of the mucous membrane. Attachment activates signaling and quorum sensing genes to eventually enable the population of *P. aeruginosa* to start synthesizing a polysaccharide biofilm composed of alginate. As the biofilm grows, the bacteria lose their flagella to become nonmotile and secrete a variety of enzymes that enable the population to obtain nutrients from the host cells. Eventually the biofilm mushrooms up and develops water channels to deliver water and nutrients to all the bacteria within the biofilm. As the biofilm begins to get too crowded with bacteria, quorum sensing enables some of the *Pseudomonas* to again produce flagella, escape the biofilm, and colonize a new location.

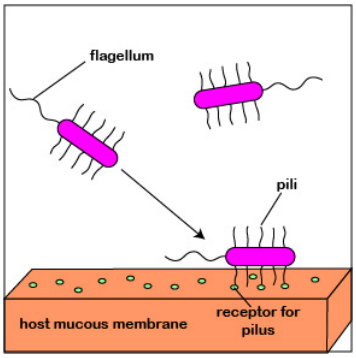


Figure 5.4.1.1.5.1.1: Development of a Biofilm by Pseudomonas aeruginosa. Planktonic Pseudomonas aeruginosa use their polar flagella and chemotaxis to swim towards host mucous membranes. Pili then bind to host cell receptors for initial but reversible bacterial attachment.

Because of their thinness, their internal flagella (axial filaments), their corkscrew shape, and their motility (Figure 5.4.1.1.5.1.2), spirochetes are more readily able to penetrate host mucous membranes, skin abrasions, etc., and enter the body. Motility and penetration may also enable the spirochetes to penetrate deeper in tissue and enter the lymphatics and bloodstream and disseminate to other body sites. Spirochetes that infect humans include *Treponema pallidum* , *Leptospira* , and *Borrelia burgdorferi*).

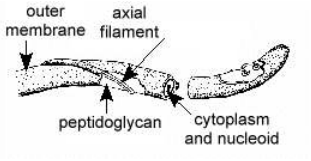


Figure **5.4.1.1.2**: Spirochete Axial Filaments

- Movie of motile *Borrelia bergdorferi*, the spirochete that causes Lyme disease. From You Tube, courtesy of CytoViva.
- Movie of motile *Borrelia bergdorferi*, the spirochete that causes Lyme disease.

Along a different line, many bacteria produce enzymes such as elastases and proteases that degrade the extracellular matrix proteins that surround cells and tissues and make it easier for those bacteria to disseminate within the body. For example, *Streptococcus pyogenes* produces streptokinase that lyses the fibrin clots produced by the body in order to localize the infection. It also produces DNase that degrades cell-free DNA found in pus and reduces the viscosity of the pus. Both of these enzymes facilitate spread of the bacterium from the localized site to new tissue.

Staphylococcus aureus, on the other hand, produces surface adhesins that bind to extracellular matrix proteins and polysaccharides surrounding host cell tissue, including fibronectin, collagen, laminin, hyaluronic acid, and elastin. *S. aureus* proteases and hyaluronidase then dissolve these components of the extracellular matrix providing food for the bacteria and enabling the bacteria to spread.

Finally, as will be seen later in this unit under toxins, some bacteria produce toxins that induce diarrhea in the host. Diarrhea is also a part of our innate immunity to flush harmful microbes and toxins out of the intestines. On one hand, diarrhea is an advantage to the body because it flushes out harmful microbes and toxins. On the other hand, it is beneficial for the bacterium inducing the diarrhea because it also flushes out a good deal of the normal flora of the intestines and this reduces the competition for nutrients between normal flora and pathogens. In addition, diarrhea enables the pathogen to more readily leave one host and enter new hosts through the fecal-oral route.

Summary

Bacteria have to make physical contact with host cells before they can adhere to those cells and resist being flushed out of the body. Motile bacteria can use their flagella and chemotaxis to swim through mucus towards mucosal epithelial cells. Because of their thinness, their internal flagella (axial filaments), their corkscrew shape, and their motility, certain spirochetes are more readily able enter lymph vessels and blood vessels and spread to other body sites. Many bacteria produce enzymes that degrade the extracellular matrix proteins that surround cells and tissues and help to localize infection, making it easier for those bacteria to spread within the body. Some bacteria produce toxins that induce diarrhea in the host enabling the pathogen to more readily leave one host and enter new hosts through the fecal-oral route.

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5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal

Learning Objectives

1. Briefly describe 3 different mechanisms by which bacteria can adhere to host cells and colonize and state how this can promote colonization.
2. State an advantage for bacteria in being able to switch the adhesive tips of their pili.
3. Define biofilm and state at least 3 benefits associated with bacteria living as a community within a biofilm.

Highlighted Bacterium

1. Read the description of *Neisseria memingitidis* andmatch the bacterium with the description of the organism and the infection it causes.

One of the body's innate immune defenses is the ability to physically remove bacteria from the body through such means as the constant shedding of surface epithelial cells from the skin and mucous membranes, the removal of bacteria by such means as coughing, sneezing, vomiting, and diarrhea, and bacterial removal by bodily fluids such as saliva, blood, mucous, and urine. Bacteria may resist this physical removal by producing pili, cell wall adhesin proteins, and/or biofilm-producing capsules. In addition, the physical attachment of bacteria to host cells can also serve as a signal for the activation of genes involved in bacterial virulence. This process is known as signal transduction.

Using Pili (fimbriae) to Adhere to Host Cells

As seen in Unit 1, pili enable some organisms to adhere to receptors on target host cells (Figure **5.4.1.2.5**.2.1) and thus colonize and resist flushing by the body. Pili are thin, protein tubes originating from the cytoplasmic membrane and are found in virtually all Gram-negative bacteria, but not in many Gram-positive bacteria.

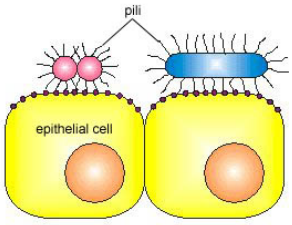


Figure **5.4.1.2.5**.2.1: Bacterial Adherence with Pili

The pilus has a shaft composed of a protein called pilin. At the end of the shaft is the adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell (Figure **5.4.1.2.5**.2.3). Because both the bacteria and the host cells have a negative charge, pili may enable the bacteria to bind to host cells without initially having to get close enough to be pushed away by electrostatic repulsion. Once attached to the host cell, the pili can depolymerize and enable adhesions in the bacterial cell wall to make more intimate contact. There is also evidence that the binding of pili to host cell receptors can serve as a trigger for activating the synthesis of some cell wall adhesins.

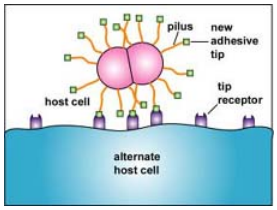


Figure **5.4.1.2.5**.2.3: By genetically altering the adhesive tips of their pili, certain bacteria are able to: 1) adhere to and colonize different cell types with different receptors, and 2) evade antibodies made against the previous pili.

Bacteria are constantly losing and reforming pili as they grow in the body and the same bacterium may switch the adhesive tips of the pili in order to adhere to different types of cells and evade immune defenses (Figure **5.4.1.2.2**.2.3). *E. coli*, for example, is able to make over 30 different types of pili.

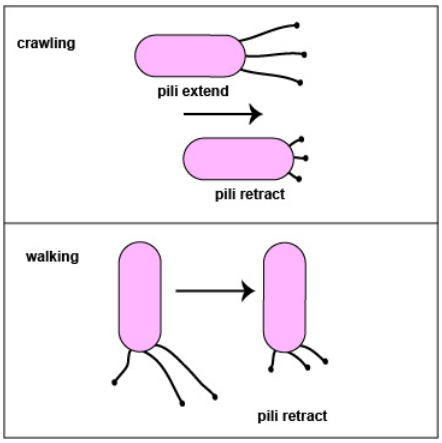


Figure5). The top illustration shows a bacterium dragging itself or "crawling" along a surface. Bacteria with polar pili are also able to pull themselves upright and "walk" along the surface as shown in the bottom illustration.

One class of pili, known as type IV pili, not only allows for attachment but also enable a twitching motility. They are located at the poles of bacilli and allow for a gliding motility along a solid surface such as a host cell. Extension and retraction of these pili allows the bacterium to drag itself along the solid surface (Figure **5.4.1.2.4**). In addition, bacteria can use their type IV pili to "slingshot" the bacterium over a cellular surface. In this case, as the pili contract they are thought to become taut like a stretched rubber band. When an anchoring pilus detaches, the taut pili "slingshot" the bacterium in the opposite direction (Figure **5.4.1.2.5**). This motion typically alternates with the twitching motility and enables a more rapid motion and direction change than with the twitching motility because the rapid slingshotting motion reduces the viscosity of the surrounding biofilm.

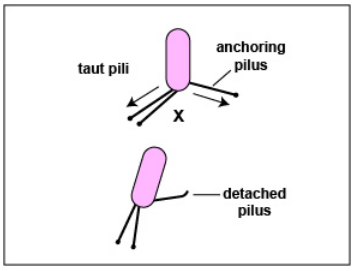


Figure **5.4.1.2.4**) also caused by type IV pili and enables a more rapid motion and direction change than with the twitching motility because the rapid "slingshotting" motion reduces the viscosity of the surrounding biofilm.

This enables bacteria with these types of pili within a biofilm to move around a cellular surface and find an optimum area on that cell for attachment and growth once they have initially bound. Bacteria with type IV pili include *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Vibrio cholerae*.

Examples of bacteria using pili to colonize:

1. To cause infection, *Neisseria gonorrhoeae* must first colonize a mucosal surface composed of columnar epithelial cells. Pili allow for this initial binding and, in fact, *N. gonorrhoeae* is able to rapidly lose pili and synthesize new ones with a different adhesive tip, enabling the bacterium to adhere to a variety of tissues and cells including sperm, the epithelial cells of the mucous membranes lining the throat, genitourinary tract, rectum, and the conjunctiva of the eye. Subsequently, the bacterium is able to make more intimate contact with the host cell surface by way of a cell wall adhesin called Opa (see below).

2. The pili of *Neisseria meningitidis* allow it to adhere to mucosal epithelial cells in the nasopharynx where it is often asymptomatic. From there, however, it sometimes enters the blood and meninges and causes septicemia and meningitis. Type IV pili are thought to help the bacterium cross the blood brain barrier.

Click on this link, read the description of *Neisseria meningitidis*, and be able to match the bacterium with its description on an exam.

3. Uropathogenic strains of *Escherichia coli* can produce pili that enable the bacterium to adhere to the urinary epithelium and cause urinary tract infections. They also produce afimbrial adhesins (see below) for attachment to epithelial cells. Enteropathogenic *E. coli* (EPEC) use pili to adhere to intestinal mucosal cells.

- To view an electron micrograph *E. coli* with pili, see Dennis Kunkel's Microscopy at the University of Hawaii-Manoa.
- To view electron micrographs of enteropathogenic *E. coli* (EPEC) adhering to intestinal cells, see Donnenberg Lab Images at the University of Maryland Medical School.

4. Pili of *Vibrio cholerae* allow it to adhere to cells of the intestinal mucosa and resist the flushing action of diarrhea.
5. Pili of *Pseudomonas aeruginosa* allow it to initially colonize wounds or the lung.

Using Adhesins to Adhere to Host Cells

Adhesins are surface proteins found in the cell wall of various bacteria that bind to specific receptor molecules on the surface of host cells and enable the bacterium to adhere intimately to that cell in order to colonize and resist physical removal (Figure **5.4.1.2.6**). Many, if not most bacteria probably use one or more adhesins to colonize host cells.

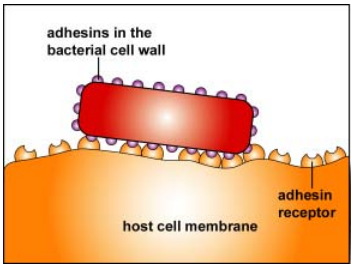


Figure **5.4.1.2.6**: Bacterial Adhesins. Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing.

For example:

1. *Streptococcus pyogenes* (see electron micrograph) (group A beta streptococci) produce a number of adhesins

   a. Protein F that binds to fibronectin , a common protein on epithelial cells. In this way it is able to adhere to the lymphatics and mucous membranes of the upper respiratory tract and cause streptococcal pharyngitis (strep throat).

   b. Lipoteichoic acid binds to fibronectin on epithelial cells.

   c. M-protein also functions as an adhesin.

2. The tip of the spirochete *Treponema pallidum* contains adhesins that are able to bind to fibronectin on epithelial cells.

   Scanning electron Micrograph of *T. pallidum* adhering to a host cell by its tip.

3. The tip of the spirochete *Borrelia burgdorferi* contains adhesins that can bind to various host cells.

4. *Escherichia coli* O157 utilizes a type 3 secretion system to inject effector proteins into intestinal epithelial cells. Some of these cause polymerization of actin at the cell surface and this pushes the host cell cytoplasmic membrane up to form a pedestal. Another effector protein inserts into the membrane of the pedestal to serve as a receptor molecule for *E. coli* adhesins (Figure **5.4.1.2.7**).

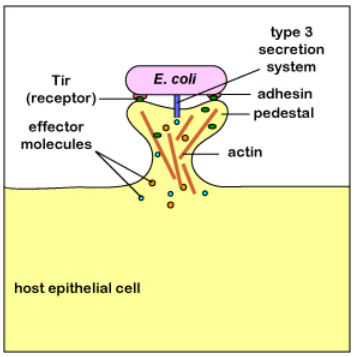


Figure 5.4.1.2.7: E. coli Using a Type 3 Secretion System to Induce Pedestal Formation in a Host Cell. Escherichia coli O157 utilizes a type 3 secretion system to inject effector proteins into intestinal epithelial cells. Some of these cause polymerization of actin at the cell surface and this pushes the host cell cytoplasmic membrane up to form a pedestal. Another effector protein inserts into the membrane of the pedestal to serve as a receptor molecule for E. coli adhesins

5. *Helicobacter pylori* use a type 4 secretion system to inject effector proteins into stomach epithelial cells to induce these host cells to display more receptors on their surface for *H. pylori* adhesins.

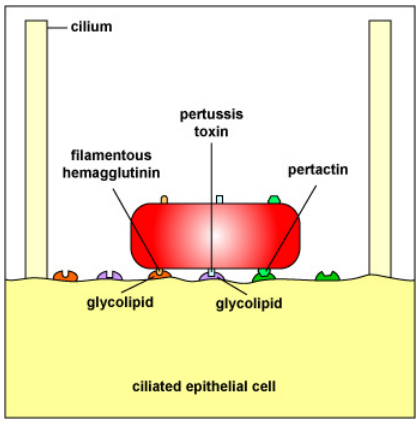


Figure 5.4.1.2.8: Bordetella pertussis using Adhesins to Adhere to a Ciliated Epithelial Cell. Bordetella pertussis produces several adhesins: (1) Filamentous hemagglutinin is an adhesin that allows the bacterium to adhere to galactose residues of the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract. (2) Pertussis toxin also functions as an adhesin. One subunit of the pertussis toxin remains bound to the bacterial cell wall while another subunit binds to the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract. (3) B. Pertussis also produces an adhesin called pertactin that further enables the bacterium to adhere to cells.

6. *Bordetella pertussis* produces several adhesins (Figure 5.4.1.2.8):

a. Filamentous hemagglutinin is an adhesin that allows the bacterium to adhere to galactose residues of the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract.

b. Pertussis toxin also functions as an adhesin. One subunit of the pertussis toxin remains bound to the bacterial cell wall while another subunit binds to the glycolipids on the membrane of ciliated epithelial cells of the respiratory tract.

c. *B. Pertussis* also produces an adhesin called pertactin that further enables the bacterium to adhere to cells.

7. *Neisseria gonorrhoeae* produces an adhesin called Opa (protein II) that enables the bacterium to make a more intimate contact with the host cell after it first adheres with its pili. Like with adhesive tips of pili, *N. gonorrhoeae* has multiple alleles for Opa protein adhesins enabling the bacterium to adhere to a variety of host cell types.

8. *Staphylococcus aureus* uses protein A as an adhesin to adhere to various host cells. It also helps the bacterium to resist phagocytosis.

Using Biofilms to Adhere to Host Cells

Many normal flora bacteria produce a capsular polysaccharide matrix or glycocalyx to form a biofilm on host tissue. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix, typically polysaccharide in nature.

Many pathogenic bacteria, as well as normal flora and many environmental bacteria, form complex bacterial communities as biofilms.

Bacteria in biofilms are often able to communicate with one another by a process called quorum sensing and are able to interact with and adapt to their environment as a population of bacteria rather than as individual bacteria. By living as a community of bacteria as a biofilm, these bacteria are better able to:

- resist attack by antibiotics;
- trap nutrients for bacterial growth and remain in a favorable niche;
- adhere to environmental surfaces and resist flushing;
- live in close association and communicate with other bacteria in the biofilm; and
- resist phagocytosis and attack by the body's complement pathways.

Biofilms are, therefore, functional, interacting, and growing bacterial communities. Biofilms even contain their own water channels for delivering water and nutrients throughout the biofilm community.

- Electron micrograph of a biofilm of *Haemophilus influenzae* from Biomedcentral.com
- Photomicrograph of a biofilm with water channels from Centers for Disease Control and Prevention Rodney M. Donlan: "Biofilms: Microbial Life on Surfaces"
- Biofilm of *Pseudomonas aeruginosa* from the Ausubel Lab, Department of Molecular Biology, Massachusetts General Hospital
- Scanning electron micrograph of *Staphylococcus aureus* forming a biofilm in an indwelling catheter courtesy of CDC.
- Biofilm of *Staphylococcus aureus* from Montana State University

For example:

1. *Streptococcus mutans*, and *Streptococcus sobrinus* , two bacteria implicated in initiating dental caries, break down sucrose into glucose and fructose. *Streptococcus mutans* can uses an enzyme called dextransucrase to convert sucrose into a sticky polysaccharide called dextran that forms a biofilm enabling the bacteria to adhere to the enamel of the tooth and initiate plaque formation.

   This dextran mesh traps the *S. mutans* and *S. sobrinus*, along with other bacteria and debris, and forms plaque. *S. mutans* and *S. sobrinus* also ferment glucose in order to produce energy. The fermentation of glucose results in the production of lactic acid that is released onto the surface of the tooth and initiates decay.

   - Scanning electron micrograph of *Streptococcus* growing in the enamel of a tooth.© Lloyd Simonson, author. Licensed for use, ASM MicrobeLibrary.
   - Scanning electron micrograph of dental plaque.© H. Busscher, H. van der Mei, W. Jongebloed, R Bos, authors. Licensed for use, ASM MicrobeLibrary.

2. Most children suffering from chronic ear infection (otitis media) have a biofilm of bacteria in their middle ear. This biofilm contains bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* and enables the bacteria to chronically colonize the middle ear as well as resist body defenses and antibiotics.

3. Planktonic *Pseudomonas aeruginosa* uses its polar flagellum to move through water or mucus and make contact with a solid surface such as the body's mucous membranes. It then can use pili and cell wall adhesins to attach to the epithelial cells of the mucous membrane. Attachment activates signaling and quorum sensing genes to eventually enable the population of *P. aeruginosa* to start synthesizing a polysaccharide biofilm composed of alginate. As the biofilm grows, the bacteria lose their flagella to become nonmotile and secrete a variety of enzymes that enable the population to obtain nutrients from the host cells. Eventually the biofilm mushrooms up and develops water channels to deliver water and nutrients to all the bacteria within the biofilm. As the biofilm begins to get too crowded with bacteria, quorum sensing enables some of the *Pseudomonas* to again produce flagella, escape the biofilm, and colonize a new location (See Figs. 9A-9H).

Many chronic and difficult-to-treat infections are caused by bacteria in biofilms. Within biofilms, bacteria grow more slowly, exhibit different gene expression than free planktonic bacteria, and are more resistant to antimicrobial agents such as antibiotics because of the reduced ability of these chemicals to penetrate the dense biofilms matrix. Biofilms have been implicated in tuberculosis, kidney stones, *Staphylococcus* infections, Legionnaires' disease, and periodontal disease. It is further estimated that as

many as 10 million people a year in the US may develop biofilm-associated infections as a result of invasive medical procedures and surgical implants.

- Scanning electron micrograph of *Listeria* growing on a stainless steel surface. © Amy Lee Wong, author. Licensed for use, ASM MicrobeLibrary.
- Scanning electron micrograph of *Pseudomonas* growing on bronchial mucosa. © Hiroyuki Kobayashi, author. Licensed for use, ASM MicrobeLibrary.
- Scanning electron micrograph of *Staphylococcus aureus* forming a biofilm in an indwelling catheter courtesy of CDC.
- Article and computer-generated model of biofilm formation courtesy of NIH.



YouTube movie and animation: What are Biofilms?

? Exercise: Think-Pair-Share Questions

Pseudomonas aeruginosa, a common cause of serious respiratory infections on people with cystic fibrosis, produces a single polar flagellum, can secrete a polysaccharide slime composed of alginate, and is able to produce both pili and cell wall adhesins. How could each of these factors contribute to the bacterium's pathogenosis and in what order might they be used?

Summary

1. One of the body's innate immune defenses is the ability to physically remove bacteria from the body.
2. Bacteria may resist physical removal by producing pili, cell wall adhesin proteins, and/or biofilm-producing capsules that enable bacteria to adhere to host cells.
3. At the end of the shaft of a bacterial pilus is an adhesive tip structure having a shape corresponding to that of specific receptor on a host cell for initial attachment. Bacteria can typically make a variety of different adhesive tips enabling them to attach to different host cell receptors.
4. Cell wall adhesins are surface proteins found in the cell wall of various bacteria that bind tightly to specific receptor molecules on the surface of host cells. Bacteria can typically make a variety of different cell wall adhesins enabling them to attach to different host cell receptors.
5. Biofilms are groups of bacteria attached to a surface and enclosed in a common secreted adhesive matrix, typically polysaccharide in nature. Many pathogenic bacteria, as well as normal flora and many environmental bacteria, form complex bacterial communities as biofilms.
6. Many chronic and difficult-to-treat infections are caused by bacteria in biofilms.

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5.4.1.3: The Ability to Evade Adaptive Immune Defenses

Learning Objectives

1. State four ways the antibody molecules made during adaptive immunity protect us against bacteria.
2. Briefly describe at least three ways a bacterium might evade our adaptive immune defenses and name a bacterium that does each.

Overview of Adaptive Immune Defenses

One of the major defenses against bacteria is the immune defenses' production of antibody molecules against the organism. The "tips" of the antibody, called the Fab portion (Figure **5.4.1.3.1**) have shapes that are complementary to portions of bacterial proteins and polysaccharides called *epitopes*. The "bottom" of the antibody, called the Fc portion (Figure **5.4.1.3.1**) binds to receptors on phagocytes and NK cells) and can activate the classical complement pathway.

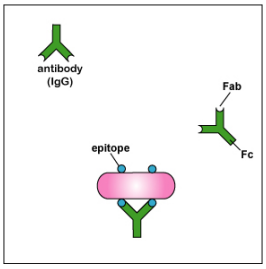


Figure **5.4.1.3.1**: Normal Antibody-Antigen Reaction. The Fab portion of the antibody has specificity for binding an epitope of an antigen. An epitope is the portion of an antigen - such as a few amino acids sticking out of a protein - to which the Fab portion of an antibody molecule fits. The Fc portion directs the biological activity of the antibody. In the case of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway. Antibodies are composed of 4 protein chains: 2 identical heavy chains and 2 identical light chains. Disulfide (S-S) bonds join the protein chains together.

There are various ways that the antibodies the body makes during adaptive immunity protect the body against bacteria:

a. As mentioned above under phagocytosis, some antibodies such as IgG and IgE function as opsonins and stick bacteria to phagocytes (Figure **5.4.1.3.2**).

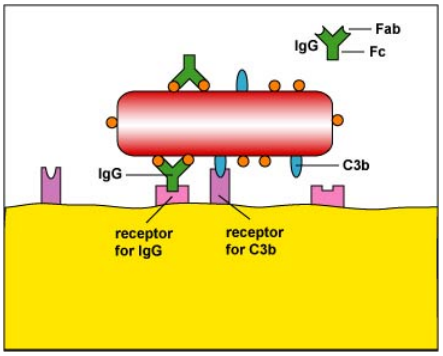


Figure **5.4.1.3.2**: An epitope is the portion of an antigen - such as a few amino acids sticking out of a protein - to which the Fab portion of an antibody molecule fits. One of the functions of certain antibody molecules known as IgG is to stick antigens such as bacterial proteins and polysaccharides to phagocytes. The tips of the antibody, the Fab portion, have a shape that fits epitopes, portions of an antigen with a complementary shape. The stalk of the antibody is called the Fc portion and is able to bind to Fc receptors on phagocytes. Also, when body defense pathways known as the complement pathways are activated, one of the beneficial defense proteins made is called C3b. C3b binds by one end to bacterial surface proteins and by the other end to C3b receptors on phagocytes. The IgG and C3b are also known as opsonins and the process of enhanced attachment is also called opsonization.

b. Antibodies, such as IgG, IgA, and IgM, can bind to bacterial adhesins, pili, and capsules and in this way block their attachment to host cells.

c. IgG and IgM can also activate the classical complement pathway providing all of its associated benefits.

d. IgA and IgM can clump bacteria together enabling them to be more readily removed by phagocytes (Figure 5.4.1.3.3).

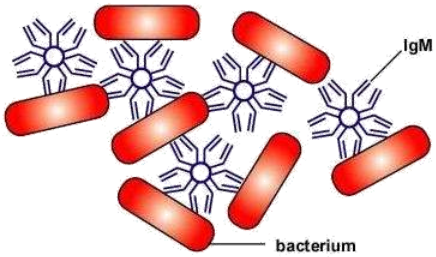


Figure 5.4.1.3.3: Agglutination of Microorganisms. The multiple Fab portions of IgM link microorganism together so out of the lymph and blood and phagocytosed more effectively.

These mechanisms will be discussed in greater detail in Unit 6.

? Exercise: Think-Pair-Share Questions

1. *Staphylococcus aureus* produces protein A, a protein that binds to the Fc portion of antibodies.

   How might this enable *S. aureus* to resist adaptive immunity?

2. Many bacteria that colonize the mucous membranes produce immunoglobulin protease, an enzyme that hydrolizes antibodies of the IgA class.

   How might this enable these bacteria to resist adaptive immunity?

Resisting Adaptive Immune Defenses

Bacteria utilize a variety of mechanisms to resist antibodies made during adaptive immunity. These include the following:

a. Certain bacteria can evade antibodies is by changing the adhesive tips of their pili as mentioned above with *Escherichia coli* and *Neisseria gonorrhoeae* (Figure 5.4.1.3.4).

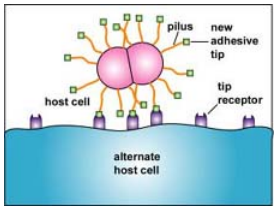


Figure 5.4.1.3.4: Bacteria Altering the Adhesive Tips of Their Pili. By genetically altering the adhesive tips of their pili, certain bacteria are able to: 1) adhere to and colonize different cell types with different receptors, and 2) evade antibodies made against the previous pili.

Bacteria can also vary other surface proteins so that antibodies previously made against those proteins will no longer "fit." (Figure 5.4.1.3.5). For example, *N. gonorrhoeae* produces Rmp protein (protein III) that protects against antibody attack by antibodies made against other surface proteins (such as adhesins) and the lipooligosaccharide (LOS) of the bacterium.

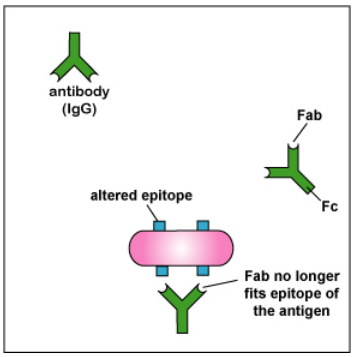


Figure 5.4.1.3.5: (A) Normal Antibody-Antigen Reaction. The Fab portion of the antibody has specificity for binding an epitope of an antigen. An epitope is the portion of an antigen - such as a few amino acids sticking out of a protein - to which the Fab portion of an antibody molecule fits. The Fc portion of an antibody directs the biological activity of the antibody. In the case of IgG, the Fc portion can bind to phagocytes for enhanced attachment (opsonization) as well as activate the classical complement pathway. (B) Altering Epitopes of an Antigen in order to Resist Antibody Molecules. The Fab portion of the antibody has specificity for binding an epitope of an antigen. By altering the molecular shape of an epitope of an antigen through mutation or genetic recombination, previous antibody molecules agains the original shaped epitope no longer fit or bind to the antigen.

b. Strains of *Neisseria meningitidis* have a capsule composed of sialic acid while strains of *Streptococcus pyogenes* (group A beta streptococci) have a capsule made of hyaluronic acid. Both of these polysaccharides closely resemble carbohydrates found in human tissue and because they are not recognized as foreign by the lymphocytes that carry out the adaptive immune responses, antibodies are not made against those capsules. Likewise, some bacteria are able to coat themselves with host proteins such as fibronectin, lactoferrin, or transferrin and in this way avoid having antibodies being made against them because they are unable to be recognized as foreign by lymphocytes.

c. *Staphylococcus aureus* produces protein A while *Streptococcus pyogenes* produces protein G. Both of these proteins bind to the Fc portion of the antibody IgG, the portion that is supposed to bind the bacterium to phagocytes during enhanced attachment (Figure 5.4.1.3.1). The bacteria become coated with antibodies in a way that does not result in opsonization (Figure 5.4.1.3.6).

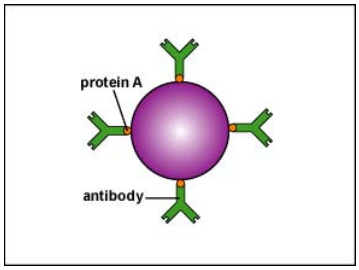


Figure 5.4.1.3.6: Staphylococcus aureus Resisting Opsonization via Protein A. The Fc portion of the antibody IgG, the portion that would normally binds to Fc receptors on phagocytes, instead binds to protein A on Staphylococcus aureus. In this way the bacterium becomes coated with a protective coat of antibodies that do not allow for opsonization.

d. *Salmonella* species can undergo phase variation of their capsular (K) and flagellar (H) antigens, that is, they can change the molecular shape of their capsular and flagellar antigens so that antibodies made against the previous form no longer fit the new form (Figure 5.4.1.3.5).

e. Bacteria such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Helicobacter pylori*, *Shigella flexneri*, *Neisseria meningitidis*, *Neisseria gonorrhoeae* and enteropathogenic *E. coli* produce immunoglobulin proteases. Immunoglobulin proteases degrade the body's protective antibodies (immunoglobulins) that are found in body secretions, a class of antibodies known as IgA.

f. Many pathogenic bacteria, as well as normal flora, form complex bacterial communities as biofilms. Bacteria in biofilms are often able to communicate with one another by a process called quorum sensing (discussed later in this unit) and are able to interact with and adapt to their environment as a population of bacteria rather than as

individual bacteria. By living as a community of bacteria as a biofilm, these bacteria are better able to resist attack by antibiotics and are better able to resist the host immune system.

Summary

1. There are various ways that the antibodies the body makes during adaptive immunity protect the body against bacteria.
2. Some antibodies such as IgG and IgE function as opsonins and stick bacteria to phagocytes (opsonization or enhanced attachment).
3. Antibodies, such as IgG, IgA, and IgM, can bind to bacterial adhesins, pili, and capsules and in this way block their attachment to host cells.
4. IgG and IgM can activate the classical complement pathway providing all of its associated benefits.
5. IgA and IgM can clump bacteria together enabling them to be more readily removed by phagocytes.
6. Antitoxin antibodies, mainly IgG, are made against bacterial exotoxins. They combine with the exotoxin molecules before they can interact with host target cells and thus neutralize the toxin.
7. Bacteria utilize a variety of mechanisms to resist antibodies made during adaptive immunity.
8. Some bacteria can vary their surface proteins or polysaccharides so that antibodies previously made against those proteins will no longer "fit."
9. Some bacteria are able to coat themselves with host proteins and in this way avoid having antibodies being made against them because they are unable to be recognized as foreign
10. Some bacteria produce immunoglobulin proteases that degrade the body's protective antibodies (immunoglobulins) that are found in body secretions.

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SECTION OVERVIEW

5.4.2: Virulence Factors that Damage the Host

Virulence factors that damage the host include: (1) the ability of PAMPs to trigger the production of inflammatory cytokines that result in an excessive inflammatory response; (2) the ability to produce harmful exotoxins; (3) and the ability to induce autoimmune responses. Most of the virulence factors we will discuss in this section that enable bacteria to harm the body are the products of quorum sensing genes. We will now look at each of these factors in greater detail.

Topic hierarchy

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5.5: Reservoirs of Infection and Disease Transmission

Learning Objectives

- Describe the different types of disease reservoirs
- Compare contact, vector, and vehicle modes of transmission
- Identify important disease vectors
- Explain the prevalence of nosocomial infections

Understanding how infectious pathogens spread is critical to preventing infectious disease. Many pathogens require a living host to survive, while others may be able to persist in a dormant state outside of a living host. But having infected one host, all pathogens must also have a mechanism of transfer from one host to another or they will die when their host dies. Pathogens often have elaborate adaptations to exploit host biology, behavior, and ecology to live in and move between hosts. Hosts have evolved defenses against pathogens, but because their rates of evolution are typically slower than their pathogens (because their generation times are longer), hosts are usually at an evolutionary disadvantage. This section will explore where pathogens survive—both inside and outside hosts—and some of the many ways they move from one host to another.

Reservoirs and Carriers

For pathogens to persist over long periods of time they require reservoirs where they normally reside. Reservoirs can be living organisms or nonliving sites. Nonliving reservoirs can include soil and water in the environment. These may naturally harbor the organism because it may grow in that environment. These environments may also become contaminated with pathogens in human feces, pathogens shed by intermediate hosts, or pathogens contained in the remains of intermediate hosts.

Pathogens may have mechanisms of dormancy or resilience that allow them to survive (but typically not to reproduce) for varying periods of time in nonliving environments. For example, *Clostridium tetani* survives in the soil and in the presence of oxygen as a resistant endospore. Although many viruses are soon destroyed once in contact with air, water, or other non-physiological conditions, certain types are capable of persisting outside of a living cell for varying amounts of time. For example, a study that looked at the ability of influenza viruses to infect a cell culture after varying amounts of time on a banknote showed survival times from 48 hours to 17 days, depending on how they were deposited on the banknote.[1] On the other hand, cold-causing rhinoviruses are somewhat fragile, typically surviving less than a day outside of physiological fluids.

A human acting as a reservoir of a pathogen may or may not be capable of transmitting the pathogen, depending on the stage of infection and the pathogen. To help prevent the spread of disease among school children, the CDC has developed guidelines based on the risk of transmission during the course of the disease. For example, children with chickenpox are considered contagious for five days from the start of the rash, whereas children with most gastrointestinal illnesses should be kept home for 24 hours after the symptoms disappear.

An individual capable of transmitting a pathogen without displaying symptoms is referred to as a carrier. A passive carrier is contaminated with the pathogen and can mechanically transmit it to another host; however, a passive carrier is not infected. For example, a health-care professional who fails to wash his hands after seeing a patient harboring an infectious agent could become a passive carrier, transmitting the pathogen to another patient who becomes infected.

By contrast, an active carrier is an infected individual who can transmit the disease to others. An active carrier may or may not exhibit signs or symptoms of infection. For example, active carriers may transmit the disease during the incubation period (before they show signs and symptoms) or the period of convalescence (after symptoms have subsided). Active carriers who do not present signs or symptoms of disease despite infection are called asymptomatic carriers. Pathogens such as hepatitis B virus, herpes simplex virus, and HIV are frequently transmitted by asymptomatic carriers. Mary Mallon, better known as Typhoid Mary, is a famous historical example of an asymptomatic carrier. An Irish immigrant, Mallon worked as a cook for households in and around New York City between 1900 and 1915. In each household, the residents developed typhoid fever (caused by *Salmonella typhi*) a few weeks after Mallon started working. Later investigations determined that Mallon was responsible for at least 122 cases of typhoid fever, five of which were fatal.[2] See Eye on Ethics: Typhoid Mary for more about the Mallon case.

A pathogen may have more than one living reservoir. In zoonotic diseases, animals act as reservoirs of human disease and transmit the infectious agent to humans through direct or indirect contact. In some cases, the disease also affects the animal, but in other cases the animal is asymptomatic.

In parasitic infections, the parasite's preferred host is called the definitive host. In parasites with complex life cycles, the definitive host is the host in which the parasite reaches sexual maturity. Some parasites may also infect one or more intermediate hosts in which the parasite goes through several immature life cycle stages or reproduces asexually.

Link to Learning

George Soper, the sanitary engineer who traced the typhoid outbreak to Mary Mallon, gives an account of his investigation, an example of descriptive epidemiology, in "The Curious Career of Typhoid Mary."

Exercise 5.5.1

1. List some nonliving reservoirs for pathogens.
2. Explain the difference between a passive carrier and an active carrier.

Transmission

Regardless of the reservoir, transmission must occur for an infection to spread. First, transmission from the reservoir to the individual must occur. Then, the individual must transmit the infectious agent to other susceptible individuals, either directly or indirectly. Pathogenic microorganisms employ diverse transmission mechanisms.

Contact Transmission

Contact transmission includes direct contact or indirect contact. Person-to-person transmission is a form of direct contact transmission. Here the agent is transmitted by physical contact between two individuals (Figure 5.5.1) through actions such as touching, kissing, sexual intercourse, or droplet sprays. Direct contact can be categorized as vertical, horizontal, or droplet transmission. Vertical direct contact transmission occurs when pathogens are transmitted from mother to child during pregnancy, birth, or breastfeeding. Other kinds of direct contact transmission are called horizontal direct contact transmission. Often, contact between mucous membranes is required for entry of the pathogen into the new host, although skin-to-skin contact can lead to mucous membrane contact if the new host subsequently touches a mucous membrane. Contact transmission may also be site-specific; for example, some diseases can be transmitted by sexual contact but not by other forms of contact.

When an individual coughs or sneezes, small droplets of mucus that may contain pathogens are ejected. This leads to direct droplet transmission, which refers to droplet transmission of a pathogen to a new host over distances of one meter or less. A wide variety of diseases are transmitted by droplets, including influenza and many forms of pneumonia. Transmission over distances greater than one meter is called airborne transmission.

Indirect contact transmission involves inanimate objects called fomites that become contaminated by pathogens from an infected individual or reservoir (Figure 5.5.2). For example, an individual with the common cold may sneeze, causing droplets to land on a fomite such as a tablecloth or carpet, or the individual may wipe her nose and then transfer mucus to a fomite such as a doorknob or towel. Transmission occurs indirectly when a new susceptible host later touches the fomite and transfers the contaminated material to a susceptible portal of entry. Fomites can also include objects used in clinical settings that are not properly sterilized, such as syringes, needles, catheters, and surgical equipment. Pathogens transmitted indirectly via such fomites are a major cause of healthcare-associated infections (see Controlling Microbial Growth).




Figure 5.5.1: Direct contact transmission of pathogens can occur through physical contact. Many pathogens require contact with a mucous membrane to enter the body, but the host may transfer the pathogen from another point of contact (e.g., hand) to a mucous membrane (e.g., mouth or eye). (credit left: modification of work by Lisa Doehnert)






Figure 5.5.2: Fomites are nonliving objects that facilitate the indirect transmission of pathogens. Contaminated doorknobs, towels, and syringes are all common examples of fomites. (credit left: modification of work by Kate Ter Haar; credit middle: modification of work by Vernon Swanepoel; credit right: modification of work by "Zaldylmg"/Flickr)

Vehicle Transmission

The term vehicle transmission refers to the transmission of pathogens through vehicles such as water, food, and air. Water contamination through poor sanitation methods leads to waterborne transmission of disease. Waterborne disease remains a serious problem in many regions throughout the world. The World Health Organization (WHO) estimates that contaminated drinking water is responsible for more than 500,000 deaths each year.[3] Similarly, food contaminated through poor handling or storage can lead to foodborne transmission of disease (Figure 5.5.3).

Dust and fine particles known as aerosols, which can float in the air, can carry pathogens and facilitate the airborne transmission of disease. For example, dust particles are the dominant mode of transmission of hantavirus to humans. Hantavirus is found in mouse feces, urine, and saliva, but when these substances dry, they can disintegrate into fine particles that can become airborne when disturbed; inhalation of these particles can lead to a serious and sometimes fatal respiratory infection.

Although droplet transmission over short distances is considered contact transmission as discussed above, longer distance transmission of droplets through the air is considered vehicle transmission. Unlike larger particles that drop quickly out of the air column, fine mucus droplets produced by coughs or sneezes can remain suspended for long periods of time, traveling considerable distances. In certain conditions, droplets desiccate quickly to produce a droplet nucleus that is capable of transmitting pathogens; air temperature and humidity can have an impact on effectiveness of airborne transmission.

Tuberculosis is often transmitted via airborne transmission when the causative agent, *Mycobacterium tuberculosis*, is released in small particles with coughs. Because tuberculosis requires as few as 10 microbes to initiate a new infection, patients with tuberculosis must be treated in rooms equipped with special ventilation, and anyone entering the room should wear a mask.



Figure 5.5.3: Food is an important vehicle of transmission for pathogens, especially of the gastrointestinal and upper respiratory systems. Notice the glass shield above the food trays, designed to prevent pathogens ejected in coughs and sneezes from entering the food. (credit: Fort George G. Meade Public Affairs Office)

Clinical Focus: Resolution

After identifying the source of the contaminated turduckens, the Florida public health office notified the CDC, which requested an expedited inspection of the facility by state inspectors. Inspectors found that a machine used to process the chicken was contaminated with *Salmonella* as a result of substandard cleaning protocols. Inspectors also found that the process of stuffing and packaging the turduckens prior to refrigeration allowed the meat to remain at temperatures conducive to bacterial growth for too long. The contamination and the delayed refrigeration led to vehicle (food) transmission of the bacteria in turduckens.

Based on these findings, the plant was shut down for a full and thorough decontamination. All turduckens produced in the plant were recalled and pulled from store shelves ahead of the December holiday season, preventing further outbreaks.

Vector Transmission

Diseases can also be transmitted by a mechanical or biological vector, an animal (typically an arthropod) that carries the disease from one host to another. Mechanical transmission is facilitated by a mechanical vector, an animal that carries a pathogen from one host to another without being infected itself. For example, a fly may land on fecal matter and later transmit bacteria from the feces to food that it lands on; a human eating the food may then become infected by the bacteria, resulting in a case of diarrhea or dysentery (Figure 5.5.4).

Biological transmission occurs when the pathogen reproduces within a biological vector that transmits the pathogen from one host to another (Figure 5.5.4). Arthropods are the main vectors responsible for biological transmission (Figure 5.5.5). Most arthropod vectors transmit the pathogen by biting the host, creating a wound that serves as a portal of entry. The pathogen may go through part of its reproductive cycle in the gut or salivary glands of the arthropod to facilitate its transmission through the bite. For example, hemipterans (called “kissing bugs” or “assassin bugs”) transmit Chagas disease to humans by defecating when they bite, after which the human scratches or rubs the infected feces into a mucous membrane or break in the skin.

Biological insect vectors include mosquitoes, which transmit malaria and other diseases, and lice, which transmit typhus. Other arthropod vectors can include arachnids, primarily ticks, which transmit Lyme disease and other diseases, and mites, which transmit scrub typhus and rickettsial pox. Biological transmission, because it involves survival and reproduction within a parasitized vector, complicates the biology of the pathogen and its transmission. There are also important non-arthropod vectors of disease, including mammals and birds. Various species of mammals can transmit rabies to humans, usually by means of a bite that transmits the rabies virus. Chickens and other domestic poultry can transmit avian influenza to humans through direct or indirect contact with avian influenza virus A shed in the birds’ saliva, mucous, and feces.

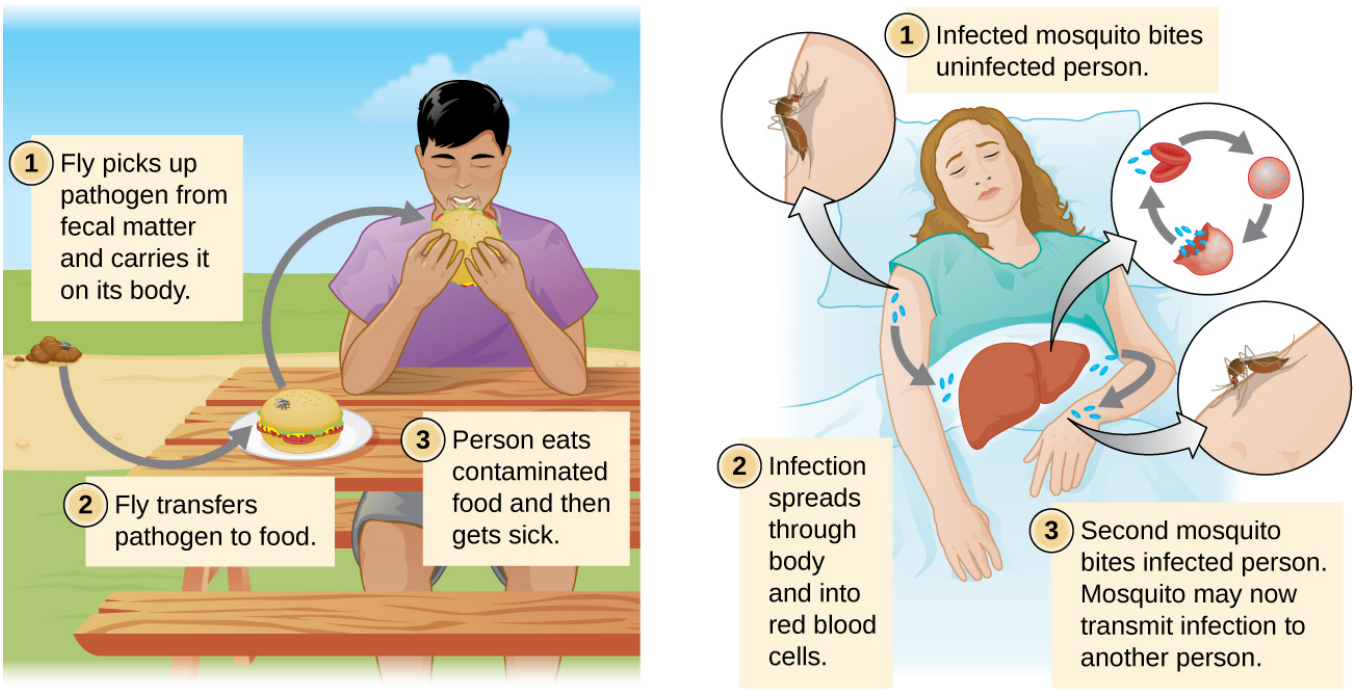


Figure 5.5.4: (a) A mechanical vector carries a pathogen on its body from one host to another, not as an infection. (b) A biological vector carries a pathogen from one host to another after becoming infected itself.

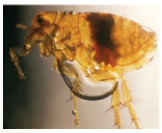
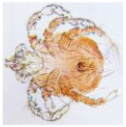
Common Arthropod Vectors and Select Pathogens			
Vector	**Species**	**Pathogen**	**Disease**
Black fly 	*Simulium* spp.	*Onchocerca volvulus*	Onchocerciasis (river blindness)
Flea 	*Xenopsylla cheopis*	*Rickettsia typhi*	Murine typhus
		Yersinia pestis	Plague
Kissing bug 	*Triatoma* spp.	*Trypanosoma cruzi*	Chagas disease
Louse 	*Pediculus humanus humanus*	*Bartonella quintana*	Trench fever
		Borrelia recurrentis	Relapsing fever
		Rickettsia prowazekii	Typhus
Mite (chigger) 	*Leptotrombidium* spp.	*Orientia tsutsugamushi*	Scrub typhus
	Liponyssoides sanguineus	*Rickettsia akari*	Rickettsialpox
Mosquito 	*Aedes* spp., *Haemagogus* spp.	*Yellow fever virus*	Yellow fever
	Anopheles spp.	*Plasmodium falciparum*	Malaria
	Culex pipiens	*West Nile virus*	West Nile disease
Sand fly 	*Phlebotomus* spp.	*Leishmania* spp.	Leishmaniasis
Tick 	*Ixodes* spp.	*Borrelia* spp.	Lyme disease
	Dermacentor spp. and others	*Rickettsia rickettsii*	Rocky Mountain spotted fever
Tsetse fly 	*Glossina* spp.	*Trypanosoma brucei*	African trypanosomiasis (sleeping sickness)

Figure 5.5.5: (credit “Black fly”, “Tick”, “Tsetse fly”: modification of work by USDA; credit: “Flea”: modification of work by Centers for Disease Control and Prevention; credit: “Louse”, “Mosquito”, “Sand fly”: modification of work by James Gathany, Centers for Disease Control and Prevention; credit “Kissing bug”: modification of work by Glenn Seplak; credit “Mite”: modification of work by Michael Wunderli)

Exercise 5.5.2

1. Describe how diseases can be transmitted through the air.
2. Explain the difference between a mechanical vector and a biological vector.

Using GMOs to Stop the Spread of Zika

In 2016, an epidemic of the Zika virus was linked to a high incidence of birth defects in South America and Central America. As winter turned to spring in the northern hemisphere, health officials correctly predicted the virus would spread to North America, coinciding with the breeding season of its major vector, the *Aedes aegypti* mosquito.

The range of the *A. aegypti* mosquito extends well into the southern United States (Figure **5.5.6**). Because these same mosquitoes serve as vectors for other problematic diseases (dengue fever, yellow fever, and others), various methods of mosquito control have been proposed as solutions. Chemical pesticides have been used effectively in the past, and are likely to be used again; but because chemical pesticides can have negative impacts on the environment, some scientists have proposed an alternative that involves genetically engineering *A. aegypti* so that it cannot reproduce. This method, however, has been the subject of some controversy.

One method that has worked in the past to control pests, with little apparent downside, has been sterile male introductions. This method controlled the screw-worm fly pest in the southwest United States and fruit fly pests of fruit crops. In this method, males of the target species are reared in the lab, sterilized with radiation, and released into the environment where they mate with wild females, who subsequently bear no live offspring. Repeated releases shrink the pest population.

A similar method, taking advantage of recombinant DNA technology,[4] introduces a dominant lethal allele into male mosquitoes that is suppressed in the presence of tetracycline (an antibiotic) during laboratory rearing. The males are released into the environment and mate with female mosquitoes. Unlike the sterile male method, these matings produce offspring, but they die as larvae from the lethal gene in the absence of tetracycline in the environment. As of 2016, this method has yet to be implemented in the United States, but a UK company tested the method in Piracicaba, Brazil, and found an 82% reduction in wild *A. aegypti* larvae and a 91% reduction in dengue cases in the treated area.[5] In August 2016, amid news of Zika infections in several Florida communities, the FDA gave the UK company permission to test this same mosquito control method in Key West, Florida, pending compliance with local and state regulations and a referendum in the affected communities.

The use of genetically modified organisms (GMOs) to control a disease vector has its advocates as well as its opponents. In theory, the system could be used to drive the *A. aegypti* mosquito extinct—a noble goal according to some, given the damage they do to human populations.[6] But opponents of the idea are concerned that the gene could escape the species boundary of *A. aegypti* and cause problems in other species, leading to unforeseen ecological consequences. Opponents are also wary of the program because it is being administered by a for-profit corporation, creating the potential for conflicts of interest that would have to be tightly regulated; and it is not clear how any unintended consequences of the program could be reversed.

There are other epidemiological considerations as well. *Aedes aegypti* is apparently not the only vector for the Zika virus. *Aedes albopictus*, the Asian tiger mosquito, is also a vector for the Zika virus.[7] *A. albopictus* is now widespread around the planet including much of the United States (Figure **5.5.6**). Many other mosquitoes have been found to harbor Zika virus, though their capacity to act as vectors is unknown.[8] Genetically modified strains of *A. aegypti* will not control the other species of vectors. Finally, the Zika virus can apparently be transmitted sexually between human hosts, from mother to child, and possibly through blood transfusion. All of these factors must be considered in any approach to controlling the spread of the virus.

Clearly there are risks and unknowns involved in conducting an open-environment experiment of an as-yet poorly understood technology. But allowing the Zika virus to spread unchecked is also risky. Does the threat of a Zika epidemic justify the ecological risk of genetically engineering mosquitos? Are current methods of mosquito control sufficiently ineffective or harmful that we need to try untested alternatives? These are the questions being put to public health officials now.

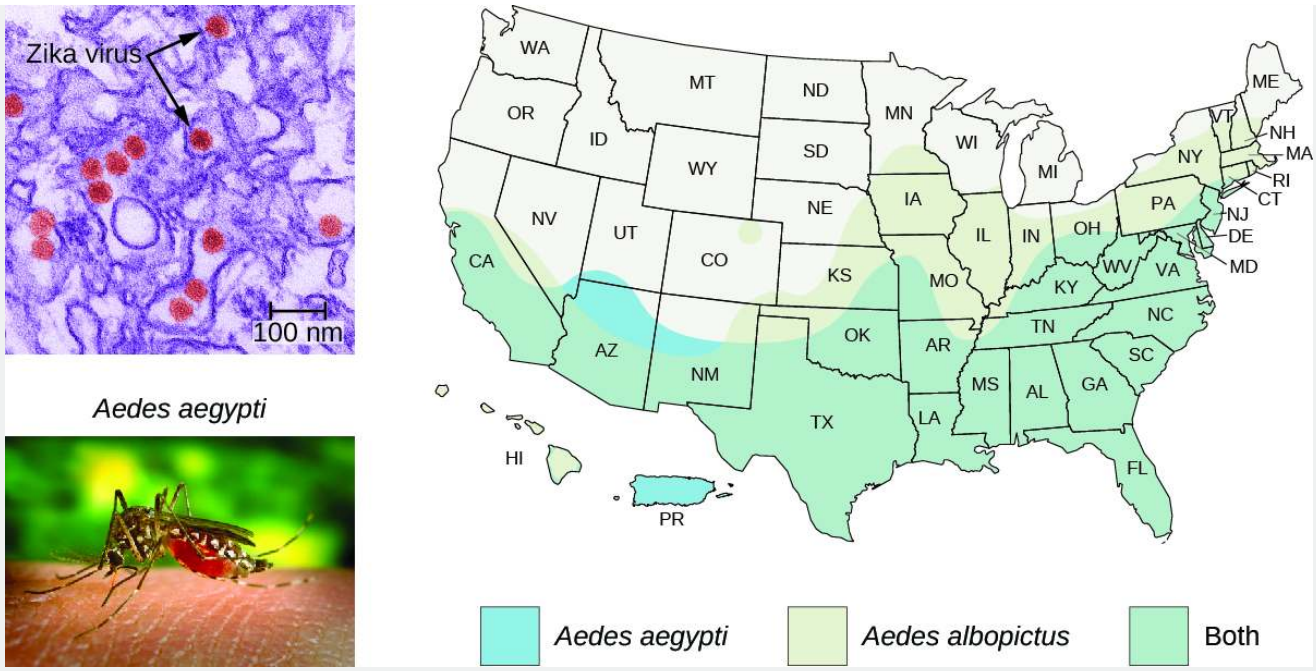


Figure 5.5.6: The Zika virus is an enveloped virus transmitted by mosquitoes, especially *Aedes aegypti.* The range of this mosquito includes much of the United States, from the Southwest and Southeast to as far north as the Mid-Atlantic. The range of *A. albopictus*, another vector, extends even farther north to New England and parts of the Midwest. (credit micrograph: modification of work by Cynthia Goldsmith, Centers for Disease Control and Prevention; credit photo: modification of work by James Gathany, Centers for Disease Control and Prevention; credit map: modification of work by Centers for Disease Control and Prevention)

Quarantining

Individuals suspected or known to have been exposed to certain contagious pathogens may be quarantined, or isolated to prevent transmission of the disease to others. Hospitals and other health-care facilities generally set up special wards to isolate patients with particularly hazardous diseases such as tuberculosis or Ebola (Figure 5.5.7). Depending on the setting, these wards may be equipped with special air-handling methods, and personnel may implement special protocols to limit the risk of transmission, such as personal protective equipment or the use of chemical disinfectant sprays upon entry and exit of medical personnel.

The duration of the quarantine depends on factors such as the incubation period of the disease and the evidence suggestive of an infection. The patient may be released if signs and symptoms fail to materialize when expected or if preventive treatment can be administered in order to limit the risk of transmission. If the infection is confirmed, the patient may be compelled to remain in isolation until the disease is no longer considered contagious.

In the United States, public health authorities may only quarantine patients for certain diseases, such as cholera, diphtheria, infectious tuberculosis, and strains of influenza capable of causing a pandemic. Individuals entering the United States or moving between states may be quarantined by the CDC if they are suspected of having been exposed to one of these diseases. Although the CDC routinely monitors entry points to the United States for crew or passengers displaying illness, quarantine is rarely implemented.



(a)



(b)

Figure 5.5.7: (a) The Aeromedical Biological Containment System (ABCS) is a module designed by the CDC and Department of Defense specifically for transporting highly contagious patients by air. (b) An isolation ward for Ebola patients in Lagos, Nigeria. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by CDC Global)

Healthcare-Associated (Nosocomial) Infections

Hospitals, retirement homes, and prisons attract the attention of epidemiologists because these settings are associated with increased incidence of certain diseases. Higher rates of transmission may be caused by characteristics of the environment itself, characteristics of the population, or both. Consequently, special efforts must be taken to limit the risks of infection in these settings.

Infections acquired in health-care facilities, including hospitals, are called nosocomial infections or healthcare-associated infections (HAI). HAIs are often connected with surgery or other invasive procedures that provide the pathogen with access to the portal of infection. For an infection to be classified as an HAI, the patient must have been admitted to the health-care facility for a reason other than the infection. In these settings, patients suffering from primary disease are often afflicted with compromised immunity and are more susceptible to secondary infection and opportunistic pathogens.

In 2011, more than 720,000 HAIs occurred in hospitals in the United States, according to the CDC. About 22% of these HAIs occurred at a surgical site, and cases of pneumonia accounted for another 22%; urinary tract infections accounted for an additional 13%, and primary bloodstream infections 10%.[9] Such HAIs often occur when pathogens are introduced to patients' bodies through contaminated surgical or medical equipment, such as catheters and respiratory ventilators. Health-care facilities seek to limit nosocomial infections through training and hygiene protocols such as those described in Control of Microbial Growth.

? Exercise 5.5.3

Give some reasons why HAIs occur.

Key Concepts and Summary

- **Reservoirs** of human disease can include the human and animal populations, soil, water, and inanimate objects or materials.
- **Contact transmission** can be **direct** or **indirect** through physical contact with either an infected host (direct) or contact with a fomite that an infected host has made contact with previously (indirect).
- Vector transmission occurs when a living organism carries an infectious agent on its body (**mechanical**) or as an infection host itself (**biological**), to a new host.
- **Vehicle transmission** occurs when a substance, such as soil, water, or air, carries an infectious agent to a new host.
- **Healthcare-associated infections (HAI)**, or **nosocomial infections**, are acquired in a clinical setting. Transmission is facilitated by medical interventions and the high concentration of susceptible, immunocompromised individuals in clinical settings.

Footnotes

1. 1 Yves Thomas, Guido Vogel, Werner Wunderli, Patricia Suter, Mark Witschi, Daniel Koch, Caroline Tapparel, and Laurent Kaiser. “Survival of Influenza Virus on Banknotes.” *Applied and Environmental Microbiology* 74, no. 10 (2008): 3002–3007.
2. 2 Filio Marineli, Gregory Tsoucalas, Marianna Karamanou, and George Androutsos. “Mary Mallon (1869–1938) and the History of Typhoid Fever.” *Annals of Gastroenterology* 26 (2013): 132–134. www.ncbi.nlm.nih.gov/pmc/arti...rol-26-132.pdf.
3. 3 World Health Organization. Fact sheet No. 391—*Drinking Water.* June 2005. www.who.int/mediacentre/factsheets/fs391/en.
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5. 5 Richard Levine. “Cases of Dengue Drop 91 Percent Due to Genetically Modified Mosquitoes.” *Entomology Today.* entomologytoday.org/2016/07/...ied-mosquitoes.
6. 6 Olivia Judson. “A Bug’s Death.” *The New York Times*, September 25, 2003. www.nytimes.com/2003/09/25/op...g-s-death.html.
7. 7 Gilda Grard, Mélanie Caron, Illich Manfred Mombo, Dieudonné Nkoghe, Statiana Mboui Ondo, Davy Jiolle, Didier Fontenille, Christophe Paupy, and Eric Maurice Leroy. “Zika Virus in Gabon (Central Africa)–2007: A New Threat from *Aedes albopictus*?” *PLOS Neglected Tropical Diseases* 8, no. 2 (2014): e2681.
8. 8 Constância F.J. Ayres. “Identification of Zika Virus Vectors and Implications for Control.” *The Lancet Infectious Diseases* 16, no. 3 (2016): 278–279.
9. 9 Centers for Disease Control and Prevention. “HAI Data and Statistics.” 2016. http://www.cdc.gov/hai/surveillance. Accessed Jan 2, 2016.

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5.6: Introduction to Epidemiology

Learning Objectives

- Explain the difference between prevalence and incidence of disease
- Distinguish the characteristics of sporadic, endemic, epidemic, and pandemic diseases
- Explain the use of Koch's postulates and their modifications to determine the etiology of disease
- Explain the relationship between epidemiology and public health

Clinical Focus: Part 1

In late November and early December, a hospital in western Florida started to see a spike in the number of cases of acute gastroenteritis-like symptoms. Patients began arriving at the emergency department complaining of excessive bouts of emesis (vomiting) and diarrhea (with no blood in the stool). They also complained of abdominal pain and cramping, and most were severely dehydrated. Alarmed by the number of cases, hospital staff made some calls and learned that other regional hospitals were also seeing 10 to 20 similar cases per day.

Exercise 5.6.1

1. What are some possible causes of this outbreak?
2. In what ways could these cases be linked, and how could any suspected links be confirmed?

The field of epidemiology concerns the geographical distribution and timing of infectious disease occurrences and how they are transmitted and maintained in nature, with the goal of recognizing and controlling outbreaks. The science of epidemiology includes etiology (the study of the causes of disease) and investigation of disease transmission (mechanisms by which a disease is spread).

Analyzing Disease in a Population

Epidemiological analyses are always carried out with reference to a population, which is the group of individuals that are at risk for the disease or condition. The population can be defined geographically, but if only a portion of the individuals in that area are susceptible, additional criteria may be required. Susceptible individuals may be defined by particular behaviors, such as intravenous drug use, owning particular pets, or membership in an institution, such as a college. Being able to define the population is important because most measures of interest in epidemiology are made with reference to the size of the population.

The state of being diseased is called morbidity. Morbidity in a population can be expressed in a few different ways. Morbidity or total morbidity is expressed in numbers of individuals without reference to the size of the population. The morbidity rate can be expressed as the number of diseased individuals out of a standard number of individuals in the population, such as 100,000, or as a percent of the population.

There are two aspects of morbidity that are relevant to an epidemiologist: a disease's prevalence and its incidence. Prevalence is the number, or proportion, of individuals with a particular illness in a given population at a point in time. For example, the Centers for Disease Control and Prevention (CDC) estimated that in 2012, there were about 1.2 million people 13 years and older with an active human immunodeficiency virus (HIV) infection. Expressed as a proportion, or rate, this is a prevalence of 467 infected persons per 100,000 in the population.[1] On the other hand, incidence is the number or proportion of *new* cases in a period of time. For the same year and population, the CDC estimates that there were 43,165 newly diagnosed cases of HIV infection, which is an incidence of 13.7 new cases per 100,000 in the population.[2] The relationship between incidence and prevalence can be seen in Figure **5.6.1**. For a chronic disease like HIV infection, prevalence will generally be higher than incidence because it represents the cumulative number of new cases over many years minus the number of cases that are no longer active (e.g., because the patient died or was cured).

In addition to morbidity rates, the incidence and prevalence of mortality (death) may also be reported. A mortality ratecan be expressed as the percentage of the population that has died from a disease or as the number of deaths per 100,000 persons (or other suitable standard number).

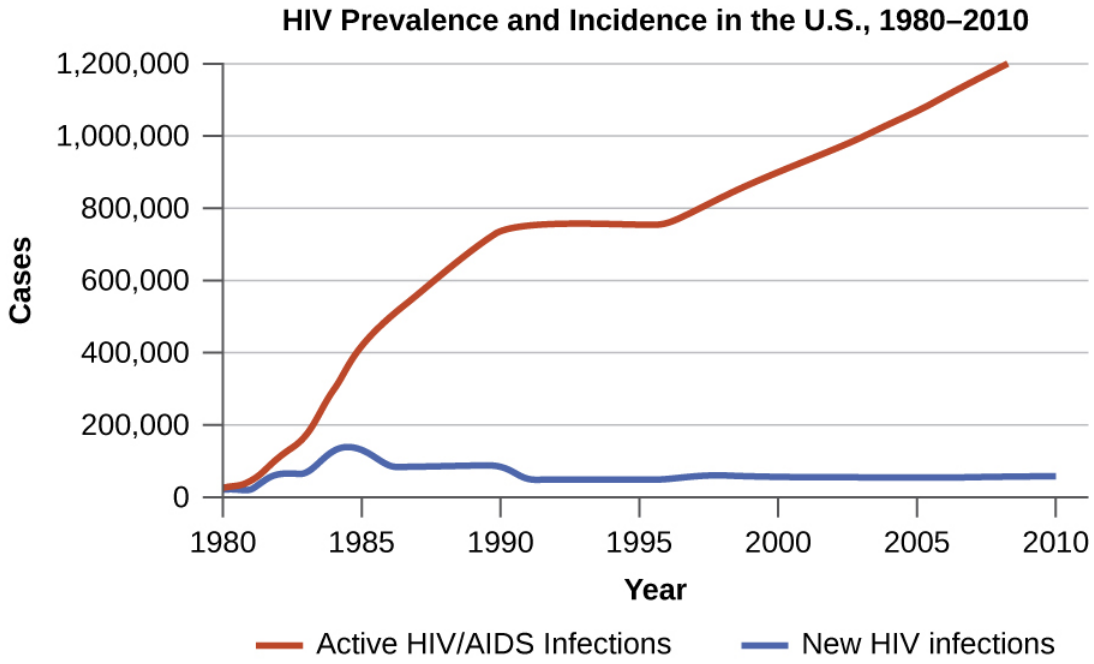


Figure **5.6.1**: This graph compares the incidence of HIV (the number of new cases reported each year) with the prevalence (the total number of cases each year). Prevalence and incidence can also be expressed as a rate or proportion for a given population.

? Exercise 5.6.2

1. Explain the difference between incidence and prevalence.
2. Describe how morbidity and mortality rates are expressed.

Patterns of Incidence

Diseases that are seen only occasionally, and usually without geographic concentration, are called sporadic diseases. Examples of sporadic diseases include tetanus, rabies, and plague. In the United States, *Clostridium tetani*, the bacterium that causes tetanus, is ubiquitous in the soil environment, but incidences of infection occur only rarely and in scattered locations because most individuals are vaccinated, clean wounds appropriately, or are only rarely in a situation that would cause infection.[3] Likewise in the United States there are a few scattered cases of plague each year, usually contracted from rodents in rural areas in the western states.[4]

Diseases that are constantly present (often at a low level) in a population within a particular geographic region are called endemic diseases. For example, malaria is endemic to some regions of Brazil, but is not endemic to the United States.

Diseases for which a larger than expected number of cases occurs in a short time within a geographic region are called epidemic diseases. Influenza is a good example of a commonly epidemic disease. Incidence patterns of influenza tend to rise each winter in the northern hemisphere. These seasonal increases are expected, so it would not be accurate to say that influenza is epidemic every winter; however, some winters have an usually large number of seasonal influenza cases in particular regions, and such situations would qualify as epidemics (Figure **5.6.2** and Figure **5.6.3**).

An epidemic disease signals the breakdown of an equilibrium in disease frequency, often resulting from some change in environmental conditions or in the population. In the case of influenza, the disruption can be due to antigenic shift or drift (see Virulence Factors of Bacterial and Viral Pathogens), which allows influenza virus strains to circumvent the acquired immunity of their human hosts.

An epidemic that occurs on a worldwide scale is called a pandemic disease. For example, HIV/AIDS is a pandemic disease and novel influenza virus strains often become pandemic.

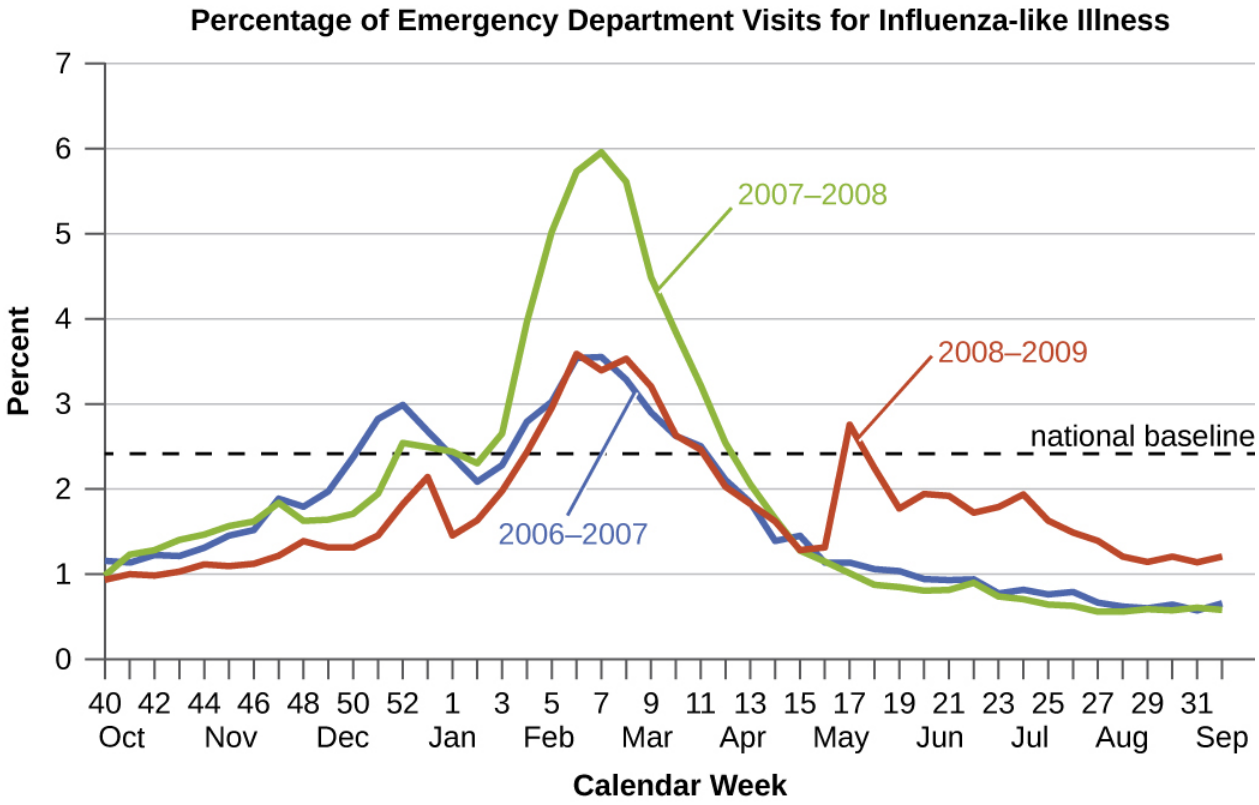


Figure **5.6.2**: The 2007–2008 influenza season in the United States saw much higher than normal numbers of visits to emergency departments for influenza-like symptoms as compared to the previous and the following years. (credit: modification of work by Centers for Disease Control and Prevention)

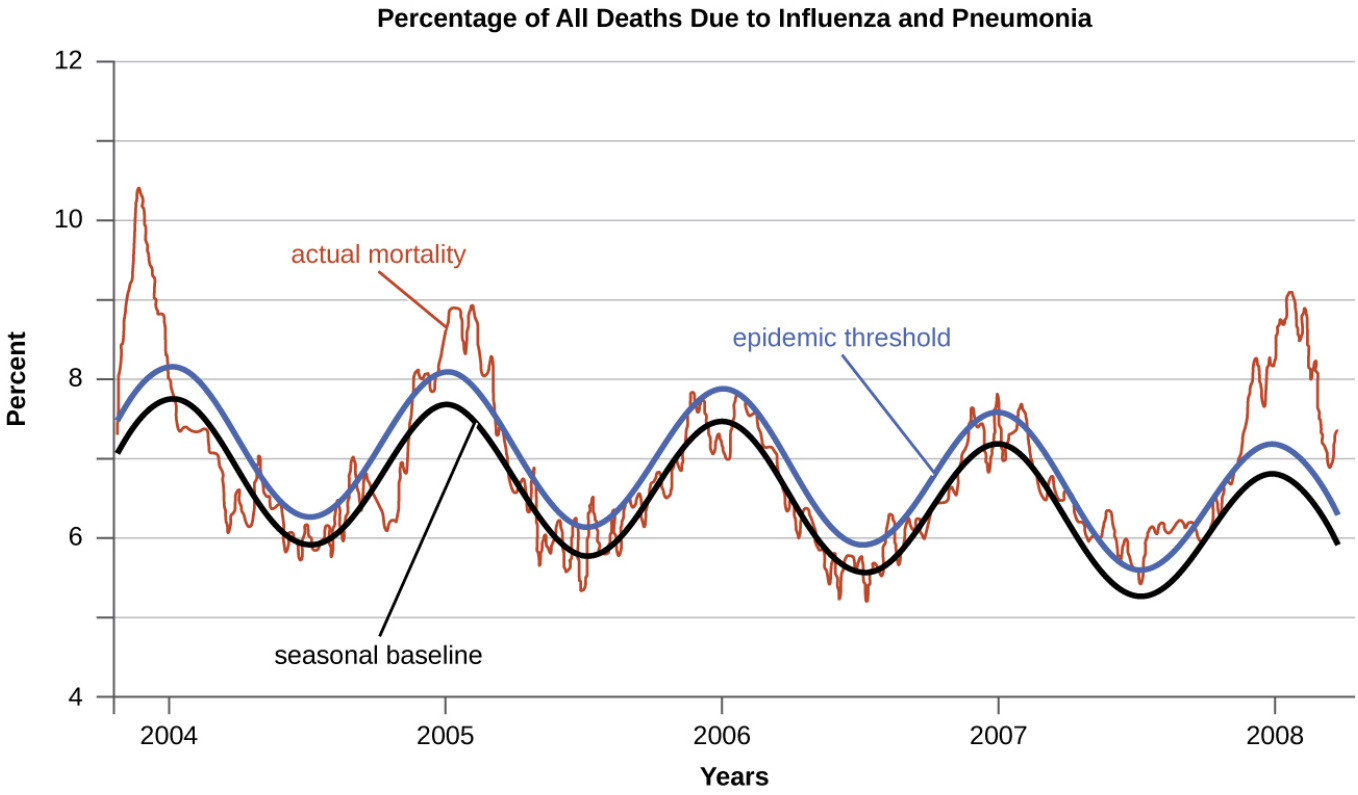


Figure **5.6.3**: The seasonal epidemic threshold (blue curve) is set by the CDC-based data from the previous five years. When actual mortality rates exceed this threshold, a disease is considered to be epidemic. As this graph shows, pneumonia- and influenza-related mortality saw pronounced epidemics during the winters of 2003–2004, 2005, and 2008. (credit: modification of work by Centers for Disease Control and Prevention)

? Exercise 5.6.3

1. Explain the difference between sporadic and endemic disease.
2. Explain the difference between endemic and epidemic disease.

Clinical Focus: Part 2

Hospital physicians suspected that some type of food poisoning was to blame for the sudden post-Thanksgiving outbreak of gastroenteritis in western Florida. Over a two-week period, 254 cases were observed, but by the end of the first week of December, the epidemic ceased just as quickly as it had started. Suspecting a link between the cases based on the localized nature of the outbreak, hospitals handed over their medical records to the regional public health office for study.

Laboratory testing of stool samples had indicated that the infections were caused by *Salmonella* bacteria. Patients ranged from children as young as three to seniors in their late eighties. Cases were nearly evenly split between males and females. Across the region, there had been three confirmed deaths in the outbreak, all due to severe dehydration. In each of the fatal cases, the patients had not sought medical care until their symptoms were severe; also, all of the deceased had preexisting medical conditions such as congestive heart failure, diabetes, or high blood pressure.

After reviewing the medical records, epidemiologists with the public health office decided to conduct interviews with a randomly selected sample of patients.

Exercise 5.6.4

1. What conclusions, if any, can be drawn from the medical records?
2. What would epidemiologists hope to learn by interviewing patients? What kinds of questions might they ask?

Etiology

When studying an epidemic, an epidemiologist's first task is to determinate the cause of the disease, called the etiologic agent or causative agent. Connecting a disease to a specific pathogen can be challenging because of the extra effort typically required to demonstrate direct causation as opposed to a simple association. It is not enough to observe an association between a disease and a suspected pathogen; controlled experiments are needed to eliminate other possible causes. In addition, pathogens are typically difficult to detect when there is no immediate clue as to what is causing the outbreak. Signs and symptoms of disease are also commonly nonspecific, meaning that many different agents can give rise to the same set of signs and symptoms. This complicates diagnosis even when a causative agent is familiar to scientists.

Robert Koch was the first scientist to specifically demonstrate the causative agent of a disease (anthrax) in the late 1800s. Koch developed four criteria, now known as Koch's postulates, which had to be met in order to positively link a disease with a pathogenic microbe. Without Koch's postulates, the Golden Age of Microbiology would not have occurred. Between 1876 and 1905, many common diseases were linked with their etiologic agents, including cholera, diphtheria, gonorrhea, meningitis, plague, syphilis, tetanus, and tuberculosis. Today, we use the molecular Koch's postulates, a variation of Koch's original postulates that can be used to establish a link between the disease state and virulence traits unique to a pathogenic strain of a microbe. Koch's original postulates and molecular Koch's postulates were described in more detail in How Pathogens Cause Disease.

? Exercise 5.6.5

List some challenges to determining the causative agent of a disease outbreak.

The Role of Public Health Organizations

The main national public health agency in the United States is the Centers for Disease Control and Prevention (CDC), an agency of the Department of Health and Human Services. The CDC is charged with protecting the public from disease and injury. One way that the CDC carries out this mission is by overseeing the National Notifiable Disease Surveillance System (NNDSS) in cooperation with regional, state, and territorial public health departments. The NNDSS monitors diseases considered to be of public health importance on a national scale. Such diseases are called notifiable diseases or reportable diseases because all cases must be reported to the CDC. A physician treating a patient with a notifiable disease is legally required to submit a report on the case. Notifiable diseases include HIV infection, measles, West Nile virus infections, and many others. Some states have their own lists of notifiable diseases that include diseases beyond those on the CDC's list.

Notifiable diseases are tracked by epidemiological studies and the data is used to inform health-care providers and the public about possible risks. The CDC publishes the *Morbidity and Mortality Weekly Report* (*MMWR*), which provides physicians and health-care workers with updates on public health issues and the latest data pertaining to notifiable diseases. Table **5.6.1** is an example of the kind of data contained in the *MMWR*.

Table **5.6.1**: Incidence of Four Notifiable Diseases in the United States, Week Ending January 2, 2016

Disease	Current Week (Jan 2, 2016)	Median of Previous 52 Weeks	Maximum of Previous 52 Weeks	Cumulative Cases 2015
Campylobacteriosis	406	869	1,385	46,618
Chlamydia trachomatis infection	11,024	28,562	31,089	1,425,303
Giardiasis	115	230	335	11,870
Gonorrhea	3,207	7,155	8,283	369,926

Link to Learning

The current *Morbidity and Mortality Weekly Report* is available online.

? Exercise 5.6.6

Describe how health agencies obtain data about the incidence of diseases of public health importance.

Key Concepts and Summary

- **Epidemiology** is the science underlying public health.
- **Morbidity** means being in a state of illness, whereas **mortality** refers to death; both **morbidity rates** and **mortality rates** are of interest to epidemiologists.
- **Incidence** is the number of new cases (morbidity or mortality), usually expressed as a proportion, during a specified time period; **prevalence** is the total number affected in the population, again usually expressed as a proportion.
- **Sporadic diseases** only occur rarely and largely without a geographic focus. **Endemic diseases** occur at a constant (and often low) level within a population. **Epidemic diseases** and **pandemic diseases** occur when an outbreak occurs on a significantly larger than expected level, either locally or globally, respectively.
- **Koch's postulates** specify the procedure for confirming a particular pathogen as the etiologic agent of a particular disease. Koch's postulates have limitations in application if the microbe cannot be isolated and cultured or if there is no animal host for the microbe. In this case, molecular Koch's postulates would be utilized.
- In the United States, the **Centers for Disease Control and Prevention** monitors **notifiable diseases** and publishes weekly updates in the *Morbidity and Mortality Weekly Report.*

Footnotes

1. 1 H. Irene Hall, Qian An, Tian Tang, Ruiguang Song, Mi Chen, Timothy Green, and Jian Kang. "Prevalence of Diagnosed and Undiagnosed HIV Infection—United States, 2008–2012." *Morbidity and Mortality Weekly Report* 64, no. 24 (2015): 657–662.
2. 2 Centers for Disease Control and Prevention. "Diagnoses of HIV Infection in the United States and Dependent Areas, 2014." *HIV Surveillance Report* 26 (2015).
3. 3 Centers for Disease Control and Prevention. "Tetanus Surveillance—United States, 2001–2008." *Morbidity and Mortality Weekly Report* 60, no. 12 (2011): 365.
4. 4 Centers for Disease Control and Prevention. "Plague in the United States." 2015. http://www.cdc.gov/plague/maps. Accessed June 1, 2016.

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5.6.1: Epidemiology

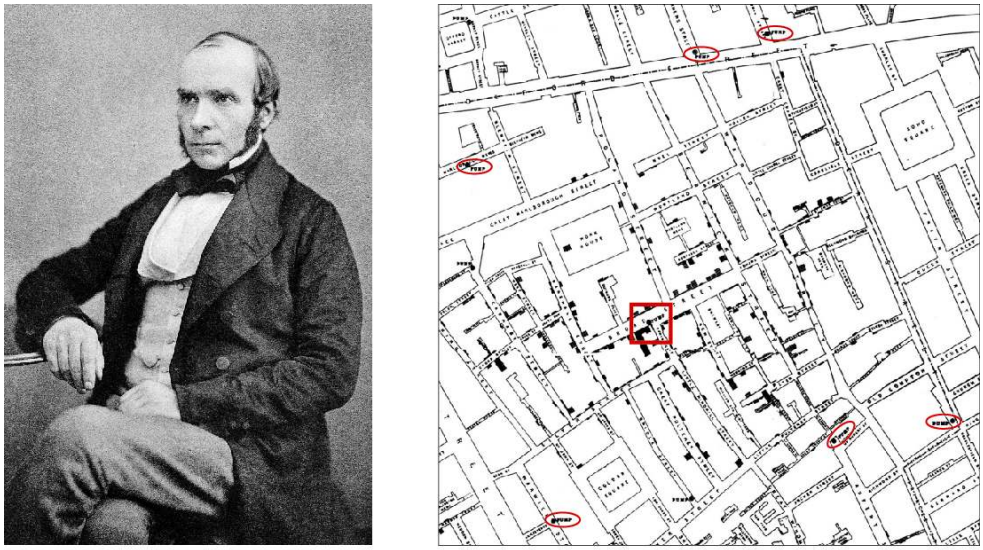
Learning Objectives

- Explain the research approaches used by the pioneers of epidemiology
- Explain how descriptive, analytical, and experimental epidemiological studies go about determining the cause of morbidity and mortality

Epidemiology has its roots in the work of physicians who looked for patterns in disease occurrence as a way to understand how to prevent it. The idea that disease could be transmitted was an important precursor to making sense of some of the patterns. In 1546, Girolamo Fracastoro first proposed the germ theory of disease in his essay *De Contagione et Contagiosis Morbis*, but this theory remained in competition with other theories, such as the miasma hypothesis, for many years (see What Our Ancestors Knew). Uncertainty about the cause of disease was not an absolute barrier to obtaining useful knowledge from patterns of disease. Some important researchers, such as Florence Nightingale, subscribed to the miasma hypothesis. The transition to acceptance of the germ theory during the 19th century provided a solid mechanistic grounding to the study of disease patterns. The studies of 19th century physicians and researchers such as John Snow, Florence Nightingale, Ignaz Semmelweis, Joseph Lister, Robert Koch, Louis Pasteur, and others sowed the seeds of modern epidemiology.

Pioneers of Epidemiology

John Snow (Figure 5.6.1.1) was a British physician known as the father of epidemiology for determining the source of the 1854 Broad Street cholera epidemic in London. Based on observations he had made during an earlier cholera outbreak (1848–1849), Snow proposed that cholera was spread through a fecal-oral route of transmission and that a microbe was the infectious agent. He investigated the 1854 cholera epidemic in two ways. First, suspecting that contaminated water was the source of the epidemic, Snow identified the source of water for those infected. He found a high frequency of cholera cases among individuals who obtained their water from the River Thames downstream from London. This water contained the refuse and sewage from London and settlements upstream. He also noted that brewery workers did not contract cholera and on investigation found the owners provided the workers with beer to drink and stated that they likely did not drink water.[1] Second, he also painstakingly mapped the incidence of cholera and found a high frequency among those individuals using a particular water pump located on Broad Street. In response to Snow's advice, local officials removed the pump's handle,[2] resulting in the containment of the Broad Street cholera epidemic.



(a) (b)

Figure 5.6.1.1: (a) John Snow (1813–1858), British physician and father of epidemiology. (b) Snow's detailed mapping of cholera incidence led to the discovery of the contaminated water pump on Broad street (red square) responsible for the 1854 cholera epidemic. (credit a: modification of work by "Rsabbatini"/Wikimedia Commons)

Snow's work represents an early epidemiological study and it resulted in the first known public health response to an epidemic. Snow's meticulous case-tracking methods are now common practice in studying disease outbreaks and in associating new diseases with their causes. His work further shed light on unsanitary sewage practices and the effects of waste dumping in the Thames. Additionally, his work supported the germ theory of disease, which argued disease could be transmitted through contaminated items, including water contaminated with fecal matter.

Snow's work illustrated what is referred to today as a common source spread of infectious disease, in which there is a single source for all of the individuals infected. In this case, the single source was the contaminated well below the Broad Street pump. Types of common source spread include point source spread, continuous common source spread, and intermittent common source spread. In point source spread of infectious disease, the common source operates for a short time period—less than the incubation period of the pathogen. An example of point source spread is a single contaminated potato salad at a group picnic. In continuous common source spread, the infection occurs for an extended period of time, longer than the incubation period. An example of continuous common source spread would be the source of London water taken downstream of the city, which was continuously contaminated with sewage from upstream. Finally, with intermittent common source spread, infections occur for a period, stop, and then begin again. This might be seen in infections from a well that was contaminated only after large rainfalls and that cleared itself of contamination after a short period.

In contrast to common source spread, propagated spread occurs through direct or indirect person-to-person contact. With propagated spread, there is no single source for infection; each infected individual becomes a source for one or more subsequent infections. With propagated spread, unless the spread is stopped immediately, infections occur for longer than the incubation period. Although point sources often lead to large-scale but localized outbreaks of short duration, propagated spread typically results in longer duration outbreaks that can vary from small to large, depending on the population and the disease (Figure **5.6.1.2**). In addition, because of person-to-person transmission, propagated spread cannot be easily stopped at a single source like point source spread.

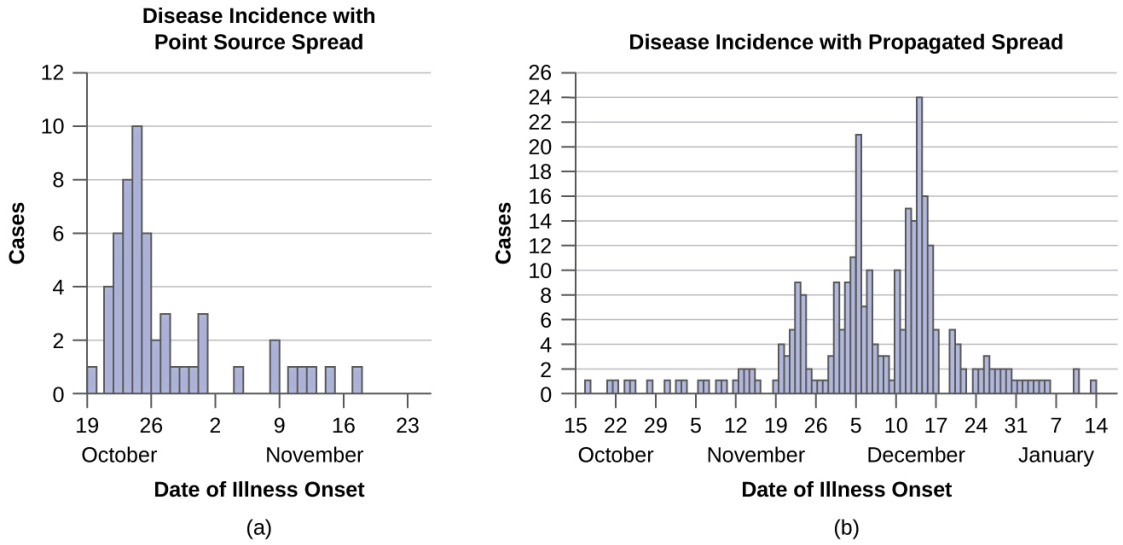
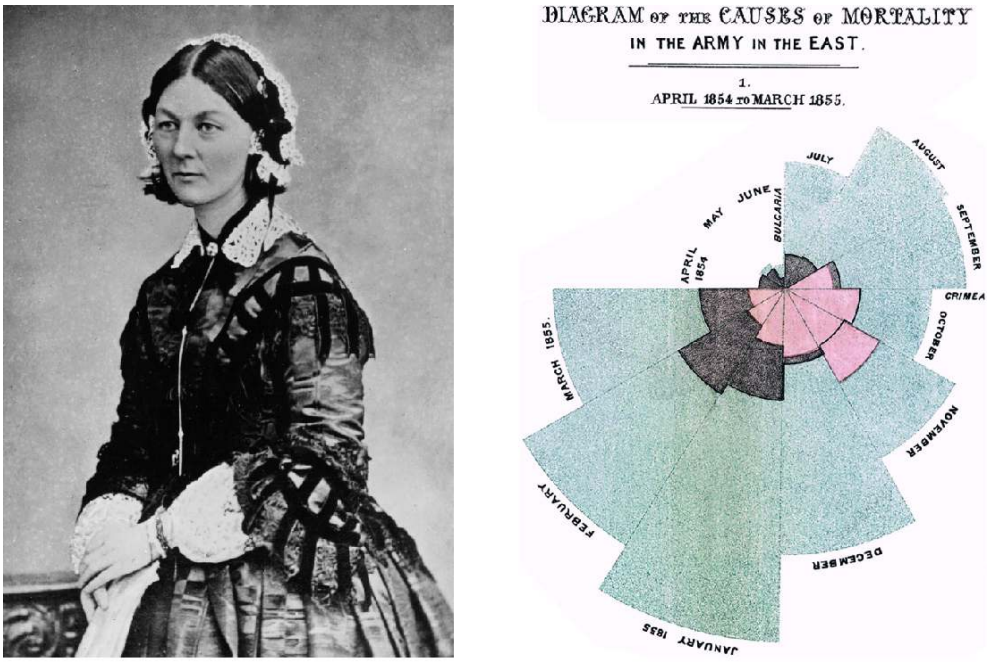


Figure **5.6.1.2**: (a) Outbreaks that can be attributed to point source spread often have a short duration. (b) Outbreaks attributed to propagated spread can have a more extended duration. (credit a, b: modification of work by Centers for Disease Control and Prevention)

Florence Nightingale's work is another example of an early epidemiological study. In 1854, Nightingale was part of a contingent of nurses dispatched by the British military to care for wounded soldiers during the Crimean War. Nightingale kept meticulous records regarding the causes of illness and death during the war. Her recordkeeping was a fundamental task of what would later become the science of epidemiology. Her analysis of the data she collected was published in 1858. In this book, she presented monthly frequency data on causes of death in a wedge chart histogram (Figure **5.6.1.3**). This graphical presentation of data, unusual at the time, powerfully illustrated that the vast majority of casualties during the war occurred not due to wounds sustained in action but to what Nightingale deemed preventable infectious diseases. Often these diseases occurred because of poor sanitation and lack of access to hospital facilities. Nightingale's findings led to many reforms in the British military's system of medical care.

Joseph Lister provided early epidemiological evidence leading to good public health practices in clinics and hospitals. These settings were notorious in the mid-1800s for fatal infections of surgical wounds at a time when the germ theory of disease was not yet widely accepted (see Foundations of Modern Cell Theory). Most physicians did not wash their hands between patient visits or clean and sterilize their surgical tools. Lister, however, discovered the disinfecting properties of carbolic acid, also known as phenol (see Using Chemicals to Control Microorganisms). He introduced several disinfection protocols that dramatically lowered post-surgical infection rates.[3] He demanded that surgeons who worked for him use a 5% carbolic acid solution to clean their surgical tools between patients, and even went so far as to spray the solution onto bandages and over the surgical site during operations (Figure 5.6.1.4). He also took precautions not to introduce sources of infection from his skin or clothing by removing his coat, rolling up his sleeves, and washing his hands in a dilute solution of carbolic acid before and during the surgery.



(a) (b)

Figure 5.6.1.3: (a) Florence Nightingale reported on the data she collected as a nurse in the Crimean War. (b) Nightingale's diagram shows the number of fatalities in soldiers by month of the conflict from various causes. The total number dead in a particular month is equal to the area of the wedge for that month. The colored sections of the wedge represent different causes of death: wounds (pink), preventable infectious diseases (gray), and all other causes (brown).

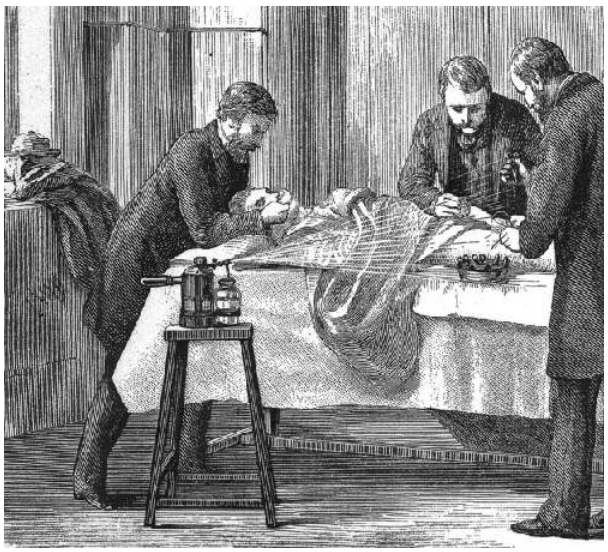


Figure 5.6.1.4: Joseph Lister initiated the use of a carbolic acid (phenol) during surgeries. This illustration of a surgery shows a pressurized canister of carbolic acid being sprayed over the surgical site.

Link to Learning

John Snow's own account of his work has additional links and information.

This CDC resource further breaks down the pattern expected from a point-source outbreak.

Learn more about Nightingale's wedge chart here.

Exercise 5.6.1.1

1. Explain the difference between common source spread and propagated spread of disease.
2. Describe how the observations of John Snow, Florence Nightingale, and Joseph Lister led to improvements in public health.

Types of Epidemiological Studies

Today, epidemiologists make use of study designs, the manner in which data are gathered to test a hypothesis, similar to those of researchers studying other phenomena that occur in populations. These approaches can be divided into observational studies (in which subjects are not manipulated) and experimental studies (in which subjects are manipulated). Collectively, these studies give modern-day epidemiologists multiple tools for exploring the connections between infectious diseases and the populations of susceptible individuals they might infect.

Observational Studies

In an observational study, data are gathered from study participants through measurements (such as physiological variables like white blood cell count), or answers to questions in interviews (such as recent travel or exercise frequency). The subjects in an observational study are typically chosen at random from a population of affected or unaffected individuals. However, the subjects in an observational study are in no way manipulated by the researcher. Observational studies are typically easier to carry out than experimental studies, and in certain situations they may be the only studies possible for ethical reasons.

Observational studies are only able to measure associations between disease occurrence and possible causative agents; they do not necessarily prove a causal relationship. For example, suppose a study finds an association between heavy coffee drinking and lower incidence of skin cancer. This might suggest that coffee prevents skin cancer, but there may be another unmeasured factor involved, such as the amount of sun exposure the participants receive. If it turns out that coffee drinkers work more in offices and spend less time outside in the sun than those who drink less coffee, then it may be possible that the lower rate of skin cancer is due to less sun exposure, not to coffee consumption. The observational study cannot distinguish between these two potential causes.

There are several useful approaches in observational studies. These include methods classified as descriptive epidemiology and analytical epidemiology. Descriptive epidemiology gathers information about a disease outbreak, the affected individuals, and how the disease has spread over time in an exploratory stage of study. This type of study will involve interviews with patients, their contacts, and their family members; examination of samples and medical records; and even histories of food and beverages consumed. Such a study might be conducted while the outbreak is still occurring. Descriptive studies might form the basis for developing a hypothesis of causation that could be tested by more rigorous observational and experimental studies.

Analytical epidemiology employs carefully selected groups of individuals in an attempt to more convincingly evaluate hypotheses about potential causes for a disease outbreak. The selection of cases is generally made at random, so the results are not biased because of some common characteristic of the study participants. Analytical studies may gather their data by going back in time (retrospective studies), or as events unfold forward in time (prospective studies).

Retrospective studies gather data from the past on present-day cases. Data can include things like the medical history, age, gender, or occupational history of the affected individuals. This type of study examines associations between factors chosen or available to the researcher and disease occurrence.

Prospective studies follow individuals and monitor their disease state during the course of the study. Data on the characteristics of the study subjects and their environments are gathered at the beginning and during the study so that subjects who become ill may be compared with those who do not. Again, the researchers can look for associations between the disease state and variables that were measured during the study to shed light on possible causes.

Analytical studies incorporate groups into their designs to assist in teasing out associations with disease. Approaches to group-based analytical studies include cohort studies, case-control studies, and cross-sectional studies. The cohort method examines groups of individuals (called cohorts) who share a particular characteristic. For example, a cohort might consist of individuals born in the same year and the same place; or it might consist of people who practice or avoid a particular behavior, e.g., smokers or nonsmokers. In a cohort study, cohorts can be followed prospectively or studied retrospectively. If only a single cohort is followed, then the affected individuals are compared with the unaffected individuals in the same group. Disease outcomes are recorded and analyzed to try to identify correlations between characteristics of individuals in the cohort and disease incidence. Cohort studies are a useful way to determine the causes of a condition without violating the ethical prohibition of exposing subjects to a risk factor. Cohorts are typically identified and defined based on suspected risk factors to which individuals have already been exposed through their own choices or circumstances.

Case-control studies are typically retrospective and compare a group of individuals with a disease to a similar group of individuals without the disease. Case-control studies are far more efficient than cohort studies because researchers can deliberately select subjects who are already affected with the disease as opposed to waiting to see which subjects from a random sample will develop a disease.

A cross-sectional study analyzes randomly selected individuals in a population and compares individuals affected by a disease or condition to those unaffected at a single point in time. Subjects are compared to look for associations between certain measurable variables and the disease or condition. Cross-sectional studies are also used to determine the prevalence of a condition.

Experimental Studies

Experimental epidemiology uses laboratory or clinical studies in which the investigator manipulates the study subjects to study the connections between diseases and potential causative agents or to assess treatments. Examples of treatments might be the administration of a drug, the inclusion or exclusion of different dietary items, physical exercise, or a particular surgical procedure. Animals or humans are used as test subjects. Because experimental studies involve manipulation of subjects, they are typically more difficult and sometimes impossible for ethical reasons.

Koch's postulates require experimental interventions to determine the causative agent for a disease. Unlike observational studies, experimental studies can provide strong evidence supporting cause because other factors are typically held constant when the researcher manipulates the subject. The outcomes for one group receiving the treatment are compared to outcomes for a group that does not receive the treatment but is treated the same in every other way. For example, one group might receive a regimen of a drug administered as a pill, while the untreated group receives a placebo (a pill that looks the same but has no active ingredient). Both groups are treated as similarly as possible except for the administration of the drug. Because other variables are held constant in both the treated and the untreated groups, the researcher is more certain that any change in the treated group is a result of the specific manipulation.

Experimental studies provide the strongest evidence for the etiology of disease, but they must also be designed carefully to eliminate subtle effects of bias. Typically, experimental studies with humans are conducted as double-blind studies, meaning neither the subjects nor the researchers know who is a treatment case and who is not. This design removes a well-known cause of bias in research called the placebo effect, in which knowledge of the treatment by either the subject or the researcher can influence the outcomes.

? Exercise 5.6.1.2

1. Describe the advantages and disadvantages of observational studies and experimental studies.
2. Explain the ways that groups of subjects can be selected for analytical studies.

Clinical Focus: Part 3

Since laboratory tests had confirmed *Salmonella*, a common foodborne pathogen, as the etiologic agent, epidemiologists suspected that the outbreak was caused by contamination at a food processing facility serving the region. Interviews with patients focused on food consumption during and after the Thanksgiving holiday, corresponding with the timing of the outbreak. During the interviews, patients were asked to list items consumed at holiday gatherings and describe how widely

each item was consumed among family members and relatives. They were also asked about the sources of food items (e.g., brand, location of purchase, date of purchase). By asking such questions, health officials hoped to identify patterns that would lead back to the source of the outbreak.

Analysis of the interview responses eventually linked almost all of the cases to consumption of a holiday dish known as the turducken—a chicken stuffed inside a duck stuffed inside a turkey. Turducken is a dish not generally consumed year-round, which would explain the spike in cases just after the Thanksgiving holiday. Additional analysis revealed that the turduckens consumed by the affected patients were purchased already stuffed and ready to be cooked. Moreover, the pre-stuffed turduckens were all sold at the same regional grocery chain under two different brand names. Upon further investigation, officials traced both brands to a single processing plant that supplied stores throughout the Florida panhandle.

Exercise 5.6.1.3

1. Is this an example of common source spread or propagated spread?
2. What next steps would the public health office likely take after identifying the source of the outbreak?

Key Concepts and Summary

- Early pioneers of epidemiology such as John Snow, Florence Nightingale, and Joseph Lister, studied disease at the population level and used data to disrupt disease transmission.
- **Descriptive epidemiology** studies rely on case analysis and patient histories to gain information about outbreaks, frequently while they are still occurring.
- **Retrospective epidemiology** studies use historical data to identify associations with the disease state of present cases. **Prospective epidemiology** studies gather data and follow cases to find associations with future disease states.
- **Analytical epidemiology** studies are observational studies that are carefully designed to compare groups and uncover associations between environmental or genetic factors and disease.
- **Experimental epidemiology** studies generate strong evidence of causation in disease or treatment by manipulating subjects and comparing them with control subjects.

Footnotes

1. John Snow. *On the Mode of Communication of Cholera. Second edition, Much Enlarged*. John Churchill, 1855.
2. John Snow. “The Cholera near Golden-Wquare, and at Deptford.” *Medical Times and Gazette* 9 (1854): 321–322. http://www.ph.ucla.edu/epi/snow/chol...densquare.html.
3. O.M. Lidwell. “Joseph Lister and Infection from the Air.” *Epidemiology and Infection* 99 (1987): 569–578. www.ncbi.nlm.nih.gov/pmc/arti...00006-0004.pdf.

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5.6.1.1: Descriptive Epidemiology

Learning Objectives

- Describe the role of a descriptive epidemiology

The goal of epidemiology is to establish causal factors for health issues in order to improve the health and safety of entire populations. A population can refer to a town, country, age group, or race. Health issues refer to anything that might impact health in the present or future. For epidemiologists, data on who is most likely to be injured in car crashes can be just as valuable as a topic of inquiry as data on what part of the population is most at risk for developing complications from the flu. In order to accomplish this, epidemiology has two main branches: descriptive and analytical.

Descriptive epidemiology evaluates and catalogs all the circumstances surrounding a person affected by a health event of interest. Analytical epidemiologists use data gathered by descriptive epidemiology experts to look for patterns suggesting causation. The end goal of both branches is to reduce the incidence of health events or diseases by understanding the risk factors for the health events or diseases. Both descriptive and analytical epidemiology often serve public health organizations by providing information that may reduce disease or reduce other kinds of events that impact people's health.

The primary considerations for descriptive epidemiology are frequency and pattern. Frequency evaluates the rate of occurrence, and pattern helps analytical epidemiologists suggest risk factors. Descriptive epidemiology evaluates frequency and pattern by examining the person, place, and time in relationship to health events.

Descriptive epidemiology examines factors like age, education, socioeconomic status, availability of health services, race, and gender. Evaluations of specific individuals may also include gathering information on behaviors like drug abuse, shift work, eating, and exercise patterns.

Key Points

- Epidemiology is the science concerned with the study of the factors that influence and determine the frequency and distribution of disease, injury, and other health -related events and their causes in a defined human population.
- The more fully a descriptive epidemiologist can describe people, places and times, and any correlations between the three, the more likely it is that patterns will emerge which can be considered as risk factors for certain kinds of health issues.
- Epidemiologists use data as an information source for communicating information to people and to influence public policy.

Key Terms

- **socioeconomic**: Of or pertaining to social and economic factors.
- **epidemiology**: The branch of a science dealing with the spread and control of diseases, computer viruses, concepts, etc., throughout populations or systems.
- **risk factor**: A variable associated with an increased risk of disease or infection.

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5.6.1.2: Analytical Epidemiology

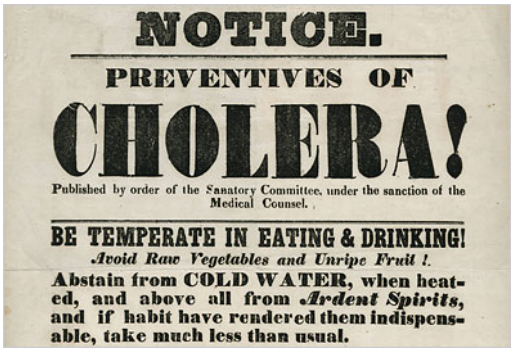
Learning Objectives

- Describe the role of an analytical epidemiologist

Epidemiology is the study (or the science of the study) of the patterns, causes, and effects of health and disease conditions in defined populations. It is the cornerstone of public health, and informs policy decisions and evidence-based medicine by identifying risk factors for disease and targets for preventive medicine. Epidemiologists help with study design, collection and statistical analysis of data, and interpretation and dissemination of results (including peer review and occasional systematic review). Epidemiology has helped develop methodology used in clinical research, public health studies and, to some extent, basic research in the biological sciences.

Epidemiologists employ a range of study designs from observational to experimental and generally categorized as descriptive, analytic (aiming to further examine known associations or hypothesized relationships), and experimental (a term often equated with clinical or community trials of treatments and other interventions).

Where descriptive epidemiology describes occurrence of disease (or of its determinants) within a population, the analytical epidemiology aims to gain knowledge on the quality and the amount of influence that determinants have on the occurrence of disease. The usual way to gain this knowledge is by group comparisons. Such a comparison starts from one or more hypotheses about how the determinant may influence occurrence of disease. Analytical epidemiology attempts to determine the cause of an outbreak. Using the case control method, the epidemiologist can look for factors that might have preceded the disease. Often, this entails comparing a group of people who have the disease with a group that is similar in age, sex, socioeconomic status, and other variables, but does not have the disease. In this way, other possible factors, e.g., genetic or environmental, might be identified as factors related to the outbreak.


NOTICE.

PREVENTIVES OF

CHOLERA!

Published by order of the Sanatory Committee, under the sanction of the Medical Counsel.

BE TEMPERATE IN EATING & DRINKING!

Avoid Raw Vegetables and Unripe Fruit !.

Abstain from COLD WATER, when heated, and above all from *Ardent Spirits*, and if habit have rendered them indispensable, take much less than usual.

Figure: **Cholera Outbreak**: The outdated public health advice demonstrates the lack of understanding of the disease and its actual causative factors in the absence of epidemiological analysis.

Key Points

- Epidemiologists employ a range of study designs from the observational to experimental and they are generally categorized as descriptive, analytic, and experimental.
- Analytic epidemiology aims to further examine known associations or hypothesized relationships.
- Analytical observations deal more with the ‘how’ of a health -related event.

Key Terms

- **analytical**: pertaining to or emanating from analysis.
- **epidemiology**: Epidemiology is the study (or the science of the study) of the patterns, causes, and effects of health and disease conditions in defined populations.

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5.6.1.3: Experimental Epidemiology

Learning Objectives

- Summarize the purpose of experimental epidemiology and the three case types: randomized control, field and community trial

Epidemiology is the study (or the science of the study) of the patterns, causes, and effects of health and disease conditions in defined populations. It is the cornerstone of public health, and informs policy decisions and evidence-based medicine by identifying risk factors for disease and targets for preventive medicine. Epidemiologists help with study design, collection and statistical analysis of data, and interpretation and dissemination of results (including peer review and occasional systematic review). Epidemiology has helped develop methodology used in clinical research, public health studies and, to a lesser extent, basic research in the biological sciences.

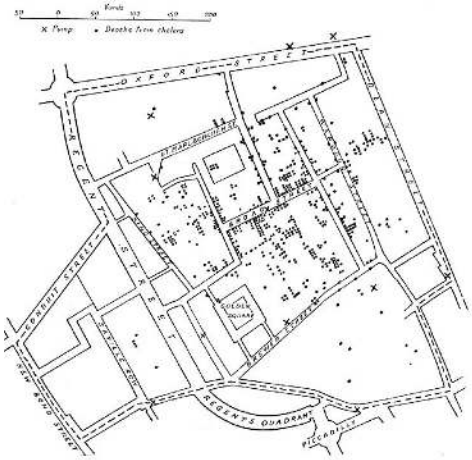


Figure: **Early epidemiology**: Original map by John Snow showing the clusters of cholera cases in the London epidemic of 1854. John Snow's investigative work was one of the first examples of epidemiology. He discovered that families that drew their water from the Broad St well became infected with cholera.

Epidemiologists employ a range of study designs from the observational to experimental and they are generally categorized as descriptive, analytic (aiming to further examine known associations or hypothesized relationships), and experimental (a term often equated with clinical or community trials of treatments and other interventions). In observational studies, nature is allowed to "take its course", as epidemiologists observe from the sidelines. Controversially, in experimental studies, the epidemiologist is the one in control of all of the factors entering a certain case study. Epidemiological studies are aimed, where possible, at revealing unbiased relationships between exposures such as alcohol or smoking, biological agents, stress, or chemicals to mortality or morbidity. The identification of causal relationships between these exposures and outcomes is an important aspect of epidemiology. Modern epidemiologists use informatics as a tool.

Experimental epidemiology contains three case types: randomized control trial (often used for new medicine or drug testing), field trial (conducted on those at a high risk of conducting a disease), and community trial (research on social originating diseases). Experimental epidemiology tests a hypothesis about a disease or disease treatment in a group of people. This strategy might be used to test whether or not a particular antibiotic is effective against a particular disease-causing organism. One group of infected individuals is divided randomly so that some receive the antibiotic and others receive a placebo—a "false" drug that is not known to have any medical effect. In this case, the antibiotic is the variable, i.e., the experimental factor being tested to see if it makes a difference between the two otherwise similar groups. If people in the group receiving the antibiotic recover more rapidly than those in the other group, it may logically be concluded that the variable—antibiotic treatment—made the difference. Thus, the antibiotic is said to be effective.

Although epidemiology is sometimes viewed as a collection of statistical tools used to elucidate the associations of exposures to health outcomes, a deeper understanding of this science is that of discovering causal relationships.

Key Points

- Experimental epidemiology is the study of the relationships of various factors determining the frequency and distribution of diseases in a community.
- Experimental epidemiology contains three case types: randomized control trial (often used for new medicine or drug testing), field trial (conducted on those at a high risk of conducting a disease), and community trial (research on social originating diseases).
- The method employs prospective population experiments designed to test epidemiological hypotheses, and usually attempt to relate the postulated cause to the observed effect. Trials of new anthelmintics are an example.

Key Terms

- **epidemiology**: Epidemiology is the study (or the science of the study) of the patterns, causes, and effects of health and disease conditions in defined populations.
- **Experimental**: An experiment is a methodical procedure carried out with the goal of verifying, falsifying, or establishing the validity of a hypothesis.
- **statistical**: of or pertaining to statistics

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SECTION OVERVIEW

5.6.2: Nosocomial Infections

A nosocomial infection is an infection that is acquired in a hospital or other health care facility. To emphasize both hospital and nonhospital settings, it is sometimes instead called a health care–associated infection. Such an infection can be acquired in hospital, nursing home, rehabilitation facility, outpatient clinic, or other clinical settings. Infection is spread to the susceptible patient in the clinical setting by various means.

Topic hierarchy

5.6.2.1: Transmission of Nosocomial Infection

5.6.2.2: Control of Nosocomial Infections

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5.6.2.1: Transmission of Nosocomial Infection

Learning Objectives

- Differentiate between the various types of transmission: air-borne, common vehicle, vector borne, direct and indirect contact transmission

The drug-resistant Gram-negative bacteria, for the most part, threaten only hospitalized patients whose immune systems are weak. They can survive for a long time on surfaces in the hospital and they enter the body through wounds, catheters, and ventilators.

The most important and frequent mode of transmission of nosocomial infections is by direct contact. Transmission occurs when droplets containing microbes from the infected person are propelled a short distance through the air and deposited on the host's body; droplets are generated from the source person mainly by coughing, sneezing, and talking, and during the performance of certain procedures, such as bronchoscopy.

Dissemination can be either airborne droplet nuclei (small-particle residue {5 µm or smaller in size} of evaporated droplets containing microorganisms that remain suspended in the air for long periods of time) or dust particles containing the infectious agent. Microorganisms carried in this manner can be dispersed widely by air currents and may become inhaled by a susceptible host within the same room or over a longer distance from the source patient, depending on environmental factors; therefore, special air-handling and ventilation are required to prevent airborne transmission. Microorganisms transmitted by airborne transmission include Legionella, Mycobacterium tuberculosis and the rubeola and varicella viruses.

Common vehicle transmission applies to microorganisms transmitted to the host by contaminated items, such as food, water, medications, devices, and equipment. Vector borne transmission occurs when vectors such as mosquitoes, flies, rats, and other vermin transmit microorganisms.



Figure: ***Cross-transmission****: Contaminated surfaces increase cross-transmission.*

Contact transmission is divided into two subgroups: direct-contact transmission and indirect-contact transmission.

Direct-contact transmission involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person, such as when a person turns a patient, gives a patient a bath, or performs other patient-care activities that require direct personal contact. Direct-contact transmission can also occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host.

Indirect-contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated gloves that are not changed between patients. In addition, the improper use of saline flush syringes, vials, and bags has been implicated in disease transmission in the US, even when healthcare workers had access to gloves, disposable needles, intravenous devices, and flushes.

Key Points

- Contact transmission is divided into two subgroups: direct-contact transmission and indirect-contact transmission.
- Direct-contact transmission involves a direct body surface-to-body surface contact and physical transfer of microorganisms.
- Indirect-contact transmission involves contact of a susceptible host with a contaminated intermediate object, usually inanimate.

Key Terms

- **microorganisms**: A microorganism or microbe is a microscopic organism that comprises either a single cell (unicellular), cell clusters, or multicellular relatively complex organisms.
- **susceptible**: likely to be affected by something; here, sensitive to growth inhibition by an antimicrobial drug.
- **transmission**: Transmission is the passing of a communicable disease from an infected host individual or group to a conspecific individual or group, regardless of whether the other individual was previously infected.

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5.6.2.2: Control of Nosocomial Infections

Learning Objectives

- Give examples of ways nosocomial infections can be controlled or prevented

Hospitals have sanitation protocols regarding uniforms, equipment sterilization, washing, and other preventive measures. Thorough hand washing and/or the use of alcohol rubs by all medical personnel before and after each patient contact is one of the most effective ways to combat nosocomial infections. More careful use of antimicrobial agents, such as antibiotics, is also considered vital.

Despite sanitation protocol, patients cannot be entirely isolated from infectious agents. Furthermore, patients are often prescribed antibiotics and other antimicrobial drugs to help treat illness; this may increase the selection pressure for the emergence of resistant strains. Sterilization goes further than just sanitizing. It kills all microorganisms on equipment and surfaces through exposure to chemicals, ionizing radiation, dry heat, or steam under pressure. Isolation precautions are designed to prevent transmission of microorganisms by common routes in hospitals. Because agent and host factors are more difficult to control, interruption of transfer of microorganisms is directed primarily at transmission.

The Importance of Handwashing

Handwashing is the single most important measure to reduce the risks of transmitting skin microorganisms from one person to another or from one site to another on the same patient. Washing hands as promptly and thoroughly as possible between patient contacts and after contact with blood, body fluids, secretions, excretions, and equipment or articles contaminated by them is an important component of infection control and isolation precautions.



Figure: ***Hand washing with soap****: Handwashing is the single most important measure to reduce the risks of transmitting skin microorganisms from one person to another or from one site to another on the same patient.*

The spread of nosocomial infections among immunocompromised patients is connected with health care workers' hand contamination in almost 40% of cases. This presents a challenging problem in the modern hospitals. The best way for workers to overcome this problem is by conducting correct hand- hygiene procedures; this is why in 2005 the WHO launched the GLOBAL Patient Safety Challenge.

Two categories of micro-organisms can be present on health care workers' hands: transient flora and resident flora. The first is represented by the micro-organisms taken by workers from the environment, and the bacteria in it. These are often capable of surviving on the human skin and sometimes to grow. The second group is represented by the permanent micro-organisms living on the skin surface, on the stratum corneum or immediately under it. They are capable of surviving on the human skin and of growing freely on it. They have low pathogenicity and infection rate, and they create a kind of protection from the colonization from other more pathogenic bacteria.

The skin of workers is colonized by 3.9 x 104 – 4.6 x 106 cfu/cm2. The microbes comprising the resident flora are: Staphylococcus epidermidis, S. hominis, and Micrococcus, Propionibacterium, Corynebacterium, Dermobacterium, and Pitosporum spp., while in the transitional could be found S. aureus, and Klebsiella pneumoniae, and Acinetobacter, Enterobacter and Candida spp. The goal of hand hygiene is to eliminate the transient flora with a careful and proper performance of hand washing, using different kinds of soap, both normal and antiseptic, and alcohol-based gels. The main problems found in the practice of hand hygiene are connected

with the lack of available sinks and the time-consuming performance of hand washing. An easy way to resolve this problem could be the use of alcohol-based hand rubs, because of faster application compared to correct hand washing.

The Second Line of Defense: Gloves

Gloves play an important role in reducing the risks of transmission of microorganisms. Gloves are worn for three important reasons in hospitals.

- They are worn to provide a protective barrier and to prevent gross contamination of the hands when touching blood, body fluids, secretions, excretions, mucous membranes, and non-intact skin. In the USA, the Occupational Safety and Health Administration (OSHA) has mandated wearing gloves to reduce the risk of blood-borne pathogen infections.
- Gloves are worn to reduce the likelihood microorganisms present on the hands of personnel will be transmitted to patients during invasive or other patient-care procedures that involve touching a patient's mucous membranes and nonintact skin.
- They are worn to reduce the likelihood the hands of personnel contaminated with micro-organisms from a patient or a fomite (contaminated object) can be transmitted to another patient. In this situation, gloves must be changed between patient contacts, and hands should be washed after gloves are removed.

Surfaces

Sanitizing surfaces is an often overlooked, yet crucial, component of the strategy for the cycle of infection in health care environments. Modern sanitizing methods such as NAV-CO2 have been effective against gastroenteritis, MRSA, and influenza agents. Use of hydrogen peroxide vapor has been clinically proven to reduce infection rates and risk of acquisition. Hydrogen peroxide is effective against endospore-forming bacteria, such as Clostridium difficile, where alcohol has been shown to be ineffective.

Microorganisms are known to survive on inanimate "touch" surfaces for extended periods of time. This can be especially troublesome in hospital environments, where patients with immunodeficiencies are at enhanced risk for contracting nosocomial infections.

Wearing an apron during patient care reduces the risk of infection. The apron should either be disposable or be used only when caring for a specific patient.

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Key Points

- Handwashing frequently is called the single most important measure to reduce the risks of transmitting skin microorganisms from one person to another or from one site to another on the same patient.
- Thorough hand washing and/or use of alcohol rubs by all medical personnel before and after each patient contact is one of the most effective ways to combat nosocomial infections.

- Sanitizing surfaces is an often overlooked, yet crucial, component of breaking the cycle of infection in health care environments.

Key Terms

- **sterilization**: Any process that eliminates or kills all forms of microbial life present on a surface, solution, or solid compound.
- **nosocomial**: contracted in a hospital, or arising from hospital treatment
- **sanitation**: The policy and practice of protecting health through hygienic measures.

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5.6.3: Global Public Health

Learning Objectives

- Describe the entities involved in international public health and their activities
- Identify and differentiate between emerging and reemerging infectious diseases

A large number of international programs and agencies are involved in efforts to promote global public health. Among their goals are developing infrastructure in health care, public sanitation, and public health capacity; monitoring infectious disease occurrences around the world; coordinating communications between national public health agencies in various countries; and coordinating international responses to major health crises. In large part, these international efforts are necessary because disease-causing microorganisms know no national boundaries.

The World Health Organization (WHO)

International public health issues are coordinated by the World Health Organization (WHO), an agency of the United Nations. Of its roughly $4 billion budget for 2015–16[1], about $1 billion was funded by member states and the remaining $3 billion by voluntary contributions. In addition to monitoring and reporting on infectious disease, WHO also develops and implements strategies for their control and prevention. WHO has had a number of successful international public health campaigns. For example, its vaccination program against smallpox, begun in the mid-1960s, resulted in the global eradication of the disease by 1980. WHO continues to be involved in infectious disease control, primarily in the developing world, with programs targeting malaria, HIV/AIDS, and tuberculosis, among others. It also runs programs to reduce illness and mortality that occur as a result of violence, accidents, lifestyle-associated illnesses such as diabetes, and poor health-care infrastructure.

WHO maintains a global alert and response system that coordinates information from member nations. In the event of a public health emergency or epidemic, it provides logistical support and coordinates international response to the emergency. The United States contributes to this effort through the CDC. The CDC carries out international monitoring and public health efforts, mainly in the service of protecting US public health in an increasingly connected world. Similarly, the European Union maintains a Health Security Committee that monitors disease outbreaks within its member countries and internationally, coordinating with WHO.

? Exercise 5.6.3.1

Name the organizations that participate in international public health monitoring.

Emerging and Reemerging Infectious Diseases

Both WHO and some national public health agencies such as the CDC monitor and prepare for emerging infectious diseases. An emerging infectious disease is either new to the human population or has shown an increase in prevalence in the previous twenty years. Whether the disease is new or conditions have changed to cause an increase in frequency, its status as emerging implies the need to apply resources to understand and control its growing impact.

Emerging diseases may change their frequency gradually over time, or they may experience sudden epidemic growth. The importance of vigilance was made clear during the Ebola hemorrhagic fever epidemic in western Africa through 2014–2015. Although health experts had been aware of the Ebola virus since the 1970s, an outbreak on such a large scale had never happened before (Figure 5.6.3.1). Previous human epidemics had been small, isolated, and contained. Indeed, the gorilla and chimpanzee populations of western Africa had suffered far worse from Ebola than the human population. The pattern of small isolated human epidemics changed in 2014. Its high transmission rate, coupled with cultural practices for treatment of the dead and perhaps its emergence in an urban setting, caused the disease to spread rapidly, and thousands of people died. The international public health community responded with a large emergency effort to treat patients and contain the epidemic.

Emerging diseases are found in all countries, both developed and developing (Table 5.6.3.1). Some nations are better equipped to deal with them. National and international public health agencies watch for epidemics like the Ebola outbreak in developing countries because those countries rarely have the health-care infrastructure and expertise to deal with large outbreaks effectively. Even with the support of international agencies, the systems in western Africa struggled to identify and care for the sick and control

spread. In addition to the altruistic goal of saving lives and assisting nations lacking in resources, the global nature of transportation means that an outbreak anywhere can spread quickly to every corner of the planet. Managing an epidemic in one location—its source—is far easier than fighting it on many fronts.

Ebola is not the only disease that needs to be monitored in the global environment. In 2015, WHO set priorities on several emerging diseases that had a high probability of causing epidemics and that were poorly understood (and thus urgently required research and development efforts).

A reemerging infectious disease is a disease that is increasing in frequency after a previous period of decline. Its reemergence may be a result of changing conditions or old prevention regimes that are no longer working. Examples of such diseases are drug-resistant forms of tuberculosis, bacterial pneumonia, and malaria. Drug-resistant strains of the bacteria causing gonorrhea and syphilis are also becoming more widespread, raising concerns of untreatable infections.

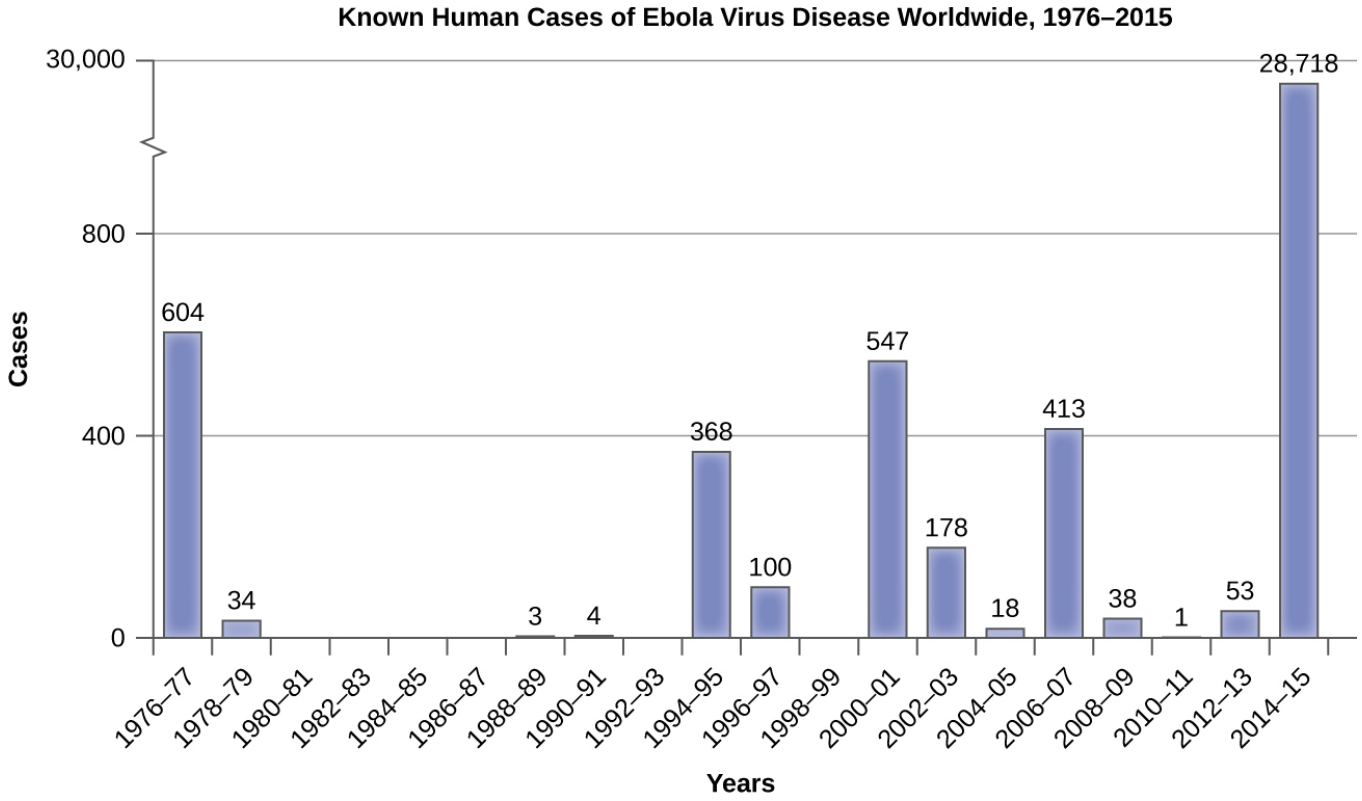


Figure **5.6.3.1**: Even before the Ebola epidemic of 2014–15, Ebola was considered an emerging disease because of several smaller outbreaks between the mid-1990s and 2000s.

Table **5.6.3.1**: Some Emerging and Reemerging Infectious Diseases

Disease	Pathogen	Year Discovered	Affected Regions	Transmission
AIDS	HIV	1981	Worldwide	Contact with infected body fluids
Chikungunya fever	Chikungunya virus	1952	Africa, Asia, India; spreading to Europe and the Americas	Mosquito-borne
Ebola virus disease	Ebola virus	1976	Central and Western Africa	Contact with infected body fluids
H1N1 Influenza (swine flu)	H1N1 virus	2009	Worldwide	Droplet transmission
Lyme disease	*Borrelia burgdorferi* bacterium	1981	Northern hemisphere	From mammal reservoirs to humans by tick vectors
West Nile virus disease	West Nile virus	1937	Africa, Australia, Canada to Venezuela, Europe, Middle East, Western Asia	Mosquito-borne

? Exercise 5.6.3.2

1. Explain why it is important to monitor emerging infectious diseases.
2. Explain how a bacterial disease could reemerge, even if it had previously been successfully treated and controlled.

SARS Outbreak and Identification

On November 16, 2002, the first case of a SARS outbreak was reported in Guangdong Province, China. The patient exhibited influenza-like symptoms such as fever, cough, myalgia, sore throat, and shortness of breath. As the number of cases grew, the Chinese government was reluctant to openly communicate information about the epidemic with the World Health Organization (WHO) and the international community. The slow reaction of Chinese public health officials to this new disease contributed to the spread of the epidemic within and later outside China. In April 2003, the Chinese government finally responded with a huge public health effort involving quarantines, medical checkpoints, and massive cleaning projects. Over 18,000 people were quarantined in Beijing alone. Large funding initiatives were created to improve health-care facilities, and dedicated outbreak teams were created to coordinate the response. By August 16, 2003, the last SARS patients were released from a hospital in Beijing nine months after the first case was reported in China.

In the meantime, SARS spread to other countries on its way to becoming a global pandemic. Though the infectious agent had yet to be identified, it was thought to be an influenza virus. The disease was named SARS, an acronym for severe acute respiratory syndrome, until the etiologic agent could be identified. Travel restrictions to Southeast Asia were enforced by many countries. By the end of the outbreak, there were 8,098 cases and 774 deaths worldwide. China and Hong Kong were hit hardest by the epidemic, but Taiwan, Singapore, and Toronto, Canada, also saw significant numbers of cases (Figure **5.6.3.2**).

Fortunately, timely public health responses in many countries effectively suppressed the outbreak and led to its eventual containment. For example, the disease was introduced to Canada in February 2003 by an infected traveler from Hong Kong, who died shortly after being hospitalized. By the end of March, hospital isolation and home quarantine procedures were in place in the Toronto area, stringent anti-infection protocols were introduced in hospitals, and the media were actively reporting on the disease. Public health officials tracked down contacts of infected individuals and quarantined them. A total of 25,000 individuals were quarantined in the city. Thanks to the vigorous response of the Canadian public health community, SARS was brought under control in Toronto by June, a mere four months after it was introduced.

In 2003, WHO established a collaborative effort to identify the causative agent of SARS, which has now been identified as a coronavirus that was associated with horseshoe bats. The genome of the SARS virus was sequenced and published by researchers at the CDC and in Canada in May 2003, and in the same month researchers in the Netherlands confirmed the etiology of the disease by fulfilling Koch's postulates for the SARS coronavirus. The last known case of SARS worldwide was reported in 2004.



Figure **5.6.3.2**: This map shows the spread of SARS as of March 28, 2003. (credit: modification of work by Central Intelligence Agency)

Link to Learning

This database of reports chronicles outbreaks of infectious disease around the world. It was on this system that the first information about the SARS outbreak in China emerged.

The CDC publishes *Emerging Infectious Diseases*, a monthly journal available online.

Key Concepts and Summary

- The **World Health Organization (WHO)** is an agency of the United Nations that collects and analyzes data on disease occurrence from member nations. WHO also coordinates public health programs and responses to international health emergencies.

- **Emerging diseases** are those that are new to human populations or that have been increasing in the past two decades. **Reemerging diseases** are those that are making a resurgence in susceptible populations after previously having been controlled in some geographic areas.

Footnotes

1. 1 World Health Organization. “Programme Budget 2014–2015.” www.who.int/about/finances-ac...lity/budget/en.

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5.6.3I: Current Epidemics

Learning Objectives

- Give examples of current epidemics

Epidemics

In epidemiology, an epidemic occurs when new cases of a certain disease, in a given human population, and during a given period, substantially exceed what is expected based on recent experience. Epidemiologists often consider the term outbreak to be synonymous to epidemic, but the general public typically perceives outbreaks to be more local and less serious than epidemics.

CAUSES

Epidemics of infectious disease are generally caused by a change in the ecology of the host population (e.g. increased stress or increase in the density of a vector species), a genetic change in the parasite population or the introduction of a new parasite to a host population (by movement of parasites or hosts). Generally, an epidemic occurs when host immunity to a parasite population is suddenly reduced below that found in the endemic equilibrium and the transmission threshold is exceeded.

EPIDEMIC VS. PANDEMIC

An epidemic may be restricted to one location; however, if it spreads to other countries or continents and affects a substantial number of people, it may be termed a *pandemic*. The declaration of an epidemic usually requires a good understanding of a baseline rate of incidence; epidemics for certain diseases, such as influenza, are defined as reaching some defined increase in incidence above this baseline. A few cases of a very rare disease may be classified as an epidemic, while many cases of a common disease (such as the common cold) would not.

INFLUENZA EPIDEMICS

Influenza is an infectious disease of birds and mammals caused by RNA viruses of the family Orthomyxoviridae, the influenza viruses. The most common symptoms are chills, fever, sore throat, muscle pains, headache (often severe), coughing, weakness/fatigue and general discomfort. Although it is often confused with other influenza-like illnesses, especially the common cold, influenza is a more severe disease caused by a different type of virus. Influenza may produce nausea and vomiting, particularly in children, but these symptoms are more common in the unrelated gastroenteritis, which is sometimes inaccurately referred to as “stomach flu” or “24-hour flu”.

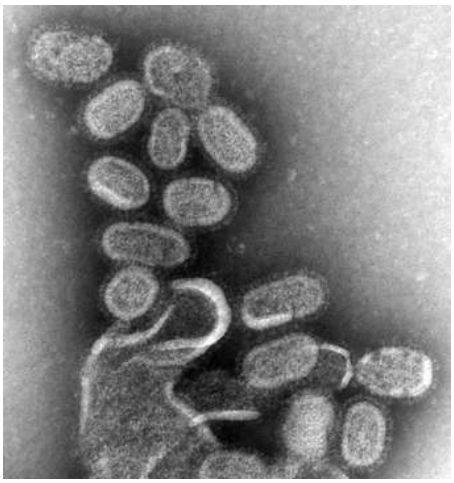


Figure: **Influenza**: TEM of negatively stained influenza virions, magnified approximately 100,000 times. Modern transport contributes in spreading diseases faster.

Typically, influenza is transmitted through the air by coughs or sneezes, creating aerosols containing the virus. Influenza can also be transmitted by direct contact with bird droppings or nasal secretions, or through contact with contaminated surfaces. Airborne

aerosols have been thought to cause most infections, although which means of transmission is most important is not absolutely clear. Influenza viruses can be inactivated by sunlight, disinfectants and detergents. As the virus can be inactivated by soap, frequent hand washing reduces the risk of infection.

Influenza spreads around the world in seasonal epidemics, resulting in about three to five million yearly cases of severe illness and about 250,000 to 500,000 yearly deaths, rising to millions in some pandemic years.

In the 20th century three influenza pandemics occurred, each caused by the appearance of a new strain of the virus in humans, and killed tens of millions of people. Often, new influenza strains appear when an existing flu virus spreads to humans from another animal species, or when an existing human strain picks up new genes from a virus that usually infects birds or pigs. An avian strain named H5N1 raised the concern of a new influenza pandemic after it emerged in Asia in the 1990s, but it has not evolved to a form that spreads easily between people.

In April 2009 a novel flu strain evolved that combined genes from human, pig, and bird flu. Initially dubbed “swine flu” and also known as influenza A/H1N1, it emerged in Mexico, the United States, and several other nations. The World Health Organization officially declared the outbreak to be a pandemic level 6 on 11 June 2009. However, the WHO’s declaration of a pandemic level 6 was an indication of spread, not severity; the strain actually having a lower mortality rate than common flu outbreaks.

VACCINATIONS

Vaccinations against influenza are usually made available to people in developed countries. Farmed poultry is often vaccinated to avoid decimation of the flocks. The most common human vaccine is the trivalent influenza vaccine (TIV) that contains purified and inactivated antigens against three viral strains. Typically, this vaccine includes material from two influenza A virus subtypes and one influenza B virus strain. The TIV carries no risk of transmitting the disease, and it has very low reactivity. A vaccine formulated for one year may be ineffective in the following year, since the influenza virus evolves rapidly, and new strains quickly replace the older ones.

Antiviral drugs such as the neuraminidase inhibitor oseltamivir (Tamiflu) have been used to treat influenza; however, their effectiveness is difficult to determine due to much of the data remaining unpublished.

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Key Points

- Epidemiologists often consider the term outbreak to be synonymous to epidemic, but the general public typically perceives outbreaks to be more local and less serious than epidemics.
- Epidemics of infectious disease are generally caused by a change in the ecology of the host population (e.g. increased stress or increase in the density of a vector species), a genetic change in the parasite population or the introduction of a new parasite to a host population.
- In the 20th century three influenza pandemics occurred, each caused by the appearance of a new strain of the virus in humans, and killed tens of millions of people.

Key Terms

- **epidemic**: A widespread disease that affects many individuals in a population.
- **population**: A collection of organisms of a particular species, sharing a particular characteristic of interest, most often that of living in a given area.
- **infectious**: Infectious diseases, also known as transmissible diseases or communicable diseases, comprise clinically evident illness (i.e., characteristic medical signs and/or symptoms of disease) resulting from the infection, presence, and growth of pathogenic biological agents in an individual host organism.

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CHAPTER OVERVIEW

6: Immunology

6.1: Overview of the Immune System

6.1.1: Cells and Organs of the Immune System

6.2: Non-Adaptive Immunity

6.2.1: Pathogen Recognition

6.2.1.1: Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)

6.2.1.2: Pattern-Recognition Receptors (PRRs)

6.2.2: The Complement System

6.2.3: Interferons

6.2.4: Natural Killer Cells

6.2.5: Dendritic Cells

6.2.6: Inflammation and Fever

6.3: Adaptive Immunity

6.3.1: Antigen Epitopes

6.3.2: Humoral Immune Response

6.3.2.1: B Lymphocytes and Antibodies

6.3.2.2: Antibody Structure

6.3.2.3: Antibody Functions

6.3.3: Cell-Mediated Immunity

6.3.3.1: T Lymphocytes

6.3.4: Immunological Memory, Primary, and Secondary Adaptive Response

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6.1: Overview of the Immune System

Learning Objectives

- Distinguish between innate and adaptive immunity

The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue.

Pathogens can rapidly evolve and adapt to avoid detection and neutralization by the immune system. As a result, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms, such as bacteria, possess a rudimentary immune system in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms including include phagocytosis, antimicrobial peptides called defensins, and the complement system, which evolved in ancient eukaryotes and remain in modern descendants, such as plants and insects. Jawed vertebrates have even more sophisticated defense mechanisms, including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.

Innate and Adaptive Immunity

The immune system protects organisms from infection with layered defenses of increasing specificity. Physical barriers prevent pathogens, such as bacteria and viruses, from entering the organism. If a pathogen breaches these barriers, the innate immune system provides an immediate, but non-specific response. Innate immune systems are found in all plants and animals. If pathogens successfully evade the innate response, vertebrates possess a second layer of protection, the adaptive immune system, which is activated by the innate response. The immune system adapts its response during an infection in order to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated, in the form of an immunological memory, and allows the adaptive immune system to mount faster and stronger attacks when this pathogen is encountered. Both innate and adaptive immunity depend on the ability of the immune system to distinguish between self and non- self molecules, where self molecules are those components of an organism's body that can be distinguished from foreign substances by the immune system.

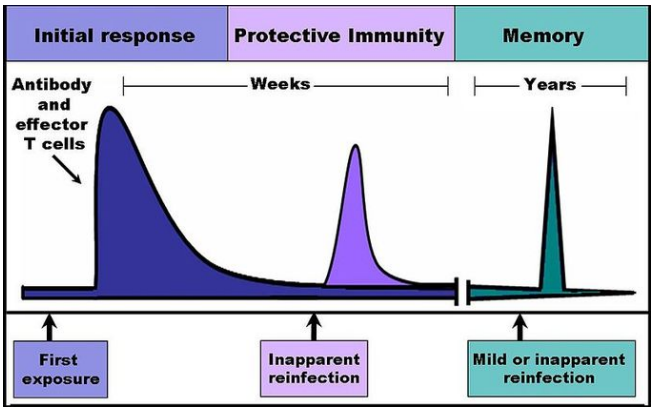


Figure: **The Time Course of an Immune Response**: Immune reactants, such as antibodies and effector T-cells, work to eliminate an infection, and their levels and activity rapidly increase following an encounter with an infectious agent, whether that agent is a pathogen or a vaccine. For several weeks these reactants remain in the serum and lymphatic tissues and provide protective immunity against reinfection by the same agent. During an early reinfection, few outward symptoms of illness are present, but the levels of immune reactants increase and are detectable in the blood and/or lymph. Following clearance of the infection, antibody level and effector T cell activity gradually declines. Because immunological memory has developed, reinfection at later times leads to a rapid increase in antibody production and effector T cell activity. These later infections can be mild or even inapparent.

Immunity is a biological term that describes a state of having sufficient biological defences to avoid infection, disease, or other unwanted biological invasion. Immunity involves both specific and non-specific components.

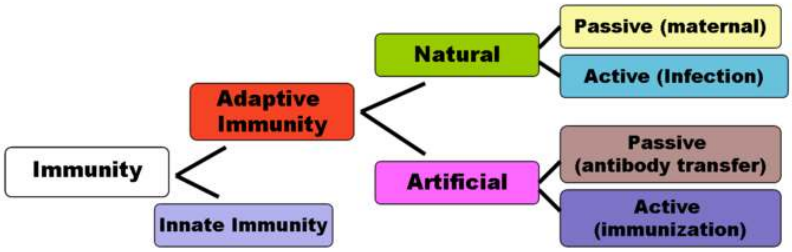


Figure: **Immunity**: Natural immunity occurs through contact with a disease causing agent, when the contact was not deliberate, where as artificial immunity develops only through deliberate actions of exposure. Both natural and artificial immunity can be further subdivided, depending on the amount of time the protection lasts. Passive immunity is short lived, and usually lasts only a few months, whereas protection via active immunity lasts much longer, and is sometimes life-long.

INNATE IMMUNITY

Innate, or nonspecific, immunity is the natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches. Microbes first encounter the epithelial layers (physical barriers that line our skin and mucous membranes). Subsequent general defenses include secreted chemical signals (cytokines), antimicrobial substances, fever, and phagocytic activity associated with the inflammatory response. The phagocytes express cell surface receptors that can bind and respond to common molecular patterns expressed on the surface of invading microbes. Through these approaches, innate immunity can prevent the colonization, entry, and spread of microbes.

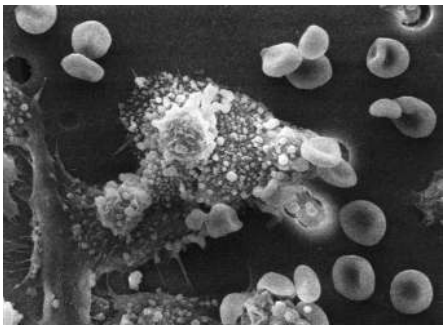


Figure: **An animal's immune response to a foreign body**: Macrophages begin to fuse with, and inject its toxins into, the cancer cell. The cell starts rounding up and loses its spikes. As the macrophage cell becomes smooth. The cancer cell appears lumpy in the last stage before it dies. These lumps are actually the macrophages fused within the cancer cell. The cancer cell then loses its morphology, shrinks up and dies. Photo magnification: 3: x8,000 Type: B & W print

ADAPTIVE IMMUNITY

Adaptive immunity is often sub-divided into two major types depending on how the immunity was introduced. Naturally acquired immunity occurs through contact with a disease causing agent, when the contact was not deliberate, whereas artificially acquired immunity develops only through deliberate actions such as vaccination. Both naturally and artificially acquired immunity can be further subdivided depending on whether immunity is induced in the host or passively transferred from an immune host. Passive immunity is acquired through transfer of antibodies or activated T cells from an immune host, and is short lived—usually lasting only a few months. Active immunity is induced in the host itself by antigen, and lasts much longer, sometimes the entire lifetime.

A further subdivision of adaptive immunity is characterized by the cells involved; humoral immunity is the aspect of immunity that is mediated by secreted antibodies, whereas the protection provided by cell-mediated immunity involves T lymphocytes alone. Humoral immunity is active when the organism generates its own antibodies, and passive when antibodies are transferred between individuals. Similarly, cell-mediated immunity is active when the organism's own T cells are stimulated and passive when T cells come from another organism.

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Key Points

- Pathogens can rapidly evolve and adapt to avoid detection and neutralization by the immune system. As a result, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. The immune system protects from infection with layered defenses of increasing specificity.
- Physical barriers prevent pathogens from entering the organism. If these barriers are breached, the innate immune system provides an immediate, non-specific response. If pathogens successfully evade the innate response, vertebrates possess a second layer of protection, the adaptive immune system.
- Immunity is a biological term that describes a state of having sufficient biological defences to avoid infection, disease, or other unwanted biological invasion.

- Innate, or nonspecific, immunity is the natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches.
- Adaptive immunity is often sub-divided into two major types acording to how the immunity was introduced. Naturally acquired immunity occurs through non-deliberate contact with a disease causing agent, whereas artificially acquired immunity develops through deliberate actions such as vaccination.
- Immunology is a branch of biomedical science that covers the study of all aspects of the immune system in all organisms. It deals with the physiological functioning of the immune system in states of both health and disease.

Key Terms

- **Adaptive (acquired) immunity**: The creation of immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.
- **Innate immunity**: The natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches.
- **Self molecules**: Those components of an organism's body that can be distinguished by the immune system from foreign substances.

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6.1.1: Cells and Organs of the Immune System

Learning Objectives

- Recognize the cells and organs of the immune system and their functions

Immune System Organs

The key primary lymphoid organs of the immune system include the thymus and bone marrow, as well as secondary lymphatic tissues including spleen, tonsils, lymph vessels, lymph nodes, adenoids, skin, and liver.

The thymus “educates” T cells and provides an inductive environment for the development of T cells from hematopoietic progenitor cells. The thymus is largest and most active during the neonatal and pre-adolescent periods of development. By the early teens, the thymus begins to atrophy and thymic stroma is replaced by adipose tissue. Nevertheless, residual T-lymphopoiesis continues throughout adult life.

Bone marrow is the flexible tissue found in the interior of bones. In humans, red blood cells are produced in the heads of long bones. The red bone marrow is a key element of the lymphatic system, being one of the primary lymphoid organs that generate lymphocytes from immature hematopoietic progenitor cells. Bone marrow and thymus constitute the primary lymphoid tissues involved in the production and early selection of lymphocytes.

The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels that carry a clear fluid, called lymph, unidirectionally towards the heart. The lymphatic system has multiple interrelated functions including the transportation of white blood cells to and from the lymph nodes into the bones, and the transportation of antigen -presenting cells (such as dendritic cells) to the lymph nodes where an immune response is stimulated. Lymphoid tissue is found in many organs, particularly the lymph nodes.

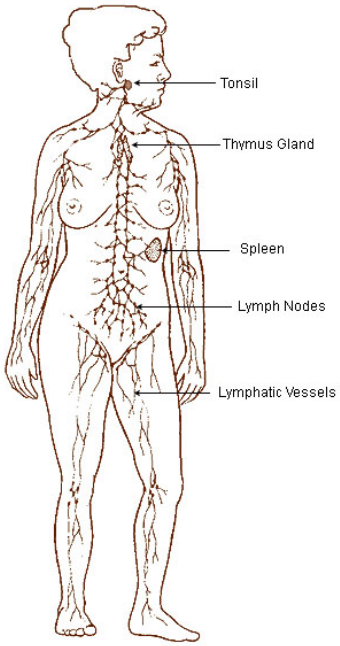


Figure: **The Lymph Nodes and Lymph Vessels in Human Beings**: The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels that carry a clear fluid called lymph.

The spleen is similar in structure to a large lymph node and acts primarily as a blood filter. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

The palatine tonsils and the nasopharyngeal tonsil are lymphoepithelial tissues located near the oropharynx and nasopharynx. These immunocompetent tissues are the immune system’s first line of defense against ingested or inhaled foreign pathogens. The fundamental immunological roles of tonsils aren’t yet understood.

Lymph nodes are distributed widely throughout areas of the body, including the armpit and stomach, and linked by lymphatic vessels. Lymph nodes are garrisons of B, T and other immune cells. Lymph nodes act as filters or traps for foreign particles and are important in the proper functioning of the immune system. They are packed tightly with the white blood cells, called lymphocytes and macrophages.

The skin is one of the most important parts of the body because it interfaces with the environment, and is the first line of defense from external factors, acting as an anatomical barrier from pathogens and damage between the internal and external environment in bodily defense. Langerhans cells in the skin are part of the adaptive immune system.

The liver has a wide range of functions, including immunological effects—the reticuloendothelial system of the liver contains many immunologically active cells, acting as a “sieve” for antigens carried to it via the portal system.

Immune System Cells

Leukocytes (white blood cells) are immune system cells involved in defending the body against infectious disease and foreign materials. Five different types of leukocytes exist, all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. The innate leukocytes include the phagocytes, mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are important mediators in the activation of the adaptive immune system.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte. During the acute phase of inflammation neutrophils migrate toward the site of inflammation and are usually the first cells to arrive at the scene of infection. Macrophages reside within tissues and produce a wide array of chemicals. They also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system. Dendritic cells are phagocytes in tissues that are in contact with the external environment, and are located mainly in the skin, nose, lungs, stomach, and intestines. These cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T-cells, one of the key cell types of the adaptive immune system.

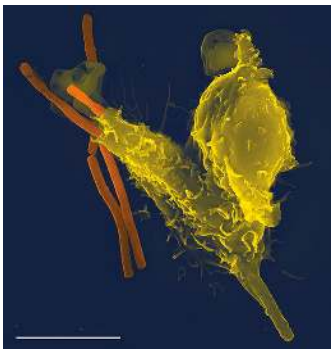


Figure: **A Phagocyte in Action**: Neutrophil engulfing anthrax bacteria. Taken with a Leo 1550 scanning electron microscope. Scale bar is 5 micrometers.

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis.

Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites, and play a role in allergic reactions, such as asthma.

Natural killer cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses.

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.

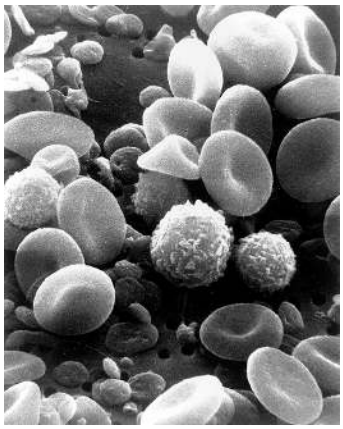


Figure: **Blood Cells**: Red blood cells, several white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.

T cells recognize a “non-self” target, such as a pathogen, only after antigens have been processed and presented in combination with a “self” receptor, called a major histocompatibility complex (MHC) molecule. There are two major subtypes of T cells: the killer T cell, which kills cells that are infected with viruses (and other pathogens) or are otherwise damaged or dysfunctional, and the helper T cell, which regulates both innate and adaptive immune responses and helps determine which immune responses the body makes to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly. A third, minor subtype are the γ T cells that recognize intact antigens not bound to MHC receptors.

In contrast, the B cell antigen-specific receptor is an antibody molecule on the B cell surface, which recognizes whole pathogens without any need for antigen processing. Each lineage of B cell expresses a different antibody, so the complete set of B cell antigen receptors represent all the antibodies that the body can manufacture.

Key Points

- The key primary lymphoid organs of the immune system are the thymus and bone marrow, and secondary lymphatic tissues such as spleen, tonsils, lymph vessels, lymph nodes, adenoids, and skin and liver.
- Leukocytes (white blood cells) act like independent, single-celled organisms and are the second arm of the innate immune system.
- The innate leukocytes include the phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are also important mediators in the activation of the adaptive immune system.
- The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.
- The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels. The lymphatic system has multiple functions such as the transportation of white blood cells to and from the lymph nodes into the bones.

Key Terms

- **lymphocytes**: A lymphocyte is a type of white blood cell in the vertebrate immune system. The three major types of lymphocyte are T cells, B cells and natural killer (NK) cells. T cells (thymus cells) and B cells (bursa-derived cells) are the major cellular components of the adaptive immune response.
- **Leukocytes**: Cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist.

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6.2: Non-Adaptive Immunity

Learning Objectives

- Describe how enzymes in body fluids provide protection against infection or disease
- List and describe the function of antimicrobial peptides, complement components, cytokines, and acute-phase proteins
- Describe similarities and differences among classic, alternate, and lectin complement pathways

In addition to physical defenses, the innate nonspecific immune system uses a number of chemical mediators that inhibit microbial invaders. The term “chemical mediators” encompasses a wide array of substances found in various body fluids and tissues throughout the body. Chemical mediators may work alone or in conjunction with each other to inhibit microbial colonization and infection.

Some chemical mediators are endogenously produced, meaning they are produced by human body cells; others are produced exogenously, meaning that they are produced by certain microbes that are part of the microbiome. Some mediators are produced continually, bathing the area in the antimicrobial substance; others are produced or activated primarily in response to some stimulus, such as the presence of microbes.

Chemical and Enzymatic Mediators Found in Body Fluids

Fluids produced by the skin include examples of both endogenous and exogenous mediators. Sebaceous glands in the dermis secrete an oil called sebum that is released onto the skin surface through hair follicles. This sebum is an endogenous mediator, providing an additional layer of defense by helping seal off the pore of the hair follicle, preventing bacteria on the skin’s surface from invading sweat glands and surrounding tissue (Figure **6.2.1**). Certain members of the microbiome, such as the bacterium *Propionibacterium acnes* and the fungus *Malassezia*, among others, can use lipase enzymes to degrade sebum, using it as a food source. This produces oleic acid, which creates a mildly acidic environment on the surface of the skin that is inhospitable to many pathogenic microbes. Oleic acid is an example of an exogenously produced mediator because it is produced by resident microbes and not directly by body cells.

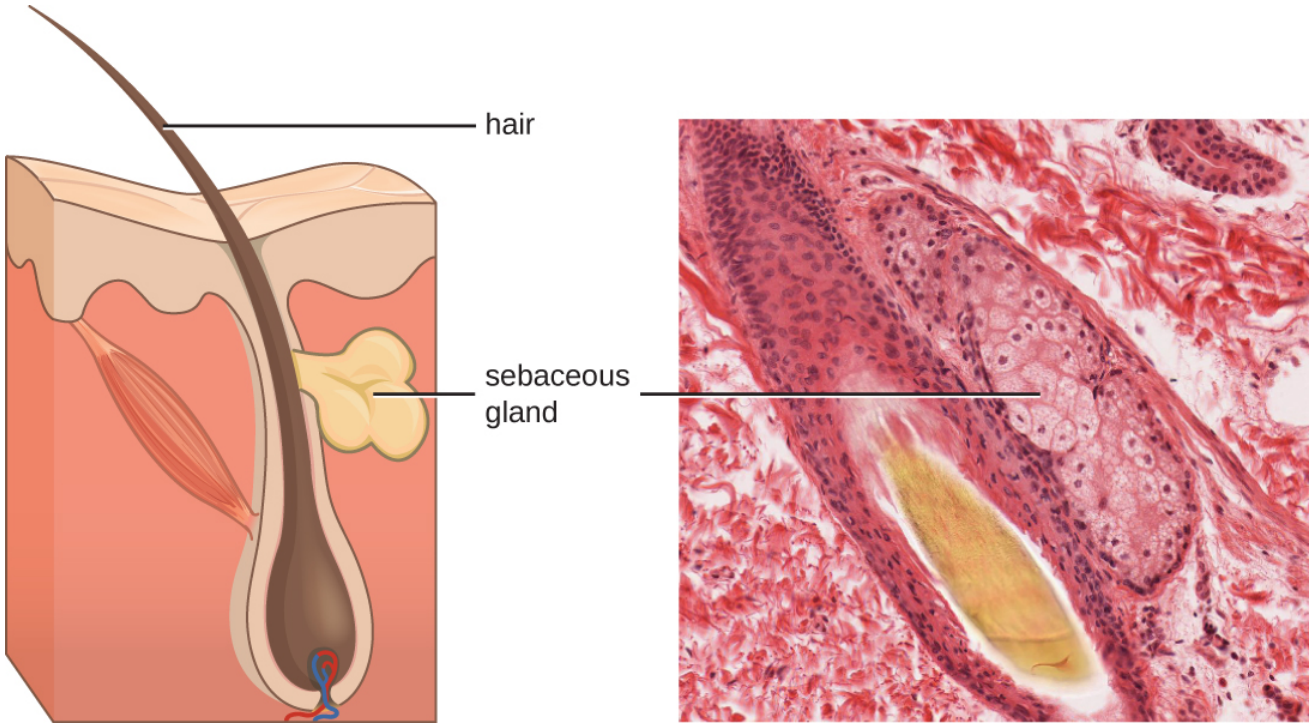


Figure **6.2.1**: Sebaceous glands secrete sebum, a chemical mediator that lubricates and protect the skin from invading microbes. Sebum is also a food source for resident microbes that produce oleic acid, an exogenously produced mediator. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Environmental factors that affect the microbiota of the skin can have a direct impact on the production of chemical mediators. Low humidity or decreased sebum production, for example, could make the skin less habitable for microbes that produce oleic acid, thus making the skin more susceptible to pathogens normally inhibited by the skin's low pH. Many skin moisturizers are formulated to counter such effects by restoring moisture and essential oils to the skin.

The digestive tract also produces a large number of chemical mediators that inhibit or kill microbes. In the oral cavity, saliva contains mediators such as lactoperoxidase enzymes, and mucus secreted by the esophagus contains the antibacterial enzyme lysozyme. In the stomach, highly acidic gastric fluid kills most microbes. In the lower digestive tract, the intestines have pancreatic and intestinal enzymes, antibacterial peptides (cryptins), bile produced from the liver, and specialized Paneth cells that produce lysozyme. Together, these mediators are able to eliminate most pathogens that manage to survive the acidic environment of the stomach.

In the urinary tract, urine flushes microbes out of the body during urination. Furthermore, the slight acidity of urine (the average pH is about 6) inhibits the growth of many microbes and potential pathogens in the urinary tract.

The female reproductive system employs lactate, an exogenously produced chemical mediator, to inhibit microbial growth. The cells and tissue layers composing the vagina produce glycogen, a branched and more complex polymer of glucose. Lactobacilli in the area ferment glycogen to produce lactate, lowering the pH in the vagina and inhibiting transient microbiota, opportunistic pathogens like *Candida* (a yeast associated with vaginal infections), and other pathogens responsible for sexually transmitted diseases.

In the eyes, tears contain the chemical mediators lysozyme and lactoferrin, both of which are capable of eliminating microbes that have found their way to the surface of the eyes. Lysozyme cleaves the bond between NAG and NAM in peptidoglycan, a component of the cell wall in bacteria. It is more effective against gram-positive bacteria, which lack the protective outer membrane associated with gram-negative bacteria. Lactoferrin inhibits microbial growth by chemically binding and sequestering iron. This effectually starves many microbes that require iron for growth.

In the ears, cerumen (earwax) exhibits antimicrobial properties due to the presence of fatty acids, which lower the pH to between 3 and 5.

The respiratory tract uses various chemical mediators in the nasal passages, trachea, and lungs. The mucus produced in the nasal passages contains a mix of antimicrobial molecules similar to those found in tears and saliva (e.g., lysozyme, lactoferrin, lactoperoxidase). Secretions in the trachea and lungs also contain lysozyme and lactoferrin, as well as a diverse group of additional chemical mediators, such as the lipoprotein complex called surfactant, which has antibacterial properties.

? Exercise 6.2.1

1. Explain the difference between endogenous and exogenous mediators
2. Describe how pH affects antimicrobial defenses

Antimicrobial Peptides

The antimicrobial peptides (AMPs) are a special class of nonspecific cell-derived mediators with broad-spectrum antimicrobial properties. Some AMPs are produced routinely by the body, whereas others are primarily produced (or produced in greater quantities) in response to the presence of an invading pathogen. Research has begun exploring how AMPs can be used in the diagnosis and treatment of disease.

AMPs may induce cell damage in microorganisms in a variety of ways, including by inflicting damage to membranes, destroying DNA and RNA, or interfering with cell-wall synthesis. Depending on the specific antimicrobial mechanism, a particular AMP may inhibit only certain groups of microbes (e.g., gram-positive or gram-negative bacteria) or it may be more broadly effective against bacteria, fungi, protozoa, and viruses. Many AMPs are found on the skin, but they can also be found in other regions of the body.

A family of AMPs called defensins can be produced by epithelial cells throughout the body as well as by cellular defenses such as macrophages and neutrophils (see Cellular Defenses). Defensins may be secreted or act inside host cells; they combat microorganisms by damaging their plasma membranes. AMPs called bacteriocins are produced exogenously by certain members of

the resident microbiota within the gastrointestinal tract. The genes coding for these types of AMPs are often carried on plasmids and can be passed between different species within the resident microbiota through lateral or horizontal gene transfer.

There are numerous other AMPs throughout the body. The characteristics of a few of the more significant AMPs are summarized in Table **6.2.1**.

Table **6.2.1**: Characteristics of Selected Antimicrobial Peptides (AMPs)

AMP	Secreted by	Body site	Pathogens inhibited	Mode of action
Bacteriocins	Resident microbiota	Gastrointestinal tract	Bacteria	Disrupt membrane
Cathelicidin	Epithelial cells, macrophages, and other cell types	Skin	Bacteria and fungi	Disrupts membrane
Defensins	Epithelial cells, macrophages, neutrophils	Throughout the body	Fungi, bacteria, and many viruses	Disrupt membrane
Dermicidin	Sweat glands	Skin	Bacteria and fungi	Disrupts membrane integrity and ion channels
Histatins	Salivary glands	Oral cavity	Fungi	Disrupt intracellular function

? Exercise 6.2.2

Why are antimicrobial peptides (AMPs) considered nonspecific defenses?

Plasma Protein Mediators

Many nonspecific innate immune factors are found in plasma, the fluid portion of blood. Plasma contains electrolytes, sugars, lipids, and proteins, each of which helps to maintain homeostasis (i.e., stable internal body functioning), and contains the proteins involved in the clotting of blood. Additional proteins found in blood plasma, such as acute-phase proteins, complement proteins, and cytokines, are involved in the nonspecific innate immune response.

Plasma versus Serum

There are two terms for the fluid portion of blood: plasma and serum. How do they differ if they are both fluid and lack cells? The fluid portion of blood left over after coagulation (blood cell clotting) has taken place is serum. Although molecules such as many vitamins, electrolytes, certain sugars, complement proteins, and antibodies are still present in serum, clotting factors are largely depleted. Plasma, conversely, still contains all the clotting elements. To obtain plasma from blood, an anticoagulant must be used to prevent clotting. Examples of anticoagulants include heparin and ethylene diamine tetraacetic acid (EDTA). Because clotting is inhibited, once obtained, the sample must be gently spun down in a centrifuge. The heavier, denser blood cells form a pellet at the bottom of a centrifuge tube, while the fluid plasma portion, which is lighter and less dense, remains above the cell pellet.

Acute-Phase Proteins

The acute-phase proteins are another class of antimicrobial mediators. Acute-phase proteins are primarily produced in the liver and secreted into the blood in response to inflammatory molecules from the immune system. Examples of acute-phase proteins include C-reactive protein, serum amyloid A, ferritin, transferrin, fibrinogen, and mannose-binding lectin. Each of these proteins has a different chemical structure and inhibits or destroys microbes in some way (Table **6.2.1**).

Table **6.2.2**: Some Acute-Phase Proteins and Their Functions

Some Acute-Phase Proteins and Their Functions	
C-reactive protein	Coats bacteria (opsonization), preparing them for ingestion by phagocytes
Serum amyloid A	
Ferritin	Bind and sequester iron, thereby inhibiting the growth of pathogens
Transferrin	
Fibrinogen	Involved in formation of blood clots that trap bacterial pathogens
Mannose-binding lectin	Activates complement cascade

The Complement System

The complement system is a group of plasma protein mediators that can act as an innate nonspecific defense while also serving to connect innate and adaptive immunity (discussed in the next chapter). The complement system is composed of more than 30 proteins (including C1 through C9) that normally circulate as precursor proteins in blood. These precursor proteins become activated when stimulated or triggered by a variety of factors, including the presence of microorganisms. Complement proteins are considered part of innate nonspecific immunity because they are always present in the blood and tissue fluids, allowing them to be activated quickly. Also, when activated through the alternative pathway (described later in this section), complement proteins target pathogens in a nonspecific manner.

The process by which circulating complement precursors become functional is called complement activation. This process is a cascade that can be triggered by one of three different mechanisms, known as the alternative, classical, and lectin pathways.

The alternative pathway is initiated by the spontaneous activation of the complement protein C3. The hydrolysis of C3 produces two products, C3a and C3b. When no invader microbes are present, C3b is very quickly degraded in a hydrolysis reaction using the water in the blood. However, if invading microbes are present, C3b attaches to the surface of these microbes. Once attached, C3b will recruit other complement proteins in a cascade (Figure **6.2.2**).

The classical pathway provides a more efficient mechanism of activating the complement cascade, but it depends upon the production of antibodies by the specific adaptive immune defenses. To initiate the classical pathway, a specific antibody must first bind to the pathogen to form an antibody-antigen complex. This activates the first protein in the complement cascade, the C1 complex. The C1 complex is a multipart protein complex, and each component participates in the full activation of the overall complex. Following recruitment and activation of the C1 complex, the remaining classical pathway complement proteins are recruited and activated in a cascading sequence (Figure **6.2.2**).

The lectin activation pathway is similar to the classical pathway, but it is triggered by the binding of mannose-binding lectin, an acute-phase protein, to carbohydrates on the microbial surface. Like other acute-phase proteins, lectins are produced by liver cells and are commonly upregulated in response to inflammatory signals received by the body during an infection (Figure **6.2.2**).

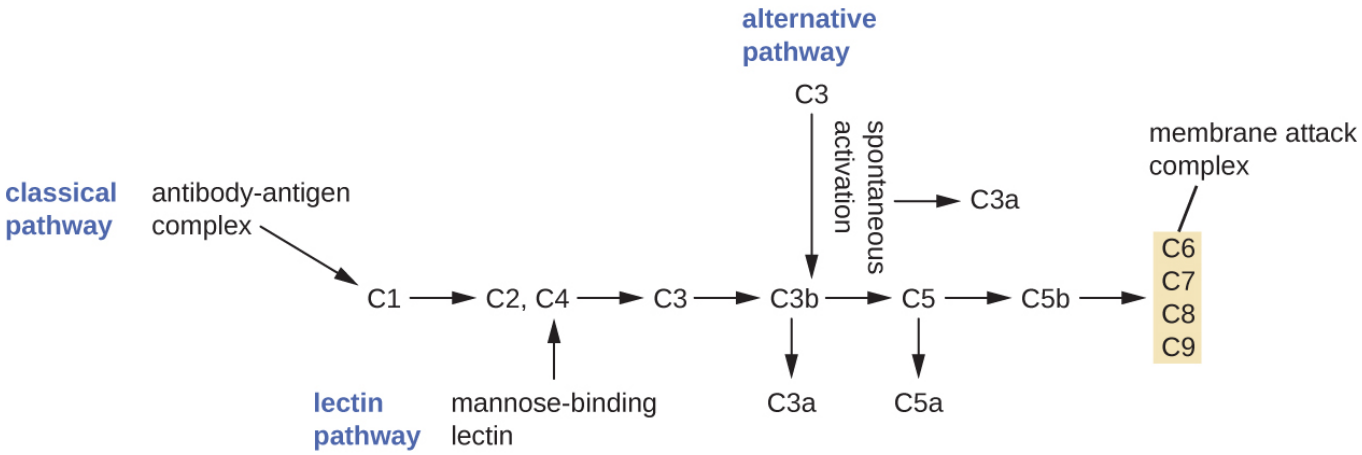


Figure 6.2.2: The three complement activation pathways have different triggers, as shown here, but all three result in the activation of the complement protein C3, which produces C3a and C3b. The latter binds to the surface of the target cell and then works with other complement proteins to cleave C5 into C5a and C5b. C5b also binds to the cell surface and then recruits C6 through C9; these molecules form a ring structure called the membrane attack complex (MAC), which punches through the cell membrane of the invading pathogen, causing it to swell and burst.

Although each complement activation pathway is initiated in a different way, they all provide the same protective outcomes: opsonization, inflammation, chemotaxis, and cytolysis. The term opsonization refers to the coating of a pathogen by a chemical substance (called an opsonin) that allows phagocytic cells to recognize, engulf, and destroy it more easily. Opsonins from the complement cascade include C1q, C3b, and C4b. Additional important opsonins include mannose-binding proteins and antibodies. The complement fragments C3a and C5a are well-characterized anaphylatoxins with potent proinflammatory functions. Anaphylatoxins activate mast cells, causing degranulation and the release of inflammatory chemical signals, including mediators that cause vasodilation and increased vascular permeability. C5a is also one of the most potent chemoattractants for neutrophils and other white blood cells, cellular defenses that will be discussed in the next section.

The complement proteins C6, C7, C8, and C9 assemble into a membrane attack complex (MAC), which allows C9 to polymerize into pores in the membranes of gram-negative bacteria. These pores allow water, ions, and other molecules to move freely in and out of the targeted cells, eventually leading to cell lysis and death of the pathogen (Figure 6.2.2). However, the MAC is only effective against gram-negative bacteria; it cannot penetrate the thick layer of peptidoglycan associated with cell walls of gram-positive bacteria. Since the MAC does not pose a lethal threat to gram-positive bacterial pathogens, complement-mediated opsonization is more important for their clearance.

Cytokines

Cytokines are soluble proteins that act as communication signals between cells. In a nonspecific innate immune response, various cytokines may be released to stimulate production of chemical mediators or other cell functions, such as cell proliferation, cell differentiation, inhibition of cell division, apoptosis, and chemotaxis.

When a cytokine binds to its target receptor, the effect can vary widely depending on the type of cytokine and the type of cell or receptor to which it has bound. The function of a particular cytokine can be described as autocrine, paracrine, or endocrine (Figure 6.2.3). In autocrine function, the same cell that releases the cytokine is the recipient of the signal; in other words, autocrine function is a form of self-stimulation by a cell. In contrast, paracrine function involves the release of cytokines from one cell to other nearby cells, stimulating some response from the recipient cells. Last, endocrine function occurs when cells release cytokines into the bloodstream to be carried to target cells much farther away.

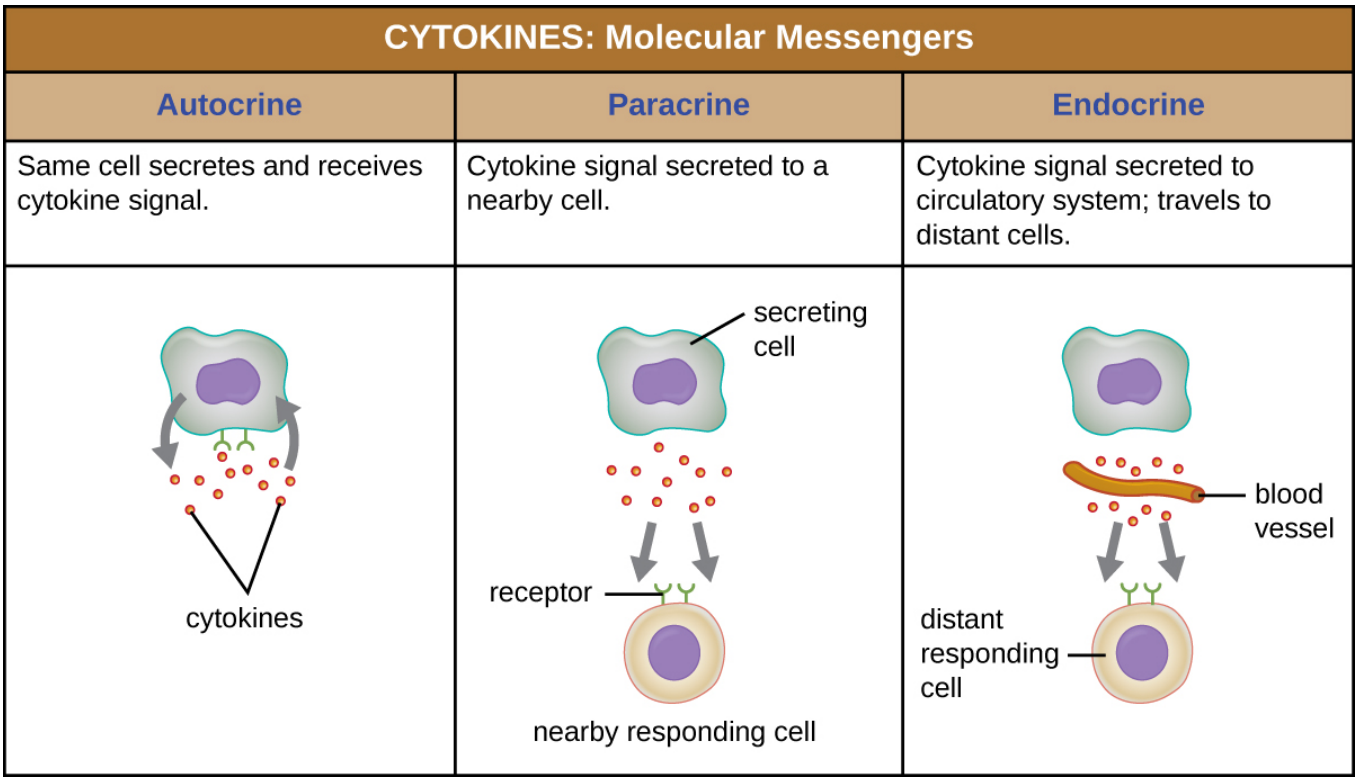


Figure 6.2.3: Autocrine, paracrine, and endocrine actions describe which cells are targeted by cytokines and how far the cytokines must travel to bind to their intended target cells' receptors.

Three important classes of cytokines are the interleukins, chemokines, and interferons. The interleukins were originally thought to be produced only by leukocytes (white blood cells) and to only stimulate leukocytes, thus the reasons for their name. Although interleukins are involved in modulating almost every function of the immune system, their role in the body is not restricted to immunity. Interleukins are also produced by and stimulate a variety of cells unrelated to immune defenses.

The chemokines are chemotactic factors that recruit leukocytes to sites of infection, tissue damage, and inflammation. In contrast to more general chemotactic factors, like complement factor C5a, chemokines are very specific in the subsets of leukocytes they recruit.

Interferons are a diverse group of immune signaling molecules and are especially important in our defense against viruses. Type I interferons (interferon-α and interferon-β) are produced and released by cells infected with virus. These interferons stimulate nearby cells to stop production of mRNA, destroy RNA already produced, and reduce protein synthesis. These cellular changes inhibit viral replication and production of mature virus, slowing the spread of the virus. Type I interferons also stimulate various immune cells involved in viral clearance to more aggressively attack virus-infected cells. Type II interferon (interferon-γ) is an important activator of immune cells (Figure 6.2.4).

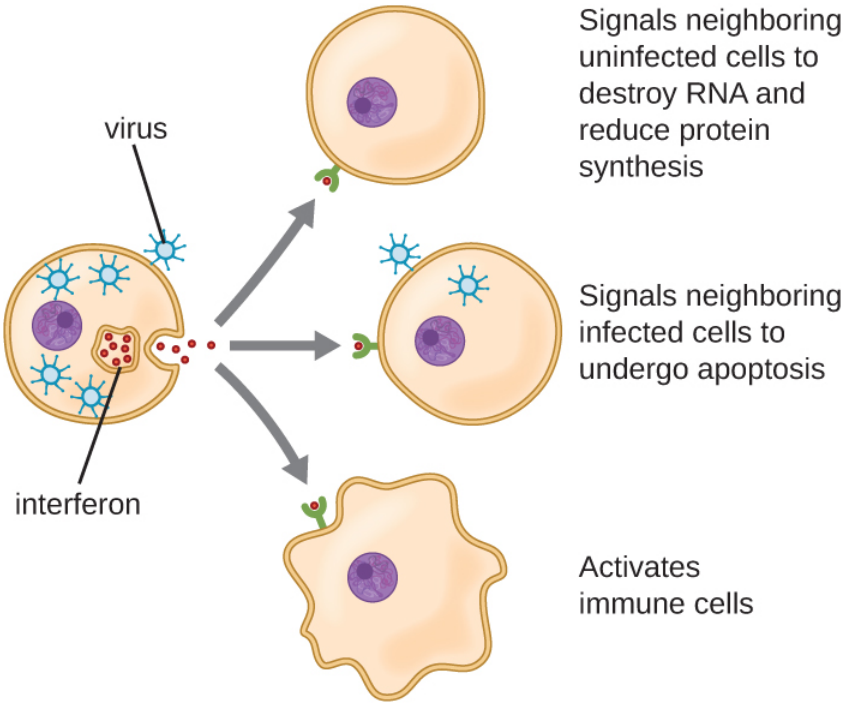


Figure **6.2.4**: Interferons are cytokines released by a cell infected with a virus. Interferon-α and interferon-β signal uninfected neighboring cells to inhibit mRNA synthesis, destroy RNA, and reduce protein synthesis (top arrow). Interferon-α and interferon-β also promote apoptosis in cells infected with the virus (middle arrow). Interferon-γ alerts neighboring immune cells to an attack (bottom arrow). Although interferons do not cure the cell releasing them or other infected cells, which will soon die, their release may prevent additional cells from becoming infected, thus stemming the infection.

Inflammation-Eliciting Mediators

Many of the chemical mediators discussed in this section contribute in some way to inflammation and fever, which are nonspecific immune responses discussed in more detail in Inflammation and Fever. Cytokines stimulate the production of acute-phase proteins such as C-reactive protein and mannose-binding lectin in the liver. These acute-phase proteins act as opsonins, activating complement cascades through the lectin pathway.

Some cytokines also bind mast cells and basophils, inducing them to release histamine, a proinflammatory compound. Histamine receptors are found on a variety of cells and mediate proinflammatory events, such as bronchoconstriction (tightening of the airways) and smooth muscle contraction.

In addition to histamine, mast cells may release other chemical mediators, such as leukotrienes. Leukotrienes are lipid-based proinflammatory mediators that are produced from the metabolism of arachidonic acid in the cell membrane of leukocytes and tissue cells. Compared with the proinflammatory effects of histamine, those of leukotrienes are more potent and longer lasting. Together, these chemical mediators can induce coughing, vomiting, and diarrhea, which serve to expel pathogens from the body.

Certain cytokines also stimulate the production of prostaglandins, chemical mediators that promote the inflammatory effects of kinins and histamines. Prostaglandins can also help to set the body temperature higher, leading to fever, which promotes the activities of white blood cells and slightly inhibits the growth of pathogenic microbes (see Inflammation and Fever).

Another inflammatory mediator, bradykinin, contributes to edema, which occurs when fluids and leukocytes leak out of the bloodstream and into tissues. It binds to receptors on cells in the capillary walls, causing the capillaries to dilate and become more permeable to fluids.

? Exercise 6.2.3

1. What do the three complement activation pathways have in common?
2. Explain autocrine, paracrine, and endocrine signals.
3. Name two important inflammation-eliciting mediators.

Clinical Focus: Part 2

To relieve the constriction of her airways, Angela is immediately treated with antihistamines and administered corticosteroids through an inhaler, and then monitored for a period of time. Though her condition does not worsen, the drugs do not seem to be alleviating her condition. She is admitted to the hospital for further observation, testing, and treatment.

Following admission, a clinician conducts allergy testing to try to determine if something in her environment might be triggering an allergic inflammatory response. A doctor orders blood analysis to check for levels of particular cytokines. A sputum sample is also taken and sent to the lab for microbial staining, culturing, and identification of pathogens that could be causing an infection.

Exercise 6.2.4

1. Which aspects of the innate immune system could be contributing to Angela's airway constriction?
2. Why was Angela treated with antihistamines?
3. Why would the doctor be interested in levels of cytokines in Angela's blood?

Table **6.2.3** provides a summary of the chemical defenses discussed in this section.

Table **6.2.3**: Chemical Defenses of Nonspecific Innate Immunity

Defense	Examples	Function
Chemicals and enzymes in body fluids	Sebum from sebaceous glands	Provides oil barrier protecting hair follicle pores from pathogens
	Oleic acid from sebum and skin microbiota	Lowers pH to inhibit pathogens
	Lysozyme in secretions	Kills bacteria by attacking cell wall
	Acid in stomach, urine, and vagina	Inhibits or kills bacteria
	Digestive enzymes and bile	Kill bacteria
	Lactoferrin and transferrin	Bind and sequester iron, inhibiting bacterial growth
	Surfactant in lungs	Kills bacteria
Antimicrobial peptides	Defensins, bacteriocins, dermicidin, cathelicidin, histatins,	Kill bacteria by attacking membranes or interfering with cell functions
Plasma protein mediators	Acute-phase proteins (C-reactive protein, serum amyloid A, ferritin, fibrinogen, transferrin, and mannose-binding lectin)	Inhibit the growth of bacteria and assist in the trapping and killing of bacteria
	Complements C3b and C4b	Opsonization of pathogens to aid phagocytosis
	Complement C5a	Chemoattractant for phagocytes
	Complements C3a and C5a	Proinflammatory anaphylatoxins
Cytokines	Interleukins	Stimulate and modulate most functions of immune system
	Chemokines	Recruit white blood cells to infected area
	Interferons	Alert cells to viral infection, induce apoptosis of virus-infected cells, induce antiviral defenses in infected and nearby uninfected cells, stimulate immune cells to attack virus-infected cells
Inflammation-eliciting mediators	Histamine	Promotes vasodilation, bronchoconstriction, smooth muscle contraction, increased secretion and mucus production
	Leukotrienes	Promote inflammation; stronger and longer lasting than histamine
	Prostaglandins	Promote inflammation and fever
	Bradykinin	Increases vasodilation and vascular permeability, leading to edema

Key Concepts and Summary

- Numerous **chemical mediators** produced endogenously and exogenously exhibit nonspecific antimicrobial functions.
- Many chemical mediators are found in body fluids such as sebum, saliva, mucus, gastric and intestinal fluids, urine, tears, cerumen, and vaginal secretions.

- **Antimicrobial peptides (AMPs)** found on the skin and in other areas of the body are largely produced in response to the presence of pathogens. These include dermcidin, cathelicidin, defensins, histatins, and bacteriocins.
- **Plasma** contains various proteins that serve as chemical mediators, including **acute-phase proteins**, **complement proteins**, and **cytokines**.
- The **complement system** involves numerous precursor proteins that circulate in plasma. These proteins become activated in a cascading sequence in the presence of microbes, resulting in the **opsonization** of pathogens, chemoattraction of leukocytes, induction of inflammation, and cytolysis through the formation of a **membrane attack complex (MAC)**.
- **Cytokines** are proteins that facilitate various nonspecific responses by innate immune cells, including production of other chemical mediators, cell proliferation, cell death, and differentiation.
- Cytokines play a key role in the inflammatory response, triggering production of inflammation-eliciting mediators such as acute-phase proteins, **histamine**, leukotrienes, **prostaglandins**, and **bradykinin**.

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6.2.1: PATHOGEN RECOGNITION

LEARNING OBJECTIVES

- Describe the role of PAMPs and PRRs, interferons, and other cytokines in innate immunity

When a pathogen enters the body, cells in the blood and lymph detect the specific pathogen-associated molecular patterns (PAMPs) on the pathogen's surface. PAMPs are carbohydrate, polypeptide, and nucleic acid "signatures" that are expressed by viruses, bacteria, and parasites, but which differ from molecules on host cells. These PAMPs allow the immune system to recognize "self" from "other" so as not to destroy the host.

The immune system has specific cells with receptors that recognize these PAMPs. A macrophage is a large, phagocytic cell that engulfs foreign particles and pathogens. Macrophages recognize PAMPs via complementary pattern recognition receptors (PRRs). PRRs are molecules on macrophages and dendritic cells which are in contact with the external environment and can thus recognize PAMPs when present. A monocyte, a type of leukocyte (white blood cell) that circulates in the blood and lymph, differentiates into macrophages after it moves into infected tissue. Dendritic cells bind molecular signatures of pathogens, promoting pathogen engulfment and destruction.

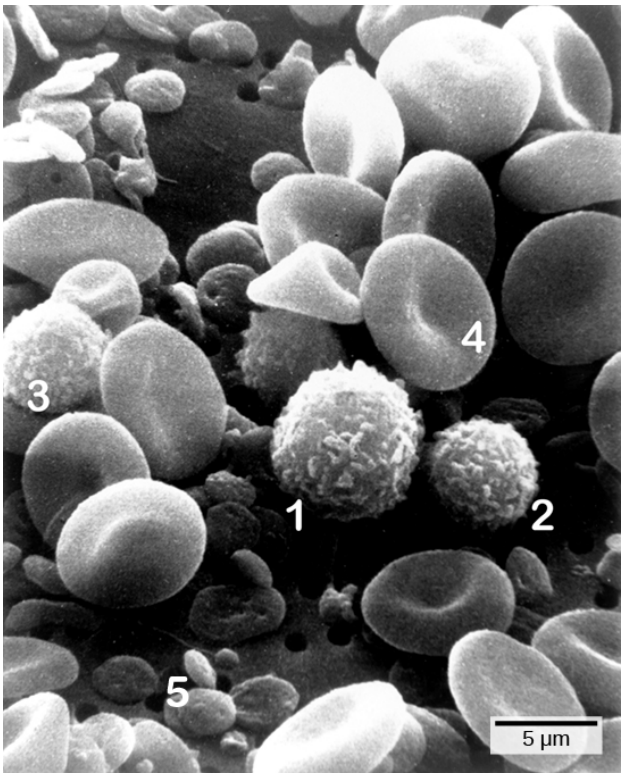


Figure **6.2.1.1**: Blood cells related to the innate immune response: Cells of the blood include (1) monocytes, (2) lymphocytes, (3) neutrophils, (4) red blood cells, and (5) platelets. Leukocytes (1, 2, 3) are white blood cells that play an important role in the body's immune system.

Cell type	Characteristics	Location	Image
Mast cell	Dilates blood vessels and induces inflammation through release of histamines and heparin. Recruits macrophages and neutrophils. Involved in wound healing and defense against pathogens but can also be responsible for allergic reactions.	Connective tissues, mucous membranes	
Macrophage	Phagocytic cell that consumes foreign pathogens and cancer cells. Stimulates response of other immune cells.	Migrates from blood vessels into tissues.	
Natural killer cell	Kills tumor cells and virus-infected cells.	Circulates in blood and migrates into tissues.	
Dendritic cell	Presents antigens on its surface, thereby triggering adaptive immunity.	Present in epithelial tissue, including skin, lung and tissues of the digestive tract. Migrates to lymph nodes upon activation.	
Monocyte	Differentiates into macrophages and dendritic cells in response to inflammation.	Stored in spleen, moves through blood vessels to infected tissues.	
Neutrophil	First responders at the site of infection or trauma, this abundant phagocytic cell represents 50-60 percent of all leukocytes. Releases toxins that kill or inhibit bacteria and fungi and recruits other immune cells to the site of infection.	Migrates from blood vessels into tissues.	
Basophil	Responsible for defense against parasites. Releases histamines that cause inflammation and may be responsible for allergic reactions.	Circulates in blood and migrates to tissues.	
Eosinophil	Releases toxins that kill bacteria and parasites but also causes tissue damage.	Circulates in blood and migrates to tissues.	

Figure **6.2.1.1**: Cells involved in the innate immune system: The immune system has specific cells whose job is to recognize pathogen-associated molecular patterns. The characteristics and location of cells involved in the innate immune system are described in this chart.

Once a pathogen is detected, the immune system must also track whether it is replicating intracellularly (inside the cell, as with most viruses and some bacteria) or extracellularly (outside of the cell, as with other bacteria, but not viruses). The innate immune system must respond accordingly by identifying the extracellular pathogen and/or by identifying host cells that have already been infected.

CYTOKINE RELEASE AFFECT

The binding of PRRs with PAMPs triggers the release of cytokines, which signal that a pathogen is present and needs to be destroyed along with any infected cells. A cytokine is a chemical messenger that regulates cell differentiation (form and function), proliferation (production), and gene expression to affect immune responses. At least 40 types of cytokines exist in humans that differ in terms of the cell type that produces them, the cell type that responds to them, and the changes they produce.

One subclass of cytokines is the interleukin (IL), which mediates interactions between leukocytes (white blood cells). Interleukins are involved in bridging the innate and adaptive immune responses. In addition to being released from cells after PAMP recognition,

cytokines are released by the infected cells which bind to nearby uninfected cells, inducing those cells to release cytokines, resulting in a cytokine burst.

A second class of cytokines is interferons, which are released by infected cells as a warning to nearby uninfected cells. A function an interferons is to inhibit viral replication, making them particularly effective against viruses. They also have other important functions, such as tumor surveillance. Interferons work by signaling neighboring uninfected cells to destroy RNA (often a very important biomolecule for viruses) and reduce protein synthesis; signaling neighboring infected cells to undergo apoptosis (programmed cell death); and activating immune cells.

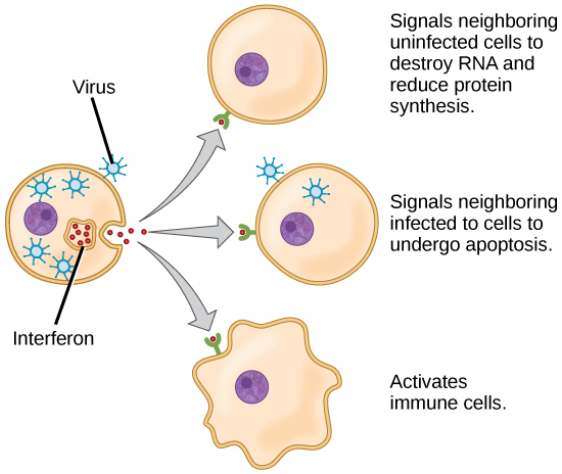


Figure **6.2.1.1**: Interferon release: Interferons are cytokines that are released by a cell infected with a virus. The response of neighboring cells to interferons helps stem the infection.

Cytokines also send feedback to cells of the nervous system to bring about the overall symptoms of feeling sick, which include lethargy, muscle pain, and nausea. These effects may have evolved because the symptoms encourage the individual to rest, preventing them from spreading the infection to others. Cytokines also increase the core body temperature, causing a fever, which causes the liver to withhold iron from the blood. Without iron, certain pathogens (such as some bacteria) are unable to replicate; this is called nutritional immunity.

PHAGOCYTOSIS AND INFLAMMATION

The first cytokines to be produced are pro-inflammatory; that is, they encourage inflammation, or the localized redness, swelling (edema), heat, loss of function, and pain that result from the movement of leukocytes and fluid through increasingly-permeable capillaries to a site of infection. The population of leukocytes that arrives at an infection site depends on the nature of the infecting pathogen. Both macrophages and dendritic cells engulf pathogens and cellular debris through phagocytosis. A neutrophil is also a phagocytic leukocyte that engulfs and digests pathogens. Neutrophils, the most-abundant leukocytes of the immune system, have a nucleus with two to five lobes and contain organelles (lysosomes) that digest engulfed pathogens. An eosinophil is a leukocyte that works with other eosinophils to surround a parasite. It is involved in the allergic response and in protection against helminthes (parasitic worms).

Neutrophils and eosinophils are particularly important leukocytes that engulf large pathogens, such as bacteria and fungi. A mast cell is a leukocyte that produces inflammatory molecules, such as histamine, in response to large pathogens. A basophil is a leukocyte that, like a neutrophil, releases chemicals to stimulate the inflammatory response. Basophils are also involved in allergy and hypersensitivity responses and induce specific types of inflammatory responses. Eosinophils and basophils produce additional inflammatory mediators to recruit more leukocytes. A hypersensitive immune response to harmless antigens, such as in pollen, often involves the release of histamine by basophils and mast cells; this is why many anti-allergy medications are anti-histamines.

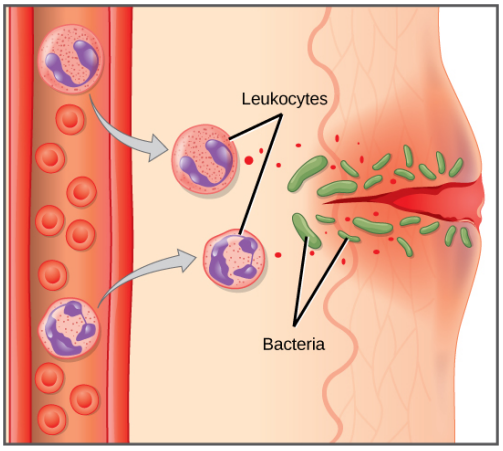


Figure **6.2.1.1**: Innate immune response to cuts: In response to a cut, mast cells secrete histamines that cause nearby capillaries to dilate. Neutrophils and monocytes leave the capillaries. Monocytes mature into macrophages. Neutrophils, dendritic cells, and macrophages release chemicals to stimulate the inflammatory response. Neutrophils and macrophages also consume invading bacteria by phagocytosis.

KEY POINTS

- Pathogens are recognized by a variety of immune cells, such as macrophages and dendritic cells, via pathogen-associated molecular patterns (PAMPs) on the pathogen surface, which interact with complementary pattern-recognition receptors (PRRs) on the immune cells' surfaces.
- Upon binding of PRRs with PAMPs (pathogen recognition), immune cells release cytokines to tell other cells to start fighting back.
- One class of cytokines, interferons, warn nearby uninfected cells of impending infection, cause cells to start cleaving RNA and reduce protein synthesis, and signal nearby infected cells to undergo apoptosis.
- Another class of cytokines, called inerleukins, mediate interactions between white blood cells (leukocytes) and help bridge the innate and adaptive immune responses.
- Inflammation (hot, red, swollen, painful tissue associated with infection) is encouraged by cytokines that are produced immediately upon pathogen recognition; the increase in blood

flow associated with inflammation allows more leukocytes (a type of innate immune cell) to reach the infected area.

KEY TERMS

- **macrophage**: a white blood cell that phagocytizes necrotic cell debris and foreign material, including viruses, bacteria, and tattoo ink; part of the innate immune system
- **phagocytosis**: the process where a cell incorporates a particle by extending pseudopodia and drawing the particle into a vacuole of its cytoplasm
- **cytokine**: any of various small regulatory proteins that regulate the cells of the immune system; they are released upon binding of PRRs to PAMPS

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6.2.1.1: Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)

State how long it takes for early induced innate immunity to become activated and what it involves.
- State what is meant by pathogen-associated molecular patterns (PAMPs), and the role PAMPs play in inducing innate immunity.
- Name at least 5 PAMPS associated with bacteria.
- Name at least 2 PAMPS associated with viruses.
- Define DAMPs and give two examples.

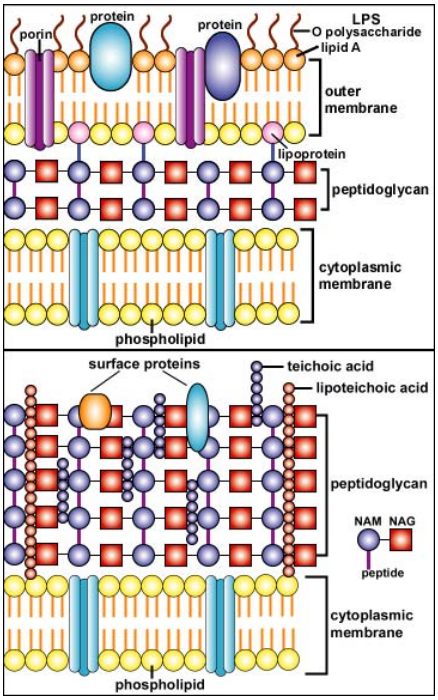


Figure **6.2.1.1.1**: (left) Structure of a Gram-Negative Cell Wall. The Gram-negative cell wall is composed of a thin, inner layer of peptidoglycan and an outer membrane consisting of molecules of phospholipids, lipopolysaccharides (LPS), lipoproteins and surface proteins. The lipopolysaccharide consists of lipid A and O polysaccharide. (right) The Gram-positive cell wall appears as dense layer typically composed of numerous rows of peptidoglycan, and molecules of lipoteichoic acid, wall teichoic acid and surface proteins.

Examples of microbial-associated PAMPs include:

a. lipopolysaccharide (LPS) from the outer membrane of the Gram-negative cell wall (see Figure **6.2.1.1.1**A);
b. bacterial lipoproteins and lipopeptides (see Figure **6.2.1.1.1**A);
c. porins in the outer membrane of the Gram-negative cell wall (see Figure **6.2.1.1.1**A);
d. peptidoglycan found abundantly in the Gram-positive cell wall and to a lesser degree in the gram-negative cell wall (see Figure **6.2.1.1.1**B);
e. lipoteichoic acids found in the Gram-positive cell wall (Figure **6.2.1.1.1**B);
f. lipoarabinomannan and mycolic acids found in acid-fast cell walls (Figure **6.2.1.1.2**B)
g. mannose-rich glycans (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar). These are common in microbial glycoproteins and glycolipids but rare in those of humans (see Figure **6.2.1.1.6**).
h. flagellin found in bacterial flagella;
i. bacterial and viral nucleic acid. Bacterial and viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide or CpG sequences (a cytosine lacking a methyl or CH_3 group and located adjacent to a guanine). Mammalian DNA has a low frequency of CpG sequences and most are methylated which may mask recognition by pattern-recognition receptors . Also, human DNA and RNA does not normally enter cellular endosomes where the pattern-recognition receptors for microbial DNA and RNA are located;
j. *N*-formylmethionine , an amino acid common to bacterial proteins;

k. double-stranded viral RNA unique to many viruses in some stage of their replication;
l. single-stranded viral RNA from many` viruses having an RNA genome;
m. lipoteichoic acids, glycolipids, and zymosan from yeast cell walls; and
n. phosphorylcholine and other lipids common to microbial membranes.

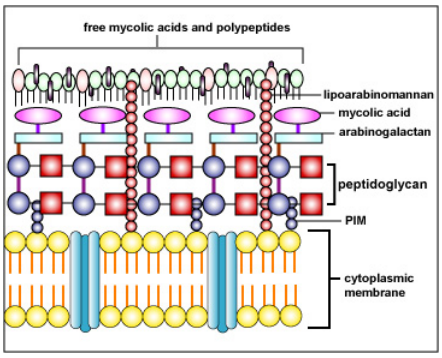


Figure 6.2.1.1.2: Structure of an Acid-Fast Cell Wall. In addition to peptidoglycan, the acid-fast cell wall of Mycobacterium contains a large amount of glycolipids, especially mycolic acids. The peptidoglycan layer is linked to arabinogalactan (D-arabinose and D-galactose) which is then linked to high-molecular weight mycolic acids. The arabinogalactan/mycolic acid layer is overlaid with a layer of polypeptides and mycolic acids consisting of free lipids, glycolipids, and peptidoglycolipids. Other glycolipids include lipoarabinomannan and phosphatidyinositol mannosides (PIM). Because of its unique cell wall, when it is stained by the acid-fast procedure, it will resist decolorization with acid-alcohol and stain red, the color of the initial stain, carbol fuchsin. With the exception of a very few other acid-fast bacteria such as Nocardia, all other bacteria will be decolorized and stain blue, the color of the methylene blue counterstain.

Examples of DAMPs associated with stressed, injured, infected, or transformed host cells and not found on normal cells include:

a. heat-shock proteins;
b. altered membrane phospholipids; and
c. molecules normally located inside phagosomes and lysosomes that enter the cytosol only when these membrane-bound compartments are damaged as a result of infection, including antibodies bound to microbes from opsonization.
d. molecules normally found within cells, such as ATP, DNA, and RNA, that spill out of damaged cells.

To recognize PAMPs such as those listed above, various body cells have a variety of corresponding receptors called pattern-recognition receptors or PRRs capable of binding specifically to conserved portions of these molecules. Cells that typically have pattern recognition receptors include macrophages , dendritic cells , endothelial cells , mucosal epithelial cells, and lymphocytes .

? What are DAMPs and why would it be an advantage for them to initiate an inflammatory response similar to PAMPs?

Summary

1. Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs.
2. Pathogen-associated molecular patterns or PAMPs are molecules shared by groups of related microbes that are essential for the survival of those organisms and are not found associated with mammalian cells. Examples include LPS, porins, peptidoglycan, lipoteichoic acids, mannose-rich glycans, flagellin, bacterial and viral genomes, mycolic acid, and lipoarabinomannan.
3. Danger-associated molecular patterns or DAMPs are unique molecules displayed on stressed, injured, infected, or transformed human cells also be recognized as a part of innate immunity. Examples include heat-shock proteins and altered membrane phospholipids.
4. PAMPs and DAMPs bind to pattern-recognition receptors or PRRs associated with body cells to induce innate immunity.

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6.2.1.2: Pattern-Recognition Receptors (PRRs)

State the function of the following as they relate to innate immunity.

a. pattern recognition receptors (PRRs)
b. endocytic pattern recognition receptors
c. signaling pattern recognition receptors
d. danger-associated molecular patterns
e. danger recognition receptors
f. inflammasome
g. pyroptosis

- Name 2 endocytic PRRs.
- Name 2 signaling PRRs found on host cell surfaces.
- Name 2 signaling PRRs found in the endosomes of phagocytic cells.
- Name 2 signaling PRRs found on the host cell cytoplasm.
- Briefly describe the major difference between the effect of the cytokines produced in response to PAMPs that bind to cell surface signaling PRRs and endosomal PRRs.

In order to recognize PAMPs, various body cells have a variety of corresponding receptors called pattern-recognition receptors or PRRs (see Figure 6.2.1.2.5) capable of binding specifically to conserved portions of these molecules. Cells that typically have pattern recognition receptors include macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, and lymphocytes.

Many pattern-recognition receptors are located on the surface of these cells where they can interact with PAMPs on the surface of microbes. Others PRRs are found within the phagolysosomes of phagocytes where they can interact with PAMPs located within microbes that have been phagocytosed. Some PRRs are found in the cytosol of the cell.

There are two functionally different major classes of pattern-recognition receptors: endocytic pattern-recognition receptors and signaling pattern-recognition receptors.

Endocytic (Phagocytic) Pattern-Recognition Receptors

Endocytic pattern-recognition receptors, also called phagocytic pattern-recognition receptors, are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes leading to their subsequent engulfment and destruction. They include:

1. Mannose receptors

Mannose receptors on the surface of phagocytes bind to various microbial carbohydrates such as those rich in mannose or fucose, and to N-acetylglucosamine (NAG). Human glycoproteins and glycolipids typically have terminal N-acetylglucosamine and sialic acid groups. C-type lectins found on the surface of phagocytes are mannose receptors (see Figure 6.2.1.2.6).

It is now thought that mannose receptors may be quite important in removing potentially harmful mannose-containing glycoproteins such as lysosomal hydrolases that are produced in increased amounts during inflammation.

2. Dectin-1

Dectin-1 recognizes beta-glucans (polymers of glucose) commonly found in fungal cell walls.

3. Scavenger receptors

Scavenger receptors found on the surface of phagocytic cells bind to bacterial cell wall components such as LPS, peptidoglycan and teichoic acids (see Figure 6.2.1.2.7). There are also scavenger receptors for certain components of other types of microorganisms, as well as for stressed, infected, or injured cells. Scavenger receptors include CD-36, CD-68, and SRB-1.

4. Opsonin receptors

Opsonins are soluble molecules produced as a part of the body's immune defenses that bind microbes to phagocytes. One portion of the opsonin binds to a PAMP on the microbial surface and another portion binds to a specific receptor on the phagocytic cell.

- Acute phase proteins circulating in the plasma, such as:
  - mannose-binding lectin (also called mannose-binding protein) that binds to various microbial carbohydrates such as those rich in mannose or fucose, and to N-acetylglucosamine (NAG); and
  - C-reactive protein (CRP) that binds to phosphorylcholine portion of teichoic acids and lipopolysaccharides of bacterial and fungal cell walls. It also binds to the phosphocholine found on the surface of damaged or dead human cells.
- Complement pathway proteins, such as C3b (see Figure 6.2.1.2.8) and C4b recognize a variety of PAMPS.
- Surfactant proteins in the alveoli of the lungs, such as SP-A and SP-D are opsonins.
- During adaptive immunity, the antibody molecule IgG can function as an opsonin (see Figure 6.2.1.2.16).

Flash animation illustrating the function of endocytic pattern-recognition receptors on phagocytes.

html5 version of animation for iPad illustrating the function of endocytic pattern-recognition receptors on phagocytes.

5. *N*-formyl Met receptors

N-formyl methionine is the first amino acid produced in bacterial proteins since the f-met-tRNA in bacteria has an anticodon complementary to the AUG start codon (see Figure 6.2.1.2.17). This form of the amino acid is not typically seen in mammalian proteins. FPR and FPRL1 are *N*-formyl receptors on neutrophils and macrophages. Binding of *N*-formyl Met to its receptor promotes the motility and the chemotaxis of these phagocytes. It also promotes phagocytosis.

Signaling Pattern-Recognition Receptors

Signaling pattern-recognition receptors bind a number of microbial molecules: LPS, peptidoglycan, teichoic acids, flagellin, pilin, unmethylated cytosine-guanine dinucleotide or CpG sequences from bacterial and viral genomes; lipoteichoic acid, glycolipids, and zymosan from fungi; double-stranded viral RNA, and certain single-stranded viral RNAs. Binding of microbial PAMPs to signaling PRRs promotes the production of:

- inflammatory cytokines, such as such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-12 (IL-12);
- antiviral cytokines called type-1 interferons (IFN), such as IFN-alpha and IFN-beta;
- chemotactic factors, such as the chemokines interleukin-8 (IL-8), MCP-1, and RANTES; and
- antimicrobial peptides, such as human defensins *)* and cathelicidins.

These molecules are crucial to initiating innate immunity and adaptive immunity.

1. Signaling PRRs found on cell surfaces (see Figure 6.2.1.2.5):

A series of signaling pattern-recognition receptors known as toll-like receptors (TLRs) are found on the surface of a variety of defense cells and other cells. These TLRs play a major role in the induction of innate immunity and contribute to the induction of adaptive immunity.

Different combinations of TLRs appear in different cell types and may occur in pairs. Different TLRs directly or indirectly bind different microbial molecules. For example:

a. TLR-2 - recognizes peptidoglycan, bacterial lipoproteins, lipoteichoic acid (Gram-positive bacteria), and porins (gram-negative bacteria).

b. TLR-4 - recognizes lipopolysaccharide (Gram-negative bacteria), fungal mannans, viral envelope proteins, parasitic phospholipids, heat-shock proteins.
c. TLR-5 - recognizes bacterial flagellin;
d. TLR-1/TLR-2 pairs - binds to bacterial lipopeptides, lipomannans (mycobacteria) lipoteichoic acids (Gram-positive bacteria), cell wall beta gucans (bacteria and fungi), zymosan (fungi) and glycosylphosphatidylinositol (GPI)-anchored proteins (protozoa).
e. TLR-2/TL6 pairs - also binds to bacterial lipopeptides, lipomannans (mycobacteria) lipoteichoic acids (Gram-positive bacteria), cell wall beta gucans (bacteria and fungi), zymosan (fungi) and glycosylphosphatidylinositol (GPI)-anchored proteins (protozoa).

Many of the TLRs, especially those that bind to bacterial and fungal cell wall components, stimulate the transcription and translation of inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-12 (IL-12), as well as chemokines such as interleukin-8 (IL-8), MCP-1, and RANTES. These cytokines trigger innate immune defenses such as inflammation, fever, and phagocytosis in order to provide an immediate response against the invading microorganism (see Figure 6.2.1.2.9). Because cytokines such as IL-I, TNF-alpha, and IL-12 that trigger an inflammatory response, they are often referred to as inflammatory cytokines. Chemokines are a group of cytokines that enable the migration of leukocytes from the blood to the tissues at the site of inflammation. To counter inflammation, anti-inflammatory cytokines such as IL-1 receptor antagonist, IL-4, and IL-10 are produced.

Another cell surface PRR is CD14. CD14 is found on monocytes, macrophages, and neutrophils and promotes the ability of TLR-4 to respond to LPS. LPS typically binds to LPS-binding protein in the plasma and tissue fluid. The LPS-binding protein promotes the binding of LPS to the CD14 receptors. At that point the LPS-binding protein comes off and the LPS-CD14 bind to TLR-4. Interaction of LPS and CD14 with TLR-4 leads to an elevated synthesis and secretion of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-alpha, and platelet-activating factor (PAF). These cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway (see Figure 6.2.1.2.9).

The signaling process for the CD14 and TLR-4 response to LPS is shown in Figure 6.2.1.2.15.

Flash animation illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.

html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.

Flash animation illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.

html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.

For More Information: Inflammation from Unit 5

TLRs also participate in adaptive immunity by triggering various secondary signals needed for humoral immunity (the production of antibodies) and cell-mediated immunity (the production of cytotoxic T-lymphocytes, activated macrophages, and additional cytokines). Without innate immune responses there could be no adaptive immunity.

a. T-independent (TI) antigens allow B-lymphocytes to mount an antibody response without the requirement of interaction with effector T4-lymphocytes. The resulting antibody molecules are generally of the IgM isotype and do

not give rise to a memory response. There are two basic types of T-independent antigens: TI-1 and TI-2. TI-1 antigens are pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) from the outer membrane of the gram-negative cell wall and lipoteichoic acids from the gram-positive cell wall. These antigens activate B-lymphocytes by binding to their specific toll-like receptors rather than to B-cell receptors (see Figure 6.2.1.2.11). Antibody molecules generated against TI-1 antigens are often called "natural antibodies" because they are always being made against bacteria present in the body.

b. The activation of naive T-lymphocytes requires co-stimulatory signals involving the interaction of accessory molecules on antigen-presenting cells or APCs with their corresponding ligands on T-lymphocytes. These co-stimulatory molecules are only synthesized when toll-like receptors on APCs bind to pathogen-associated molecular patterns of microbes (see Figure 6.2.1.2.12).

2. Signaling PRRs found in the membranes of the endosomes (phagolysosomes) used to degrade pathogens (see Figure 6.2.1.2.5):

a. TLR-3 - binds double-stranded viral RNA;
b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
c. TLR-8 - binds single-stranded viral RNA;
d. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

Most of the TLRs that bind to viral components trigger the synthesis of cytokines called interferons that block viral replication within infected host cells as well as inflammatory cytokines.

Flash animation showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.

html5 version of animation for iPad showing showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.

GIF animation showing the antiviral nature of interferon.

3. Signaling PRRs and DRRs found in the cytoplasm (see Figure 6.2.1.2.5)

Pattern-recognition receptors or PRRs found in the cytoplasm include:

a. NODs (nucleotide-binding oligomerization domain)

NOD proteins, including NOD-1 and NOD-2, are cytostolic proteins that allow intracellular recognition of peptidoglycan components.

1. NOD-1 recognizes peptidoglycan containing the muramyl dipeptide NAG-NAM-gamma-D-glutamyl-meso diaminopimelic acid, part of the peptidoglycan monomer in common gram-negative bacteria and just a few gram-positive bacteria.

2. NOD-2 recognizes peptidoglycan containing the muramyl dipeptide NAG-NAM-L-alanyl-isoglutamine found in practically all bacteria (see Figure 6.2.1.2.5).

As macrophages phagocytose either whole bacteria or peptidoglycan fragments released during bacterial growth, the peptidoglycan is broken down into muramyl dipeptides. Binding of the muramyl dipetides to NOD-1 or NOD-2 leads to the activation of genes coding for inflammatory cytokines such as IL-1, TNF-alpha, IL-8, and IL-12 in a manner similar to the cell surface TLRs. Activation of NOD-2 also induces the production of antimicrobial peptides such as defensins as well as microbicidal reactive oxygen species (ROS).

b. CARD-containing proteins

CARD (caspase activating and recruitment domain)-containing proteins, such as RIG-1 (retinoic acid-inducible gene-1) and MDA-5 (melanoma differentiation-associated gene-5), are cytoplasmic sensors of viral RNA molecules that trigger the synthesis of type-1 interferons, antiviral cytokines that block viral replication within infected host cells in a manner similar to the endosomal TLRs. RIG-1 recognizes 5'-PPPs on viral RNAs. The 5'-PPPs on host cell RNAs are either capped or removed and are not recognized by RIG-1. Rig-1 and MDA-5 can also, through another regulatory pathway, stimulate the production of inflammatory cytokines.

Detection of PAMPs by PRRs in the cytosol trigger the formation of multi-protein complexes called inflammasomes which, in turn, leads to the activation of caspase-1. Caspase-1 triggers the formation of inflammatory cytokines and can also result in an inflammatory response-induced cell suicide called pyroptosis. Pyroptosis, unlike apoptosis, leads to the release of PAMPS as well as inflammatory cytokines from the lysed cell.

Pyroptosis is initiated by PAMPs binding to pattern-recognition receptors (PRRs) on various defense cells which then triggers the production of inflammatory cytokines and type-1 interferons. Other PRRs, called nod-like receptors (NLRs) located in the cytosol of these defense cells recognize PAMPs and DAMPs that have entered the host cell's cytosol. Some NLRs trigger the production of inflammatory cytokines while others activate caspase 1-dependent pyroptosis of the cell causing the release of its intracellular inflammatory cytokines (see Figure 6.2.1.2.1). The binding of PAMPs or DAMPs to their respective NLRs triggers the assembly of multiprotein complexes called inflammasomes in the cytosol of the host cell. It is these inflammasomes that activate caspase 1 and induce inflammation and pyroptosis. Pyroptosis results in production of proinflammatory cytokines, rupture of the cell's plasma membrane, and subsequent release of proinflammatory intracellular contents. It plays an essential role in innate immunity by promoting inflammation to control microbial infections. At highly elevated levels, however, it can cause considerable harm to the body and even death.

c. Danger recognition receptors or DRRs

Danger recognition receptors or DRRs found in the cytoplasm recognize danger-associated molecular patterns (DAMPS) in the cytosol such as altered membrane phospholipids, and materials released from damaged phagosomes and damaged lysosomes, including antibodies bound to microbes from opsonization. DAMPs are also produced as a result of tissue injury during cancer, heart attack, and stroke. Detection of DAMPs by DRRs in the cytosol also triggers the activation of inflammasomes, release of inflammatory cytokines, and pyroptosis.

4. Secreted signaling PRRs found in plasma and tissue fluid

In addition to the PRRs found on or within cells, there are also secreted pattern-recognition receptors. These PRRs bind to microbial cell walls and enable them to activate the complement pathways, as well as by phagocytes. For example, mannan-binding lectin -also known as mannan-binding protein - is synthesized by the liver and released into the bloodstream as part of the acute phase response discussed later in Unit 4. Here it can bind to the carbohydrates on bacteria, yeast, some viruses, and some parasites (see Figure 6.2.1.2.6). This, in turn, activates the lectin complement pathway (discussed later in Unit 4) and results in the production of a variety of activated complement proteins that are able to trigger inflammation, chemotactically attract phagocytes to the infection site, promote the attachment of antigens to phagocytes via enhanced attachment or opsonization, and cause lysis of gram-negative bacteria and infected or transformed human cells.

Other secreted PRRs include C-reactive protein (CRP), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), and ficolins.

Flash animation showing activation of the lectin pathway, formation of C3 convertase, and formation of C5 convertase.

html5 version of animation for iPad showing activation of the lectin pathway, formation of C3 convertase, and formation of C5 convertase.

Compare and contrast the functions of endocytic pattern-recognition receptors and signaling pattern-recognition receptors.

- Compare and contrast signaling pattern-recognition receptors found on cell surfaces with those found in the membranes of endosomes (phagolysosomes).

Concept Map for PRRs and DRRs

Summary

1. Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs and danger-associated molecular patterns or DAMPs binding to danger-recognition receptors or DRRs.
2. Endocytic pattern-recognition receptors are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes leading to their subsequent engulfment and destruction. They include mannose receptors, scavenger receptors, and opsonin receptors.
3. Binding of microbial PAMPs to signaling PRRs promotes the production of inflammatory cytokines, antiviral cytokines called type-1 interferons (IFN), chemotactic factors, and antimicrobial peptides. They include toll-like receptors (TLRs) and NODs.
4. PRRs found on the surface of the body's cells typically bind to surface PAMPs on microbes and stimulate the production of inflammatory cytokines.
5. PRRs found within cellular phagolysosomes (endosomes) typically detect nucleic acid PAMPs released during the phagocytic destruction of viruses and stimulate the production of antiviral cytokines called type-1 interferons.
6. PRRs and DRRs found within the cytoplasm of host cells typically trigger the formation of multi-protein complexes called inflammasomes which, in turn, triggers the formation of inflammatory cytokines and can also leads to an inflammatory response-induced cell suicide called pyroptosis.
7. PRRs circulating in the blood and tissue fluid activate the complement pathways and may function as opsonins.

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6.2.2: The Complement System

Learning Objectives

- Describe the function of the complement system

The Complement System

The serum complement system, which represents a chief component of innate immunity, not only participates in inflammation but also acts to enhance the adaptive immune response. Specific activation of the complement via innate recognition proteins or secreted antibody releases cleavage products that interact with a wide range of cell surface receptors found on myeloid, lymphoid, and stromal cells. This intricate interaction among complement activation products and cell surface receptors provides a basis for the regulation of both B and T cell responses.

The complement system plays a crucial role in the innate defense against common pathogens. Activation of the complement leads to robust and efficient proteolytic cascades, which terminate in opsonization and lysis of the pathogen as well as in the generation of the classical inflammatory response through the production of potent proinflammatory molecules. More recently, however, the role of the complement in the immune response has been expanded due to observations that link complement activation to adaptive immune responses. It is now understood that the complement is a functional bridge between innate and adaptive immune responses that allows an integrated host defense to pathogenic challenges.

Activation of the Complement System

The complement system can be activated through three major pathways: classical, lectin, and alternative. Initiation of the classical pathway occurs when C1q, in complex with C1r and C1s serine proteases (the C1 complex), binds to the Fc region of complement-fixing antibodies (generally $IgG_1$and IgM) attached to pathogenic surfaces. Autocatalytic activation of C1r and C1s in turn cleaves C4 and C2 into larger (C4b, C2a) and smaller (C4a, C2b) fragments. The larger fragments associate to form C4bC2a on pathogenic surfaces, and the complex gains the ability to cleave C3 and is termed the C3 convertase.

Generation of the C3 convertase, which cleaves C3 into the anaphylatoxin C3a and the opsonin C3b, is the point at which all complement activation cascades converge. When C3 is cleaved into C3b, it exposes an internal thioester bond that allows stable covalent binding of C3b to hydroxyl groups on proximate carbohydrates and proteins. This activity underpins the entire complement system by effectively “tagging” microorganisms as foreign, leading to further complement activation on and around the opsonized surface and terminating in the production of anaphylatoxins and assembly of membrane attack complexes.

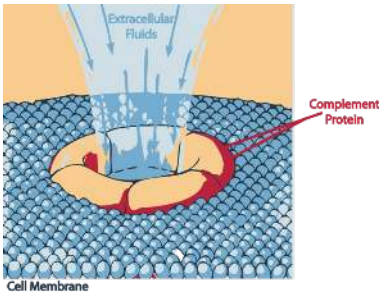


Figure: **Complement death**: A complement protein attacking an invader.

Functions of the Complement System

The functions of the complement system, oposonization, lysis, and generation of the inflammatory response through soluble mediators, are paradigmatic and represent a well-characterized component of an innate host defense. It has become increasingly understood that complement functions in host defense extend beyond innate immune responses. The finding that B lymphocytes bound C3 raised the question as early as in the 1970s as to whether the complement system was involved in adaptive immune responses. Subsequent work demonstrated that depletion of C3 impaired humoral immune responses and provided direct evidence that efficient adaptive responses were contingent on an intact complement system in some cases.

Further study in animals bearing natural complement deficiencies implicated the classical pathway as a crucial mechanism for efficient antigen trapping and retention in lymphoid tissues (e.g., splenic follicles), suggesting that a major function of the complement system was to localize foreign antigens into immune sites important for lymphocytes responses.

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Key Points

- The complement system has originally been identified as the part of the immune system called the innate immune system.
- The complement system can also be recruited and brought into action by the adaptive immune system.
- The three biochemical pathways that activate the complement system are the classical complement pathway, the alternative complement pathway, and the lectin pathway.
- The complement system consists of small proteins found in the blood, generally synthesized by the liver, and normally circulating as inactive precursors. When stimulated by a trigger, proteases in the system cleave specific proteins to release cytokines that amplify further cleavages.
- The end-result of this activation cascade is the massive amplification of the response and activation of the cell-killing membrane attack complex.

Key Terms

- **opsonization**: the process of an antigen bound by antibody or complement to attract phagocytic cells.

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6.2.3: Interferons

Learning Objectives

- Identify interferons and their effects

Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites, or tumor cells. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to "interfere" with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages, they increase recognition of infection or tumor cells by up-regulating antigen presentation to T lymphocytes, and they increase the ability of uninfected host cells to resist new infection by virus. Certain symptoms, such as aching muscles and fever, are related to the production of IFNs during infection.

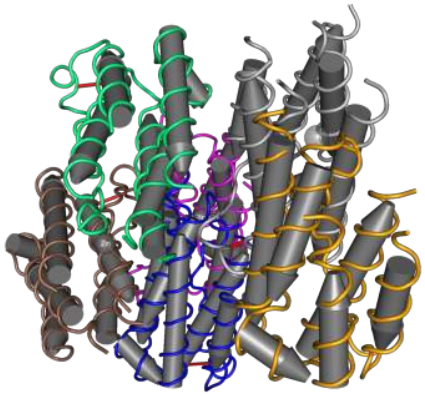


Figure: ***Interferon****: The molecular structure of human interferon-alpha.*

About ten distinct IFNs have been identified in mammals; seven of these have been described for humans. They are typically divided among three IFN classes: type I IFN, type II IFN, and type III IFN. IFNs belonging to all IFN classes are very important for fighting viral infections.

Based on the type of receptor through which they signal, human interferons have been classified into three major types:

- Interferon type I: All type I IFNs bind to a specific cell surface receptor complex, known as the IFN-α receptor (IFNAR) that consists of IFNAR1 and IFNAR2 chains. The type I interferons present in humans are IFN-α, IFN-β and IFN-ω.
- Interferon type II: These bind to IFNGR that consist of IFNGR1 and IFNGR2 chains. In humans this is IFN-γ.
- Interferon type III: These signal through a receptor complex consisting of IL10R2 (also called CRF2-4) and IFNLR1 (also called CRF2-12). Acceptance of this classification is less universal than that of type I and type II, and unlike the other two, it is not currently included in *Medical Subject Headings*.

Effects of Interferons

All interferons share several common effects; they are antiviral agents and can fight tumors. As an infected cell dies from a cytolytic virus, viral particles are released that can infect nearby cells. In addition, interferons induce production of hundreds of other proteins—known collectively as interferon-stimulated genes (ISGs)—that have roles in combating viruses. They also limit viral spread by increasing p53 activity, which kills virus-infected cells by promoting apoptosis. The effect of IFN on p53 is also linked to its protective role against certain cancers. Another function of interferons is to upregulate major histocompatibility complex molecules, MHC I and MHC II, and increase immunoproteasome activity. Interferons, such as interferon gamma, directly activate other immune cells, such as macrophages and natural killer cells. Interferons can inflame the tongue and cause dysfunction in taste bud cells, restructuring or killing taste buds entirely.

Key Points

- Interferons are named after their ability to "interfere" with viral replication within host cells.
- IFNs are divided into three classes: type I IFN, type II IFN, and type III IFNs.

- IFNs activate immune cells (natural killer cells and macrophages), increase recognition of infection and tumor cells by up-regulating antigen presentation to T lymphocytes, and increase the ability of uninfected host cells to resist new infection by virus.

Key Terms

- **Interferons**: Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites or tumor cells. They allow for communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors.
- **pathogens**: A pathogen or infectious agent (colloquially known as a germ) is a microorganism (in the widest sense, such as a virus, bacterium, prion, or fungus) that causes disease in its host. The host may be an animal (including humans), a plant, or even another microorganism.
- **immune cells**: White blood cells, or leukocytes, are cells of the immune system involved in defending the body against both infectious disease and foreign materials.

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6.2.4: NATURAL KILLER CELLS

LEARNING OBJECTIVES

- Describe the role of natural killer cells in the immune response

Lymphocytes are leukocytes (white blood cells) that are histologically identifiable by their large, darkly-staining nuclei; they are small cells with very little cytoplasm. After a pathogen enters the body, infected cells are identified and destroyed by natural killer (NK) cells, which are a type of lymphocyte that can kill cells infected with viruses or tumor cells (abnormal cells that uncontrollably divide and invade other tissue). While NK cells are part of the innate immune response, they are best understood relative to their counterparts in the adaptive immune response,T cells, which are also classified as lymphocytes.

T cells are lymphocytes that mature in the thymus gland and identify intracellular infections, especially from viruses, by the altered expression of major histocompatibility class (MHC) I molecules on the surface of infected cells. MHC I molecules are proteins on the surfaces of all nucleated cells which help the immune system distinguish between “self” and “non-self.” If the cell is infected, the MHC I molecules display fragments of proteins from the infectious agents to T-cells. Healthy cells do not display any proteins and will be ignored by the immune system, while the cells identified as “non-self” by foreign proteins will be attacked by the immune system.

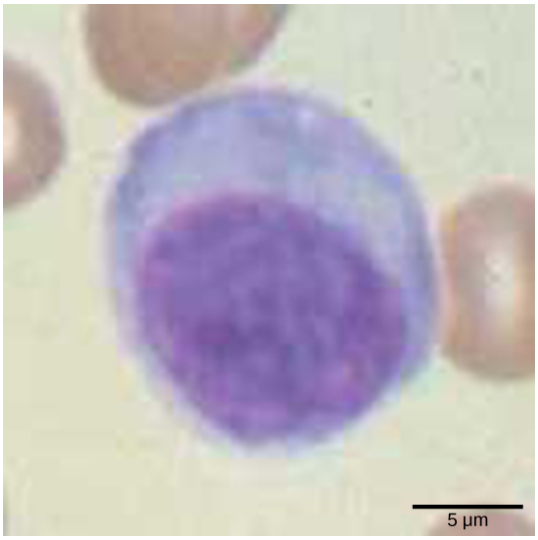


Figure 6.2.4.1: Lymphocytes: Lymphocytes, such as NK cells, are characterized by their large nuclei that actively absorb Wright stain and, therefore, appear dark colored under a microscope.

An infected cell (or a tumor cell) is often incapable of synthesizing and displaying MHC I molecules appropriately. The metabolic resources of cells infected by some viruses produce proteins that interfere with MHC I processing and/or trafficking to the cell surface. The reduced MHC I on host cells varies from virus to virus and results from active inhibitors being produced by the viruses. This process can deplete host MHC I molecules on the cell surface, which prevents T-cells from recognizing them, but which NK cells detect as “unhealthy” or “abnormal” while searching for cellular MHC I molecules. As such, NK cells offer a complementary check for unhealthy cells, relative to T cells. Similarly, the dramatically-altered gene expression of tumor cells leads to expression of extremely- deformed or absent MHC I molecules that also signal “unhealthy” or “abnormal.”

NK cells are always active; an interaction with normal, intact MHC I molecules on a healthy cell disables the killing sequence, causing the NK cell to move on. After the NK cell detects an infected or tumor cell, its cytoplasm secretes granules comprised of perforin: a destructive protein that creates a pore in the target cell. Granzymes are released along with the perforin in the immunological synapse. A granzyme, a protease that digests cellular proteins, induces the target cell to undergo programmed cell death, or apoptosis. Phagocytic cells then digest the cell debris left behind. NK cells are constantly patrolling the body. They are an effective mechanism for controlling potential infections and preventing cancer progression.

KEY POINTS

- Natural killer (NK) cells are lymphocytes (a subclass of white blood cells) that recognize infected or tumorogenic cells and kill them.
- Unlike the related T cells, NK cells do not recognize fragments of the infecting particle, but rather the incorrect display of major histocompatibility complex (MHC) I molecules.
- NK cells are always active, but will not perform their killing function on cells with intact MHC I molecules.
- When NK cells detect an infected or tumor cell, they secrete granules that contain perforin, creating a pore in the target cell; granzymes then pass through these pores, degrading cellular proteins, causing cells to undergo apoptosis.

KEY TERMS

- **lymphocyte**: a type of white blood cell or leukocyte that is divided into two principal groups and a null group: B-cells, T-cells, and natural killer (NK) cells
- **major histocompatibility complex**: a protein present on the extracellular surface of the cell that displays portions of the proteins that are degraded inside the cell
- **T cell**: a lymphocyte, from the thymus, that can recognize specific antigens and can activate or deactivate other immune cells

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6.2.5: Dendritic Cells

Learning Objectives

- Discuss the mechanism of action for dendritic cells

Dendritic cells are present in lymphoid organs, the epithelia of the skin, the gastrointestinal and respiratory tracts, and in most parenchymal organs. These cells are identified morphologically by their membranous projections that resemble spines. All dendritic cells are thought to arise from bone marrow precursors. Most, called myeloid dendritic cells, are related in lineage to mononuclear phagocytes. Immature dendritic cells (e.g. Langerhans cells of the epidermis) are located in main portals of entry of microbes (skin and gut epithelia). The function of epithelial dendritic cells is to capture microbial protein antigens and to transport the antigens to draining lymph nodes. During their migration to the lymph nodes, the dendritic cells mature to become extremely efficient at presenting antigens and stimulating naive T cells, hence their classification as antigen presenting cells. Mature dendritic cells reside in the T cell zones of the lymph nodes, and in this location they display antigens to T cells. Subsets of dendritic cells can be distinguished by the expression of cell surface markers. Different subpopulations of dendritic cells may stimulate distinct types of T cell effector responses. Some may even inhibit T cell activation.

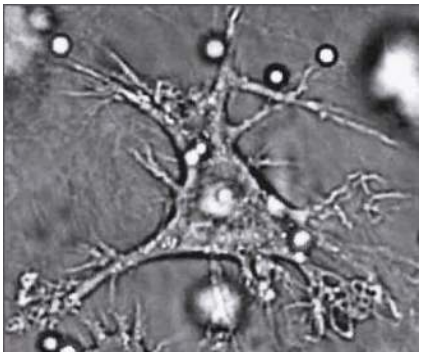


Figure: **Dendritic cell**: Dendritic cell characterized by membranous projections that resemble spines.

Dendritic cells are constantly in communication with other cells in the body. This communication can take the form of direct cell-to-cell contact based on the interaction of cell-surface proteins. An example of this includes the interaction of the membrane proteins of the B7 family of the dendritic cell with a CD28 cell surface molecule present on the lymphocyte. However, the cell-cell interaction can also take place at a distance via soluble factors such as cytokines. For example, stimulating dendritic cells *in vivo* with microbial extracts causes the dendritic cells to rapidly begin producing interleukin 12 (IL-12). IL-12 is a signal that helps differentiate naive CD4 T cells into a helper T cell phenotype. The ultimate consequence is priming and activation of the immune system for attack against the antigens which the dendritic cell presents on its surface.

Key Points

- Dendritic cells function as antigen presenting cells.
- Dendritic cells are present in small quantities in tissues that are in contact with the external environment, mainly the skin (where there is a specialized dendritic cell type called Langerhans cells) and the inner lining of the nose, lungs, stomach and intestines.
- Once activated, dendritic cells migrate to the lymphoid tissues where they interact with T cells and B cells to initiate and shape the adaptive immune response.

Key Terms

- **lymphoid organs**: lymph nodes, spleen, and gut-associated lymphoid tissue where lymphocytes reside.

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6.2.6: Inflammation and Fever

Learning Objectives

- Identify the signs of inflammation and fever and explain why they occur
- Explain the advantages and risks posed by inflammatory responses

The inflammatory response, or inflammation, is triggered by a cascade of chemical mediators and cellular responses that may occur when cells are damaged and stressed or when pathogens successfully breach the physical barriers of the innate immune system. Although inflammation is typically associated with negative consequences of injury or disease, it is a necessary process insofar as it allows for recruitment of the cellular defenses needed to eliminate pathogens, remove damaged and dead cells, and initiate repair mechanisms. Excessive inflammation, however, can result in local tissue damage and, in severe cases, may even become deadly.

Acute Inflammation

An early, if not immediate, response to tissue injury is acute inflammation. Immediately following an injury, vasoconstriction of blood vessels will occur to minimize blood loss. The amount of vasoconstriction is related to the amount of vascular injury, but it is usually brief. Vasoconstriction is followed by vasodilation and increased vascular permeability, as a direct result of the release of histamine from resident mast cells. Increased blood flow and vascular permeability can dilute toxins and bacterial products at the site of injury or infection. They also contribute to the five observable signs associated with the inflammatory response: erythema (redness), edema (swelling), heat, pain, and altered function. Vasodilation and increased vascular permeability are also associated with an influx of phagocytes at the site of injury and/or infection. This can enhance the inflammatory response because phagocytes may release proinflammatory chemicals when they are activated by cellular distress signals released from damaged cells, by PAMPs, or by opsonins on the surface of pathogens. Activation of the complement system can further enhance the inflammatory response through the production of the anaphylatoxin C5a. Figure **6.2.6.1** illustrates a typical case of acute inflammation at the site of a skin wound.

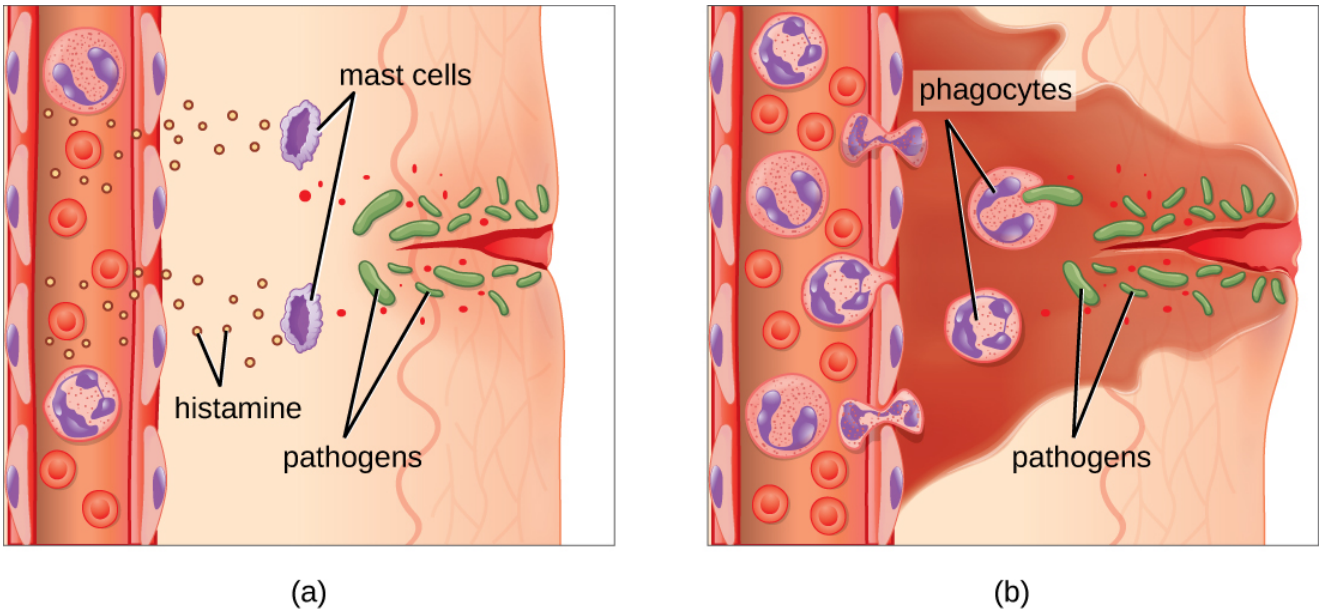


Figure **6.2.6.1**: (a) Mast cells detect injury to nearby cells and release histamine, initiating an inflammatory response. (b) Histamine increases blood flow to the wound site, and increased vascular permeability allows fluid, proteins, phagocytes, and other immune cells to enter infected tissue. These events result in the swelling and reddening of the injured site, and the increased blood flow to the injured site causes it to feel warm. Inflammation is also associated with pain due to these events stimulating nerve pain receptors in the tissue. The interaction of phagocyte PRRs with cellular distress signals and PAMPs and opsonins on the surface of pathogens leads to the release of more proinflammatory chemicals, enhancing the inflammatory response.

During the period of inflammation, the release of bradykinin causes capillaries to remain dilated, flooding tissues with fluids and leading to edema. Increasing numbers of neutrophils are recruited to the area to fight pathogens. As the fight rages on, pus forms

from the accumulation of neutrophils, dead cells, tissue fluids, and lymph. Typically, after a few days, macrophages will help to clear out this pus. Eventually, tissue repair can begin in the wounded area.

Chronic Inflammation

When acute inflammation is unable to clear an infectious pathogen, chronic inflammation may occur. This often results in an ongoing (and sometimes futile) lower-level battle between the host organism and the pathogen. The wounded area may heal at a superficial level, but pathogens may still be present in deeper tissues, stimulating ongoing inflammation. Additionally, chronic inflammation may be involved in the progression of degenerative neurological diseases such as Alzheimer's and Parkinson's, heart disease, and metastatic cancer.

Chronic inflammation may lead to the formation of granulomas, pockets of infected tissue walled off and surrounded by WBCs. Macrophages and other phagocytes wage an unsuccessful battle to eliminate the pathogens and dead cellular materials within a granuloma. One example of a disease that produces chronic inflammation is tuberculosis, which results in the formation of granulomas in lung tissues. A tubercular granuloma is called a tubercle (Figure **6.2.6.2**). Tuberculosis will be covered in more detail in Bacterial Infections of the Respiratory Tract.

Chronic inflammation is not just associated with bacterial infections. Chronic inflammation can be an important cause of tissue damage from viral infections. The extensive scarring observed with hepatitis C infections and liver cirrhosis is the result of chronic inflammation.

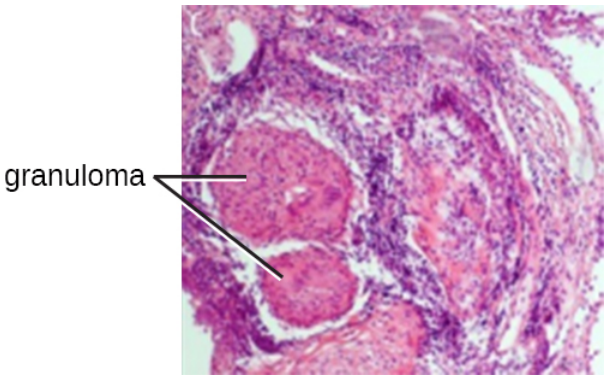


Figure **6.2.6.2**: A tubercle is a granuloma in the lung tissue of a patient with tuberculosis. In this micrograph, white blood cells (stained purple) have walled off a pocket of tissue infected with *Mycobacterium tuberculosis*. Granulomas also occur in many other forms of disease. (credit: modification of work by Piotrowski WJ, Górski P, Duda-Szymańska J, Kwiatkowska S)

? Exercise 6.2.6.1

1. Name the five signs of inflammation.
2. Is a granuloma an acute or chronic form of inflammation? Explain

Fever

A fever is an inflammatory response that extends beyond the site of infection and affects the entire body, resulting in an overall increase in body temperature. Body temperature is normally regulated and maintained by the hypothalamus, an anatomical section of the brain that functions to maintain homeostasis in the body. However, certain bacterial or viral infections can result in the production of pyrogens, chemicals that effectively alter the "thermostat setting" of the hypothalamus to elevate body temperature and cause fever. Pyrogens may be exogenous or endogenous. For example, the endotoxin lipopolysaccharide (LPS), produced by gram-negative bacteria, is an exogenous pyrogen that may induce the leukocytes to release endogenous pyrogens such as interleukin-1 (IL-1), IL-6, interferon-γ (IFN-γ), and tumor necrosis factor (TNF). In a cascading effect, these molecules can then lead to the release of prostaglandin E2 (PGE_2) from other cells, resetting the hypothalamus to initiate fever (Figure **6.2.6.4**).



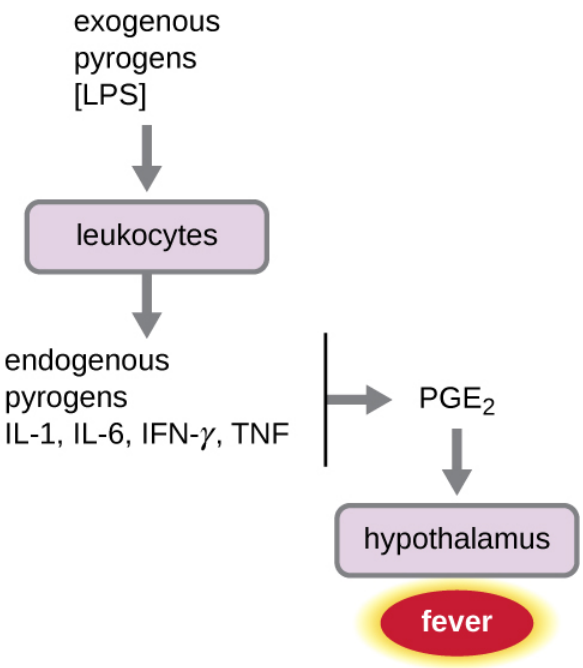


Figure 6.2.6.4: The role of the hypothalamus in the inflammatory response. Macrophages recognize pathogens in an area and release cytokines that trigger inflammation. The cytokines also send a signal up the vagus nerve to the hypothalamus.

Like other forms of inflammation, a fever enhances the innate immune defenses by stimulating leukocytes to kill pathogens. The rise in body temperature also may inhibit the growth of many pathogens since human pathogens are mesophiles with optimum growth occurring around 35 °C (95 °F). In addition, some studies suggest that fever may also stimulate release of iron-sequestering compounds from the liver, thereby starving out microbes that rely on iron for growth.[2]

During fever, the skin may appear pale due to vasoconstriction of the blood vessels in the skin, which is mediated by the hypothalamus to divert blood flow away from extremities, minimizing the loss of heat and raising the core temperature. The hypothalamus will also stimulate shivering of muscles, another effective mechanism of generating heat and raising the core temperature.

The crisis phase occurs when the fever breaks. The hypothalamus stimulates vasodilation, resulting in a return of blood flow to the skin and a subsequent release of heat from the body. The hypothalamus also stimulates sweating, which cools the skin as the sweat evaporates.

Although a low-level fever may help an individual overcome an illness, in some instances, this immune response can be too strong, causing tissue and organ damage and, in severe cases, even death. The inflammatory response to bacterial superantigens is one scenario in which a life-threatening fever may develop. Superantigens are bacterial or viral proteins that can cause an excessive activation of T cells from the specific adaptive immune defense, as well as an excessive release of cytokines that overstimulates the inflammatory response. For example, *Staphylococcus aureus* and *Streptococcus pyogenes* are capable of producing superantigens that cause toxic shock syndrome and scarlet fever, respectively. Both of these conditions can be associated with very high, life-threatening fevers in excess of 42 °C (108 °F).

? Exercise 6.2.6.2

1. Explain the difference between exogenous and endogenous pyrogens.
2. How does a fever inhibit pathogens?

Key Concepts and Summary

- **Inflammation** results from the collective response of chemical mediators and cellular defenses to an injury or infection.
- **Acute inflammation** is short lived and localized to the site of injury or infection. **Chronic inflammation** occurs when the inflammatory response is unsuccessful, and may result in the formation of **granulomas** (e.g., with tuberculosis) and scarring

(e.g., with hepatitis C viral infections and liver cirrhosis).

- The five cardinal signs of inflammation are **erythema**, **edema**, heat, pain, and altered function. These largely result from innate responses that draw increased blood flow to the injured or infected tissue.
- **Fever** is a system-wide sign of inflammation that raises the body temperature and stimulates the immune response.
- Both inflammation and fever can be harmful if the inflammatory response is too severe.

Footnotes

1. 1 Centers for Disease Control and Prevention. “Parasites–Lymphatic Filiariasis.” 2016. http://www.cdc.gov/parasites/lymphat...info/faqs.html.
2. 2 N. Parrow et al. “Sequestration and Scavenging of Iron in Infection.” *Infection and Immunity* 81 no. 10 (2013):3503–3514

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6.3: Adaptive Immunity

Skills to Develop

- Explain adaptive immunity
- Compare and contrast adaptive and innate immunity
- Describe cell-mediated immune response and humoral immune response
- Describe immune tolerance

The adaptive, or acquired, immune response takes days or even weeks to become established—much longer than the innate response; however, adaptive immunity is more specific to pathogens and has memory. Adaptive immunity is an immunity that occurs after exposure to an antigen either from a pathogen or a vaccination. This part of the immune system is activated when the innate immune response is insufficient to control an infection. In fact, without information from the innate immune system, the adaptive response could not be mobilized. There are two types of adaptive responses: the cell-mediated immune response, which is carried out by T cells, and the humoral immune response, which is controlled by activated B cells and antibodies. Activated T cells and B cells that are specific to molecular structures on the pathogen proliferate and attack the invading pathogen. Their attack can kill pathogens directly or secrete antibodies that enhance the phagocytosis of pathogens and disrupt the infection. Adaptive immunity also involves a memory to provide the host with long-term protection from reinfection with the same type of pathogen; on re-exposure, this memory will facilitate an efficient and quick response.

Antigen-presenting Cells

Unlike NK cells of the innate immune system, B cells (B lymphocytes) are a type of white blood cell that gives rise to antibodies, whereas T cells (T lymphocytes) are a type of white blood cell that plays an important role in the immune response. T cells are a key component in the cell-mediated response—the specific immune response that utilizes T cells to neutralize cells that have been infected with viruses and certain bacteria. There are three types of T cells: cytotoxic, helper, and suppressor T cells. Cytotoxic T cells destroy virus-infected cells in the cell-mediated immune response, and helper T cells play a part in activating both the antibody and the cell-mediated immune responses. Suppressor T cells deactivate T cells and B cells when needed, and thus prevent the immune response from becoming too intense.

An antigen is a foreign or “non-self” macromolecule that reacts with cells of the immune system. Not all antigens will provoke a response. For instance, individuals produce innumerable “self” antigens and are constantly exposed to harmless foreign antigens, such as food proteins, pollen, or dust components. The suppression of immune responses to harmless macromolecules is highly regulated and typically prevents processes that could be damaging to the host, known as tolerance.

The innate immune system contains cells that detect potentially harmful antigens, and then inform the adaptive immune response about the presence of these antigens. An antigen-presenting cell (APC) is an immune cell that detects, engulfs, and informs the adaptive immune response about an infection. When a pathogen is detected, these APCs will phagocytose the pathogen and digest it to form many different fragments of the antigen. Antigen fragments will then be transported to the surface of the APC, where they will serve as an indicator to other immune cells. Dendritic cells are immune cells that process antigen material; they are present in the skin (Langerhans cells) and the lining of the nose, lungs, stomach, and intestines. Sometimes a dendritic cell presents on the surface of other cells to induce an immune response, thus functioning as an antigen-presenting cell. Macrophages also function as APCs. Before activation and differentiation, B cells can also function as APCs.

After phagocytosis by APCs, the phagocytic vesicle fuses with an intracellular lysosome forming phagolysosome. Within the phagolysosome, the components are broken down into fragments; the fragments are then loaded onto MHC class I or MHC class II molecules and are transported to the cell surface for antigen presentation, as illustrated in Figure **6.3.1**. Note that T lymphocytes cannot properly respond to the antigen unless it is processed and embedded in an MHC II molecule. APCs express MHC on their surfaces, and when combined with a foreign antigen, these complexes signal a “non-self” invader. Once the fragment of antigen is embedded in the MHC II molecule, the immune cell can respond. Helper T- cells are one of the main lymphocytes that respond to antigen-presenting cells. Recall that all other nucleated cells of the body expressed MHC I molecules, which signal “healthy” or “normal.”

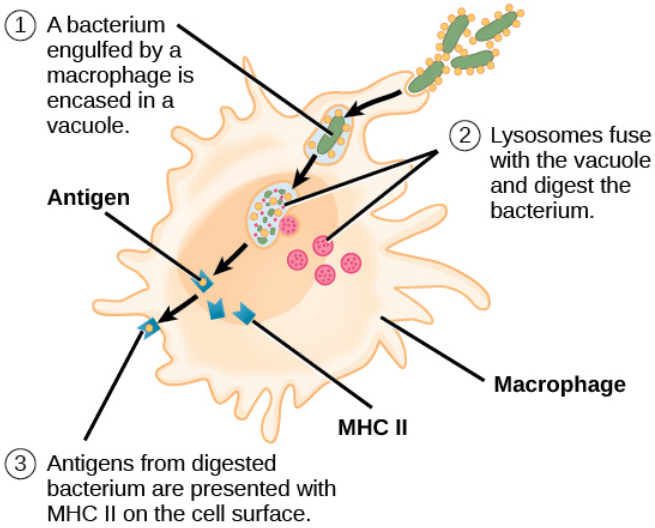


Figure **6.3.1**: An APC, such as a macrophage, engulfs and digests a foreign bacterium. An antigen from the bacterium is presented on the cell surface in conjunction with an MHC II molecule Lymphocytes of the adaptive immune response interact with antigen-embedded MHC II molecules to mature into functional immune cells.

T and B Lymphocytes

Lymphocytes in human circulating blood are approximately 80 to 90 percent T cells, shown in Figure **6.3.2**, and 10 to 20 percent B cells. Recall that the T cells are involved in the cell-mediated immune response, whereas B cells are part of the humoral immune response.

T cells encompass a heterogeneous population of cells with extremely diverse functions. Some T cells respond to APCs of the innate immune system, and indirectly induce immune responses by releasing cytokines. Other T cells stimulate B cells to prepare their own response. Another population of T cells detects APC signals and directly kills the infected cells. Other T cells are involved in suppressing inappropriate immune reactions to harmless or “self” antigens.

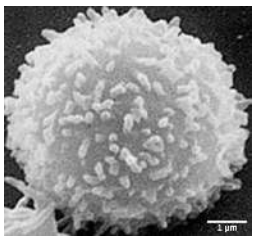


Figure **6.3.2**: This scanning electron micrograph shows a T lymphocyte, which is responsible for the cell-mediated immune response. T cells are able to recognize antigens. (credit: modification of work by NCI; scale-bar data from Matt Russell)

T and B cells exhibit a common theme of recognition/binding of specific antigens via a complementary receptor, followed by activation and self-amplification/maturation to specifically bind to the particular antigen of the infecting pathogen. T and B lymphocytes are also similar in that each cell only expresses one type of antigen receptor. Any individual may possess a population of T and B cells that together express a near limitless variety of antigen receptors that are capable of recognizing virtually any infecting pathogen. T and B cells are activated when they recognize small components of antigens, called epitopes, presented by APCs, illustrated in Figure **6.3.3**. Note that recognition occurs at a specific epitope rather than on the entire antigen; for this reason, epitopes are known as “antigenic determinants.” In the absence of information from APCs, T and B cells remain inactive, or naïve, and are unable to prepare an immune response. The requirement for information from the APCs of innate immunity to trigger B cell or T cell activation illustrates the essential nature of the innate immune response to the functioning of the entire immune system.

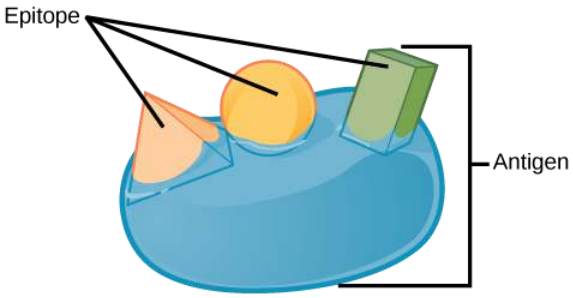


Figure **6.3.3**: An antigen is a macromolecule that reacts with components of the immune system. A given antigen may contain several motifs that are recognized by immune cells. Each motif is an epitope. In this figure, the entire structure is an antigen, and the orange, salmon and green components projecting from it represent potential epitopes.

Naïve T cells can express one of two different molecules, CD4 or CD8, on their surface, as shown in Figure **6.3.4**, and are accordingly classified as $CD4^+$ or $CD8^+$ cells. These molecules are important because they regulate how a T cell will interact with and respond to an APC. Naïve $CD4^+$ cells bind APCs via their antigen-embedded MHC II molecules and are stimulated to become helper T (T_H) lymphocytes, cells that go on to stimulate B cells (or cytotoxic T cells) directly or secrete cytokines to inform more and various target cells about the pathogenic threat. In contrast, $CD8^+$ cells engage antigen-embedded MHC I molecules on APCs and are stimulated to become cytotoxic T lymphocytes (CTLs), which directly kill infected cells by apoptosis and emit cytokines to amplify the immune response. The two populations of T cells have different mechanisms of immune protection, but both bind MHC molecules via their antigen receptors called T cell receptors (TCRs). The CD4 or CD8 surface molecules differentiate whether the TCR will engage an MHC II or an MHC I molecule. Because they assist in binding specificity, the CD4 and CD8 molecules are described as coreceptors.

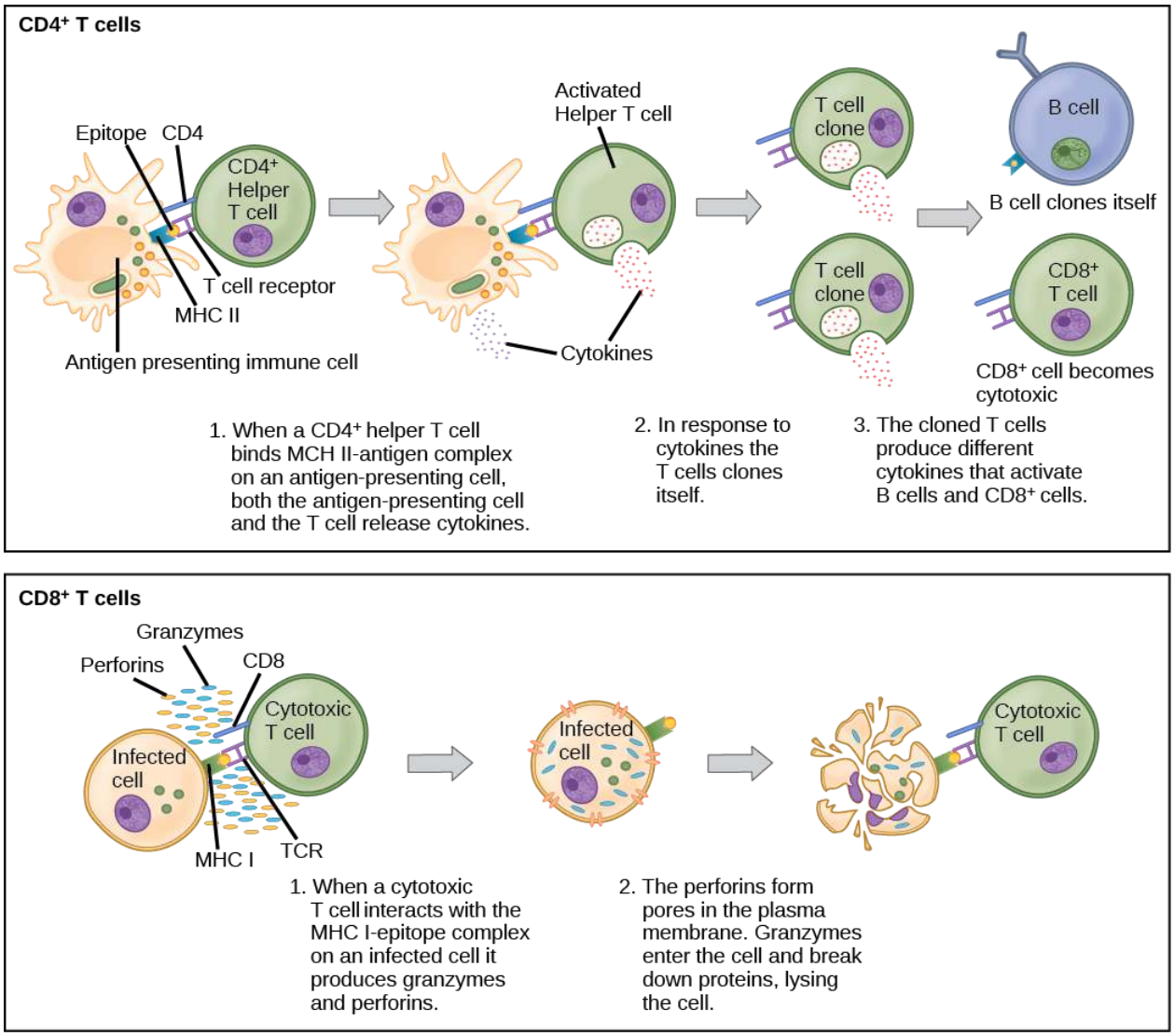


Figure **6.3.4**: Naïve $CD4^+$ T cells engage MHC II molecules on antigen-presenting cells (APCs) and become activated. Clones of the activated helper T cell, in turn, activate B cells and $CD8^+$ T cells, which become cytotoxic T cells. Cytotoxic T cells kill infected cells.

Exercise 6.3.1

Which of the following statements about T cells is false?

a. Helper T cells release cytokines while cytotoxic T cells kill the infected cell.
b. Helper T cells are $CD4^+$, while cytotoxic T cells are $CD8^+$.
c. MHC II is a receptor found on most body cells, while MHC I is a receptor found on immune cells only.
d. The T cell receptor is found on both $CD4^+$ and $CD8^+$ T cells.

Answer

C

Consider the innumerable possible antigens that an individual will be exposed to during a lifetime. The mammalian adaptive immune system is adept in responding appropriately to each antigen. Mammals have an enormous diversity of T cell populations, resulting from the diversity of TCRs. Each TCR consists of two polypeptide chains that span the T cell membrane, as illustrated in Figure **6.3.5**; the chains are linked by a disulfide bridge. Each polypeptide chain is comprised of a constant domain and a variable domain: a domain, in this sense, is a specific region of a protein that may be regulatory or structural. The intracellular domain is

involved in intracellular signaling. A single T cell will express thousands of identical copies of one specific TCR variant on its cell surface. The specificity of the adaptive immune system occurs because it synthesizes millions of different T cell populations, each expressing a TCR that differs in its variable domain. This TCR diversity is achieved by the mutation and recombination of genes that encode these receptors in stem cell precursors of T cells. The binding between an antigen-displaying MHC molecule and a complementary TCR "match" indicates that the adaptive immune system needs to activate and produce that specific T cell because its structure is appropriate to recognize and destroy the invading pathogen.

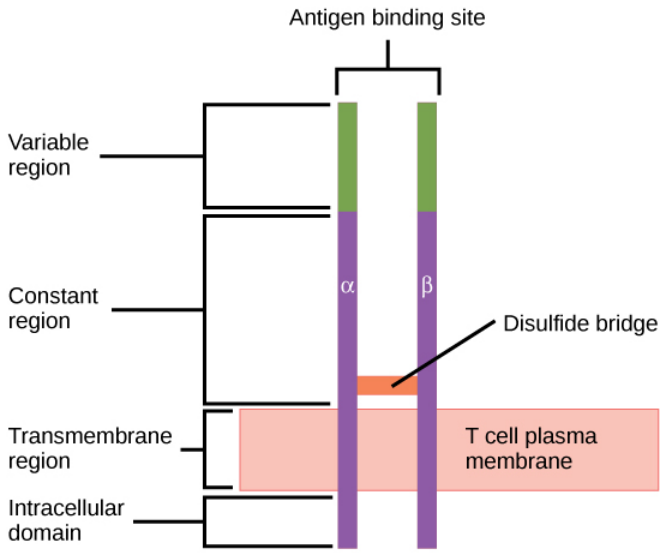


Figure **6.3.5**: A T cell receptor spans the membrane and projects variable binding regions into the extracellular space to bind processed antigens via MHC molecules on APCs.

Helper T Lymphocytes

The T_H lymphocytes function indirectly to identify potential pathogens for other cells of the immune system. These cells are important for extracellular infections, such as those caused by certain bacteria, helminths, and protozoa. T_H lymphocytes recognize specific antigens displayed in the MHC II complexes of APCs. There are two major populations of T_H cells: T_H1 and T_H2. T_H1 cells secrete cytokines to enhance the activities of macrophages and other T cells. T_H1 cells activate the action of cyotoxic T cells, as well as macrophages. T_H2 cells stimulate naïve B cells to destroy foreign invaders via antibody secretion. Whether a T_H1 or a T_H2 immune response develops depends on the specific types of cytokines secreted by cells of the innate immune system, which in turn depends on the nature of the invading pathogen.

The T_H1-mediated response involves macrophages and is associated with inflammation. Recall the frontline defenses of macrophages involved in the innate immune response. Some intracellular bacteria, such as *Mycobacterium tuberculosis*, have evolved to multiply in macrophages after they have been engulfed. These pathogens evade attempts by macrophages to destroy and digest the pathogen. When *M. tuberculosis* infection occurs, macrophages can stimulate naïve T cells to become T_H1 cells. These stimulated T cells secrete specific cytokines that send feedback to the macrophage to stimulate its digestive capabilities and allow it to destroy the colonizing *M. tuberculosis*. In the same manner, T_H1-activated macrophages also become better suited to ingest and kill tumor cells. In summary; T_H1 responses are directed toward intracellular invaders while T_H2 responses are aimed at those that are extracellular.

B Lymphocytes

When stimulated by the T_H2 pathway, naïve B cells differentiate into antibody-secreting plasma cells. A plasma cell is an immune cell that secrets antibodies; these cells arise from B cells that were stimulated by antigens. Similar to T cells, naïve B cells initially are coated in thousands of B cell receptors (BCRs), which are membrane-bound forms of Ig (immunoglobulin, or an antibody). The B cell receptor has two heavy chains and two light chains connected by disulfide linkages. Each chain has a constant and a variable region; the latter is involved in antigen binding. Two other membrane proteins, Ig alpha and Ig beta, are involved in signaling. The receptors of any particular B cell, as shown in Figure **6.3.6** are all the same, but the hundreds of millions of different B cells in an individual have distinct recognition domains that contribute to extensive diversity in the types of molecular structures to which they

can bind. In this state, B cells function as APCs. They bind and engulf foreign antigens via their BCRs and then display processed antigens in the context of MHC II molecules to T_H2 cells. When a T_H2 cell detects that a B cell is bound to a relevant antigen, it secretes specific cytokines that induce the B cell to proliferate rapidly, which makes thousands of identical (clonal) copies of it, and then it synthesizes and secretes antibodies with the same antigen recognition pattern as the BCRs. The activation of B cells corresponding to one specific BCR variant and the dramatic proliferation of that variant is known as clonal selection. This phenomenon drastically, but briefly, changes the proportions of BCR variants expressed by the immune system, and shifts the balance toward BCRs specific to the infecting pathogen.

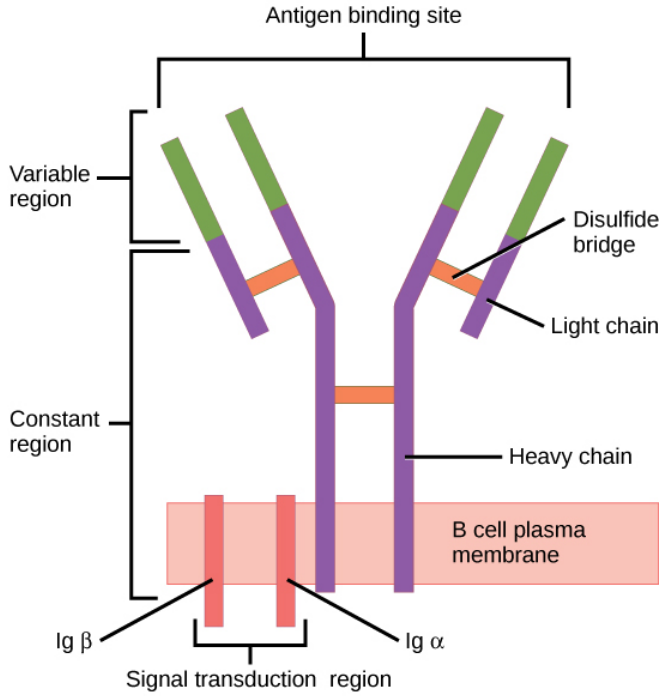


Figure **6.3.6**: B cell receptors are embedded in the membranes of B cells and bind a variety of antigens through their variable regions. The signal transduction region transfers the signal into the cell.

T and B cells differ in one fundamental way: whereas T cells bind antigens that have been digested and embedded in MHC molecules by APCs, B cells function as APCs that bind intact antigens that have not been processed. Although T and B cells both react with molecules that are termed “antigens,” these lymphocytes actually respond to very different types of molecules. B cells must be able to bind intact antigens because they secrete antibodies that must recognize the pathogen directly, rather than digested remnants of the pathogen. Bacterial carbohydrate and lipid molecules can activate B cells independently from the T cells.

Cytotoxic T Lymphocytes

CTLs, a subclass of T cells, function to clear infections directly. The cell-mediated part of the adaptive immune system consists of CTLs that attack and destroy infected cells. CTLs are particularly important in protecting against viral infections; this is because viruses replicate within cells where they are shielded from extracellular contact with circulating antibodies. When APCs phagocytize pathogens and present MHC I-embedded antigens to naïve $CD8^+$ T cells that express complementary TCRs, the $CD8^+$ T cells become activated to proliferate according to clonal selection. These resulting CTLs then identify non-APCs displaying the same MHC I-embedded antigens (for example, viral proteins)—for example, the CTLs identify infected host cells.

Intracellularly, infected cells typically die after the infecting pathogen replicates to a sufficient concentration and lyses the cell, as many viruses do. CTLs attempt to identify and destroy infected cells before the pathogen can replicate and escape, thereby halting the progression of intracellular infections. CTLs also support NK lymphocytes to destroy early cancers. Cytokines secreted by the T_H1 response that stimulates macrophages also stimulate CTLs and enhance their ability to identify and destroy infected cells and tumors.

CTLs sense MHC I-embedded antigens by directly interacting with infected cells via their TCRs. Binding of TCRs with antigens activates CTLs to release perforin and granzyme, degradative enzymes that will induce apoptosis of the infected cell. Recall that

this is a similar destruction mechanism to that used by NK cells. In this process, the CTL does not become infected and is not harmed by the secretion of perforin and granzymes. In fact, the functions of NK cells and CTLs are complementary and maximize the removal of infected cells, as illustrated in Figure **6.3.7**. If the NK cell cannot identify the "missing self" pattern of down-regulated MHC I molecules, then the CTL can identify it by the complex of MHC I with foreign antigens, which signals "altered self." Similarly, if the CTL cannot detect antigen-embedded MHC I because the receptors are depleted from the cell surface, NK cells will destroy the cell instead. CTLs also emit cytokines, such as interferons, that alter surface protein expression in other infected cells, such that the infected cells can be easily identified and destroyed. Moreover, these interferons can also prevent virally infected cells from releasing virus particles.

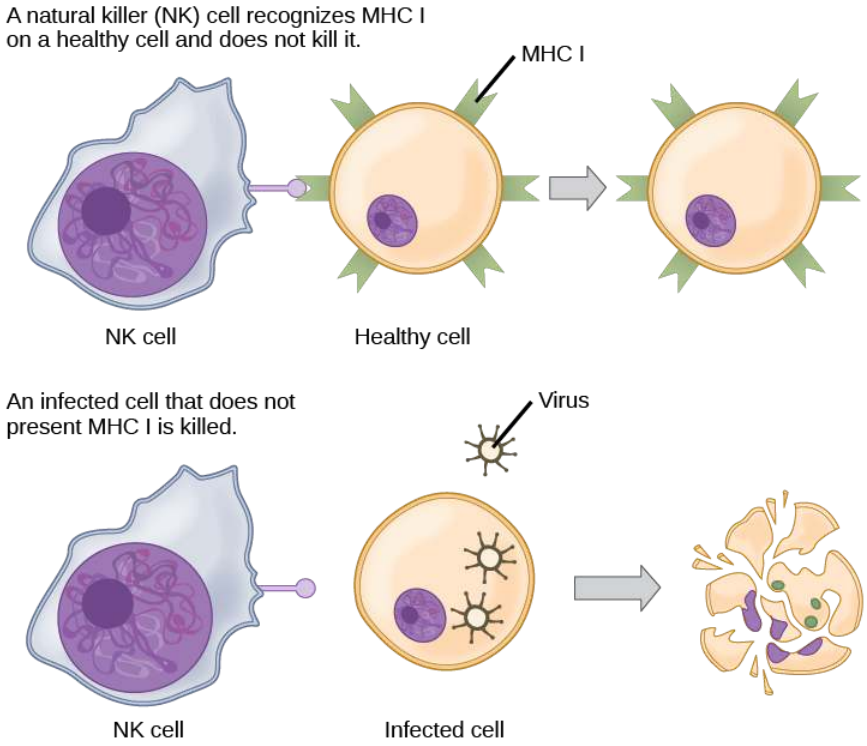


Figure **6.3.7**: Natural killer (NK) cells recognize the MHC I receptor on healthy cells. If MHC I is absent, the cell is lysed.

Exercise 6.3.2

Based on what you know about MHC receptors, why do you think an organ transplanted from an incompatible donor to a recipient will be rejected?

Answer

MHC receptors differ from person to person. Thus, MHC receptors on an incompatible donor are considered "non-self" and are rejected by the immune system.

Plasma cells and CTLs are collectively called effector cells: they represent differentiated versions of their naïve counterparts, and they are involved in bringing about the immune defense of killing pathogens and infected host cells.

Mucosal Surfaces and Immune Tolerance

The innate and adaptive immune responses discussed thus far comprise the systemic immune system (affecting the whole body), which is distinct from the mucosal immune system. Mucosal immunity is formed by mucosa-associated lymphoid tissue, which functions independently of the systemic immune system, and which has its own innate and adaptive components. Mucosa-associated lymphoid tissue (MALT), illustrated in Figure **6.3.8**, is a collection of lymphatic tissue that combines with epithelial tissue lining the mucosa throughout the body. This tissue functions as the immune barrier and response in areas of the body with direct contact to the external environment. The systemic and mucosal immune systems use many of the same cell types. Foreign

particles that make their way to MALT are taken up by absorptive epithelial cells called M cells and delivered to APCs located directly below the mucosal tissue. M cells function in the transport described, and are located in the Peyer's patch, a lymphoid nodule. APCs of the mucosal immune system are primarily dendritic cells, with B cells and macrophages having minor roles. Processed antigens displayed on APCs are detected by T cells in the MALT and at various mucosal induction sites, such as the tonsils, adenoids, appendix, or the mesenteric lymph nodes of the intestine. Activated T cells then migrate through the lymphatic system and into the circulatory system to mucosal sites of infection.

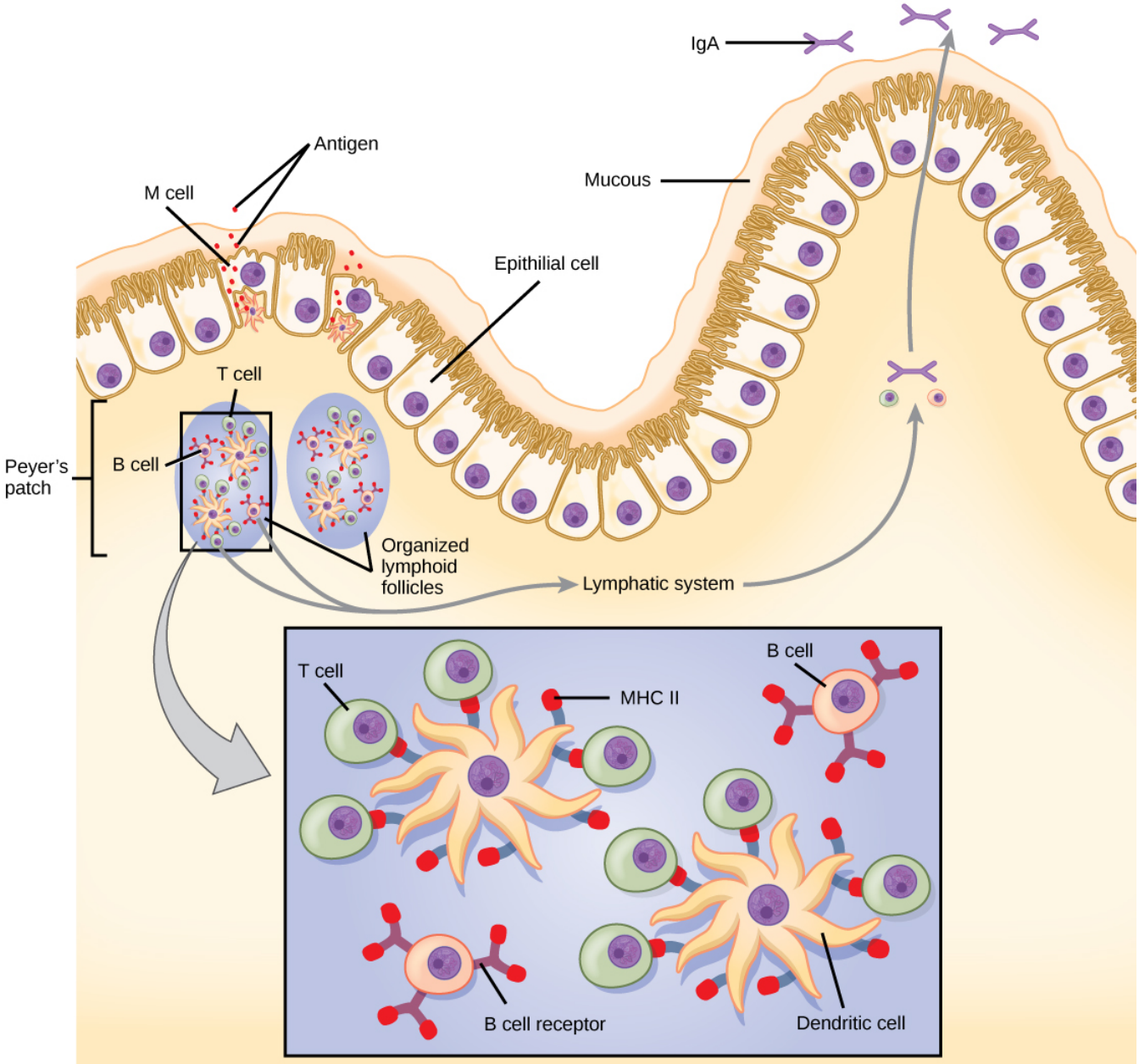


Figure 6.3.8: The topology and function of intestinal MALT is shown. Pathogens are taken up by M cells in the intestinal epithelium and excreted into a pocket formed by the inner surface of the cell. The pocket contains antigen-presenting cells such as dendritic cells, which engulf the antigens, then present them with MHC II molecules on the cell surface. The dendritic cells migrate to an underlying tissue called a Peyer's patch. Antigen-presenting cells, T cells, and B cells aggregate within the Peyer's patch, forming organized lymphoid follicles. There, some T cells and B cells are activated. Other antigen-loaded dendritic cells migrate through the lymphatic system where they activate B cells, T cells, and plasma cells in the lymph nodes. The activated cells then return to MALT tissue effector sites. IgA and other antibodies are secreted into the intestinal lumen.

MALT is a crucial component of a functional immune system because mucosal surfaces, such as the nasal passages, are the first tissues onto which inhaled or ingested pathogens are deposited. The mucosal tissue includes the mouth, pharynx, and esophagus,

and the gastrointestinal, respiratory, and urogenital tracts.

The immune system has to be regulated to prevent wasteful, unnecessary responses to harmless substances, and more importantly so that it does not attack “self.” The acquired ability to prevent an unnecessary or harmful immune response to a detected foreign substance known not to cause disease is described as immune tolerance. Immune tolerance is crucial for maintaining mucosal homeostasis given the tremendous number of foreign substances (such as food proteins) that APCs of the oral cavity, pharynx, and gastrointestinal mucosa encounter. Immune tolerance is brought about by specialized APCs in the liver, lymph nodes, small intestine, and lung that present harmless antigens to an exceptionally diverse population of regulatory T (T_{reg}) cells, specialized lymphocytes that suppress local inflammation and inhibit the secretion of stimulatory immune factors. The combined result of T_{reg} cells is to prevent immunologic activation and inflammation in undesired tissue compartments and to allow the immune system to focus on pathogens instead. In addition to promoting immune tolerance of harmless antigens, other subsets of T_{reg} cells are involved in the prevention of the autoimmune response, which is an inappropriate immune response to host cells or self-antigens. Another T_{reg} class suppresses immune responses to harmful pathogens after the infection has cleared to minimize host cell damage induced by inflammation and cell lysis.

Immunological Memory

The adaptive immune system possesses a memory component that allows for an efficient and dramatic response upon reinvasion of the same pathogen. Memory is handled by the adaptive immune system with little reliance on cues from the innate response. During the adaptive immune response to a pathogen that has not been encountered before, called a primary response, plasma cells secreting antibodies and differentiated T cells increase, then plateau over time. As B and T cells mature into effector cells, a subset of the naïve populations differentiates into B and T memory cells with the same antigen specificities, as illustrated in Figure **6.3.9**.

A memory cell is an antigen-specific B or T lymphocyte that does not differentiate into effector cells during the primary immune response, but that can immediately become effector cells upon re-exposure to the same pathogen. During the primary immune response, memory cells do not respond to antigens and do not contribute to host defenses. As the infection is cleared and pathogenic stimuli subside, the effectors are no longer needed, and they undergo apoptosis. In contrast, the memory cells persist in the circulation.

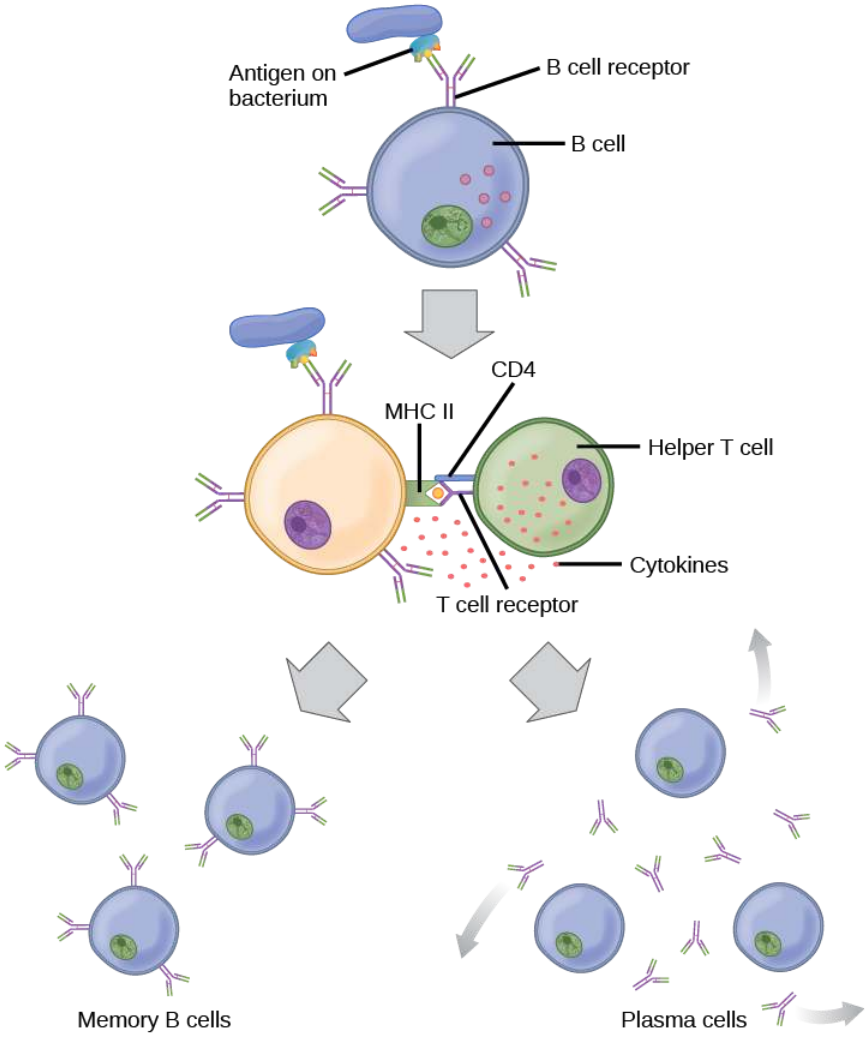


Figure 6.3.9: After initially binding an antigen to the B cell receptor (BCR), a B cell internalizes the antigen and presents it on MHC II. A helper T cell recognizes the MHC II–antigen complex and activates the B cell. As a result, memory B cells and plasma cells are made.

Exercise 6.3.3

The Rh antigen is found on Rh-positive red blood cells. An Rh-negative female can usually carry an Rh-positive fetus to term without difficulty. However, if she has a second Rh-positive fetus, her body may launch an immune attack that causes hemolytic disease of the newborn. Why do you think hemolytic disease is only a problem during the second or subsequent pregnancies?

Answer

If the blood of the mother and fetus mixes, memory cells that recognize the Rh antigen can form late in the first pregnancy. During subsequent pregnancies, these memory cells launch an immune attack on the fetal blood cells. Injection of anti-Rh antibody during the first pregnancy prevents the immune response from occurring.

If the pathogen is never encountered again during the individual's lifetime, B and T memory cells will circulate for a few years or even several decades and will gradually die off, having never functioned as effector cells. However, if the host is re-exposed to the same pathogen type, circulating memory cells will immediately differentiate into plasma cells and CTLs without input from APCs or T_H cells. One reason the adaptive immune response is delayed is because it takes time for naïve B and T cells with the appropriate antigen specificities to be identified and activated. Upon reinfection, this step is skipped, and the result is a more rapid production of immune defenses. Memory B cells that differentiate into plasma cells output tens to hundreds-fold greater antibody

amounts than were secreted during the primary response, as the graph in Figure **6.3.10** illustrates. This rapid and dramatic antibody response may stop the infection before it can even become established, and the individual may not realize they had been exposed.

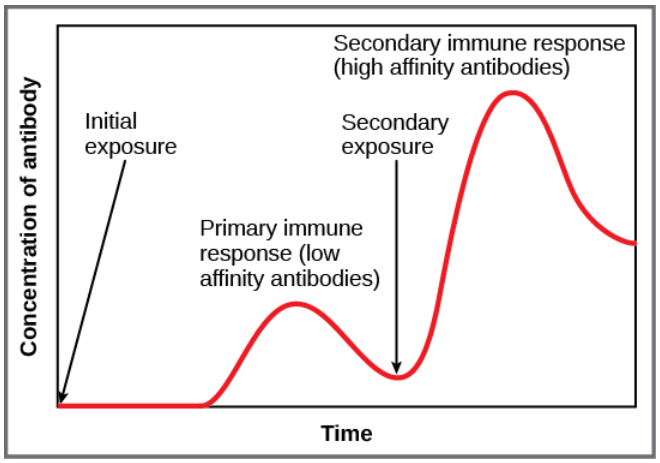


Figure **6.3.10**: In the primary response to infection, antibodies are secreted first from plasma cells. Upon re-exposure to the same pathogen, memory cells differentiate into antibody-secreting plasma cells that output a greater amount of antibody for a longer period of time.

Vaccination is based on the knowledge that exposure to noninfectious antigens, derived from known pathogens, generates a mild primary immune response. The immune response to vaccination may not be perceived by the host as illness but still confers immune memory. When exposed to the corresponding pathogen to which an individual was vaccinated, the reaction is similar to a secondary exposure. Because each reinfection generates more memory cells and increased resistance to the pathogen, and because some memory cells die, certain vaccine courses involve one or more booster vaccinations to mimic repeat exposures: for instance, tetanus boosters are necessary every ten years because the memory cells only live that long.

Mucosal Immune Memory

A subset of T and B cells of the mucosal immune system differentiates into memory cells just as in the systemic immune system. Upon reinvasion of the same pathogen type, a pronounced immune response occurs at the mucosal site where the original pathogen deposited, but a collective defense is also organized within interconnected or adjacent mucosal tissue. For instance, the immune memory of an infection in the oral cavity would also elicit a response in the pharynx if the oral cavity was exposed to the same pathogen.

Career Connection: Vaccinologist

Vaccination (or immunization) involves the delivery, usually by injection as shown in Figure **6.3.11**, of noninfectious antigen(s) derived from known pathogens. Other components, called adjuvants, are delivered in parallel to help stimulate the immune response. Immunological memory is the reason vaccines work. Ideally, the effect of vaccination is to elicit immunological memory, and thus resistance to specific pathogens without the individual having to experience an infection.



Figure **6.3.11**: Vaccines are often delivered by injection into the arm. (credit: U.S. Navy Photographer's Mate Airman Apprentice Christopher D. Blachly)

Vaccinologists are involved in the process of vaccine development from the initial idea to the availability of the completed vaccine. This process can take decades, can cost millions of dollars, and can involve many obstacles along the way. For instance, injected vaccines stimulate the systemic immune system, eliciting humoral and cell-mediated immunity, but have little effect on the mucosal response, which presents a challenge because many pathogens are deposited and replicate in mucosal compartments, and the injection does not provide the most efficient immune memory for these disease agents. For this reason, vaccinologists are actively involved in developing new vaccines that are applied via intranasal, aerosol, oral, or transcutaneous (absorbed through the skin) delivery methods. Importantly, mucosal-administered vaccines elicit both mucosal and systemic immunity and produce the same level of disease resistance as injected vaccines.



Figure **6.3.12**: The polio vaccine can be administered orally. (credit: modification of work by UNICEF Sverige)

Currently, a version of intranasal influenza vaccine is available, and the polio and typhoid vaccines can be administered orally, as shown in Figure **6.3.12**. Similarly, the measles and rubella vaccines are being adapted to aerosol delivery using inhalation devices. Eventually, transgenic plants may be engineered to produce vaccine antigens that can be eaten to confer disease resistance. Other vaccines may be adapted to rectal or vaginal application to elicit immune responses in rectal, genitourinary, or reproductive mucosa. Finally, vaccine antigens may be adapted to transdermal application in which the skin is lightly scraped and microneedles are used to pierce the outermost layer. In addition to mobilizing the mucosal immune response, this new generation of vaccines may end the anxiety associated with injections and, in turn, improve patient participation.

Primary Centers of the Immune System

Although the immune system is characterized by circulating cells throughout the body, the regulation, maturation, and intercommunication of immune factors occur at specific sites. The blood circulates immune cells, proteins, and other factors through the body. Approximately 0.1 percent of all cells in the blood are leukocytes, which encompass monocytes (the precursor of macrophages) and lymphocytes. The majority of cells in the blood are erythrocytes (red blood cells). Lymph is a watery fluid that bathes tissues and organs with protective white blood cells and does not contain erythrocytes. Cells of the immune system can travel between the distinct lymphatic and blood circulatory systems, which are separated by interstitial space, by a process called extravasation (passing through to surrounding tissue).

The cells of the immune system originate from hematopoietic stem cells in the bone marrow. Cytokines stimulate these stem cells to differentiate into immune cells. B cell maturation occurs in the bone marrow, whereas naïve T cells transit from the bone marrow to the thymus for maturation. In the thymus, immature T cells that express TCRs complementary to self-antigens are destroyed. This process helps prevent autoimmune responses.

On maturation, T and B lymphocytes circulate to various destinations. Lymph nodes scattered throughout the body, as illustrated in Figure **6.3.13**, house large populations of T and B cells, dendritic cells, and macrophages. Lymph gathers antigens as it drains from tissues. These antigens then are filtered through lymph nodes before the lymph is returned to circulation. APCs in the lymph nodes capture and process antigens and inform nearby lymphocytes about potential pathogens.

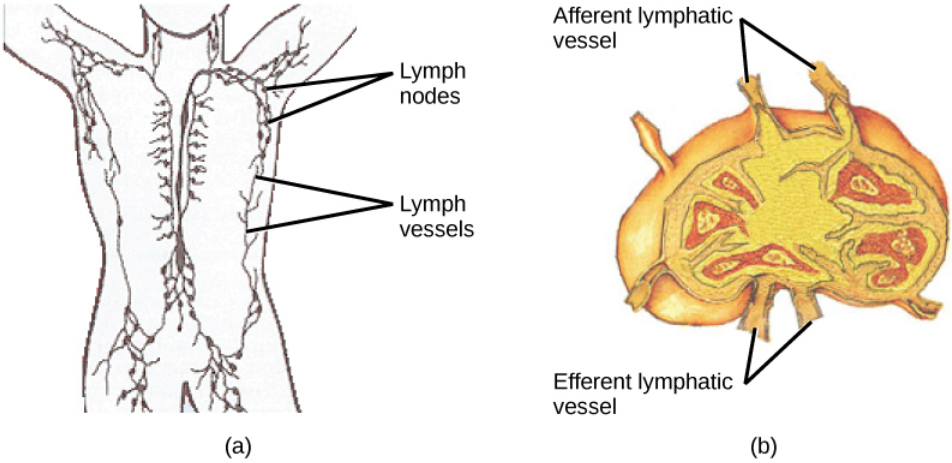


Figure **6.3.13**: (a) Lymphatic vessels carry a clear fluid called lymph throughout the body. The liquid enters (b) lymph nodes through afferent vessels. Lymph nodes are filled with lymphocytes that purge infecting cells. The lymph then exits through efferent vessels. (credit: modification of work by NIH, NCI)

The spleen houses B and T cells, macrophages, dendritic cells, and NK cells. The spleen, shown in Figure **6.3.14**, is the site where APCs that have trapped foreign particles in the blood can communicate with lymphocytes. Antibodies are synthesized and secreted by activated plasma cells in the spleen, and the spleen filters foreign substances and antibody-complexed pathogens from the blood. Functionally, the spleen is to the blood as lymph nodes are to the lymph.

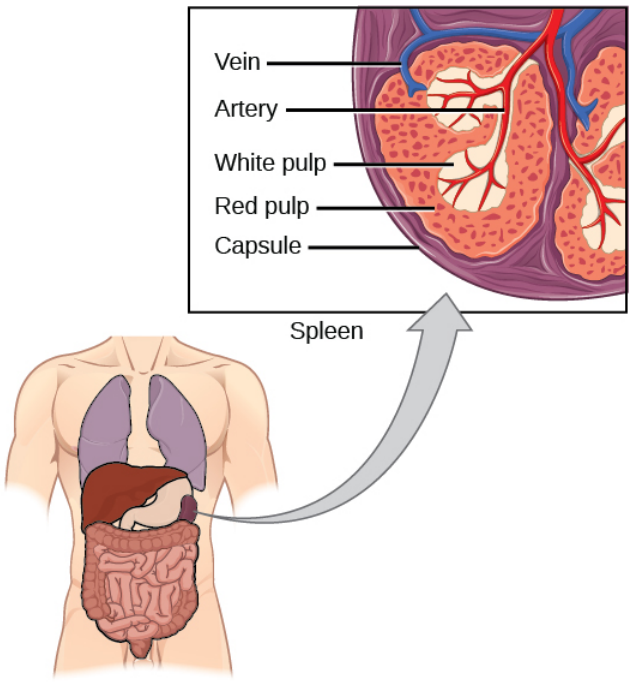


Figure **6.3.14**: The spleen is similar to a lymph node but is much larger and filters blood instead of lymph. Blood enters the spleen through arteries and exits through veins. The spleen contains two types of tissue: red pulp and white pulp. Red pulp consists of cavities that store blood. Within the red pulp, damaged red blood cells are removed and replaced by new ones. White pulp is rich in lymphocytes that remove antigen-coated bacteria from the blood. (credit: modification of work by NCI)

Summary

The adaptive immune response is a slower-acting, longer-lasting, and more specific response than the innate response. However, the adaptive response requires information from the innate immune system to function. APCs display antigens via MHC molecules to complementary naïve T cells. In response, the T cells differentiate and proliferate, becoming T_H cells or CTLs. T_H cells stimulate B cells that have engulfed and presented pathogen-derived antigens. B cells differentiate into plasma cells that secrete antibodies, whereas CTLs induce apoptosis in intracellularly infected or cancerous cells. Memory cells persist after a primary exposure to a pathogen. If re-exposure occurs, memory cells differentiate into effector cells without input from the innate immune system. The mucosal immune system is largely independent from the systemic immune system but functions in a parallel fashion to protect the extensive mucosal surfaces of the body.

Glossary

adaptive immunity
: immunity that has memory and occurs after exposure to an antigen either from a pathogen or a vaccination

antigen
: foreign or “non-self” protein that triggers the immune response

antigen-presenting cell (APC)
: immune cell that detects, engulfs, and informs the adaptive immune response about an infection by presenting the processed antigen on the cell surface

autoimmune response
: inappropriate immune response to host cells or self-antigens

cell-mediated immune response
: adaptive immune response that is carried out by T cells

clonal selection
activation of B cells corresponding to one specific BCR variant and the dramatic proliferation of that variant

cytotoxic T lymphocyte (CTL)
adaptive immune cell that directly kills infected cells via perforin and granzymes, and releases cytokines to enhance the immune response

dendritic cell
immune cell that processes antigen material and presents it on the surface of other cells to induce an immune response

effector cell
lymphocyte that has differentiated, such as a B cell, plasma cell, or cytotoxic T lymphocyte

epitope
small component of an antigen that is specifically recognized by antibodies, B cells, and T cells; the antigenic determinant

helper T lymphocyte (T_H)
cell of the adaptive immune system that binds APCs via MHC II molecules and stimulates B cells or secretes cytokines to initiate the immune response

humoral immune response
adaptive immune response that is controlled by activated B cells and antibodies

immune tolerance
acquired ability to prevent an unnecessary or harmful immune response to a detected foreign body known not to cause disease or to self-antigens

lymph
watery fluid that bathes tissues and organs with protective white blood cells and does not contain erythrocytes

mucosa-associated lymphoid tissue (MALT)
collection of lymphatic tissue that combines with epithelial tissue lining the mucosa throughout the body

memory cell
antigen-specific B or T lymphocyte that does not differentiate into effector cells during the primary immune response but that can immediately become an effector cell upon re-exposure to the same pathogen

plasma cell
immune cell that secrets antibodies; these cells arise from B cells that were stimulated by antigens

regulatory T (T_{reg}) cell
specialized lymphocyte that suppresses local inflammation and inhibits the secretion of cytokines, antibodies, and other stimulatory immune factors; involved in immune tolerance

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6.3.1: Antigen Epitopes

Learning Objectives

1. Define antigen and immunogen.
2. State what antigens are composed of chemically.
3. List 3 characteristics an antigen must have to be immunogenic.
4. Define epitope.
5. Briefly describe how the body recognizes an antigen as foreign.
6. Compare B-cell receptors and T-cell receptors in terms of how they recognize epitopes.
7. In terms of infectious diseases, list 2 categories of microbial materials that may act as an antigen.
8. List 3 groups of noninfectious materials that may act as an antigen.
9. Define the following:
    a. endogenous antigen
    b. exogenous antigen
    c. autoantigen
    d. hapten

An antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes. An immunogen is an antigen that is recognized by the body as non-self and stimulates an adaptive immune response. For simplicity, both antigens and immunogens are usually referred to as antigens.

To be immunogenic, an antigen must possess three characteristics:

- be of high molecular weight,
- exhibit chemical complexity, and
- exhibit foreignness (recognized as non-self by the body).

Chemically, antigens are large molecular weight proteins (including conjugated proteins such as glycoproteins, lipoproteins, and nucleoproteins) and polysaccharides (including lipopolysaccharides). These protein and polysaccharide antigens are found on the surfaces of viruses and cells, including microbial cells (bacteria, fungi, protozoans) and human cells.

Epitopes of an antigen

The actual portions or fragments of an antigen that react with receptors on B-lymphocytes and T-lymphocytes, as well as with free antibody molecules, are called epitopes or antigenic determinants. The size of an epitope is generally thought to be equivalent to 5-15 amino acids or 3-4 sugar residues.

Some antigens, such as polysaccharides, usually have many epitopes, but all of the same specificity. This is because polysaccharides may be composed of hundreds of sugars with branching sugar side chains, but usually contain only one or two different sugars. As a result, most "shapes" along the polysaccharide are the same (see Figure **6.3.1.1**).

Other antigens such as proteins usually have many epitopes of different specificities. This is because proteins are usually hundreds of amino acids long and are composed of 20 different amino acids. Certain amino acids are able to interact with other amino acids in the protein chain and this causes the protein to fold over upon itself and assume a complex three-dimensional shape. As a result, there are many different "shapes" on the protein (see Figure **6.3.1.2**). That is why proteins are more immunogenic than polysaccharides; they are chemically more complex.

A microbe, such as a single bacterium, has many different proteins (and polysaccharides) on its surface that collectively form its various structures, and each different protein may have many different epitopes. Therefore, immune responses are directed against many different epitopes of many different antigens of the same microbe. (For example,

a bacterial cell wall alone may contain over 100 different epitopes.) Even simple viruses possess many different epitopes. (see Figure **6.3.1.3**).

Substances that act as antigens

In terms of infectious diseases, the following may act as antigens:

a. microbial structures, such as bacterial and fungal cell walls, protozoan cell membranes, bacterial and fungal capsules, microbial flagella, bacterial pili, viral capsids, viral envelope-associated glycoproteins, etc.; and

b. microbial toxins

Certain non-infectious materials may also act as antigens if they are recognized as "nonself" by the body. These include:

a. allergens, including dust, pollen, hair, foods, dander, bee venom, drugs, and other agents causing allergic reactions;

b. foreign tissues and cells from transplants and transfusions; and

c. the body's own cells that the body fails to recognize as "normal self," such as cancer cells, infected cells, cells involved in autoimmune diseases.

There are three broad categories of antigens: endogenous antigens, exogenous antigens, and autoantigens.

1. Endogenous antigens are proteins found within the cytosol of human cells. Examples of endogenous antigens include:

a. viral proteins produced during viral replication;
b. proteins produced by intracellular bacteria such as Rickettsias and Chlamydias during their replication;
c. proteins that have escaped into the cytosol from the phagosome of phagocytes such as antigen-presenting cells;
d. tumor antigens produced by cancer cells; and
e. self-peptides from host cellular proteins.

2. Exogenous antigens are antigens that enter from outside the body, such as bacteria, fungi, protozoa, and free viruses. These exogenous antigens enter macrophages, dendritic cells, and B-lymphocytes through phagocytosis or pinocytosis.

3. Autoantigens are any of an organism’s own antigens (self-antigens) that stimulate an autoimmune reaction, that is humoral immunity or cell-mediated against self.

A hapten is a small molecule that by itself is not immunogenic but can act as an antigen when it binds to a larger protein molecule. The hapten then acts as an epitope on the protein. For example with penicillin and poison ivy allergies, the penicillin molecules and the oil urushiol from the poison ivy plant function as haptens, binding to tissue proteins to form an antigen and stimulating an allergic immune response.

? Exercise: Think-Pair-Share Questions

1. How is the body able to distinguish epitopes of microorganisms from “self” epitopes present as a part of our body?
2. What is the difference between how B-lymphocytes and T-lymphocytes recognize antigens?

Summary

1. An antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes.
2. An immunogen is an antigen that is recognized by the body as non-self and stimulates an adaptive immune response.
3. Chemically, antigens are large molecular weight proteins and polysaccharides.
4. The actual portions or fragments of an antigen that react with receptors on B-lymphocytes and T-lymphocytes, as well as with free antibody molecules, are called epitopes.

5. The size of an epitope is generally thought to be equivalent to 5-15 amino acids or 3-4 sugar residues.
6. Polysaccharides antigens usually have many epitopes but all of the same specificity.
7. Proteins antigens usually have many epitopes of different specificities.
8. Immune responses are directed against many different epitopes of many different antigens of the same microbe.
9. The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope.
10. The antigen receptors on the cytoplasmic membrane of B-lymphocytes are called B-cell receptors and are actually antibody molecules made by that cell and anchored to the outer surface of its cytoplasmic membrane and is composed of composed of four interconnected glycoprotein chains.
11. The receptors on the membrane of T-lymphocytes are called T-cell receptors or TCRs and are composed of just two glycoprotein chains.
12. During its development, each different B-lymphocyte and T-lymphocyte becomes genetically programmed to produce a B-cell receptor or T-cell receptor with a unique three-dimensional shape.
13. The body produces 107 or more distinct clones of both B-lymphocytes and T-lymphocytes, each with a unique B-cell receptor or T-cell receptor and with this large variety of B-cell receptors and T-cell receptors there is bound to be at least one that has an epitope-binding site able to fit, at least to some degree, any antigen the immune system eventually encounters.
14. In terms of infectious diseases, microbial structures and microbial toxins act as antigens.
15. Certain noninfectious materials also act as antigens, including allergens, foreign tissues and cells from transplants and transfusions, and the body's own cells that the body fails to recognize as "normal self," such as cancer cells, infected cells, and cells involved in autoimmune diseases.
16. Endogenous antigens are antigens found within the cytosol of human cells such as viral proteins, proteins from intracellular bacteria, and tumor antigens.
17. Exogenous antigens are antigens that enter from outside the body, such as bacteria, fungi, protozoa, and free viruses.
18. Autoantigens are any of an organism's own antigens (self-antigens) that stimulate an autoimmune reaction.

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6.3.2: HUMORAL IMMUNE RESPONSE

LEARNING OBJECTIVES

- Summarize the humoral immune response

The humoral immune response fights pathogens that are free in the bodily fluids, or “humours”. It relies on antigens (which are also often free in the humours) to detect these pathogens. An antigen is a biomolecule, such as a protein or sugar, that binds to a specific antibody. An antibody/antigen interaction may stimulate an immune response. Not every biomolecule is antigenic and not all antigens produce an immune response. B cells are the major cell type involved in the humoral immune response. When a foreign antigen (one coming from a pathogen, for example) is detected, B cells in the body that recognize that antigen will begin to produce antibodies as a means of fighting off the foreign invader.

B CELL MATURATION

During maturation, B cells gain antigen receptor molecules, termed B cell receptors (BCRs), which are displayed in large numbers, extracellularly on their membrane. These membrane-bound protein complexes contain antibodies, which enable specific antigen recognition. Each B cell initially produced has only one kind of antibody (antigen receptor), which makes every B cell unique. It is the immense number of B cells in the body, each of which produces a unique antibody, that allows the immune system to detect such a wide variety of pathogenic antigens. B cells containing antibodies that recognize “self” antigens are destroyed before they can mature, preventing the immune system from attacking the host. Once B cells mature in the bone marrow, they migrate to lymph nodes or other lymphatic organs, where they may begin to encounter pathogens.

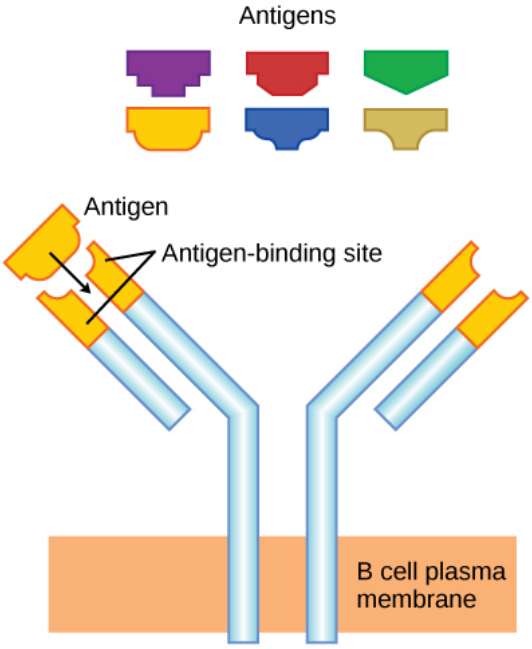


Figure **6.3.2.1**: B cell receptors: B cell receptors, containing antibodies (termed antigen-binding site in the picture) are embedded in the membranes of B cells and bind a variety of antigens through their variable regions.

B CELL ACTIVATION

When a B cell encounters the antigen that binds to its receptor, the antigen molecule is brought into the cell by endocytosis, reappearing on the surface of the cell bound to an MHC class II molecule. When this process is complete, the B cell is sensitized. In most cases, the sensitized B cell must then encounter a specific kind of T cell, called a helper T cell, before it is activated. This activation of the helper T cell occurs when a dendritic cell presents an antigen on its MHC II molecule, allowing the T cell to recognize it and mature.

The helper T cell binds to the antigen-MHC class II complex and is induced to release cytokines that induce the B cell to divide rapidly, making thousands of identical (clonal) cells. These daughter cells become either plasma cells or memory B cells. The memory B cells remain inactive at this point. A later encounter with the antigen, caused by a reinfection by the same bacteria or virus, will result in them dividing into a new population of plasma cells. The plasma cells, on the other hand, produce and secrete large quantities, up to 100 million molecules per hour, of antibody molecules. An antibody, also known as an immunoglobulin (Ig), is a protein that is produced by plasma cells after stimulation by an antigen. Antibodies are the agents of humoral immunity. Antibodies occur in the blood, in gastric and mucus secretions, and in breast milk. Antibodies in these bodily fluids can bind pathogens and mark them for destruction by phagocytes before they are able to infect cells.

ANTIBODIES

These antibodies circulate in the blood stream and lymphatic system, binding with the antigen whenever it is encountered. The binding can fight infection in several ways. Antibodies can bind to viruses or bacteria, which interferes with the chemical interactions required for them to infect or bind to other cells. The antibodies may create bridges between different particles containing antigenic sites, clumping them all together and preventing their proper functioning. Antibody neutralization can prevent pathogens from entering and infecting host cells. The neutralized antibody-coated pathogens can then be filtered by the spleen to be eliminated in urine or feces. The antigen-antibody complex stimulates the complement system described previously, destroying the cell bearing the antigen. Antibodies also opsonize pathogen cells, wherein they mark them for destruction by phagocytic cells, such as macrophages or neutrophils. Additionally, antibodies stimulate inflammation, while their presence in mucus and on the skin prevents pathogen attack.

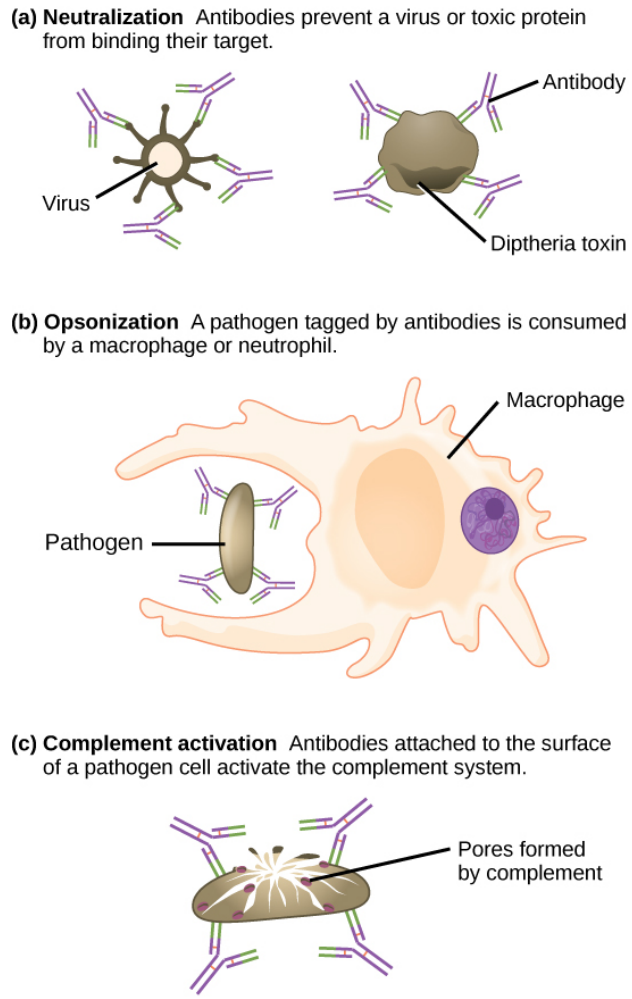


Figure **6.3.2.1**: Methods by which antibodies inhibit infection: Antibodies may inhibit infection by (a) preventing the antigen from binding its target, (b) tagging a pathogen for destruction by macrophages or neutrophils, or (c) activating the complement cascade.

The production of antibodies by plasma cells in response to an antigen is called active immunity. This describes the host's active response of the immune system to an infection or to a vaccination. There is also a passive immune response wherein antibodies are introduced into the host from an outside source, instead of the individual's own plasma cells. For example, antibodies circulating in a pregnant woman's body move across the placenta into the developing fetus. The child benefits from the presence of these antibodies for up to several months after birth. In addition, a passive immune response is possible by injecting antibodies into an individual in the form of an antivenom to a snake-bite toxin or antibodies in blood serum to help fight a hepatitis infection, giving immediate relief.

KEY POINTS

- Antigens are proteins and other macromolecules that bind to a specific antibody and are used by the immune system to recognize pathogens.
- B cells express receptors (BCRs) on their membrane which contain antibodies; these antibodies allow B cells to detect pathogens and release further antibodies to fight the infection.
- Antibodies fight infections in three ways: they mark pathogens for destruction by phagocytic cells in a process known as opsonization, they coat key sites on pathogens necessary for infection, and they induce the complement cascade to occur against antibody-bound pathogens.
- Once the adaptive immune response has encountered an antigen, B cells will divide to produce plasma cells, which rapidly secrete antibodies to that antigen in a process called active immunity.

KEY TERMS

- **antibody**: a protein produced by B-lymphocytes that binds to a specific antigen
- **opsonize**: to make (bacteria or other cells) more susceptible to the action of phagocytes by use of opsonins
- **MHC**: an acronym for major histocompatibility complex; these extracellular protein receptors display antigens derived from extracellular (class I) or intracellular (class II) proteins and other biomolecules

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6.3.2.1: B Lymphocytes and Antibodies

Learning Objectives

- Describe the production and maturation of B cells
- Compare the structure of B-cell receptors and T-cell receptors
- Compare T-dependent and T-independent activation of B cells
- Compare the primary and secondary antibody responses

Humoral immunity refers to mechanisms of the adaptive immune defenses that are mediated by antibodies secreted by B lymphocytes, or B cells. This section will focus on B cells and discuss their production and maturation, receptors, and mechanisms of activation.

B Cell Production and Maturation

Like T cells, B cells are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow and follow a pathway through lymphoid stem cell and lymphoblast (see Figure 17.3.1). Unlike T cells, however, lymphoblasts destined to become B cells do not leave the bone marrow and travel to the thymus for maturation. Rather, eventual B cells continue to mature in the bone marrow.

The first step of B cell maturation is an assessment of the functionality of their antigen-binding receptors. This occurs through positive selection for B cells with normal functional receptors. A mechanism of negative selection is then used to eliminate self-reacting B cells and minimize the risk of autoimmunity. Negative selection of self-reacting B cells can involve elimination by apoptosis, editing or modification of the receptors so they are no longer self-reactive, or induction of anergy in the B cell. Immature B cells that pass the selection in the bone marrow then travel to the spleenfor their final stages of maturation. There they become naïve mature B cells, i.e., mature B cells that have not yet been activated.

Exercise 6.3.2.1.1

Compare the maturation of B cells with the maturation of T cells.

B-Cell Receptors

Like T cells, B cells possess antigen-specific receptors with diverse specificities. Although they rely on T cells for optimum function, B cells can be activated without help from T cells. B-cell receptors (BCRs) for naïve mature B cells are membrane-bound monomeric forms of IgD and IgM. They have two identical heavy chains and two identical light chains connected by disulfide bonds into a basic “Y” shape (Figure **6.3.2.1.1**). The trunk of the Y-shaped molecule, the constant region of the two heavy chains, spans the B cell membrane. The two antigen-binding sites exposed to the exterior of the B cell are involved in the binding of specific pathogen epitopes to initiate the activation process. It is estimated that each naïve mature B cell has upwards of 100,000 BCRs on its membrane, and each of these BCRs has an identical epitope-binding specificity.

In order to be prepared to react to a wide range of microbial epitopes, B cells, like T cells, use genetic rearrangementof hundreds of gene segments to provide the necessary diversity of receptor specificities. The variable region of the BCR heavy chain is made up of V, D, and J segments, similar to the β chain of the TCR. The variable region of the BCR light chain is made up of V and J segments, similar to the α chain of the TCR. Genetic rearrangement of all possible combinations of V-J-D (heavy chain) and V-J (light chain) provides for millions of unique antigen-binding sites for the BCR and for the antibodies secreted after activation.

One important difference between BCRs and TCRs is the way they can interact with antigenic epitopes. Whereas TCRs can only interact with antigenic epitopes that are presented within the antigen-binding cleft of MHC I or MHC II, BCRs do not require antigen presentation with MHC; they can interact with epitopes on free antigens or with epitopesdisplayed on the surface of intact pathogens. Another important difference is that TCRs only recognize protein epitopes, whereas BCRs can recognize epitopes associated with different molecular classes (e.g., proteins, polysaccharides, lipopolysaccharides).

Activation of B cells occurs through different mechanisms depending on the molecular class of the antigen. Activation of a B cell by a protein antigen requires the B cell to function as an APC, presenting the protein epitopes with MHC II to helper T cells.

Because of their dependence on T cells for activation of B cells, protein antigens are classified as T-dependent antigens. In contrast, polysaccharides, lipopolysaccharides, and other nonprotein antigens are considered T-independent antigens because they can activate B cells without antigen processing and presentation to T cells.

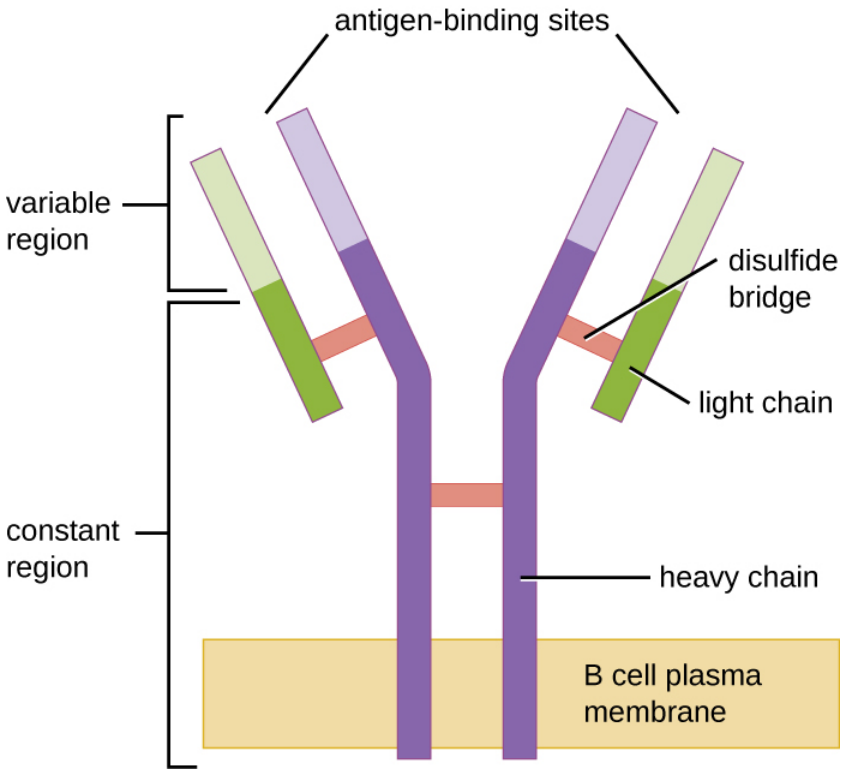


Figure **6.3.2.1.1**: B-cell receptors are embedded in the membranes of B cells. The variable regions of all of the receptors on a single cell bind the same specific antigen.

? Exercise 6.3.2.1.2

1. What types of molecules serve as the BCR?
2. What are the differences between TCRs and BCRs with respect to antigen recognition?
3. Which molecule classes are T-dependent antigens and which are T-independent antigens?

T Cell-Independent Activation of B cells

Activation of B cells without the cooperation of helper T cells is referred to as T cell-independent activation and occurs when BCRs interact with T-independent antigens. T-independent antigens (e.g., polysaccharide capsules, lipopolysaccharide) have repetitive epitope units within their structure, and this repetition allows for the cross-linkageof multiple BCRs, providing the first signal for activation (Figure **6.3.2.1.2**). Because T cells are not involved, the second signal has to come from other sources, such as interactions of toll-like receptors with PAMPs or interactions with factors from the complement system.

Once a B cell is activated, it undergoes clonal proliferation and daughter cells differentiate into plasma cells. Plasma cells are antibody factories that secrete large quantities of antibodies. After differentiation, the surface BCRs disappear and the plasma cell secretes pentameric IgM molecules that have the same antigen specificity as the BCRs (Figure **6.3.2.1.2**).

The T cell-independent response is short-lived and does not result in the production of memory B cells. Thus it will not result in a secondary response to subsequent exposures to T-independent antigens.

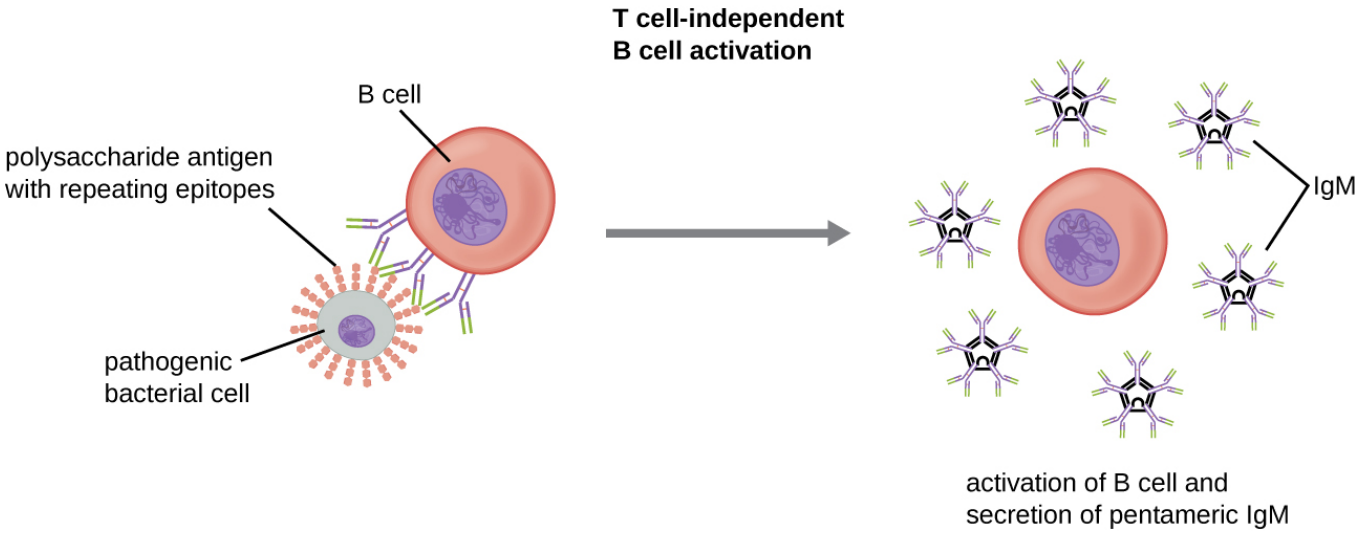


Figure **6.3.2.1.2**: T-independent antigens have repeating epitopes that can induce B cell recognition and activation without involvement from T cells. A second signal, such as interaction of TLRs with PAMPs (not shown), is also required for activation of the B cell. Once activated, the B cell proliferates and differentiates into antibody-secreting plasma cells.

? Exercise 6.3.2.1.3

1. What are the two signals required for T cell-independent activation of B cells?
2. What is the function of a plasma cell?

T Cell-Dependent Activation of B cells

T cell-dependent activation of B cells is more complex than T cell-independent activation, but the resulting immune response is stronger and develops memory. T cell-dependent activation can occur either in response to free protein antigens or to protein antigens associated with an intact pathogen. Interaction between the BCRs on a naïve mature B cell and a free protein antigen stimulate internalization of the antigen, whereas interaction with antigens associated with an intact pathogen initiates the extraction of the antigen from the pathogen before internalization. Once internalized inside the B cell, the protein antigen is processed and presented with MHC II. The presented antigen is then recognized by helper T cells specific to the same antigen. The TCR of the helper T cell recognizes the foreign antigen, and the T cell's CD4 molecule interacts with MHC II on the B cell. The coordination between B cells and helper T cells that are specific to the same antigen is referred to as linked recognition.

Once activated by linked recognition, T_H2 cells produce and secrete cytokines that activate the B cell and cause proliferation into clonal daughter cells. After several rounds of proliferation, additional cytokines provided by the T_H2 cells stimulate the differentiation of activated B cell clones into memory B cells, which will quickly respond to subsequent exposures to the same protein epitope, and plasma cells that lose their membrane BCRs and initially secrete pentameric IgM (Figure **6.3.2.1.3**).

After initial secretion of IgM, cytokines secreted by T_H2 cells stimulate the plasma cells to switch from IgM production to production of IgG, IgA, or IgE. This process, called class switching or isotype switching, allows plasma cellscloned from the same activated B cell to produce a variety of antibody classes with the same epitope specificity. Class switching is accomplished by genetic rearrangement of gene segments encoding the constant region, which determines an antibody's class. The variable region is not changed, so the new class of antibody retains the original epitope specificity.

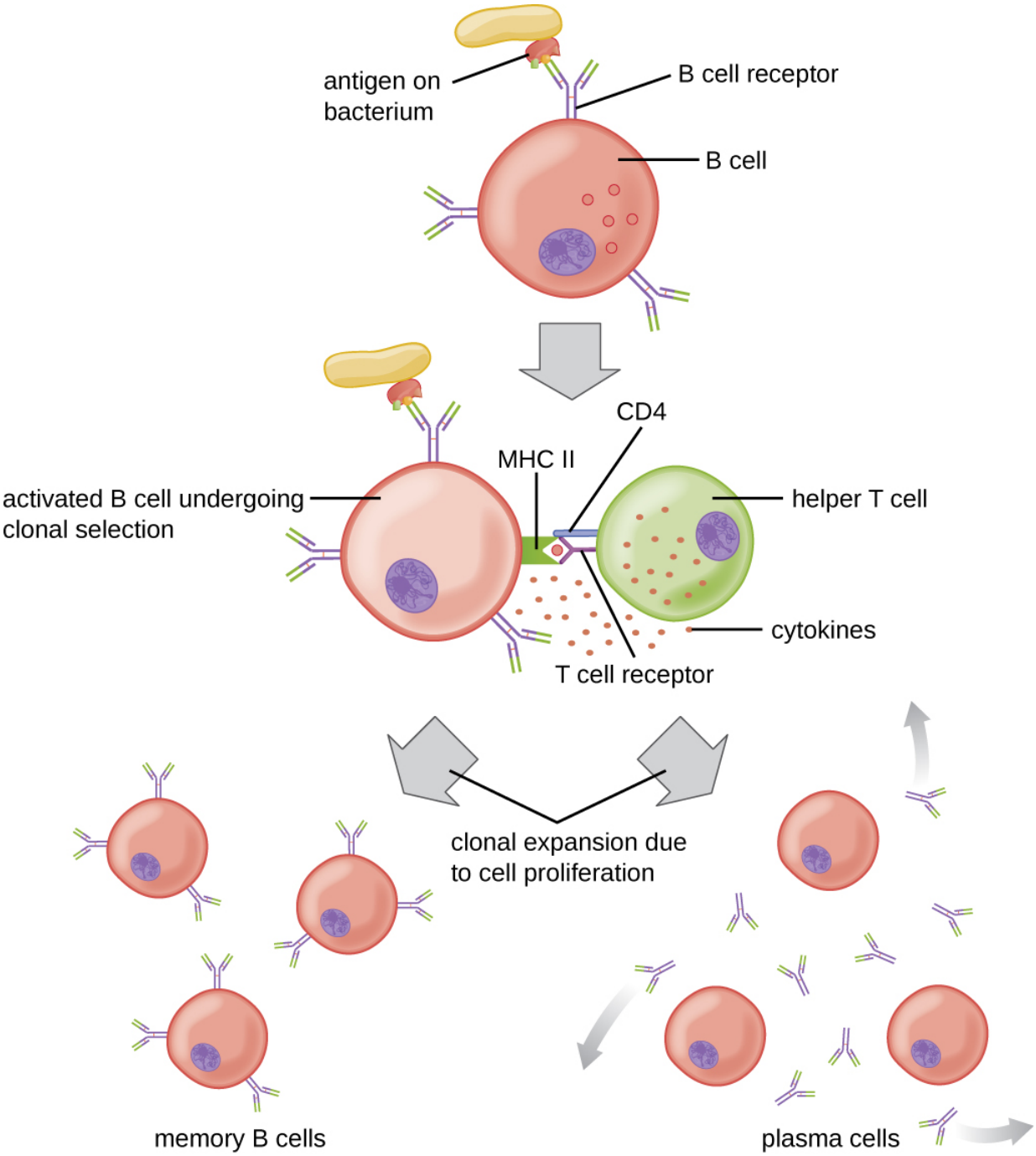


Figure **6.3.2.1.3**: In T cell-dependent activation of B cells, the B cell recognizes and internalizes an antigen and presents it to a helper T cell that is specific to the same antigen. The helper T cell interacts with the antigen presented by the B cell, which activates the T cell and stimulates the release of cytokines that then activate the B cell. Activation of the B cell triggers proliferation and differentiation into B cells and plasma cells.

? Exercise 6.3.2.1.4

1. What steps are required for T cell-dependent activation of B cells?
2. What is antibody class switching and why is it important?

Primary and Secondary Responses

T cell-dependent activation of B cells plays an important role in both the primary and secondary responses associated with adaptive immunity. With the first exposure to a protein antigen, a T cell-dependent primary antibody responseoccurs. The initial stage of the

primary response is a lag period, or latent period, of approximately 10 days, during which no antibody can be detected in serum. This lag period is the time required for all of the steps of the primary response, including naïve mature B cell binding of antigen with BCRs, antigen processing and presentation, helper T cell activation, B cell activation, and clonal proliferation. The end of the lag period is characterized by a rise in IgM levels in the serum, as T_H2 cells stimulate B cell differentiation into plasma cells. IgM levels reach their peak around 14 days after primary antigen exposure; at about this same time, T_H2 stimulates antibody class switching, and IgM levels in serum begin to decline. Meanwhile, levels of IgG increase until they reach a peak about three weeks into the primary response (Figure **6.3.2.1.4**).

During the primary response, some of the cloned B cells are differentiated into memory B cells programmed to respond to subsequent exposures. This secondary response occurs more quickly and forcefully than the primary response. The lag period is decreased to only a few days and the production of IgG is significantly higher than observed for the primary response (Figure **6.3.2.1.4**). In addition, the antibodies produced during the secondary response are more effective and bind with higher affinity to the targeted epitopes. Plasma cells produced during secondary responses live longer than those produced during the primary response, so levels of specific antibody remain elevated for a longer period of time.

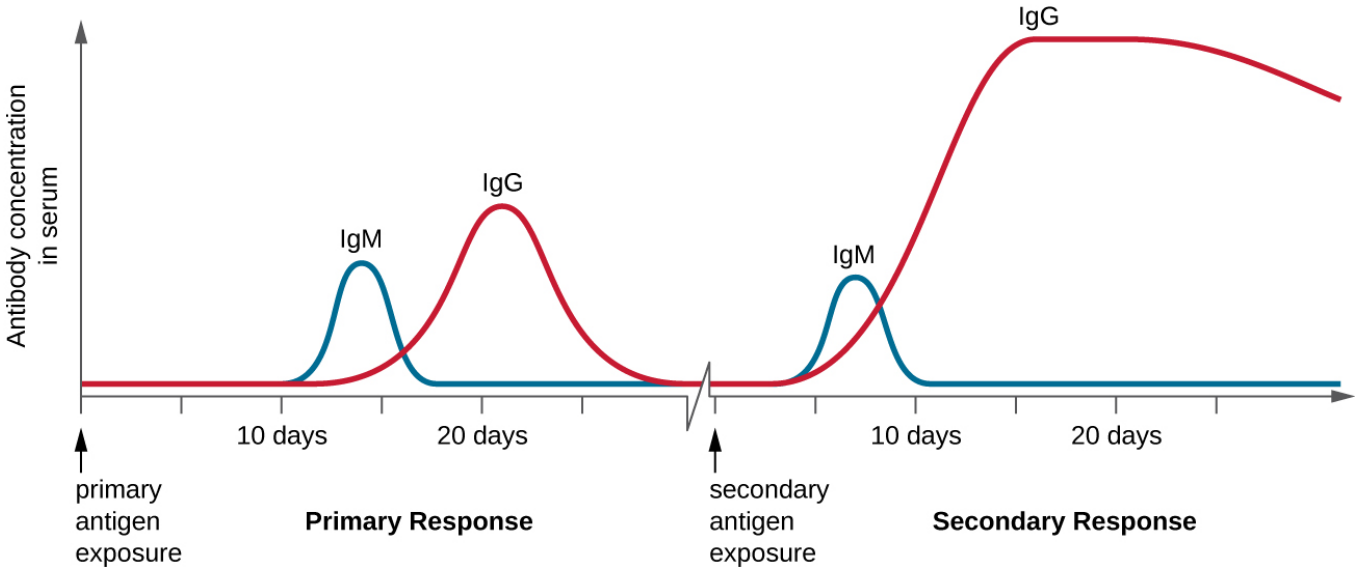


Figure **6.3.2.1.4**: Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. The secondary response mostly involves IgG.

? Exercise 6.3.2.1.5

1. What events occur during the lag period of the primary antibody response?
2. Why do antibody levels remain elevated longer during the secondary antibody response?

Key Concepts and Summary

- **B lymphocytes** or **B cells** produce antibodies involved in humoral immunity. B cells are produced in the bone marrow, where the initial stages of maturation occur, and travel to the spleen for final steps of maturation into naïve mature B cells.
- **B-cell receptors (BCRs)** are membrane-bound monomeric forms of IgD and IgM that bind specific antigen epitopes with their Fab antigen-binding regions. Diversity of antigen binding specificity is created by genetic rearrangement of V, D, and J segments similar to the mechanism used for TCR diversity.
- Protein antigens are called **T-dependent antigens** because they can only activate B cells with the cooperation of helper T cells. Other molecule classes do not require T cell cooperation and are called **T-independent antigens**.
- **T cell-independent activation** of B cells involves cross-linkage of BCRs by repetitive nonprotein antigen epitopes. It is characterized by the production of IgM by **plasma cells** and does not produce memory B cells.
- **T cell-dependent activation** of B cells involves processing and presentation of protein antigens to helper T cells, activation of the B cells by cytokines secreted from activated T_H2 cells, and plasma cells that produce different classes of antibodies as a result of **class switching**. **Memory B cells** are also produced.

- Secondary exposures to T-dependent antigens result in a secondary antibody response initiated by memory B cells. The secondary response develops more quickly and produces higher and more sustained levels of antibody with higher affinity for the specific antigen.

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6.3.2.2: ANTIBODY STRUCTURE

LEARNING OBJECTIVES

- Differentiate among the classes of antibodies

An antibody is a molecule that recognizes a specific antigen; this recognition is a vital component of the adaptive immune response. Antibodies are composed of four polypeptides: two identical heavy chains (large peptide units) that are partially bound to each other in a "Y" formation, which are flanked by two identical light chains (small peptide units). The area where the antigen is recognized on the antibody is known as the variable domain or variable region. This is why there are numerous antibodies that can each recognize a different antigen. The antibody base is known as the constant domain or constant region. The portion of an antigen that is recognized by the antibody is known as the epitope.

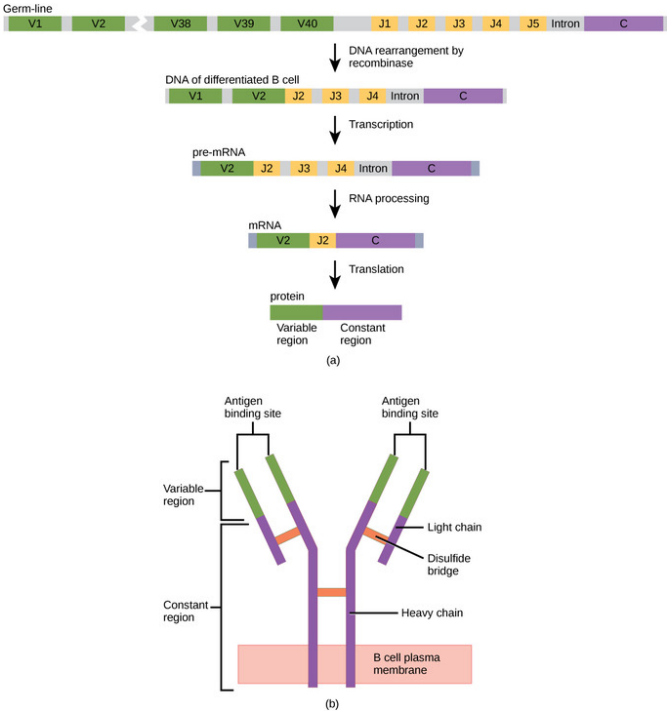


Figure 6.3.2.2.1: Antibodies: (a) As a germ-line B cell matures, an enzyme called DNA recombinase randomly excises V and J segments from the light chain gene. Splicing at the mRNA level results in further gene rearrangement. As a result, (b) each antibody has a unique variable region capable of binding a different antigen.

ANTIBODY VARIATION

In B cells, the variable region of the light chain gene has 40 variable (V) and five joining (J) segments. An enzyme called DNA recombinase randomly excises most of these segments out of the gene, splicing one V segment to one J segment. During RNA processing, all but one V and J segment are spliced out. Recombination and splicing may result in over 10^6 possible VJ combinations. As a result, each differentiated B cell in the human body typically has a unique variable chain. The constant domain, which does not bind to an antibody, is the same for all antibodies. The large diversity of antibody structure translates into the large diversity of antigens that antibodies can bind and recognize.

Similar to TCRs (T cell receptors) and BCRs (B cell receptors), antibody diversity is produced by the mutation and recombination of approximately 300 different gene segments encoding the light and heavy chain variable domains in precursor cells that are destined to become B cells. The variable domains from the heavy and light chains interact to form the binding site through which an antibody can bind a specific epitope on an antigen. The numbers of repeated constant domains in Ig classes (discussed below) are the same for all antibodies corresponding to a specific class. Antibodies are structurally similar to the extracellular component of the BCRs. The maturation of B cells into plasma cells occurs when the cells gain the ability to secrete the antibody portion of its BCR in large quantities.

ANTIBODY CLASSES

Antibodies can be divided into five classes (IgM, IgG, IgA, IgD, and IgE) based on their physiochemical, structural, and immunological properties. Ig stands for immunoglobulin, another term for an antibody. IgGs, which make up about 80 percent of all antibodies in circulation, have heavy chains that consist of one variable domain and three identical constant domains. IgA and IgD also have three constant domains per heavy chain, whereas IgM and IgE each have four constant domains per heavy chain. The variable domain determines binding specificity, while the constant domain of the heavy chain determines the immunological mechanism of action of the corresponding antibody class. It is possible for two antibodies to have the same binding specificities, but be in different classes and, therefore, to be involved in different functions.

After an adaptive defense is produced against a pathogen, typically plasma cells first secrete IgM into the blood. BCRs on naïve B cells are of the IgM class and, occasionally, the IgD class. IgM molecules comprise approximately ten percent of all antibodies. Prior to antibody secretion, plasma cells assemble IgM molecules into pentamers (five individual antibodies) linked by a joining (J) chain. The pentamer arrangement means that these macromolecules can bind ten identical antigens. However, IgM molecules released early in the adaptive immune response do not bind to antigens as stably as do IgGs, which are one of the possible types of antibodies secreted in large quantities upon re-exposure to the same pathogen. The properties of immunoglobulins and their basic structures are shown in the table.

Name	Properties	Structure
IgA	Found in mucous, saliva, tears, and breast milk. Protects against pathogens.	
IgD	Part of the B cell receptor. Activates basophils and mast cells.	
IgE	Protects against parasitic worms. Responsible for allergic reactions.	
IgG	Secreted by plasma cells in the blood. Able to cross the placenta into the fetus.	
IgM	May be attached to the surface of a B cell or secreted into the blood. Responsible for early stages of immunity.	

Figure **6.3.2.2.1**: Classes of antibodies: Immunoglobulins (antibody classes) have different functions, but all are composed of light and heavy chains that form a Y-shaped structure.

IgAs populate the saliva, tears, breast milk, and mucus secretions of the gastrointestinal, respiratory, and genitourinary tracts. Collectively, these bodily fluids coat and protect the extensive mucosa (4000 square feet in humans). The total number of IgA molecules in these bodily secretions is greater than the number of IgG molecules in the blood serum. A small amount of IgA is also secreted into the serum in monomeric form. Conversely, some IgM is secreted into bodily fluids of the mucosa. Similarly to IgM, IgA molecules are secreted as polymeric structures linked with a J chain. However, IgAs are secreted mostly as dimeric molecules, not pentamers.

IgE is present in the serum in small quantities and is best characterized in its role as an allergy mediator. IgD is also present in small quantities. Similarly to IgM, BCRs containing the IgD class of antibodies are found on the surface of naïve B cells. This class supports antigen recognition and subsequent maturation of B cells to plasma cells.

KEY POINTS

- Antibodies contain four polypeptides: two identical (to each other) heavy chains in a “Y” formation and two idenitical (to each other) light chains on the outside of the top of the “Y” portion.
- Each antibody has a unique variable region, which is responsible for antigen detection and specificity.
- There are five classes of antibodies, each utilized by the body under different conditions, including IgM, IgG, IgA, IgD, and IgE; Ig stands for immunoglobulin.
- IgAs, secreted in the milk, tears and mucous, are the most numerous antibodies produced; inside of the body, circulating IgGs are the most abundant.

KEY TERMS

- **immunoglobulin**: any of the glycoproteins in blood serum that respond to invasion by foreign antigens and that protect the host by removing pathogens; also known as an antibody
- **antigen**: a substance that binds to a specific antibody; may cause an immune response
- **B cell**: a lymphocyte, developed in the bursa of birds and the bone marrow of other animals, that produces antibodies and is responsible for the immune system
- **epitope**: that part of a biomolecule (such as a protein) that is the target of an immune response; the part of the antigen recognized by the immune system

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6.3.2.3: ANTIBODY FUNCTIONS

LEARNING OBJECTIVES

- Differentiate among affinity, avidity, and cross-reactivity in antibodies

Differentiated plasma cells are crucial players in the humoral immunity response. The antibodies they secrete are particularly significant against extracellular pathogens and toxins. Once secreted, antibodies circulate freely and act independently of plasma cells. Sometimes, antibodies can be transferred from one individual to another. For instance, a person who has recently produced a successful immune response against a particular disease agent can donate blood to a non-immune recipient, confering temporary immunity through antibodies in the donor's blood serum. This phenomenon, called passive immunity, also occurs naturally during breastfeeding, which makes breastfed infants highly resistant to infections during the first few months of life.

Antibodies coat extracellular pathogens and neutralize them by blocking key sites on the pathogen that enhance their infectivity, such as receptors that "dock" pathogens on host cells. Antibody neutralization can prevent pathogens from entering and infecting host cells, as opposed to the cytotoxic T-cell-mediated approach of killing cells that are already infected to prevent progression of an established infection. The neutralized antibody-coated pathogens can then be filtered by the spleen and eliminated in urine or feces.

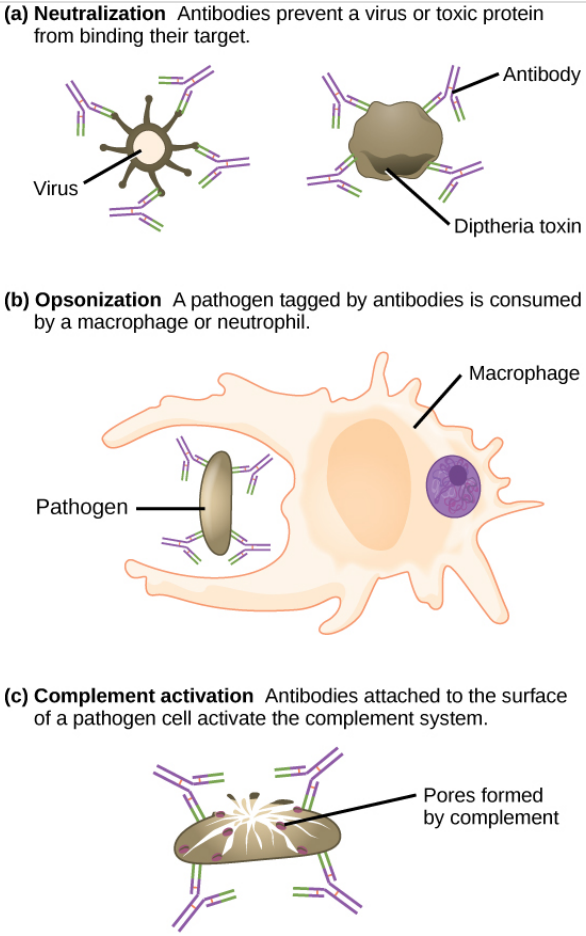


Figure 6.3.2.3.1: Mechanisms of antibody action: Antibodies may inhibit infection by (a) preventing the antigen from binding to its target, (b) tagging a pathogen for destruction by macrophages or neutrophils, or (c) activating the complement cascade.

Antibodies also mark pathogens for destruction by phagocytic cells, such as macrophages or neutrophils, because they are highly attracted to macromolecules complexed with antibodies. Phagocytic enhancement by antibodies is called opsonization. In another process, complement fixation, IgM and IgG in serum bind to antigens, providing docking sites onto which sequential complement proteins can bind. The combination of antibodies and complement enhances opsonization even further, promoting rapid clearing of pathogens.

AFFINITY, AVIDITY, AND CROSS REACTIVITY

Not all antibodies bind with the same strength, specificity, and stability. In fact, antibodies exhibit different affinities (attraction) depending on the molecular complementarity between antigen and antibody molecules. An antibody with a higher affinity for a particular antigen would bind more strongly and stably. It would be expected to present a more challenging defense against the pathogen corresponding to the specific antigen.

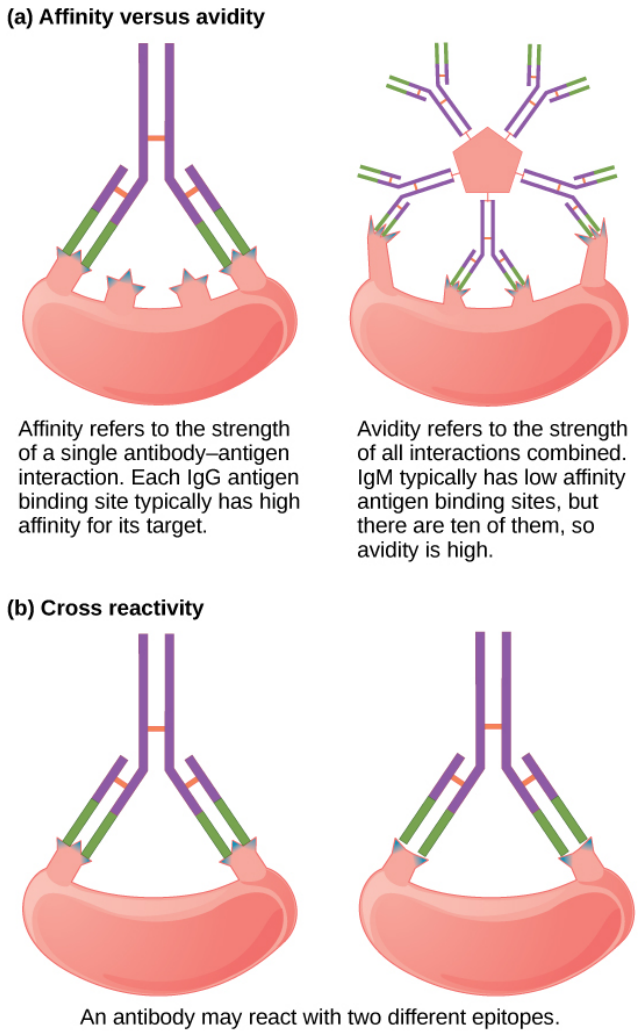


Figure **6.3.2.3.1**: Antibody affinity, avidity, and cross reactivity: (a) Affinity refers to the strength of single interactions between antigen and antibody, while avidity refers to the strength of all interactions combined. (b) An antibody may cross-react with different epitopes.

The term avidity describes binding by antibody classes that are secreted as joined, multivalent structures (such as IgM and IgA). Although avidity measures the strength of binding, just as affinity does, the avidity is not simply the sum of the affinities of the antibodies in a multimeric structure. The avidity depends on the number of identical binding sites on the antigen being detected, as well as other physical and chemical factors. Typically, multimeric antibodies, such as pentameric IgM, are classified as having lower affinity than monomeric antibodies, but high avidity. Essentially, the fact that multimeric antibodies can bind many antigens simultaneously balances their slightly-lower-binding strength for each antibody/antigen interaction.

Antibodies secreted after binding to one epitope on an antigen may exhibit cross reactivity for the same or similar epitopes on different antigens. Cross reactivity occurs when an antibody binds not to the antigen that elicited its synthesis and secretion, but to a different antigen. Because an epitope corresponds to such a small region (the surface area of about four to six amino acids), it is possible for different macromolecules to exhibit the same molecular identities and orientations over short regions.

Cross reactivity can be beneficial if an individual develops immunity to several related pathogens despite having been exposed to or vaccinated against only one of them. For instance, antibody cross reactivity may occur against the similar surface structures of various Gram-negative bacteria. Conversely, antibodies raised against pathogenic molecular components that resemble self molecules may incorrectly mark host cells for destruction, causing autoimmune damage. Patients who develop systemic lupus erythematosus (SLE) commonly exhibit antibodies that react with their own DNA. These antibodies may have been initially raised against the nucleic acid of microorganisms, but later cross-reacted with self-antigens. This phenomenon is also called molecular mimicry.

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KEY POINTS

- Antibodies are produced by plasma cells, but, once secreted, can act independently against extracellular pathogen and toxins.
- Antibodies bind to specific antigens on pathogens; this binding can inhibit pathogen infectivity by blocking key extracellular sites, such as receptors involved in host cell entry.

- Antibodies can also induce the innate immune response to destroy a pathogen, by activating phagocytes such as macrophages or neutrophils, which are attracted to antibody-bound cells.
- Affinity describes how strongly a single antibody binds a given antigen, while avidity describes the binding of a multimeric antibody to multiple antigens.
- A multimeric antibody may have individual arms with low affinity, but have high overall avidity due to synergistic effects between binding sites.
- Cross reactivity occurs when an antibody binds to a different-but-similar antigen than the one for which it was raised; this can increase pathogen resistance or result in an autoimmune reaction.

KEY TERMS

- **avidity**: the measure of the synergism of the strength individual interactions between proteins
- **affinity**: the attraction between an antibody and an antigen

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6.3.3: CELL-MEDIATED IMMUNITY

LEARNING OBJECTIVES

- Summarize the cell-mediated immune response

T CELLS

Just as the humoral immune response has B cells which mediate its response, the cellular immune response has T cells, which recognize infected cells and destroy them before the pathogen inside can replicate and spread to infect other cells. Unlike B cells, T lymphocytes (T cells) are unable to recognize pathogens without assistance. First, an antigen-presenting cell (APC, such as a dendritic cell or a macrophage) detects, engulfs (via phagocytosis in the case of macrophages or by entry of the pathogen of its own accord in the case of dendritic cells), and digests pathogens into hundreds or thousands of antigen fragments. These fragments are then transported to the surface of the APC, where they are presented on proteins known as Major Histocompatibility Complexes class II (MHC II, see). T cells become activated towards a certain antigen once they encounter it displayed on an MHC II. After a virus or bacteria enters a cell, it can no longer be detected by the humoral immune response. Instead, the cellular immune response must take over. To do so, a T cell will become activated by interacting with an antigen of the infecting cell or virus presented on the MHC II of an APC.

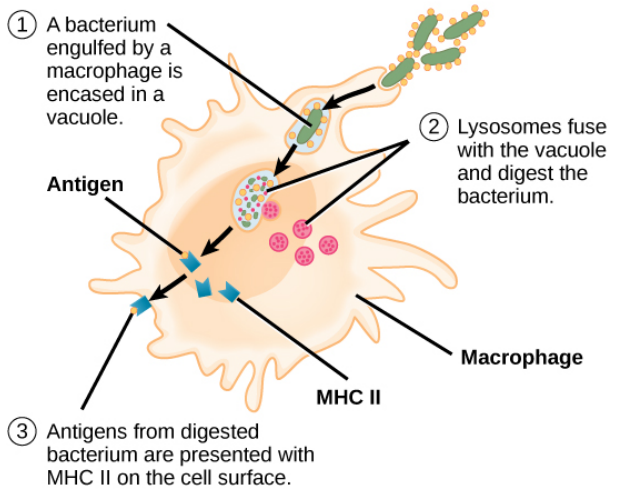


Figure **6.3.3.1**: APCs, MHCs and lymphocytes: An antigen-presenting cell (APC), such as a macrophage, engulfs a foreign antigen, partially digests it in a lysosome, and then embeds it in an MHC class II molecule for presentation at the cell surface. Lymphocytes of the adaptive immune response must interact with antigen-embedded MHC class II molecules to mature into functional immune cells.

CYTOTOXIC T CELLS MEDIATE ONE ARM OF THE CELLULAR IMMUNE RESPONSE

There are two main types of T cells: helper T lymphocytes (T_H) and the cytotoxic T lymphocytes (T_C). The T_H lymphocytes function indirectly to tell other immune cells about potential pathogens, while cytotoxic T cells (T_C) are the key component of the cell-mediated part of the adaptive immune system which attacks and destroys infected cells. T_C cells are particularly important in protecting against viral infections because viruses replicate within cells where they are shielded from extracellular contact with circulating antibodies. Once activated, the T_C creates a large clone of cells with one specific set of cell-surface receptors, similar to the proliferation of activated B cells. As with B cells, the clone includes active T_C cells and inactive memory T_C cells. The resulting active T_C cells then identify infected host cells.

T_C cells attempt to identify and destroy infected cells by triggering apoptosis (programmed cell death) before the pathogen can replicate and escape, thereby halting the progression of intracellular infections. To recognize which cells to pursue, T_C recognize antigens presented on MHC I complexes, which are present on all nucleated cells. MHC I complexes display a current readout of intracellular proteins inside a cell and will present pathogen antigens if the pathogen is present in the cell. T_C cells also support NK lymphocytes to destroy early cancers.

CYTOKINES RELEASED BY T_H CELLS RECRUIT NK CELLS AND PHAGOCYTES

Cytokines are signaling molecules secreted by a T_H cell in response to a pathogen-infected cell; they stimulate natural killer cells and phagocytes such as macrophages. Phagocytes will then engulf infected cells and destroy them. Cytokines are also involved in stimulating T_C cells, enhancing their ability to identify and destroy infected cells and tumors. A summary of how the humoral and cell-mediated immune responses are activated appears in. B plasma cells and T_C cells are collectively called effector cells because they are involved in “effecting” (bringing about) the immune response of killing pathogens and infected host cells.

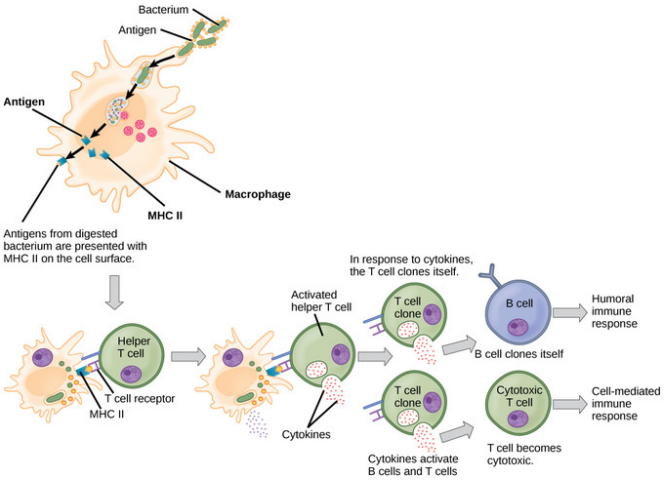


Figure **6.3.3.1**: Helper T cells in the immune response: A helper T cell becomes activated by binding to an antigen presented by an APC via the MHCII receptor, causing it to release cytokines. Depending on the cytokines released, this activates either the humoral or the cell-mediated immune response.

KEY POINTS

- Once a pathogen enters a cell, it can no longer be detected by the humoral immune response; instead, the cell-mediated immune response must take over to kill the infected cell before it can allow the virus or bacteria to replicate and spread.
- T cells recognize infected cells by interacting with antigen present on their MHC II molecules; before a T cell can do so, it must be activated via interaction with an antigen presenting cell, or APC.
- Once a cytotoxic T cell (T_C) is activated, it will clone itself, producing many T_C cells with the correct receptors; some portion of the cells are active and will help destroy infected cells, while others are inactive memory cells that will create more active T_C cells if the infection returns.
- Helper T cells (T_H cells) also aid in cell-mediated immunity by releasing signaling molecules known as cytokines which can recruit natural killer cells and phagocytes to destroy infected cells and further activate T_C cells; they do not directly destroy pathogens.

KEY TERMS

- **cytotoxic T cell**: a subgroup of lymphocytes (white blood cells) that are capable of inducing death to infected somatic or tumor cells; part of cell-mediated immunity
- **cytokine**: any of various small regulatory proteins that regulate the cells of the immune system; they are released upon binding of PRRs to PAMPS

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6.3.3.1: T Lymphocytes

Learning Objectives

- Describe the process of T-cell maturation and thymic selection
- Explain the genetic events that lead to diversity of T-cell receptors
- Compare and contrast the various classes and subtypes of T cells in terms of activation and function
- Explain the mechanism by which superantigens effect unregulated T-cell activation

As explained in Overview of Specific Adaptive Immunity, the antibodies involved in humoral immunity often bind pathogens and toxins before they can attach to and invade host cells. Thus, humoral immunity is primarily concerned with fighting pathogens in extracellular spaces. However, pathogens that have already gained entry to host cells are largely protected from the humoral antibody-mediated defenses. Cellular immunity, on the other hand, targets and eliminates intracellular pathogens through the actions of T lymphocytes, or T cells (Figure **6.3.3.1.1**). T cells also play a more central role in orchestrating the overall adaptive immune response (humoral as well as cellular) along with the cellular defenses of innate immunity.

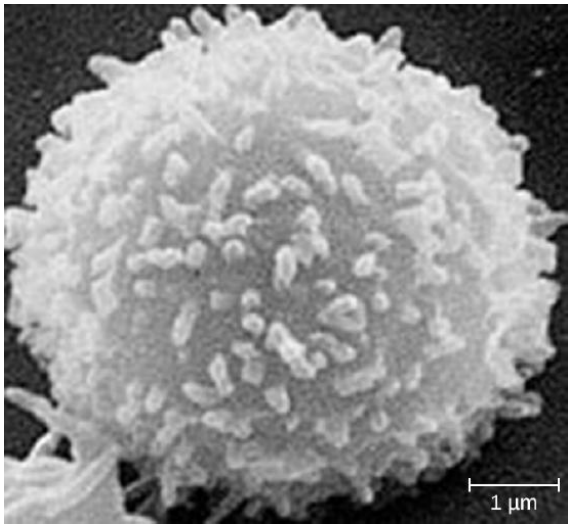


Figure **6.3.3.1.1**: This scanning electron micrograph shows a T lymphocyte, which is responsible for the cell-mediated immune response. The spike-like membrane structures increase surface area, allowing for greater interaction with other cell types and their signals. (credit: modification of work by NCI)

T Cell Production and Maturation

T cells, like all other white blood cells involved in innate and adaptive immunity, are formed from multipotent hematopoietic stem cells (HSCs) in the bone marrow (see Figure 17.3.1). However, unlike the white blood cells of innate immunity, eventual T cells differentiate first into lymphoid stem cells that then become small, immature lymphocytes, sometimes called lymphoblasts. The first steps of differentiation occur in the red marrow of bones (Figure **6.3.3.1.2**), after which immature T lymphocytes enter the bloodstream and travel to the thymus for the final steps of maturation (Figure **6.3.3.1.3**). Once in the thymus, the immature T lymphocytes are referred to as thymocytes.

The maturation of thymocytes within the thymus can be divided into tree critical steps of positive and negative selection, collectively referred to as thymic selection. The first step of thymic selection occurs in the cortex of the thymus and involves the development of a functional T-cell receptor (TCR) that is required for activation by APCs. Thymocytes with defective TCRs are removed by negative selection through the induction of apoptosis (programmed controlled cell death). The second step of thymic selection also occurs in the cortex and involves the positive selection of thymocytes that will interact appropriately with MHC molecules. Thymocytes that can interact appropriately with MHC molecules receive a positive stimulation that moves them further through the process of maturation, whereas thymocytes that do not interact appropriately are not stimulated and are eliminated by apoptosis. The third and final step of thymic selection occurs in both the cortex and medulla and involves negative selection to remove self-reacting thymocytes, those that react to self-antigens, by apoptosis. This final step is sometimes referred to as central

tolerance because it prevents self-reacting T cells from reaching the bloodstream and potentially causing autoimmune disease, which occurs when the immune system attacks healthy “self” cells.

Despite central tolerance, some self-reactive T cells generally escape the thymus and enter the peripheral bloodstream. Therefore, a second line of defense called peripheral tolerance is needed to protect against autoimmune disease. Peripheral tolerance involves mechanisms of anergy and inhibition of self-reactive T cells by regulatory T cells. Anergy refers to a state of nonresponsiveness to antigen stimulation. In the case of self-reactive T cells that escape the thymus, lack of an essential co-stimulatory signal required for activation causes anergy and prevents autoimmune activation. Regulatory T cells participate in peripheral tolerance by inhibiting the activation and function of self-reactive T cells and by secreting anti-inflammatory cytokines.

It is not completely understood what events specifically direct maturation of thymocytes into regulatory T cells. Current theories suggest the critical events may occur during the third step of thymic selection, when most self-reactive T cells are eliminated. Regulatory T cells may receive a unique signal that is below the threshold required to target them for negative selection and apoptosis. Consequently, these cells continue to mature and then exit the thymus, armed to inhibit the activation of self-reactive T cells.

It has been estimated that the three steps of thymic selection eliminate 98% of thymocytes. The remaining 2% that exit the thymus migrate through the bloodstream and lymphatic system to sites of secondary lymphoid organs/tissues, such as the lymph nodes, spleen, and tonsils (Figure **6.3.3.1.3**), where they await activation through the presentation of specific antigens by APCs. Until they are activated, they are known as mature naïve T cells.

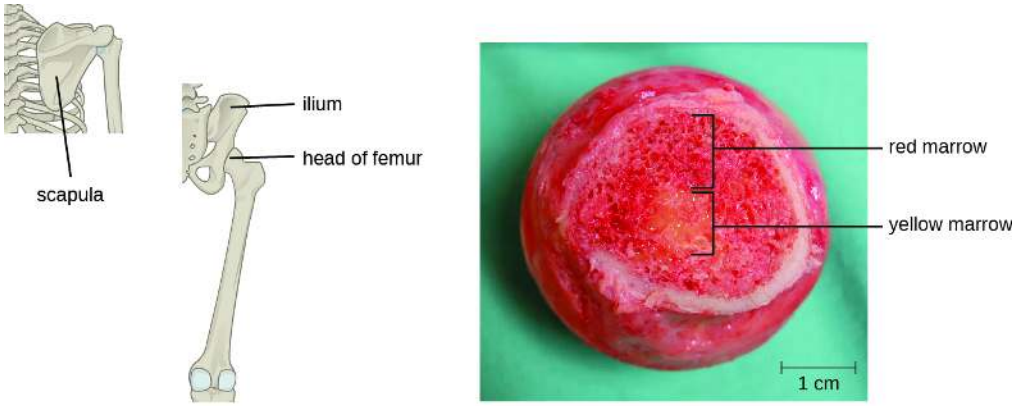


Figure **6.3.3.1.2**: (a) Red bone marrow can be found in the head of the femur (thighbone) and is also present in the flat bones of the body, such as the ilium and the scapula. (b) Red bone marrow is the site of production and differentiation of many formed elements of blood, including erythrocytes, leukocytes, and platelets. The yellow bone marrow is populated primarily with adipose cells.

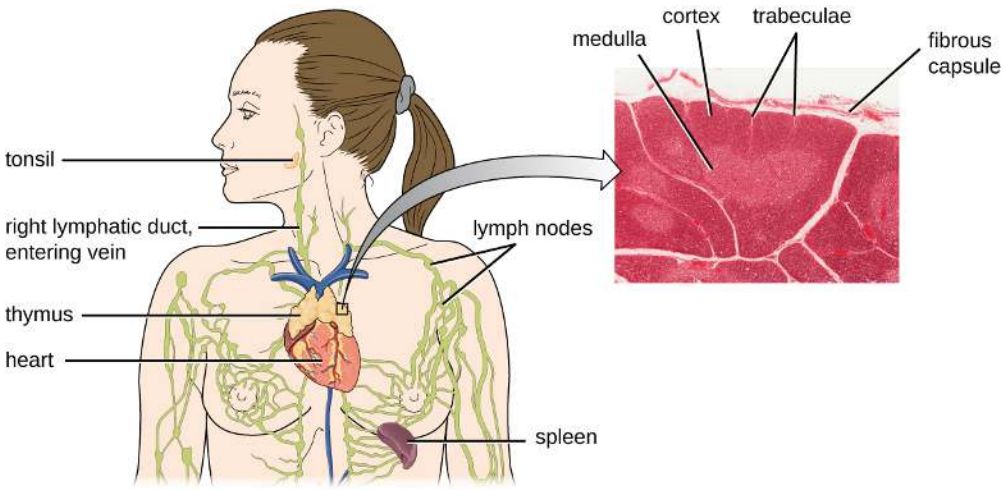


Figure **6.3.3.1.3**: The thymus is a bi-lobed, H-shaped glandular organ that is located just above the heart. It is surrounded by a fibrous capsule of connective tissue. The darkly staining cortex and the lighter staining medulla of individual lobules are clearly visible in the light micrograph of the thymus of a newborn (top right, LM × 100). (credit micrograph: modification of micrograph provided by the Regents of University of Michigan Medical School © 2012)

? Exercise 6.3.3.1.1

1. What anatomical sites are involved in T cell production and maturation?
2. What are the three steps involved in thymic selection?
3. Why are central tolerance and peripheral tolerance important? What do they prevent?

Classes of T Cells

T cells can be categorized into three distinct classes: helper T cells, regulatory T cells, and cytotoxic T cells. These classes are differentiated based on their expression of certain surface molecules, their mode of activation, and their functional roles in adaptive immunity (Table **6.3.3.1.1**).

All T cells produce cluster of differentiation (CD) molecules, cell surface glycoproteins that can be used to identify and distinguish between the various types of white blood cells. Although T cells can produce a variety of CD molecules, CD4and CD8 are the two most important used for differentiation of the classes. Helper T cells and regulatory T cells are characterized by the expression of CD4 on their surface, whereas cytotoxic T cells are characterized by the expression of CD8.

Classes of T cells can also be distinguished by the specific MHC molecules and APCs with which they interact for activation. Helper T cells and regulatory T cells can only be activated by APCs presenting antigens associated with MHC II. In contrast, cytotoxic T cells recognize antigens presented in association with MHC I, either by APCs or by nucleated cells infected with an intracellular pathogen.

The different classes of T cells also play different functional roles in the immune system. Helper T cells serve as the central orchestrators that help activate and direct functions of humoral and cellular immunity. In addition, helper T cells enhance the pathogen-killing functions of macrophages and NK cells of innate immunity. In contrast, the primary role of regulatory T cells is to prevent undesirable and potentially damaging immune responses. Their role in peripheral tolerance, for example, protects against autoimmune disorders, as discussed earlier. Finally, cytotoxic T cells are the primary effector cells for cellular immunity. They recognize and target cells that have been infected by intracellular pathogens, destroying infected cells along with the pathogens inside.

Table **6.3.3.1.1**: Classes of T Cells

Class	Surface CD Molecules	Activation	Functions
Helper T cells	CD4	APCs presenting antigens associated with MHC II	Orchestrate humoral and cellular immunity
			Involved in the activation of macrophages and NK cells
Regulatory T cells	CD4	APCs presenting antigens associated with MHC II	Involved in peripheral tolerance and prevention of autoimmune responses
Cytotoxic T cells	CD8	APCs or infected nucleated cells presenting antigens associated with MHC I	Destroy cells infected with intracellular pathogens

? Exercise 6.3.3.1.2

1. What are the unique functions of the three classes of T cells?
2. Which T cells can be activated by antigens presented by cells other than APCs?

T-Cell Receptors

For both helper T cells and cytotoxic T cells, activation is a complex process that requires the interactions of multiple molecules and exposure to cytokines. The T-cell receptor (TCR) is involved in the first step of pathogen epitope recognition during the activation process.

The TCR comes from the same receptor family as the antibodies IgD and IgM, the antigen receptors on the B cell membrane surface, and thus shares common structural elements. Similar to antibodies, the TCR has a variable regionand a constant region, and the variable region provides the antigen-binding site (Figure **6.3.3.1.4**). However, the structure of TCR is smaller and less complex than the immunoglobulin molecules (Figure 18.1.4). Whereas immunoglobulins have four peptide chains and Y-shaped structures, the TCR consists of just two peptide chains (α and β chains), both of which span the cytoplasmic membrane of the T cell.

TCRs are epitope-specific, and it has been estimated that 25 million T cells with unique epitope-binding TCRs are required to protect an individual against a wide range of microbial pathogens. Because the human genome only contains about 25,000 genes, we know that each specific TCR cannot be encoded by its own set of genes. This raises the question of how such a vast population of T cells with millions of specific TCRs can be achieved. The answer is a process called genetic rearrangement, which occurs in the thymus during the first step of thymic selection.

The genes that code for the variable regions of the TCR are divided into distinct gene segments called variable (V), diversity (D), and joining (J) segments. The genes segments associated with the α chain of the TCR consist 70 or more different V_α segments and 61 different J_α segments. The gene segments associated with the β chain of the TCR consist of 52 different V_β segments, two different D_β segments, and 13 different J_β segments. During the development of the functional TCR in the thymus, genetic rearrangement in a T cell brings together one V_α segment and one J_α segment to code for the variable region of the α chain. Similarly, genetic rearrangement brings one of the V_β segments together with one of the D_β segments and one of theJ_β segments to code for the variable region of the β chain. All the possible combinations of rearrangements between different segments of V, D, and J provide the genetic diversity required to produce millions of TCRs with unique epitope-specific variable regions.

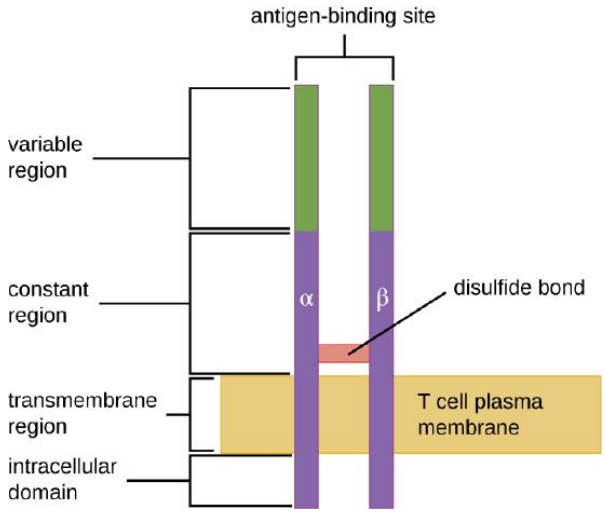


Figure **6.3.3.1.4**: A T-cell receptor spans the cytoplasmic membrane and projects variable binding regions into the extracellular space to bind processed antigens associated with MHC I or MHC II molecules.

? Exercise 6.3.3.1.3

1. What are the similarities and differences between TCRs and immunoglobulins?
2. What process is used to provide millions of unique TCR binding sites?

Activation and Differentiation of Helper T Cells

Helper T cells can only be activated by APCs presenting processed foreign epitopes in association with MHC II. The first step in the activation process is TCR recognition of the specific foreign epitope presented within the MHC II antigen-binding cleft. The second step involves the interaction of CD4 on the helper T cell with a region of the MHC II molecule separate from the antigen-binding cleft. This second interaction anchors the MHC II-TCR complex and ensures that the helper T cell is recognizing both the foreign ("nonself") epitope and "self" antigen of the APC; both recognitions are required for activation of the cell. In the third step, the APC and T cell secrete cytokines that activate the helper T cell. The activated helper T cell then proliferates, dividing by mitosis to produce clonal naïve helper T cells that differentiate into subtypes with different functions (Figure **6.3.3.1.5**).

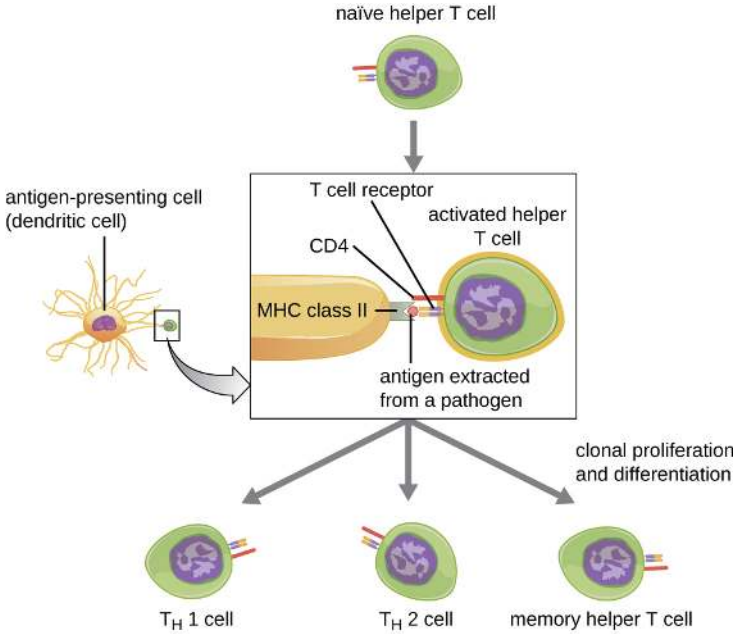


Figure **6.3.3.1.5**: This illustration depicts the activation of a naïve (unactivated) helper T cell by an antigen-presenting cell and the subsequent proliferation and differentiation of the activated T cell into different subtypes.

Activated helper T cells can differentiate into one of four distinct subtypes, summarized in Table **6.3.3.1.2**. The differentiation process is directed by APC-secreted cytokines. Depending on which APC-secreted cytokines interact with an activated helper T cell, the cell may differentiate into a T helper 1 (T_H1) cell, a T helper 2 (T_H2) cell, or a memory helper T cell. The two types of helper T cells are relatively short-lived effector cells, meaning that they perform various functions of the immediate immune response. In contrast, memory helper T cells are relatively long lived; they are programmed to "remember" a specific antigen or epitope in order to mount a rapid, strong, secondary response to subsequent exposures.

T_H1 cells secrete their own cytokines that are involved in stimulating and orchestrating other cells involved in adaptive and innate immunity. For example, they stimulate cytotoxic T cells, enhancing their killing of infected cells and promoting differentiation into memory cytotoxic T cells. T_H1 cells also stimulate macrophages and neutrophils to become more effective in their killing of intracellular bacteria. They can also stimulate NK cells to become more effective at killing target cells.

T_H2 cells play an important role in orchestrating the humoral immune response through their secretion of cytokines that activate B cells and direct B cell differentiation and antibody production. Various cytokines produced by T_H2 cells orchestrate antibody class switching, which allows B cells to switch between the production of IgM, IgG, IgA, and IgE as needed to carry out specific antibody functions and to provide pathogen-specific humoral immune responses.

A third subtype of helper T cells called T_H17 cells was discovered through observations that immunity to some infections is not associated with T_H1 or T_H2 cells. T_H17 cells and the cytokines they produce appear to be specifically responsible for the body's defense against chronic mucocutaneous infections. Patients who lack sufficient T_H17 cells in the mucosa (e.g., HIV patients) may be more susceptible to bacteremia and gastrointestinal infections.[1]

Table **6.3.3.1.2**: Subtypes of Helper T Cells

Subtype	Functions
T_H1 cells	Stimulate cytotoxic T cells and produce memory cytotoxic T cells
	Stimulate macrophages and neutrophils (PMNs) for more effective intracellular killing of pathogens
	Stimulate NK cells to kill more effectively
T_H2 cells	Stimulate B cell activation and differentiation into plasma cells and memory B cells
	Direct antibody class switching in B cells
T_H17 cells	Stimulate immunity to specific infections such as chronic mucocutaneous infections
Memory helper T cells	"Remember" a specific pathogen and mount a strong, rapid secondary response upon re-exposure

Activation and Differentiation of Cytotoxic T Cells

Cytotoxic T cells (also referred to as cytotoxic T lymphocytes, or CTLs) are activated by APCs in a three-step process similar to that of helper T cells. The key difference is that the activation of cytotoxic T cells involves recognition of an antigen presented with MHC I (as opposed to MHC II) and interaction of CD8 (as opposed to CD4) with the receptor complex. After the successful co-recognition of foreign epitope and self-antigen, the production of cytokines by the APC and the cytotoxic T cell activate clonal proliferation and differentiation. Activated cytotoxic T cells can differentiate into effector cytotoxic T cells that target pathogens for destruction or memory cells that are ready to respond to subsequent exposures.

As noted, proliferation and differentiation of cytotoxic T cells is also stimulated by cytokines secreted from T_H1 cells activated by the same foreign epitope. The co-stimulation that comes from these T_H1 cells is provided by secreted cytokines. Although it is possible for activation of cytotoxic T cells to occur without stimulation from T_H1 cells, the activation is not as effective or long-lasting.

Once activated, cytotoxic T cells serve as the effector cells of cellular immunity, recognizing and kill cells infected with intracellular pathogens through a mechanism very similar to that of NK cells. However, whereas NK cells recognize nonspecific

signals of cell stress or abnormality, cytotoxic T cells recognize infected cells through antigen presentation of pathogen-specific epitopes associated with MHC I. Once an infected cell is recognized, the TCR of the cytotoxic T cell binds to the epitope and releases perforin and granzymes that destroy the infected cell (Figure **6.3.3.1.6**). Perforin is a protein that creates pores in the target cell, and granzymes are proteases that enter the pores and induce apoptosis. This mechanism of programmed cell death is a controlled and efficient means of destroying and removing infected cells without releasing the pathogens inside to infect neighboring cells, as might occur if the infected cells were simply lysed.

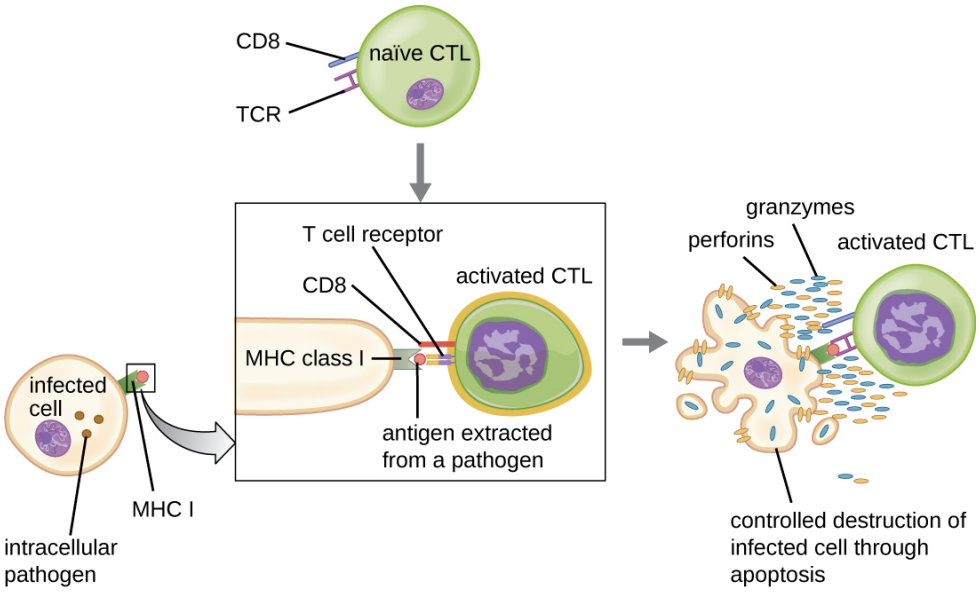


Figure **6.3.3.1.6**: This figure illustrates the activation of a naïve (unactivated) cytotoxic T cell (CTL) by an antigen-presenting MHC I molecule on an infected body cell. Once activated, the CTL releases perforin and granzymes that invade the infected cell and induce controlled cell death, or apoptosis.

Link to Learning

In this video, you can see a cytotoxic T cell inducing apoptosis in a target cell.

? Exercise 6.3.3.1.4

1. Compare and contrast the activation of helper T cells and cytotoxic T cells.
2. What are the different functions of helper T cell subtypes?
3. What is the mechanism of CTL-mediated destruction of infected cells?

Superantigens and Unregulated Activation of T Cells

When T cell activation is controlled and regulated, the result is a protective response that is effective in combating infections. However, if T cell activation is unregulated and excessive, the result can be a life-threatening. Certain bacterial and viral pathogens produce toxins known as superantigens (see Virulence Factors of Bacterial and Viral Pathogens) that can trigger such an unregulated response. Known bacterial superantigens include toxic shock syndrome toxin (TSST), staphylococcal enterotoxins, streptococcal pyrogenic toxins, streptococcal superantigen, and the streptococcal mitogenic exotoxin. Viruses known to produce superantigens include Epstein-Barr virus (human herpesvirus 4), cytomegalovirus (human herpesvirus 5), and others.

The mechanism of T cell activation by superantigens involves their simultaneous binding to MHC II molecules of APCs and the variable region of the TCR β chain. This binding occurs outside of the antigen-binding cleft of MHC II, so the superantigen will bridge together and activate MHC II and TCR without specific foreign epitope recognition (Figure **6.3.3.1.7**). The result is an excessive, uncontrolled release of cytokines, often called a cytokine storm, which stimulates an excessive inflammatory response. This can lead to a dangerous decrease in blood pressure, shock, multi-organ failure, and potentially, death.

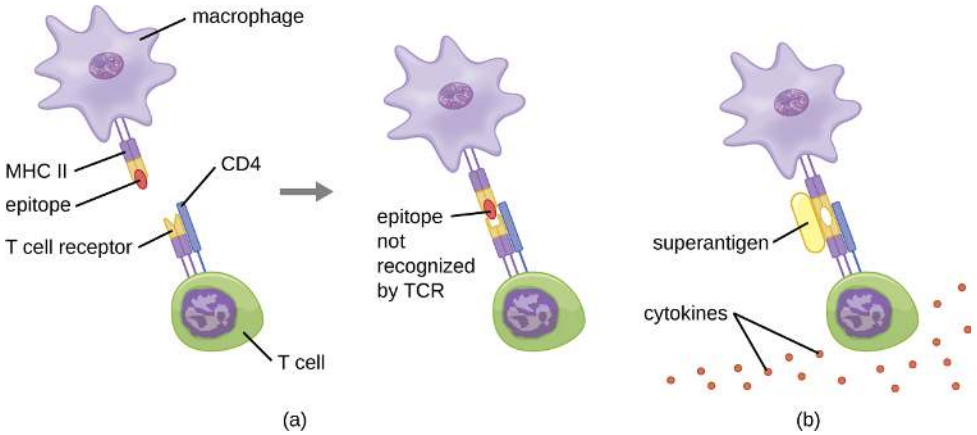


Figure **6.3.3.1.7**: (a) The macrophage in this figure is presenting a foreign epitope that does not match the TCR of the T cell. Because the T cell does not recognize the epitope, it is not activated. (b) The macrophage in this figure is presenting a superantigen that is not recognized by the TCR of the T cell, yet the superantigen still is able to bridge and bind the MHC II and TCR molecules. This nonspecific, uncontrolled activation of the T cell results in an excessive release of cytokines that activate other T cells and cause excessive inflammation. (credit: modification of work by "Microbiotic"/YouTube)

? Exercise 6.3.3.1.5

1. What are examples of superantigens?
2. How does a superantigen activate a helper T cell?
3. What effect does a superantigen have on a T cell?

Case in Point: Superantigens

Melissa, an otherwise healthy 22-year-old woman, is brought to the emergency room by her concerned boyfriend. She complains of a sudden onset of high fever, vomiting, diarrhea, and muscle aches. In her initial interview, she tells the attending physician that she is on hormonal birth control and also is two days into the menstruation portion of her cycle. She is on no other medications and is not abusing any drugs or alcohol. She is not a smoker. She is not diabetic and does not currently have an infection of any kind to her knowledge.

While waiting in the emergency room, Melissa's blood pressure begins to drop dramatically and her mental state deteriorates to general confusion. The physician believes she is likely suffering from toxic shock syndrome (TSS). TSS is caused by the toxin TSST-1, a superantigen associated with *Staphylococcus aureus*, and improper tampon use is a common cause of infections leading to TSS. The superantigen inappropriately stimulates widespread T cell activation and excessive cytokine release, resulting in a massive and systemic inflammatory response that can be fatal.

Vaginal or cervical swabs may be taken to confirm the presence of the microbe, but these tests are not critical to perform based on Melissa's symptoms and medical history. The physician prescribes rehydration, supportive therapy, and antibiotics to stem the bacterial infection. She also prescribes drugs to increase Melissa's blood pressure. Melissa spends three days in the hospital undergoing treatment; in addition, her kidney function is monitored because of the high risk of kidney failure associated with TSS. After 72 hours, Melissa is well enough to be discharged to continue her recovery at home.

Exercise 6.3.3.1.6

In what way would antibiotic therapy help to combat a superantigen?

Clinical Focus: Part 2

Olivia's swollen lymph nodes, abdomen, and spleen suggest a strong immune response to a systemic infection in progress. In addition, little Olivia is reluctant to turn her head and appears to be experiencing severe neck pain. The physician orders a complete blood count, blood culture, and lumbar puncture. The cerebrospinal fluid (CSF) obtained appears cloudy and is

further evaluated by Gram stain assessment and culturing for potential bacterial pathogens. The complete blood count indicates elevated numbers of white blood cells in Olivia's bloodstream. The white blood cell increases are recorded at 28.5 K/µL (normal range: 6.0–17.5 K/µL). The neutrophil percentage was recorded as 60% (normal range: 23–45%). Glucose levels in the CSF were registered at 30 mg/100 mL (normal range: 50–80 mg/100 mL). The WBC count in the CSF was 1,163/mm3 (normal range: 5–20/mm^3).

Exercise 6.3.3.1.7

1. Based on these results, do you have a preliminary diagnosis?
2. What is a recommended treatment based on this preliminary diagnosis?

Key Concepts and Summary

- Immature T lymphocytes are produced in the red bone marrow and travel to the thymus for maturation.
- **Thymic selection** is a three-step process of negative and positive selection that determines which T cells will mature and exit the thymus into the peripheral bloodstream.
- **Central tolerance** involves negative selection of self-reactive T cells in the thymus, and **peripheral tolerance**involves **anergy** and **regulatory T cells** that prevent self-reactive immune responses and autoimmunity.
- The **TCR** is similar in structure to immunoglobulins, but less complex. Millions of unique epitope-binding TCRs are encoded through a process of genetic rearrangement of V, D, and J gene segments.
- T cells can be divided into three classes—**helper T cells, cytotoxic T cells,** and **regulatory T cells—**based on their expression of CD4 or CD8, the MHC molecules with which they interact for activation, and their respective functions.
- Activated helper T cells differentiate into **T_H1, T_H2, T_H17**, or **memory T cell subtypes**. Differentiation is directed by the specific cytokines to which they are exposed. T_H1, T_H2, and T_H17 perform different functions related to stimulation of adaptive and innate immune defenses. Memory T cells are long-lived cells that can respond quickly to secondary exposures.
- Once activated, cytotoxic T cells target and kill cells infected with intracellular pathogens. Killing requires recognition of specific pathogen epitopes presented on the cell surface using MHC I molecules. Killing is mediated by **perforin** and **granzymes** that induce apoptosis.
- **Superantigens** are bacterial or viral proteins that cause a nonspecific activation of helper T cells, leading to an excessive release of cytokines (**cytokine storm**) and a systemic, potentially fatal inflammatory response.

Footnotes

1. 1 Blaschitz C., Raffatellu M. "Th17 cytokines and the gut mucosal barrier." J Clin Immunol. 2010 Mar; 30(2):196-203. doi: 10.1007/s10875-010-9368-7.

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6.3.4: IMMUNOLOGICAL MEMORY, PRIMARY, AND SECONDARY ADAPTIVE RESPONSE

LEARNING OBJECTIVES

- Describe the role of memory B and T cells in immunological memory

The adaptive immune system has a memory component that allows for a rapid and large response upon re-invasion of the same pathogen. During the adaptive immune response to a pathogen that has not been encountered before, known as the primary immune response, plasma cells secreting antibodies and differentiated T cells increase, then plateau over time. As B cells and T cells mature into effector cells, a subset of the naïve populations differentiates into B and T memory cells with the same antigen specificities. A memory cell is an antigen-specific B or T lymphocyte that does not differentiate into an effector cell during the primary immune response, but that can immediately become an effector cell on re-exposure to the same pathogen. As the infection is cleared and pathogenic stimuli subside, the effector cells are no longer needed; they undergo apoptosis. In contrast, the memory cells persist in circulation.

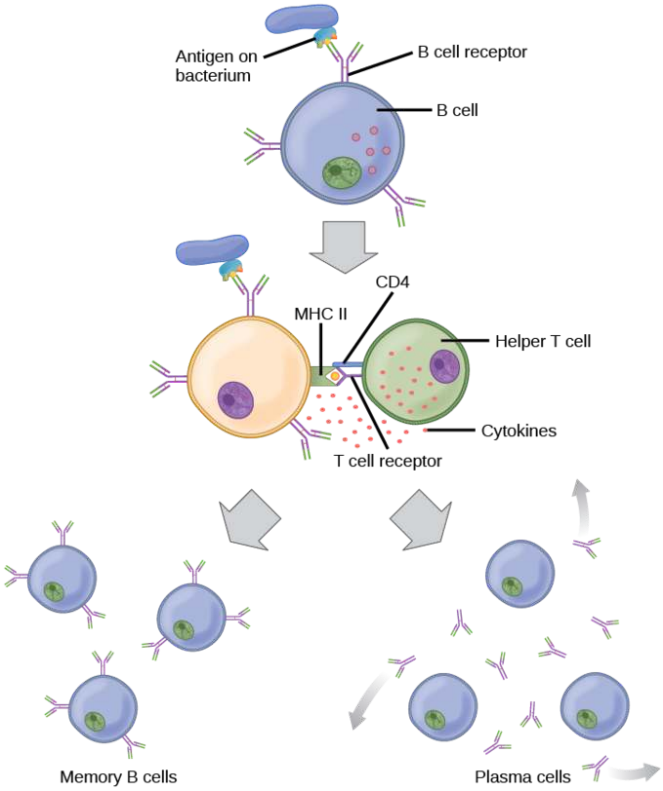


Figure **6.3.4.1**: Formation of memory B cells and plasma cells: After initially binding an antigen to the B cell receptor (BCR), a B cell internalizes the antigen and presents it on MHC II. A helper T cell recognizes the MHC II–antigen complex and activates the B cell. As a result, memory B cells and plasma cells are made.

If the pathogen is not encountered again during the individual's lifetime, B and T memory cells will circulate for a few years or even several decades, gradually dying off, having never functioned as effector cells. However, if the host is re-exposed to the same pathogen type, circulating memory cells will immediately differentiate into plasma cells and T_C cells without input from APCs or T_H cells. This is known as the secondary immune response. One reason why the adaptive immune response is delayed is that it takes time for naïve B and T cells with the appropriate antigen specificities to be identified, activated, and proliferate. On reinfection, this step is skipped. The result is a more rapid production of immune defenses. Memory B cells that differentiate into plasma cells output ten to hundred-fold greater antibody amounts than were secreted during the primary response. This rapid and dramatic antibody response may stop the infection before it can even become established. Individuals may not realize they had been exposed.

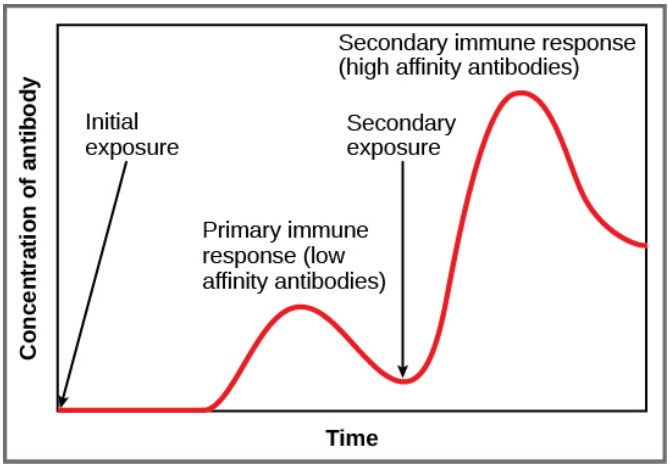


Figure **6.3.4.1**: Primary versus secondary immune response: In the primary response to infection, antibodies are secreted first from plasma cells. Upon re-exposure to the same pathogen, memory cells differentiate into antibody-secreting plasma cells that output a greater amount of antibody for a longer period of time.

Vaccination is based on the knowledge that exposure to noninfectious antigens, derived from known pathogens, generates a mild primary immune response. The immune response to vaccination may not be perceived by the host as illness, but still confers immune memory. When exposed to the corresponding pathogen to which an individual was vaccinated, the reaction is similar to a secondary exposure. Because each reinfection generates more memory cells and increased resistance to the pathogen, some vaccine courses involve one or more booster vaccinations to mimic repeat exposures.



Figure **6.3.4.1**: Vaccination: Vaccines, often delivered by injection into the arm, result in a secondary immune response if the vaccinated individual is later exposed to that pathogen.

KEY POINTS

- During the adaptive immune response to a pathogen that the body has not previously encountered (the primary immune response), the level of plasma cells that secrete antibodies to the new pathogen and T cells that recognize the pathogen will increase at a modest pace.
- If a pathogen is re-encountered, memory B and T cells can immediately differentiate into plasma cells and cytotoxic T cells without input from APCs or T_H cells; this secondary immune response occurs much more rapidly than the primary immune response.
- Vaccination can be used to generate a mild, primary immune response against an inactivated pathogen, which will allow the secondary immune response to function the first time the immune system encounters the actual pathogen.

KEY TERMS

- **vaccination**: inoculation in order to protect against a particular disease or strain of disease; causes a primary immune response without illness, allowing the secondary response to destroy subsequent infection
- **effector cell**: a plasma B cell or cytotoxic T cell, which are the main types of cells responsible for the humoral and cellular immune responses, respectively

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CHAPTER OVERVIEW

7: Microbial Metabolism

7.1: Introduction to Metabolism and Enzymes

7.1.1: Factors Affecting Enzyme Activity

7.2: Catabolism of Glucose (1)- Stages 1-3 of Cellular Respiration

7.2A: Alternative to Glycolysis- the Entner-Doudoroff Pathway

7.2B: Alternative to Glycolysis- the Pentose Phosphate Shunt

7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation

7.4: Catabolism of Glucose (3)- In the Absence of Oxygen

7.4.1: Electron Donors and Acceptors in Anaerobic Respiration

7.4.2: Fermentation

7.5: Catabolism of Lipids and Proteins

7.6: Anabolism of Glucose- Photosynthesis

7.6.1: The Two Parts of Photosynthesis

7.6.2: The Process of Photosynthesis

7.7: Other Anabolism

7.7.1: Amino Acid Synthesis

7.7.2: Polysaccharide Biosynthesis

7.7.3: Lipid Biosynthesis

7.E: Microbial Metabolism (Exercises)

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7.1: Introduction to Metabolism and Enzymes

Learning Objectives

- Define and describe metabolism
- Compare and contrast autotrophs and heterotrophs
- Describe the importance of oxidation-reduction reactions in metabolism
- Describe why ATP, FAD, NAD^+, and $NADP^+$ are important in a cell
- Identify the structure and structural components of an enzyme
- Describe the differences between competitive and noncompetitive enzyme inhibitors

Clinical Focus: Part 1

Hannah is a 15-month-old girl from Washington state. She is spending the summer in Gambia, where her parents are working for a nongovernmental organization. About 3 weeks after her arrival in Gambia, Hannah's appetite began to diminish and her parents noticed that she seemed unusually sluggish, fatigued, and confused. She also seemed very irritable when she was outdoors, especially during the day. When she began vomiting, her parents figured she had caught a 24-hour virus, but when her symptoms persisted, they took her to a clinic. The local physician noticed that Hannah's reflexes seemed abnormally slow, and when he examined her eyes with a light, she seemed unusually light sensitive. She also seemed to be experiencing a stiff neck.

Exercise 7.1.1

What are some possible causes of Hannah's symptoms?

The term used to describe all of the chemical reactions inside a cell is metabolism (Figure **7.1.1**). Cellular processes such as the building or breaking down of complex molecules occur through series of stepwise, interconnected chemical reactions called metabolic pathways. Reactions that are spontaneous and release energy are exergonic reactions, whereas endergonic reactions require energy to proceed. The term anabolism refers to those endergonic metabolic pathways involved in biosynthesis, converting simple molecular building blocks into more complex molecules, and fueled by the use of cellular energy. Conversely, the term catabolism refers to exergonic pathways that break down complex molecules into simpler ones. Molecular energy stored in the bonds of complex molecules is released in catabolic pathways and harvested in such a way that it can be used to produce high-energy molecules, which are used to drive anabolic pathways. Thus, in terms of energy and molecules, cells are continually balancing catabolism with anabolism.

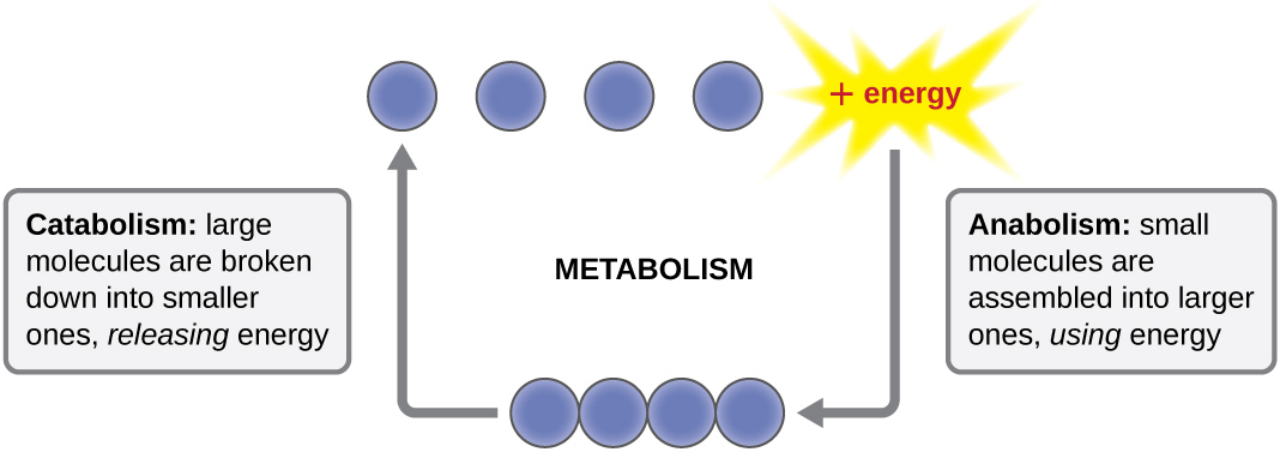


Figure **7.1.1**: Metabolism includes catabolism and anabolism. Anabolic pathways require energy to synthesize larger molecules. Catabolic pathways generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance.

Classification by Carbon and Energy Source

Organisms can be identified according to the source of carbon they use for metabolism as well as their energy source. The prefixes auto- (“self”) and hetero- (“other”) refer to the origins of the carbon sources various organisms can use. Organisms that convert inorganic carbon dioxide (CO_2) into organic carbon compounds are autotrophs. Plants and cyanobacteria are well-known examples of autotrophs. Conversely, heterotrophs rely on more complex organic carbon compounds as nutrients; these are provided to them initially by autotrophs. Many organisms, ranging from humans to many prokaryotes, including the well-studied *Escherichia coli*, are heterotrophic.

Organisms can also be identified by the energy source they use. All energy is derived from the transfer of electrons, but the source of electrons differs between various types of organisms. The prefixes photo- (“light”) and chemo- (“chemical”) refer to the energy sources that various organisms use. Those that get their energy for electron transfer from light are phototrophs, whereas chemotrophs obtain energy for electron transfer by breaking chemical bonds. There are two types of chemotrophs: organotrophs and lithotrophs. Organotrophs, including humans, fungi, and many prokaryotes, are chemotrophs that obtain energy from organic compounds. Lithotrophs (“litho” means “rock”) are chemotrophs that get energy from inorganic compounds, including hydrogen sulfide (H_2S) and reduced iron. Lithotrophy is unique to the microbial world.

The strategies used to obtain both carbon and energy can be combined for the classification of organisms according to nutritional type. Most organisms are chemoheterotrophs because they use organic molecules as both their electron and carbon sources. Table **7.1.1** summarizes this and the other classifications.

Table **7.1.1**: Classifications of Organisms by Energy and Carbon Source

Classifications		Energy Source	Carbon Source	Examples
Chemotrophs	Chemoautotrophs	Chemical	Inorganic	Hydrogen-, sulfur-, iron-, nitrogen-, and carbon monoxide-oxidizing bacteria
	Chemoheterotrophs	Chemical	Organic compounds	All animals, most fungi, protozoa, and bacteria
Phototrophs	Photoautotrophs	Light	Inorganic	All plants, algae, cyanobacteria, and green and purple sulfur bacteria
	Photoheterotrophs	Light	Organic compounds	Green and purple nonsulfur bacteria, heliobacteria

? Exercise 7.1.2

1. Explain the difference between catabolism and anabolism.
2. Explain the difference between autotrophs and heterotrophs.

Oxidation and Reduction in Metabolism

The transfer of electrons between molecules is important because most of the energy stored in atoms and used to fuel cell functions is in the form of high-energy electrons. The transfer of energy in the form of electrons allows the cell to transfer and use energy incrementally; that is, in small packages rather than a single, destructive burst. Reactions that remove electrons from donor molecules, leaving them oxidized, are oxidation reactions; those that add electrons to acceptor molecules, leaving them reduced, are reduction reactions. Because electrons can move from one molecule to another, oxidation and reduction occur in tandem. These pairs of reactions are called oxidation-reduction reactions, or redox reactions.

Energy Carriers: NAD^+, $NADP^+$, FAD, and ATP

The energy released from the breakdown of the chemical bonds within nutrients can be stored either through the reduction of electron carriers or in the bonds of adenosine triphosphate (ATP). In living systems, a small class of compounds functions as mobile electron carriers, molecules that bind to and shuttle high-energy electrons between compounds in pathways. The principal electron carriers we will consider originate from the B vitamin group and are derivatives of nucleotides; they are nicotinamide adenine dinucleotide, nicotine adenine dinucleotide phosphate, and flavin adenine dinucleotide. These compounds can be easily reduced or oxidized. Nicotinamide adenine dinucleotide (NAD^+/NADH) is the most common mobile electron carrier used in catabolism. NAD^+ is the oxidized form of the molecule; NADH is the reduced form of the molecule. Nicotine adenine dinucleotide phosphate ($NADP^+$), the oxidized form of an NAD^+ variant that contains an extra phosphate group, is another important electron carrier; it forms NADPH when reduced. The oxidized form of flavin adenine dinucleotide is FAD, and its reduced form is $FADH_2$. Both NAD^+/NADH and FAD/$FADH_2$ are extensively used in energy extraction from sugars during catabolism in chemoheterotrophs, whereas $NADP^+$/NADPH plays an important role in anabolic reactions and photosynthesis. Collectively, $FADH_2$, NADH, and NADPH are often referred to as having reducing power due to their ability to donate electrons to various chemical reactions.

A living cell must be able to handle the energy released during catabolism in a way that enables the cell to store energy safely and release it for use only as needed. Living cells accomplish this by using the compound adenosine triphosphate (ATP). ATP is often called the “energy currency” of the cell, and, like currency, this versatile compound can be used to fill any energy need of the cell. At the heart of ATP is a molecule of adenosine monophosphate (AMP), which is composed of an adenine molecule bonded to a ribose molecule and a single phosphate group. Ribose is a five-carbon sugar found in RNA, and AMP is one of the nucleotides in RNA. The addition of a second phosphate group to this core molecule results in the formation of adenosine diphosphate (ADP); the addition of a third phosphate group forms ATP (Figure **7.1.2**). Adding a phosphate group to a molecule, a process called phosphorylation, requires energy. Phosphate groups are negatively charged and thus repel one another when they are arranged in series, as they are in ADP and ATP. This repulsion makes the ADP and ATP molecules inherently unstable. Thus, the bonds between phosphate groups (one in ADP and two in ATP) are called high-energy phosphate bonds. When these high-energy bonds are broken to release one phosphate (called inorganic phosphate [P_i]) or two connected phosphate groups (called pyrophosphate [PP_i]) from ATP through a process called dephosphorylation, energy is released to drive endergonic reactions (Figure **7.1.3**).

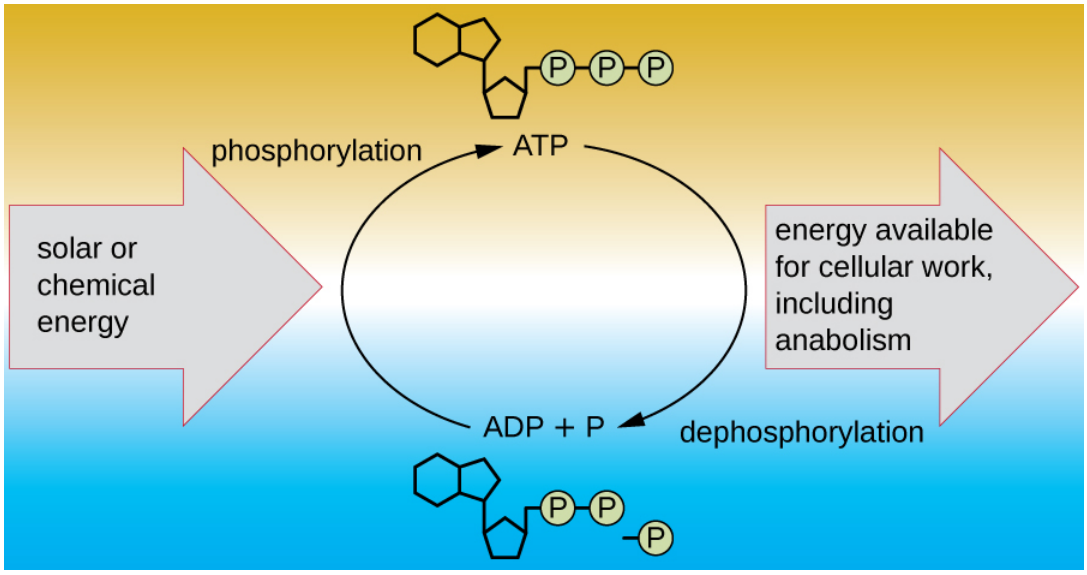


Figure **7.1.2**: The energy released from dephosphorylation of ATP is used to drive cellular work, including anabolic pathways. ATP is regenerated through phosphorylation, harnessing the energy found in chemicals or from sunlight. (credit: modification of work by Robert Bear, David Rintoul)

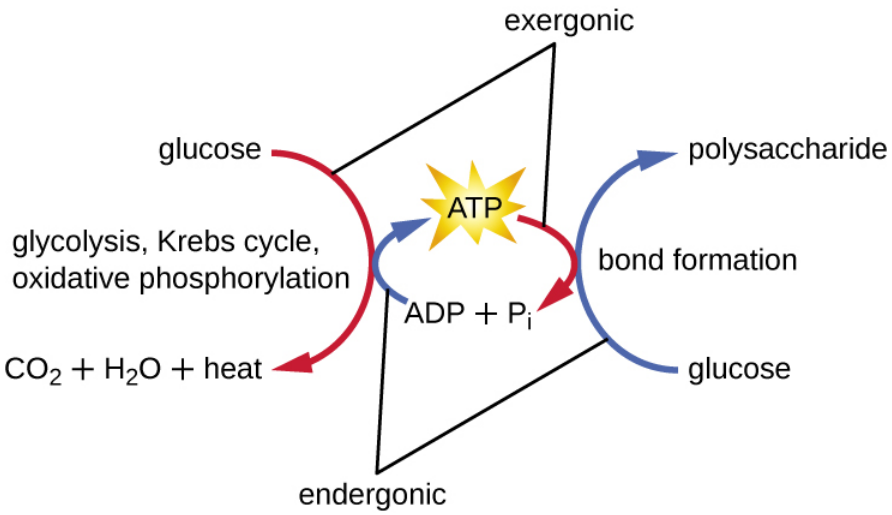


Figure **7.1.3**: Exergonic reactions are coupled to endergonic ones, making the combination favorable. Here, the endergonic reaction of ATP phosphorylation is coupled to the exergonic reactions of catabolism. Similarly, the exergonic reaction of ATP dephosphorylation is coupled to the endergonic reaction of polypeptide formation, an example of anabolism.

? Exercise 7.1.3

What is the function of an electron carrier?

Enzyme Structure and Function

A substance that helps speed up a chemical reaction is a catalyst. Catalysts are not used or changed during chemical reactions and, therefore, are reusable. Whereas inorganic molecules may serve as catalysts for a wide range of chemical reactions, proteins called enzymes serve as catalysts for biochemical reactions inside cells. Enzymes thus play an important role in controlling cellular metabolism.

An enzyme functions by lowering the activation energy of a chemical reaction inside the cell. Activation energy is the energy needed to form or break chemical bonds and convert reactants to products (Figure **7.1.4**). Enzymes lower the activation energy by binding to the reactant molecules and holding them in such a way as to speed up the reaction.

The chemical reactants to which an enzyme binds are called substrates, and the location within the enzyme where the substrate binds is called the enzyme's active site. The characteristics of the amino acids near the active site create a very specific chemical environment within the active site that induces suitability to binding, albeit briefly, to a specific substrate (or substrates). Due to this jigsaw puzzle-like match between an enzyme and its substrates, enzymes are known for their specificity. In fact, as an enzyme binds to its substrate(s), the enzyme structure changes slightly to find the best fit between the transition state (a structural intermediate between the substrate and product) and the active site, just as a rubber glove molds to a hand inserted into it. This active-site modification in the presence of substrate, along with the simultaneous formation of the transition state, is called induced fit (Figure **7.1.5**). Overall, there is a specifically matched enzyme for each substrate and, thus, for each chemical reaction; however, there is some flexibility as well. Some enzymes have the ability to act on several different structurally related substrates.

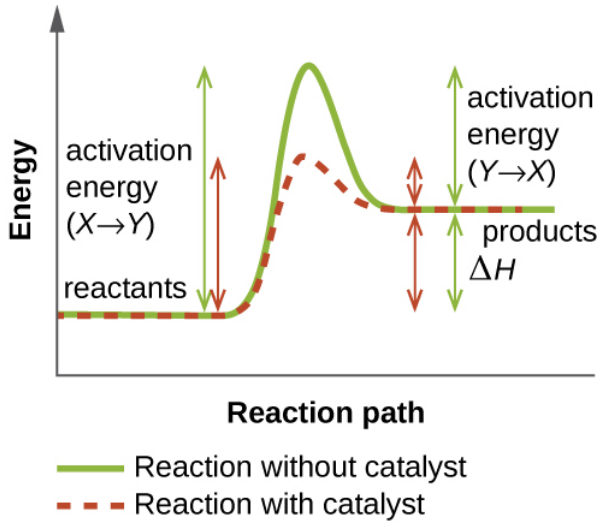


Figure **7.1.4**: Enzymes lower the activation energy of a chemical reaction.

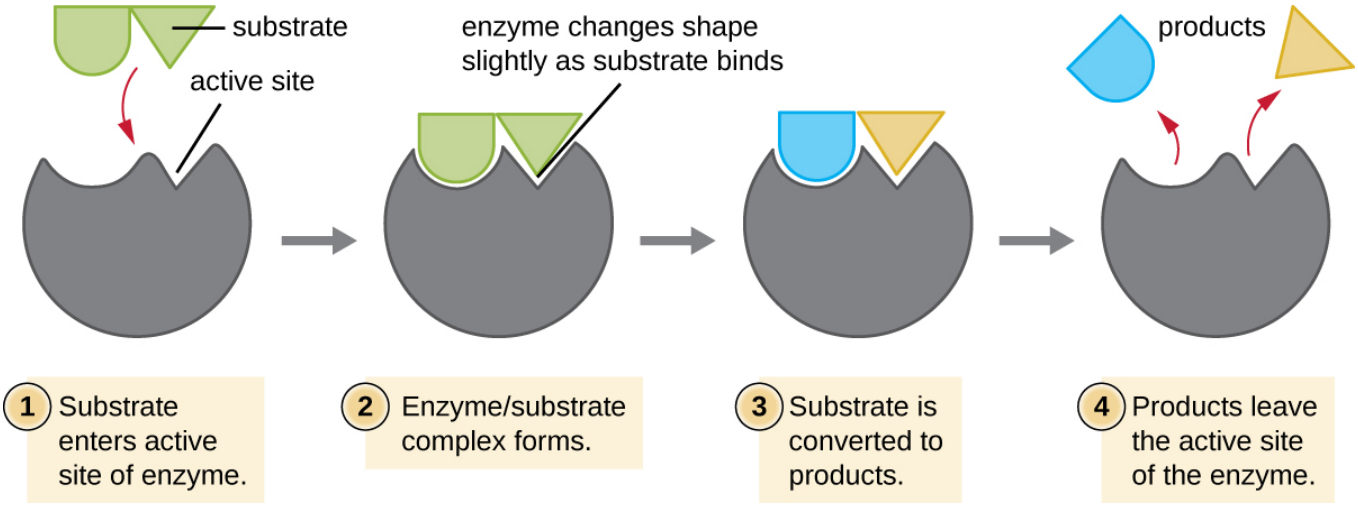


Figure **7.1.5**: According to the induced-fit model, the active site of the enzyme undergoes conformational changes upon binding with the substrate.

Enzymes are subject to influences by local environmental conditions such as pH, substrate concentration, and temperature. Although increasing the environmental temperature generally increases reaction rates, enzyme catalyzed or otherwise, increasing or decreasing the temperature outside of an optimal range can affect chemical bonds within the active site, making them less well suited to bind substrates. High temperatures will eventually cause enzymes, like other biological molecules, to denature, losing their three-dimensional structure and function. Enzymes are also suited to function best within a certain pH range, and, as with temperature, extreme environmental pH values (acidic or basic) can cause enzymes to denature. Active-site amino-acid side chains have their own acidic or basic properties that are optimal for catalysis and, therefore, are sensitive to changes in pH.

Another factor that influences enzyme activity is substrate concentration: Enzyme activity is increased at higher concentrations of substrate until it reaches a saturation point at which the enzyme can bind no additional substrate. Overall, enzymes are optimized to work best under the environmental conditions in which the organisms that produce them live. For example, while microbes that inhabit hot springs have enzymes that work best at high temperatures, human pathogens have enzymes that work best at 37°C. Similarly, while enzymes produced by most organisms work best at a neutral pH, microbes growing in acidic environments make enzymes optimized to low pH conditions, allowing for their growth at those conditions.

Many enzymes do not work optimally, or even at all, unless bound to other specific nonprotein helper molecules, either temporarily through ionic or hydrogen bonds or permanently through stronger covalent bonds. Binding to these molecules promotes optimal conformation and function for their respective enzymes. Two types of helper molecules are cofactors and coenzymes. Cofactors are inorganic ions such as iron (Fe^{2+}) and magnesium (Mg^{2+}) that help stabilize enzyme conformation and function. One example of an

enzyme that requires a metal ion as a cofactor is the enzyme that builds DNA molecules, DNA polymerase, which requires a bound zinc ion (Zn^{2+}) to function.

Coenzymes are organic helper molecules that are required for enzyme action. Like enzymes, they are not consumed and, hence, are reusable. The most common sources of coenzymes are dietary vitamins. Some vitamins are precursors to coenzymes and others act directly as coenzymes.

Some cofactors and coenzymes, like coenzyme A (CoA), often bind to the enzyme's active site, aiding in the chemistry of the transition of a substrate to a product (Figure **7.1.6**). In such cases, an enzyme lacking a necessary cofactor or coenzyme is called an apoenzyme and is inactive. Conversely, an enzyme with the necessary associated cofactor or coenzyme is called a holoenzyme and is active. NADH and ATP are also both examples of commonly used coenzymes that provide high-energy electrons or phosphate groups, respectively, which bind to enzymes, thereby activating them.

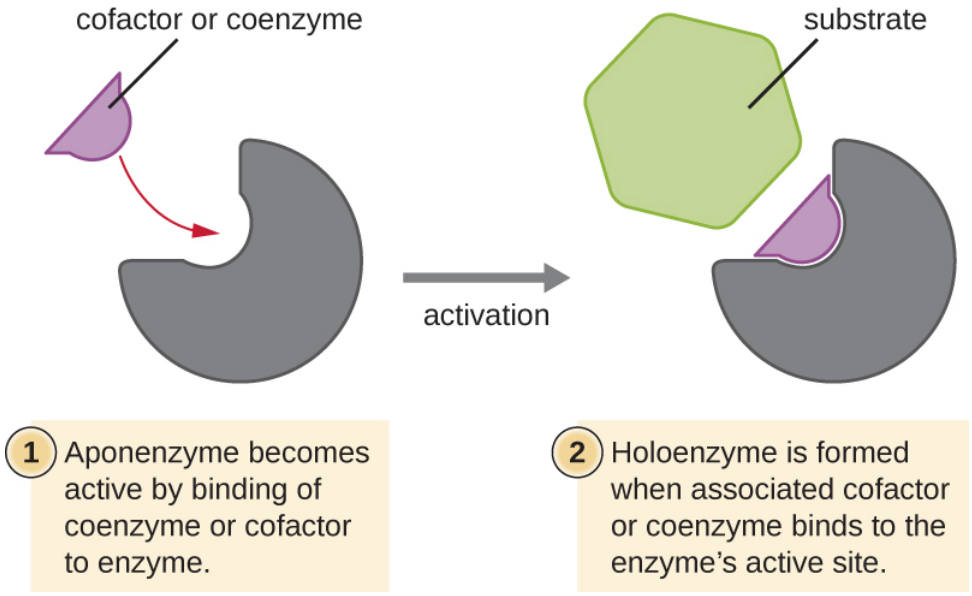


Figure **7.1.6**: The binding of a coenzyme or cofactor to an apoenzyme is often required to form an active holoenzyme.

? Exercise 7.1.4

What role do enzymes play in a chemical reaction?

Enzyme Inhibitors

Enzymes can be regulated in ways that either promote or reduce their activity. There are many different kinds of molecules that inhibit or promote enzyme function, and various mechanisms exist for doing so (Figure **7.1.7**). A competitive inhibitor is a molecule similar enough to a substrate that it can compete with the substrate for binding to the active site by simply blocking the substrate from binding. For a competitive inhibitor to be effective, the inhibitor concentration needs to be approximately equal to the substrate concentration. Sulfa drugs provide a good example of competitive competition. They are used to treat bacterial infections because they bind to the active site of an enzyme within the bacterial folic acid synthesis pathway. When present in a sufficient dose, a sulfa drug prevents folic acid synthesis, and bacteria are unable to grow because they cannot synthesize DNA, RNA, and proteins. Humans are unaffected because we obtain folic acid from our diets.

On the other hand, a noncompetitive (allosteric) inhibitor binds to the enzyme at an allosteric site, a location other than the active site, and still manages to block substrate binding to the active site by inducing a conformational change that reduces the affinity of the enzyme for its substrate (Figure **7.1.8**). Because only one inhibitor molecule is needed per enzyme for effective inhibition, the concentration of inhibitors needed for noncompetitive inhibition is typically much lower than the substrate concentration.

In addition to allosteric inhibitors, there are allosteric activators that bind to locations on an enzyme away from the active site, inducing a conformational change that increases the affinity of the enzyme's active site(s) for its substrate(s).

Allosteric control is an important mechanism of regulation of metabolic pathways involved in both catabolism and anabolism. In a most efficient and elegant way, cells have evolved also to use the products of their own metabolic reactions for feedback inhibition of enzyme activity. Feedback inhibition involves the use of a pathway product to regulate its own further production. The cell responds to the abundance of specific products by slowing production during anabolic or catabolic reactions (Figure **7.1.8**).

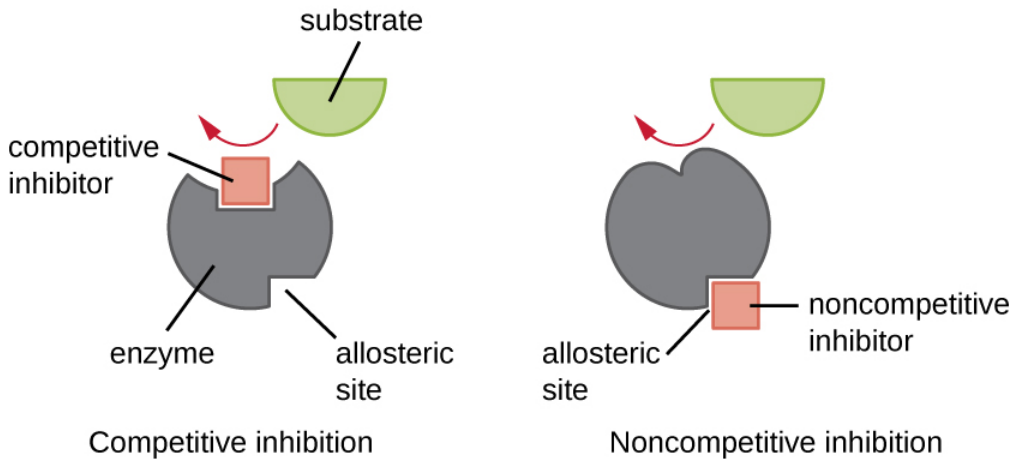


Figure **7.1.7**: Enzyme activity can be regulated by either competitive inhibitors, which bind to the active site, or noncompetitive inhibitors, which bind to an allosteric site.

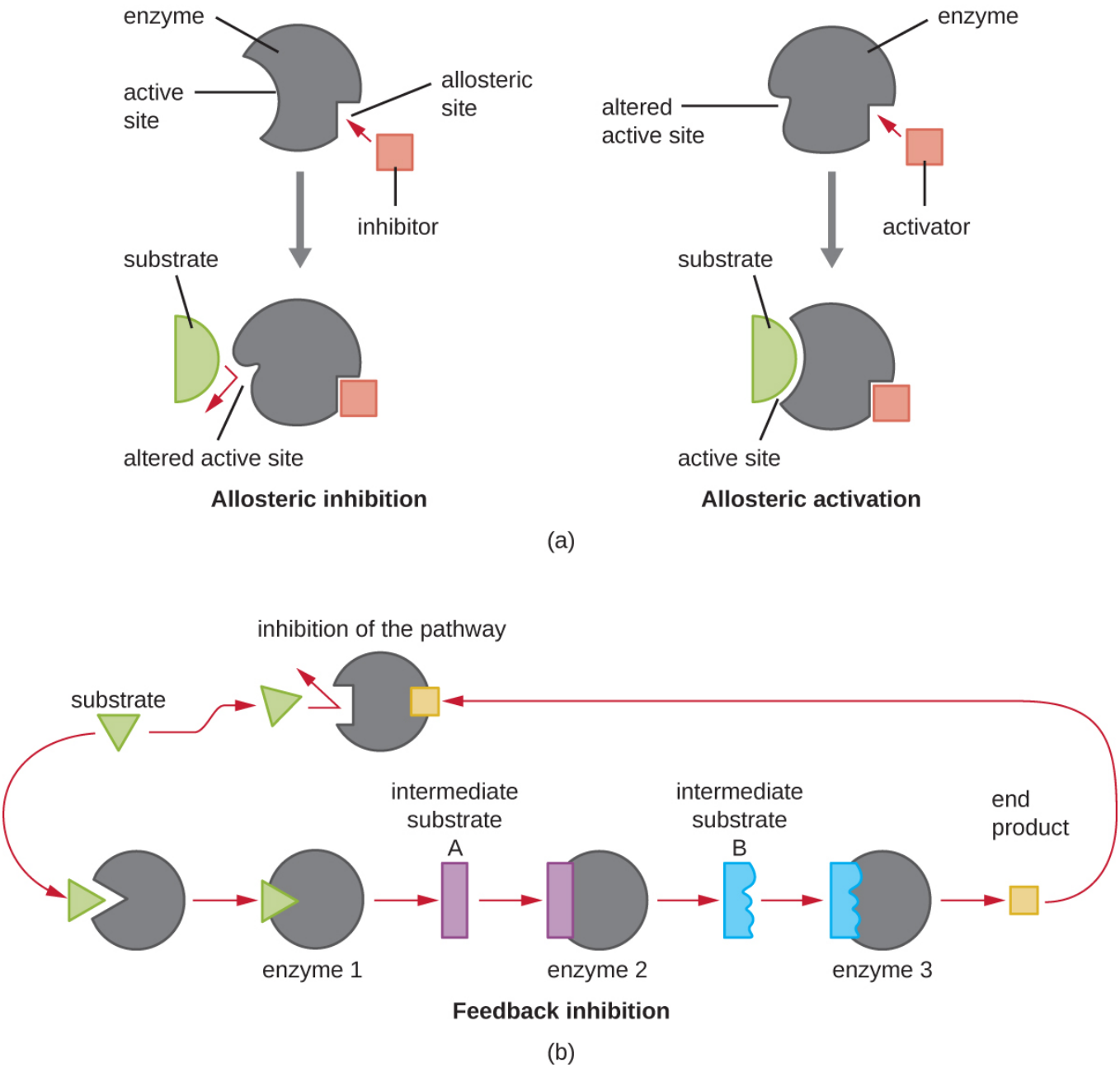


Figure **7.1.8**: (a) Binding of an allosteric inhibitor reduces enzyme activity, but binding of an allosteric activator increases enzyme activity. (b) Feedback inhibition, where the end product of the pathway serves as a noncompetitive inhibitor to an enzyme early in the pathway, is an important mechanism of allosteric regulation in cells.

? Exercise 7.1.5

Explain the difference between a competitive inhibitor and a noncompetitive inhibitor.

Key Concepts and Summary

- **Metabolism** includes chemical reactions that break down complex molecules (**catabolism**) and those that build complex molecules (**anabolism**).
- Organisms may be classified according to their source of carbon. **Autotrophs** convert inorganic carbon dioxide into organic carbon; **heterotrophs** use fixed organic carbon compounds.

- Organisms may also be classified according to their energy source. **Phototrophs** obtain their energy from light. **Chemotrophs** get their energy from chemical compounds. **Organotrophs** use organic molecules, and **lithotrophs** use inorganic chemicals.
- Cellular **electron carriers** accept high-energy electrons from foods and later serve as electron donors in subsequent **redox reactions**. **FAD/$FADH_2$, NAD^+/NADH, and $NADP^+$/NADPH** are important electron carriers.
- **Adenosine triphosphate (ATP)** serves as the energy currency of the cell, safely storing chemical energy in its two **high-energy phosphate bonds** for later use to drive processes requiring energy.
- **Enzymes** are biological **catalysts** that increase the rate of chemical reactions inside cells by lowering the activation energy required for the reaction to proceed.
- In nature, **exergonic reactions** do not require energy beyond activation energy to proceed, and they release energy. They may proceed without enzymes, but at a slow rate. Conversely, **endergonic reactions** require energy beyond activation energy to occur. In cells, endergonic reactions are coupled to exergonic reactions, making the combination energetically favorable.
- **Substrates** bind to the enzyme's **active site**. This process typically alters the structures of both the active site and the substrate, favoring transition-state formation; this is known as **induced fit**.
- **Cofactors** are inorganic ions that stabilize enzyme conformation and function. **Coenzymes** are organic molecules required for proper enzyme function and are often derived from vitamins. An enzyme lacking a cofactor or coenzyme is an **apoenzyme;** an enzyme with a bound cofactor or coenzyme is a **holoenzyme**.
- **Competitive inhibitors** regulate enzymes by binding to an enzyme's active site, preventing substrate binding. **Noncompetitive (allosteric) inhibitors** bind to **allosteric sites**, inducing a conformational change in the enzyme that prevents it from functioning. **Feedback inhibition** occurs when the product of a metabolic pathway noncompetitively binds to an enzyme early on in the pathway, ultimately preventing the synthesis of the product.

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7.1.1: Factors Affecting Enzyme Activity

Learning Objectives

1. Define or describe the following:
    a. metabolism
    b. catabolic reaction
    c. anabolic reaction
    d. enzyme
    e. substrate
    f. apoenzyme
    g. haloenzyme
    h. cofactor (coenzyme)
2. State how enzymes are able to speed up the rate of chemical reactions.
3. Briefly describe a generalized enzyme-substrate reaction, state the function of an enzyme's active site, and describe how an enzyme is able to speed up chemical reactions.
4. State four characteristics of enzymes.
5. State how the following affect the rate of an enzyme reaction.
    a. enzyme concentration
    b. substrate concentration
    c. temperature
    d. pH
    e. salt concentration
6. State how chemicals such as chlorine, iodine, iodophores, mercurials, and ethylene oxide inhibit or kill bacteria.
7. State how high temperature and low temperature exert their effect on bacteria.

To live, grow, and reproduce, microorganisms undergo a variety of chemical changes. They alter nutrients so they can enter the cell and they change them once they enter in order to synthesize cell parts and obtain energy. Metabolism refers to all of the organized chemical reactions in a cell. Reactions in which chemical compounds are broken down are called catabolic reactions while reactions in which chemical compounds are synthesized are termed anabolic reactions. All of these reactions are under the control of enzymes.

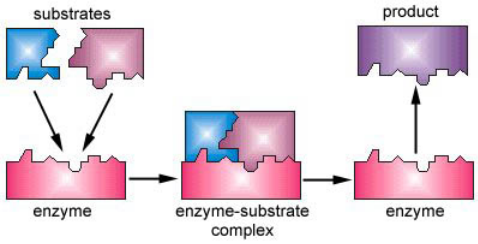


Figure **7.1.1.1**: Enzymesare substances present in the cell in small amounts which speed up or catalyze chemical reactions. Enzymes speed up the rate of chemical reactions because they lower the energy of activation, the energy that must be supplied in order for molecules to react with one another. Enzymes lower the energy of activation by forming an enzyme-substrate complex.

Enzymes are substances present in the cell in small amounts that function to speed up or catalyze chemical reactions. On the surface of the enzyme is usually a small crevice that functions as an active site or catalytic site to which one or two specific substrates are able to bind. (Anything that an enzyme normally combines with is called a substrate.) The binding of the substrate to the enzyme causes the flexible enzyme to change its shape slightly through a process called induced fit to form a tempore intermediate called an enzyme-substrate complex (Figure **7.1.1.1**).

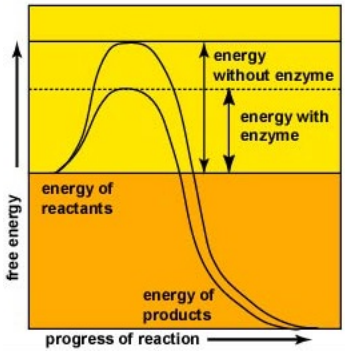


Figure 7.1.1.2: An enzyme speeds up a chemical reaction by lowering its energy of activation, the energy that must be supplied in order for molecules to react with one another.

Enzymes speed up the rate of chemical reactions because they lower the energy of activation, the energy that must be supplied in order for molecules to react with one another (Figure 7.1.1.2). Enzymes lower the energy of activation by forming an enzyme-substrate complex allowing products of the enzyme reaction to be formed and released (Figure 7.1.1.3).

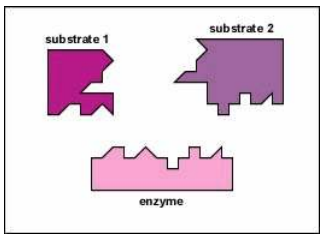


Figure 7.1.1.3: Enzyme-Substrate Reaction. Enzymesare substances present in the cell in small amounts which speed up or catalyze chemical reactions. Enzymes speed up the rate of chemical reactions because they lower the energy of activation, the energy that must be supplied in order for molecules to react with one another. Enzymes lower the energy of activation by forming an enzyme-substrate complex.

Many enzymes require a nonprotein cofactor to assist them in their reaction. In this case, the protein portion of the enzyme, called an apoenzyme, combines with the cofactor to form the whole enzyme or haloenzyme (Figure 7.1.1.3). Some cofactors are ions such as Ca^{++}, Mg^{++}, and K^{+}; other cofactors are organic molecules called coenzymes which serve as carriers for chemical groups or electrons. NAD^{+}, $NADP^{+}$, FAD, and coenzyme A (CoA) are examples of coenzymes.

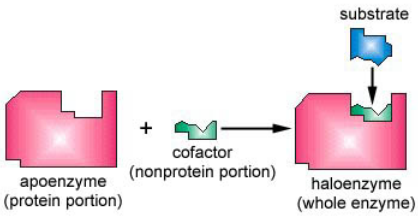


Figure 7.1.1.4: An apoenzyme and cofactor combine to form a haloenzyme. If the cofactor is an organic molecule, it is called a coenzyme.

Characteristics of Enzymes

Chemically, enzymes are generally globular proteins. (Some RNA molecules called ribozymes can also be enzymes. These are usually found in the nuclear region of cells and catalyze the splitting of RNA molecules). Enzymes are catalysts that breakdown or synthesize more complex chemical compounds. They allow chemical reactions to occur fast enough to support life. Enzymes speed up the rate of chemical reactions because they lower the energy of activation, the energy that must be supplied in order for molecules to react with one another. Anything that an enzyme normally combines with is called a substrate. Enzymes are very efficient. An enzyme generally can typically catalyze between 1 and 10,000 molecules of substrate per second. Enzymes are only present in small amounts in the cell since they are not altered during their reactions. and they are highly specific for their substrate. Generally there is one specific enzyme for each specific chemical reaction.

Enzyme Activity

Enzyme activity is affected by a number of factors including:

- **The concentration of enzyme**: Assuming a sufficient concentration of substrate is available, increasing enzyme concentration will increase the enzyme reaction rate.
- **The concentration of substrate**: At a constant enzyme concentration and at lower concentrations of substrates, the substrate concentration is the limiting factor. As the substrate concentration increases, the enzyme reaction rate increases. However, at very high substrate concentrations, the enzymes become saturated with substrate and a higher concentration of substrate does not increase the reaction rate.
- **The temperature**: Each enzyme has an optimum temperature at which it works best. A higher temperature generally results in an increase in enzyme activity. As the temperature increases, molecular motion increases resulting in more molecular collisions. If, however, the temperature rises above a certain point, the heat will denature the enzyme, causing it to lose its three-dimensional functional shape by denaturing its hydrogen bonds. Cold temperature, on the other hand, slows down enzyme activity by decreasing molecular motion.
- **The pH**: Each enzyme has an optimal pH that helps maintain its three-dimensional shape. Changes in pH may denature enzymes by altering the enzyme's charge. This alters the ionic bonds of the enzyme that contribute to its functional shape.
- **The salt concentration**: Each enzyme has an optimal salt concentration. Changes in the salt concentration may also denature enzymes.

Some relationships between bacterial enzymes and the use of disinfectants and extremes of temperature to control bacteria.

1. Many disinfectants, such as chlorine, iodine, iodophores, mercurials, silver nitrate, formaldehyde, and ethylene oxide, inactivate bacterial enzymes and thus block metabolism.
2. High temperatures, such as autoclaving, boiling, and pasteurization, denature proteins and enzymes.
3. Cold temperatures, such as refrigeration and freezing, slow down or stop enzyme reactions.

Summary

1. Enzymes are substances present in the cell in small amounts that function to speed up or catalyze chemical reactions so they occur fast enough to support life.
2. On the surface of the enzyme is typically a small crevice that functions as an active site or catalytic site to which one or two specific substrates are able to bind.
3. Anything that an enzyme normally combines with is called a substrate.
4. The binding of the substrate to the enzyme causes the flexible enzyme to change its shape slightly through a process called induced fit to form a temporary intermediate called an enzyme-substrate complex.
5. Enzymes speed up the rate of chemical reactions because they lower the energy of activation, the energy that must be supplied in order for molecules to react with one another.
6. Many enzymes require a nonprotein cofactor to assist them in their reaction. In this case, the protein portion of the enzyme, called an apoenzyme, combines with the cofactor to form the whole enzyme or haloenzyme.
7. Some cofactors are ions such as Ca^{++}, Mg^{++}, and K^{+}; other cofactors are organic molecules called coenzymes which serve as carriers for chemical groups or electrons. NAD^{+}, $NADP^{+}$, FAD, and coenzyme A (CoA) are examples of coenzymes.
8. Chemically, enzymes are generally globular proteins. Some RNA molecules called ribozymes can also be enzymes, usually functioning to cleave RNA molecules.
9. Enzymes are only present in small amounts in the cell since they are not altered during their reactions and are highly specific for their substrate.
10. Enzyme activity is affected by a number of factors including the concentration of the enzyme, the concentration of the substrate, the temperature, the pH, and the salt concentration.

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7.2: Catabolism of Glucose (1)- Stages 1-3 of Cellular Respiration

Learning Objectives

- Describe why glycolysis is not oxygen dependent
- Define and describe the net yield of three-carbon molecules, ATP, and NADH from glycolysis
- Explain how three-carbon pyruvate molecules are converted into two-carbon acetyl groups that can be funneled into the Krebs cycle.
- Define and describe the net yield of CO_2, GTP/ATP, $FADH_2$, and NADH from the Krebs cycle
- Explain how intermediate carbon molecules of the Krebs cycle can be used in a cell

Extensive enzyme pathways exist for breaking down carbohydrates to capture energy in ATP bonds. In addition, many catabolic pathways produce intermediate molecules that are also used as building blocks for anabolism. Understanding these processes is important for several reasons. First, because the main metabolic processes involved are common to a wide range of chemoheterotrophic organisms, we can learn a great deal about human metabolism by studying metabolism in more easily manipulated bacteria like *E. coli*. Second, because animal and human pathogens are also chemoheterotrophs, learning about the details of metabolism in these bacteria, including possible differences between bacterial and human pathways, is useful for the diagnosis of pathogens as well as for the discovery of antimicrobial therapies targeting specific pathogens. Last, learning specifically about the pathways involved in chemoheterotrophic metabolism also serves as a basis for comparing other more unusual metabolic strategies used by microbes. Although the chemical source of electrons initiating electron transfer is different between chemoheterorophs and chemoautotrophs, many similar processes are used in both types of organisms.

The typical example used to introduce concepts of metabolism to students is carbohydrate catabolism. For chemoheterotrophs, our examples of metabolism start with the catabolism of polysaccharides such as glycogen, starch, or cellulose. Enzymes such as amylase, which breaks down glycogen or starch, and cellulases, which break down cellulose, can cause the hydrolysis of glycosidic bonds between the glucose monomers in these polymers, releasing glucose for further catabolism.

Glycolysis

For bacteria, eukaryotes, and most archaea, glycolysis is the most common pathway for the catabolism of glucose; it produces energy, reduced electron carriers, and precursor molecules for cellular metabolism. Every living organism carries out some form of glycolysis, suggesting this mechanism is an ancient universal metabolic process. The process itself does not use oxygen; however, glycolysis can be coupled with additional metabolic processes that are either aerobic or anaerobic. Glycolysis takes place in the cytoplasm of prokaryotic and eukaryotic cells. It begins with a single six-carbon glucose molecule and ends with two molecules of a three-carbon sugar called pyruvate. Pyruvate may be broken down further after glycolysis to harness more energy through aerobic or anaerobic respiration, but many organisms, including many microbes, may be unable to respire; for these organisms, glycolysis may be their only source of generating ATP.

The type of glycolysis found in animals and that is most common in microbes is the Embden-Meyerhof-Parnas (EMP) pathway, named after Gustav Embden (1874–1933), Otto Meyerhof (1884–1951), and Jakub Parnas (1884–1949). Glycolysis using the EMP pathway consists of two distinct phases (Figure **7.2.1**). The first part of the pathway, called the energy investment phase, uses energy from two ATP molecules to modify a glucose molecule so that the six-carbon sugar molecule can be split evenly into two phosphorylated three-carbon molecules called glyceraldehyde 3-phosphate (G3P). The second part of the pathway, called the energy payoff phase, extracts energy by oxidizing G3P to pyruvate, producing four ATP molecules and reducing two molecules of NAD^+ to two molecules of NADH, using electrons that originated from glucose. (A discussion and illustration of the full EMP pathway with chemical structures and enzyme names appear in Appendix C.)

The ATP molecules produced during the energy payoff phase of glycolysis are formed by substrate-level phosphorylation (Figure **7.2.1**), one of two mechanisms for producing ATP. In substrate-level phosphorylation, a phosphate group is removed from an organic molecule and is directly transferred to an available ADP molecule, producing ATP. During glycolysis, high-energy phosphate groups from the intermediate molecules are added to ADP to make ATP.

Overall, in this process of glycolysis, the net gain from the breakdown of a single glucose molecule is:

- two ATP molecules

- two NADH molecule, and
- two pyruvate molecules.

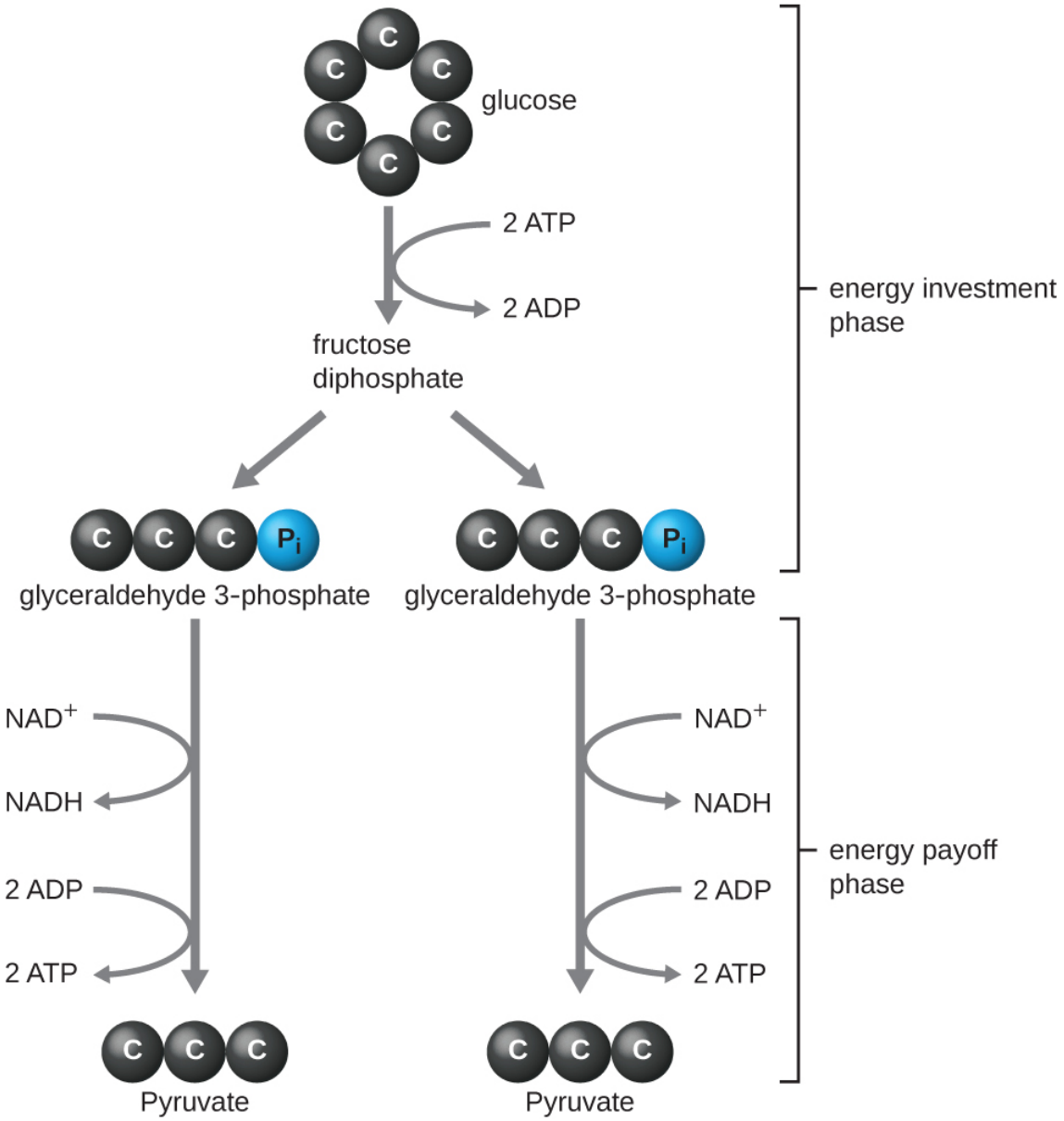


Figure **7.2.1**: The energy investment phase of the Embden-Meyerhof-Parnas glycolysis pathway uses two ATP molecules to phosphorylate glucose, forming two glyceraldehyde 3-phosphate (G3P) molecules. The energy payoff phase harnesses the energy in the G3P molecules, producing four ATP molecules, two NADH molecules, and two pyruvates.

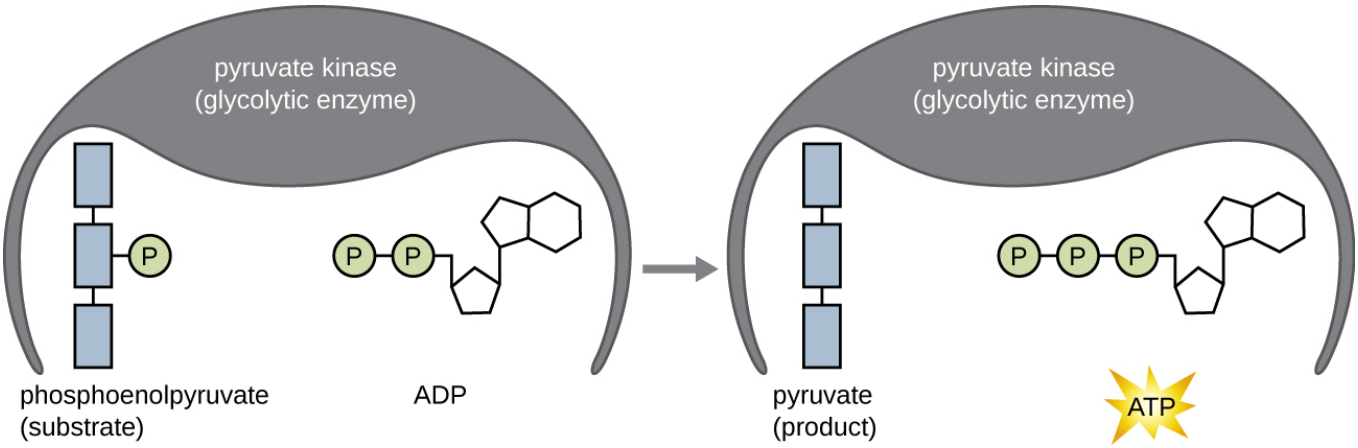


Figure **7.2.2**: The ATP made during glycolysis is a result of substrate-level phosphorylation. One of the two enzymatic reactions in the energy payoff phase of Embden Meyerhof-Parnas glycolysis that produce ATP in this way is shown here.

Other Glycolytic Pathways

When we refer to glycolysis, unless otherwise indicated, we are referring to the EMP pathway used by animals and many bacteria. However, some prokaryotes use alternative glycolytic pathways. One important alternative is the Entner-Doudoroff (ED) pathway, named after its discoverers Nathan Entner and Michael Doudoroff (1911–1975). Although some bacteria, including the opportunistic gram-negative pathogen *Pseudomonas aeruginosa*, contain only the ED pathway for glycolysis, other bacteria, like *E. coli*, have the ability to use either the ED pathway or the EMP pathway.

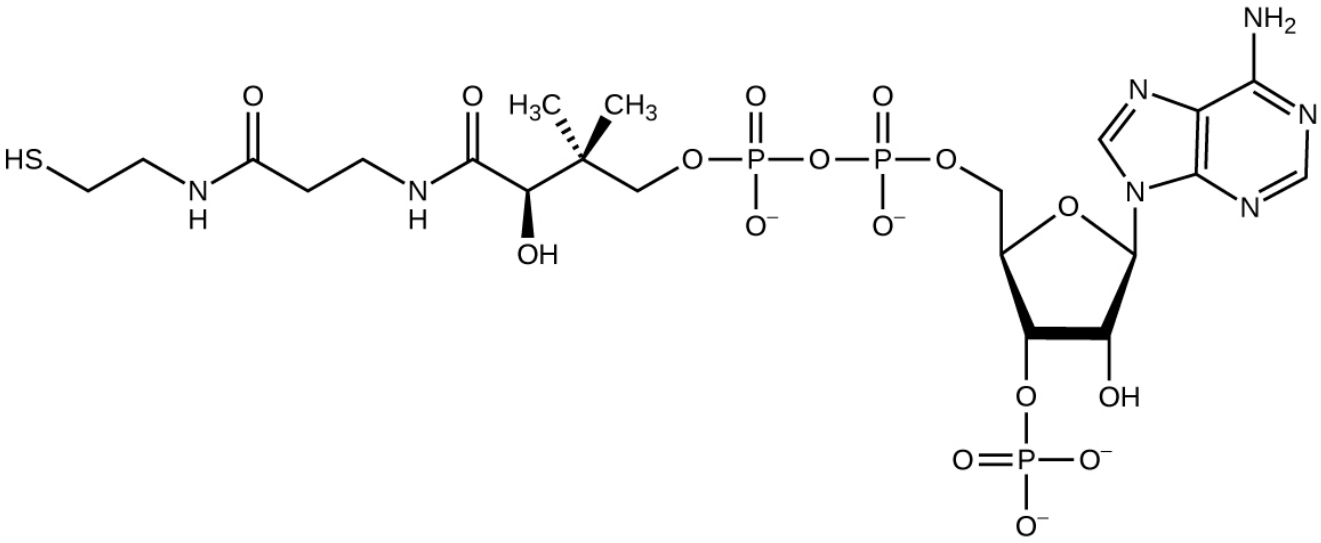
A third type of glycolytic pathway that occurs in all cells, which is quite different from the previous two pathways, is the pentose phosphate pathway (PPP) also called the phosphogluconate pathway or the hexose monophosphate shunt. Evidence suggests that the PPP may be the most ancient universal glycolytic pathway. The intermediates from the PPP are used for the biosynthesis of nucleotides and amino acids. Therefore, this glycolytic pathway may be favored when the cell has need for nucleic acid and/or protein synthesis, respectively. A discussion and illustration of the complete ED pathway and PPP with chemical structures and enzyme names appear in Appendix C.

? Exercise 7.2.1

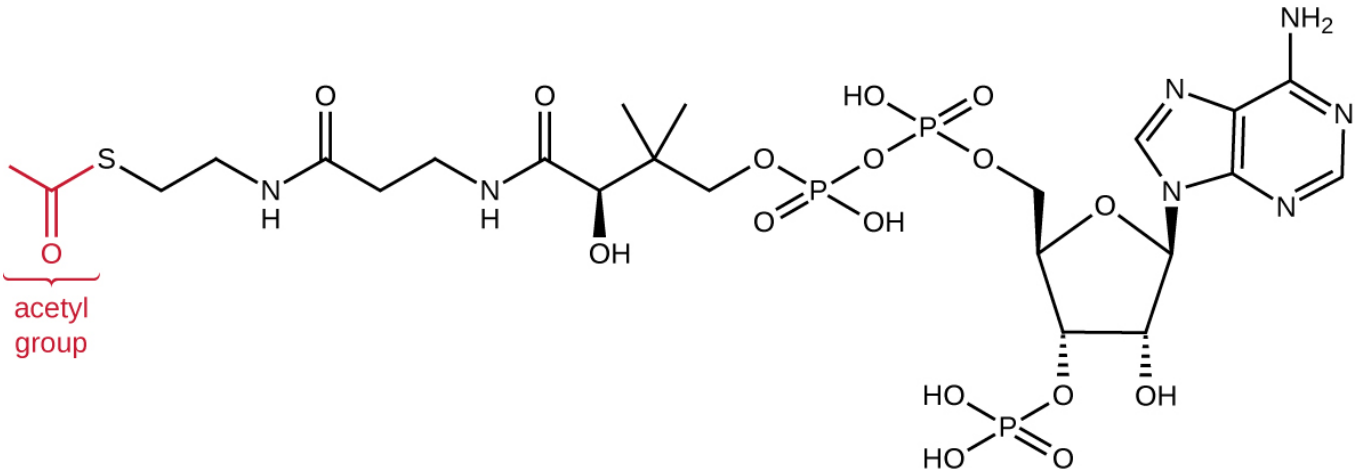
When might an organism use the ED pathway or the PPP for glycolysis?

Transition Reaction, Coenzyme A, and the Krebs Cycle

Glycolysis produces pyruvate, which can be further oxidized to capture more energy. For pyruvate to enter the next oxidative pathway, it must first be decarboxylated by the enzyme complex pyruvate dehydrogenase to a two-carbon acetyl group in the transition reaction, also called the bridge reaction (see Appendix C and Figure **7.2.3**). In the transition reaction, electrons are also transferred to NAD^+ to form NADH. To proceed to the next phase of this metabolic process, the comparatively tiny two-carbon acetyl must be attached to a very large carrier compound called coenzyme A (CoA). The transition reaction occurs in the mitochondrial matrix of eukaryotes; in prokaryotes, it occurs in the cytoplasm because prokaryotes lack membrane-enclosed organelles.



(a) coenzyme A without an attached acetyl group



(b) coenzyme A with an attached acetyl group

Figure **7.2.3**: (a) Coenzyme A is shown here without an attached acetyl group. (b) Coenzyme A is shown here with an attached acetyl group.

The Krebs cycle transfers remaining electrons from the acetyl group produced during the transition reaction to electron carrier molecules, thus reducing them. The Krebs cycle also occurs in the cytoplasm of prokaryotes along with glycolysis and the transition reaction, but it takes place in the mitochondrial matrix of eukaryotic cells where the transition reaction also occurs. The Krebs cycle is named after its discoverer, British scientist Hans Adolf Krebs (1900–1981) and is also called the citric acid cycle, or the tricarboxylic acid cycle (TCA) because citric acid has three carboxyl groups in its structure. Unlike glycolysis, the Krebs cycle is a closed loop: The last part of the pathway regenerates the compound used in the first step (Figure **7.2.4**). The eight steps of the cycle are a series of chemical reactions that capture the two-carbon acetyl group (the CoA carrier does not enter the Krebs cycle) from the transition reaction, which is added to a four-carbon intermediate in the Krebs cycle, producing the six-carbon intermediate citric acid (giving the alternate name for this cycle). As one turn of the cycle returns to the starting point of the four-carbon intermediate, the cycle produces two CO_2 molecules, one ATP molecule (or an equivalent, such as guanosine triphosphate [GTP]) produced by substrate-level phosphorylation, and three molecules of NADH and one of $FADH_2$. (A discussion and detailed illustration of the full Krebs cycle appear in Appendix C.)

Although many organisms use the Krebs cycle as described as part of glucose metabolism, several of the intermediate compounds in the Krebs cycle can be used in synthesizing a wide variety of important cellular molecules, including amino acids, chlorophylls,

fatty acids, and nucleotides; therefore, the cycle is both anabolic and catabolic (Figure 7.2.5).

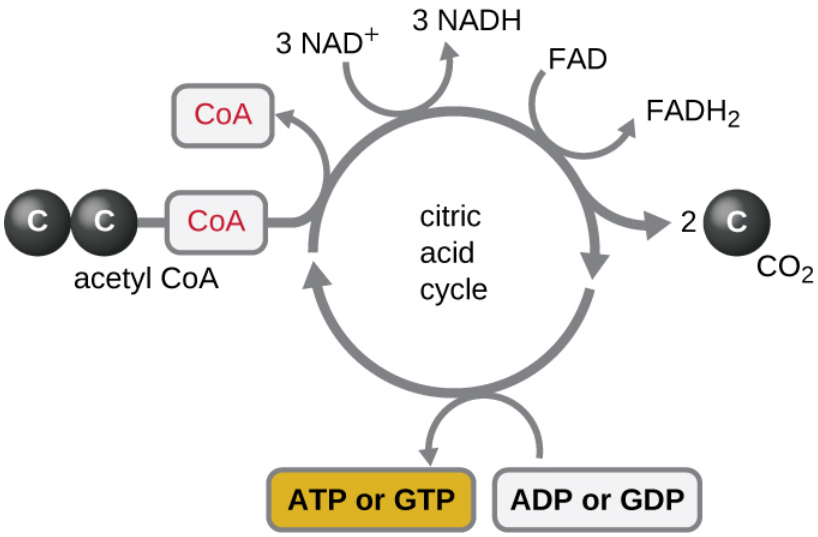


Figure 7.2.4: The Krebs cycle, also known as the citric acid cycle, is summarized here. Note incoming two-carbon acetyl results in the main outputs per turn of two CO_2, three NADH, one $FADH_2$, and one ATP (or GTP) molecules made by substrate-level phosphorylation. Two turns of the Krebs cycle are required to process all of the carbon from one glucose molecule.

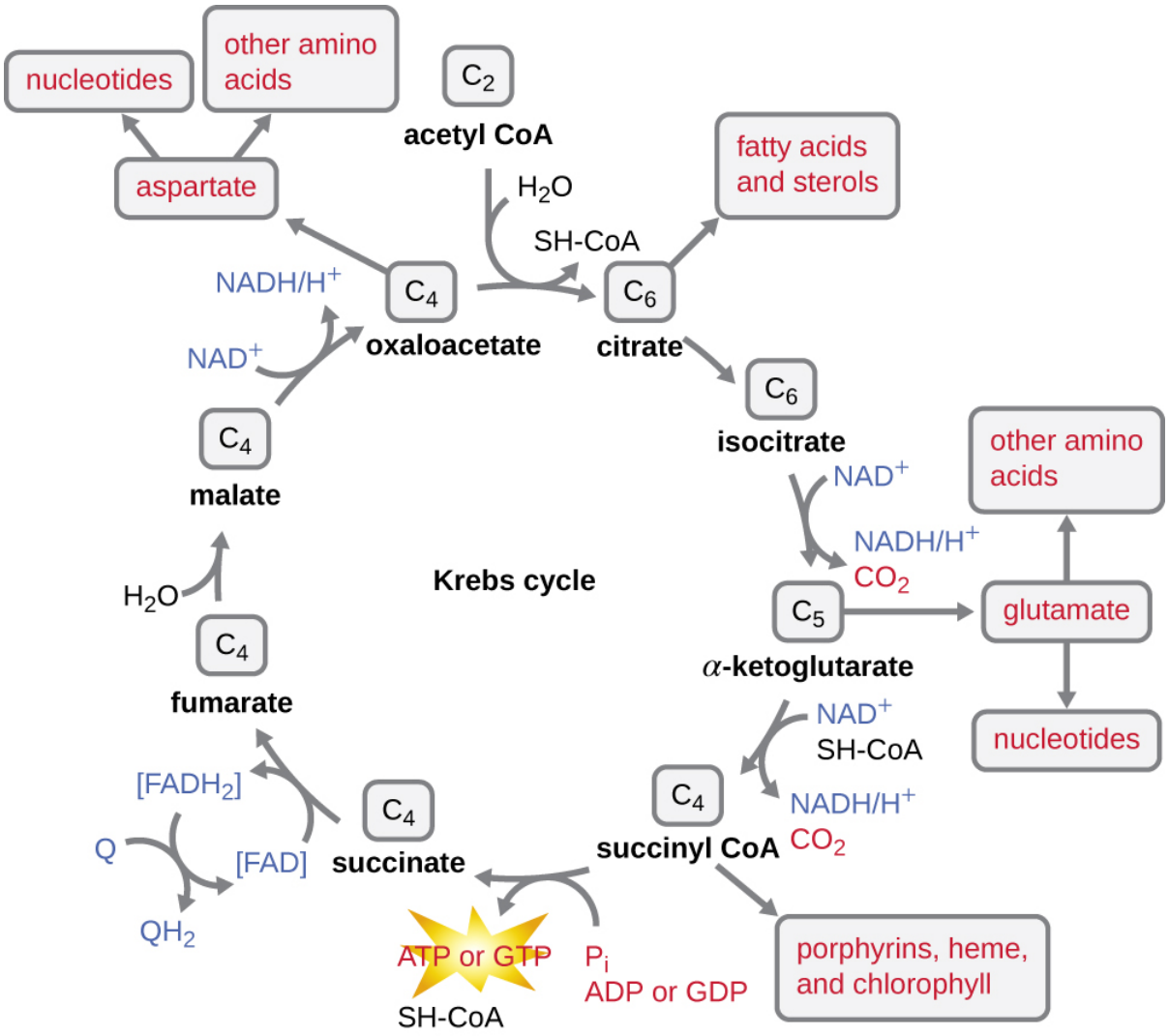


Figure **7.2.5**: Many organisms use intermediates from the Krebs cycle, such as amino acids, fatty acids, and nucleotides, as building blocks for biosynthesis.

Key Concepts and Summary

- **Glycolysis** is the first step in the breakdown of glucose, resulting in the formation of ATP, which is produced by **substrate-level phosphorylation**; NADH; and two pyruvate molecules. Glycolysis does not use oxygen and is not oxygen dependent.
- After glycolysis, a three-carbon pyruvate is decarboxylated to form a two-carbon acetyl group, coupled with the formation of NADH. The acetyl group is attached to a large carrier compound called coenzyme A.
- After the transition step, coenzyme A transports the two-carbon acetyl to the **Krebs cycle**, where the two carbons enter the cycle. Per turn of the cycle, one acetyl group derived from glycolysis is further oxidized, producing three NADH molecules, one $FADH_2$, and one ATP by **substrate-level phosphorylation**, and releasing two CO_2 molecules.
- The Krebs cycle may be used for other purposes. Many of the intermediates are used to synthesize important cellular molecules, including amino acids, chlorophylls, fatty acids, and nucleotides.

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7.2A: Alternative to Glycolysis- the Entner-Doudoroff Pathway

Learning Objectives

- Distinguish between the Entner-Doudoroff pathway and glycolysis

The Entner–Doudoroff pathway describes an alternate series of reactions that catabolize glucose to pyruvate using a set of enzymes different from those used in either glycolysis or the pentose phosphate pathway. Glycolysis (from glycose, an older term for glucose + -lysis degradation) is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, CH_3COCOO^-. The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide). Most bacteria use glycolysis and the pentose phosphate pathway. This pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.

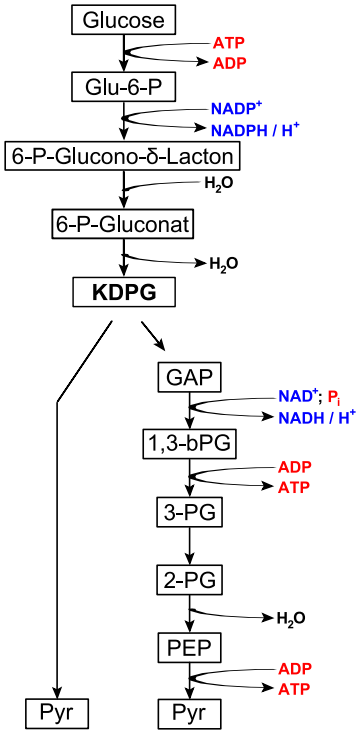


Figure: ***The Entner–Doudoroff Pathway****: This is a diagram of the Entner-Doudoroff pathway (KDPG: 2-keto-3-deoxy-6-phosphogluconate).*

Distinct features of the Entner–Doudoroff pathway are that it occurs only in prokaryotes and it uses 6-phosphogluconate dehydratase and 2-keto-3-deoxyphosphogluconate aldolase to create pyruvate from glucose. The Entner–Doudoroff pathway has a net yield of 1 ATP for every glucose molecule processed, as well as 1 NADH and 1 NADPH. By comparison, glycolysis has a net yield of 2 ATP and 2 NADH for every one glucose molecule processed.

There are a few bacteria that substitute classic glycolysis with the Entner-Doudoroff pathway. They may lack enzymes essential for glycolysis, such as phosphofructokinase-1. This pathway is generally found in *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Agrobacterium*, and a few other Gram-negative genera. Very few Gram-positive bacteria have this pathway, with *Enterococcus faecalis* being a rare exception. Most organisms that use the pathway are aerobes due to the low ATP yield per glucose such as Pseudomonas, a genus of Gram-negative bacteria, and Azotobacter, a genus of Gram-negative bacteria.

Key Points

- Glycolysis is the metabolic pathway that converts glucose into pyruvate and hydrogen ions.
- The Entner-Doudoroff pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.
- There are a few bacteria that substitute classic glycolysis with the Entner-Doudoroff pathway.

Key Terms

- **glycolysis**: The metabolic pathway that converts glucose into pyruvate and hydrogen ions.
- **ATP**: Adenosine-5′-triphosphate (ATP) is a multifunctional nucleoside triphosphate used in cells as a coenzyme. It is often called the “molecular unit of currency” of intracellular energy transfer. ATP transports chemical energy within cells for metabolism.

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7.2B: Alternative to Glycolysis- the Pentose Phosphate Shunt

Learning Objectives

- Outline the two major phases of the pentose phosphate shunt: oxidative and non-oxidative phases

The pentose phosphate pathway (PPP; also called the phosphogluconate pathway and the hexose monophosphate shunt) is a process that breaks down glucose-6-phosphate into NADPH and pentoses (5-carbon sugars) for use in downstream biological processes. There are two distinct phases in the pathway: the oxidative phase and the non-oxidative phase. The first is the oxidative phase in which glucose-6-phosphate is converted to ribulose-5-phosphate. During this process two molecules of $NADP^+$are reduced to NADPH. The overall reaction for this process is:

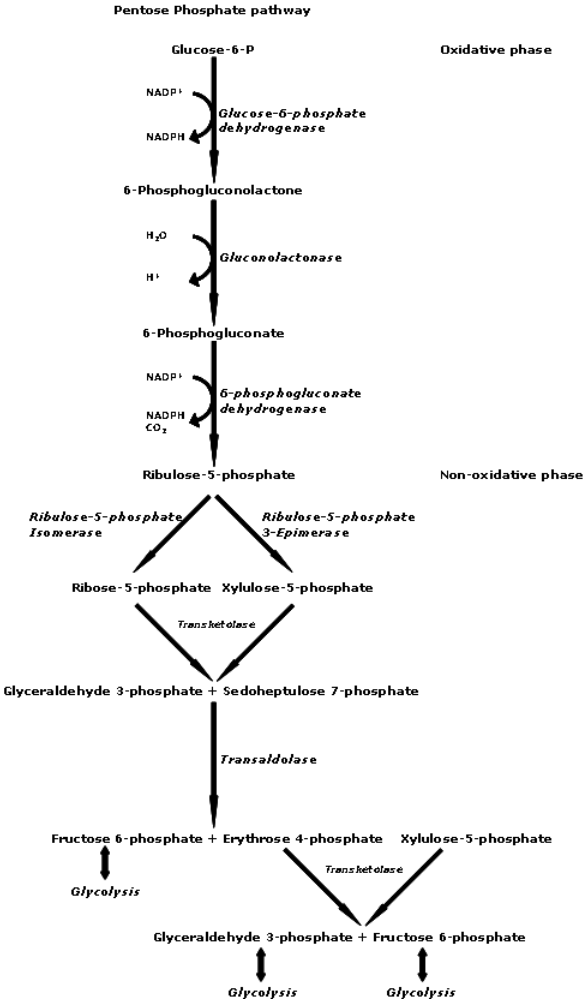


Figure: ***Figure 1 The Pentose Phosphate Pathway****: The pentose phosphate pathway generates reducing equivalents in the form of NADPH. It is used in reductive biosynthesis reactions within cells (e.g. fatty acid synthesis). It produces ribulose-5-phosphate, used in the synthesis of nucleotides. It also produces nucleic acids and erythrose-4-phosphate, used in the synthesis of aromatic amino acids.*

$$\text{Glucose 6-phosphate} + 2\ NADP^+ + H_2O \rightarrow \text{ribulose-5-phosphate} + 2\ NADPH + 2\ H^+ + CO_2$$

The second phase of this pathway is the non-oxidative synthesis of 5-carbon sugars. Depending on the body's state, ribulose-5-phosphate can reversibly isomerize to ribose-5-phosphate. Ribulose-5-phosphate can alternatively undergo a series of isomerizations as well as transaldolations and transketolations that result in the production of other pentose phosphates including fructose-6-phosphate, erythrose-4-phosphate, and glyceraldehyde-3-phosphate (both intermediates in glycolysis). These

compounds are used in a variety of different biological processes including production of nucleotides and nucleic acids (ribose-5-phosphate), as well as synthesis of aromatic amino acids (erythrose-4-phosphate).

Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme in this pathway. It is allosterically stimulated by $NADP^+$. NADPH-utilizing pathways, such as fatty acid synthesis, generate $NADP^+$, which stimulates glucose-6-phosphate dehydrogenase to produce more NADPH. In mammals, the PPP occurs exclusively in the cytoplasm; it is found to be most active in the liver, mammary gland, and adrenal cortex. The ratio of NADPH:$NADP^+$ is normally about 100:1 in liver cytosol, making the cytosol a highly-reducing environment.

The PPP is one of the three main ways the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in humans. While the PPP does involve oxidation of glucose, its primary role is anabolic rather than catabolic, using the energy stored in NADPH to synthesize large, complex molecules from small precursors.

Additionally, NADPH can be used by cells to prevent oxidative stress. NADPH reduces glutathione via glutathione reductase, which converts reactive H_2O_2 into H_2O by glutathione peroxidase. For example, erythrocytes generate a large amount of NADPH through the pentose phosphate pathway to use in the reduction of glutathione.

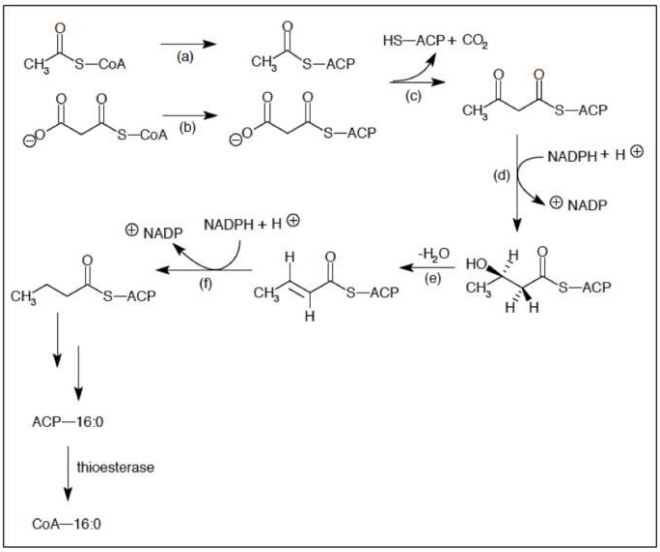


Figure: **Fatty Acid Synthesis**: Synthesis of Straight-Chain Saturated Fatty Acids

Key Points

- There are two distinct phases in the pathway: the oxidative phase and the non-oxidative phase.
- In the oxidative phase, two molecules of $NADP^+$ are reduced to NADPH, utilizing the energy from the conversion of glucose-6-phosphate into ribulose-5-phosphate. These NADPH molecules can then be used as an energy source in elsewhere in the cell.
- The non-oxidative phase generates 5-carbon sugars, which can be used in the synthesis of nucleotides, nucleic acids, and amino acids.
- The pentose phosphate pathway is an alternative to glycolysis.

Key Terms

- **glycolysis**: The cellular degradation of the simple sugar glucose to yield pyruvic acid and ATP as an energy source.
- **NADPH**: Nicotinamide adenine dinucleotide phosphate (NADP) carrying electrons and bonded with a hydrogen (H) ion; the reduced form of NADP+.
- **oxidative stress**: Damage caused to cells or tissue by reactive oxygen species.

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7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation

Learning Objectives

- Compare and contrast the electron transport system location and function in a prokaryotic cell and a eukaryotic cell
- Compare and contrast the differences between substrate-level and oxidative phosphorylation
- Explain the relationship between chemiosmosis and proton motive force
- Describe the function and location of ATP synthase in a prokaryotic versus eukaryotic cell
- Compare and contrast aerobic and anaerobic respiration

We have just discussed two pathways in glucose catabolism—glycolysis and the Krebs cycle—that generate ATP by substrate-level phosphorylation. Most ATP, however, is generated during a separate process called oxidative phosphorylation, which occurs during cellular respiration. Cellular respiration begins when electrons are transferred from NADH and $FADH_2$—made in glycolysis, the transition reaction, and the Krebs cycle—through a series of chemical reactions to a final inorganic electron acceptor (either oxygen in aerobic respiration or non-oxygen inorganic molecules in anaerobic respiration). These electron transfers take place on the inner part of the cell membrane of prokaryotic cells or in specialized protein complexes in the inner membrane of the mitochondria of eukaryotic cells. The energy of the electrons is harvested to generate an electrochemical gradient across the membrane, which is used to make ATP by oxidative phosphorylation.

Electron Transport System

The electron transport system (ETS) is the last component involved in the process of cellular respiration; it comprises a series of membrane-associated protein complexes and associated mobile accessory electron carriers. Electron transport is a series of chemical reactions that resembles a bucket brigade in that electrons from NADH and $FADH_2$ are passed rapidly from one ETS electron carrier to the next. These carriers can pass electrons along in the ETS because of their redox potential. For a protein or chemical to accept electrons, it must have a more positive redox potential than the electron donor. Therefore, electrons move from electron carriers with more negative redox potential to those with more positive redox potential. The four major classes of electron carriers involved in both eukaryotic and prokaryotic electron transport systems are the cytochromes, flavoproteins, iron-sulfur proteins, and the quinones.

In aerobic respiration, the final electron acceptor (i.e., the one having the most positive redox potential) at the end of the ETS is an oxygen molecule (O_2) that becomes reduced to water (H_2O) by the final ETS carrier. This electron carrier, cytochrome oxidase, differs between bacterial types and can be used to differentiate closely related bacteria for diagnoses. For example, the gram-negative opportunist *Pseudomonas aeruginosa* and the gram-negative cholera-causing *Vibrio cholerae* use cytochrome c oxidase, which can be detected by the oxidase test, whereas other gram-negative Enterobacteriaceae, like *E. coli*, are negative for this test because they produce different cytochrome oxidase types.

There are many circumstances under which aerobic respiration is not possible, including any one or more of the following:

- The cell lacks genes encoding an appropriate cytochrome oxidase for transferring electrons to oxygen at the end of the electron transport system.
- The cell lacks genes encoding enzymes to minimize the severely damaging effects of dangerous oxygen radicals produced during aerobic respiration, such as hydrogen peroxide (H_2O_2) or superoxide (O_2^-).
- The cell lacks a sufficient amount of oxygen to carry out aerobic respiration.

One possible alternative to aerobic respiration is anaerobic respiration, using an inorganic molecule other than oxygen as a final electron acceptor. There are many types of anaerobic respiration found in bacteria and archaea. Denitrifiers are important soil bacteria that use nitrate (NO_3^-) and nitrite (NO_2^-) as final electron acceptors, producing nitrogen gas (N_2). Many aerobically respiring bacteria, including *E. coli*, switch to using nitrate as a final electron acceptor and producing nitrite when oxygen levels have been depleted.

Microbes using anaerobic respiration commonly have an intact Krebs cycle, so these organisms can access the energy of the NADH and $FADH_2$ molecules formed. However, anaerobic respirers use altered ETS carriers encoded by their genomes, including

distinct complexes for electron transfer to their final electron acceptors. Smaller electrochemical gradients are generated from these electron transfer systems, so less ATP is formed through anaerobic respiration.

? Exercise 7.3.1

Do both aerobic respiration and anaerobic respiration use an electron transport chain?

Chemiosmosis, Proton Motive Force, and Oxidative Phosphorylation

In each transfer of an electron through the ETS, the electron loses energy, but with some transfers, the energy is stored as potential energy by using it to pump hydrogen ions (H^+) across a membrane. In prokaryotic cells, H^+ is pumped to the outside of the cytoplasmic membrane (called the periplasmic space in gram-negative and gram-positive bacteria), and in eukaryotic cells, they are pumped from the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space. There is an uneven distribution of H^+ across the membrane that establishes an electrochemical gradient because H^+ ions are positively charged (electrical) and there is a higher concentration (chemical) on one side of the membrane. This electrochemical gradient formed by the accumulation of H^+ (also known as a proton) on one side of the membrane compared with the other is referred to as the proton motive force (PMF). Because the ions involved are H^+, a pH gradient is also established, with the side of the membrane having the higher concentration of H^+ being more acidic. Beyond the use of the PMF to make ATP, as discussed in this chapter, the PMF can also be used to drive other energetically unfavorable processes, including nutrient transport and flagella rotation for motility.

The potential energy of this electrochemical gradient generated by the ETS causes the H^+ to diffuse across a membrane (the plasma membrane in prokaryotic cells and the inner membrane in mitochondria in eukaryotic cells). This flow of hydrogen ions across the membrane, called chemiosmosis, must occur through a channel in the membrane via a membrane-bound enzyme complex called ATP synthase (Figure **7.3.1**). The tendency for movement in this way is much like water accumulated on one side of a dam, moving through the dam when opened. ATP synthase (like a combination of the intake and generator of a hydroelectric dam) is a complex protein that acts as a tiny generator, turning by the force of the H^+ diffusing through the enzyme, down their electrochemical gradient from where there are many mutually repelling H^+ to where there are fewer H^+. In prokaryotic cells, H^+ flows from the outside of the cytoplasmic membrane into the cytoplasm, whereas in eukaryotic mitochondria, H^+ flows from the intermembrane space to the mitochondrial matrix. The turning of the parts of this molecular machine regenerates ATP from ADP and inorganic phosphate (P_i) by oxidative phosphorylation, a second mechanism for making ATP that harvests the potential energy stored within an electrochemical gradient.

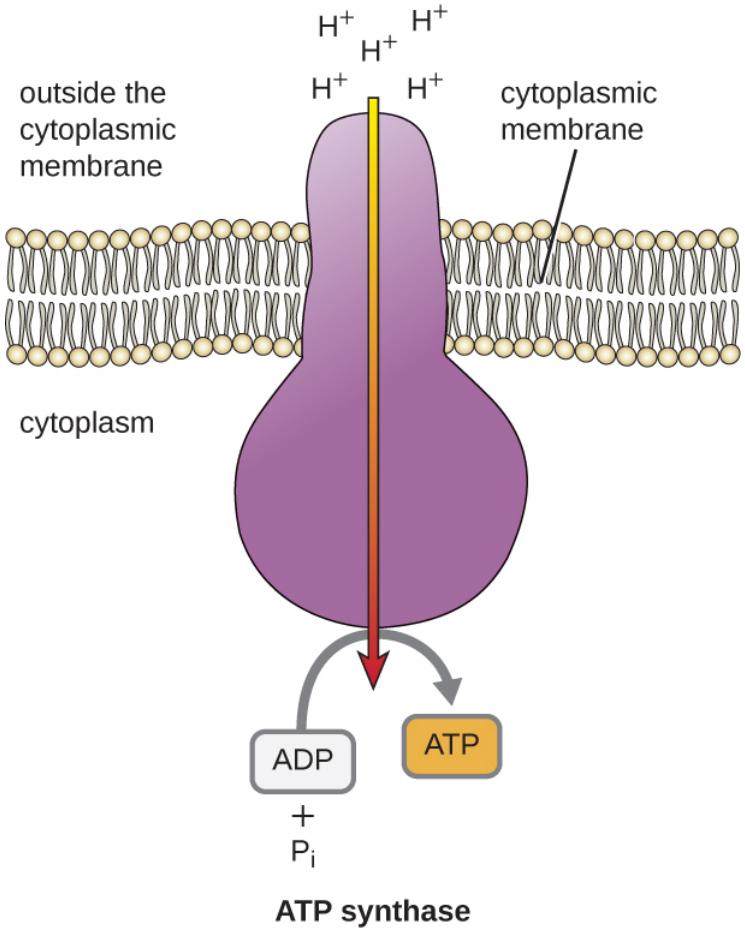


Figure **7.3.1**: ATP synthase is a complex integral membrane protein through which H^+ flows down an electrochemical gradient, providing the energy for ATP production by oxidative phosphorylation. (credit: modification of work by Klaus Hoffmeier)

The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport system complexes can pump through the membrane varies between different species of organisms. In aerobic respiration in mitochondria, the passage of electrons from one molecule of NADH generates enough proton motive force to make three ATP molecules by oxidative phosphorylation, whereas the passage of electrons from one molecule of $FADH_2$ generates enough proton motive force to make only two ATP molecules. Thus, the 10 NADH molecules made per glucose during glycolysis, the transition reaction, and the Krebs cycle carry enough energy to make 30 ATP molecules, whereas the two $FADH_2$ molecules made per glucose during these processes provide enough energy to make four ATP molecules. Overall, the theoretical maximum yield of ATP made during the complete aerobic respiration of glucose is 38 molecules, with four being made by substrate-level phosphorylation and 34 being made by oxidative phosphorylation (Figure **7.3.2**). In reality, the total ATP yield is usually less, ranging from one to 34 ATP molecules, depending on whether the cell is using aerobic respiration or anaerobic respiration; in eukaryotic cells, some energy is expended to transport intermediates from the cytoplasm into the mitochondria, affecting ATP yield.

Figure **7.3.2** summarizes the theoretical maximum yields of ATP from various processes during the complete aerobic respiration of one glucose molecule.

Source	Carbon Flow	Molecules of Reduced Coenzymes Produced	Net ATP Molecules Made by Substrate-Level Phosphory-lation	Net ATP Molecules Made by Oxidative Phosphory-lation	Theoretical Maximum Yield of ATP Molecules
Glycolysis (EMP)	Glucose (6C) ⟶ 2 pyruvates (2C)	2 NADH	2 ATP	6 ATP from 2 NADH	8
Transition reaction	2 pyruvates (3C) ⟶ 2 acetyl (2C) + 2 CO_2	2 NADH		6 ATP from 2 NADH	6
Krebs cycle	2 acetyl (2C) ⟶ 4 CO_2	6 NADH 2 $FADH_2$	2 ATP	18 ATP from 6 NADH 4 ATP from 2 $FADH_2$	24
Total:	glucose (6C) ⟶ 6 CO_2	10 NADH 2 $FADH_2$	4 ATP	34 ATP	38 ATP

Figure **7.3.2**: Theoretical maximum yields of ATP from various processes during the complete aerobic respiration of one glucose molecule.

? Exercise 7.3.1

What are the functions of the proton motive force?

Summary

- Most ATP generated during the cellular respiration of glucose is made by **oxidative phosphorylation**.
- An **electron transport system (ETS)** is composed of a series of membrane-associated protein complexes and associated mobile accessory electron carriers. The ETS is embedded in the cytoplasmic membrane of prokaryotes and the inner mitochondrial membrane of eukaryotes.
- Each ETS complex has a different redox potential, and electrons move from electron carriers with more negative redox potential to those with more positive redox potential.
- To carry out **aerobic respiration**, a cell requires oxygen as the final electron acceptor. A cell also needs a complete Krebs cycle, an appropriate cytochrome oxidase, and oxygen detoxification enzymes to prevent the harmful effects of oxygen radicals produced during aerobic respiration.
- Organisms performing **anaerobic respiration** use alternative electron transport system carriers for the ultimate transfer of electrons to the final non-oxygen electron acceptors.
- Microbes show great variation in the composition of their electron transport systems, which can be used for diagnostic purposes to help identify certain pathogens.
- As electrons are passed from NADH and $FADH_2$ through an ETS, the electron loses energy. This energy is stored through the pumping of H^+ across the membrane, generating a **proton motive force**.
- The energy of this proton motive force can be harnessed by allowing hydrogen ions to diffuse back through the membrane by **chemiosmosis** using **ATP synthase**. As hydrogen ions diffuse through down their electrochemical gradient, components of ATP synthase spin, making ATP from ADP and P_i by oxidative phosphorylation.

- Aerobic respiration forms more ATP (a maximum of 34 ATP molecules) during oxidative phosphorylation than does anaerobic respiration (between one and 32 ATP molecules).

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7.4: Catabolism of Glucose (3)- In the Absence of Oxygen

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7.4.1: Electron Donors and Acceptors in Anaerobic Respiration

In anaerobic respiration, a molecule other than oxygen is used as the terminal electron acceptor in the electron transport chain.

Learning Objectives

- Describe various types of electron acceptors and donors including: nitrate, sulfate, hydrgoen, carbon dioxide and ferric iron

Key Points

- Both inorganic and organic compounds may be used as electron acceptors in anaerobic respiration. Inorganic compounds include sulfate (SO_4^{2-}), nitrate (NO_3^-), and ferric iron (Fe^{3+}). Organic compounds include DMSO.
- These molecules have a lower reduction potential than oxygen. Therefore, less energy is formed per molecule of glucose in anaerobic versus aerobic conditions.
- The reduction of certain inorganic compounds by anaerobic microbes is often ecologically significant.

Key Terms

- **anaerobic**: Without oxygen; especially of an environment or organism.
- **reduction**: A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- **anaerobic respiration**: metabolic reactions and processes that take place in the cells of organisms that use electron acceptors other than oxygen

Anaerobic respiration is the formation of ATP without oxygen. This method still incorporates the respiratory electron transport chain, but without using oxygen as the terminal electron acceptor. Instead, molecules such as sulfate (SO_4^{2-}), nitrate (NO_3^-), or sulfur (S) are used as electron acceptors. These molecules have a lower reduction potential than oxygen; thus, less energy is formed per molecule of glucose in anaerobic versus aerobic conditions.

Many different types of electron acceptors may be used for anaerobic respiration. Denitrification is the utilization of nitrate (NO_3^-) as the terminal electron acceptor. Nitrate, like oxygen, has a high reduction potential. This process is widespread, and used by many members of Proteobacteria. Many denitrifying bacteria can also use ferric iron (Fe^{3+}) and different organic electron acceptors.

Sulfate reduction uses sulfate (SO_2^{-4}) as the electron acceptor, producing hydrogen sulfide (H_2S) as a metabolic end product. Sulfate reduction is a relatively energetically poor process, and is used by many Gram negative bacteria found within the δ-Proteobacteria. It is also used in Gram-positive organisms related to Desulfotomaculum or the archaeon Archaeoglobus.

Sulfate reduction requires the use of electron donors, such as the carbon compounds lactate and pyruvate (organotrophic reducers), or hydrogen gas (lithotrophic reducers). Some unusual autotrophic sulfate-reducing bacteria, such as *Desulfotignum phosphitoxidans*, can use phosphite (HPO_3^-) as an electron donor. Others, such as certain *Desulfovibrio* species, are capable of sulfur disproportionation (splitting one compound into an electron donor and an electron acceptor) using elemental sulfur (S0), sulfite (SO_3^{-2}), and thiosulfate ($S_2O_3^{2-}$) to produce both hydrogen sulfide (H_2S) and sulfate (SO^{2-}).

Acetogenesis is a type of microbial metabolism that uses hydrogen (H_2) as an electron donor and carbon dioxide (CO_2) as an electron acceptor to produce acetate, the same electron donors and acceptors used in methanogenesis.

Ferric iron (Fe^{3+}) is a widespread anaerobic terminal electron acceptor used by both autotrophic and heterotrophic organisms. Electron flow in these organisms is similar to those in electron transport, ending in oxygen or nitrate, except that in ferric iron-reducing organisms the final enzyme in this system is a ferric iron reductase. Since some ferric iron-reducing bacteria (*e.g.G. metallireducens*) can use toxic hydrocarbons (*e.g.* toluene) as a carbon source, there is significant interest in using these organisms as bioremediation agents in ferric iron contaminated aquifers.

Other inorganic electron acceptors include the reduction of Manganic ion (Mn^{4+}) to manganous (Mn^{2+}), Selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}) to selenium (Se), Arsenate (AsO_4^{3-}) to arsenite (AsO_3^{3-}), and Uranyl (UO_2^{2+}) to uranium dioxide (UO_2)

Organic compounds may also be used as electron acceptors in anaerobic respiration. These include the reduction of fumarate to succinate, Trimethylamine N-oxide (TMAO) to trimethylamine (TMA), and Dimethyl sulfoxide (DMSO) to Dimethyl sulfide

(DMS).

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7.4.2: Fermentation

Learning Objectives

- Define fermentation and explain why it does not require oxygen
- Describe the fermentation pathways and their end products and give examples of microorganisms that use these pathways
- Compare and contrast fermentation and anaerobic respiration

Many cells are unable to carry out respiration because of one or more of the following circumstances:

1. The cell lacks a sufficient amount of any appropriate, inorganic, final electron acceptor to carry out cellular respiration.
2. The cell lacks genes to make appropriate complexes and electron carriers in the electron transport system.
3. The cell lacks genes to make one or more enzymes in the Krebs cycle.

Whereas lack of an appropriate inorganic final electron acceptor is environmentally dependent, the other two conditions are genetically determined. Thus, many prokaryotes, including members of the clinically important genus *Streptococcus*, are permanently incapable of respiration, even in the presence of oxygen. Conversely, many prokaryotes are facultative, meaning that, should the environmental conditions change to provide an appropriate inorganic final electron acceptor for respiration, organisms containing all the genes required to do so will switch to cellular respiration for glucose metabolism because respiration allows for much greater ATP production per glucose molecule.

If respiration does not occur, NADH must be reoxidized to NAD^+ for reuse as an electron carrier for glycolysis, the cell's only mechanism for producing any ATP, to continue. Some living systems use an organic molecule (commonly pyruvate) as a final electron acceptor through a process called fermentation. Fermentation does not involve an electron transport system and does not directly produce any additional ATP beyond that produced during glycolysis by substrate-level phosphorylation. Organisms carrying out fermentation, called fermenters, produce a maximum of two ATP molecules per glucose during glycolysis. Table **7.4.2.1** compares the final electron acceptors and methods of ATP synthesis in aerobic respiration, anaerobic respiration, and fermentation. Note that the number of ATP molecules shown for glycolysis assumes the Embden-Meyerhof-Parnas pathway. The number of ATP molecules made by substrate-level phosphorylation (SLP) versus oxidative phosphorylation (OP) are indicated.

Table **7.4.2.1**: Comparison of Respiration Versus Fermentation

Type of Metabolism	Example	Final Electron Acceptor	Pathways Involved in ATP Synthesis (Type of Phosphorylation)	Maximum Yield of ATP Molecules
Aerobic respiration	*Pseudomonas aeruginosa*	O_2	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 34
			Total	**38**
Anaerobic respiration	*Paracoccus denitrificans*	NO_3^-, SO_4^{-2}, Fe^{+3}, CO_2, other inorganics	EMP glycolysis (SLP) Krebs cycle (SLP) Electron transport and chemiosmosis (OP):	2 2 1–32
			Total	**5–36**
Fermentation	*Candida albicans*	Organics (usually pyruvate)	EMP glycolysis (SLP) Fermentation	2 0
			Total	**2**

Microbial fermentation processes have been manipulated by humans and are used extensively in the production of various foods and other commercial products, including pharmaceuticals. Microbial fermentation can also be useful for identifying microbes for

diagnostic purposes.

Fermentation by some bacteria, like those in yogurt and other soured food products, and by animals in muscles during oxygen depletion, is lactic acid fermentation. The chemical reaction of lactic acid fermentation is as follows:

$$\text{Pyruvate} + \text{NADH} \leftrightarrow \text{lactic acid} + \text{NAD}^+ \quad (7.4.2.1)$$

Bacteria of several gram-positive genera, including *Lactobacillus*, *Leuconostoc*, and *Streptococcus*, are collectively known as the lactic acid bacteria (LAB), and various strains are important in food production. During yogurt and cheese production, the highly acidic environment generated by lactic acid fermentation denatures proteins contained in milk, causing it to solidify. When lactic acid is the only fermentation product, the process is said to be homolactic fermentation; such is the case for *Lactobacillus delbrueckii* and *S. thermophiles* used in yogurt production. However, many bacteria perform heterolactic fermentation, producing a mixture of lactic acid, ethanol and/or acetic acid, and CO_2 as a result, because of their use of the branched pentose phosphate pathway instead of the EMP pathway for glycolysis. One important heterolactic fermenter is *Leuconostoc mesenteroides*, which is used for souring vegetables like cucumbers and cabbage, producing pickles and sauerkraut, respectively.

Lactic acid bacteria are also important medically. The production of low pH environments within the body inhibits the establishment and growth of pathogens in these areas. For example, the vaginal microbiota is composed largely of lactic acid bacteria, but when these bacteria are reduced, yeast can proliferate, causing a yeast infection. Additionally, lactic acid bacteria are important in maintaining the health of the gastrointestinal tract and, as such, are the primary component of probiotics.

Another familiar fermentation process is alcohol fermentation, which produces ethanol. The ethanol fermentation reaction is shown in Figure **7.4.2.1**. In the first reaction, the enzyme pyruvate decarboxylase removes a carboxyl group from pyruvate, releasing CO_2 gas while producing the two-carbon molecule acetaldehyde. The second reaction, catalyzed by the enzyme alcohol dehydrogenase, transfers an electron from NADH to acetaldehyde, producing ethanol and NAD^+. The ethanol fermentation of pyruvate by the yeast *Saccharomyces cerevisiae* is used in the production of alcoholic beverages and also makes bread products rise due to CO_2 production. Outside of the food industry, ethanol fermentation of plant products is important in biofuel production.

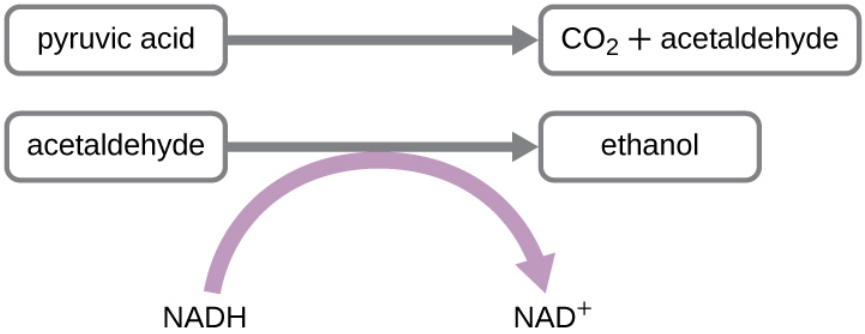


Figure **7.4.2.1**: The chemical reactions of alcohol fermentation are shown here. Ethanol fermentation is important in the production of alcoholic beverages and bread.

Beyond lactic acid fermentation and alcohol fermentation, many other fermentation methods occur in prokaryotes, all for the purpose of ensuring an adequate supply of NAD^+ for glycolysis (Table **7.4.2.2**). Without these pathways, glycolysis would not occur and no ATP would be harvested from the breakdown of glucose. It should be noted that most forms of fermentation besides homolactic fermentation produce gas, commonly CO_2 and/or hydrogen gas. Many of these different types of fermentation pathways are also used in food production and each results in the production of different organic acids, contributing to the unique flavor of a particular fermented food product. The propionic acid produced during propionic acid fermentation contributes to the distinctive flavor of Swiss cheese, for example.

Several fermentation products are important commercially outside of the food industry. For example, chemical solvents such as acetone and butanol are produced during acetone-butanol-ethanol fermentation. Complex organic pharmaceutical compounds used in antibiotics (e.g., penicillin), vaccines, and vitamins are produced through mixed acid fermentation. Fermentation products are used in the laboratory to differentiate various bacteria for diagnostic purposes. For example, enteric bacteria are known for their ability to perform mixed acid fermentation, reducing the pH, which can be detected using a pH indicator. Similarly, the bacterial production of acetoin during butanediol fermentation can also be detected. Gas production from fermentation can also be seen in an inverted Durham tube that traps produced gas in a broth culture.

Microbes can also be differentiated according to the substrates they can ferment. For example, *E. coli* can ferment lactose, forming gas, whereas some of its close gram-negative relatives cannot. The ability to ferment the sugar alcohol sorbitol is used to identify the pathogenic enterohemorrhagic O157:H7 strain of *E. coli* because, unlike other *E. coli* strains, it is unable to ferment sorbitol. Last, mannitol fermentation differentiates the mannitol-fermenting *Staphylococcus aureus* from other non–mannitol-fermenting staphylococci.

Table **7.4.2.2**: Common Fermentation Pathways

Pathway	End Products	Example Microbes	Commercial Products
Acetone-butanol-ethanol	Acetone, butanol, ethanol, CO_2	*Clostridium acetobutylicum*	Commercial solvents, gasoline alternative
Alcohol	Ethanol, CO_2	*Candida*, *Saccharomyces*	Beer, bread
Butanediol	Formic and lactic acid; ethanol; acetoin; 2,3 butanediol; CO_2; hydrogen gas	*Klebsiella*, *Enterobacter*	Chardonnay wine
Butyric acid	Butyric acid, CO_2, hydrogen gas	*Clostridium butyricum*	Butter
Lactic acid	Lactic acid	*Streptococcus*, *Lactobacillus*	Sauerkraut, yogurt, cheese
Mixed acid	Acetic, formic, lactic, and succinic acids; ethanol, CO_2, hydrogen gas	*Escherichia*, *Shigella*	Vinegar, cosmetics, pharmaceuticals
Propionic acid	Acetic acid, propionic acid, CO_2	*Propionibacterium*, *Bifidobacterium*	Swiss cheese

? Exercise 7.4.2.1

When would a metabolically versatile microbe perform fermentation rather than cellular respiration?

Identifying Bacteria by Using API Test Panels

Identification of a microbial isolate is essential for the proper diagnosis and appropriate treatment of patients. Scientists have developed techniques that identify bacteria according to their biochemical characteristics. Typically, they either examine the use of specific carbon sources as substrates for fermentation or other metabolic reactions, or they identify fermentation products or specific enzymes present in reactions. In the past, microbiologists have used individual test tubes and plates to conduct biochemical testing. However, scientists, especially those in clinical laboratories, now more frequently use plastic, disposable, multitest panels that contain a number of miniature reaction tubes, each typically including a specific substrate and pH indicator. After inoculation of the test panel with a small sample of the microbe in question and incubation, scientists can compare the results to a database that includes the expected results for specific biochemical reactions for known microbes, thus enabling rapid identification of a sample microbe. These test panels have allowed scientists to reduce costs while improving efficiency and reproducibility by performing a larger number of tests simultaneously.

Many commercial, miniaturized biochemical test panels cover a number of clinically important groups of bacteria and yeasts. One of the earliest and most popular test panels is the Analytical Profile Index (API) panel invented in the 1970s. Once some basic laboratory characterization of a given strain has been performed, such as determining the strain's Gram morphology, an appropriate test strip that contains 10 to 20 different biochemical tests for differentiating strains within that microbial group can be used. Currently, the various API strips can be used to quickly and easily identify more than 600 species of bacteria, both aerobic and anaerobic, and approximately 100 different types of yeasts. Based on the colors of the reactions when metabolic end products are present, due to the presence of pH indicators, a metabolic profile is created from the results (Figure **7.4.2.2**). Microbiologists can then compare the sample's profile to the database to identify the specific microbe.



Figure 7.4.2.2: The API 20NE test strip is used to identify specific strains of gram-negative bacteria outside the Enterobacteriaceae. Here is an API 20NE test strip result for *Photobacterium damselae* ssp. *piscicida*.

Clinical Focus: Part 2

Many of Hannah's symptoms are consistent with several different infections, including influenza and pneumonia. However, her sluggish reflexes along with her light sensitivity and stiff neck suggest some possible involvement of the central nervous system, perhaps indicating meningitis. Meningitis is an infection of the cerebrospinal fluid (CSF) around the brain and spinal cord that causes inflammation of the meninges, the protective layers covering the brain. Meningitis can be caused by viruses, bacteria, or fungi. Although all forms of meningitis are serious, bacterial meningitis is particularly serious. Bacterial meningitis may be caused by several different bacteria, but the bacterium *Neisseria meningitidis*, a gram-negative, bean-shaped diplococcus, is a common cause and leads to death within 1 to 2 days in 5% to 10% of patients.

Given the potential seriousness of Hannah's conditions, her physician advised her parents to take her to the hospital in the Gambian capital of Banjul and there have her tested and treated for possible meningitis. After a 3-hour drive to the hospital, Hannah was immediately admitted. Physicians took a blood sample and performed a lumbar puncture to test her CSF. They also immediately started her on a course of the antibiotic ceftriaxone, the drug of choice for treatment of meningitis caused by *N. meningitidis*, without waiting for laboratory test results.

Exercise 7.4.2.2

1. How might biochemical testing be used to confirm the identity of *N. meningitidis*?
2. Why did Hannah's doctors decide to administer antibiotics without waiting for the test results?

Key Concepts and Summary

- Fermentation uses an organic molecule as a final electron acceptor to regenerate NAD^+ from NADH so that glycolysis can continue.
- Fermentation does not involve an electron transport system, and no ATP is made by the fermentation process directly. Fermenters make very little ATP—only two ATP molecules per glucose molecule during glycolysis.
- Microbial fermentation processes have been used for the production of foods and pharmaceuticals, and for the identification of microbes.
- During lactic acid fermentation, pyruvate accepts electrons from NADH and is reduced to lactic acid. Microbes performing **homolactic fermentation** produce only lactic acid as the fermentation product; microbes performing **heterolactic fermentation** produce a mixture of lactic acid, ethanol and/or acetic acid, and CO_2.
- Lactic acid production by the normal microbiota prevents growth of pathogens in certain body regions and is important for the health of the gastrointestinal tract.
- During ethanol fermentation, pyruvate is first decarboxylated (releasing CO_2) to acetaldehyde, which then accepts electrons from NADH, reducing acetaldehyde to ethanol. Ethanol fermentation is used for the production of alcoholic beverages, for making bread products rise, and for biofuel production.
- Fermentation products of pathways (e.g., propionic acid fermentation) provide distinctive flavors to food products. Fermentation is used to produce chemical solvents (acetone-butanol-ethanol fermentation) and pharmaceuticals (mixed acid fermentation).
- Specific types of microbes may be distinguished by their fermentation pathways and products. Microbes may also be differentiated according to the substrates they are able to ferment.

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7.5: Catabolism of Lipids and Proteins

Learning Objectives

- Describe how lipids are catabolized
- Describe how lipid catabolism can be used to identify microbes
- Describe how proteins are catabolized
- Describe how protein catabolism can be used to identify bacteria

Previous sections have discussed the catabolism of glucose, which provides energy to living cells, as well as how polysaccharides like glycogen, starch, and cellulose are degraded to glucose monomers. But microbes consume more than just carbohydrates for food. In fact, the microbial world is known for its ability to degrade a wide range of molecules, both naturally occurring and those made by human processes, for use as carbon sources. In this section, we will see that the pathways for both lipid and protein catabolism connect to those used for carbohydrate catabolism, eventually leading into glycolysis, the transition reaction, and the Krebs cycle pathways. Metabolic pathways should be considered to be porous—that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways.

Lipid Catabolism

Triglycerides are a form of long-term energy storage in animals. They are made of glycerol and three fatty acids (see Figure 7.3.1). Phospholipids compose the cell and organelle membranes of all organisms except the archaea. Phospholipid structure is similar to triglycerides except that one of the fatty acids is replaced by a phosphorylated head group (see Figure 7.3.2). Triglycerides and phospholipids are broken down first by releasing fatty acid chains (and/or the phosphorylated head group, in the case of phospholipids) from the three-carbon glycerol backbone. The reactions breaking down triglycerides are catalyzed by lipases and those involving phospholipids are catalyzed by phospholipases. These enzymes contribute to the virulence of certain microbes, such as the bacterium *Staphylococcus aureus* and the fungus *Cryptococcus neoformans*. These microbes use phospholipases to destroy lipids and phospholipids in host cells and then use the catabolic products for energy (see Virulence Factors of Bacterial and Viral Pathogens).

The resulting products of lipid catabolism, glycerol and fatty acids, can be further degraded. Glycerol can be phosphorylated to glycerol-3-phosphate and easily converted to glyceraldehyde 3-phosphate, which continues through glycolysis. The released fatty acids are catabolized in a process called β-oxidation, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains, reducing NAD^+ and FAD to produce NADH and $FADH_2$, respectively, whose electrons can be used to make ATP by oxidative phosphorylation. The acetyl groups produced during β-oxidation are carried by coenzyme A to the Krebs cycle, and their movement through this cycle results in their degradation to CO_2, producing ATP by substrate-level phosphorylation and additional NADH and $FADH_2$ molecules (see Appendix C for a detailed illustration of β-oxidation).

Other types of lipids can also be degraded by certain microbes. For example, the ability of certain pathogens, like *Mycobacterium tuberculosis*, to degrade cholesterol contributes to their virulence. The side chains of cholesterol can be easily removed enzymatically, but degradation of the remaining fused rings is more problematic. The four fused rings are sequentially broken in a multistep process facilitated by specific enzymes, and the resulting products, including pyruvate, can be further catabolized in the Krebs cycle.

Exercise 7.5.1

How can lipases and phospholipases contribute to virulence in microbes?

Protein Catabolism

Proteins are degraded through the concerted action of a variety of microbial protease enzymes. Extracellular proteases cut proteins internally at specific amino acid sequences, breaking them down into smaller peptides that can then be taken up by cells. Some clinically important pathogens can be identified by their ability to produce a specific type of extracellular protease. For example,

the production of the extracellular protease gelatinase by members of the genera *Proteus* and *Serratia* can be used to distinguish them from other gram-negative enteric bacteria. Following inoculation and growth of microbes in gelatin broth, degradation of the gelatin protein due to gelatinase production prevents solidification of gelatin when refrigerated. Other pathogens can be distinguished by their ability to degrade casein, the main protein found in milk. When grown on skim milk agar, production of the extracellular protease caseinase causes degradation of casein, which appears as a zone of clearing around the microbial growth. Caseinase production by the opportunist pathogen *Pseudomonas aeruginosa* can be used to distinguish it from other related gram-negative bacteria.

After extracellular protease degradation and uptake of peptides in the cell, the peptides can then be broken down further into individual amino acids by additional intracellular proteases, and each amino acid can be enzymatically deaminated to remove the amino group. The remaining molecules can then enter the transition reaction or the Krebs cycle.

? Exercise 7.5.2

How can protein catabolism help identify microbes?

Clinical Focus: Part 3

Because bacterial meningitis progresses so rapidly, Hannah's doctors had decided to treat her aggressively with antibiotics, based on empirical observation of her symptoms. However, laboratory testing to confirm the cause of Hannah's meningitis was still important for several reasons. *N. meningitidis* is an infectious pathogen that can be spread from person to person through close contact; therefore, if tests confirm *N. meningitidis* as the cause of Hannah's symptoms, Hannah's parents and others who came into close contact with her might need to be vaccinated or receive prophylactic antibiotics to lower their risk of contracting the disease. On the other hand, if it turns out that *N. meningitidis* is not the cause, Hannah's doctors might need to change her treatment.

The clinical laboratory performed a Gram stain on Hannah's blood and CSF samples. The Gram stain showed the presence of a bean-shaped gram-negative diplococcus. The technician in the hospital lab cultured Hannah's blood sample on both blood agar and chocolate agar, and the bacterium that grew on both media formed gray, nonhemolytic colonies. Next, he performed an oxidase test on this bacterium and determined that it was oxidase positive. Last, he examined the repertoire of sugars that the bacterium could use as a carbon source and found that the bacterium was positive for glucose and maltose use but negative for lactose and sucrose use. All of these test results are consistent with characteristics of *N. meningitidis*.

Exercise 7.5.3

1. What do these test results tell us about the metabolic pathways of *N. meningitidis*?
2. Why do you think that the hospital used these biochemical tests for identification in lieu of molecular analysis by DNA testing?

Key Concepts and Summary

- Collectively, microbes have the ability to degrade a wide variety of carbon sources besides carbohydrates, including lipids and proteins. The catabolic pathways for all of these molecules eventually connect into glycolysis and the Krebs cycle.
- Several types of lipids can be microbially degraded. Triglycerides are degraded by extracellular **lipases**, releasing fatty acids from the glycerol backbone. Phospholipids are degraded by **phospholipases**, releasing fatty acids and the phosphorylated head group from the glycerol backbone. Lipases and phospholipases act as virulence factors for certain pathogenic microbes.
- Fatty acids can be further degraded inside the cell through **β-oxidation**, which sequentially removes two-carbon acetyl groups from the ends of fatty acid chains.
- Protein degradation involves extracellular **proteases** that degrade large proteins into smaller peptides. Detection of the extracellular proteases gelatinase and caseinase can be used to differentiate clinically relevant bacteria.

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7.6: Anabolism of Glucose- Photosynthesis

Learning Objectives

- Describe the process of photosynthesis

The Importance of Photosynthesis

The processes of all organisms—from bacteria to humans—require energy. To get this energy, many organisms access stored energy by eating food. Carnivores eat other animals and herbivores eat plants. But where does the stored energy in food originate? All of this energy can be traced back to the process of photosynthesis and light energy from the sun.

Photosynthesis is essential to all life on earth. It is the only biological process that captures energy from outer space (sunlight) and converts it into chemical energy in the form of G3P (
Glyceraldehyde 3-phosphate) which in turn can be made into sugars and other molecular compounds. Plants use these compounds in all of their metabolic processes; plants do not need to consume other organisms for food because they build all the molecules they need. Unlike plants, animals need to consume other organisms to consume the molecules they need for their metabolic processes.

The Process of Photosynthesis

During photosynthesis, molecules in leaves capture sunlight and energize electrons, which are then stored in the covalent bonds of carbohydrate molecules. That energy within those covalent bonds will be released when they are broken during cell respiration. How long lasting and stable are those covalent bonds? The energy extracted today by the burning of coal and petroleum products represents sunlight energy captured and stored by photosynthesis almost 200 million years ago.

Plants, algae, and a group of bacteria called cyanobacteria are the only organisms capable of performing photosynthesis. Because they use light to manufacture their own food, they are called photoautotrophs (“self-feeders using light”). Other organisms, such as animals, fungi, and most other bacteria, are termed heterotrophs (“other feeders”) because they must rely on the sugars produced by photosynthetic organisms for their energy needs. A third very interesting group of bacteria synthesize sugars, not by using sunlight’s energy, but by extracting energy from inorganic chemical compounds; hence, they are referred to as chemoautotrophs.

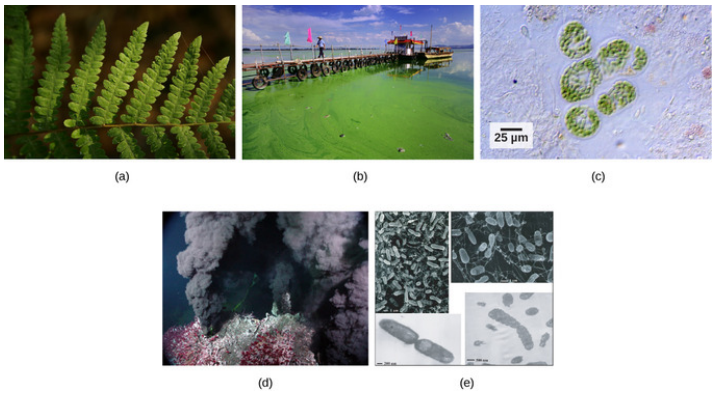


Figure: **Photosynthetic and Chemosynthetic Organisms**: Photoautotrophs, including (a) plants, (b) algae, and (c) cyanobacteria, synthesize their organic compounds via photosynthesis using sunlight as an energy source. Cyanobacteria and planktonic algae can grow over enormous areas in water, at times completely covering the surface. In a (d) deep sea vent, chemoautotrophs, such as these (e) thermophilic bacteria, capture energy from inorganic compounds to produce organic compounds. The ecosystem surrounding the vents has a diverse array of animals, such as tubeworms, crustaceans, and octopi that derive energy from the bacteria.

The importance of photosynthesis is not just that it can capture sunlight’s energy. A lizard sunning itself on a cold day can use the sun’s energy to warm up. Photosynthesis is vital because it evolved as a way to store the energy in solar radiation (the “photo-” part) as high-energy electrons in the carbon-carbon bonds of carbohydrate molecules (the “-synthesis” part). Those carbohydrates are the energy source that heterotrophs use to power the synthesis of ATP via respiration. Therefore, photosynthesis powers 99

percent of Earth's ecosystems. When a top predator, such as a wolf, preys on a deer, the wolf is at the end of an energy path that went from nuclear reactions on the surface of the sun, to light, to photosynthesis, to vegetation, to deer, and finally to wolf.

Key Points

- Photosynthesis evolved as a way to store the energy in solar radiation as high-energy electrons in carbohydrate molecules.
- Plants, algae, and cyanobacteria, known as photoautotrophs, are the only organisms capable of performing photosynthesis.
- Heterotrophs, unable to produce their own food, rely on the carbohydrates produced by photosynthetic organisms for their energy needs.

Key Terms

- **photosynthesis**: the process by which plants and other photoautotrophs generate carbohydrates and oxygen from carbon dioxide, water, and light energy in chloroplasts
- **photoautotroph**: an organism that can synthesize its own food by using light as a source of energy
- **chemoautotroph**: a simple organism, such as a protozoan, that derives its energy from chemical processes rather than photosynthesis

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7.6.1: The Two Parts of Photosynthesis

Learning Objectives

- Distinguish between the two parts of photosynthesis

Photosynthesis takes place in two sequential stages:

1. The light-dependent reactions;
2. The light-independent reactions, or Calvin Cycle.

Light-Dependent Reactions

Just as the name implies, light-dependent reactions require sunlight. In the light-dependent reactions, energy from sunlight is absorbed by chlorophyll and converted into stored chemical energy, in the form of the electron carrier molecule NADPH (nicotinamide adenine dinucleotide phosphate) and the energy currency molecule ATP (adenosine triphosphate). The light-dependent reactions take place in the thylakoid membranes in the granum (stack of thylakoids), within the chloroplast.

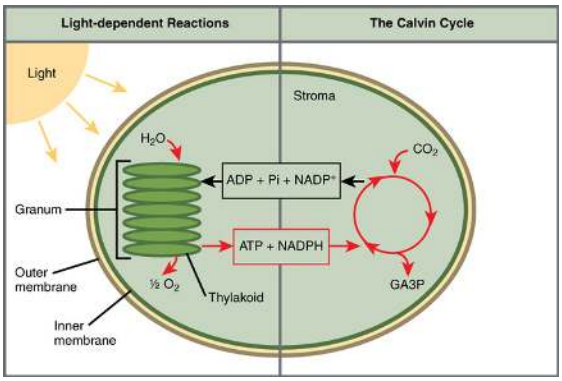


Figure: **The two stages of photosynthesis**: Photosynthesis takes place in two stages: light-dependent reactions and the Calvin cycle (light-independent reactions). Light-dependent reactions, which take place in the thylakoid membrane, use light energy to make ATP and NADPH. The Calvin cycle, which takes place in the stroma, uses energy derived from these compounds to make GA3P from CO2.

Photosystems

The process that converts light energy into chemical energy takes place in a multi-protein complex called a photosystem. Two types of photosystems are embedded in the thylakoid membrane: photosystem II (PSII) and photosystem I (PSI). Each photosystem plays a key role in capturing the energy from sunlight by exciting electrons. These energized electrons are transported by “energy carrier” molecules, which power the light-independent reactions.

Photosystems consist of a light-harvesting complex and a reaction center. Pigments in the light-harvesting complex pass light energy to two special chlorophyll *a* molecules in the reaction center. The light excites an electron from the chlorophyll *a* pair, which passes to the primary electron acceptor. The excited electron must then be replaced. In photosystem II, the electron comes from the splitting of water, which releases oxygen as a waste product. In photosystem I, the electron comes from the chloroplast electron transport chain.

The two photosystems oxidize different sources of the low-energy electron supply, deliver their energized electrons to different places, and respond to different wavelengths of light.

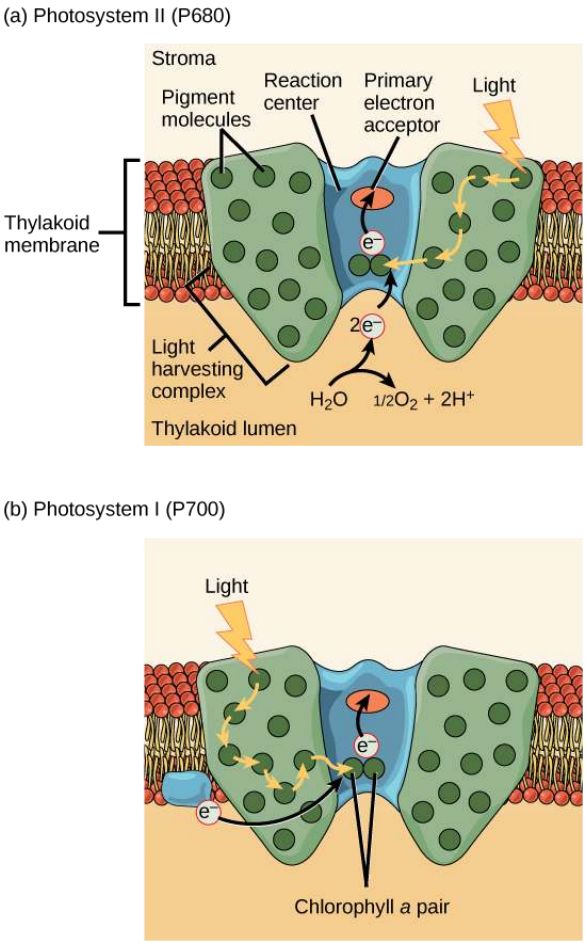


Figure: **Photosystems I & II**: As explained above, the photosystems manipulate electrons with energy harvested from light.

Light-Independent Reactions

In the light-independent reactions or Calvin cycle, the energized electrons from the light-dependent reactions provide the energy to form carbohydrates from carbon dioxide molecules. The light-independent reactions are sometimes called the Calvin cycle because of the cyclical nature of the process.

Although the light-independent reactions do not use light as a reactant (and as a result can take place at day or night), they require the products of the light-dependent reactions to function. The light-independent molecules depend on the energy carrier molecules, ATP and NADPH, to drive the construction of new carbohydrate molecules. After the energy is transferred, the energy carrier molecules return to the light-dependent reactions to obtain more energized electrons. In addition, several enzymes of the light-independent reactions are activated by light.

Key Points

- In light-dependent reactions, the energy from sunlight is absorbed by chlorophyll and converted into chemical energy in the form of electron carrier molecules like ATP and NADPH.
- Light energy is harnessed in Photosystems I and II, both of which are present in the thylakoid membranes of chloroplasts.
- In light-independent reactions (the Calvin cycle), carbohydrate molecules are assembled from carbon dioxide using the chemical energy harvested during the light-dependent reactions.

Key Terms

- **photosystem**: Either of two biochemical systems active in chloroplasts that are part of photosynthesis.

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7.6.2: The Process of Photosynthesis

Learning Objectives

- Describe the function and locations of photosynthetic pigments in eukaryotes and prokaryotes
- Describe the major products of the light-dependent and light-independent reactions
- Describe the reactions that produce glucose in a photosynthetic cell
- Compare and contrast cyclic and noncyclic photophosphorylation

Heterotrophic organisms ranging from *E. coli* to humans rely on the chemical energy found mainly in carbohydrate molecules. Many of these carbohydrates are produced by photosynthesis, the biochemical process by which phototrophic organisms convert solar energy (sunlight) into chemical energy. Although photosynthesis is most commonly associated with plants, microbial photosynthesis is also a significant supplier of chemical energy, fueling many diverse ecosystems. In this section, we will focus on microbial photosynthesis.

Photosynthesis takes place in two sequential stages: the light-dependent reactions and the light-independent reactions (Figure **7.6.2.1**). In the light-dependent reactions, energy from sunlight is absorbed by pigment molecules in photosynthetic membranes and converted into stored chemical energy. In the light-independent reactions, the chemical ener gy produced by the light-dependent reactions is used to drive the assembly of sugar molecules using CO_2; however, these reactions are still light dependent because the products of the light-dependent reactions necessary for driving them are short-lived. The light-dependent reactions produce ATP and either NADPH or NADH to temporarily store energy. These energy carriers are used in the light-independent reactions to drive the energetically unfavorable process of "fixing" inorganic CO_2 in an organic form, sugar.

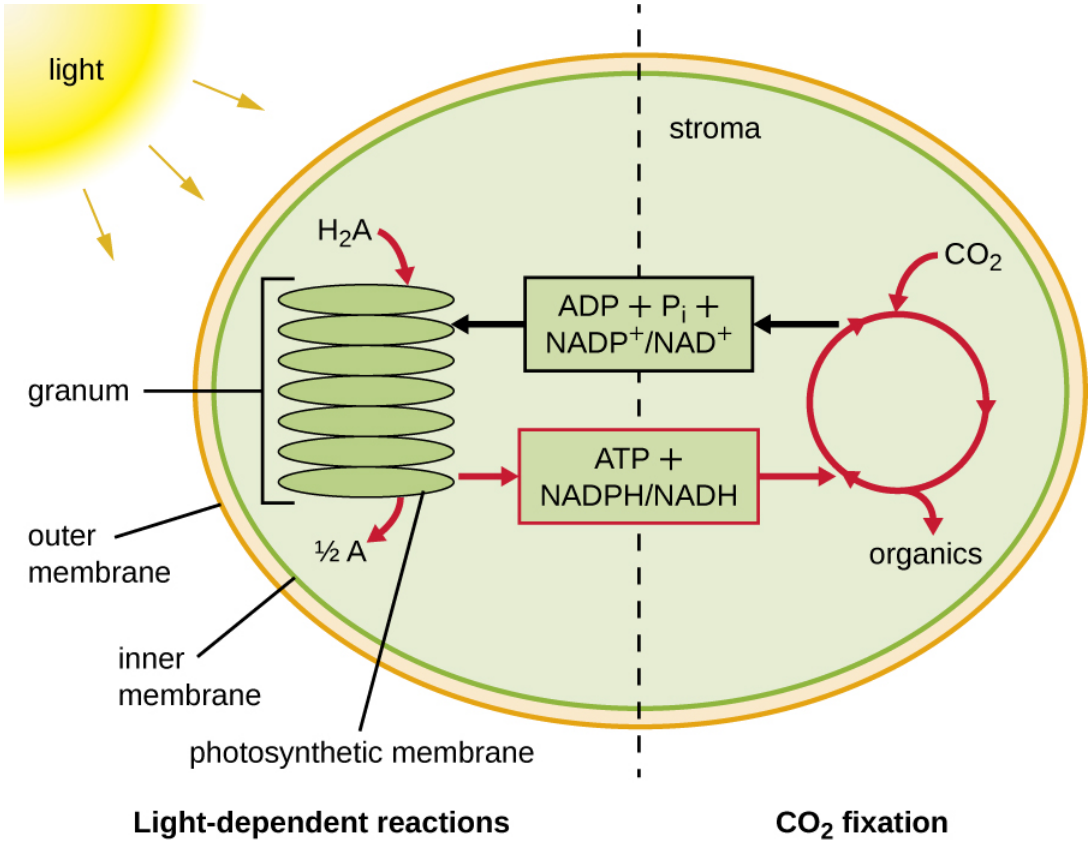


Figure **7.6.2.1**: The light-dependent reactions of photosynthesis (left) convert light energy into chemical energy, forming ATP and NADPH. These products are used by the light-independent reactions to fix CO_2, producing organic carbon molecules.

Photosynthetic Structures in Eukaryotes and Prokaryotes

In all phototrophic eukaryotes, photosynthesis takes place inside a chloroplast, an organelle that arose in eukaryotes by endosymbiosis of a photosynthetic bacterium (see Unique Characteristics of Eukaryotic Cells). These chloroplasts are enclosed by a double membrane with inner and outer layers. Within the chloroplast is a third membrane that forms stacked, disc-shaped photosynthetic structures called thylakoids (Figure **7.6.2.2**). A stack of thylakoids is called a granum, and the space surrounding the granum within the chloroplast is called stroma.

Photosynthetic membranes in prokaryotes, by contrast, are not organized into distinct membrane-enclosed organelles; rather, they are infolded regions of the plasma membrane. In cyanobacteria, for example, these infolded regions are also referred to as thylakoids. In either case, embedded within the thylakoid membranes or other photosynthetic bacterial membranes are photosynthetic pigment molecules organized into one or more photosystems, where light energy is actually converted into chemical energy.

Photosynthetic pigments within the photosynthetic membranes are organized into photosystems, each of which is composed of a light-harvesting (antennae) complex and a reaction center. The light-harvesting complex consists of multiple proteins and associated pigments that each may absorb light energy and, thus, become excited. This energy is transferred from one pigment molecule to another until eventually (after about a millionth of a second) it is delivered to the reaction center. Up to this point, only energy—not electrons—has been transferred between molecules. The reaction center contains a pigment molecule that can undergo oxidation upon excitation, actually giving up an electron. It is at this step in photosynthesis that light energy is converted into an excited electron.

Different kinds of light-harvesting pigments absorb unique patterns of wavelengths (colors) of visible light. Pigments reflect or transmit the wavelengths they cannot absorb, making them appear the corresponding color. Examples of photosynthetic pigments (molecules used to absorb solar energy) are bacteriochlorophylls (green, purple, or red), carotenoids (orange, red, or yellow), chlorophylls (green), phycocyanins (blue), and phycoerythrins (red). By having mixtures of pigments, an organism can absorb energy from more wavelengths. Because photosynthetic bacteria commonly grow in competition for sunlight, each type of photosynthetic bacteria is optimized for harvesting the wavelengths of light to which it is commonly exposed, leading to stratification of microbial communities in aquatic and soil ecosystems by light quality and penetration.

Once the light harvesting complex transfers the energy to the reaction center, the reaction center delivers its high-energy electrons, one by one, to an electron carrier in an electron transport system, and electron transfer through the ETS is initiated. The ETS is similar to that used in cellular respiration and is embedded within the photosynthetic membrane. Ultimately, the electron is used to produce NADH or NADPH. The electrochemical gradient that forms across the photosynthetic membrane is used to generate ATP by chemiosmosis through the process of photophosphorylation, another example of oxidative phosphorylation (Figure **7.6.2.3**).

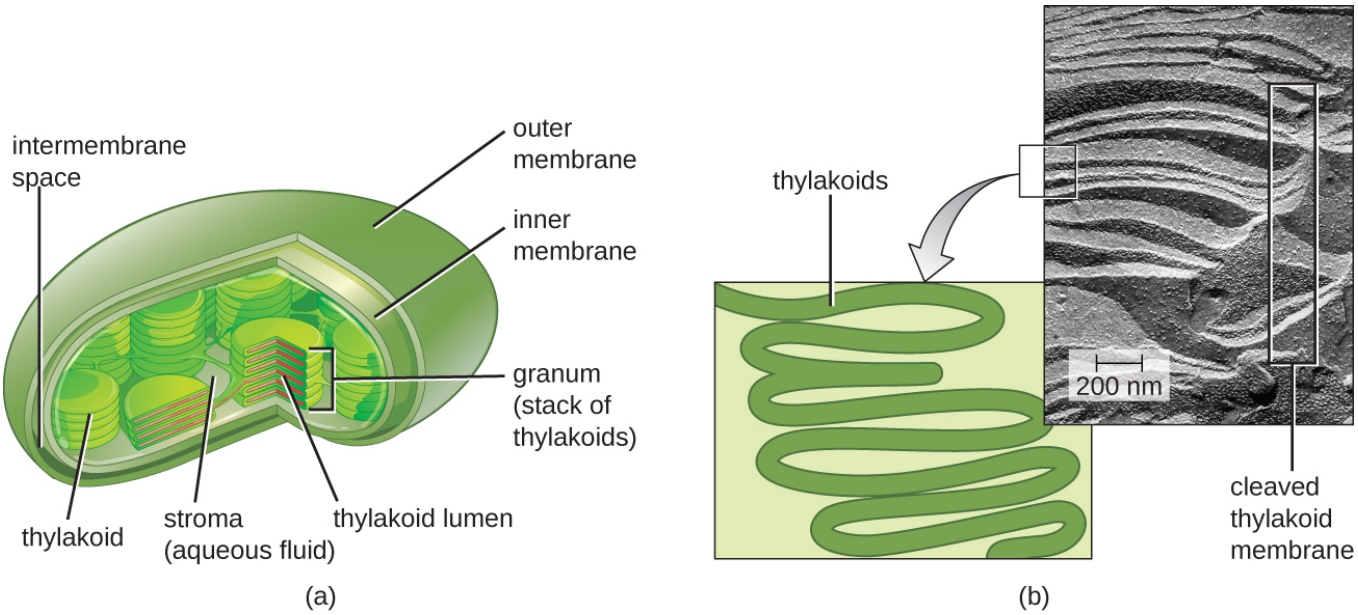


Figure **7.6.2.2**: (a) Photosynthesis in eukaryotes takes place in chloroplasts, which contain thylakoids stacked into grana. (b) A photosynthetic prokaryote has infolded regions of the plasma membrane that function like thylakoids. (credit: scale bar data from Matt Russell.)

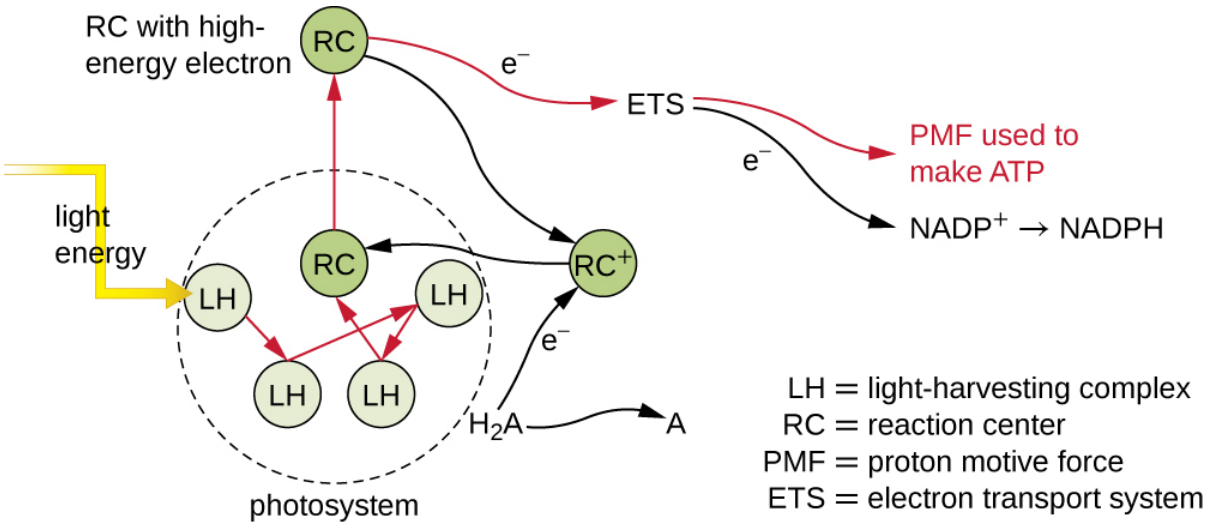


Figure **7.6.2.3**: This figure summarizes how a photosystem works. Light harvesting (LH) pigments absorb light energy, converting it to chemical energy. The energy is passed from one LH pigment to another until it reaches a reaction center (RC) pigment, exciting an electron. This high-energy electron is lost from the RC pigment and passed through an electron transport system (ETS), ultimately producing NADH or NADPH and ATP. A reduced molecule (H_2A) donates an electron, replacing electrons to the electron-deficient RC pigment.

? Exercise 7.6.2.1

In a phototrophic eukaryote, where does photosynthesis take place?

Oxygenic and Anoxygenic Photosynthesis

For photosynthesis to continue, the electron lost from the reaction center pigment must be replaced. The source of this electron (H_2A) differentiates the oxygenic photosynthesis of plants and cyanobacteria from anoxygenic photosynthesis carried out by other types of bacterial phototrophs (Figure **7.6.2.4**). In oxygenic photosynthesis, H_2O is split and supplies the electron to the reaction center. Because oxygen is generated as a byproduct and is released, this type of photosynthesis is referred to as oxygenic photosynthesis. However, when other reduced compounds serve as the electron donor, oxygen is not generated; these types of

photosynthesis are called anoxygenic photosynthesis. Hydrogen sulfide (H_2S) or thiosulfate ($S_2O_3^{2-}$) can serve as the electron donor, generating elemental sulfur and sulfate (SO_4^{2-}) ions, respectively, as a result.

Photosystems have been classified into two types: photosystem I (PSI) and photosystem II (PSII) (Figure **7.6.2.5**). Cyanobacteria and plant chloroplasts have both photosystems, whereas anoxygenic photosynthetic bacteria use only one of the photosystems. Both photosystems are excited by light energy simultaneously. If the cell requires both ATP and NADPH for biosynthesis, then it will carry out noncyclic photophosphorylation. Upon passing of the PSII reaction center electron to the ETS that connects PSII and PSI, the lost electron from the PSII reaction center is replaced by the splitting of water. The excited PSI reaction center electron is used to reduce $NADP^+$ to NADPH and is replaced by the electron exiting the ETS. The flow of electrons in this way is called the Z-scheme.

If a cell's need for ATP is significantly greater than its need for NADPH, it may bypass the production of reducing power through cyclic photophosphorylation. Only PSI is used during cyclic photophosphorylation; the high-energy electron of the PSI reaction center is passed to an ETS carrier and then ultimately returns to the oxidized PSI reaction center pigment, thereby reducing it.

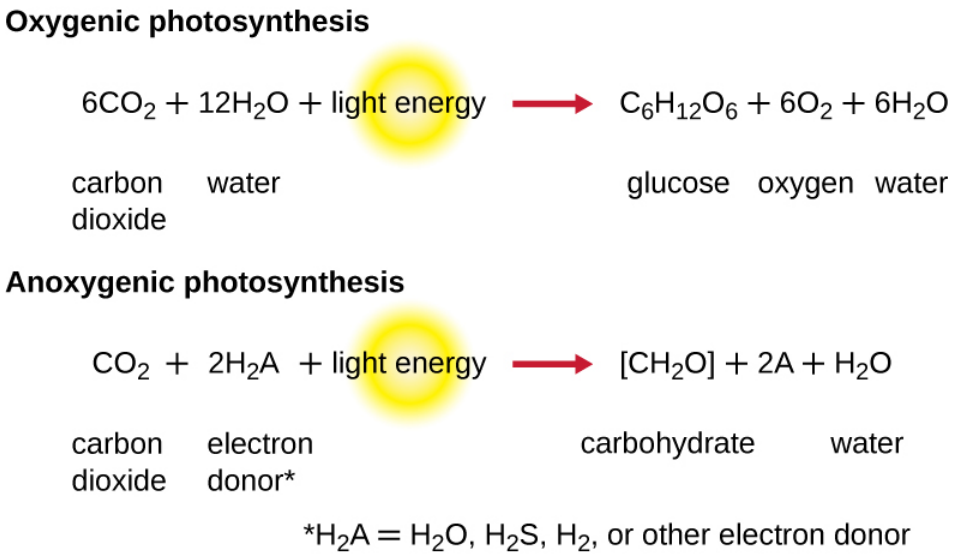
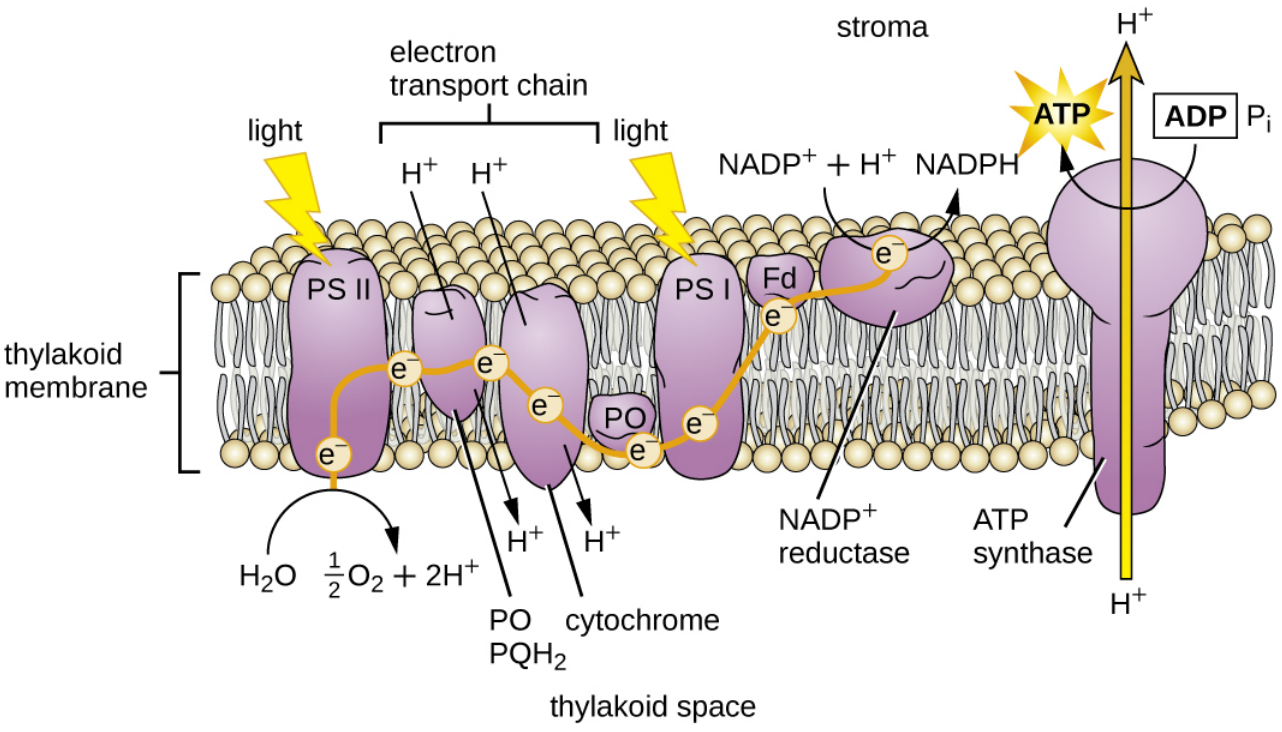
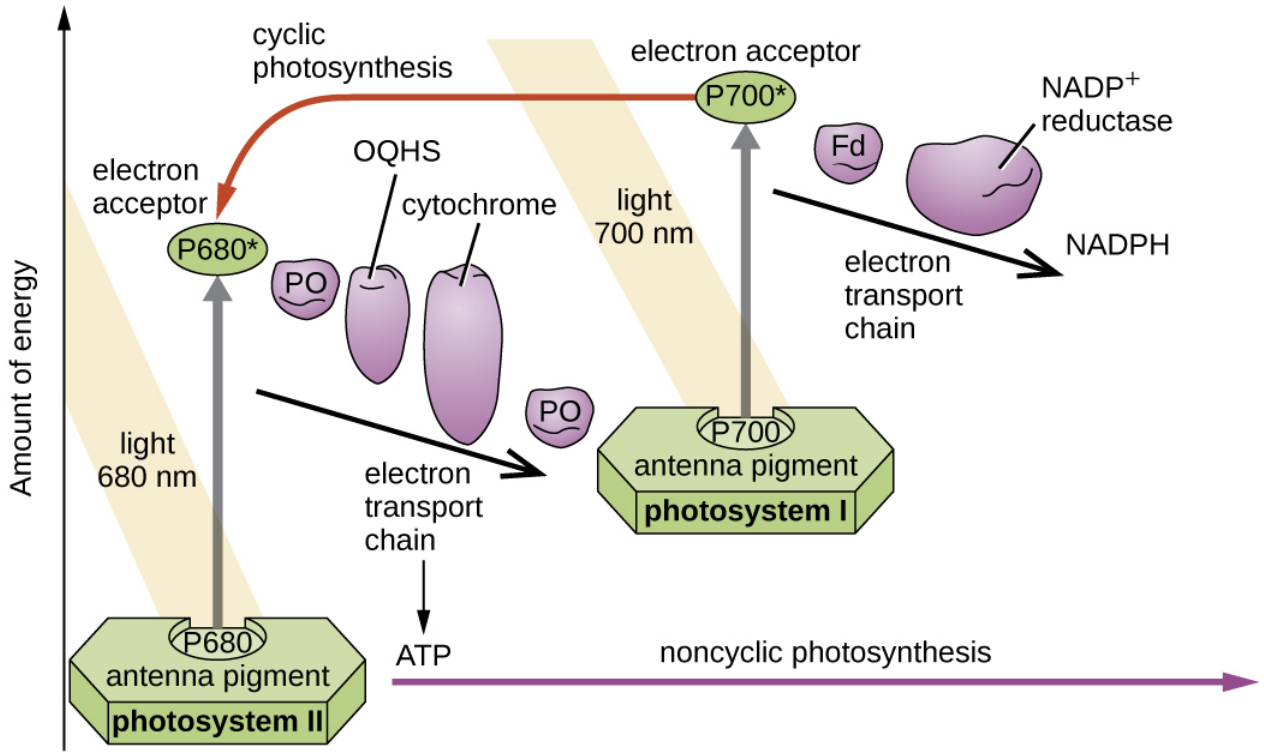


Figure **7.6.2.4**: Eukaryotes and cyanobacteria carry out oxygenic photosynthesis, producing oxygen, whereas other bacteria carry out anoxygenic photosynthesis, which does not produce oxygen.


stroma
electron
transport chain
light
light
H+
H+
NADP+ + H+
NADPH
ATP
ADP
Pi
H+
PS II
PS I
Fd
thylakoid
membrane
e−
PO
H2O
½O2 + 2H+
H+
H+
NADP+
reductase
ATP
synthase
H+
PO
PQH2
cytochrome
thylakoid space

(a)


cyclic
photosynthesis
electron acceptor
P700*
NADP+
reductase
Fd
OQHS
electron
acceptor
cytochrome
light
700 nm
P680*
PO
NADPH
electron
transport
chain
Amount of energy
PO
light
680 nm
P700
antenna pigment
photosystem I
electron
transport
chain
P680
antenna pigment
photosystem II
ATP
noncyclic photosynthesis

(b)

Figure 7.6.2.5: (a) PSI and PSII are found on the thylakoid membrane. The high-energy electron from PSII is passed to an ETS, which generates a proton motive force for ATP synthesis by chemiosmosis, and ultimately replaces the electron lost by the PSI reaction center. The PSI reaction center electron is used to make NADPH. (b) When both ATP and NADPH are required, noncyclic photophosphorylation (in cyanobacteria and plants) provides both. The electron flow described here is referred to as the Z-scheme (shown in yellow in [a]). When the cell's ATP needs outweigh those for NADPH, cyanobacteria and plants will use only PSI, and its reaction center electron is passed to the ETS to generate a proton motive force used for ATP synthesis.

? Exercise 7.6.2.2

Why would a photosynthetic bacterium have different pigments?

Light-Independent Reactions

After the energy from the sun is converted into chemical energy and temporarily stored in ATP and NADPH molecules (having lifespans of millionths of a second), photoautotrophs have the fuel needed to build multicarbon carbohydrate molecules, which can survive for hundreds of millions of years, for long-term energy storage. The carbon comes from CO_2, the gas that is a waste product of cellular respiration.

The Calvin-Benson cycle (named for Melvin Calvin [1911–1997] and Andrew Benson [1917–2015]), the biochemical pathway used for fixation of CO_2, is located within the cytoplasm of photosynthetic bacteria and in the stroma of eukaryotic chloroplasts. The light-independent reactions of the Calvin cycle can be organized into three basic stages: fixation, reduction, and regeneration (see Appendix C for a detailed illustration of the Calvin cycle).

- **Fixation**: The enzyme ribulose bisphosphate carboxylase (RuBisCO) catalyzes the addition of a CO_2 to ribulose bisphosphate (RuBP). This results in the production of 3-phosphoglycerate (3-PGA).
- **Reduction**: Six molecules of both ATP and NADPH (from the light-dependent reactions) are used to convert 3-PGA into glyceraldehyde 3-phosphate (G3P). Some G3P is then used to build glucose.
- **Regeneration**: The remaining G3P not used to synthesize glucose is used to regenerate RuBP, enabling the system to continue CO_2 fixation. Three more molecules of ATP are used in these regeneration reactions.

The Calvin cycle is used extensively by plants and photoautotrophic bacteria, and the enzyme RuBisCO is said to be the most plentiful enzyme on earth, composing 30%–50% of the total soluble protein in plant chloroplasts.[1] However, besides its prevalent use in photoautotrophs, the Calvin cycle is also used by many nonphotosynthetic chemoautotrophs to fix CO_2. Additionally, other bacteria and archaea use alternative systems for CO_2 fixation. Although most bacteria using Calvin cycle alternatives are chemoautotrophic, certain green sulfur photoautotrophic bacteria have been also shown to use an alternative CO_2 fixation pathway.

? Exercise 7.6.2.3

Describe the three stages of the Calvin cycle.

Key Concepts and Summary

- Heterotrophs depend on the carbohydrates produced by autotrophs, many of which are photosynthetic, converting solar energy into chemical energy.
- Different photosynthetic organisms use different mixtures of **photosynthetic pigments**, which increase the range of the wavelengths of light an organism can absorb.
- **Photosystems** (PSI and PSII) each contain a **light-harvesting complex**, composed of multiple proteins and associated pigments that absorb light energy. The **light-dependent reactions** of photosynthesis convert solar energy into chemical energy, producing ATP and NADPH or NADH to temporarily store this energy.
- In **oxygenic photosynthesis**, H_2O serves as the electron donor to replace the reaction center electron, and oxygen is formed as a byproduct. In **anoxygenic photosynthesis**, other reduced molecules like H_2S or thiosulfate may be used as the electron donor; as such, oxygen is not formed as a byproduct.
- **Noncyclic photophosphorylation** is used in oxygenic photosynthesis when there is a need for both ATP and NADPH production. If a cell's needs for ATP outweigh its needs for NADPH, then it may carry out **cyclic photophosphorylation** instead, producing only ATP.

- The **light-independent reactions** of photosynthesis use the ATP and NADPH from the light-dependent reactions to fix CO_2 into organic sugar molecules.

Footnotes

1. 1 A. Dhingra et al. “Enhanced Translation of a Chloroplast-Expressed *Rbc*S Gene Restores Small Subunit Levels and Photosynthesis in Nuclear *Rbc*S Antisense Plants.” *Proceedings of the National Academy of Sciences of the United States of America* 101 no. 16 (2004):6315–6320.

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7.7: Other Anabolism

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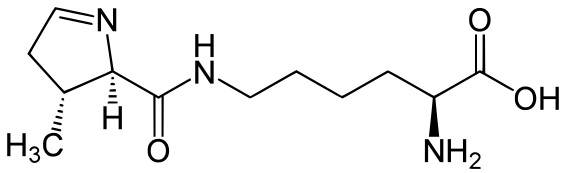
7.7.1: Amino Acid Synthesis

Learning Objectives

- Recognize the factors involved in amino acid synthesis

Amino acids are the structural units that make up proteins. They join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins. These polymers are linear and unbranched, with each amino acid within the chain attached to two neighboring amino acids. The process of making proteins is called translation and involves the step-by-step addition of amino acids to a growing protein chain by a ribozyme that is called a ribosome. The order in which the amino acids are added is read through the genetic code from an mRNA template, which is a RNA copy of one of the organism ‘s genes.

Twenty-two amino acids are naturally incorporated into polypeptides and are called proteinogenic or natural amino acids. Of these, 20 are encoded by the universal genetic code. The remaining two, selenocysteine and pyrrolysine, are incorporated into proteins by unique synthetic mechanisms. Selenocysteine is incorporated when the mRNA being translated includes a SECIS element, which causes the UGA codon to encode selenocysteine instead of a stop codon. Pyrrolysine is used by some methanogenic archaea in enzymes that they use to produce methane. It is coded with the codon UAG, which is normally a stop codon in other organisms. Pyrrolysine (abbreviated as Pyl or O) is a naturally occurring amino acid similar to lysine, but with an added pyrroline ring linked to the end of the lysine side chain. Produced by a specific tRNA and aminoacyl tRNA synthetase, it forms part of an unusual genetic code in these organisms. It is considered the 22nd proteinogenic amino acid. This UAG codon is followed by a PYLIS downstream sequence.



Structure of Pyrrolysine: Pyrrolysine (abbreviated as Pyl or O) is a naturally occurring, genetically coded amino acid used by some methanogenic archaea and one known bacterium in enzymes that are part of their methane-producing metabolism.

Organisms vary in their ability to synthesize the 20 common amino acids. Most bacteria and plants can synthesize all 20. Some simple parasites, such as the bacteria *Mycoplasma pneumoniae*, lack all amino acid synthesis and take their amino acids directly from their hosts. All amino acids are synthesized from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway. Nitrogen is provided by glutamate and glutamine. Amino acid synthesis depends on the formation of the appropriate alpha-keto acid, which is then transaminated to form an amino acid. Amino acids are made into proteins by being joined together in a chain by peptide bonds. Each different protein has a unique sequence of amino acid residues: this is its primary structure. Just as the letters of the alphabet can be combined to form an almost endless variety of words, amino acids can be linked in varying sequences to form a huge variety of proteins. Proteins are made from amino acids that have been activated by attachment to a transfer RNA molecule through an ester bond.

Aside from the 22 standard amino acids, there are many other amino acids that are called non-proteinogenic or non-standard. Those either are not found in proteins (for example carnitine, GABA) or are not produced directly and in isolation by standard cellular machinery (for example, hydroxyproline and selenomethionine). Non-standard amino acids that are found in proteins are formed by post-translational modification, which is modification after translation during protein synthesis. These modifications are often essential for the function or regulation of a protein. For example, the carboxylation of glutamate allows for better binding of calcium cations. The hydroxylation of proline is critical for maintaining connective tissues. Another example is the formation of hypusine in the translation initiation factor EIF5A, through modification of a lysine residue. Such modifications can also determine the localization of the protein, e.g., the addition of long hydrophobic groups can cause a protein to bind to a phospholipid membrane. Some nonstandard amino acids are not found in proteins. Examples include lanthionine, 2-aminoisobutyric acid, dehydroalanine, and the neurotransmitter gamma-aminobutyric acid. Nonstandard amino acids often occur as intermediates in the metabolic pathways for standard amino acids — for example, ornithine and citrulline occur in the urea cycle, part of amino acid catabolism.

Key Points

- All amino acids are synthesized from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway.
- Amino acid synthesis depends on the formation of the appropriate alpha-keto acid, which is then transaminated to form an amino acid.
- Of the 22 amino acids naturally incorporated into proteins, 20 are encoded by the universal genetic code and the remaining two, selenocysteine and pyrrolysine, are incorporated into proteins by unique synthetic mechanisms.

Key Terms

- **pyrrolysine**: An amino acid found in methanogenic bacteria.
- **selenocysteine**: A naturally-occurring amino acid, present in several enzymes, whose structure is that of cysteine but with the sulfur atom replaced by one of selenium.
- **genetic code**: The set of rules by which the sequence of bases in DNA are translated into the amino acid sequence of proteins

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7.7.2: Polysaccharide Biosynthesis

Learning Objectives

- Describe the mechanism of polysaccharide biosynthesis and its importance in bacteria

Polysaccharides are long carbohydrate molecules of repeated monomer units joined together by glycosidic bonds. They range in structure from linear to highly branched. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water.

One of the most common building block of polysaccharides is glucose. However, glucose has to be in its activated forms. There are two forms of activated glucose: UDP-glucose and ADP-glucose.

Uridine diphosphate glucose (uracil-diphosphate glucose, UDP-glucose) is a nucleotide sugar. Components UDP-glucose consists of the pyrophosphate group, the pentose sugar ribose, glucose, and the nucleobase uracil. It is used in nucleotide sugars metabolism as an activated form of glucose as a substrate for enzymes called glucosyltransferases. UDP-glucose can also be used as a precursor of lipopolysaccharides, and peptidoglycan. ADP-glucose is usually the precursor for glycogen production in bacteria.

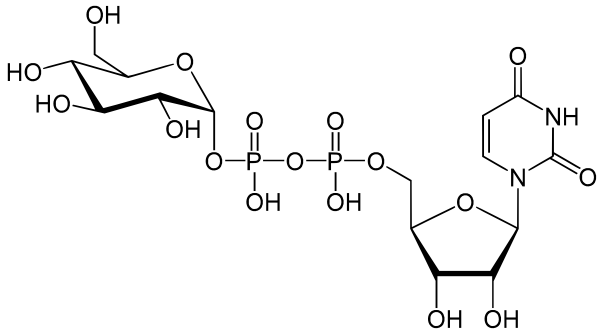


Figure: **Structure of UDP-glucose**: UDP-Glucose consists of the pyrophosphate group, the pentose sugar ribose, glucose, and the nucleobase uracil.

When the cells are grown on a carbon source different than glucose, then polysaccharides are synthesized using a different pathway. Gluconeogenesis (abbreviated GNG) is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as phosphoenolpyruvate (PEP). PEP is formed from the decarboxylation of oxaloacetate and hydrolysis of one guanosine triphosphate molecule. This reaction is a rate-limiting step in gluconeogenesis.

Pathogenic bacteria commonly produce a thick, mucous-like, layer of polysaccharide. This “capsule” cloaks antigenic proteins on the bacterial surface that would otherwise provoke an immune response and thereby lead to the destruction of the bacteria. Bacteria and many other microbes, including fungi and algae, often secrete polysaccharides as an evolutionary adaptation to help them adhere to surfaces and to prevent them from drying out. Humans have developed some of these polysaccharides into useful products including xanthan gum, dextran, welan gum, gellan gum, diutan gum, and pullulan.

Key Points

- Uridine diphosphate glucose (UDP-glucose) is a nucleotide sugar. UDP-glucose consists of the pyrophosphate group, the pentose sugar ribose, glucose, and the nucleobase uracil. It is used as a substrate for enzymes called glucosyltransferases.
- UDP-glucose can also be used as a precursor of lipopolysaccharides, and peptidoglycan. ADP-glucose is usually the precursor for glycogen production in bacteria.
- When the cells are grown on a carbon source different than glucose, gluconeogenesis (abbreviated GNG) is the metabolic pathway used to produce glucose from non- carbohydrate carbon substrates such as phosphoenolpyruvate (PEP).
- Pathogenic bacteria can produce a thick, mucous-like, layer of polysaccharide. This “capsule” cloaks antigenic proteins on the bacterial surface. Bacteria and other microbes, secrete polysaccharides as an evolutionary adaptation to help them adhere to surfaces and to prevent them from drying out.

Key Terms

- **Polysaccharides**: Polysaccharides are long, carbohydrate molecules of repeated monomer units joined together by glycosidic bonds. They range in structure from linear to highly branched. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit.
- **gluconeogenesis**: Gluconeogenesis (abbreviated GNG) is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.
- **glucosyltransferases**: Glucosyltransferases are a type of glycosyltransferase that enable the transfer of glucose such as glycogen synthesis.

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7.7.3: Lipid Biosynthesis

Learning Objectives

- Outline the characteristics and processes of lipid biosynthesis, including:; lipogenesis and fatty acid biosynthesis

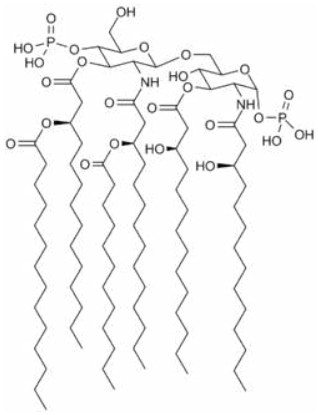


Figure: **Chemical structure of lipid A as found in E. coli**: Chemical structure of lipid A as found in E. coli

Lipids constitute a broad group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules.

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules. The amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or “building-blocks”: ketoacyl and isoprene groups. Using this approach, lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits).

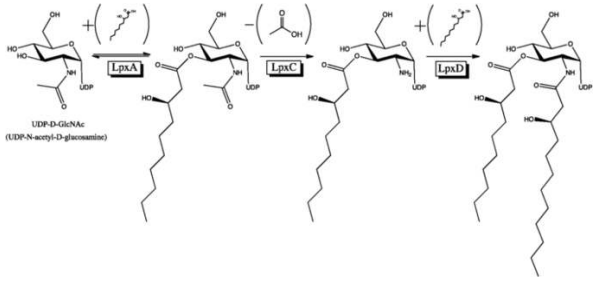


Figure: **Synthesis of the UDP-diacylglucosamine precursor of Lipid A**: Synthesis of the UDP-diacylglucosamine precursor of Lipid AThe synthesis of unsaturated fatty acids involves a desaturation reaction, whereby a double bond is introduced into the fatty acyl chain. For example, in humans, the desaturation of stearic acid by stearoyl-CoA desaturase-1 produces oleic acid. The doubly unsaturated fatty acid linoleic acid as well as the triply unsaturated α-linolenic acid cannot be synthesized in mammalian tissues, and are therefore essential fatty acids and must be obtained from the diet. Triglyceride synthesis takes place in the endoplasmic reticulum by metabolic pathways in which acyl groups in fatty acyl-CoAs are transferred to the hydroxyl groups of glycerol-3-phosphate and diacylglycerol. Terpenes and isoprenoids, including the carotenoids, are made by the assembly and modification of isoprene units donated from the reactive precursors isopentenyl pyrophosphate and dimethylallyl pyrophosphate. These precursors can be made in different ways. In archaea, the mevalonate pathway produces these compounds from acetyl-CoA, while in plants and bacteria the non-mevalonate pathway uses pyruvate and glyceraldehyde 3-phosphate as substrates.

Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

In animals, when there is an oversupply of dietary carbohydrate, the excess carbohydrate is converted to triglycerides. This involves the synthesis of fatty acids from acetyl-CoA and the esterification of fatty acids in the production of triglycerides, a process called lipogenesis. Fatty acids are made by fatty acid synthases that polymerize and then reduce acetyl-CoA units. The acyl

chains in the fatty acids are extended by a cycle of reactions that add the acetyl group, reduce it to an alcohol, dehydrate it to an alkene group and then reduce it again to an alkane group.

The enzymes of fatty acid biosynthesis are divided into two groups, in animals and fungi all these fatty acid synthase reactions are carried out by a single multifunctional protein, while in plant plastids and bacteria separate enzymes perform each step in the pathway. The fatty acids may be subsequently converted to triglycerides that are packaged in lipoproteins and secreted from the liver.

One important reaction that uses these activated isoprene donors is steroid biosynthesis. Here, the isoprene units are joined together to make squalene and then folded up and formed into a set of rings to make lanosterol. Lanosterol can then be converted into other steroids such as cholesterol and ergosterol.

Key Points

- Lipid A is a lipid component of an endotoxin held responsible for toxicity of Gram-negative bacteria.
- The synthesis of unsaturated fatty acids involves a desaturation reaction, whereby a double bond is introduced into the fatty acyl chain.
- In archaea, the mevalonate pathway produces reactive precursors isopentenyl pyrophosphate and dimethylallyl pyrophosphate from acetyl-CoA, while in plants and bacteria the non-mevalonate pathway uses pyruvate and glyceraldehyde 3-phosphate as substrates.

Key Terms

- **lipid A**: Lipid A is a lipid component of an endotoxin held responsible for toxicity of Gram-negative bacteria. It is the innermost of the three regions of the lipopolysaccharide (LPS, also called endotoxin) molecule, and its hydrophobic nature allows it to anchor the LPS to the outer membrane.
- **endotoxin**: Any toxin secreted by a microorganism and released into the surrounding environment only when it dies.
- **lipogenesis**: The biochemical production of fat, especially the conversion of carbohydrate into fat so that it may be stored as a long-term source of energy when food is scarce.

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7.E: Microbial Metabolism (Exercises)

8.1: Energy, Matter, and Enzymes

Cellular processes such as the building or breaking down of complex molecules occur through series of stepwise, interconnected chemical reactions called metabolic pathways. The term anabolism refers to those endergonic metabolic pathways involved in biosynthesis, converting simple molecular building blocks into more complex molecules, and fueled by the use of cellular energy.

Multiple Choice

Which of the following is an organism that obtains its energy from the transfer of electrons originating from chemical compounds and its carbon from an inorganic source?

A. chemoautotroph
B. chemoheterotroph
C. photoheterotroph
D. photoautotroph

Answer

A

Which of the following molecules is reduced?

A. NAD^+
B. FAD
C. O_2
D. NADPH

Answer

D

Enzymes work by which of the following?

A. increasing the activation energy
B. reducing the activation energy
C. making exergonic reactions endergonic
D. making endergonic reactions exergonic

Answer

B

To which of the following does a competitive inhibitor most structurally resemble?

A. the active site
B. the allosteric site
C. the substrate
D. a coenzyme

Answer

C

Which of the following are organic molecules that help enzymes work correctly?

A. cofactors
B. coenzymes
C. holoenzymes

D. apoenzymes

Answer

B

Fill in the Blank

Processes in which cellular energy is used to make complex molecules from simpler ones are described as _____.

Answer

anabolic

The loss of an electron from a molecule is called _____.

Answer

oxidation

The part of an enzyme to which a substrate binds is called the _____.

Answer

active site

True/False

Competitive inhibitors bind to allosteric sites.

Answer

False

Short Answer

In cells, can an oxidation reaction happen in the absence of a reduction reaction? Explain.

What is the function of molecules like NAD^+/NADH and FAD/$FADH_2$ in cells?

8.2: Catabolism of Carbohydrates

Glycolysis is the first step in the breakdown of glucose, resulting in the formation of ATP, which is produced by substrate-level phosphorylation; NADH; and two pyruvate molecules. Glycolysis does not use oxygen and is not oxygen dependent. After glycolysis, a three-carbon pyruvate is decarboxylated to form a two-carbon acetyl group, coupled with the formation of NADH. The acetyl group is attached to a large carrier compound called coenzyme A.

Multiple Choice

During which of the following is ATP not made by substrate-level phosphorylation?

A. Embden-Meyerhof pathway
B. Transition reaction
C. Krebs cycle
D. Entner-Doudoroff pathway

Answer

B

Which of the following products is made during Embden-Meyerhof glycolysis?

A. NAD^+
B. pyruvate

C. CO_2
D. two-carbon acetyl

Answer

B

During the catabolism of glucose, which of the following is produced only in the Krebs cycle?

A. ATP
B. NADH
C. NADPH
D. $FADH_2$

Answer

D

Which of the following is not a name for the cycle resulting in the conversion of a two-carbon acetyl to one ATP, two CO_2, one $FADH_2$, and three NADH molecules?

A. Krebs cycle
B. tricarboxylic acid cycle
C. Calvin cycle
D. citric acid cycle

Answer

C

True/False

Glycolysis requires oxygen or another inorganic final electron acceptor to proceed.

Answer

False

Fill in the Blank

Per turn of the Krebs cycle, one acetyl is oxidized, forming ____ CO_2, ____ ATP, ____ NADH, and ____ $FADH_2$molecules.

Answer

2; 1; 3; 1

Most commonly, glycolysis occurs by the _____ pathway.

Answer

Embden-Meyerhof

Short Answer

What is substrate-level phosphorylation? When does it occur during the breakdown of glucose to CO_2?

Why is the Krebs cycle important in both catabolism and anabolism?

Critical Thinking

What would be the consequences to a cell of having a mutation that knocks out coenzyme A synthesis?

8.3: Cellular Respiration

Cellular respiration begins when electrons are transferred from NADH and $FADH_2$—through a series of chemical reactions to a final inorganic electron acceptor (either oxygen in aerobic respiration or non-oxygen inorganic molecules in anaerobic respiration). These electron transfers take place on the inner part of the cell membrane of prokaryotic cells or in specialized protein complexes in the inner membrane of the mitochondria of eukaryotic cells.

Multiple Choice

Which is the location of electron transports systems in prokaryotes?

A. the outer mitochondrial membrane
B. the cytoplasm
C. the inner mitochondrial membrane
D. the cytoplasmic membrane

Answer

D

Which is the source of the energy used to make ATP by oxidative phosphorylation?

A. oxygen
B. high-energy phosphate bonds
C. the proton motive force
D. P_i

Answer

C

A cell might perform anaerobic respiration for which of the following reasons?

A. It lacks glucose for degradation.
B. It lacks the transition reaction to convert pyruvate to acetyl-CoA.
C. It lacks Krebs cycle enzymes for processing acetyl-CoA to CO_2.
D. It lacks a cytochrome oxidase for passing electrons to oxygen.

Answer

D

In prokaryotes, which of the following is true?

A. As electrons are transferred through an ETS, H^+ is pumped out of the cell.
B. As electrons are transferred through an ETS, H^+ is pumped into the cell.
C. As protons are transferred through an ETS, electrons are pumped out of the cell.
D. As protons are transferred through an ETS, electrons are pumped into the cell.

Answer

A

Which of the following is not an electron carrier within an electron transport system?

A. flavoprotein
B. ATP synthase
C. ubiquinone
D. cytochrome oxidase

Answer

B

Fill in the Blank

The final ETS complex used in aerobic respiration that transfers energy-depleted electrons to oxygen to form H_2O is called _____.

Answer

cytochrome oxidase

The passage of hydrogen ions through _____ down their electrochemical gradient harnesses the energy needed for ATP synthesis by oxidative phosphorylation.

Answer

ATP synthase

True/False

All organisms that use aerobic cellular respiration have cytochrome oxidase.

Answer

True

Short Answer

What is the relationship between chemiosmosis and the proton motive force?

How does oxidative phosphorylation differ from substrate-level phosphorylation?

How does the location of ATP synthase differ between prokaryotes and eukaryotes? Where do protons accumulate as a result of the ETS in each cell type?

8.4: Fermentation

Fermentation uses an organic molecule as a final electron acceptor to regenerate NAD^+ from NADH so that glycolysis can continue. Fermentation does not involve an electron transport system, and no ATP is made by the fermentation process directly. Fermenters make very little ATP—only two ATP molecules per glucose molecule during glycolysis. Microbial fermentation processes have been used for the production of foods and pharmaceuticals, and for the identification of microbes.

Multiple Choice

Which of the following is the purpose of fermentation?

A. to make ATP
B. to make carbon molecule intermediates for anabolism
C. to make NADH
D. to make NAD^+

Answer

D

Which molecule typically serves as the final electron acceptor during fermentation?

A. oxygen
B. NAD^+
C. pyruvate
D. CO_2

Answer

C

Which fermentation product is important for making bread rise?

A. ethanol
B. CO_2
C. lactic acid
D. hydrogen gas

Answer

B

Which of the following is not a commercially important fermentation product?

A. ethanol
B. pyruvate
C. butanol
D. penicillin

Answer

B

Fill in the Blank

The microbe responsible for ethanol fermentation for the purpose of producing alcoholic beverages is _____.

Answer

yeast (*Saccharomyces cerevisiae*)

_____ results in the production of a mixture of fermentation products, including lactic acid, ethanol and/or acetic acid, and CO_2.

Answer

Heterolactic fermentation

Fermenting organisms make ATP through the process of _____.

Answer

glycolysis

Matching

Match the fermentation pathway with the correct commercial product it is used to produce:

___acetone-butanol-ethanol fermentation	a. bread
___alcohol fermentation	b. pharmaceuticals
___lactic acid fermentation	c. Swiss cheese
___mixed acid fermentation	d. yogurt
___propionic acid fermentation	e. industrial solvents

Answer

e; 2. a; 3. d; 4. b; 5. c

Short Answer

Why are some microbes, including *Streptococcus* spp., unable to perform aerobic respiration, even in the presence of oxygen?

How can fermentation be used to differentiate various types of microbes?

Critical Thinking

The bacterium *E. coli* is capable of performing aerobic respiration, anaerobic respiration, and fermentation. When would it perform each process and why? How is ATP made in each case?

8.5: Catabolism of Lipids and Proteins

Collectively, microbes have the ability to degrade a wide variety of carbon sources besides carbohydrates, including lipids and proteins. The catabolic pathways for all of these molecules eventually connect into glycolysis and the Krebs cycle. Several types of lipids can be microbially degraded. Triglycerides are degraded by extracellular lipases, releasing fatty acids from the glycerol backbone. Phospholipids are degraded by phospholipases, releasing fatty acids and phosphorylated head groups.

Multiple Choice

Which of the following molecules is not produced during the breakdown of phospholipids?

A. glucose
B. glycerol
C. acetyl groups
D. fatty acids

Answer

A

Caseinase is which type of enzyme?

A. phospholipase
B. lipase
C. extracellular protease
D. intracellular protease

Answer

C

Which of the following is the first step in triglyceride degradation?

A. removal of fatty acids
B. β-oxidation
C. breakage of fused rings
D. formation of smaller peptides

Answer

A

Fill in the Blank

The process by which two-carbon units are sequentially removed from fatty acids, producing acetyl-CoA, $FADH_2$, and NADH is called _____.

Answer

β-oxidation

The NADH and $FADH_2$ produced during β-oxidation are used to make _____.

Answer

ATP by oxidative phosphorylation

_____ is a type of medium used to detect the production of an extracellular protease called caseinase.

Answer

Skim milk agar

Short Answer

How are the products of lipid and protein degradation connected to glucose metabolism pathways?

What is the general strategy used by microbes for the degradation of macromolecules?

Critical Thinking

Do you think that β-oxidation can occur in an organism incapable of cellular respiration? Why or why not?

8.6: Photosynthesis and the Importance of Light

Heterotrophic organisms ranging from *E. coli* to humans rely on the chemical energy found mainly in carbohydrate molecules. Many of these carbohydrates are produced by photosynthesis, the biochemical process by which phototrophic organisms convert solar energy (sunlight) into chemical energy. Although photosynthesis is most commonly associated with plants, microbial photosynthesis is also a significant supplier of chemical energy, fueling many diverse ecosystems.

Multiple Choice

During the light-dependent reactions, which molecule loses an electron?

A. a light-harvesting pigment molecule
B. a reaction center pigment molecule
C. NADPH
D. 3-phosphoglycerate

Answer

B

In prokaryotes, in which direction are hydrogen ions pumped by the electron transport system of photosynthetic membranes?

A. to the outside of the plasma membrane
B. to the inside (cytoplasm) of the cell
C. to the stroma
D. to the intermembrane space of the chloroplast

Answer

A

Which of the following does not occur during cyclic photophosphorylation in cyanobacteria?

A. electron transport through an ETS
B. photosystem I use
C. ATP synthesis
D. NADPH formation

Answer

D

Which are two products of the light-dependent reactions are _____.

A. glucose and NADPH
B. NADPH and ATP
C. glyceraldehyde 3-phosphate and CO_2
D. glucose and oxygen

Answer

B

True/False

Photosynthesis always results in the formation of oxygen.

Answer

False

Fill in the Blank

The enzyme responsible for CO_2 fixation during the Calvin cycle is called _____.

Answer

ribulose bisphosphate carboxylase (RuBisCO)

The types of pigment molecules found in plants, algae, and cyanobacteria are _____ and _____.

Answer

chlorophylls and carotenoids

Short Answer

Why would an organism perform cyclic phosphorylation instead of noncyclic phosphorylation?

What is the function of photosynthetic pigments in the light-harvesting complex?

Critical Thinking

Is life dependent on the carbon fixation that occurs during the light-independent reactions of photosynthesis? Explain.

8.7: Biogeochemical Cycles

Energy flows directionally through ecosystems, entering as sunlight for phototrophs or as inorganic molecules for chemoautotrophs. The six most common elements associated with organic molecules—carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur—take a variety of chemical forms and may exist for long periods in the atmosphere, on land, in water, or beneath earth's surface.

Multiple Choice

Which of the following is the group of archaea that can use CO_2 as their final electron acceptor during anaerobic respiration, producing CH_4?

A. methylotrophs
B. methanotrophs
C. methanogens
D. anoxygenic photosynthesizers

Answer

C

Which of the following processes is not involved in the conversion of organic nitrogen to nitrogen gas?

A. nitrogen fixation
B. ammonification
C. nitrification
D. denitrification

Answer

A

Which of the following processes produces hydrogen sulfide?

A. anoxygenic photosynthesis
B. oxygenic photosynthesis
C. anaerobic respiration
D. chemoautrophy

Answer

C

The biogeochemical cycle of which of the following elements is based on changes in solubility rather than redox chemistry?

A. carbon
B. sulfur
C. nitrogen
D. phosphorus

Answer

D

Fill in the Blank

The molecule central to the carbon cycle that is exchanged within and between ecosystems, being produced by heterotrophs and used by autotrophs, is _____.

Answer

carbon dioxide

The use of microbes to remove pollutants from a contaminated system is called _____.

Answer

bioremediation

True/False

There are many naturally occurring microbes that have the ability to degrade several of the compounds found in oil.

Answer

True

Short Answer

Why must autotrophic organisms also respire or ferment in addition to fixing CO_2?

How can human activity lead to eutrophication?

Critical Thinking

In considering the symbiotic relationship between *Rhizobium* species and their plant hosts, what metabolic activity does each organism perform that benefits the other member of the pair?

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CHAPTER OVERVIEW

8: Microbial Growth

We are all familiar with the slimy layer on a pond surface or that makes rocks slippery. These are examples of biofilms—microorganisms embedded in thin layers of matrix material (Figure 8.1). Biofilms were long considered random assemblages of cells and had little attention from researchers. Recently, progress in visualization and biochemical methods has revealed that biofilms are an organized ecosystem within which many cells, usually of different species of bacteria, fungi, and algae, interact through cell signaling and coordinated responses. The biofilm provides a protected environment in harsh conditions and aids colonization by microorganisms. Biofilms also have clinical importance. They form on medical devices, resist routine cleaning and sterilization, and cause health-acquired infections. Within the body, biofilms form on the teeth as plaque, in the lungs of patients with cystic fibrosis, and on the cardiac tissue of patients with endocarditis. The slime layer helps protect the cells from host immune defenses and antibiotic treatments.

Studying biofilms requires new approaches. Because of the cells' adhesion properties, many of the methods for culturing and counting cells that are explored in this chapter are not easily applied to biofilms. This is the beginning of a new era of challenges and rewarding insight into the ways that microorganisms grow and thrive in nature.

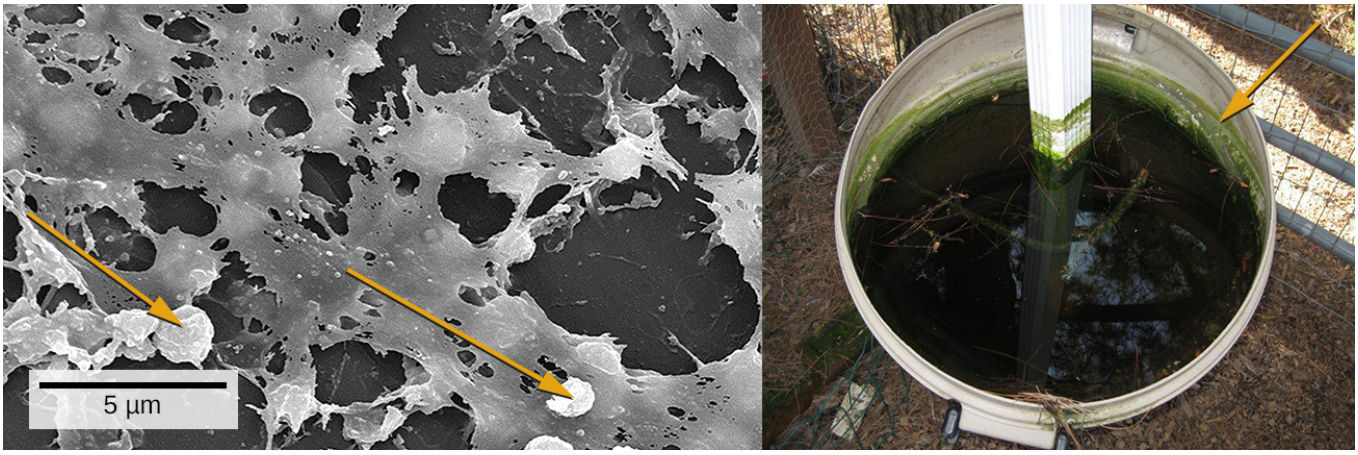


Figure 8.1: Medical devices that are inserted into a patient's body often become contaminated with a thin biofilm of microorganisms enmeshed in the sticky material they secrete. The electron micrograph (left) shows the inside walls of an indwelling catheter. Arrows point to the round cells of *Staphylococcus aureus* bacteria attached to the layers of extracellular substrate. The garbage can (right) served as a rain collector. The arrow points to a green biofilm on the sides of the container. (credit left: modification of work by Centers for Disease Control and Prevention; credit right: modification of work by NASA)

8.1: How Bacteria Grow

8.2: Factors that Affect Bacterial Growth

8.2.1: Temperature Effects on Bacterial Growth

8.2.2: pH Effects on Bacterial Growth

8.2.3: Nitrogen Effects on Bacterial Growth

8.2.4: Oxygen Effects on Bacterial Growth

8.2.5: Other Environmental Effects on Bacterial Growth

8.3: Bacterial Quorum Sensing

8.4: Culturing Bacteria

8.4.1: Media Used for Bacterial Growth

8.4.2: Culture Media

8.4.3: Complex and Synthetic Media

8.4.4: Selective and Differential Media

8.4.5: Special Culture Techniques
8.5: Growth Characteristics
8.5.1: Generation Time

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8.1: How Bacteria Grow

Learning Objectives

- Define the generation time for growth based on binary fission
- Identify and describe the activities of microorganisms undergoing typical phases of binary fission (simple cell division) in a growth curve
- Explain several laboratory methods used to determine viable and total cell counts in populations undergoing exponential growth
- Describe examples of cell division not involving binary fission, such as budding or fragmentation
- Describe the formation and characteristics of biofilms
- Identify health risks associated with biofilms and how they are addressed
- Describe quorum sensing and its role in cell-to-cell communication and coordination of cellular activities

Clinical Focus: Part 1

Jeni, a 24-year-old pregnant woman in her second trimester, visits a clinic with complaints of high fever, 38.9 °C (102 °F), fatigue, and muscle aches—typical flu-like signs and symptoms. Jeni exercises regularly and follows a nutritious diet with emphasis on organic foods, including raw milk that she purchases from a local farmer's market. All of her immunizations are up to date. However, the health-care provider who sees Jeni is concerned and orders a blood sample to be sent for testing by the microbiology laboratory.

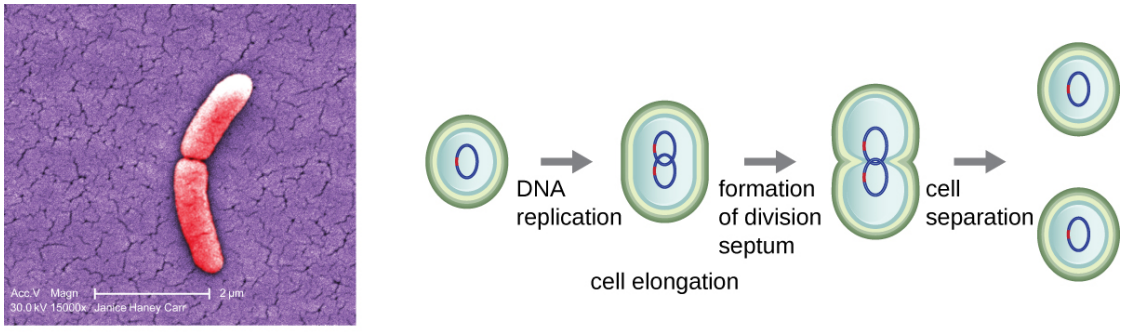
Exercise 8.1.1

Why is the health-care provider concerned about Jeni's signs and symptoms?

The bacterial cell cycle involves the formation of new cells through the replication of DNA and partitioning of cellular components into two daughter cells. In prokaryotes, reproduction is always asexual, although extensive genetic recombination in the form of horizontal gene transfer takes place, as will be explored in a different chapter. Most bacteria have a single circular chromosome; however, some exceptions exist. For example, *Borrelia burgdorferi*, the causative agent of Lyme disease, has a linear chromosome.

Binary Fission

The most common mechanism of cell replication in bacteria is a process called binary fission, which is depicted in Figure 8.1.1. Before dividing, the cell grows and increases its number of cellular components. Next, the replication of DNA starts at a location on the circular chromosome called the origin of replication, where the chromosome is attached to the inner cell membrane. Replication continues in opposite directions along the chromosome until the terminus is reached.



(a) (b)

Figure 8.1.1: (a) The electron micrograph depicts two cells of *Salmonella typhimurium* after a binary fission event. (b) Binary fission in bacteria starts with the replication of DNA as the cell elongates. A division septum forms in the center of the cell. Two daughter cells of similar size form and separate, each receiving a copy of the original chromosome. (credit a: modification of work by Centers for Disease Control and Prevention).

The center of the enlarged cell constricts until two daughter cells are formed, each offspring receiving a complete copy of the parental genome and a division of the cytoplasm (cytokinesis). This process of cytokinesis and cell division is directed by a protein called FtsZ. FtsZ assembles into a Z ring on the cytoplasmic membrane (Figure **8.1.2**). The Z ring is anchored by FtsZ-binding proteins and defines the division plane between the two daughter cells. Additional proteins required for cell division are added to the Z ring to form a structure called the divisome. The divisome activates to produce a peptidoglycan cell wall and build a septum that divides the two daughter cells. The daughter cells are separated by the division septum, where all of the cells' outer layers (the cell wall and outer membranes, if present) must be remodeled to complete division. For example, we know that specific enzymes break bonds between the monomers in peptidoglycans and allow addition of new subunits along the division septum.

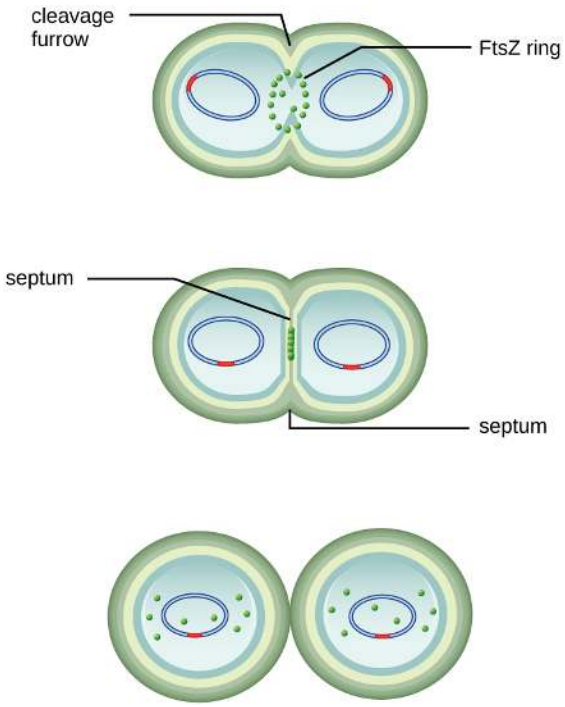


Figure **8.1.2**: FtsZ proteins assemble to form a Z ring that is anchored to the plasma membrane. The Z ring pinches the cell envelope to separate the cytoplasm of the new cells.

? Exercise 8.1.2

What is the name of the protein that assembles into a Z ring to initiate cytokinesis and cell division?

Generation Time

In eukaryotic organisms, the generation time is the time between the same points of the life cycle in two successive generations. For example, the typical generation time for the human population is 25 years. This definition is not practical for bacteria, which may reproduce rapidly or remain dormant for thousands of years. In prokaryotes (Bacteria and Archaea), the generation time is also called the doubling time and is defined as the time it takes for the population to double through one round of binary fission. Bacterial doubling times vary enormously. Whereas *Escherichia coli* can double in as little as 20 minutes under optimal growth conditions in the laboratory, bacteria of the same species may need several days to double in especially harsh environments. Most pathogens grow rapidly, like *E. coli*, but there are exceptions. For example, *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has a generation time of between 15 and 20 hours. On the other hand, *M. leprae*, which causes Hansen's disease (leprosy), grows much more slowly, with a doubling time of 14 days.

Calculating Number of Cells

It is possible to predict the number of cells in a population when they divide by binary fission at a constant rate. As an example, consider what happens if a single cell divides every 30 minutes for 24 hours. The diagram in Figure **8.1.3** shows the increase in cell numbers for the first three generations.

The number of cells increases exponentially and can be expressed as 2^n, where n is the number of generations. If cells divide every 30 minutes, after 24 hours, 48 divisions would have taken place. If we apply the formula 2^n, where n is equal to 48, the single cell would give rise to 2^{48} or 281,474,976,710,656 cells at 48 generations (24 hours). When dealing with such huge numbers, it is more practical to use scientific notation. Therefore, we express the number of cells as 2.8×10^{14} cells.

In our example, we used one cell as the initial number of cells. For any number of starting cells, the formula is adapted as follows:

$$N_n = N_0 2^n \tag{8.1.1}$$

N_n is the number of cells at any generation n, N_0 is the initial number of cells, and n is the number of generations.

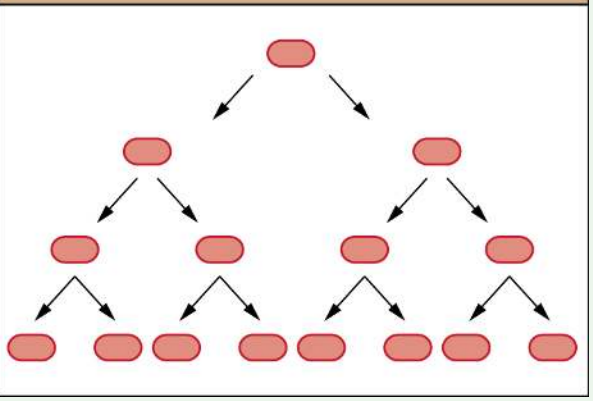
Number of generations (n)	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

Figure **8.1.3**: The parental cell divides and gives rise to two daughter cells. Each of the daughter cells, in turn, divides, giving a total of four cells in the second generation and eight cells in the third generation. Each division doubles the number of cells.

? Exercise 8.1.3

With a doubling time of 30 minutes and a starting population size of 1×10^5 cells, how many cells will be present after 2 hours, assuming no cell death?

The Growth Curve

Microorganisms grown in closed culture (also known as a batch culture), in which no nutrients are added and most waste is not removed, follow a reproducible growth pattern referred to as the growth curve. An example of a batch culture in nature is a pond in which a small number of cells grow in a closed environment. The culture density is defined as the number of cells per unit volume. In a closed environment, the culture density is also a measure of the number of cells in the population. Infections of the body do not always follow the growth curve, but correlations can exist depending upon the site and type of infection. When the number of live cells is plotted against time, distinct phases can be observed in the curve (Figure **8.1.4**).

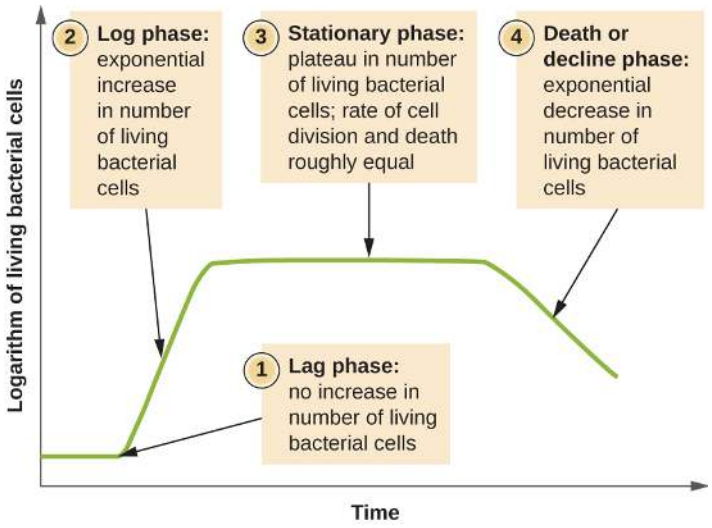


Figure **8.1.4**: The growth curve of a bacterial culture is represented by the logarithm of the number of live cells plotted as a function of time. The graph can be divided into four phases according to the slope, each of which matches events in the cell. The four phases are lag, log, stationary, and death.

The Lag Phase

The beginning of the growth curve represents a small number of cells, referred to as an inoculum, that are added to a fresh culture medium, a nutritional broth that supports growth. The initial phase of the growth curve is called the lag phase, during which cells are gearing up for the next phase of growth. The number of cells does not change during the lag phase; however, cells grow larger and are metabolically active, synthesizing proteins needed to grow within the medium. If any cells were damaged or shocked during the transfer to the new medium, repair takes place during the lag phase. The duration of the lag phase is determined by many factors, including the species and genetic make-up of the cells, the composition of the medium, and the size of the original inoculum.

The Log Phase

In the logarithmic (log) growth phase, sometimes called exponential growth phase, the cells are actively dividing by binary fission and their number increases exponentially. For any given bacterial species, the generation time under specific growth conditions (nutrients, temperature, pH, and so forth) is genetically determined, and this generation time is called the intrinsic growth rate. During the log phase, the relationship between time and number of cells is not linear but exponential; however, the growth curve is often plotted on a semilogarithmic graph, as shown in Figure **8.1.5**, which gives the appearance of a linear relationship.

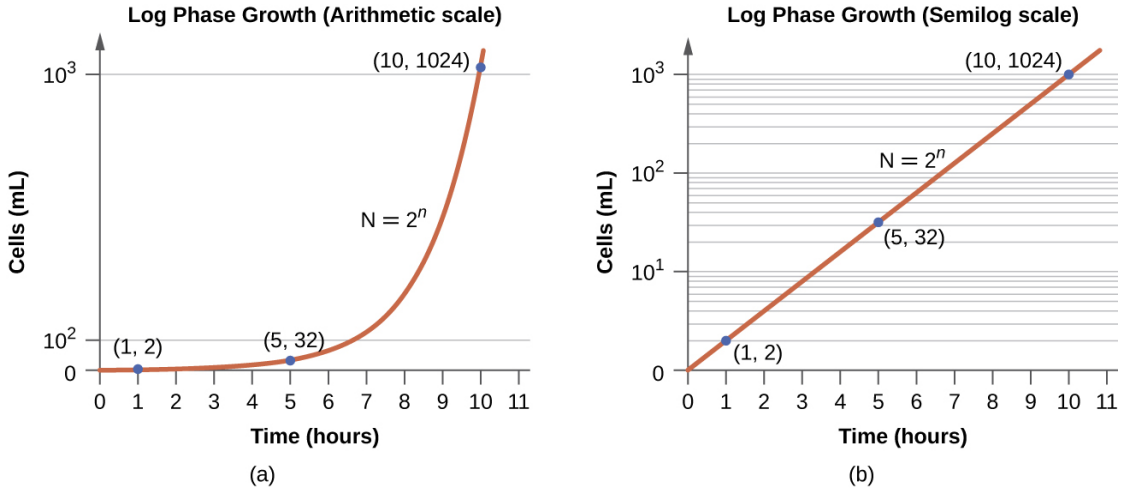


Figure **8.1.5**: Both graphs illustrate population growth during the log phase for a bacterial sample with an initial population of one cell and a doubling time of 1 hour. (a) When plotted on an arithmetic scale, the growth rate resembles a curve. (b) When plotted on a semilogarithmic scale (meaning the values on the y-axis are logarithmic), the growth rate appears linear.

Cells in the log phase show constant growth rate and uniform metabolic activity. For this reason, cells in the log phase are preferentially used for industrial applications and research work. The log phase is also the stage where bacteria are the most susceptible to the action of disinfectants and common antibiotics that affect protein, DNA, and cell-wall synthesis.

Stationary Phase

As the number of cells increases through the log phase, several factors contribute to a slowing of the growth rate. Waste products accumulate and nutrients are gradually used up. In addition, gradual depletion of oxygen begins to limit aerobic cell growth. This combination of unfavorable conditions slows and finally stalls population growth. The total number of live cells reaches a plateau referred to as the stationary phase (Figure **8.1.4**). In this phase, the number of new cells created by cell division is now equivalent to the number of cells dying; thus, the total population of living cells is relatively stagnant. The culture density in a stationary culture is constant. The culture's carrying capacity, or maximum culture density, depends on the types of microorganisms in the culture and the specific conditions of the culture; however, carrying capacity is constant for a given organism grown under the same conditions.

During the stationary phase, cells switch to a survival mode of metabolism. As growth slows, so too does the synthesis of peptidoglycans, proteins, and nucleic-acids; thus, stationary cultures are less susceptible to antibiotics that disrupt these processes. In bacteria capable of producing endospores, many cells undergo sporulation during the stationary phase. Secondary metabolites, including antibiotics, are synthesized in the stationary phase. In certain pathogenic bacteria, the stationary phase is also associated with the expression of virulence factors, products that contribute to a microbe's ability to survive, reproduce, and cause disease in a host organism. For example, quorum sensing in *Staphylococcus aureus* initiates the production of enzymes that can break down human tissue and cellular debris, clearing the way for bacteria to spread to new tissue where nutrients are more plentiful.

The Death Phase

As a culture medium accumulates toxic waste and nutrients are exhausted, cells die in greater and greater numbers. Soon, the number of dying cells exceeds the number of dividing cells, leading to an exponential decrease in the number of cells (Figure **8.1.4**). This is the aptly named death phase, sometimes called the decline phase. Many cells lyse and release nutrients into the medium, allowing surviving cells to maintain viability and form endospores. A few cells, the so-called persisters, are characterized by a slow metabolic rate. Persister cells are medically important because they are associated with certain chronic infections, such as tuberculosis, that do not respond to antibiotic treatment.

Sustaining Microbial Growth

The growth pattern shown in Figure **8.1.4** takes place in a closed environment; nutrients are not added and waste and dead cells are not removed. In many cases, though, it is advantageous to maintain cells in the logarithmic phase of growth. One example is in industries that harvest microbial products. A chemostat (Figure **8.1.6**) is used to maintain a continuous culture in which nutrients are supplied at a steady rate. A controlled amount of air is mixed in for aerobic processes. Bacterial suspension is removed at the same rate as nutrients flow in to maintain an optimal growth environment.

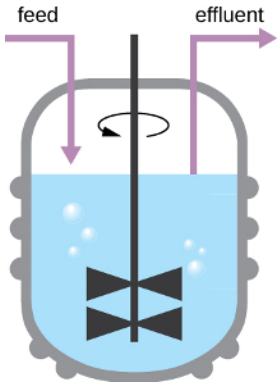


Figure **8.1.6**: A chemostat is a culture vessel fitted with an opening to add nutrients (feed) and an outlet to remove contents (effluent), effectively diluting toxic wastes and dead cells. The addition and removal of fluids is adjusted to maintain the culture in the logarithmic phase of growth. If aerobic bacteria are grown, suitable oxygen levels are maintained.

? Exercise 8.1.4

1. During which phase does growth occur at the fastest rate?
2. Name two factors that limit microbial growth.

Measurement of Bacterial Growth

Estimating the number of bacterial cells in a sample, known as a bacterial count, is a common task performed by microbiologists. The number of bacteria in a clinical sample serves as an indication of the extent of an infection. Quality control of drinking water, food, medication, and even cosmetics relies on estimates of bacterial counts to detect contamination and prevent the spread of disease. Two major approaches are used to measure cell number. The direct methods involve counting cells, whereas the indirect methods depend on the measurement of cell presence or activity without actually counting individual cells. Both direct and indirect methods have advantages and disadvantages for specific applications.

Direct Cell Count

Direct cell count refers to counting the cells in a liquid culture or colonies on a plate. It is a direct way of estimating how many organisms are present in a sample. Let's look first at a simple and fast method that requires only a specialized slide and a compound microscope.

The simplest way to count bacteria is called the direct microscopic cell count, which involves transferring a known volume of a culture to a calibrated slide and counting the cells under a light microscope. The calibrated slide is called a Petroff-Hausser chamber (Figure **8.1.7**) and is similar to a hemocytometer used to count red blood cells. The central area of the counting chamber is etched into squares of various sizes. A sample of the culture suspension is added to the chamber under a coverslip that is placed at a specific height from the surface of the grid. It is possible to estimate the concentration of cells in the original sample by counting individual cells in a number of squares and determining the volume of the sample observed. The area of the squares and the height at which the coverslip is positioned are specified for the chamber. The concentration must be corrected for dilution if the sample was diluted before enumeration.

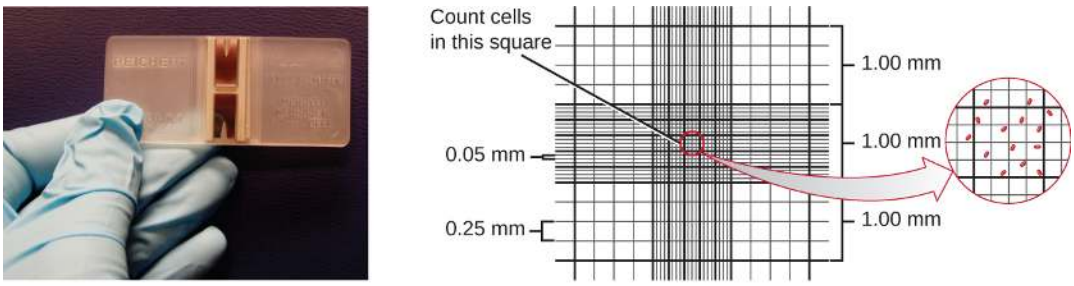


Figure **8.1.7**: (a) A Petroff-Hausser chamber is a special slide designed for counting the bacterial cells in a measured volume of a sample. A grid is etched on the slide to facilitate precision in counting. (b) This diagram illustrates the grid of a Petroff-Hausser chamber, which is made up of squares of known areas. The enlarged view shows the square within which bacteria (red cells) are counted. If the coverslip is 0.2 mm above the grid and the square has an area of 0.04 mm^2, then the volume is 0.008 mm^3, or 0.000008 mL. Since there are 10 cells inside the square, the density of bacteria is 10 cells/0.000008 mL, which equates to 1,250,000 cells/mL. (credit a: modification of work by Jeffrey M. Vinocur)

Cells in several small squares must be counted and the average taken to obtain a reliable measurement. The advantages of the chamber are that the method is easy to use, relatively fast, and inexpensive. On the downside, the counting chamber does not work well with dilute cultures because there may not be enough cells to count.

Using a counting chamber does not necessarily yield an accurate count of the number of live cells because it is not always possible to distinguish between live cells, dead cells, and debris of the same size under the microscope. However, newly developed fluorescence staining techniques make it possible to distinguish viable and dead bacteria. These viability stains (or live stains) bind to nucleic acids, but the primary and secondary stains differ in their ability to cross the cytoplasmic membrane. The primary stain, which fluoresces green, can penetrate intact cytoplasmic membranes, staining both live and dead cells. The secondary stain, which fluoresces red, can stain a cell only if the cytoplasmic membrane is considerably damaged. Thus, live cells fluoresce green because

they only absorb the green stain, whereas dead cells appear red because the red stain displaces the green stain on their nucleic acids (Figure 8.1.8).

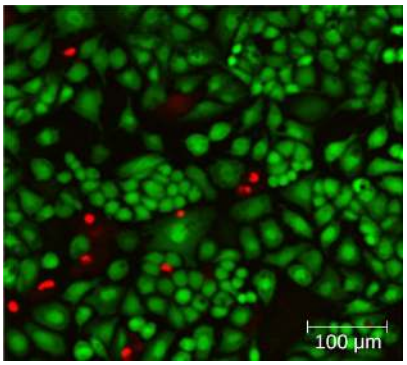
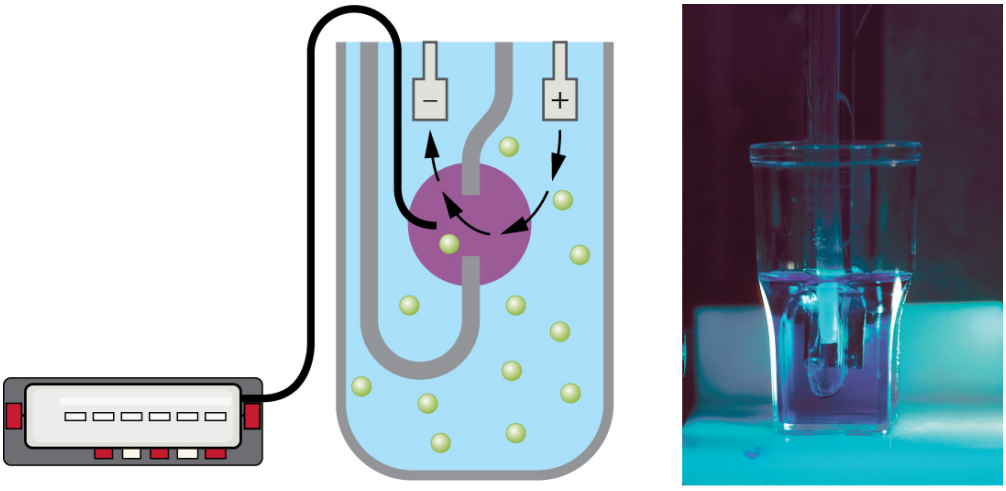


Figure 8.1.8: Fluorescence staining can be used to differentiate between viable and dead bacterial cells in a sample for purposes of counting. Viable cells are stained green, whereas dead cells are stained red. (credit: modification of work by Panseri S, Cunha C, D'Alessandro T, Sandri M, Giavaresi G, Maracci M, Hung CT, Tampieri A)

Another technique uses an electronic cell counting device (Coulter counter) to detect and count the changes in electrical resistance in a saline solution. A glass tube with a small opening is immersed in an electrolyte solution. A first electrode is suspended in the glass tube. A second electrode is located outside of the tube. As cells are drawn through the small aperture in the glass tube, they briefly change the resistance measured between the two electrodes and the change is recorded by an electronic sensor (Figure 8.1.9); each resistance change represents a cell. The method is rapid and accurate within a range of concentrations; however, if the culture is too concentrated, more than one cell may pass through the aperture at any given time and skew the results. This method also does not differentiate between live and dead cells.

Direct counts provide an estimate of the total number of cells in a sample. However, in many situations, it is important to know the number of live, or viable, cells. Counts of live cells are needed when assessing the extent of an infection, the effectiveness of antimicrobial compounds and medication, or contamination of food and water.



(a) (b)

Figure 8.1.9: A Coulter counter is an electronic device that counts cells. It measures the change in resistance in an electrolyte solution that takes place when a cell passes through a small opening in the inside container wall. A detector automatically counts the number of cells passing through the opening. (credit b: modification of work by National Institutes of Health)

? Exercise 8.1.5

1. Why would you count the number of cells in more than one square in the Petroff-Hausser chamber to estimate cell numbers?
2. In the viability staining method, why do dead cells appear red?

Plate Count

The viable plate count, or simply plate count, is a count of viable or live cells. It is based on the principle that viable cells replicate and give rise to visible colonies when incubated under suitable conditions for the specimen. The results are usually expressed as colony-forming units per milliliter (CFU/mL) rather than cells per milliliter because more than one cell may have landed on the same spot to give rise to a single colony. Furthermore, samples of bacteria that grow in clusters or chains are difficult to disperse and a single colony may represent several cells. Some cells are described as viable but nonculturable and will not form colonies on solid media. For all these reasons, the viable plate count is considered a low estimate of the actual number of live cells. These limitations do not detract from the usefulness of the method, which provides estimates of live bacterial numbers.

Microbiologists typically count plates with 30–300 colonies. Samples with too few colonies (<30) do not give statistically reliable numbers, and overcrowded plates (>300 colonies) make it difficult to accurately count individual colonies. Also, counts in this range minimize occurrences of more than one bacterial cell forming a single colony. Thus, the calculated CFU is closer to the true number of live bacteria in the population.

There are two common approaches to inoculating plates for viable counts: the pour plate and the spread plate methods. Although the final inoculation procedure differs between these two methods, they both start with a serial dilution of the culture.

Serial Dilution

The serial dilution of a culture is an important first step before proceeding to either the pour plate or spread plate method. The goal of the serial dilution process is to obtain plates with CFUs in the range of 30–300, and the process usually involves several dilutions in multiples of 10 to simplify calculation. The number of serial dilutions is chosen according to a preliminary estimate of the culture density. Figure 8.1.10 illustrates the serial dilution method.

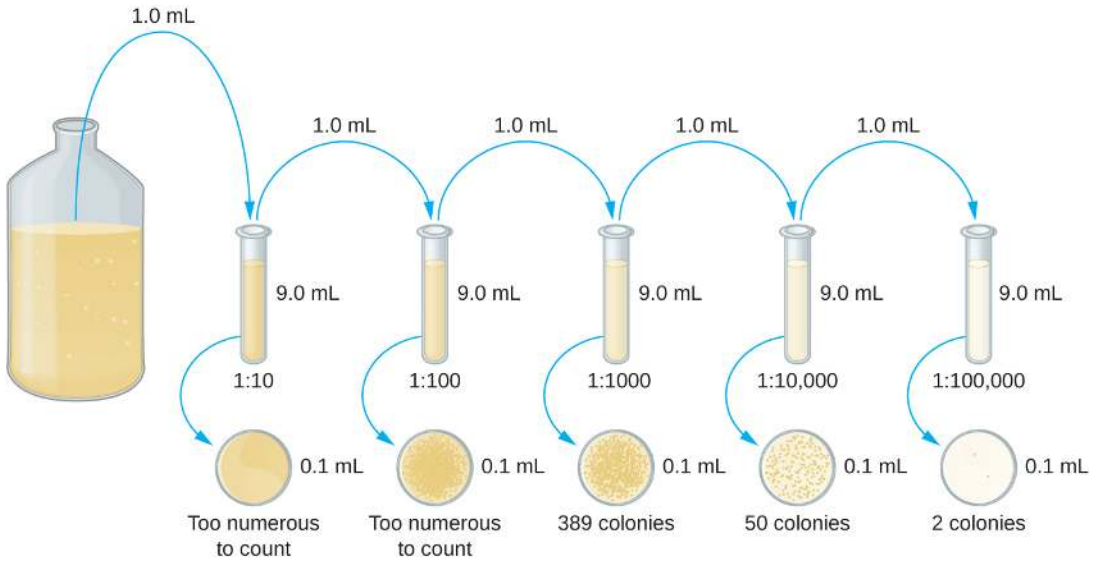


Figure 8.1.10: Serial dilution involves diluting a fixed volume of cells mixed with dilution solution using the previous dilution as an inoculum. The result is dilution of the original culture by an exponentially growing factor. (credit: modification of work by "Leberechtc"/Wikimedia Commons)

A fixed volume of the original culture, 1.0 mL, is added to and thoroughly mixed with the first dilution tube solution, which contains 9.0 mL of sterile broth. This step represents a dilution factor of 10, or 1:10, compared with the original culture. From this first dilution, the same volume, 1.0 mL, is withdrawn and mixed with a fresh tube of 9.0 mL of dilution solution. The dilution factor is now 1:100 compared with the original culture. This process continues until a series of dilutions is produced that will bracket the desired cell concentration for accurate counting. From each tube, a sample is plated on solid medium using either the pour plate method (Figure 8.1.11) or the spread plate method (Figure 8.1.12). The plates are incubated until colonies appear. Two to three plates are usually prepared from each dilution and the numbers of colonies counted on each plate are averaged. In all cases, thorough mixing of samples with the dilution medium (to ensure the cell distribution in the tube is random) is paramount to obtaining reliable results.

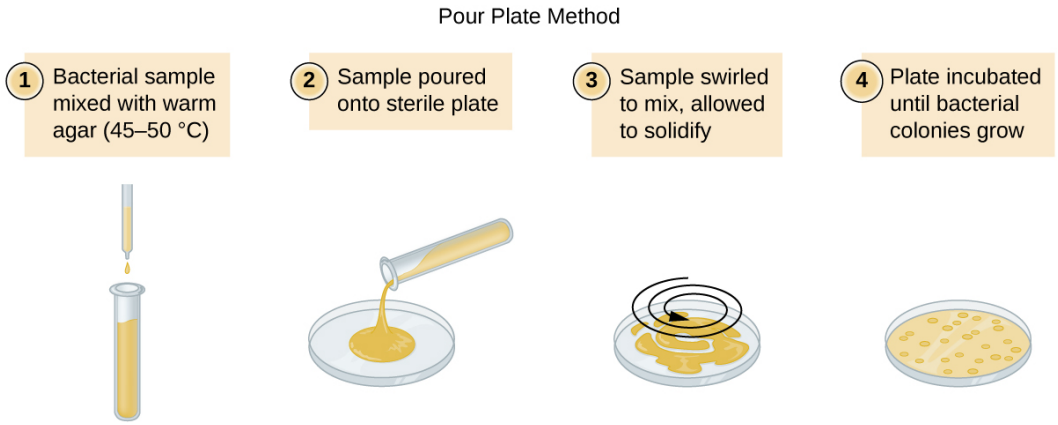


Figure **8.1.11**: In the pour plate method of cell counting, the sample is mixed in liquid warm agar (45–50 °C) poured into a sterile Petri dish and further mixed by swirling. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number of cells in the original volume sampled.

The dilution factor is used to calculate the number of cells in the original cell culture. In our example, an average of 50 colonies was counted on the plates obtained from the 1:10,000 dilution. Because only 0.1 mL of suspension was pipetted on the plate, the multiplier required to reconstitute the original concentration is 10 × 10,000. The number of CFU per mL is equal to 50 × 100 × 10,000 = 5,000,000. The number of bacteria in the culture is estimated as 5 million cells/mL. The colony count obtained from the 1:1000 dilution was 389, well below the expected 500 for a 10-fold difference in dilutions. This highlights the issue of inaccuracy when colony counts are greater than 300 and more than one bacterial cell grows into a single colony.

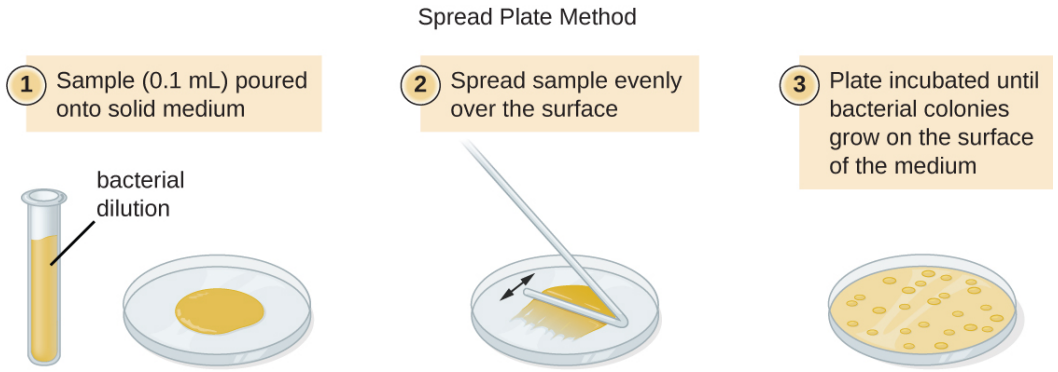


Figure **8.1.12**: In the spread plate method of cell counting, the sample is poured onto solid agar and then spread using a sterile spreader. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number of cells in the original volume samples.

A very dilute sample—drinking water, for example—may not contain enough organisms to use either of the plate count methods described. In such cases, the original sample must be concentrated rather than diluted before plating. This can be accomplished using a modification of the plate count technique called the membrane filtration technique. Known volumes are vacuum-filtered aseptically through a membrane with a pore size small enough to trap microorganisms. The membrane is transferred to a Petri plate containing an appropriate growth medium. Colonies are counted after incubation. Calculation of the cell density is made by dividing the cell count by the volume of filtered liquid.

Link to Learning

Watch this video for demonstrations of serial dilutions and spread plate techniques.

The Most Probable Number

The number of microorganisms in dilute samples is usually too low to be detected by the plate count methods described thus far. For these specimens, microbiologists routinely use the most probable number (MPN) method, a statistical procedure for estimating of the number of viable microorganisms in a sample. Often used for water and food samples, the MPN method evaluates detectable growth by observing changes in turbidity or color due to metabolic activity.

A typical application of MPN method is the estimation of the number of coliforms in a sample of pond water. Coliforms are gram-negative rod bacteria that ferment lactose. The presence of coliforms in water is considered a sign of contamination by fecal matter. For the method illustrated in Figure **8.1.13**, a series of three dilutions of the water sample is tested by inoculating five lactose broth tubes with 10 mL of sample, five lactose broth tubes with 1 mL of sample, and five lactose broth tubes with 0.1 mL of sample. The lactose broth tubes contain a pH indicator that changes color from red to yellow when the lactose is fermented. After inoculation and incubation, the tubes are examined for an indication of coliform growth by a color change in media from red to yellow. The first set of tubes (10-mL sample) showed growth in all the tubes; the second set of tubes (1 mL) showed growth in two tubes out of five; in the third set of tubes, no growth is observed in any of the tubes (0.1-mL dilution). The numbers 5, 2, and 0 are compared with Figure B1 in Appendix B, which has been constructed using a probability model of the sampling procedure. From our reading of the table, we conclude that 49 is the most probable number of bacteria per 100 mL of pond water.

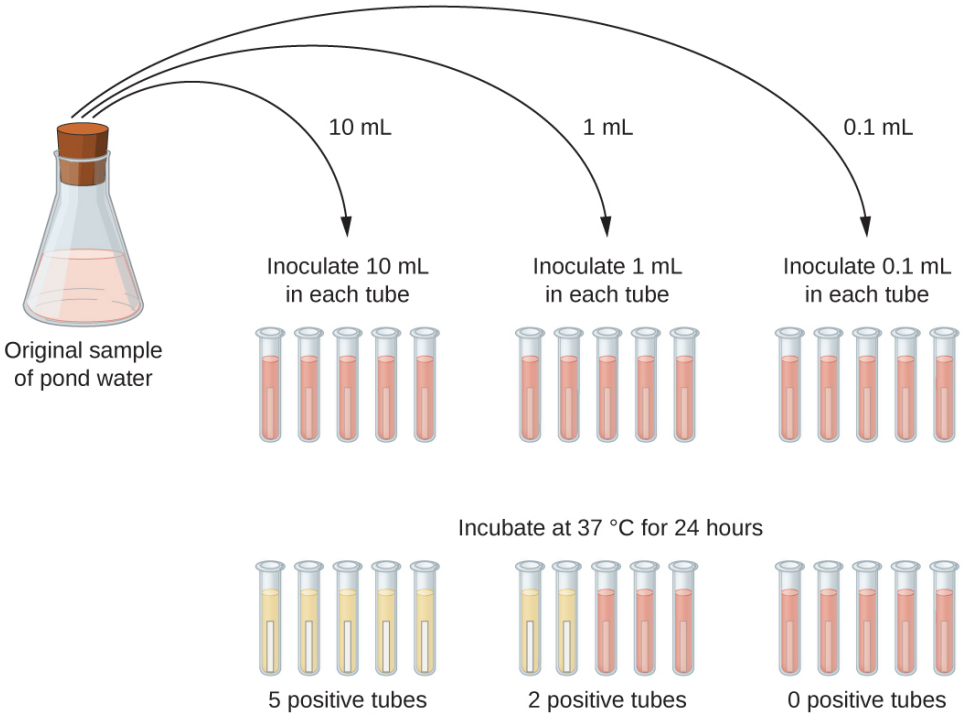


Figure **8.1.13**: In the most probable number method, sets of five lactose broth tubes are inoculated with three different volumes of pond water: 10 mL, 1 mL, and 0.1mL. Bacterial growth is assessed through a change in the color of the broth from red to yellow as lactose is fermented.

? Exercise 8.1.6

1. What is a colony-forming unit?
2. What two methods are frequently used to estimate bacterial numbers in water samples?

Indirect Cell Counts

Besides direct methods of counting cells, other methods, based on an indirect detection of cell density, are commonly used to estimate and compare cell densities in a culture. The foremost approach is to measure the turbidity (cloudiness) of a sample of bacteria in a liquid suspension. The laboratory instrument used to measure turbidity is called a spectrophotometer (Figure **8.1.14**). In a spectrophotometer, a light beam is transmitted through a bacterial suspension, the light passing through the suspension is measured by a detector, and the amount of light passing through the sample and reaching the detector is converted to either percent transmission or a logarithmic value called absorbance (optical density). As the numbers of bacteria in a suspension increase, the turbidity also increases and causes less light to reach the detector. The decrease in light passing through the sample and reaching the detector is associated with a decrease in percent transmission and increase in absorbance measured by the spectrophotometer.

Measuring turbidity is a fast method to estimate cell density as long as there are enough cells in a sample to produce turbidity. It is possible to correlate turbidity readings to the actual number of cells by performing a viable plate count of samples taken from

cultures having a range of absorbance values. Using these values, a calibration curve is generated by plotting turbidity as a function of cell density. Once the calibration curve has been produced, it can be used to estimate cell counts for all samples obtained or cultured under similar conditions and with densities within the range of values used to construct the curve.

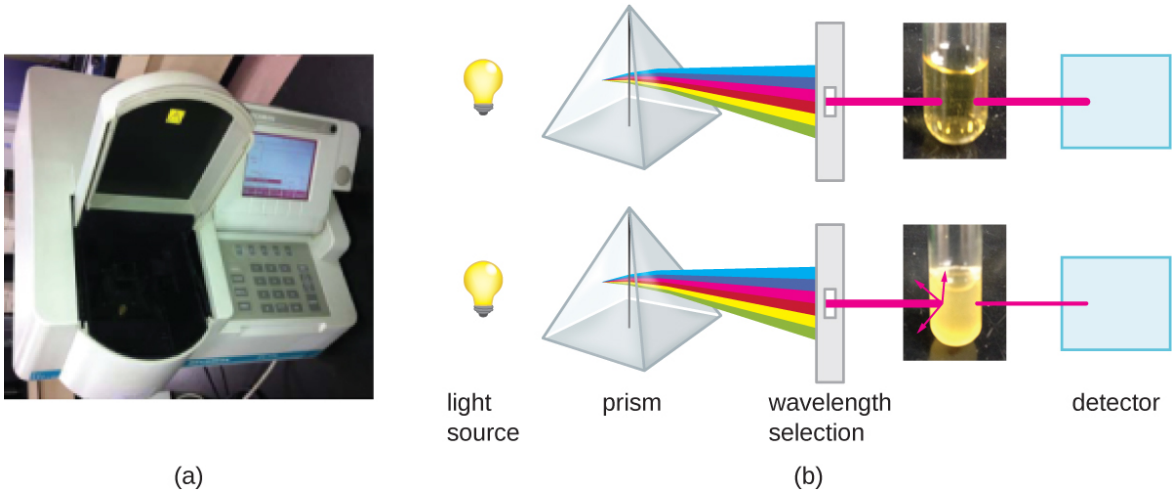


Figure 8.1.14: (a) A spectrophotometer is commonly used to measure the turbidity of a bacterial cell suspension as an indirect measure of cell density. (b) A spectrophotometer works by splitting white light from a source into a spectrum. The spectrophotometer allows choice of the wavelength of light to use for the measurement. The optical density (turbidity) of the sample will depend on the wavelength, so once one wavelength is chosen, it must be used consistently. The filtered light passes through the sample (or a control with only medium) and the light intensity is measured by a detector. The light passing into a suspension of bacteria is scattered by the cells in such a way that some fraction of it never reaches the detector. This scattering happens to a far lesser degree in the control tube with only the medium. (credit a: modification of work by Hwang HS, Kim MS; credit b "test tube photos": modification of work by Suzanne Wakim)

Measuring dry weight of a culture sample is another indirect method of evaluating culture density without directly measuring cell counts. The cell suspension used for weighing must be concentrated by filtration or centrifugation, washed, and then dried before the measurements are taken. The degree of drying must be standardized to account for residual water content. This method is especially useful for filamentous microorganisms, which are difficult to enumerate by direct or viable plate count.

As we have seen, methods to estimate viable cell numbers can be labor intensive and take time because cells must be grown. Recently, indirect ways of measuring live cells have been developed that are both fast and easy to implement. These methods measure cell activity by following the production of metabolic products or disappearance of reactants. Adenosine triphosphate (ATP) formation, biosynthesis of proteins and nucleic acids, and consumption of oxygen can all be monitored to estimate the number of cells.

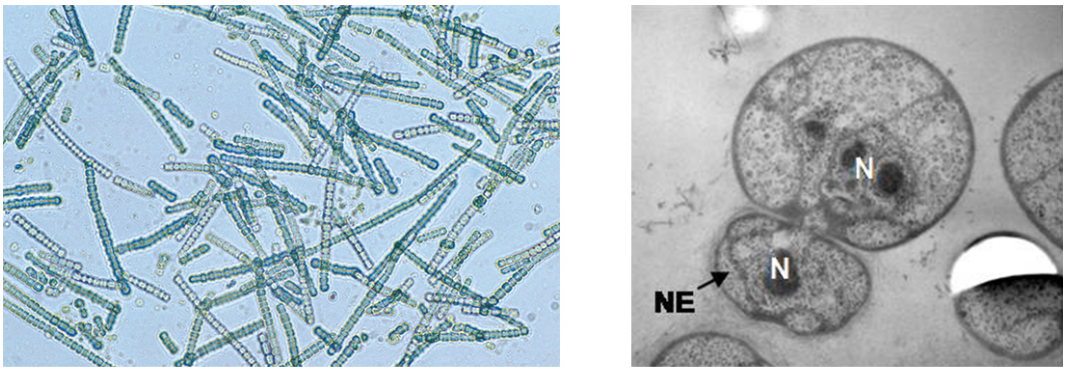
? Exercise 8.1.7

1. What is the purpose of a calibration curve when estimating cell count from turbidity measurements?
2. What are the newer indirect methods of counting live cells?

Alternative Patterns of Cell Division

Binary fission is the most common pattern of cell division in prokaryotes, but it is not the only one. Other mechanisms usually involve asymmetrical division (as in budding) or production of spores in aerial filaments.

In some cyanobacteria, many nucleoids may accumulate in an enlarged round cell or along a filament, leading to the generation of many new cells at once. The new cells often split from the parent filament and float away in a process called fragmentation (Figure 8.1.15). Fragmentation is commonly observed in the Actinomycetes, a group of gram-positive, anaerobic bacteria commonly found in soil. Another curious example of cell division in prokaryotes, reminiscent of live birth in animals, is exhibited by the giant bacterium *Epulopiscium*. Several daughter cells grow fully in the parent cell, which eventually disintegrates, releasing the new cells to the environment. Other species may form a long narrow extension at one pole in a process called budding. The tip of the extension swells and forms a smaller cell, the bud that eventually detaches from the parent cell. Budding is most common in yeast (Figure 8.1.15), but it is also observed in prosthecate bacteria and some cyanobacteria.



(a) (b)

Figure **8.1.15**: (a) Filamentous cyanobacteria, like those pictured here, replicate by fragmentation. (b) In this electron micrograph, cells of the bacterium *Gemmata obscuriglobus* are budding. The larger cell is the mother cell. Labels indicate the nucleoids (N) and the still-forming nuclear envelope (NE) of the daughter cell. (credit a: modification of work by CSIRO; credit b: modification of work by Kuo-Chang Lee, Rick I Webb and John A Fuerst)

The soil bacteria *Actinomyces* grow in long filaments divided by septa, similar to the mycelia seen in fungi, resulting in long cells with multiple nucleoids. Environmental signals, probably related to low nutrient availability, lead to the formation of aerial filaments. Within these aerial filaments, elongated cells divide simultaneously. The new cells, which contain a single nucleoid, develop into spores that give rise to new colonies.

? Exercise 8.1.8

Identify at least one difference between fragmentation and budding.

Biofilms

In nature, microorganisms grow mainly in biofilms, complex and dynamic ecosystems that form on a variety of environmental surfaces, from industrial conduits and water treatment pipelines to rocks in river beds. Biofilms are not restricted to solid surface substrates, however. Almost any surface in a liquid environment containing some minimal nutrients will eventually develop a biofilm. Microbial mats that float on water, for example, are biofilms that contain large populations of photosynthetic microorganisms. Biofilms found in the human mouth may contain hundreds of bacterial species. Regardless of the environment where they occur, biofilms are not random collections of microorganisms; rather, they are highly structured communities that provide a selective advantage to their constituent microorganisms.

Biofilm Structure

Observations using confocal microscopy have shown that environmental conditions influence the overall structure of biofilms. Filamentous biofilms called streamers form in rapidly flowing water, such as freshwater streams, eddies, and specially designed laboratory flow cells that replicate growth conditions in fast-moving fluids. The streamers are anchored to the substrate by a “head” and the “tail” floats downstream in the current. In still or slow-moving water, biofilms mainly assume a mushroom-like shape. The structure of biofilms may also change with other environmental conditions such as nutrient availability.

Detailed observations of biofilms under confocal laser and scanning electron microscopes reveal clusters of microorganisms embedded in a matrix interspersed with open water channels. The extracellular matrix consists of extracellular polymeric substances (EPS) secreted by the organisms in the biofilm. The extracellular matrix represents a large fraction of the biofilm, accounting for 50%–90% of the total dry mass. The properties of the EPS vary according to the resident organisms and environmental conditions.

EPS is a hydrated gel composed primarily of polysaccharides and containing other macromolecules such as proteins, nucleic acids, and lipids. It plays a key role in maintaining the integrity and function of the biofilm. Channels in the EPS allow movement of nutrients, waste, and gases throughout the biofilm. This keeps the cells hydrated, preventing desiccation. EPS also shelters organisms in the biofilm from predation by other microbes or cells (e.g., protozoans, white blood cells in the human body).

Biofilm Formation

Free-floating microbial cells that live in an aquatic environment are called planktonic cells. The formation of a biofilm essentially involves the attachment of planktonic cells to a substrate, where they become sessile (attached to a surface). This occurs in stages, as depicted in Figure 8.1.16. The first stage involves the attachment of planktonic cells to a surface coated with a conditioning film of organic material. At this point, attachment to the substrate is reversible, but as cells express new phenotypes that facilitate the formation of EPS, they transition from a planktonic to a sessile lifestyle. The biofilm develops characteristic structures, including an extensive matrix and water channels. Appendages such as fimbriae, pili, and flagella interact with the EPS, and microscopy and genetic analysis suggest that such structures are required for the establishment of a mature biofilm. In the last stage of the biofilm life cycle, cells on the periphery of the biofilm revert to a planktonic lifestyle, sloughing off the mature biofilm to colonize new sites. This stage is referred to as dispersal.

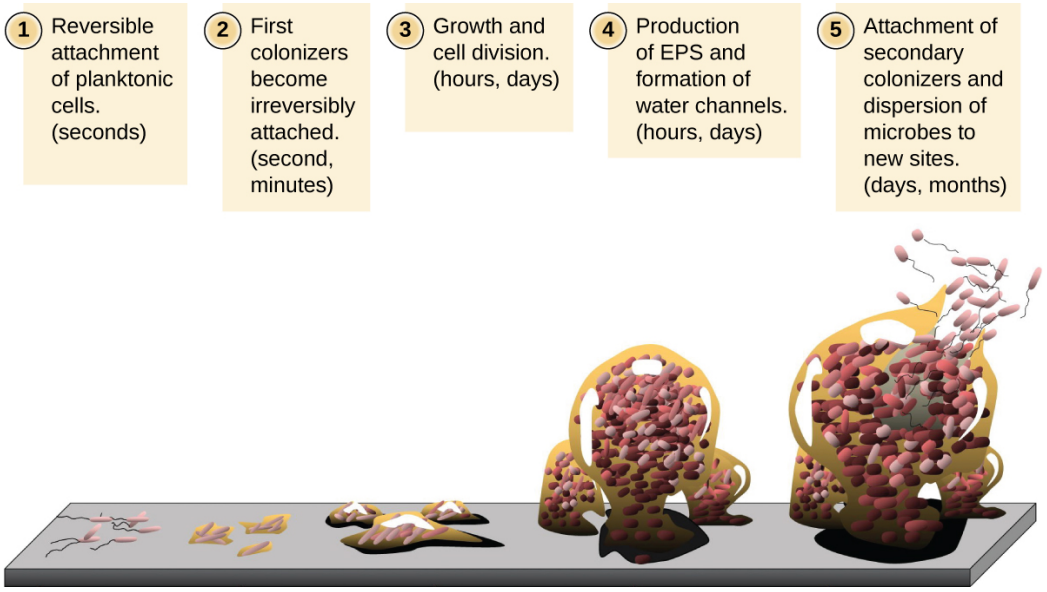


Figure 8.1.16: Stages in the formation and life cycle of a biofilm. (credit: modification of work by Public Library of Science and American Society for Microbiology)

Within a biofilm, different species of microorganisms establish metabolic collaborations in which the waste product of one organism becomes the nutrient for another. For example, aerobic microorganisms consume oxygen, creating anaerobic regions that promote the growth of anaerobes. This occurs in many polymicrobial infections that involve both aerobic and anaerobic pathogens.

The mechanism by which cells in a biofilm coordinate their activities in response to environmental stimuli is called quorum sensing. Quorum sensing—which can occur between cells of different species within a biofilm—enables microorganisms to detect their cell density through the release and binding of small, diffusible molecules called autoinducers. When the cell population reaches a critical threshold (a quorum), these autoinducers initiate a cascade of reactions that activate genes associated with cellular functions that are beneficial only when the population reaches a critical density. For example, in some pathogens, synthesis of virulence factors only begins when enough cells are present to overwhelm the immune defenses of the host. Although mostly studied in bacterial populations, quorum sensing takes place between bacteria and eukaryotes and between eukaryotic cells such as the fungus *Candida albicans*, a common member of the human microbiota that can cause infections in immunocompromised individuals.

The signaling molecules in quorum sensing belong to two major classes. Gram-negative bacteria communicate mainly using N-acylated homoserine lactones, whereas gram-positive bacteria mostly use small peptides (Figure 8.1.17). In all cases, the first step in quorum sensing consists of the binding of the autoinducer to its specific receptor only when a threshold concentration of signaling molecules is reached. Once binding to the receptor takes place, a cascade of signaling events leads to changes in gene expression. The result is the activation of biological responses linked to quorum sensing, notably an increase in the production of signaling molecules themselves, hence the term autoinducer.

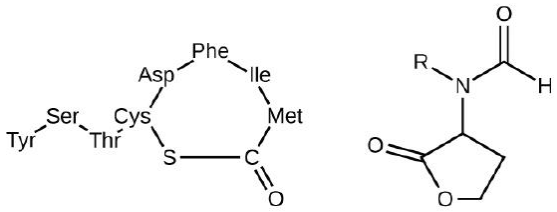


Figure 8.1.17: Short peptides in gram-positive bacteria and N-acetylated homoserine lactones in gram-negative bacteria act as autoinducers in quorum sensing and mediate the coordinated response of bacterial cells. The R side chain of the N-acetylated homoserine lactone is specific for the species of gram-negative bacteria. Some secreted homoserine lactones are recognized by more than one species.

Biofilms and Human Health

The human body harbors many types of biofilms, some beneficial and some harmful. For example, the layers of normal microbiota lining the intestinal and respiratory mucosa play a role in warding off infections by pathogens. However, other biofilms in the body can have a detrimental effect on health. For example, the plaque that forms on teeth is a biofilm that can contribute to dental and periodontal disease. Biofilms can also form in wounds, sometimes causing serious infections that can spread. The bacterium *Pseudomonas aeruginosa* often colonizes biofilms in the airways of patients with cystic fibrosis, causing chronic and sometimes fatal infections of the lungs. Biofilms can also form on medical devices used in or on the body, causing infections in patients with in-dwelling catheters, artificial joints, or contact lenses.

Pathogens embedded within biofilms exhibit a higher resistance to antibiotics than their free-floating counterparts. Several hypotheses have been proposed to explain why. Cells in the deep layers of a biofilm are metabolically inactive and may be less susceptible to the action of antibiotics that disrupt metabolic activities. The EPS may also slow the diffusion of antibiotics and antiseptics, preventing them from reaching cells in the deeper layers of the biofilm. Phenotypic changes may also contribute to the increased resistance exhibited by bacterial cells in biofilms. For example, the increased production of efflux pumps, membrane-embedded proteins that actively extrude antibiotics out of bacterial cells, have been shown to be an important mechanism of antibiotic resistance among biofilm-associated bacteria. Finally, biofilms provide an ideal environment for the exchange of extrachromosomal DNA, which often includes genes that confer antibiotic resistance.

? Exercise 8.1.9

1. What is the matrix of a biofilm composed of?
2. What is the role of quorum sensing in a biofilm?

Key Concepts and Summary

- Most bacterial cells divide by **binary fission**. **Generation time** in bacterial growth is defined as the **doubling time**of the population.
- Cells in a closed system follow a pattern of growth with four phases: **lag**, **logarithmic (exponential)**, **stationary**, and **death**.
- Cells can be counted by **direct viable cell count**. The **pour plate** and **spread plate** methods are used to plate **serial dilutions** into or onto, respectively, agar to allow counting of viable cells that give rise to **colony-forming units**. **Membrane filtration** is used to count live cells in dilute solutions. The **most probable cell number (MPN)**method allows estimation of cell numbers in cultures without using solid media.
- Indirect methods can be used to estimate **culture density** by measuring **turbidity** of a culture or live cell density by measuring metabolic activity.
- Other patterns of cell division include multiple nucleoid formation in cells; asymmetric division, as in **budding**; and the formation of hyphae and terminal spores.
- **Biofilms** are communities of microorganisms enmeshed in a matrix of **extracellular polymeric substance**. The formation of a biofilm occurs when **planktonic** cells attach to a substrate and become **sessile**. Cells in biofilms coordinate their activity by communicating through **quorum sensing**.

- Biofilms are commonly found on surfaces in nature and in the human body, where they may be beneficial or cause severe infections. Pathogens associated with biofilms are often more resistant to antibiotics and disinfectants.

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8.2: Factors that Affect Bacterial Growth

Define the following:

a. psychrophile
b. psychrotroph
c. mesophile
d. thermophile
e. obligate aerobe
f. obligate anaerobe
g. aerotolerant anaerobe
h. facultative anaerobe

- State the optimum pH range for most bacteria and compare this range with the optimum pH for fungi.
- Define the following:

a. phototroph
b. chemotroph
c. autotroph
d. heterotroph
e. fastidious

Physical requirements

a. Temperature

Bacteria have a minimum, optimum, and maximum temperature for growth and can be divided into 3 groups based on their optimum growth temperature:

1. Psychrophiles are cold-loving bacteria. Their optimum growth temperature is between -5C and 15C. They are usually found in the Arctic and Antarctic regions and in streams fed by glaciers.

2. Mesophiles are bacteria that grow best at moderate temperatures. Their optimum growth temperature is between 25C and 45C. Most bacteria are mesophilic and include common soil bacteria and bacteria that live in and on the body.

3. Thermophiles are heat-loving bacteria. Their optimum growth temperature is between 45C and 70C and are commonly found in hot springs and in compost heaps.

4. Hyperthermophiles are bacteria that grow at very high temperatures. Their optimum growth temperature is between 70C and 110C. They are usually members of the Archaea and are found growing near hydrothermal vents at great depths in the ocean.

b. Oxygen requirements

Bacteria show a great deal of variation in their requirements for gaseous oxygen. Most can be placed in one of the following groups:

1. Obligate aerobes are organisms that grow only in the presence of oxygen. They obtain their energy through aerobic respiration .

2. Microaerophils are organisms that require a low concentration of oxygen (2% to 10%) for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration .

3. Obligate anaerobes are organisms that grow only in the absence of oxygen and, in fact, are often inhibited or killed by its presence. They obtain their energy through anaerobic respiration or fermentation .

4. Aerotolerant anaerobes , like obligate anaerobes, cannot use oxygen to transform energy but can grow in its presence. They obtain energy only by fermentation and are known as obligate fermenters.

5. Facultative anaerobes are organisms that grow with or without oxygen, but generally better with oxygen. They obtain their energy through aerobic respiration if oxygen is present, but use fermentation or anaerobic respiration if it is absent. Most bacteria are facultative anaerobes.

c. pH

Microorganisms can be placed in one of the following groups based on their optimum pH requirements:

1. Neutrophiles grow best at a pH range of 5 to 8.
2. Acidophiles grow best at a pH below 5.5.
3. Alkaliphiles grow best at a pH above 8.5.

d. Osmosis

Osmosis is the diffusion of water across a membrane from an area of higher water concentration (lower solute concentration) to lower water concentration (higher solute concentration). Osmosis is powered by the potential energy of a concentration gradient and does not require the expenditure of metabolic energy. While water molecules are small enough to pass between the phospholipids in the cytoplasmic membrane, their transport can be enhanced by water transporting transport proteins known as aquaporins . The aquaporins form channels that span the cytoplasmic membrane and transport water in and out of the cytoplasm.

To understand osmosis, one must understand what is meant by a solution . A solution consists of a solute dissolved in a solvent . In terms of osmosis, solute refers to all the molecules or ions dissolved in the water (the solvent). When a solute such as sugar dissolves in water, it forms weak hydrogen bonds with water molecules. While free, unbound water molecules are small enough to pass through membrane pores, water molecules bound to solute are not (see Figure 8.2.4C and Figure 8.2.4D).Therefore, the higher the solute concentration, the lower the concentration of free water molecules capable of passing through the membrane.

A cell can find itself in one of three environments: isotonic , hypertonic , or hypotonic . (The prefixes iso-, hyper-, and hypo- refer to the solute concentration).

- In an isotonic environment (see Figure 8.2.5A), both the water and solute concentration are the same inside and outside the cell and water goes into and out of the cell at an equal rate.

Flash animation showing osmosis in an isotonic environment.

http5 version of animation for iPad showing osmosis in an isotonic environment.

- If the environment is hypertonic (see Figure 8.2.5B), the water concentration is greater inside the cell while the solute concentration is higher outside (the interior of the cell is hypotonic to the surrounding hypertonic environment). Water goes out of the cell.

Flash animation showing osmosis in a hypertonic environment.

html5 version of animation for iPad showing osmosis in a hypertonic environment.

- In an environment that is hypotonic (see Figure 8.2.5C), the water concentration is greater outside the cell and the solute concentration is higher inside (the interior of the cell is hypertonic to the hypotonic surroundings). Water goes into the cell.

Flash animation showing osmosis in a hypotonic environment.

html5 version of animation for iPad showing osmosis in a hypotonic environment.

Most bacteria require an isotonic environment or a hypotonic environment for optimum growth. Organisms that can grow at relatively high salt concentration (up to 10%) are said to be osmotolerant . Those that require relatively high salt concentrations for growth, like some of the Archaea that require sodium chloride concentrations of 20 % or higher halophiles .

Nutritional requirements

In addition to a proper physical environment, microorganisms also depend on an available source of chemical nutrients. Microorganisms are often grouped according to their energy source and their source of carbon.

a. Energy source

1. Phototrophs use radiant energy (light) as their primary energy source.

2. Chemotrophs use the oxidation and reduction of chemical compounds as their primary energy source.

b. Carbon source

Carbon is the structural backbone of the organic compounds that make up a living cell. Based on their source of carbon bacteria can be classified as autotrophs or heterotrophs.

1. Autotrophs : require only carbon dioxide as a carbon source. An autotroph can synthesize organic molecules from inorganic nutrients.

2. Heterotrophs : require organic forms of carbon. A Heterotroph cannot synthesize organic molecules from inorganic nutrients.

Combining their nutritional patterns, all organisms in nature can be placed into one of four separate groups: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs.

1. Photoautotrophs use light as an energy source and carbon dioxide as their main carbon source. They include photosynthetic bacteria (green sulfur bacteria, purple sulfur bacteria, and cyanobacteria), algae, and green plants. Photoautotrophs transform carbon dioxide and water into carbohydrates and oxygen gas through photosynthesis .

   Cyanobacteria, as well as algae and green plants, use hydrogen atoms from water to reduce carbon dioxide to form carbohydrates, and during this process oxygen gas is given off (an oxygenic process). Other photosynthetic bacteria (the green sulfur bacteria and purple sulfur bacteria) carry out an anoxygenic process, using sulfur, sulfur compounds or hydrogen gas to reduce carbon dioxide and form organic compounds.

2. Photoheterotrophs use light as an energy source but cannot convert carbon dioxide into energy. Instead they use organic compounds as a carbon source. They include the green nonsulfur bacteria and the purple nonsulfur bacteria.

3. Chemolithoautotrophs use inorganic compounds such as hydrogen sulfide, sulfur, ammonia, nitrites, hydrogen gas, or iron as an energy source and carbon dioxide as their main carbon source.

4. Chemooganoheterotrophs use organic compounds as both an energy source and a carbon source. Saprophytes live on dead organic matter while parasites get their nutrients from a living host. Most bacteria, and all protozoans, fungi, and animals are chemoorganoheterotrophs.

c. Nitrogen source

Nitrogen is needed for the synthesis of such molecules as amino acids, DNA, RNA and ATP . Depending on the organism, nitrogen, nitrates, ammonia, or organic nitrogen compounds may be used as a nitrogen source.

d. Minerals

1. Sulfur

Sulfur is needed to synthesizes sulfur-containing amino acids and certain vitamins. Depending on the organism, sulfates, hydrogen sulfide, or sulfur-containing amino acids may be used as a sulfur source.

2. Phosphorus

Phosphorus is needed to synthesize phospholipids , DNA, RNA, and ATP . Phosphate ions are the primary source of phosphorus.

3. Potassium, magnesium, and calcium

These are required for certain enzymes to function as well as additional functions.

4. Iron

Iron is a part of certain enzymes.

5. Trace elements

Trace elements are elements required in very minute amounts, and like potassium, magnesium, calcium, and iron, they usually function as cofactors in enzyme reactions. They include sodium, zinc, copper,molybdenum, manganese, and cobalt ions. Cofactors usually function as electron donors or electron acceptors during enzyme reactions.

e. Water

f. Growth factors

Growth factors are organic compounds such as amino acids , purines , pyrimidines , and vitamins that a cell must have for growth but cannot synthesize itself. Organisms having complex nutritional requirements and needing many growth factors are said to be fastidious .

Summary

1. Bacteria have a minimum, optimum, and maximum temperature for growth and can be divided into 3 groups based on their optimum growth temperature: psychrophils, mesophils, thermophils, or hyperthermophils.
2. Bacteria show a great deal of variation in their requirements for gaseous oxygen. Most can be placed in one of the following groups: obligate aerobes, microaerophils, obligate anaerobes, aerotolerant anaerobes, or facultative anaerobes.
3. Microorganisms can be placed in one of the following groups based on their optimum pH requirements: neutrophiles, acidophiles, or alkaliphiles.
4. A bacterium's osmotic environment can affect bacterial growth.
5. Bacteria can be grouped according to their energy source as phototrophs or chemotrophs.
6. Bacteria can be grouped according to their carbon source as autotrophs or heterotrophs.
7. Combining their nutritional patterns, all organisms in nature can be placed into one of four separate groups: photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs.
8. Bacteria also need a nitrogen source, various minerals, and water for growth.

9. Organisms having complex nutritional requirements and needing many growth factors are said to be fastidious.

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8.2.1: Temperature Effects on Bacterial Growth

Learning Objectives

- Illustrate and briefly describe minimum, optimum, and maximum temperature requirements for growth
- Identify and describe different categories of microbes with temperature requirements for growth: psychrophile, psychrotrophs, mesophile, thermophile, hyperthermophile
- Give examples of microorganisms in each category of temperature tolerance

When the exploration of Lake Whillans started in Antarctica, researchers did not expect to find much life. Constant subzero temperatures and lack of obvious sources of nutrients did not seem to be conditions that would support a thriving ecosystem. To their surprise, the samples retrieved from the lake showed abundant microbial life. In a different but equally harsh setting, bacteria grow at the bottom of the ocean in sea vents (Figure 8.2.1.1), where temperatures can reach 340 °C (700 °F).

Microbes can be roughly classified according to the range of temperature at which they can grow. The growth rates are the highest at the optimum growth temperature for the organism. The lowest temperature at which the organism can survive and replicate is its minimum growth temperature. The highest temperature at which growth can occur is its maximum growth temperature. The following ranges of permissive growth temperatures are approximate only and can vary according to other environmental factors.

Organisms categorized as mesophiles (“middle loving”) are adapted to moderate temperatures, with optimal growth temperatures ranging from room temperature (about 20 °C) to about 45 °C. As would be expected from the core temperature of the human body, 37 °C (98.6 °F), normal human microbiota and pathogens (e.g., *E. coli*, *Salmonella* spp., and *Lactobacillus* spp.) are mesophiles.

Organisms called psychrotrophs, also known as psychrotolerant, prefer cooler environments, from a high temperature of 25 °C to refrigeration temperature about 4 °C. They are found in many natural environments in temperate climates. They are also responsible for the spoilage of refrigerated food.

Clinical Focus: Resolution

The presence of *Listeria* in Jeni’s blood suggests that her symptoms are due to listeriosis, an infection caused by *L. monocytogenes*. Listeriosis is a serious infection with a 20% mortality rate and is a particular risk to Jeni’s fetus. A sample from the amniotic fluid cultured for the presence of *Listeria* gave negative results. Because the absence of organisms does not rule out the possibility of infection, a molecular test based on the nucleic acid amplification of the 16S ribosomal RNA of *Listeria* was performed to confirm that no bacteria crossed the placenta. Fortunately, the results from the molecular test were also negative.

Jeni was admitted to the hospital for treatment and recovery. She received a high dose of two antibiotics intravenously for 2 weeks. The preferred drugs for the treatment of listeriosis are ampicillin or penicillin G with an aminoglycoside antibiotic. Resistance to common antibiotics is still rare in *Listeria* and antibiotic treatment is usually successful. She was released to home care after a week and fully recovered from her infection.

L. monocytogenes is a gram-positive short rod found in soil, water, and food. It is classified as a psychrophile and is halotolerant. Its ability to multiply at refrigeration temperatures (4–10 °C) and its tolerance for high concentrations of salt (up to 10% sodium chloride [NaCl]) make it a frequent source of food poisoning. Because *Listeria* can infect animals, it often contaminates food such as meat, fish, or dairy products. Contamination of commercial foods can often be traced to persistent biofilms that form on manufacturing equipment that is not sufficiently cleaned.

Listeria infection is relatively common among pregnant women because the elevated levels of progesterone downregulate the immune system, making them more vulnerable to infection. The pathogen can cross the placenta and infect the fetus, often resulting in miscarriage, stillbirth, or fatal neonatal infection. Pregnant women are thus advised to avoid consumption of soft cheeses, refrigerated cold cuts, smoked seafood, and unpasteurized dairy products. Because *Listeria* bacteria can easily be confused with diphtheroids, another common group of gram-positive rods, it is important to alert the laboratory when listeriosis is suspected.

The organisms retrieved from arctic lakes such as Lake Whillans are considered extreme psychrophiles (cold loving). Psychrophiles are microorganisms that can grow at 0 °C and below, have an optimum growth temperature close to 15 °C, and usually do not survive at temperatures above 20 °C. They are found in permanently cold environments such as the deep waters of the oceans. Because they are active at low temperature, psychrophiles and psychrotrophs are important decomposers in cold climates.

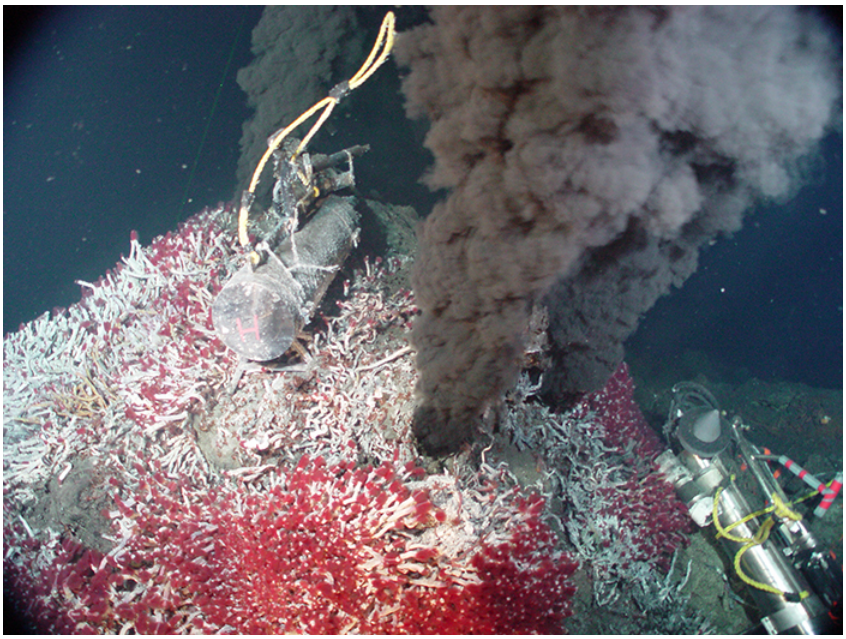


Figure 8.2.1.1: A black smoker at the bottom of the ocean belches hot, chemical-rich water, and heats the surrounding waters. Sea vents provide an extreme environment that is nonetheless teeming with macroscopic life (the red tubeworms) supported by an abundant microbial ecosystem. (credit: NOAA)

Organisms that grow at optimum temperatures of 50 °C to a maximum of 80 °C are called thermophiles ("heat loving"). They do not multiply at room temperature. Thermophiles are widely distributed in hot springs, geothermal soils, and manmade environments such as garden compost piles where the microbes break down kitchen scraps and vegetal material. Examples of thermophiles include *Thermus aquaticus* and *Geobacillus* spp. Higher up on the extreme temperature scale we find the hyperthermophiles, which are characterized by growth ranges from 80 °C to a maximum of 110 °C, with some extreme examples that survive temperatures above 121 °C, the average temperature of an autoclave. The hydrothermal vents at the bottom of the ocean are a prime example of extreme environments, with temperatures reaching an estimated 340 °C (Figure 8.2.1.1). Microbes isolated from the vents achieve optimal growth at temperatures higher than 100 °C. Noteworthy examples are *Pyrobolus* and *Pyrodictium*, archaea that grow at 105 °C and survive autoclaving. Figure 8.2.1.2 shows the typical skewed curves of temperature-dependent growth for the categories of microorganisms we have discussed.

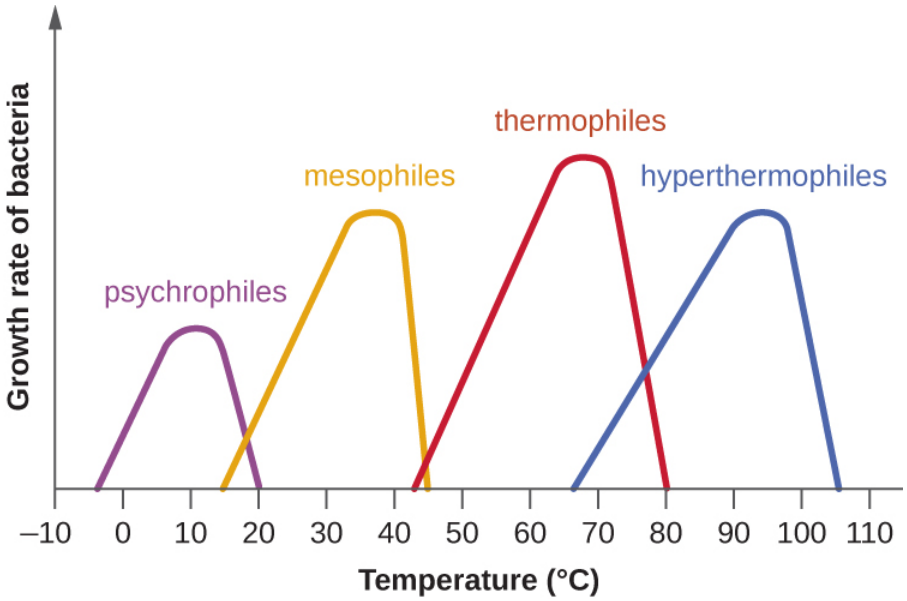


Figure **8.2.1.2**: The graph shows growth rate of bacteria as a function of temperature. Notice that the curves are skewed toward the optimum temperature. The skewing of the growth curve is thought to reflect the rapid denaturation of proteins as the temperature rises past the optimum for growth of the microorganism.

Life in extreme environments raises fascinating questions about the adaptation of macromolecules and metabolic processes. Very low temperatures affect cells in many ways. Membranes lose their fluidity and are damaged by ice crystal formation. Chemical reactions and diffusion slow considerably. Proteins become too rigid to catalyze reactions and may undergo denaturation. At the opposite end of the temperature spectrum, heat denatures proteins and nucleic acids. Increased fluidity impairs metabolic processes in membranes. Some of the practical applications of the destructive effects of heat on microbes are sterilization by steam, pasteurization, and incineration of inoculating loops. Proteins in psychrophiles are, in general, rich in hydrophobic residues, display an increase in flexibility, and have a lower number of secondary stabilizing bonds when compared with homologous proteins from mesophiles. Antifreeze proteins and solutes that decrease the freezing temperature of the cytoplasm are common. The lipids in the membranes tend to be unsaturated to increase fluidity. Growth rates are much slower than those encountered at moderate temperatures. Under appropriate conditions, mesophiles and even thermophiles can survive freezing. Liquid cultures of bacteria are mixed with sterile glycerol solutions and frozen to −80 °C for long-term storage as stocks. Cultures can withstand freeze drying (lyophilization) and then be stored as powders in sealed ampules to be reconstituted with broth when needed.

Macromolecules in thermophiles and hyperthermophiles show some notable structural differences from what is observed in the mesophiles. The ratio of saturated to polyunsaturated lipids increases to limit the fluidity of the cell membranes. Their DNA sequences show a higher proportion of guanine–cytosine nitrogenous bases, which are held together by three hydrogen bonds in contrast to adenine and thymine, which are connected in the double helix by two hydrogen bonds. Additional secondary ionic and covalent bonds, as well as the replacement of key amino acids to stabilize folding, contribute to the resistance of proteins to denaturation. The so-called thermoenzymes purified from thermophiles have important practical applications. For example, amplification of nucleic acids in the polymerase chain reaction (PCR) depends on the thermal stability of *Taq* polymerase, an enzyme isolated from *T. aquaticus*. Degradation enzymes from thermophiles are added as ingredients in hot-water detergents, increasing their effectiveness.

? Exercise 8.2.1.1

1. What temperature requirements do most bacterial human pathogens have?
2. What DNA adaptation do thermophiles exhibit?

Feeding the World…and the World's Algae

Artificial fertilizers have become an important tool in food production around the world. They are responsible for many of the gains of the so-called green revolution of the 20th century, which has allowed the planet to feed many of its more than 7 billion people. Artificial fertilizers provide nitrogen and phosphorus, key limiting nutrients, to crop plants, removing the normal barriers that would otherwise limit the rate of growth. Thus, fertilized crops grow much faster, and farms that use fertilizer produce higher crop yields.

However, careless use and overuse of artificial fertilizers have been demonstrated to have significant negative impacts on aquatic ecosystems, both freshwater and marine. Fertilizers that are applied at inappropriate times or in too-large quantities allow nitrogen and phosphorus compounds to escape use by crop plants and enter drainage systems. Inappropriate use of fertilizers in residential settings can also contribute to nutrient loads, which find their way to lakes and coastal marine ecosystems. As water warms and nutrients are plentiful, microscopic algae bloom, often changing the color of the water because of the high cell density.

Most algal blooms are not directly harmful to humans or wildlife; however, they can cause harm indirectly. As the algal population expands and then dies, it provides a large increase in organic matter to the bacteria that live in deep water. With this large supply of nutrients, the population of nonphotosynthetic microorganisms explodes, consuming available oxygen and creating "dead zones" where animal life has virtually disappeared.

Depletion of oxygen in the water is not the only damaging consequence of some algal blooms. The algae that produce red tides in the Gulf of Mexico, *Karenia brevis*, secrete potent toxins that can kill fish and other organisms and also accumulate in shellfish. Consumption of contaminated shellfish can cause severe neurological and gastrointestinal symptoms in humans. Shellfish beds must be regularly monitored for the presence of the toxins, and harvests are often shut down when it is present, incurring economic costs to the fishery. Cyanobacteria, which can form blooms in marine and freshwater ecosystems, produce toxins called microcystins, which can cause allergic reactions and liver damage when ingested in drinking water or during swimming. Recurring cyanobacterial algal blooms in Lake Erie (Figure **8.2.1.3**) have forced municipalities to issue drinking water bans for days at a time because of unacceptable toxin levels.

This is just a small sampling of the negative consequences of algal blooms, red tides, and dead zones. Yet the benefits of crop fertilizer—the main cause of such blooms—are difficult to dispute. There is no easy solution to this dilemma, as a ban on fertilizers is not politically or economically feasible. In lieu of this, we must advocate for responsible use and regulation in agricultural and residential contexts, as well as the restoration of wetlands, which can absorb excess fertilizers before they reach lakes and oceans.



Figure **8.2.1.3**: Heavy rains cause runoff of fertilizers into Lake Erie, triggering extensive algal blooms, which can be observed along the shoreline. Notice the brown unplanted and green planted agricultural land on the shore. (credit: NASA)

Link to Learning

This video discusses algal blooms and dead zones in more depth.

Key Concepts and Summary

- Microorganisms thrive at a wide range of temperatures; they have colonized different natural environments and have adapted to extreme temperatures. Both extreme cold and hot temperatures require evolutionary adjustments to macromolecules and biological processes.
- **Psychrophiles** grow best in the temperature range of 0–15 °C whereas **psychrotrophs** thrive between 4 °C and 25 °C.
- **Mesophiles** grow best at moderate temperatures in the range of 20 °C to about 45 °C. Pathogens are usually mesophiles.
- **Thermophiles** and **hyperthemophiles** are adapted to life at temperatures above 50 °C.
- Adaptations to cold and hot temperatures require changes in the composition of membrane lipids and proteins.

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8.2.2: pH Effects on Bacterial Growth

Learning Objectives

- Illustrate and briefly describe minimum, optimum, and maximum pH requirements for growth
- Identify and describe the different categories of microbes with pH requirements for growth: acidophiles, neutrophiles, and alkaliphiles
- Give examples of microorganisms for each category of pH requirement

Yogurt, pickles, sauerkraut, and lime-seasoned dishes all owe their tangy taste to a high acid content (Figure **8.2.2.1**). Recall that acidity is a function of the concentration of hydrogen ions [H^+] and is measured as pH. Environments with pH values below 7.0 are considered acidic, whereas those with pH values above 7.0 are considered basic. Extreme pH affects the structure of all macromolecules. The hydrogen bonds holding together strands of DNA break up at high pH. Lipids are hydrolyzed by an extremely basic pH. The proton motive force responsible for production of ATP in cellular respiration depends on the concentration gradient of H^+ across the plasma membrane (see Cellular Respiration). If H^+ ions are neutralized by hydroxide ions, the concentration gradient collapses and impairs energy production. But the component most sensitive to pH in the cell is its workhorse, the protein. Moderate changes in pH modify the ionization of amino-acid functional groups and disrupt hydrogen bonding, which, in turn, promotes changes in the folding of the molecule, promoting denaturation and destroying activity.



Figure **8.2.2.1**: Lactic acid bacteria that ferment milk into yogurt or transform vegetables in pickles thrive at a pH close to 4.0. Sauerkraut and dishes such as pico de gallo owe their tangy flavor to their acidity. Acidic foods have been a mainstay of the human diet for centuries, partly because most microbes that cause food spoilage grow best at a near neutral pH and do not tolerate acidity well. (credit “yogurt”: modification of work by “nina.jsc”/Flickr; credit “pickles”: modification of work by Noah Sussman; credit “sauerkraut”: modification of work by Jesse LaBuff; credit “pico de gallo”: modification of work by “regan76”/Flickr)

The optimum growth pH is the most favorable pH for the growth of an organism. The lowest pH value that an organism can tolerate is called the minimum growth pH and the highest pH is the maximum growth pH. These values can cover a wide range, which is important for the preservation of food and to microorganisms’ survival in the stomach. For example, the optimum growth pH of *Salmonella* spp. is 7.0–7.5, but the minimum growth pH is closer to 4.2.

Most bacteria are neutrophiles, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7 (see Figure **8.2.2.2**). Most familiar bacteria, like *Escherichia coli*, staphylococci, and *Salmonella* spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli*, *S. typhi*, and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0–6.0.

Microorganisms that grow optimally at pH less than 5.55 are called acidophiles. For example, the sulfur-oxidizing *Sulfolobus* spp. isolated from sulfur mud fields and hot springs in Yellowstone National Park are extreme acidophiles. These archaea survive at pH values of 2.5–3.5. Species of the archaean genus *Ferroplasma* live in acid mine drainage at pH values of 0–2.9. *Lactobacillus* bacteria, which are an important part of the normal microbiota of the vagina, can tolerate acidic environments at pH values 3.5–6.8 and also contribute to the acidity of the vagina (pH of 4, except at the onset of menstruation) through their metabolic production of lactic acid. The vagina’s acidity plays an important role in inhibiting other microbes that are less tolerant of acidity. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H^+ ions out of the cells. The changes in the composition of membrane phospholipids probably reflect the need to maintain membrane fluidity at low pH.

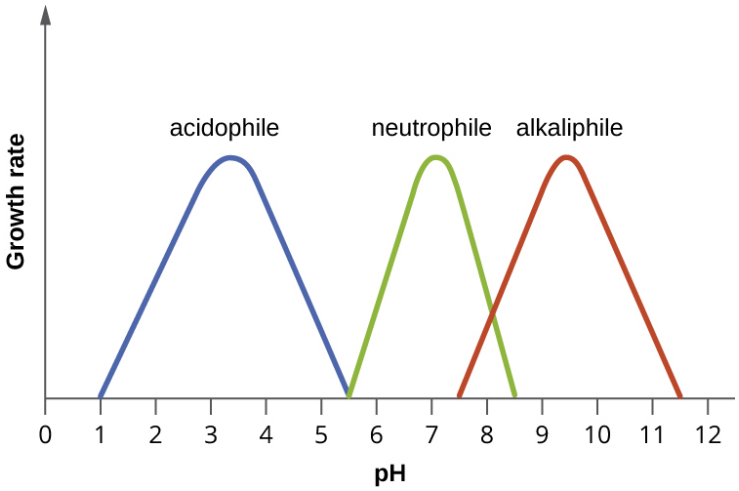


Figure 8.2.2.2: The curves show the approximate pH ranges for the growth of the different classes of pH-specific prokaryotes. Each curve has an optimal pH and extreme pH values at which growth is much reduced. Most bacteria are neutrophiles and grow best at near-neutral pH (center curve). Acidophiles have optimal growth at pH values near 3 and alkaliphiles have optimal growth at pH values above 9.

At the other end of the spectrum are alkaliphiles, microorganisms that grow best at pH between 8.0 and 10.5. *Vibrio cholerae*, the pathogenic agent of cholera, grows best at the slightly basic pH of 8.0; it can survive pH values of 11.0 but is inactivated by the acid of the stomach. When it comes to survival at high pH, the bright pink archaean *Natronobacterium*, found in the soda lakes of the African Rift Valley, may hold the record at a pH of 10.5 (Figure 8.2.2.3). Extreme alkaliphiles have adapted to their harsh environment through evolutionary modification of lipid and protein structure and compensatory mechanisms to maintain the proton motive force in an alkaline environment. For example, the alkaliphile *Bacillus firmus* derives the energy for transport reactions and motility from a Na^+ ion gradient rather than a proton motive force. Many enzymes from alkaliphiles have a higher isoelectric point, due to an increase in the number of basic amino acids, than homologous enzymes from neutrophiles.



Figure 8.2.2.3: View from space of Lake Natron in Tanzania. The pink color is due to the pigmentation of the extreme alkaliphilic and halophilic microbes that colonize the lake. (credit: NASA)

Survival at the Low pH of the Stomach

Peptic ulcers (or stomach ulcers) are painful sores on the stomach lining. Until the 1980s, they were believed to be caused by spicy foods, stress, or a combination of both. Patients were typically advised to eat bland foods, take anti-acid medications, and avoid stress. These remedies were not particularly effective, and the condition often recurred. This all changed dramatically when the real cause of most peptic ulcers was discovered to be a slim, corkscrew-shaped bacterium, *Helicobacter pylori*. This organism was identified and isolated by Barry Marshall and Robin Warren, whose discovery earned them the Nobel Prize in Medicine in 2005.

The ability of *H. pylori* to survive the low pH of the stomach would seem to suggest that it is an extreme acidophile. As it turns out, this is not the case. In fact, *H. pylori* is a neutrophile. So, how does it survive in the stomach? Remarkably, *H. pylori* creates a microenvironment in which the pH is nearly neutral. It achieves this by producing large amounts of the enzyme urease, which breaks down urea to form NH_4^+ and CO_2. The ammonium ion raises the pH of the immediate environment.

This metabolic capability of *H. pylori* is the basis of an accurate, noninvasive test for infection. The patient is given a solution of urea containing radioactively labeled carbon atoms. If *H. pylori* is present in the stomach, it will rapidly break down the urea, producing radioactive CO_2 that can be detected in the patient's breath. Because peptic ulcers may lead to gastric cancer, patients who are determined to have *H. pylori* infections are treated with antibiotics.

? Exercise 8.2.2.1

1. What effect do extremes of pH have on proteins?
2. What pH-adaptive type of bacteria would most human pathogens be?

Key Concepts and Summary

- Bacteria are generally **neutrophiles**. They grow best at neutral pH close to 7.0.
- **Acidophiles** grow optimally at a pH near 3.0. **Alkaliphiles** are organisms that grow optimally between a pH of 8 and 10.5. Extreme acidophiles and alkaliphiles grow slowly or not at all near neutral pH.
- Microorganisms grow best at their **optimum growth pH**. Growth occurs slowly or not at all below the **minimum growth pH** and above the **maximum growth pH**.

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8.2.3: Nitrogen Effects on Bacterial Growth

Learning Objectives

- Distinguish between component I and II of the nitrogenase enzyme and its role in biological nitrogen fixation

Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The reaction for BNF is:

$$\mathbf{N_2 + 8\,H^+ + 8\,e^- \longrightarrow 2\,NH_3 + H_2.} \qquad (8.2.3.1)$$

This type of reaction results in N_2 gaining electrons (see above equation) and is thus termed a reduction reaction. The exact mechanism of catalysis is unknown due to the technical difficulties biochemists have in actually visualizing this reaction in vitro, so the exact sequence of the steps of this reaction are not completely understood. Despite this, a great deal is known of the process. While the equilibrium formation of ammonia from molecular hydrogen and nitrogen has an overall negative enthalpy of reaction (i.e. it gives off energy), the energy barrier to activation is very high without the assistance of catalysis, which is done by nitrogenases. The enzymatic reduction of N_2 to ammonia therefore requires an input of chemical energy, released from ATP hydrolysis, to overcome the activation energy barrier.

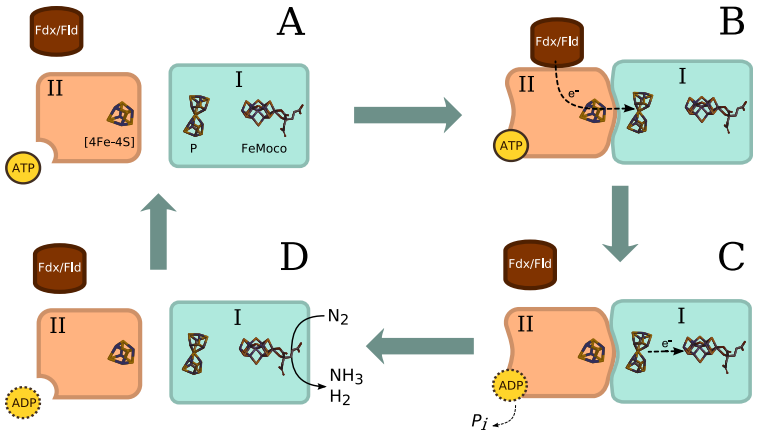


Figure: ***A General Catalytic Mechanism Scheme for Nitrogenase****: A) Components I and II are dissociated; II is ready for reduction. B) ATP binds to component II, which receives electrons from an electron donor (ferredoxin or flavodoxin); binding of ATP induces an allosteric conformational change which allows association of the two proteins. Electrons flow from the [4Fe-4S] cluster on II to the P cluster on I. C) Electrons are further shuttled to the iron-molybdenum cofactor (FeMoco), and ATP is hydrolised to ADP. This step is repeated several times before a molecule of N2 can bind to FeMoco. D) The protein complex dissociates, and nitrogenase reduces dinitrogen to ammonia and dihydrogen. Legend:I: component I (dinitrogenase; MoFe protein); II: component II (dinitrogen reductase; Fe protein); ATP: adenosine triphosphate; ADP: adenosine diphosphate; Fdx: ferredoxin; Fld: flavodoxin.*

Nitrogenase is made up of two soluble proteins: component I and II. Component I known as MoFe protein or nitrogenase contains 2 Mo atoms, 28 to 34 Fe atoms, and 26 to 28 acid-labile sulfides, also known as a iron-molybdenum cofactor (FeMoco). Component I is composed of two copies each of two subunits (α and β); each subunit's stability depends on the other in vivo. Component II known as Fe protein or nitrogenase reductase is composed of two copies of a single subunit. This protein has four non-heme Fe atoms and four acid-labile sulfides (4Fe-4S). Substrate binding and reduction takes place on component I, which binds to ATP and ferredoxin or flavodoxin proteins (Fdx or Fld) (see step B).

The hydrolysis of ATP supplies the energy for the reaction while the Fdx/Fld proteins supply the electrons. Note this is a reduction reaction which means that electrons must be added to the N_2 to reduce it to NH_4. Thus, the role of component II is to supply electrons, one at a time to component I. ATP is not hydrolyzed to ADP until component II transfers an electron to component I (see step C and D). 21-25 ATPs are required for each N_2 fixed. The association of nitrogenase component I and II and later dissociation occurs several times to allow the fixation of one N_2 molecule (see step B and D).

Nitrogenase ultimately bonds each atom of nitrogen to three hydrogen atoms to form ammonia (NH_3). The nitrogenase reaction additionally produces molecular hydrogen as a side product, which is of special interest for people trying to produce H_2 as an

alternative energy source to fossil fuels.

Key Points

- Nitrogen fixation does result in the release of energy, but the activation of this reaction takes energy in the form of ATP hydrolysis.
- Nitrogenases are metalloenzymes, which are proteins that have metalic molecules as subunits.
- While a great deal is known about how nitrogenases reduce nitrogen, some steps are unknown.

Key Terms

- **sulfide**: Any compound of sulfur and a metal or other electropositive element or group.
- **reduction**: A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- **enthalpy**: In thermodynamics, a measure of the heat content of a chemical or physical system.

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8.2.4: Oxygen Effects on Bacterial Growth

Learning Objectives

- Interpret visual data demonstrating minimum, optimum, and maximum oxygen or carbon dioxide requirements for growth
- Identify and describe different categories of microbes with requirements for growth with or without oxygen: obligate aerobe, obligate anaerobe, facultative anaerobe, aerotolerant anaerobe, microaerophile, and capnophile
- Give examples of microorganisms for each category of growth requirements

Ask most people "What are the major requirements for life?" and the answers are likely to include water and oxygen. Few would argue about the need for water, but what about oxygen? Can there be life without oxygen?

The answer is that molecular oxygen (O_2) is not always needed. The earliest signs of life are dated to a period when conditions on earth were highly reducing and free oxygen gas was essentially nonexistent. Only after cyanobacteria started releasing oxygen as a byproduct of photosynthesis and the capacity of iron in the oceans for taking up oxygen was exhausted did oxygen levels increase in the atmosphere. This event, often referred to as the Great Oxygenation Event or the Oxygen Revolution, caused a massive extinction. Most organisms could not survive the powerful oxidative properties of reactive oxygen species (ROS), highly unstable ions and molecules derived from partial reduction of oxygen that can damage virtually any macromolecule or structure with which they come in contact. Singlet oxygen (O_2•), superoxide (O_2^-), peroxides (H_2O_2), hydroxyl radical (OH•), and hypochlorite ion (OCl^-), the active ingredient of household bleach, are all examples of ROS. The organisms that were able to detoxify reactive oxygen species harnessed the high electronegativity of oxygen to produce free energy for their metabolism and thrived in the new environment.

Oxygen Requirements of Microorganisms

Many ecosystems are still free of molecular oxygen. Some are found in extreme locations, such as deep in the ocean or in earth's crust; others are part of our everyday landscape, such as marshes, bogs, and sewers. Within the bodies of humans and other animals, regions with little or no oxygen provide an anaerobic environment for microorganisms. (Figure 8.2.4.1).



(a) (b)

Figure 8.2.4.1: Anaerobic environments are still common on earth. They include environments like (a) a bog where undisturbed dense sediments are virtually devoid of oxygen, and (b) the rumen (the first compartment of a cow's stomach), which provides an oxygen-free incubator for methanogens and other obligate anaerobic bacteria. (credit a: modification of work by National Park Service; credit b: modification of work by US Department of Agriculture)

We can easily observe different requirements for molecular oxygen by growing bacteria in thioglycolate tube cultures. A test-tube culture starts with autoclaved thioglycolate medium containing a low percentage of agar to allow motile bacteria to move throughout the medium. Thioglycolate has strong reducing properties and autoclaving flushes out most of the oxygen. The tubes are inoculated with the bacterial cultures to be tested and incubated at an appropriate temperature. Over time, oxygen slowly diffuses throughout the thioglycolate tube culture from the top. Bacterial density increases in the area where oxygen concentration is best suited for the growth of that particular organism.

The growth of bacteria with varying oxygen requirements in thioglycolate tubes is illustrated in Figure 8.2.4.2. In tube A, all the growth is seen at the top of the tube. The bacteria are obligate (strict) aerobes that cannot grow without an abundant supply of

oxygen. Tube B looks like the opposite of tube A. Bacteria grow at the bottom of tube B. Those are obligate anaerobes, which are killed by oxygen. Tube C shows heavy growth at the top of the tube and growth throughout the tube, a typical result with facultative anaerobes. Facultative anaerobes are organisms that thrive in the presence of oxygen but also grow in its absence by relying on fermentation or anaerobic respiration, if there is a suitable electron acceptor other than oxygen and the organism is able to perform anaerobic respiration. The aerotolerant anaerobes in tube D are indifferent to the presence of oxygen. They do not use oxygen because they usually have a fermentative metabolism, but they are not harmed by the presence of oxygen as obligate anaerobes are. Tube E on the right shows a "Goldilocks" culture. The oxygen level has to be just right for growth, not too much and not too little. These microaerophiles are bacteria that require a minimum level of oxygen for growth, about 1%–10%, well below the 21% found in the atmosphere.

Examples of obligate aerobes are *Mycobacterium tuberculosis*, the causative agent of tuberculosis and *Micrococcus luteus*, a gram-positive bacterium that colonizes the skin. *Neisseria meningitidis*, the causative agent of severe bacterial meningitis, and *N. gonorrheae*, the causative agent of sexually transmitted gonorrhea, are also obligate aerobes.

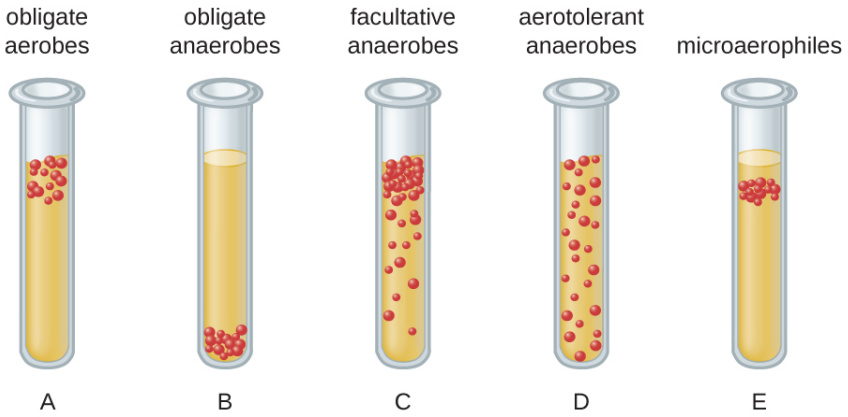


Figure **8.2.4.2**: Diagram of bacterial cell distribution in thioglycolate tubes.

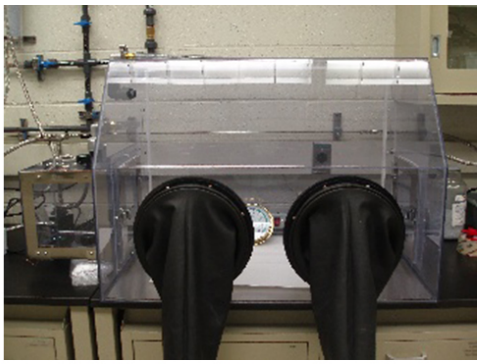
Many obligate anaerobes are found in the environment where anaerobic conditions exist, such as in deep sediments of soil, still waters, and at the bottom of the deep ocean where there is no photosynthetic life. Anaerobic conditions also exist naturally in the intestinal tract of animals. Obligate anaerobes, mainly *Bacteroidetes*, represent a large fraction of the microbes in the human gut. Transient anaerobic conditions exist when tissues are not supplied with blood circulation; they die and become an ideal breeding ground for obligate anaerobes. Another type of obligate anaerobe encountered in the human body is the gram-positive, rod-shaped *Clostridium* spp. Their ability to form endospores allows them to survive in the presence of oxygen. One of the major causes of health-acquired infections is *C. difficile*, known as C. diff. Prolonged use of antibiotics for other infections increases the probability of a patient developing a secondary *C. difficile* infection. Antibiotic treatment disrupts the balance of microorganisms in the intestine and allows the colonization of the gut by *C. difficile*, causing a significant inflammation of the colon.

Other clostridia responsible for serious infections include *C. tetani*, the agent of tetanus, and *C. perfringens*, which causes gas gangrene. In both cases, the infection starts in necrotic tissue (dead tissue that is not supplied with oxygen by blood circulation). This is the reason that deep puncture wounds are associated with tetanus. When tissue death is accompanied by lack of circulation, gangrene is always a danger.

The study of obligate anaerobes requires special equipment. Obligate anaerobic bacteria must be grown under conditions devoid of oxygen. The most common approach is culture in an anaerobic jar (Figure **8.2.4.3**). Anaerobic jars include chemical packs that remove oxygen and release carbon dioxide (CO_2). An anaerobic chamber is an enclosed box from which all oxygen is removed. Gloves sealed to openings in the box allow handling of the cultures without exposing the culture to air (Figure **8.2.4.3**).



(a)



(b)

Figure **8.2.4.3**: (a) An anaerobic jar is pictured that is holding nine Petri plates supporting cultures. (b) Openings in the side of an anaerobic box are sealed by glove-like sleeves that allow for the handling of cultures inside the box. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by NIST)

Staphylococci and Enterobacteriaceae are examples of facultative anaerobes. Staphylococci are found on the skin and upper respiratory tract. Enterobacteriaceae are found primarily in the gut and upper respiratory tract but can sometimes spread to the urinary tract, where they are capable of causing infections. It is not unusual to see mixed bacterial infections in which the facultative anaerobes use up the oxygen, creating an environment for the obligate anaerobes to flourish.

Examples of aerotolerant anaerobes include lactobacilli and streptococci, both found in the oral microbiota. *Campylobacter jejuni*, which causes gastrointestinal infections, is an example of a microaerophile and is grown under low-oxygen conditions.

The optimum oxygen concentration, as the name implies, is the ideal concentration of oxygen for a particular microorganism. The lowest concentration of oxygen that allows growth is called the minimum permissive oxygen concentration. The highest tolerated concentration of oxygen is the maximum permissive oxygen concentration. The organism will not grow outside the range of oxygen levels found between the minimum and maximum permissive oxygen concentrations.

? Exercise 8.2.4.1

1. Would you expect the oldest bacterial lineages to be aerobic or anaerobic?
2. Which bacteria grow at the top of a thioglycolate tube, and which grow at the bottom of the tube?

An Unwelcome Anaerobe

Charles is a retired bus driver who developed type 2 diabetes over 10 years ago. Since his retirement, his lifestyle has become very sedentary and he has put on a substantial amount of weight. Although he has felt tingling and numbness in his left foot for a while, he has not been worried because he thought his foot was simply “falling asleep.” Recently, a scratch on his foot does not seem to be healing and is becoming increasingly ugly. Because the sore did not bother him much, Charles figured it could not be serious until his daughter noticed a purplish discoloration spreading on the skin and oozing (Figure **8.2.4.4**). When he was finally seen by his physician, Charles was rushed to the operating room. His open sore, or ulcer, is the result of a diabetic foot.

The concern here is that gas gangrene may have taken hold in the dead tissue. The most likely agent of gas gangrene is *Clostridium perfringens*, an endospore-forming, gram-positive bacterium. It is an obligate anaerobe that grows in tissue devoid of oxygen. Since dead tissue is no longer supplied with oxygen by the circulatory system, the dead tissue provides pockets of ideal environment for the growth of *C. perfringens*.

A surgeon examines the ulcer and radiographs of Charles’s foot and determines that the bone is not yet infected. The wound will have to be surgically debrided (debridement refers to the removal of dead and infected tissue) and a sample sent for microbiological lab analysis, but Charles will not have to have his foot amputated. Many diabetic patients are not so lucky. In 2008, nearly 70,000 diabetic patients in the United States lost a foot or limb to amputation, according to statistics from the Centers for Disease Control and Prevention.



Figure 8.2.4.4: This clinical photo depicts ulcers on the foot of a diabetic patient. Dead tissue accumulating in ulcers can provide an ideal growth environment for the anaerobe *C. perfringens*, a causative agent of gas gangrene. (credit: Shigeo Kono, Reiko Nakagawachi, Jun Arata, Benjamin A Lipsky)

Exercise 8.2.4.2

Which growth conditions would you recommend for the detection of *C. perfringens*?

Detoxification of Reactive Oxygen Species

Aerobic respiration constantly generates reactive oxygen species (ROS), byproducts that must be detoxified. Even organisms that do not use aerobic respiration need some way to break down some of the ROS that may form from atmospheric oxygen. Three main enzymes break down those toxic byproducts: superoxide dismutase, peroxidase, and catalase. Each one catalyzes a different reaction. Reactions of type seen in Reaction 1 are catalyzed by peroxidases.

$$\mathrm{X} - (2\mathrm{H}^+) + \mathrm{H_2O_2} \rightarrow \text{oxidized-X} + 2\mathrm{H_2O} \tag{8.2.4.1}$$

In these reactions, an electron donor (reduced compound; e.g., reduced nicotinamide adenine dinucleotide [NADH]) oxidizes hydrogen peroxide, or other peroxides, to water. The enzymes play an important role by limiting the damage caused by peroxidation of membrane lipids. Reaction 2 is mediated by the enzyme superoxide dismutase (SOD) and breaks down the powerful superoxide anions generated by aerobic metabolism:

$$2\mathrm{O_2^-} + 2\mathrm{H^+} \rightarrow \mathrm{H_2O_2} + \mathrm{O_2} \tag{8.2.4.2}$$

The enzyme catalase converts hydrogen peroxide to water and oxygen as shown in Reaction 3.

$$2\mathrm{H_2O_2} \rightarrow 2\mathrm{H_2O} + \mathrm{O_2} \tag{8.2.4.3}$$

Obligate anaerobes usually lack all three enzymes. Aerotolerant anaerobes do have SOD but no catalase. Reaction 3, shown occurring in Figure 8.2.4.5, is the basis of a useful and rapid test to distinguish streptococci, which are aerotolerant and do not possess catalase, from staphylococci, which are facultative anaerobes. A sample of culture rapidly mixed in a drop of 3% hydrogen peroxide will release bubbles if the culture is catalase positive.

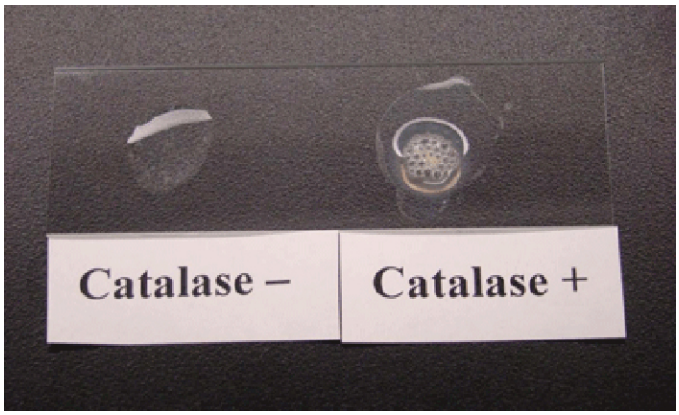


Figure **8.2.4.5**: The catalase test detects the presence of the enzyme catalase by noting whether bubbles are released when hydrogen peroxide is added to a culture sample. Compare the positive result (right) with the negative result (left). (credit: Centers for Disease Control and Prevention)

Bacteria that grow best in a higher concentration of CO_2 and a lower concentration of oxygen than present in the atmosphere are called capnophiles. One common approach to grow capnophiles is to use a candle jar. A candle jar consists of a jar with a tight-fitting lid that can accommodate the cultures and a candle. After the cultures are added to the jar, the candle is lit and the lid closed. As the candle burns, it consumes most of the oxygen present and releases CO_2.

? Exercise 8.2.4.3

1. What substance is added to a sample to detect catalase?
2. What is the function of the candle in a candle jar?

Clinical Focus: Part 2

The health-care provider who saw Jeni was concerned primarily because of her pregnancy. Her condition enhances the risk for infections and makes her more vulnerable to those infections. The immune system is downregulated during pregnancy, and pathogens that cross the placenta can be very dangerous for the fetus. A note on the provider's order to the microbiology lab mentions a suspicion of infection by *Listeria monocytogenes*, based on the signs and symptoms exhibited by the patient.

Jeni's blood samples are streaked directly on sheep blood agar, a medium containing tryptic soy agar enriched with 5% sheep blood. (Blood is considered sterile; therefore, competing microorganisms are not expected in the medium.) The inoculated plates are incubated at 37 °C for 24 to 48 hours. Small grayish colonies surrounded by a clear zone emerge. Such colonies are typical of *Listeria* and other pathogens such as streptococci; the clear zone surrounding the colonies indicates complete lysis of blood in the medium, referred to as beta-hemolysis (Figure **8.2.4.6**). When tested for the presence of catalase, the colonies give a positive response, eliminating *Streptococcus* as a possible cause. Furthermore, a Gram stain shows short gram-positive bacilli. Cells from a broth culture grown at room temperature displayed the tumbling motility characteristic of *Listeria* (Figure **8.2.4.6**). All of these clues lead the lab to positively confirm the presence of *Listeria* in Jeni's blood samples.



Figure **8.2.4.6**: (a) A sample blood agar test showing beta-hemolysis. (b) A sample motility test showing both positive and negative results. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by "VeeDunn"/Flickr)

Exercise 8.2.4.4

How serious is Jeni's condition and what is the appropriate treatment?

Key Concepts and Summary

- Aerobic and anaerobic environments can be found in diverse niches throughout nature, including different sites within and on the human body.
- Microorganisms vary in their requirements for molecular oxygen. **Obligate aerobes** depend on aerobic respiration and use oxygen as a terminal electron acceptor. They cannot grow without oxygen.
- **Obligate anaerobes** cannot grow in the presence of oxygen. They depend on fermentation and anaerobic respiration using a final electron acceptor other than oxygen.
- **Facultative anaerobes** show better growth in the presence of oxygen but will also grow without it.
- Although **aerotolerant anaerobes** do not perform aerobic respiration, they can grow in the presence of oxygen. Most aerotolerant anaerobes test negative for the enzyme **catalase**.
- **Microaerophiles** need oxygen to grow, albeit at a lower concentration than 21% oxygen in air.
- **Optimum oxygen concentration** for an organism is the oxygen level that promotes the fastest growth rate. The **minimum permissive oxygen concentration** and the **maximum permissive oxygen concentration** are, respectively, the lowest and the highest oxygen levels that the organism will tolerate.
- **Peroxidase**, **superoxide dismutase**, and **catalase** are the main enzymes involved in the detoxification of the **reactive oxygen species**. Superoxide dismutase is usually present in a cell that can tolerate oxygen. All three enzymes are usually detectable in cells that perform aerobic respiration and produce more ROS.
- A **capnophile** is an organism that requires a higher than atmospheric concentration of CO_2 to grow.

Footnotes

1. 1 Centers for Disease Control and Prevention. "Living With Diabetes: Keep Your Feet Healthy." http://www.cdc.gov/Features/DiabetesFootHealth/

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8.2.5: Other Environmental Effects on Bacterial Growth

Learning Objectives

- Identify and describe different categories of microbes with specific growth requirements other than oxygen, pH, and temperature, such as altered barometric pressure, osmotic pressure, humidity, and light
- Give at least one example microorganism for each category of growth requirement

Microorganisms interact with their environment along more dimensions than pH, temperature, and free oxygen levels, although these factors require significant adaptations. We also find microorganisms adapted to varying levels of salinity, barometric pressure, humidity, and light.

Osmotic and Barometric Pressure

Most natural environments tend to have lower solute concentrations than the cytoplasm of most microorganisms. Rigid cell walls protect the cells from bursting in a dilute environment. Not much protection is available against high osmotic pressure. In this case, water, following its concentration gradient, flows out of the cell. This results in plasmolysis (the shrinking of the protoplasm away from the intact cell wall) and cell death. This fact explains why brines and layering meat and fish in salt are time-honored methods of preserving food. Microorganisms called halophiles ("salt loving") actually require high salt concentrations for growth. These organisms are found in marine environments where salt concentrations hover at 3.5%. Extreme halophilic microorganisms, such as the red alga *Dunaliella salina* and the archaeal species *Halobacterium* in Figure **8.2.5.1**, grow in hypersaline lakes such as the Great Salt Lake, which is 3.5–8 times saltier than the ocean, and the Dead Sea, which is 10 times saltier than the ocean.

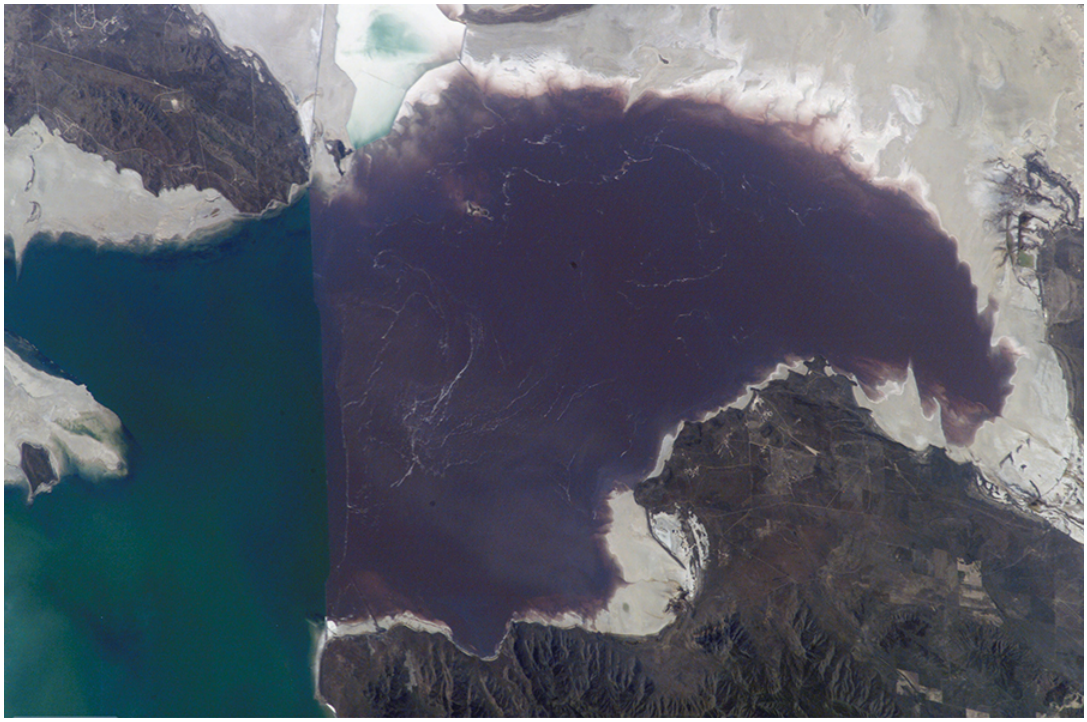


Figure **8.2.5.1**: Photograph taken from space of the Great Salt Lake in Utah. The purple color is caused by high density of the alga *Dunaliella* and the archaean *Halobacterium* spp. (credit: NASA)

Dunaliella spp. counters the tremendous osmotic pressure of the environment with a high cytoplasmic concentration of glycerol and by actively pumping out salt ions. *Halobacterium* spp. accumulates large concentrations of K^+ and other ions in its cytoplasm. Its proteins are designed for high salt concentrations and lose activity at salt concentrations below 1–2 M. Although most halotolerant organisms, for example *Halomonas* spp. in salt marshes, do not need high concentrations of salt for growth, they will survive and divide in the presence of high salt. Not surprisingly, the staphylococci, micrococci, and corynebacteria that colonize our skin tolerate salt in their environment. Halotolerant pathogens are an important cause of food-borne illnesses because they

survive and multiply in salty food. For example, the halotolerant bacteria *S. aureus*, *Bacillus cereus*, and *V. cholerae* produce dangerous enterotoxins and are major causes of food poisoning.

Microorganisms depend on available water to grow. Available moisture is measured as water activity (a_w), which is the ratio of the vapor pressure of the medium of interest to the vapor pressure of pure distilled water; therefore, the a_w of water is equal to 1.0. Bacteria require high a_w (0.97–0.99), whereas fungi can tolerate drier environments; for example, the range of a_w for growth of *Aspergillus* spp. is 0.8–0.75. Decreasing the water content of foods by drying, as in jerky, or through freeze-drying or by increasing osmotic pressure, as in brine and jams, are common methods of preventing spoilage.

Microorganisms that require high atmospheric pressure for growth are called barophiles. The bacteria that live at the bottom of the ocean must be able to withstand great pressures. Because it is difficult to retrieve intact specimens and reproduce such growth conditions in the laboratory, the characteristics of these microorganisms are largely unknown.

Light

Photoautotrophs, such as cyanobacteria or green sulfur bacteria, and photoheterotrophs, such as purple nonsulfur bacteria, depend on sufficient light intensity at the wavelengths absorbed by their pigments to grow and multiply. Energy from light is captured by pigments and converted into chemical energy that drives carbon fixation and other metabolic processes. The portion of the electromagnetic spectrum that is absorbed by these organisms is defined as photosynthetically active radiation (PAR). It lies within the visible light spectrum ranging from 400 to 700 nanometers (nm) and extends in the near infrared for some photosynthetic bacteria. A number of accessory pigments, such as fucoxanthin in brown algae and phycobilins in cyanobacteria, widen the useful range of wavelengths for photosynthesis and compensate for the low light levels available at greater depths of water. Other microorganisms, such as the archaea of the class Halobacteria, use light energy to drive their proton and sodium pumps. The light is absorbed by a pigment protein complex called bacteriorhodopsin, which is similar to the eye pigment rhodopsin. Photosynthetic bacteria are present not only in aquatic environments but also in soil and in symbiosis with fungi in lichens. The peculiar watermelon snow is caused by a microalga *Chlamydomonas nivalis*, a green alga rich in a secondary red carotenoid pigment (astaxanthin) which gives the pink hue to the snow where the alga grows.

? Exercise 8.2.5.1

1. Which photosynthetic pigments were described in this section?
2. What is the fundamental stress of a hypersaline environment for a cell?

Key Concepts and Summary

- **Halophiles** require high salt concentration in the medium, whereas **halotolerant** organisms can grow and multiply in the presence of high salt but do not require it for growth.
- Halotolerant pathogens are an important source of foodborne illnesses because they contaminate foods preserved in salt.
- Photosynthetic bacteria depend on visible light for energy.
- Most bacteria, with few exceptions, require high moisture to grow.

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8.3: Bacterial Quorum Sensing

Learning Objectives

1. Define the following:
   a. pathogenicity
   b. virulence
2. Even though a microorganism may be considered pathogenic, it still may not be able to cause disease upon entering the body. Discuss why.
3. Define and briefly describe the overall process of quorum sensing in bacteria and how it may enable bacteria to behave as a multicellular population.
4. State at least two possible advantages of individual bacterial behavior.
5. State at least two possible advantages of multicellular bacterial behavior.
6. State what is meant by intraspecies, interspecies, and interkingdom communication.
7. State the function of bacterial secretions systems (injectisomes) such as the type 3 and type 6 secretion systems in bacterial pathogenicity.

In this Learning Object we are going to look at several aspects of bacterial genetics that are directly related to bacterial pathogenicity, namely, quorum sensing, pathogenicity islands, and secretion systems. Pathogenicity and virulence are terms that refer to an organism's ability to cause disease. Pathogenicity is the ability of a microbe to cause disease and inflict damage upon its host, whereas virulence is the degree of pathogenicity within a group or species of microbes as indicated by case fatality rates and/or the ability of the organism to invade the tissues of the host. The pathogenicity of an organism, that is its ability to cause disease, is determined by its virulence factors .

Many of the virulence factors that enable bacteria to colonize the body and/or harm the body are the products of quorum sensing genes. Many bacteria are able to sense their own population density, communicate with each other by way of secreted chemical factors, and behave as a population rather than as individual bacteria . This plays an important role in pathogenicity and survival for many bacteria.

Bacterial Quorum Sensing

Bacteria can behave either as individual single-celled organisms or as multicellular populations. Bacteria exhibit these behaviors by chemically "talking" to one another through a process called quorum sensing. Quorum sensing involves the production, release, and community-wide sensing of molecules called autoinducers that modulate gene expression, and ultimately bacterial behavior, in response to the density of a bacterial population.

To initiate the process of quorum sensing, bacterial genes code for the production of signaling molecules called autoinducers that are released into the bacterium's surrounding environment. These signaling molecules then bind to signaling receptors either on the bacterial surface or in the cytoplasm. When these autoinducers reach a critical, threshold level, they activate bacterial quorum sensing genes that enable the bacteria to behave as a multicellular population rather than as individual single-celled organisms (Figure **8.3.3**.2.2). The autoinducer/receptor complex is able to bind to DNA promoters and activate the transcription of quorum sensing-controlled genes in the bacterium. In this way, individual bacteria within a group are able to benefit from the activity of the entire group.

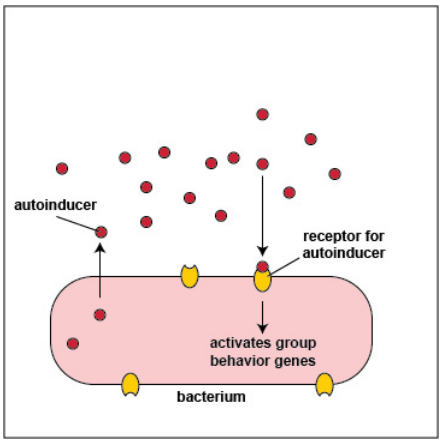
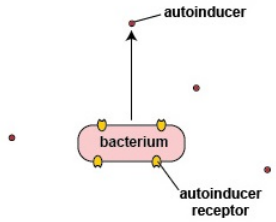


Figure 8.3.3.2.1: Mechanism for Quorum Sensing. Bacteria "talk" to one another through a process called quorum sensing. Bacterial genes code for the production of signaling molecules called autoinducers that are released into the surrounding environment. These signaling molecules then bind to signaling receptors either on the bacterial surface or in the cytoplasm, in this case, on the surface. When these autoinducers reach a critical, threshold level, they activate bacterial quorum sensing genes that enable the bacteria to behave as a multicellular population rather than as individual single-celled organism. The autoinducer/receptor complex is able to bind to DNA promoters and activate the transcription of quorum sensing-controlled genes in the bacterium. In this way, individual bacteria within a group are able to benefit from the activity of the entire group.

1. In Gram-negative bacteria, the autoinducers are typically molecules called acyl-homoserine lactones or AHL. AHLs diffuse readily out of and into bacterial cells where they bind to AHL receptors in the cytoplasm of the bacteria. When a critical level of AHL is reached, the cytoplasmic autoinducer/receptor complex functions as a DNA-binding transcriptional activator.
2. In Gram-positive bacteria, the autoinducers are oligopeptides, short peptides typically 8-10 amino acids long. Oligopeptides cannot diffuse in and out of bacteria like AHLs, but rather leave bacteria via specific exporters. They then bind to autoinducer receptors on the surface of the bacterium. When a critical level of oligopeptide is reached, the binding of the oligopeptide to its receptor starts a phosphorylation cascade that activates DNA-binding transcriptional regulatory proteins called response regulators.

The outcomes of bacteria-host interaction are often related to bacterial population density. Bacterial virulence, that is its ability to cause disease, is largely based on the bacterium's ability to produce gene products called virulence factors that enable that bacterium to colonize the host, resist body defenses, and harm the body.

At a low density of bacteria, the autoinducers diffuse away from the bacteria (Figure 8.3.3.2.2). Sufficient quantities of these molecules are unable to bind to the signaling receptors on the bacterial surface and the quorum sensing genes that enable the bacteria to act as a population are not activated. This enables the bacteria to behave as individual, single-celled organisms.



Low density of bacteria:
autoinducers diffuse away from bacteria
promoting individual cell behavior.

Figure 8.3.3.2.2: Quorum Sensing with a Low Density of Bacterial Cells At a low density of bacteria, the signaling molecules (autoinducers) diffuse away from the bacteria. Sufficient quantities of these molecules are unavailable for binding to the signaling receptors on the bacterial surface (Gram-positive bacteria) or in the cytoplasm (Gram-negative bacteria), and the quorum sensing genes that enable the bacteria to act as a population are not activated. The bacterium then utilizes genes that enable the bacterium to act as an indiviual organism rather than as a multicellular population. Acting as individual organisms may better enable that low density of bacteria to gain a better foothold in their new environment.

Possible advantages of individual bacterial behavior seen at low bacterial density

If a relatively small number of a specific bacterium were to enter the body and immediately start producing their virulence factors, chances are the body's immune systems would have sufficient time to recognize and counter those virulence factors and remove the bacteria before there was sufficient quantity to cause harm. The bacterium instead utilizes genes that enable it to act as an individual organism rather than as part of a multicellular population.

Acting as individual organisms may better enable that low density of bacteria to gain a better foothold in their new environment in the following ways:

1. Many bacteria are capable of motility and motility serves to keep bacteria in an optimum environment via taxis .

Motility and chemotaxis probably help some intestinal and urinary pathogens to move through the mucous layer so they can attach to the epithelial cells of the mucous membranes. In fact, many bacteria that can colonize the mucous membranes of the bladder and the intestines are motile. Motility probably helps these bacteria move through the mucus in places where it is less viscous.

2. One of the body's innate defenses is the ability to physically remove bacteria from the body through such means as the constant shedding of surface epithelial cells from the skin and mucous membranes, the removal of bacteria by such means as coughing, sneezing, vomiting, and diarrhea, and bacterial removal by bodily fluids such as saliva, blood, mucous, and urine. Bacteria may resist this physical removal by producing pili (see Figure 8.3.3), cell wall adhesin proteins (Figure **8.3.3**.2.4), and/or biofilm-producing capsules . Some pili, called type IV pili also allow some bacteria to "walk" or "crawl" along surfaces to spread out and eventually form microcolonies.

Figure **8.3.3***.2.3: Adhesive Tip of Bacterial Pili Binding to Host Cell Receptors*

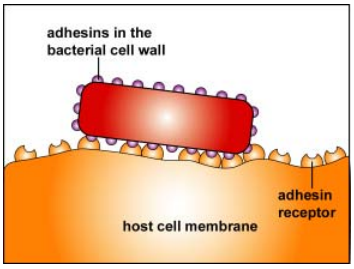


Figure **8.3.3.2.4**: Bacterial Adhesins. Surface proteins called adhesins in the bacterial cell wall bind to receptor molecules on the surface of a susceptible host cell enabling the bacterium to make intimate contact with the host cell, adhere, colonize, and resist flushing.

3. Many bacteria secrete an extracellular polysaccharide or polypeptide matrix called a *capsule* or *glycocalyx* that enables the bacteria to adhere to host cells, resist phagocytosis, and form microcolonies. As the bacteria geometrically increase in number by binary fission, so does the amount of their secreted autoinducers, and production of high levels of autoinducers then enables the population of bacteria to communicate with one another by quorum sensing. At a high density of bacteria, large quantities of autoinducers are produced (Figure **8.3.3**.2.5) and are able to bind to the signaling receptors on the bacterial surface in sufficient quantity so as to activate the quorum sensing genes that enable the bacteria to behave as a multicellular population (Figure **8.3.3**.2.1).

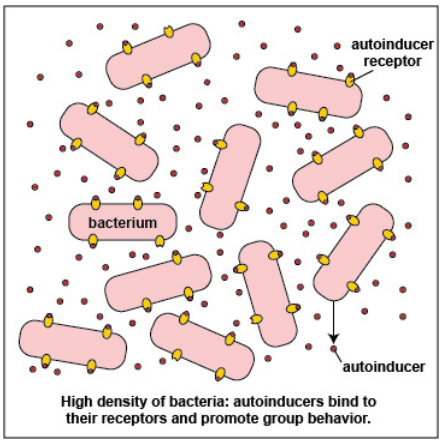


Figure **8.3.3.2.5**: Quorum Sensing with a High Density of Bacterial Cells. At a high density of bacteria, sufficient quantities of signaling molecules (autoinducers) are available for binding to the signaling receptors on the bacterial surface (Gram-positive bacteria) or in the cytoplasm (Gram-negative bacteria), and the quorum sensing genes that enable the bacteria to act as a population become activated. The outcomes of bacteria-host interaction are often related to bacterial population density. Bacterial virulence, that is its ability to cause disease, is largely based on the bacterium's ability to produce gene products called virulence factors that enable that bacterium to colonize the host, resist body defenses, and harm the body.

Advantages of Multicellular Behavior seen at High Bacterial Density

1. By behaving as a multicellular population, individual bacteria within a group are able to benefit from the activity of the entire group. As the entire population of bacteria simultaneously turn on their virulence genes, the body's immune systems are much less likely to have enough time to counter those virulence factors before harm is done.
2. This triggers production of an extracellular adhesive matrix (glycocalyx) enabling the bacteria to form microcolonies and irreversibly attachment to the mucous membranes . Biofilm formation begins.
3. Virulence factors such as exoenzymes and toxins can damage host cells enabling the bacteria in the biofilm to obtain nutrients. The biofilm continues to develop and mature.
4. As the area becomes over-populated with bacteria, quorum sensing enables some of the bacteria to escape the biofilm, often by again producing flagella, and return to individual single-celled organism behavior in order to find a new sight to colonize.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an example of a quorum sensing bacterium. *P. aeruginosa* causes severe hospital-acquired infections, chronic infections in people with cystic fibrosis, and potentially fatal infections in those who are immunocompromised.

1. When *P. aeruginosa* first enters the body, they are at a low density of bacteria. The autoinducers diffuse away from the bacteria (Figure **8.3.3**.2.2), sufficient quantities of these molecules are unable to bind to the signaling receptors, and the quorum sensing genes that enable the bacteria to act as a population are not activated. The *P. aeruginosa* continue to function as individual bacteria. Motility genes (coding for flagella) and adhesin genes (coding for pili and cell wall adhesins) are expressed. The flagella enable the initial bacteria to swim through mucus towards host tissues such as mucous membranes. Pili then enable the bacteria to reversibly attach to host cells in order to resist flushing and begin colonization (Figure **8.3.3**.2.6; left). Type IV pili, which enable a twitching motility in some bacteria, then enable the bacteria as they replicate to crawl along and spread out over the mucous membranes (Figure **8.3.3**.2.6; middle). The pili subsequently retract and bacterial cell wall adhesins enable a more intimate attachment of the bacterium to the mucous membranes (Figure **8.3.3**.2.6; right).

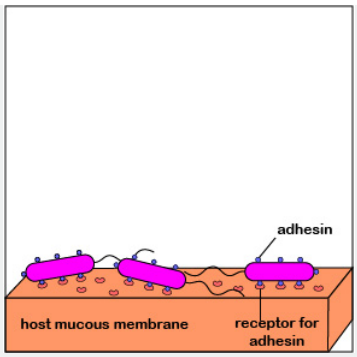


Figure 8.3.3.2.6: Development of a Biofilm by Pseudomonas aeruginosa. Step 1 (left): Planktonic Pseudomonas aeruginosa use their polar flagella and chemotaxis to swim towards host mucous membranes. Pili then bind to host cell receptors for initial but reversible bacterial attachment. Step 2 (middle): As the bacteria begin to replicate, type IV pili enable the bacteria, by way of twitching motility, to crawl along the surface of the mucous membranes and spread out. Step 3 (right): The pili retract and bacterial cell wall adhesins enable a more intimate attachment of the bacterium

2. Once *P. aeruginosa* has colonized, it is able to replicate geometrically and achieve a high population density. Quorum sensing genes are activated and the bacteria function as a population. This triggers production of an extracellular polysaccharide called *alginate* to form microcolonies and enables irreversible attachment to the mucous membranes (Figure 3.2.7; left). Biofilm formation begins.

3. Quorum sensing genes coding for enzymes and toxins that damage host cells are produced. These are injected into the host cells by way of an injectosome. This releases nutrients for the bacteria in the biofilm. The bacteria continue to replicate as the biofilm continues to develop, mushroom up, and mature (Figure 3.2. 7; middle).

4. As the bacteria replicate, the biofilm continues to mature (Figure 3.2.7; right). Water channels form within the biofilm to deliver water, oxygen, and nutrients to the growing population of *P. aeruginosa.* The high density of bacteria bacteria are now acting as a multicellular population rather than as individual bacteria.

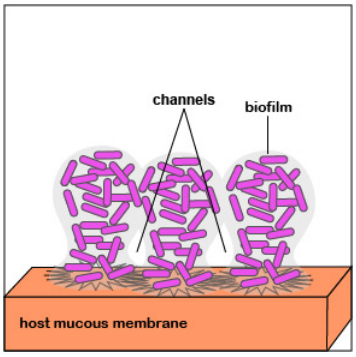


Figure 8.3.3.2.7: Development of a Biofilm by Pseudomonas aeruginosa: Step 4 (left): As the bacteria replicate, quorum sensing genes trigger production of an extracellular polysaccharide called alginate to form microcolonies and enable irreversible attachment to the mucous membranes. Biofilm formation begins. Step 5 (middle): Quorum sensing genes coding for enzymes and toxins that damage host cells are produced. This releases nutrients for the bacteria in the biofilm. The bacteria continue to replicate as the biofilm continues to develop, mushroom up, and mature. Step 6 (right): As the bacteria replicate, the biofilm continues to mature. Water channels form within the biofilm to deliver water, oxygen, and nutrients to the growing population of P. aeruginosa.

The biofilm enables bacteria to:

- resist attack by antibiotics;
- trap nutrients for bacterial growth and remain in a favorable niche;
- adhere to environmental surfaces and resist flushing;
- live in close association and communicate with other bacteria in the biofilm; and
- resist phagocytosis and attack by the body's complement pathways.

5. When the population of *P. aeruginosa* begins to outgrow their local environment, quorum sensing enables them to turn off adhesin genes and turn on flagella genes that allow some of the bacteria to spread out of the biofilm to new location within that environment via motility (Figure **8.3.3**.2.8).

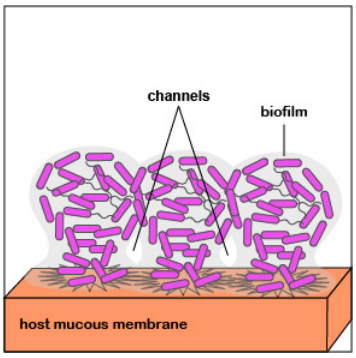


Figure **8.3.3.2.8**: Development of a Biofilm by Pseudomonas aeruginosa: Step 7 (left): As the population begins to overgrow the area and nutrients become limited, quorum sensing genes trigger some of the P. aeruginosa in the biofilm to again produce flagella. Step 8 (right): Planktonic P. aeruginosa leave the biofilm and move to a new location to begin new biofilms.

It turns out that bacteria are multilingual. They use quorum sensing not only to "talk" to members their own species (intraspecies communication), but also to "talk" to bacteria that are not of their genus and species (interspecies communication). Intraspecies autoinducers and receptors enable bacteria to communicate with others of their own species while interspecies autoinducers and receptors enable bacteria to communicate with bacteria of a different species or genus (Figure **8.3.3**.2.9). The autoinducers for interspecies communications are referred to as AI-2 family autoinducers and are different from the intraspecies (AI-1) autoinducers. In some cases bacteria use interspeciecies communication to work cooperatively with various other bacteria in their biofilm to the benefit all involved; in other cases, bacteria may use interspecies communication in such a way that one group benefits at the expense of another.

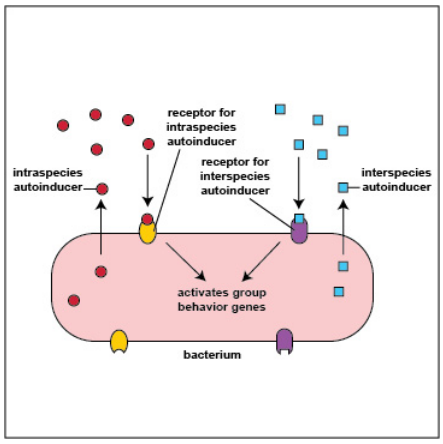


Figure **8.3.3.2.9**: Intraspecies and Interspecies Communication. Intraspecies autoinducers and receptors enable bacteria to communicate with others of their own species while interspecies autoinducers and receptors enable bacteria to communicate with bacteria of a different species or genus.

Furthermore, bacteria are capable of interkingdom communication, communication between bacteria and their animal or plant host. Increasing numbers of bacteria are being found that have signaling receptors that recognize human hormones. For example, a number of bacteria that are pathogens of the human intestinal tract have a sensing molecule called QseC that binds the human hormones adrenaline and noradrenaline. This, in turn, activates various virulence genes of the bacteria. On the other hand, some bacterial autoinducers can enter human host cells and regulate human cellular function. For example, at low concentration some bacterial autoinducers suppress host immune responses thus better enabling those bacteria to better establish themselves in the body. At high concentrations, however, they stimulate an inflammatory response in the host to help the bacteria to spread from the initial infection site. One bacterial autoinducer has been found to initiate apoptosis (cell suicide) in phagocytes such as neutrophils and macrophages.

Bacterial Pathogenicity Islands

The genomes of pathogenic bacteria, when compared with those of similar nonpathogenic species or strains, often show extra genes coding for virulence factors , that is, molecules expressed and secreted by the bacterium that enable them to colonize the host, evade or inhibit the immune responses of the host, enter into or out of a host cell, and/or obtain nutrition from the host. These include virulence factors such as capsules, adhesins, type 3 secretion systems, invasins, and toxins.

Most genes coding for virulence factors in bacteria are located in pathogenicity islands or PAIs and are usually acquired by horizontal gene transfer . These PAIs may be located in the bacterial chromosome, in plasmids, or even in bacteriophage genomes that have entered the bacterium. The genomes of most pathogenic bacteria typically contain multiple PAIs that can account for up to 10 transpoases ,- 20% of the bacterium's genome. PAIs carry genes such as integrases , or insertion sequences that enable them to insert into host bacterial DNA. Transfer RNA (tRNA) genes are often the target site for integration of PAIs. Conjugative plasmids are the most frequent means of transfer of PAIs from one bacterium to another and the transfer of PAIs can then confer virulence to a previously nonpathogenic bacterium.

Type 3 Secretion Systems (T3SS or Injectisomes) and Type 6 Secretion Systems (T6SS)

Many bacteria involved in infection have the ability to co-opt the functions of host cells for the bacterium's own benefit. This is done by way of bacterial secretions systems that enable the bacterium to directly inject bacterial effector molecules into the cytoplasm of the host cell in order to alter its cellular machinery or cellular communication to the benefit of the bacteria.

The most common type is the type 3 secretion system or T3SS (Figure **8.3.3**.2.10). A secretion apparatus in the cytoplasmic membrane and cell wall of the bacterium polymerizes a hollow needle that is lowered to the cytoplasmic membrane of the host cell and a *translocon* protein is then delivered to anchor the needle to the host cell. Effector proteins in the bacterium can now be injected into the cytoplasm of the host cell. The delivery system is sometimes called an *injectisome*. (A type 4 secretion system can transfer effector proteins and/or DNA into the host cell because it is similar to the conjugation transfer system initiated by *tra* genes discussed under horizontal gene transfer.)

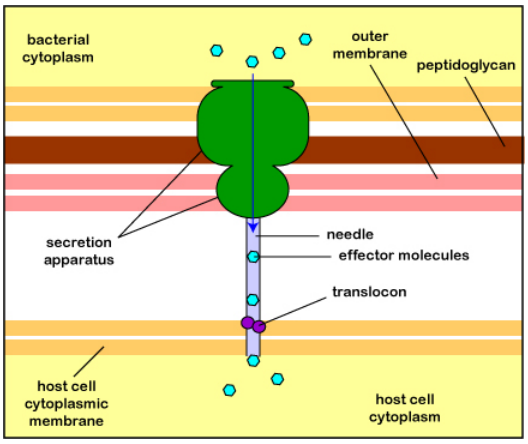


Figure **8.3.3**.2.10: The Bacterial Type 3 Secretion System. Many bacteria involved in infection have the ability to co-opt the functions of the host cell for the bacterium's own benefit. This is done by way of bacterial secretions systems that enable the bacterium to directly inject bacterial effector molecules into the cytoplasm of the host cell in order to alter its cellular machinery or cellular communication. The most common type is the type 3 secretion system. A secretion apparatus in the cytoplasmic membrane and cell wall of the bacterium polymerizes a hollow needle that is lowered to the cytoplasmic membrane of the host cell and a translocon protein is then delivered to anchor the needle to the host cell. Effector proteins in the bacterium can now be injected into the cytoplasm of the host cell. The delivery system is sometimes called an injectisome.



Electron micrograph of an injectisome. A transmission electron-microscope image of isolated T3SS needle complexes from Salmonella typhimurium. (CC BY-SA 2.5; Schraidt O, Lefebre MD, Brunner MJ, Schmied WH, Schmidt A, Radics J, Mechtler K, Galán JE, Marlovits TC - Cropped image from Schraidt et al. (2010), Topology and Organization of the Salmonella typhimurium Type III Secretion Needle Complex Components. PLoS Pathog 6(4): e1000824.doi:10.1371/journal.ppat.1000824)

Some bacteria, such as *Pseudomonas aeruginosa* and *Vibrio cholerae*, produce a type 6 secretion system, or T6SS, that consists of a protein tube surrounded by a contractile sheath, similar to the tail of T4-bacteriophages (a bacteriophage is a virus that only infects bacteria.) The type 6 secretion system not only injects effector molecules into eukaryotic cells, but also is able to inject antibacterial effector molecules into other bacteria in order to kill those bacteria. Predator bacteria can use their T6SS to kill prey bacteria. In fact, *V. cholerae* and *P. aeruginosa* have been shown to "duel" with one another via their respective T6SSs.

V. cholerae also uses its T6SS to promote horizontal gene transfer by way of transformation. Individual *V. cholerae* cells also use their T6SS to attack one another upon cell-to-cell contact. Most members of the population, however, produce immunity proteins that protect them from being killed by the effector molecules that are injected. Not all strains of *V. cholerae* in the population, however, produce these immunity proteins and these non-immune cells are subsequently lysed, releasing their DNA into the environment. This DNA can then be taken up by neighboring competent *V. cholerae* via transformation.

? Exercise 8.3.1: Think-Pair-Share Questions

Briefly describe how bacterial quorum sensing may play a role in pathogenicity by:

a. Promoting colonization of a new host by bacteria that have just entered the body.
b. Enabling the bacterium to persist within that host once they have colonized.
c. Allowing some of the bacteria to spread to a new location within a host or to a new host.

? Exercise 8.3.2: Think-Pair-Share Questions

Briefly describe how the ability to produce a type 3 secretion system might play a role in a pathogen colonizing the body and causing an infection.

Summary

1. Pathogenicity is the ability of a microbe to cause disease and inflict damage upon its host; virulence is the degree of pathogenicity within a group or species of microbes.
2. The pathogenicity of an organism is determined by its virulence factors.
3. Virulence factors enable that bacterium to colonize the host, resist body defenses, and harm the body.
4. Most of the virulence factors are the products of quorum sensing genes.
5. Quorum sensing involves the production, release, and community-wide sensing of molecules called autoinducers that modulate gene expression, and ultimately bacterial behavior, in response to the density of a bacterial population.
6. The outcomes of bacteria-host interaction are often related to bacterial population density.
7. At a low density of bacteria, the autoinducers diffuse away from the bacteria and there are insufficient quantities of these molecules to activate the quorum sensing genes that enable the bacteria to act as a population. As a result the bacteria behave as individual, single-celled organisms.

8. Acting as individual organisms may enable a low density of bacteria to gain a better foothold in their new environment by enabling bacteria to use motility and taxis to contact host cells, use pili to initially adhere to and crawl over host cell surfaces, use adhesins to adhere to host cells and resist flushing, and secrete a glycocalyx to form microcolonies.
9. As the bacteria increase in numbers geometrically as a result of binary fission and reach high density, large quantities of autoinducers are produced and are able to bind to the signaling receptors on the bacterial surface in sufficient quantity so as to activate the quorum sensing genes that enable the bacteria to now behave as a multicellular population.
10. By behaving as a multicellular population, individual bacteria within a group are able to benefit from the activity of the entire group.
11. As the entire population of bacteria simultaneously turn on their virulence genes, the body's immune systems are much less likely to have enough time to counter those virulence factors before harm is done. Virulence factors such as exoenzymes and toxins can damage host cells enabling the bacteria in the biofilm to obtain nutrients.
12. As the area becomes over-populated with bacteria, quorum sensing enables some of the bacteria to escape the biofilm and return to individual single-celled organism behavior in order to find a new sight to colonize.
13. Quorum sensing enables bacteria to communicate with members of their own species, with other species of bacteria, and with their eukaryotic host cells.
14. Most genes coding for virulence factors in bacteria are located in pathogenicity islands or PAIs and are usually acquired by horizontal gene transfer.
15. Many bacteria involved in infection have the ability to co-opt the functions of the host cell for the bacterium’s own benefit by producing secretions systems that enable the bacterium to directly inject bacterial effector molecules into the cytoplasm of the host cell in order to alter the host cell’s cellular machinery, cellular function, or cellular communication.

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SECTION OVERVIEW

8.4: Culturing Bacteria

Topic hierarchy

8.4.1: Media Used for Bacterial Growth

8.4.2: Culture Media

8.4.3: Complex and Synthetic Media

8.4.4: Selective and Differential Media

8.4.5: Special Culture Techniques

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8.4.1: Media Used for Bacterial Growth

Learning Objectives

- Identify and describe culture media for the growth of bacteria, including examples of all-purpose media, enriched, selective, differential, defined, and enrichment media

The study of microorganisms is greatly facilitated if we are able to culture them, that is, to keep reproducing populations alive under laboratory conditions. Culturing many microorganisms is challenging because of highly specific nutritional and environmental requirements and the diversity of these requirements among different species.

Nutritional Requirements

The number of available media to grow bacteria is considerable. Some media are considered general all-purpose media and support growth of a large variety of organisms. A prime example of an all-purpose medium is tryptic soy broth (TSB). Specialized media are used in the identification of bacteria and are supplemented with dyes, pH indicators, or antibiotics. One type, enriched media, contains growth factors, vitamins, and other essential nutrients to promote the growth of fastidious organisms, organisms that cannot make certain nutrients and require them to be added to the medium. When the complete chemical composition of a medium is known, it is called a chemically defined medium. For example, in EZ medium, all individual chemical components are identified and the exact amounts of each is known. In complex media, which contain extracts and digests of yeasts, meat, or plants, the precise chemical composition of the medium is not known. Amounts of individual components are undetermined and variable. Nutrient broth, tryptic soy broth, and brain heart infusion, are all examples of complex media.

Media that inhibit the growth of unwanted microorganisms and support the growth of the organism of interest by supplying nutrients and reducing competition are called selective media. An example of a selective medium is MacConkey agar. It contains bile salts and crystal violet, which interfere with the growth of many gram-positive bacteria and favor the growth of gram-negative bacteria, particularly the Enterobacteriaceae. These species are commonly named enterics, reside in the intestine, and are adapted to the presence of bile salts. The enrichment cultures foster the preferential growth of a desired microorganism that represents a fraction of the organisms present in an inoculum. For example, if we want to isolate bacteria that break down crude oil, hydrocarbonoclastic bacteria, sequential subculturing in a medium that supplies carbon only in the form of crude oil will enrich the cultures with oil-eating bacteria. The differential media make it easy to distinguish colonies of different bacteria by a change in the color of the colonies or the color of the medium. Color changes are the result of end products created by interaction of bacterial enzymes with differential substrates in the medium or, in the case of hemolytic reactions, the lysis of red blood cells in the medium. In Figure **8.4.1.1**, the differential fermentation of lactose can be observed on MacConkey agar. The lactose fermenters produce acid, which turns the medium and the colonies of strong fermenters hot pink. The medium is supplemented with the pH indicator neutral red, which turns to hot pink at low pH. Selective and differential media can be combined and play an important role in the identification of bacteria by biochemical methods.



Figure **8.4.1.1**: On this MacConkey agar plate, the lactose-fermenter *E. coli* colonies are bright pink. *Serratia marcescens*, which does not ferment lactose, forms a cream-colored streak on the tan medium. (credit: American Society for Microbiology)

? Exercise 8.4.1.1

1. Distinguish complex and chemically defined media.
2. Distinguish selective and enrichment media.

Link to Learning

Compare the compositions of EZ medium and sheep blood agar.

The End-of-Year Picnic

The microbiology department is celebrating the end of the school year in May by holding its traditional picnic on the green. The speeches drag on for a couple of hours, but finally all the faculty and students can dig into the food: chicken salad, tomatoes, onions, salad, and custard pie. By evening, the whole department, except for two vegetarian students who did not eat the chicken salad, is stricken with nausea, vomiting, retching, and abdominal cramping. Several individuals complain of diarrhea. One patient shows signs of shock (low blood pressure). Blood and stool samples are collected from patients, and an analysis of all foods served at the meal is conducted.

Bacteria can cause gastroenteritis (inflammation of the stomach and intestinal tract) either by colonizing and replicating in the host, which is considered an infection, or by secreting toxins, which is considered intoxication. Signs and symptoms of infections are typically delayed, whereas intoxication manifests within hours, as happened after the picnic.

Blood samples from the patients showed no signs of bacterial infection, which further suggests that this was a case of intoxication. Since intoxication is due to secreted toxins, bacteria are not usually detected in blood or stool samples. MacConkey agar and sorbitol-MacConkey agar plates and xylose-lysine-deoxycholate (XLD) plates were inoculated with stool samples and did not reveal any unusually colored colonies, and no black colonies or white colonies were observed on XLD. All lactose fermenters on MacConkey agar also ferment sorbitol. These results ruled out common agents of food-borne illnesses: *E. coli*, *Salmonella* spp., and *Shigella* spp.

Analysis of the chicken salad revealed an abnormal number of gram-positive cocci arranged in clusters (Figure **8.4.1.2**). A culture of the gram-positive cocci releases bubbles when mixed with hydrogen peroxide. The culture turned mannitol salt agar yellow after a 24-hour incubation.

All the tests point to *Staphylococcus aureus* as the organism that secreted the toxin. Samples from the salad showed the presence of gram-positive cocci bacteria in clusters. The colonies were positive for catalase. The bacteria grew on mannitol salt agar fermenting mannitol, as shown by the change to yellow of the medium. The pH indicator in mannitol salt agar is phenol red, which turns to yellow when the medium is acidified by the products of fermentation.

The toxin secreted by *S. aureus* is known to cause severe gastroenteritis. The organism was probably introduced into the salad during preparation by the food handler and multiplied while the salad was kept in the warm ambient temperature during the speeches.

Exercise 8.4.1.2

1. What are some other factors that might have contributed to rapid growth of *S. aureus* in the chicken salad?
2. Why would *S. aureus* not be inhibited by the presence of salt in the chicken salad?

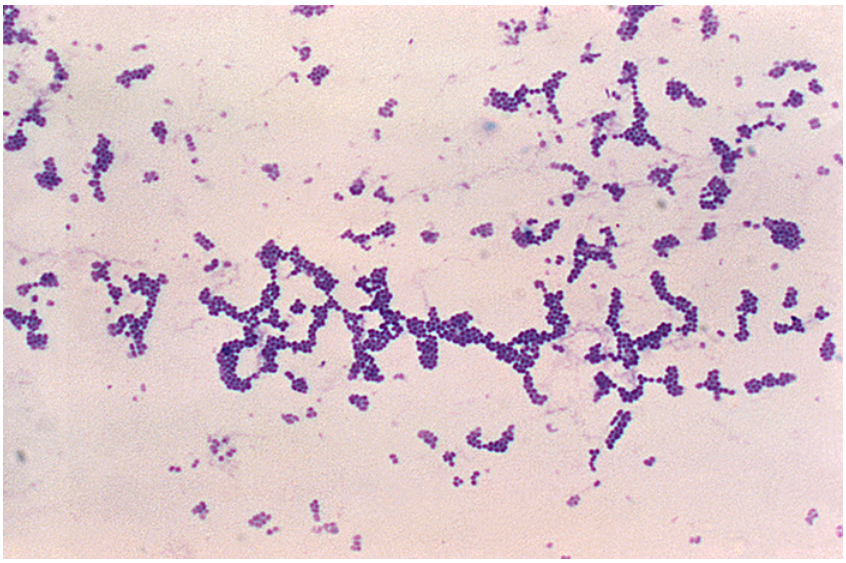


Figure **8.4.1.2**: Gram-positive cocci in clusters. (credit: Centers for Disease Control and Prevention)

Key Concepts and Summary

- **Chemically defined media** contain only chemically known components.
- **Selective media** favor the growth of some microorganisms while inhibiting others.
- **Enriched media** contain added essential nutrients a specific organism needs to grow
- **Differential media** help distinguish bacteria by the color of the colonies or the change in the medium.

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8.4.2: Culture Media

Learning Objectives

- Classify culture media

Culture medium or growth medium is a liquid or gel designed to support the growth of microorganisms. There are different types of media suitable for growing different types of cells. Here, we will discuss microbiological cultures used for growing microbes, such as bacteria or yeast.

NUTRIENT BROTHS AND AGAR PLATES

These are the most common growth media, although specialized media are sometimes required for microorganism and cell culture growth. Some organisms, termed fastidious organisms, need specialized environments due to complex nutritional requirements. Viruses, for example, are obligate intracellular parasites and require a growth medium containing living cells. Many human microbial pathogens also require the use of human cells or cell lysates to grow on a media.

The most common growth media nutrient broths (liquid nutrient medium) or LB medium (Lysogeny Broth) are liquid. These are often mixed with agar and poured into Petri dishes to solidify. These agar plates provide a solid medium on which microbes may be cultured. They remain solid, as very few bacteria are able to decompose agar. Many microbes can also be grown in liquid cultures comprised of liquid nutrient media without agar.



Figure: **Microbial pathogen growing on blood-agar plate**: Red blood cells are used to make an agar plate. Different pathogens that can use red blood cells to grow are shown on these plates. On the left is staphylococcus and the right streptococcus.

DEFINED VS UNDEFINED MEDIA

This is an important distinction between growth media types. A *defined* medium will have known quantities of all ingredients. For microorganisms, it provides trace elements and vitamins required by the microbe and especially a defined carbon and nitrogen source. Glucose or glycerol are often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources. An *undefined* medium has some complex ingredients, such as yeast extract, which consists of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.

There are many different types of media that can be used to grow specific microbes, and even promote certain cellular processes; such as wort, the medium which is the growth media for the yeast that makes beer. Without wort in certain conditions, fermentation cannot occur and the beer will not contain alcohol or be carbonated (bubbly).

COMMON BROADLY-DEFINED CULTURE MEDIA

Nutrient media – A source of amino acids and nitrogen (e.g., beef, yeast extract). This is an undefined medium because the amino acid source contains a variety of compounds with the exact composition being unknown. These media contain all the elements that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory-culture collections.

Minimal media – Media that contains the minimum nutrients possible for colony growth, generally without the presence of amino acids, and are often used by microbiologists and geneticists to grow "wild type" microorganisms. These media can also be used to select for or against the growth of specific microbes. Usually a fair amount of information must be known about the microbe to determine its minimal media requirements.

Selective media – Used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing.

Differential media – Also known as indicator media, are used to distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection and identification of microorganisms.

These few examples of general media types provide some indication only; there are a myriad of different types of media that can be used to grow and control microbes.

Key Points

- Culture media contains the nutrients needed to sustain a microbe.
- Culture media can vary in many ingredients allowing the media to select for or against microbes.
- Glucose or glycerol are often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources in culture media.

Key Terms

- **culture**: The process of growing a bacterial or other biological entity in an artificial medium.
- **lysogeny broth**: Lysogeny broth (LB) is a nutritionally-rich medium; primarily used for the growth of bacteria.

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8.4.3: Complex and Synthetic Media

Learning Objectives

- Differentiate complex and synthetic medias

There are many types of culture media, which is food that microbes can live on. Two major sub types of media are complex and synthetic medias, known as undefined and defined media.



Figure: **Undefined Media**: Luria Broth as shown here is made with yeast extract, as yeast extract is not completely chemically defined Luria Broth is therefore an undefined media. By Lilly_M [GFDL (http://www.gnu.org/copyleft/fdl.html), CC-BY-SA, via Wikimedia Commons

An undefined medium has some complex ingredients, such as yeast extract or casein hydrolysate, which consist of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.A defined medium (also known as chemically defined medium or synthetic medium) is a medium in which all the chemicals used are known, no yeast, animal, or plant tissue is present. A chemically defined medium is a growth medium suitable for the culture of microbes or animal cells (including human) of which all of the chemical components are known. The term chemically defined medium was defined by Jayme and Smith as a ‘Basal formulation which may also be protein-free and is comprised solely of biochemically-defined low molecular weight constituents.

A chemically defined medium is entirely free of animal-derived components (including microbial derived components such as yeast extract) and represents the purest and most consistent cell culture environment. By definition chemically defined media cannot contain either fetal bovine serum, bovine serum albumin, or human serum albumin as these products are derived from bovine or human sources and contain complex mixes of albumins and lipids. The term ‘chemically defined media’ is often misused in the literature to refer to serum albumin-containing media. Animal serum or albumin is routinely added to culture media as a source of nutrients and other ill-defined factors, despite technical disadvantages to its inclusion and its high cost. Technical disadvantages to using serum include the undefined nature of serum, batch-to-batch variability in composition, and the risk of contamination. There are increasing concerns about animal suffering inflicted during serum collection that add an ethical imperative to move away from the use of serum wherever possible. Chemically defined media differ from serum-free media in that bovine serum albumin or human serum albumin with either a chemically defined recombinant version (which lacks the albumin associated lipids) or synthetic chemical such as the polymer polyvinyl alcohol which can reproduce some of the functions of serums.

Key Points

- Defined media is made from constituents that are completely understood.
- Undefined media has some part of which is not entirely defined.
- The presence of extracts from animals or other microbes makes a media undefined as the entire chemical composition of extracts are not completely known.

Key Terms

- **recombinant**: This term refers to something formed by combining existing elements in a new combination. Thus, the phrase recombinant DNA refers to an organism created in the lab by adding DNA from another species.
- **serum**: The clear yellowish fluid obtained upon separating whole blood into its solid and liquid components after it has been allowed to clot. Also called blood serum.

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8.4.4: Selective and Differential Media

Learning Objectives

- Compare selective and differential media

There are many types of media used in the studies of microbes. Two types of media with similar implying names but very different functions, referred to as selective and differential media, are defined as follows.

Selective media are used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing. Media lacking an amino acid such as proline in conjunction with *E. coli* unable to synthesize it were commonly used by geneticists before the emergence of genomics to map bacterial chromosomes. Selective growth media are also used in cell culture to ensure the survival or proliferation of cells with certain properties, such as antibiotic resistance or the ability to synthesize a certain metabolite. Normally, the presence of a specific gene or an allele of a gene confers upon the cell the ability to grow in the selective medium. In such cases, the gene is termed a marker. Selective growth media for eukaryotic cells commonly contain neomycin to select cells that have been successfully transfected with a plasmid carrying the neomycin resistance gene as a marker. Gancyclovir is an exception to the rule as it is used to specifically kill cells that carry its respective marker, the Herpes simplex virus thymidine kinase (HSV TK). Some examples of selective media include:

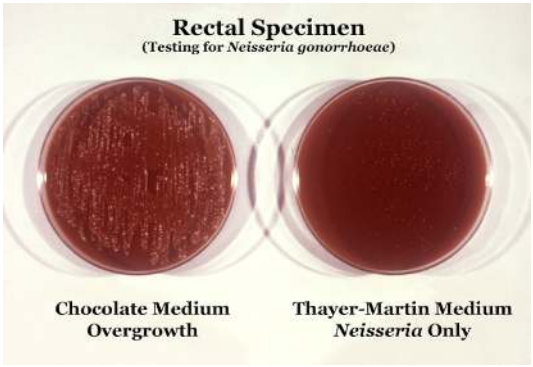


Figure: **Non-selective versus selective media.**: The non-selective media on the right allows of the growth of several different bactarial species and is overgrown with bacteria (whitish lines). While the plate on the right selectively only allows the bacteria Neisseria gonorrhoeae, to grow (white dots).

- Eosin methylene blue (EMB) that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram negative bacteria.
- YM (yeast and mold) which has a low pH, deterring bacterial growth.
- MacConkey agar for Gram-negative bacteria.
- Hektoen enteric agar (HE) which is selective for Gram-negative bacteria.
- Mannitol salt agar (MSA) which is selective for Gram-positive bacteria and differential for mannitol. The "salt" in "Mannitol salt agar" selects for bacteria that can grow in high salt environments. *Staphylococci* thrive on the medium, largely because of their adaptation to salty habitats such as human skin.
- Terrific Broth (TB) is used with glycerol in cultivating recombinant strains of Escherichia coli.
- Xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria buffered charcoal yeast extract agar, which is selective for certain gram-negative bacteria, especially Legionella pneumophila.

Differential media or indicator media distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria. Examples of differential media include:

- Blood agar (used in strep tests), which contains bovine heart blood that becomes transparent in the presence of hemolytic.

- Streptococcuseosin methylene blue (EMB), which is differential for lactose and sucrose fermentation.
- MacConkey (MCK), which is differential for lactose fermentationmannitol salt agar (MSA), which is differential for mannitol fermentation.
- X-gal plates, which are differential for lac operon mutants.

Key Points

- Selective media generally selects for the growth of a desired organism, stopping the growth of or altogether killing non-desired organisms.
- Differential media takes advantage of biochemical properties of target organisms, often leading to a visible change when growth of target organisms are present.
- Differential media, unlike selective media, does not kill organisms. It indicates if a target organism is present.

Key Terms

- **recombinant**: This term refers to something formed by combining existing elements in a new combination. Thus, the phrase recombinant DNA refers to an organism created in the lab by adding DNA from another species.
- **gene**: A unit of heredity; a segment of DNA or RNA that is transmitted from one generation to the next. It carries genetic information such as the sequence of amino acids for a protein.
- **allele**: One of a number of alternative forms of the same gene occupying a given position on a chromosome.

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8.4.5: Special Culture Techniques

Learning Objectives

- Evaluate special culture techniques

Microbiologists would prefer to use well-defined media to grow a microbe, making the microbe easier to control. However, microbes are incredibly varied in what they use as a food source, the environments they live in, and the danger levels they may have for humans and other organisms they may compete with. Therefore they need special nutrient and growth environments. To grow these difficult microbes, microbiologists often turn to undefined media which is chosen based on price and more-so in this case by necessity as some microorganisms have never been cultured on defined media. Some special culture conditions are relatively simple as demonstrated by microaerophile.

A microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere (~20% concentration). Many microphiles are also capnophiles, as they require an elevated concentration of carbon dioxide. In the laboratory they can be easily cultivated in a candle jar. A candle jar is a container into which a lit candle is introduced before sealing the container's airtight lid. The candle's flame burns until extinguished by oxygen deprivation, which creates a carbon dioxide-rich, oxygen-poor atmosphere in the jar. Many labs also have access directly to carbon dioxide and can add the desired carbon dioxide levels directly to incubators where they want to grow microaerophiles.

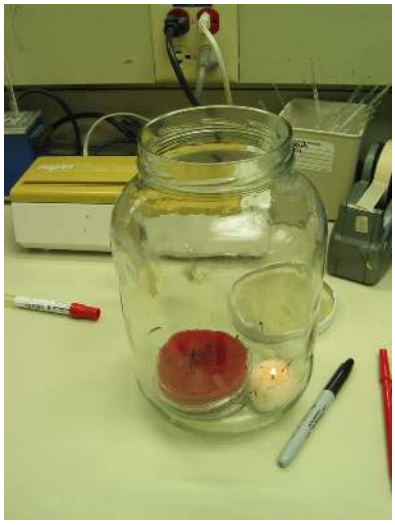


Figure: ***Candle jar****: A candle is lit in a jar with a culture plate. The lid is put on, as the burns it increases the carbon dioxide levels in the jar.*

Animals can often be used to culture microbes. For example, armadillos are often used in the study of leprosy. They are particularly susceptible due to their unusually low body temperature, which is hospitable to the leprosy bacterium, Mycobacterium leprae. The leprosy bacterium is difficult to culture and armadillos have a body temperature of 34°C, similar to human skin. Likewise, humans can acquire a leprosy infection from armadillos by handling them or consuming armadillo meat. Additionally, Syphillis which is caused by the bacteria *Treponema pallidum* is difficult to grow with defined media, so rabbits are used to culture *Treponema pallidum*. *Treponema pallidum* belongs to the Spirochaetesphylum of bacteria.

To date Spirochaetes are very difficult if not impossible to rear in a controlled laboratory environment. This also includes other human pathogens like the bacterium that causes Lyme disease. Using animals to culture human-pathogens has problems. First, the use of animals is always difficult for technical and ethical reasons. Also, a microbe growing on animal other than a human may behave very differently from how that same microbe will behave on a human. Some human pathogens are grown directly on cells cultured from humans. Exemplified by the bacteria *Chlamydia trachomatis*, the bacteria responsible for the sexually transmitted infection (STI) in humans known as Chlamydia. As *Chlamydia trachomatis* only grows in humans. The human cell culture known as McCoy cell culture is used to culture this bacteria.

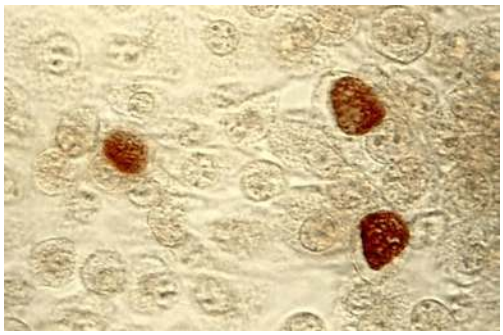


Figure: ***Chlamydias bacteria group****: Light microscope view of cells infected with chlamydiae as shown by the brown inclusion bodies.*

A large concern of microbiology is trying to find ways in which humans can avoid or get rid of microbrial infections. As typified by some of the above examples, some microbes have to be grown in the lab, and some of them can infect humans. To deal with this, microbiologists use a classification of biosafety levels. A biosafety level is the level of the biocontainment precautions required to isolate dangerous biological agents in an enclosed facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4). In the United States, the Centers for Disease Control and Prevention (CDC) have specified these levels.

- **Biosafety Level 1**: This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, with minimal potential hazard to laboratory personnel and the environment.
- **Biosafety Level 2**: This level is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It includes various bacteria and viruses that cause only mild disease to humans or are difficult to contract via aerosol in a lab setting such as chlamydia.
- **Biosafety Level 3**: This level is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease after inhalation. It includes various bacteria, parasites, and viruses that can cause severe to fatal disease in humans, but for which treatments exist (eg. yellow fever).
- **Biosafety Level 4**: This level is reserved for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, agents that cause severe to fatal disease in humans for which vaccines or other treatments are not available, such as Bolivian and Argentine hemorrhagic fevers, Marburg virus, and the Ebola virus. Very few laboratories are biosafety level 4.



Figure: **Positive pressure suit**: A scientist puts on a positive pressure suit, something needed to work with the most dangerous human pathogens in a biosafety level 4 laboratory.

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Key Points

- Microbes, often those that we know little about, have to be cultured with undefined media or growth conditions.
- The use of animals to culture animals is sometimes necessary as no simple media can be used, this presents technical and ethical issues.
- As human pathogens are often studied by microbiologists, special safety conditions know as biosafety levels are used to keep researches free of infection from the pathogens they study.

Key Terms

- **yellow fever**: An acute febrile illness of tropical regions, caused by a flavivirus and spread by mosquitoes, characterized by jaundice, black vomit, and the absence of urination.
- **Lyme disease**: Infection by a bacterium of the genus Borrelia which is transmitted by ticks. Symptoms include a rash followed by fever, joint pain, and headaches.

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8.5: Growth Characteristics

Provided with the right conditions (food, correct temperature, etc) microbes can grow very quickly. Depending on the situation, this could be a good thing for humans (yeast growing in wort to make beer) or a bad thing (bacteria growing in your throat causing strep throat). It's important to have knowledge of their growth, so we can predict or control their growth under particular conditions. While growth for muticelluar organisms is typically measured in terms of the increase in size of a single organism, microbial growth is measured by the increase in population, either by measuring the increase in cell number or the increase in overall mass.

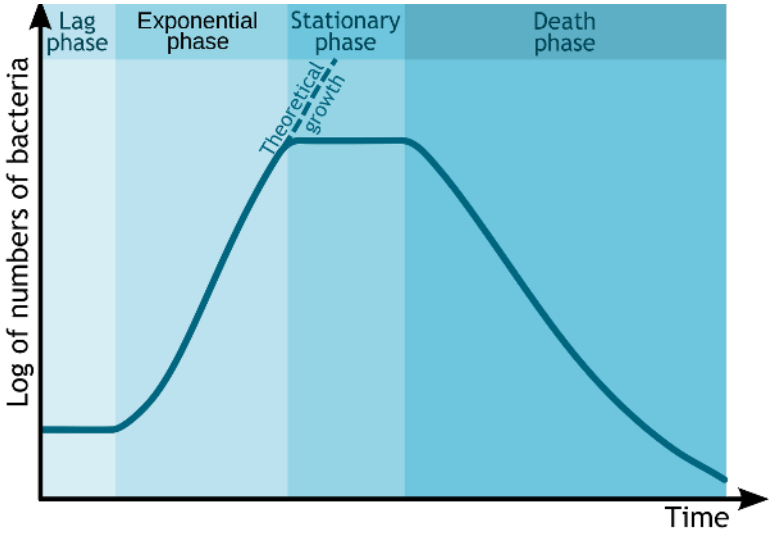
Bacterial Division

Bacteria and archaea reproduce asexually only, while eukartyotic microbes can engage in either sexual or asexual reproduction. Bacteria and archaea most commonly engage in a process known as **binary fission**, where a single cell splits into two equally sized cells. Other, less common processes can include **multiple fission**, **budding**, and the production of **spores**.

The process begins with cell elongation, which requires careful enlargement of the cell membrane and the cell wall, in addition to an increase in cell volume. The cell starts to replicate its DNA, in preparation for having two copies of its chromosome, one for each newly formed cell. The protein FtsZ is essential for the formation of a septum, which initially manifests as a ring in the middle of the elongated cell. After the nucleoids are segregated to each end of the elongated cell, septum formation is completed, dividing the elongated cell into two equally sized daughter cells. The entire process or **cell cycle**can take as little as 20 minutes for an active culture of *E. coli* bacteria.

Growth Curve

Since bacteria are easy to grow in the lab, their growth has been studied extensively. It has been determined that in a **closed system** or **batch culture** (no food added, no wastes removed) bacteria will grow in a predictable pattern, resulting in a **growth curve** composed of four distinct phases of growth: the lag phase, the exponential or log phase, the stationary phase, and the death or decline phase. Additionally, this growth curve can yield generation time for a particular organism – the amount of time it takes for the population to double.



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The details associated with each growth curve (number of cells, length of each phase, rapidness of growth or death, overall amount of time) will vary from organism to organism or even with different conditions for the same organism. But the pattern of four distinct phases of growth will typically remain.

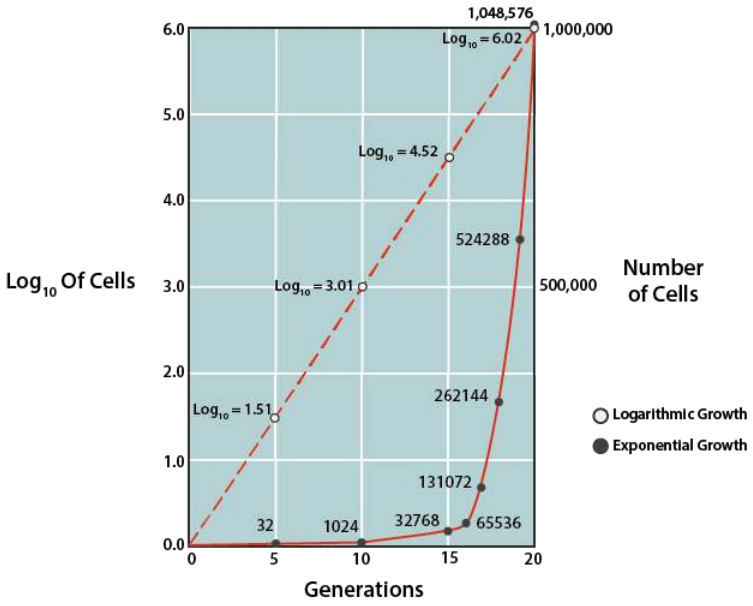
Lag phase

The **lag phase** is an adaptation period, where the bacteria are adjusting to their new conditions. The length of the lag phase can vary considerably, based on how different the conditions are from the conditions that the bacteria came from, as well as the condition of the bacterial cells themselves. Actively growing cells transferred from one type of media into the same type of media, with the same environmental conditions, will have the shortest lag period. Damaged cells will have a long lag period, since they must repair themselves before they can engage in reproduction.

Typically cells in the lag period are synthesizing RNA, enzymes, and essential metabolites that might be missing from their new environment (such as growth factors or macromolecules), as well as adjusting to environmental changes such as changes in temperature, pH, or oxygen availability. They can also be undertaking any necessary repair of injured cells.

Exponential or Log phase

Once cells have accumulated all that they need for growth, they proceed into cell division. The **exponential or log phase** of growth is marked by predictable doublings of the population, where 1 cell become 2 cells, becomes 4, becomes 8 etc. Conditions that are optimal for the cells will result in very rapid growth (and a steeper slope on the growth curve), while less than ideal conditions will result in slower growth. Cells in the exponential phase of growth are the healthiest and most uniform, which explains why most experiments utilize cells from this phase.



Bacterial Growth Rates.

Due to the predictability of growth in this phase, this phase can be used to mathematically calculate the time it takes for the bacterial population to double in number, known as the **generation time (*g*)**. This information is used by microbiologists in basic research, as well as in industry. In order to determine generation time, the natural logarithm of cell number can be plotted against time (where the units can vary, depending upon speed of growth for the particular population), using a semilogarithmic graph to generate a line with a predictable slope.

The slope of the line is equal to 0.301/*g*. Alternatively one can rely on the fixed relationship between the initial number of cells at the start of the exponential phase and the number of cells after some period of time, which can be expressed by:

$$N = N_0 2^n \tag{8.5.1}$$

where N is the final cell concentration, N_0 is the initial cell concentration, and n is the number of generations that occurred between the specified period of time.

Generation time (g) can be represented by t/n, with t being the specified period of time in minutes, hours, days, or months. Thus, if one knows the cell concentration at the start of the exponential phase of growth and the cell concentration after some period of time

of exponential growth, the number of generations can be calculated. Then, using the amount of time that growth was allowed to proceed (*t*), one can calculate *g*.

Stationary Phase

All good things must come to an end (otherwise bacteria would equal the mass of the Earth in 7 days!). At some point the bacterial population runs out of an essential nutrient/chemical or its growth is inhibited by its own waste products (it is a closed container, remember?) or lack of physical space, causing the cells to enter into the **stationary phase**. At this point the number of new cells being produced is equal to the number of cells dying off or growth has entirely ceased, resulting in a flattening out of growth on the growth curve.

Physiologically the cells become quite different at this stage, as they try to adapt to their new starvation conditions. The few new cells that are produced are smaller in size, with bacilli becoming almost spherical in shape. Their plasma membrane becomes less fluid and permeable, with more hydrophobic molecules on the surface that promote cell adhesion and aggregation. The nucleoid condenses and the DNA becomes bound with **DNA-binding proteins from starved cells (DPS)**, to protect the DNA from damage. The changes are designed to allow the cell to survive for a longer period of time in adverse conditions, while waiting for more optimal conditions (such as an infusion of nutrients) to occur. These same strategies are used by cells in **oligotrophic** or low-nutrient environments. It has been hypothesized that cells in the natural world (i.e. outside of the laboratory) typically exist for long periods of time in oligotrophic environments, with only sporadic infusions of nutrients that return them to exponential growth for very brief periods of time.

During the stationary phase cells are also prone to producing **secondary metabolites**, or metabolites produced after active growth, such as antibiotics. Cells that are capable of making an endospore will activate the necessary genes during this stage, in order to initiate the sporulation process.

Death or Decline phase

In the last phase of the growth curve, the **death or decline phase**, the number of viable cells decreases in a predictable (or exponential) fashion. The steepness of the slope corresponds to how fast cells are losing viability. It is thought that the culture conditions have deteriorated to a point where the cells are irreparably harmed, since cells collected from this phase fail to show growth when transferred to fresh medium. It is important to note that if the turbidity of a culture is being measured as a way to determine cell density, measurements might not decrease during this phase, since cells could still be intact.

It has been suggested that the cells thought to be dead might be revived under specific conditions, a condition described as **viable but nonculturable (VBNC)**. This state might be of importance for pathogens, where they enter a state of very low metabolism and lack of cellular division, only to resume growth at a later time, when conditions improve.

It has also been shown that 100% cell death is unlikely, for any cell population, as the cells mutate to adapt to their environmental conditions, however harsh. Often there is a tailing effect observed, where a small population of the cells cannot be killed off. In addition, these cells might benefit from their death of their fellow cells, which provide nutrients to the environment as they lyse and release their cellular contents.

Key Words

binary fission, multiple fission, budding, spores, cell cycle, closed system, batch culture, growth curve, lag phase, exponential or log phase, generation time (*g*), N, N0, *n*, *t*, stationary phase, DNA-binding proteins from starved cells (DPS), oligotrophic, secondary metabolites, death or decline phase, viable but nonculturable (VBNC).

Essential Questions/Objectives

1. How is growth measured in microbial populations?
2. How do eukaryotes and bacteria/archaea differ in their reproductive methods?
3. What are the steps of binary fission? What is happening at each step?
4. Know what the growth curve of an organism grown in a closed system looks like. Know the various stages and what is occurring at each stage, physiologically. What can influence lag phase? What are the 2 differing explanations for cell loss in the death or senescence phase?

5. Understand generation time and how can it be determined on a log number of cells vs. time graph. Know the advantage of plotting the log number of cells vs. time instead of the number of cells vs. time. What factors affect the generation time of an organism?
6. Practice problem: Six *Staphylococcus aureus* are inoculated into a cream pie by the hands of a pastry chef. The generation time of *S. aureus* in cream pie at room temperature is 30 minutes. a) How many *S. aureus* are in the pie after 4 hours at RT? b) After 24 hours?

Exploratory Questions (OPTIONAL)

1. In what situation would the VBNC occurrence benefit cells? How could this pose a public health threat?

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8.5.1: Generation Time

Briefly describe the process of binary fission in bacteria, stating the functions of Par proteins, the divisome, and FtsZ proteins.

- Define the following:

a. generation time

b. geometric progression

- Draw a generalized bacterial growth curve, label the phases, and briefly describe what is happening during each phase.

Bacterial Growth

Bacteria replicate by binary fission, a process by which one bacterium splits into two. Therefore, bacteria increase their numbers by geometric progression whereby their population doubles every generation time.Generation time is the time it takes for a population of bacteria to double in number. For many common bacteria, the generation time is quite short, 20-60 minutes under optimum conditions. For most common pathogens in the body, the generation time is probably closer to 5-10 hours. Because bacteria grow by geometric progression and most have a short generation time, they can astronomically increase their number in a short period of time.

The relationship between the number of bacteria in a population at a given time (N_t), the original number of bacterial cells in the population (N_o), and the number of divisions those bacteria have undergone during that time (n) can be expressed by the following equation:

$$N_t = N_o \times 2^n \tag{8.5.1.1}$$

For example, *Escherichia coli*, under optimum conditions, has a generation time of 20 minutes. If one started with only 10 *E. coli* (N_o = 10) and allowed them to grow for 12 hours (n = 36; with a generation time of 20 minutes they would divide 3 times in one hour and 36 times in 12 hours), then plugging the numbers in the formula, the number of bacteria after 12 hours (N_t) would be

$$10 \times 2^{36} = N_t = 687,194,767,360\ E.\ coli \tag{8.5.1.2}$$

In general it is thought that during DNA replication (discussed in Unit 6), each strand of the replicating bacterial DNA attaches to proteins at what will become the cell division plane. For example, Par proteins function to separate bacterial chromosomes to opposite poles of the cell during cell division. They bind to the origin of replication of the DNA and physically pull or push the chromosomes apart, similar to the mitotic apparatus of eukaryotic cells.

In the center of the bacterium, a group of proteins called Fts (filamentous temperature sensitive) proteins interact to form a ring at the cell division plane. These proteins form the cell division apparatus known as the divisome and are directly involved in bacterial cell division by binary fission (see Figure 8.5.1.1 and Figure 8.5.1.2).

- electron micrograph of a divisome: see under Bacterial Cell Division, Jon Beckwith's Lab.

The divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum. The function of a number of divisome proteins have been identified, including:

- MinE: Directs formation of the FtsZ ring and divisome complex at the bacterium's division plane.
- FtsZ: Similar to tubulin in eukaryotic cells, FtsZ forms a constricting ring at the division site. As FtsZ depolymerizes, it directs an inward growth of the cell wall to form the division septum. It is found in both *Bacteria* and *Archaea*, as well as in mitochondria and chloroplasts.
- ZipA: A protein that connects the FtsZ ring to the bacterial cytoplasmic membrane.
- FtsA: An ATPase that breaks down ATP to provide energy for cell division and also helps connect the FtsZ ring to the bacterial cytoplasmic membrane.
- FtsK: Helps in separating the replicated bacterial chromosome.

- FtsI: Needed for peptidoglycan synthesis.

YouTube movie of binary fission in bacteria, #1.

YouTube movie of binary fission in bacteria, #2.

YouTube movie of fluorescing imaging of binary fission in bacteria.

- Scanning electron micrograph of dividing *Escherichia coli*; courtesy of CDC.

- Scanning electron micrograph of dividing *Salmonella typhimurium*; courtesy of CDC.

- To view an transmission electron micrograph of dividing streptococci, see the Rockefeller University home page.

The Population Growth Curve

Although bacteria are capable of replicating geometrically as a result of binary fission, in reality this only occurs as long as their is space to grow, sufficient nutrients, and a way to dispose of waste products. Because these factors limit the ability to replicate geometrically, over time in a closed growth system a bacterial population usually exhibits a predictable pattern of growth - its growth curve - that follows several stages or phases:

1. The lag phase

During the lag phase growth is relatively flat and the population appears either not to be growing or growing quite slowly (see Figure 8.5.1.3). During this phase the newly inoculated cells are adapting to their new environment and synthesizing the molecules they will need in order to grow rapidly.

2. The exponential growth phase (also called the logarithmic or log phase)

This is the phase where the population increases geometrically as long as there is sufficient food and space for growth (see Figure 8.5.1.3).

3. The stationary growth phase

Here the population grows slowly or stops growing (see Figure 8.5.1.3) because of decreasing food, increasing waste, and lack of space. The rate of replication is balanced out by the rate of inhibition or death.

4. The decline or death phase

Here the population dies exponentially from the accumulation of waste products (see Figure 8.5.1.3), although the rate of death depends on the degree of toxicity and the resistance of the species and viable cells may remain for weeks to months.

For more information: Lab 4, Enumeration of Microorganisms

Summary

1. Bacteria replicate by binary fission, a process by which one bacterium splits into two.
2. Generation time is the time it takes for a population of bacteria to double in number. For many bacteria the generation time ranges from minutes to hours.
3. Because of binary fission, bacteria increase their numbers by geometric progression whereby their population doubles every generation time.
4. Par proteins function to separate bacterial chromosomes to opposite poles of the cell during bacterial cell division.

5. The bacterial divisome is responsible for directing the synthesis of new cytoplasmic membrane and new peptidoglycan to form the division septum.
6. Although bacteria are capable of replicating geometrically as a result of binary fission, this only occurs as long as their is space to grow, sufficient nutrients, and a way to dispose of waste products.
7. In a closed growth system, a bacterial population usually exhibits a predictable pattern of growth - its growth curve - that follows several stages or phases.
8. During the lag phase growth is relatively flat and the population appears either not to be growing or growing quite slowly as newly inoculated cells are adapt to their new environment.
9. During the exponential growth phase (log phase) the population increases geometrically as long as there is sufficient food and space for growth.
10. During the stationary phase the population grows slowly or stops growing because of decreasing food, increasing waste, and lack of space.
11. During the death (decline) phase the population dies exponentially from the accumulation of waste products.

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CHAPTER OVERVIEW

9: Microbial Genetics

9.1: Genome, DNA, and Genes

9.1.1: The Structure and Function of Cellular Genomes

9.1.2: Plasmids

9.1.2A: Introduction to Plasmids

9.1.2B: Types of Plasmids and Their Biological Significance

9.1.3: Structure and Function of DNA

9.1.4: Structure and Function of RNA

9.1.5: What Are Genes?

9.1.E: Biochemistry of the Genome (Exercises)

9.2: DNA Replication

9.3: RNA Transcription

9.3A: mRNA Processing

9.4: Protein Synthesis (Translation)

9.5: Mutations

9.5A: DNA Repair

9.6: Gene Regulation - Operon Theory

9.7: How Asexual Prokaryotes Achieve Genetic Diversity

9.E: Mechanisms of Microbial Genetics (Exercises)

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9.1: Genome, DNA, and Genes

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9.1.1: The Structure and Function of Cellular Genomes

Learning Objectives

- Define gene and genotype and differentiate genotype from phenotype
- Describe chromosome structure and packaging
- Compare prokaryotic and eukaryotic chromosomes
- Explain why extrachromosomal DNA is important in a cell

Thus far, we have discussed the structure and function of individual pieces of DNA and RNA. In this section, we will discuss how all of an organism's genetic material—collectively referred to as its genome—is organized inside of the cell. Since an organism's genetics to a large extent dictate its characteristics, it should not be surprising that organisms differ in the arrangement of their DNA and RNA.

Genotype versus Phenotype

All cellular activities are encoded within a cell's DNA. The sequence of bases within a DNA molecule represents the genetic information of the cell. Segments of DNA molecules are called genes, and individual genes contain the instructional code necessary for synthesizing various proteins, enzymes, or stable RNA molecules.

The full collection of genes that a cell contains within its genome is called its genotype. However, a cell does not express all of its genes simultaneously. Instead, it turns on (expresses) or turns off certain genes when necessary. The set of genes being expressed at any given point in time determines the cell's activities and its observable characteristics, referred to as its phenotype. Genes that are always expressed are known as constitutive genes; some constitutive genes are known as housekeeping genes because they are necessary for the basic functions of the cell.

While the genotype of a cell remains constant, the phenotype may change in response to environmental signals (e.g., changes in temperature or nutrient availability) that affect which nonconstitutive genes are expressed. For example, the oral bacterium *Streptococcus mutans* produces a sticky slime layer that allows it to adhere to teeth, forming dental plaque; however, the genes that control the production of the slime layer are only expressed in the presence of sucrose (table sugar). Thus, while the genotype of *S. mutans* is constant, its phenotype changes depending on the presence and absence of sugar in its environment. Temperature can also regulate gene expression. For example, the gram-negative bacterium *Serratia marcescens*, a pathogen frequently associated with hospital-acquired infections, produces a red pigment at 28 °C but not at 37 °C, the normal internal temperature of the human body (Figure **9.1.1.1**).

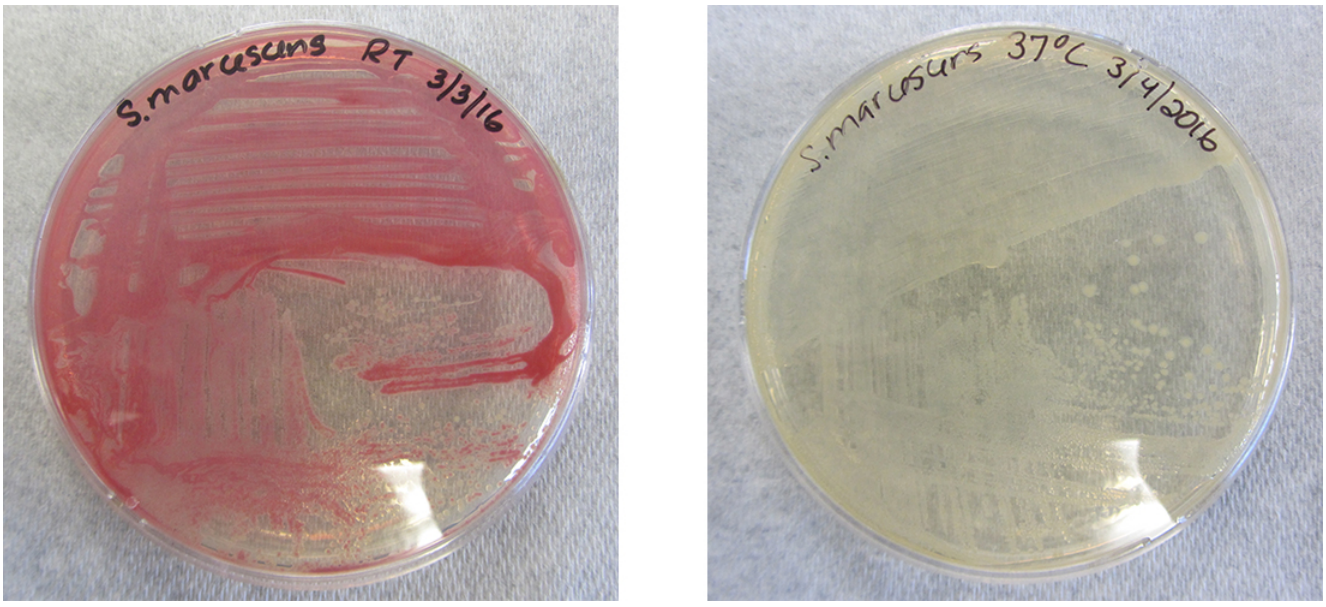


Figure **9.1.1.1**: Both plates contain strains of *Serratia marcescens* that have the gene for red pigment. However, this gene is expressed at 28 °C (left) but not at 37 °C (right). (credit: modification of work by Ann Auman)

Organization of Genetic Material

The vast majority of an organism's genome is organized into the cell's chromosomes, which are discrete DNA structures within cells that control cellular activity. Recall that while eukaryotic chromosomes are housed in the membrane-bound nucleus, most prokaryotes contain a single, circular chromosome that is found in an area of the cytoplasm called the nucleoid (see Unique Characteristics of Prokaryotic Cells). A chromosome may contain several thousand genes.

Organization of Eukaryotic Chromosome

Chromosome structure differs somewhat between eukaryotic and prokaryotic cells. Eukaryotic chromosomes are typically linear, and eukaryotic cells contain multiple distinct chromosomes. Many eukaryotic cells contain two copies of each chromosome and, therefore, are diploid.

The length of a chromosome greatly exceeds the length of the cell, so a chromosome needs to be packaged into a very small space to fit within the cell. For example, the combined length of all of the 3 billion base pairs[1] of DNA of the human genome would measure approximately 2 meters if completely stretched out, and some eukaryotic genomes are many times larger than the human genome. DNA supercoiling refers to the process by which DNA is twisted to fit inside the cell. Supercoiling may result in DNA that is either underwound (less than one turn of the helix per 10 base pairs) or overwound (more than one turn per 10 base pairs) from its normal relaxed state. Proteins known to be involved in supercoiling include topoisomerases; these enzymes help maintain the structure of supercoiled chromosomes, preventing overwinding of DNA during certain cellular processes like DNA replication.

During DNA packaging, DNA-binding proteins called histones perform various levels of DNA wrapping and attachment to scaffolding proteins. The combination of DNA with these attached proteins is referred to as chromatin. In eukaryotes, the packaging of DNA by histones may be influenced by environmental factors that affect the presence of methyl groups on certain cytosine nucleotides of DNA. The influence of environmental factors on DNA packaging is called epigenetics. Epigenetics is another mechanism for regulating gene expression without altering the sequence of nucleotides. Epigenetic changes can be maintained through multiple rounds of cell division and, therefore, can be heritable.

Link to Learning

View this animation from the DNA Learning Center to learn more about on DNA packaging in eukaryotes.

Organization of Prokaryotic Chromosomes

Chromosomes in bacteria and archaea are usually circular, and a prokaryotic cell typically contains only a single chromosome within the nucleoid. Because the chromosome contains only one copy of each gene, prokaryotes are haploid. As in eukaryotic cells, DNA supercoiling is necessary for the genome to fit within the prokaryotic cell. The DNA in the bacterial chromosome is arranged in several supercoiled domains. As with eukaryotes, topoisomerases are involved in supercoiling DNA. DNA gyrase is a type of topoisomerase, found in bacteria and some archaea, that helps prevent the overwinding of DNA. (Some antibiotics kill bacteria by targeting DNA gyrase.) In addition, histone-like proteins bind DNA and aid in DNA packaging. Other proteins bind to the origin of replication, the location in the chromosome where DNA replication initiates. Because different regions of DNA are packaged differently, some regions of chromosomal DNA are more accessible to enzymes and thus may be used more readily as templates for gene expression. Interestingly, several bacteria, including *Helicobacter pylori* and *Shigella flexneri*, have been shown to induce epigenetic changes in their hosts upon infection, leading to chromatin remodeling that may cause long-term effects on host immunity.[2]

? Exercise 9.1.1.1

1. What is the difference between a cell's genotype and its phenotype?
2. How does DNA fit inside cells?

Noncoding DNA

In addition to genes, a genome also contains many regions of noncoding DNA that do not encode proteins or stable RNA products. Noncoding DNA is commonly found in areas prior to the start of coding sequences of genes as well as in intergenic regions (i.e., DNA sequences located between genes) (Figure **9.1.1.2**).

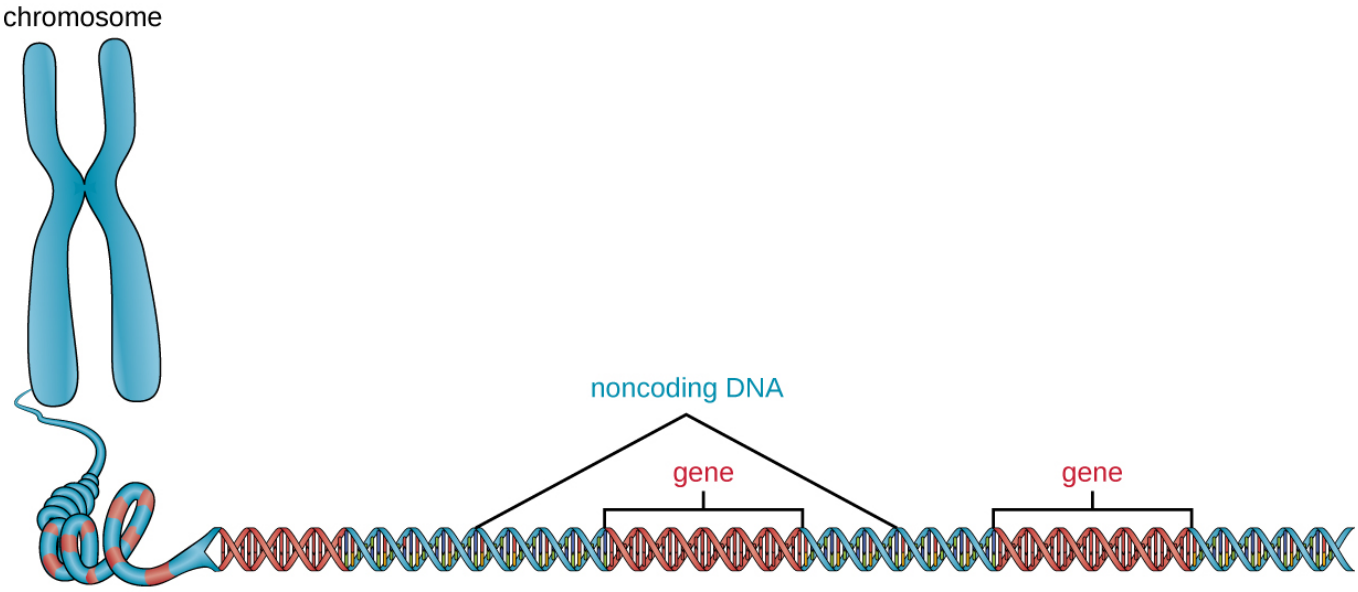


Figure **9.1.1.2**: Chromosomes typically have a significant amount of noncoding DNA, often found in intergenic regions.

Prokaryotes appear to use their genomes very efficiently, with only an average of 12% of the genome being taken up by noncoding sequences. In contrast, noncoding DNA can represent about 98% of the genome in eukaryotes, as seen in humans, but the percentage of noncoding DNA varies between species.[3] These noncoding DNA regions were once referred to as "junk DNA"; however, this terminology is no longer widely accepted because scientists have since found roles for some of these regions, many of which contribute to the regulation of transcription or translation through the production of small noncoding RNA molecules, DNA packaging, and chromosomal stability. Although scientists may not fully understand the roles of all noncoding regions of DNA, it is generally believed that they do have purposes within the cell.

Exercise 9.1.1.2

What is the role of noncoding DNA?

Extrachromosomal DNA

Although most DNA is contained within a cell's chromosomes, many cells have additional molecules of DNA outside the chromosomes, called extrachromosomal DNA, that are also part of its genome. The genomes of eukaryotic cells would also include the chromosomes from any organelles such as mitochondria and/or chloroplasts that these cells maintain (Figure **9.1.1.3**). The maintenance of circular chromosomes in these organelles is a vestige of their prokaryotic origins and supports the endosymbiotic theory (see Foundations of Modern Cell Theory). In some cases, genomes of certain DNA viruses can also be maintained independently in host cells during latent viral infection. In these cases, these viruses are another form of extrachromosomal DNA. For example, the human papillomavirus (HPV) may be maintained in infected cells in this way.

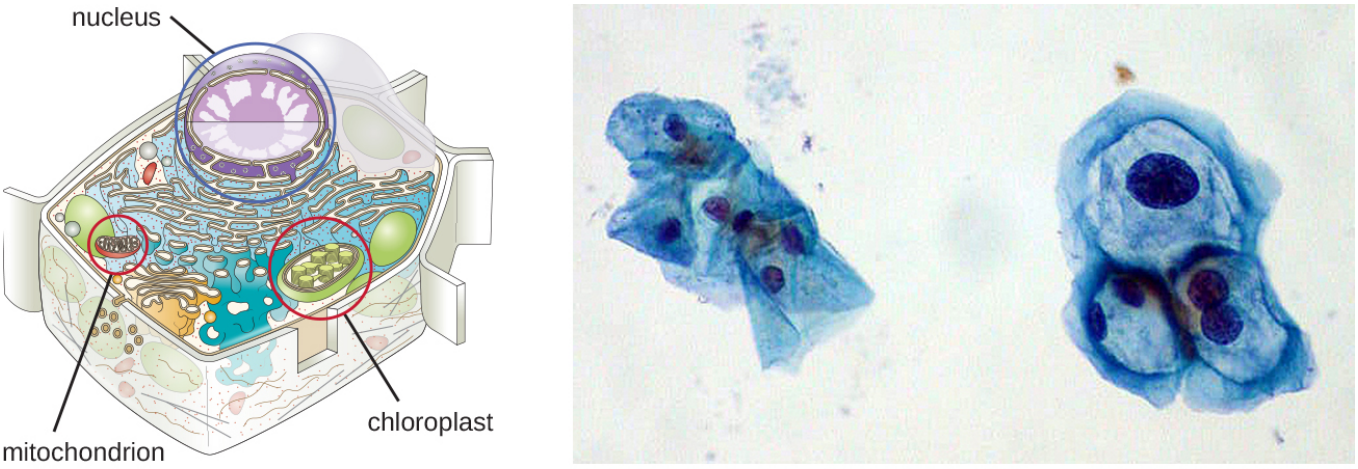


Figure **9.1.1.3**: The genome of a eukaryotic cell consists of the chromosome housed in the nucleus, and extrachromosomal DNA found in the mitochondria (all cells) and chloroplasts (plants and algae).

Besides chromosomes, some prokaryotes also have smaller loops of DNA called plasmids that may contain one or a few genes not essential for normal growth (Figure 3.3.1). Bacteria can exchange these plasmids with other bacteria in a process known as horizontal gene transfer (HGT). The exchange of genetic material on plasmids sometimes provides microbes with new genes beneficial for growth and survival under special conditions. In some cases, genes obtained from plasmids may have clinical implications, encoding virulence factors that give a microbe the ability to cause disease or make a microbe resistant to certain antibiotics. Plasmids are also used heavily in genetic engineering and biotechnology as a way to move genes from one cell to another. The role of plasmids in horizontal gene transfer and biotechnology will be discussed further in Mechanisms of Microbial Genetics and Modern Applications of Microbial Genetics.

Exercise 9.1.1.3

How are plasmids involved in antibiotic resistance?

Lethal Plasmids

Maria, a 20-year-old anthropology student from Texas, recently became ill in the African nation of Botswana, where she was conducting research as part of a study-abroad program. Maria's research was focused on traditional African methods of tanning hides for the production of leather. Over a period of three weeks, she visited a tannery daily for several hours to observe and participate in the tanning process. One day, after returning from the tannery, Maria developed a fever, chills, and a headache, along with chest pain, muscle aches, nausea, and other flu-like symptoms. Initially, she was not concerned, but when her fever spiked and she began to cough up blood, her African host family became alarmed and rushed her to the hospital, where her condition continued to worsen.

After learning about her recent work at the tannery, the physician suspected that Maria had been exposed to anthrax. He ordered a chest X-ray, a blood sample, and a spinal tap, and immediately started her on a course of intravenous penicillin. Unfortunately, lab tests confirmed the physician's presumptive diagnosis. Maria's chest X-ray exhibited pleural effusion, the accumulation of fluid in the space between the pleural membranes, and a Gram stain of her blood revealed the presence of gram-positive, rod-shaped bacteria in short chains, consistent with *Bacillus anthracis*. Blood and bacteria were also shown to be present in her cerebrospinal fluid, indicating that the infection had progressed to meningitis. Despite supportive treatment and aggressive antibiotic therapy, Maria slipped into an unresponsive state and died three days later.

Anthrax is a disease caused by the introduction of endospores from the gram-positive bacterium *B. anthracis* into the body. Once infected, patients typically develop meningitis, often with fatal results. In Maria's case, she inhaled the endospores while handling the hides of animals that had been infected.

The genome of *B. anthracis* illustrates how small structural differences can lead to major differences in virulence. In 2003, the genomes of *B. anthracis* and *Bacillus cereus*, a similar but less pathogenic bacterium of the same genus, were sequenced and compared.[4] Researchers discovered that the 16S rRNA gene sequences of these bacteria are more than 99% identical, meaning that they are actually members of the same species despite their traditional classification as separate species. Although their chromosomal sequences also revealed a great deal of similarity, several virulence factors of *B. anthracis* were found to be encoded on two large plasmids not found in *B. cereus*. The plasmid pX01 encodes a three-part toxin that suppresses the host immune system, whereas the plasmid pX02 encodes a capsular polysaccharide that further protects the bacterium from the host immune system (Figure **9.1.1.4**). Since *B. cereus* lacks these plasmids, it does not produce these virulence factors, and although it is still pathogenic, it is typically associated with mild cases of diarrhea from which the body can quickly recover. Unfortunately for Maria, the presence of these toxin-encoding plasmids in *B. anthracis* gives it its lethal virulence.

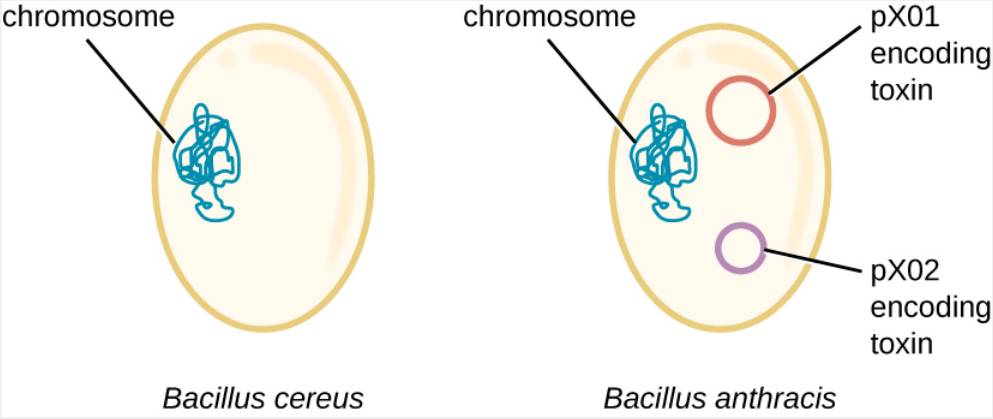


Figure **9.1.1.4**: Genome sequencing of Bacillus anthracis and its close relative *B. cereus* reveals that the pathogenicity of *B. anthracis* is due to the maintenance of two plasmids, pX01 and pX02, which encode virulence factors.

Exercise 9.1.1.4

What do you think would happen to the pathogenicity of *B. anthracis* if it lost one or both of its plasmids?

Clinical Focus: Resolution

Within 24 hours, the results of the diagnostic test analysis of Alex's stool sample revealed that it was positive for heat-labile enterotoxin (LT), heat-stabile enterotoxin (ST), and colonization factor (CF), confirming the hospital physician's suspicion of ETEC. During a follow-up with Alex's family physician, this physician noted that Alex's symptoms were not resolving quickly and he was experiencing discomfort that was preventing him from returning to classes. The family physician prescribed Alex a course of ciprofloxacin to resolve his symptoms. Fortunately, the ciprofloxacin resolved Alex's symptoms within a few days.

Alex likely got his infection from ingesting contaminated food or water. Emerging industrialized countries like Mexico are still developing sanitation practices that prevent the contamination of water with fecal material. Travelers in such countries should avoid the ingestion of undercooked foods, especially meats, seafood, vegetables, and unpasteurized dairy products. They

should also avoid use of water that has not been treated; this includes drinking water, ice cubes, and even water used for brushing teeth. Using bottled water for these purposes is a good alternative. Good hygiene (handwashing) can also aid the prevention of an ETEC infection. Alex had not been careful about his food or water consumption, which led to his illness.

Alex's symptoms were very similar to those of cholera, caused by the gram-negative bacterium *Vibrio cholerae*, which also produces a toxin similar to ST and LT. At some point in the evolutionary history of ETEC, a nonpathogenic strain of *E. coli* similar to those typically found in the gut may have acquired the genes encoding the ST and LT toxins from *V. cholerae*. The fact that the genes encoding those toxins are encoded on extrachromosomal plasmids in ETEC supports the idea that these genes were acquired by *E. coli* and are likely maintained in bacterial populations through horizontal gene transfer.

Viral Genomes

Viral genomes exhibit significant diversity in structure. Some viruses have genomes that consist of DNA as their genetic material. This DNA may be single stranded, as exemplified by human parvoviruses, or double stranded, as seen in the herpesviruses and poxviruses. Additionally, although all cellular life uses DNA as its genetic material, some viral genomes are made of either single-stranded or double-stranded RNA molecules, as we have discussed. Viral genomes are typically smaller than most bacterial genomes, encoding only a few genes, because they rely on their hosts to carry out many of the functions required for their replication. The diversity of viral genome structures and their implications for viral replication life cycles are discussed in more detail in The Viral Life Cycle.

? Exercise 9.1.1.5

Why do viral genomes vary widely among viruses?

Genome Size Matters

There is great variation in size of genomes among different organisms. Most eukaryotes maintain multiple chromosomes; humans, for example have 23 pairs, giving them 46 chromosomes. Despite being large at 3 billion base pairs, the human genome is far from the largest genome. Plants often maintain very large genomes, up to 150 billion base pairs, and commonly are polyploid, having multiple copies of each chromosome.

The size of bacterial genomes also varies considerably, although they tend to be smaller than eukaryotic genomes (Figure **9.1.1.5**). Some bacterial genomes may be as small as only 112,000 base pairs. Often, the size of a bacterium's genome directly relates to how much the bacterium depends on its host for survival. When a bacterium relies on the host cell to carry out certain functions, it loses the genes encoding the abilities to carry out those functions itself. These types of bacterial endosymbionts are reminiscent of the prokaryotic origins of mitochondria and chloroplasts.

From a clinical perspective, obligate intracellular pathogens also tend to have small genomes (some around 1 million base pairs). Because host cells supply most of their nutrients, they tend to have a reduced number of genes encoding metabolic functions. Due to their small sizes, the genomes of organisms like *Mycoplasma genitalium* (580,000 base pairs), *Chlamydia trachomatis* (1.0 million), *Rickettsia prowazekii* (1.1 million), and *Treponema pallidum* (1.1 million) were some of the earlier bacterial genomes sequenced. Respectively, these pathogens cause urethritis and pelvic inflammation, chlamydia, typhus, and syphilis.

Whereas obligate intracellular pathogens have unusually small genomes, other bacteria with a great variety of metabolic and enzymatic capabilities have unusually large bacterial genomes. *Pseudomonas aeruginosa*, for example, is a bacterium commonly found in the environment and is able to grow on a wide range of substrates. Its genome contains 6.3 million base pairs, giving it a high metabolic ability and the ability to produce virulence factors that cause several types of opportunistic infections.

Interestingly, there has been significant variability in genome size in viruses as well, ranging from 3,500 base pairs to 2.5 million base pairs, significantly exceeding the size of many bacterial genomes. The great variation observed in viral genome sizes further contributes to the great diversity of viral genome characteristics already discussed.

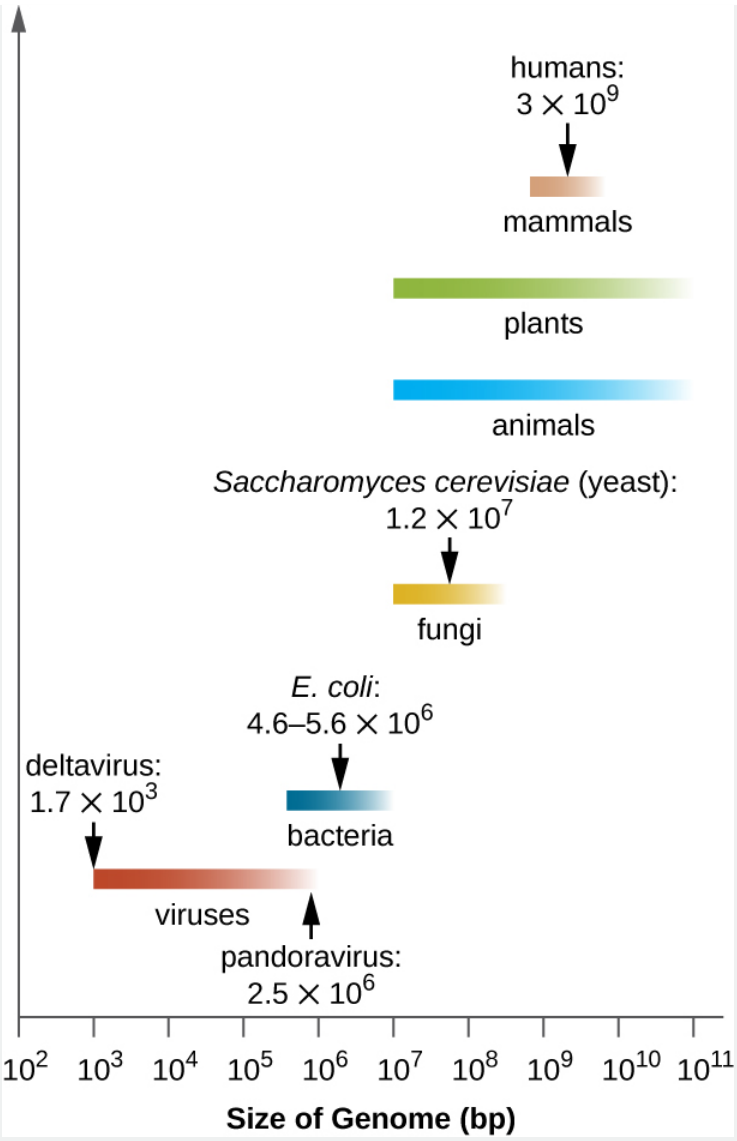


Figure **9.1.1.5**: There is great variability as well as overlap among the genome sizes of various groups of organisms and viruses.

Link to Learning

Visit the genome database of the National Center for Biotechnology Information (NCBI) to see the genomes that have been sequenced and their sizes.

Key Concepts and Summary

- The entire genetic content of a cell is its **genome**.
- **Genes** code for proteins, or stable RNA molecules, each of which carries out a specific function in the cell.
- Although the **genotype** that a cell possesses remains constant, expression of genes is dependent on environmental conditions.
- A **phenotype** is the observable characteristics of a cell (or organism) at a given point in time and results from the complement of genes currently being used.
- The majority of genetic material is organized into **chromosomes** that contain the DNA that controls cellular activities.
- Prokaryotes are typically haploid, usually having a single circular chromosome found in the nucleoid. Eukaryotes are diploid; DNA is organized into multiple linear chromosomes found in the nucleus.
- Supercoiling and DNA packaging using DNA binding proteins allows lengthy molecules to fit inside a cell. Eukaryotes and archaea use histone proteins, and bacteria use different proteins with similar function.

- Prokaryotic and eukaryotic genomes both contain **noncoding DNA**, the function of which is not well understood. Some noncoding DNA appears to participate in the formation of small noncoding RNA molecules that influence gene expression; some appears to play a role in maintaining chromosomal structure and in DNA packaging.
- **Extrachromosomal DNA** in eukaryotes includes the chromosomes found within organelles of prokaryotic origin (mitochondria and chloroplasts) that evolved by endosymbiosis. Some viruses may also maintain themselves extrachromosomally.
- Extrachromosomal DNA in prokaryotes is commonly maintained as **plasmids** that encode a few nonessential genes that may be helpful under specific conditions. Plasmids can be spread through a bacterial community by horizontal gene transfer.
- Viral genomes show extensive variation and may be composed of either RNA or DNA, and may be either double or single stranded.

Footnotes

1. 1 National Human Genome Research Institute. “The Human Genome Project Completion: Frequently Asked Questions.” https://www.genome.gov/11006943. Accessed June 10, 2016
2. 2 H. Bierne et al. “Epigenetics and Bacterial Infections.” *Cold Spring Harbor Perspectives in Medicine* 2 no. 12 (2012):a010272.
3. 3 R.J. Taft et al. “The Relationship between Non-Protein-Coding DNA and Eukaryotic Complexity.” *Bioessays* 29 no. 3 (2007):288–299.
4. 4 N. Ivanova et al. “Genome Sequence of *Bacillus cereus* and Comparative Analysis with *Bacillus anthracis.*” *Nature* 423 no. 6935 (2003):87–91.

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SECTION OVERVIEW

9.1.2: Plasmids

Topic hierarchy

9.1.2A: Introduction to Plasmids

9.1.2B: Types of Plasmids and Their Biological Significance

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9.1.2A: Introduction to Plasmids

Learning Objectives

- Outline the utility of plasmids

The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952. A plasmid is a DNA molecule that is separate from, and can replicate independently of the chromosomal DNA. They are double-stranded and, in many cases, circular. Plasmids usually occur naturally in bacteria, but are sometimes found in archaea, and even in eukaryotic organisms (e.g., the 2-micrometre ring in *Saccharomyces cerevisiae*). Plasmid sizes vary from 1 to over 1,000 kbp. The number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances. Plasmids can be considered part of the mobilome because they are often associated with conjugation, a mechanism of horizontal gene transfer.

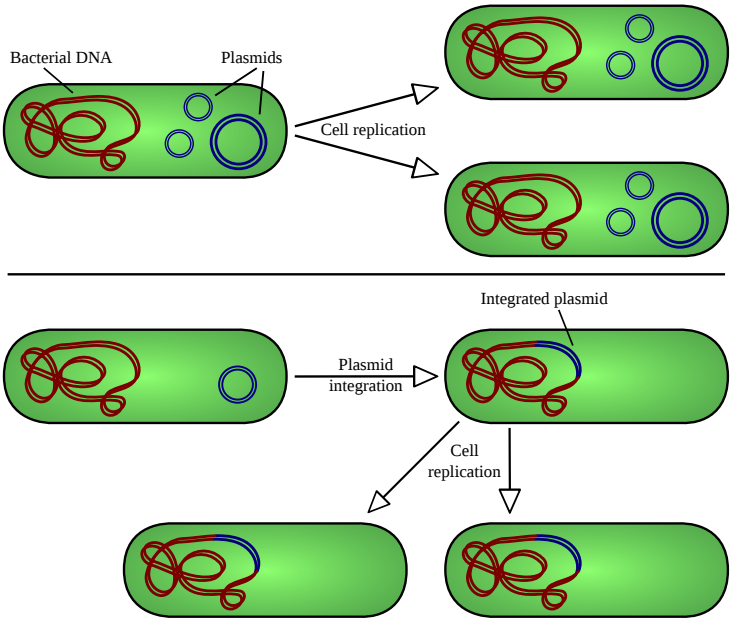


Figure: ***Step by step of cloning a gene using a plasmid****: This image shows a line drawing that compares the activity of non-integrating plasmids, on the top, with episomes, on the bottom, during cell division. The upper half of the image shows a bacterium with its chromosomal DNA and plasmids dividing into two identical bacteria, each with their chromosomal DNA and plasmids. The lower half of the image shows a bacterium with its chromosomal DNA, but with an episome. Next to this bacterium, we see the same bacterium, but after the episome has integrated into the chromosomal DNA and has become a part of it. This second bacterium now divides into two bacteria identical to it, each with an episome integrated into it.*

Plasmids are considered replicons. They can be found in all three major domains: Archaea, Bacteria, and Eukarya. Similar to viruses, plasmids are not considered by some to be a form of life. Unlike viruses, they are naked DNA and do not encode genes necessary to encase the genetic material for transfer to a new host, though some classes of plasmids encode the sex pilus necessary for their own transfer. Plasmid host-to-host transfer requires direct mechanical transfer by conjugation, or changes in incipient host gene expression allowing the intentional uptake of the genetic element by transformation. Microbial transformation with plasmid DNA is neither parasitic nor symbiotic in nature, because each implies the presence of an independent species living in a commensal or detrimental state with the host organism. Rather, plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or the proteins produced may act as toxins under similar circumstances. Plasmids can also provide bacteria with the ability to fix elemental nitrogen or to degrade recalcitrant organic compounds that provide an advantage when nutrients are scarce.

Key Points

- Plasmids can be found in all three major domains: Archaea, Bacteria, and Eukarya. Similar to viruses, plasmids are not considered by some to be a form of life.
- Plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state.
- Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or the proteins produced may act as toxins under similar circumstances.

Key Terms

- **plasmid**: A circle of double-stranded DNA that is separate from the chromosomes, which is found in bacteria and protozoa.
- **mobilome**: The entirety of the mobile (transposable) elements of a genome.
- **replicons**: a region of DNA or RNA, that replicates from a single origin of replication.

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9.1.2B: Types of Plasmids and Their Biological Significance

Plasmids are commonly used to multiply (make many copies of) or express particular genes.

LEARNING OBJECTIVES

Recognize the characteristics of, and thus the functions, of plasmids

Key Takeaways

Types of Plasmids

Plasmids used in genetic engineering are called vectors. Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to multiply (make many copies of) or express particular genes. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics. The gene is also inserted into a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments.

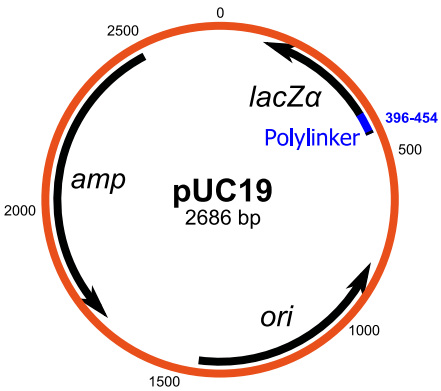


Figure: **A Plasmid Map of pUC19**: pUC19 is one of a series of plasmid cloning vectors created by Messing and co-workers in the University of California. The p in its name stands for plasmid and UC represents the University in which it was created. It is a circular double stranded DNA and has 2686 base pairs. pUC19 is one of the most widely used vector molecules as the recombinants, or the cells into which foreign DNA has been introduced, can be easily distinguished from the non-recombinants based on color differences of colonies on growth media. pUC18 is similar to pUC19, but the multiple cloning site region is reversed.

Next, the plasmids are inserted into bacteria by a process called transformation. Then, the bacteria are exposed to the particular antibiotics. Only bacteria that take up copies of the plasmid survive, since the plasmid makes them resistant. In particular, the protecting genes are expressed (used to make a protein) and the expressed protein breaks down the antibiotics. In this way, the antibiotics act as a filter, selecting only the modified bacteria. Finally, these bacteria can be grown in large amounts, harvested, and lysed (often using the alkaline lysis method) to isolate the plasmid of interest.

Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for; for example, insulin or even antibiotics.

One way of grouping plasmids is by their ability to transfer to other bacteria. Conjugative plasmids contain tra genes, which perform the complex process of conjugation, the transfer of plasmids to another bacterium. Non-conjugative plasmids are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids. An intermediate class of plasmids are mobilizable, and carry only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence. Plasmids are now being used to manipulate DNA, and may possibly be a tool for curing many diseases.

It is possible for plasmids of different types to coexist in a single cell. Several different plasmids have been found in E. coli. However, related plasmids are often incompatible, in the sense that only one of them survives in the cell line, due to the regulation

of vital plasmid functions. Thus, plasmids can be assigned into incompatibility groups.

Another way to classify plasmids is by function. There are five main classes:

- Fertility F-plasmids, which contain tra genes. They are capable of conjugation and result in the expression of sex pilli.
- Resistance plasmids, which contain genes that provide resistance against antibiotics or poisons. They were historically known as R-factors, before the nature of plasmids was understood.
- Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
- Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
- Virulence plasmids, which turn the bacterium into a pathogen.

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Key Points

- The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics, and into a multiple cloning site (MCS, or polylinker), allowing the easy insertion of DNA fragments.
- A major use of plasmids is to make large amounts of proteins. Bacterium can be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for; for example, insulin or even antibiotics.
- It is possible for plasmids of different types to coexist in a single cell. Several different plasmids have been found in E. coli. However, related plasmids are often incompatible, in the sense that only one of them survives in the cell line, due to the regulation of vital plasmid functions.

Key Terms

- **Col plasmids**: These plasmids contain genes that code for bacteriocins, proteins that can kill other bacteria.
- **F-plasmid**: Fertility F-plasmids contain tra genes and are capable of conjugation resulting in the expression of sex pilli.
- **Resistance plasmids**: These plasmids contain genes that provide resistance against antibiotics or poisons.

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9.1.3: Structure and Function of DNA

Learning Objectives

- Describe the biochemical structure of deoxyribonucleotides
- Identify the base pairs used in the synthesis of deoxyribonucleotides
- Explain why the double helix of DNA is described as antiparallel

In Microbial Metabolism, we discussed three classes of macromolecules: proteins, lipids, and carbohydrates. In this chapter, we will discuss a fourth class of macromolecules: nucleic acids. Like other macromolecules, nucleic acids are composed of monomers, called nucleotides, which are polymerized to form large strands. Each nucleic acid strand contains certain nucleotides that appear in a certain order within the strand, called its base sequence. The base sequence of deoxyribonucleic acid (DNA) is responsible for carrying and retaining the hereditary information in a cell. In Mechanisms of Microbial Genetics, we will discuss in detail the ways in which DNA uses its own base sequence to direct its own synthesis, as well as the synthesis of RNA and proteins, which, in turn, gives rise to products with diverse structure and function. In this section, we will discuss the basic structure and function of DNA.

DNA Nucleotides

The building blocks of nucleic acids are nucleotides. Nucleotides that compose DNA are called deoxyribonucleotides. The three components of a deoxyribonucleotide are a five-carbon sugar called deoxyribose, a phosphate group, and a nitrogenous base, a nitrogen-containing ring structure that is responsible for complementary base pairing between nucleic acid strands (Figure **9.1.3.1**). The carbon atoms of the five-carbon deoxyribose are numbered 1′, 2′, 3′, 4′, and 5′ (1′ is read as “one prime”). A nucleoside comprises the five-carbon sugar and nitrogenous base.

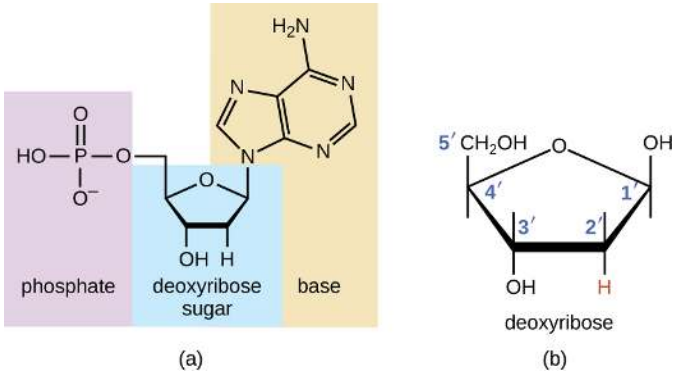


Figure **9.1.3.1**: (a) Each deoxyribonucleotide is made up of a sugar called deoxyribose, a phosphate group, and a nitrogenous base—in this case, adenine. (b) The five carbons within deoxyribose are designated as 1′, 2′, 3′, 4′, and 5′.

The deoxyribonucleotide is named according to the nitrogenous bases (Figure **9.1.3.2**). The nitrogenous bases adenine (A) and guanine (G) are the purines; they have a double-ring structure with a six-carbon ring fused to a five-carbon ring. The pyrimidines, cytosine (C) and thymine (T), are smaller nitrogenous bases that have only a six-carbon ring structure.

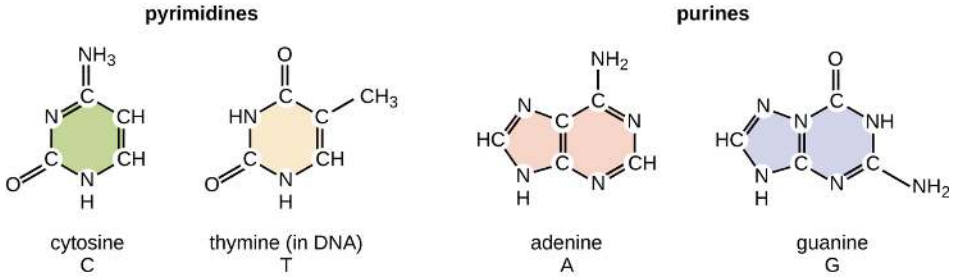


Figure **9.1.3.2**: Nitrogenous bases within DNA are categorized into the two-ringed purines adenine and guanine and the single-ringed pyrimidines cytosine and thymine. Thymine is unique to DNA.

Individual nucleoside triphosphates combine with each other by covalent bonds known as 5′-3′ phosphodiester bonds, or linkages whereby the phosphate group attached to the 5′ carbon of the sugar of one nucleotide bonds to the hydroxyl group of the 3′ carbon

of the sugar of the next nucleotide. Phosphodiester bonding between nucleotides forms the sugar-phosphate backbone, the alternating sugar-phosphate structure composing the framework of a nucleic acid strand (Figure **9.1.3.3**). During the polymerization process, deoxynucleotide triphosphates (dNTP) are used. To construct the sugar-phosphate backbone, the two terminal phosphates are released from the dNTP as a pyrophosphate. The resulting strand of nucleic acid has a free phosphate group at the 5′ carbon end and a free hydroxyl group at the 3′ carbon end. The two unused phosphate groups from the nucleotide triphosphate are released as pyrophosphate during phosphodiester bond formation. Pyrophosphate is subsequently hydrolyzed, releasing the energy used to drive nucleotide polymerization.

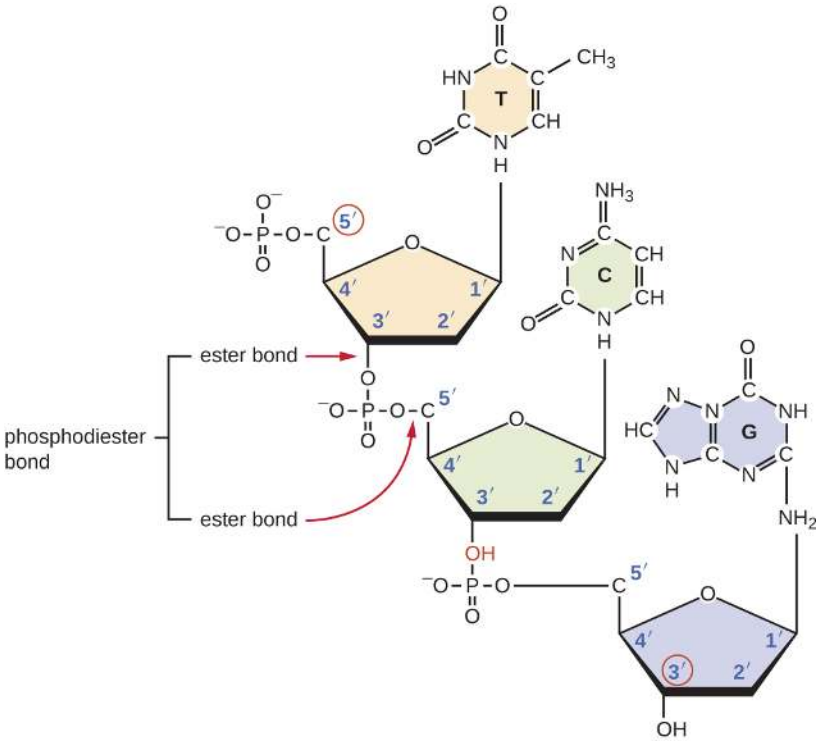


Figure **9.1.3.3**: Phosphodiester bonds form between the phosphate group attached to the 5′ carbon of one nucleotide and the hydroxyl group of the 3′ carbon in the next nucleotide, bringing about polymerization of nucleotides in to nucleic acid strands. Note the 5′ and 3′ ends of this nucleic acid strand.

? Exercise 9.1.3.1

What is meant by the 5′ and 3′ ends of a nucleic acid strand?

Discovering the Double Helix

By the early 1950s, considerable evidence had accumulated indicating that DNA was the genetic material of cells, and now the race was on to discover its three-dimensional structure. Around this time, Austrian biochemist Erwin Chargaff[1](1905–2002) examined the content of DNA in different species and discovered that adenine, thymine, guanine, and cytosine were not found in equal quantities, and that it varied from species to species, but not between individuals of the same species. He found that the amount of adenine was very close to equaling the amount of thymine, and the amount of cytosine was very close to equaling the amount of guanine, or A = T and G = C. These relationships are also known as Chargaff's rules.

Other scientists were also actively exploring this field during the mid-20th century. In 1952, American scientist Linus Pauling (1901–1994) was the world's leading structural chemist and odds-on favorite to solve the structure of DNA. Pauling had earlier discovered the structure of protein α helices, using X-ray diffraction, and, based upon X-ray diffraction images of DNA made in his laboratory, he proposed a triple-stranded model of DNA.[2] At the same time, British researchers Rosalind Franklin (1920–1958) and her graduate student R.G. Gosling were also using X-ray diffraction to understand the structure of DNA (Figure **9.1.3.4**). It was Franklin's scientific expertise that resulted in the production of more well-defined X-ray diffraction images of DNA that would clearly show the overall double-helix structure of DNA.

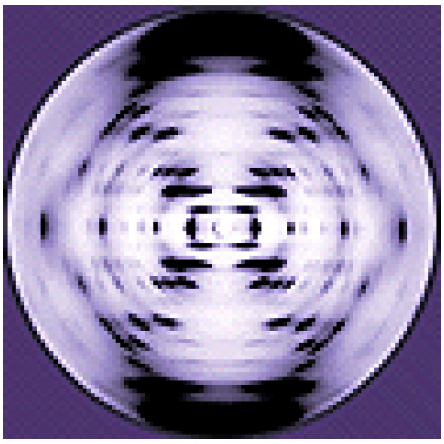


Figure **9.1.3.4**: The X-ray diffraction pattern of DNA shows its helical nature. (credit: National Institutes of Health)

James Watson (1928–), an American scientist, and Francis Crick (1916–2004), a British scientist, were working together in the 1950s to discover DNA's structure. They used Chargaff's rules and Franklin and Wilkins' X-ray diffractionimages of DNA fibers to piece together the purine-pyrimidine pairing of the double helical DNA molecule (Figure **9.1.3.5**). In April 1953, Watson and Crick published their model of the DNA double helix in *Nature*.[3] The same issue additionally included papers by Wilkins and colleagues,[4] as well as by Franklin and Gosling,[5] each describing different aspects of the molecular structure of DNA. In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Physiology and Medicine. Unfortunately, by then Franklin had died, and Nobel prizes at the time were not awarded posthumously. Work continued, however, on learning about the structure of DNA. In 1973, Alexander Rich(1924–2015) and colleagues were able to analyze DNA crystals to confirm and further elucidate DNA structure.[6]



Figure **9.1.3.5**: In 1953, James Watson and Francis Crick built this model of the structure of DNA, shown here on display at the Science Museum in London.

? Exercise 9.1.3.2

Which scientists are given most of the credit for describing the molecular structure of DNA?

DNA Structure

Watson and Crick proposed that DNA is made up of two strands that are twisted around each other to form a right-handed helix. The two DNA strands are antiparallel, such that the 3′ end of one strand faces the 5′ end of the other (Figure **9.1.3.6**). The 3′ end of each strand has a free hydroxyl group, while the 5′ end of each strand has a free phosphate group. The sugar and phosphate of the polymerized nucleotides form the backbone of the structure, whereas the nitrogenous bases are stacked inside. These nitrogenous bases on the interior of the molecule interact with each other, base pairing.

Analysis of the diffraction patterns of DNA has determined that there are approximately 10 bases per turn in DNA. The asymmetrical spacing of the sugar-phosphate backbones generates major grooves (where the backbone is far apart) and minor grooves (where the backbone is close together) (Figure **9.1.3.6**). These grooves are locations where proteins can bind to DNA. The binding of these proteins can alter the structure of DNA, regulate replication, or regulate transcription of DNA into RNA.

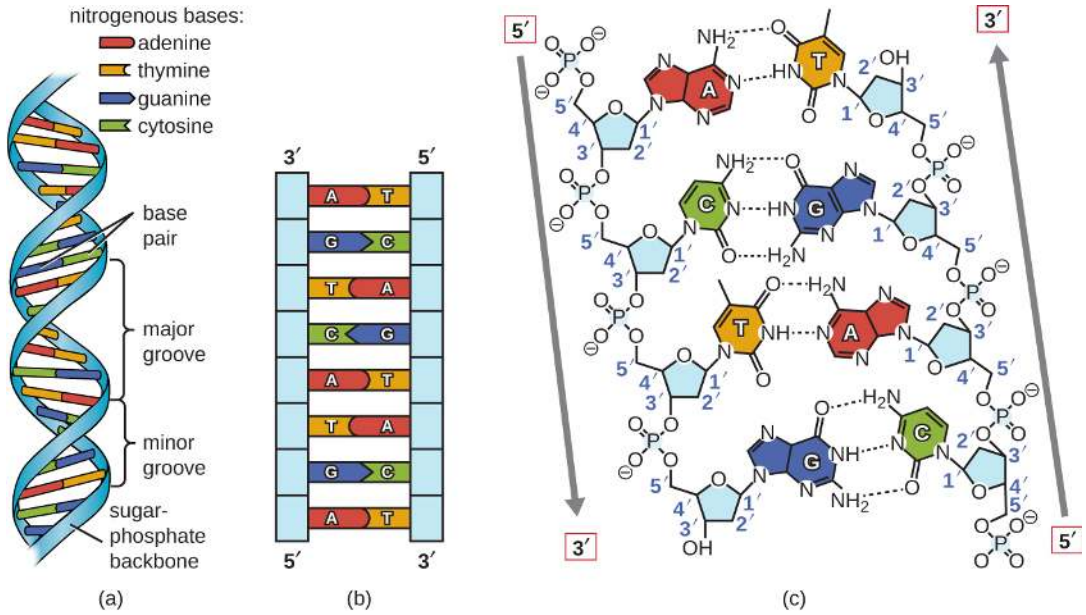


Figure **9.1.3.6**: Watson and Crick proposed the double helix model for DNA. (a) The sugar-phosphate backbones are on the outside of the double helix and purines and pyrimidines form the "rungs" of the DNA helix ladder. (b) The two DNA strands are antiparallel to each other. (c) The direction of each strand is identified by numbering the carbons (1 through 5) in each sugar molecule. The 5′ end is the one where carbon #5 is not bound to another nucleotide; the 3′ end is the one where carbon #3 is not bound to another nucleotide.

Base pairing takes place between a purine and pyrimidine. In DNA, adenine (A) and thymine (T) are complementary base pairs, and cytosine (C) and guanine (G) are also complementary base pairs, explaining Chargaff's rules (Figure **9.1.3.7**). The base pairs are stabilized by hydrogen bonds; adenine and thymine form two hydrogen bonds between them, whereas cytosine and guanine form three hydrogen bonds between them.

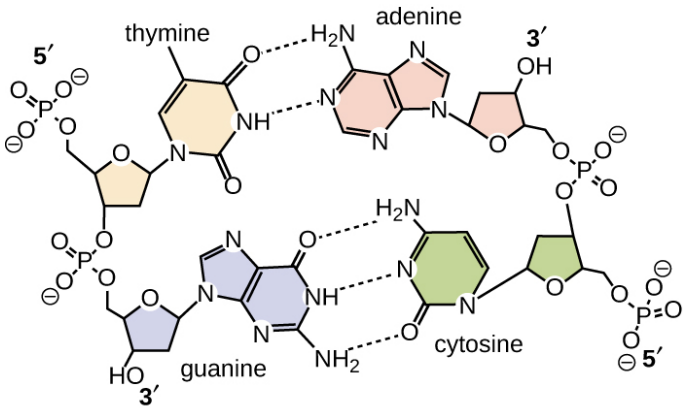


Figure **9.1.3.7**: Hydrogen bonds form between complementary nitrogenous bases on the interior of DNA.

In the laboratory, exposing the two DNA strands of the double helix to high temperatures or to certain chemicals can break the hydrogen bonds between complementary bases, thus separating the strands into two separate single strands of DNA (single-stranded DNA [ssDNA]). This process is called DNA denaturation and is analogous to protein denaturation, as described in Proteins. The ssDNA strands can also be put back together as double-stranded DNA (dsDNA), through reannealing or renaturing by cooling or removing the chemical denaturants, allowing these hydrogen bonds to reform. The ability to artificially manipulate DNA in this way is the basis for several important techniques in biotechnology (Figure **9.1.3.8**). Because of the additional hydrogen

bonding between the C = G base pair, DNA with a high GC content is more difficult to denature than DNA with a lower GC content.

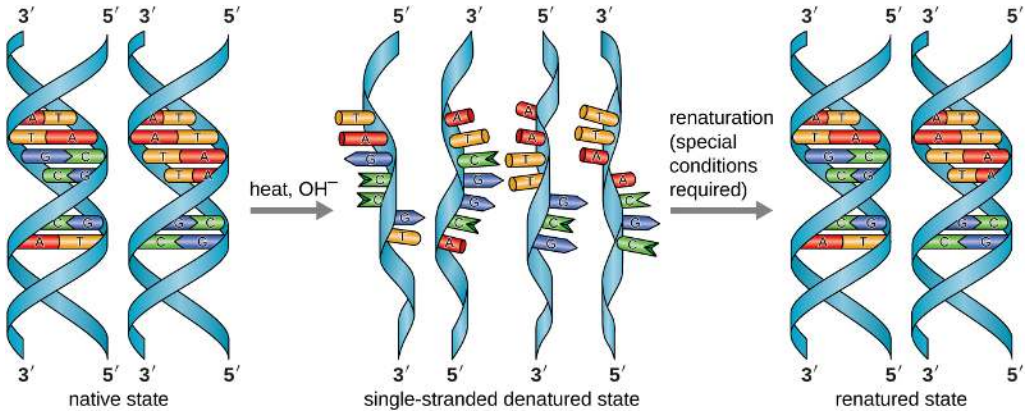


Figure **9.1.3.8**: In the laboratory, the double helix can be denatured to single-stranded DNA through exposure to heat or chemicals, and then renatured through cooling or removal of chemical denaturants to allow the DNA strands to reanneal. (credit: modification of work by Hernández-Lemus E, Nicasio-Collazo LA, Castañeda-Priego R)

Link to Learning

View an animation on DNA structure from the DNA Learning Center to learn more.

Exercise 9.1.3.3

What are the two complementary base pairs of DNA and how are they bonded together?

DNA Function

DNA stores the information needed to build and control the cell. The transmission of this information from mother to daughter cells is called vertical gene transfer and it occurs through the process of DNA replication. DNA is replicated when a cell makes a duplicate copy of its DNA, then the cell divides, resulting in the correct distribution of one DNA copy to each resulting cell. DNA can also be enzymatically degraded and used as a source of nucleosides and nucleotides for the cell. Unlike other macromolecules, DNA does not serve a structural role in cells.

Exercise 9.1.3.4

How does DNA transmit genetic information to offspring?

Paving the Way for Women in Science and Health Professions

Historically, women have been underrepresented in the sciences and in medicine, and often their pioneering contributions have gone relatively unnoticed. For example, although Rosalind Franklin performed the X-ray diffraction studies demonstrating the double helical structure of DNA, it is Watson and Crick who became famous for this discovery, building on her data. There still remains great controversy over whether their acquisition of her data was appropriate and whether personality conflicts and gender bias contributed to the delayed recognition of her significant contributions. Similarly, Barbara McClintock did pioneering work in maize (corn) genetics from the 1930s through 1950s, discovering transposons (jumping genes), but she was not recognized until much later, receiving a Nobel Prize in Physiology or Medicine in 1983 (Figure **9.1.3.9**).

Today, women still remain underrepresented in many fields of science and medicine. While more than half of the undergraduate degrees in science are awarded to women, only 46% of doctoral degrees in science are awarded to women. In academia, the number of women at each level of career advancement continues to decrease, with women holding less than one-third of the positions of Ph.D.-level scientists in tenure-track positions, and less than one-quarter of the full professorships at 4-year colleges and universities.[7] Even in the health professions, like nearly all other fields, women are often underrepresented in

many medical careers and earn significantly less than their male counterparts, as shown in a 2013 study published by the *Journal of the American Medical Association*.[8]

Why do such disparities continue to exist and how do we break these cycles? The situation is complex and likely results from the combination of various factors, including how society conditions the behaviors of girls from a young age and supports their interests, both professionally and personally. Some have suggested that women do not belong in the laboratory, including Nobel Prize winner Tim Hunt, whose 2015 public comments suggesting that women are too emotional for science[9] were met with widespread condemnation.

Perhaps girls should be supported more from a young age in the areas of science and math (Figure **9.1.3.9**). Science, technology, engineering, and mathematics (STEM) programs sponsored by the American Association of University Women (AAUW)[10] and National Aeronautics and Space Administration (NASA)[11] are excellent examples of programs that offer such support. Contributions by women in science should be made known more widely to the public, and marketing targeted to young girls should include more images of historically and professionally successful female scientists and medical professionals, encouraging all bright young minds, including girls and women, to pursue careers in science and medicine.



Figure **9.1.3.9**: (a) Barbara McClintock's work on maize genetics in the 1930s through 1950s resulted in the discovery of transposons, but its significance was not recognized at the time. (b) Efforts to appropriately mentor and to provide continued societal support for women in science and medicine may someday help alleviate some of the issues preventing gender equality at all levels in science and medicine. (credit a: modification of work by Smithsonian Institution; credit b: modification of work by Haynie SL, Hinkle AS, Jones NL, Martin CA, Olsiewski PJ, Roberts MF)

Clinical Focus: Part 2

Based upon his symptoms, Alex's physician suspects that he is suffering from a foodborne illness that he acquired during his travels. Possibilities include bacterial infection (e.g., enterotoxigenic *E. coli*, *Vibrio cholerae*, *Campylobacter jejuni*, *Salmonella*), viral infection (rotavirus or norovirus), or protozoan infection (*Giardia lamblia*, *Cryptosporidium parvum*, or *Entamoeba histolytica*).

His physician orders a stool sample to identify possible causative agents (e.g., bacteria, cysts) and to look for the presence of blood because certain types of infectious agents (like *C. jejuni*, *Salmonella*, and *E. histolytica*) are associated with the production of bloody stools.

Alex's stool sample showed neither blood nor cysts. Following analysis of his stool sample and based upon his recent travel history, the hospital physician suspected that Alex was suffering from traveler's diarrhea caused by enterotoxigenic *E. coli* (ETEC), the causative agent of most traveler's diarrhea. To verify the diagnosis and rule out other possibilities, Alex's physician ordered a diagnostic lab test of his stool sample to look for DNA sequences encoding specific virulence factors of ETEC. The physician instructed Alex to drink lots of fluids to replace what he was losing and discharged him from the hospital.

ETEC produces several plasmid-encoded virulence factors that make it pathogenic compared with typical *E. coli*. These include the secreted toxins heat-labile enterotoxin (LT) and heat-stabile enterotoxin (ST), as well as colonization factor (CF). Both LT and ST cause the excretion of chloride ions from intestinal cells to the intestinal lumen, causing a consequent loss of

water from intestinal cells, resulting in diarrhea. CF encodes a bacterial protein that aids in allowing the bacterium to adhere to the lining of the small intestine.

Exercise 9.1.3.5

Why did Alex's physician use genetic analysis instead of either isolation of bacteria from the stool sample or direct Gram stain of the stool sample alone?

Key Concepts and Summary

- **Nucleic acids** are composed of **nucleotides**, each of which contains a pentose sugar, a phosphate group, and a **nitrogenous base**. **Deoxyribonucleotides** within DNA contain **deoxyribose** as the pentose sugar.
- DNA contains the **pyrimidines cytosine** and **thymine**, and the **purines adenine** and **guanine**.
- **Nucleotides** are linked together by phosphodiester bonds between the 5′ phosphate group of one nucleotide and the 3′ hydroxyl group of another. A **nucleic acid strand** has a free phosphate group at the 5′ end and a free hydroxyl group at the 3′ end.
- Chargaff discovered that the amount of **adenine** is approximately equal to the amount of **thymine** in DNA, and that the amount of the **guanine** is approximately equal to **cytosine**. These relationships were later determined to be due to complementary base pairing.
- Watson and Crick, building on the work of Chargaff, Franklin and Gosling, and Wilkins, proposed the double helix model and base pairing for DNA structure.
- DNA is composed of two complementary strands oriented **antiparallel** to each other with the **phosphodiester backbones** on the exterior of the molecule. The nitrogenous bases of each strand face each other and complementary bases hydrogen bond to each other, stabilizing the double helix.
- Heat or chemicals can break the hydrogen bonds between complementary bases, denaturing DNA. Cooling or removing chemicals can lead to renaturation or reannealing of DNA by allowing hydrogen bonds to reform between complementary bases.
- DNA stores the instructions needed to build and control the cell. This information is transmitted from parent to offspring through **vertical gene transfer**.

Footnotes

1. 1 N. Kresge et al. "Chargaff's Rules: The Work of Erwin Chargaff." *Journal of Biological Chemistry* 280 (2005):e21.
2. 2 L. Pauling, "A Proposed Structure for the Nucleic Acids." *Proceedings of the National Academy of Science of the United States of America* 39 no. 2 (1953):84–97.
3. 3 J.D. Watson, F.H.C. Crick. "A Structure for Deoxyribose Nucleic Acid." *Nature* 171 no. 4356 (1953):737–738.
4. 4 M.H.F. Wilkins et al. "Molecular Structure of Deoxypentose Nucleic Acids." *Nature* 171 no. 4356 (1953):738–740.
5. 5 R. Franklin, R.G. Gosling. "Molecular Configuration in Sodium Thymonucleate." *Nature* 171 no. 4356 (1953):740–741.
6. 6 R.O. Day et al. "A Crystalline Fragment of the Double Helix: The Structure of the Dinucleoside Phosphate Guanylyl-3',5'-Cytidine." *Proceedings of the National Academy of Sciences of the United States of America* 70 no. 3 (1973):849–853.
7. 7 N.H. Wolfinger "For Female Scientists, There's No Good Time to Have Children." *The Atlantic* July 29, 2013. www.theatlantic.com/sexes/arc...ildren/278165/.
8. 8 S.A. Seabury et al. "Trends in the Earnings of Male and Female Health Care Professionals in the United States, 1987 to 2010." *Journal of the American Medical Association Internal Medicine* 173 no. 18 (2013):1748–1750.
9. 9 E. Chung. "Tim Hunt, Sexism and Science: The Real 'Trouble With Girls' in Labs." *CBC News Technology and Science*, June 12, 2015. http://www.cbc.ca/news/technology/ti...labs-1.3110133. Accessed 8/4/2016.
10. 10 American Association of University Women. "Building a STEM Pipeline for Girls and Women." www.aauw.org/what-we-do/stem-education/. Accessed June 10, 2016.
11. 11 National Aeronautics and Space Administration. "Outreach Programs: Women and Girls Initiative." http://women.nasa.gov/outreach-programs/. Accessed June 10, 2016.

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9.1.4: Structure and Function of RNA

Learning Objectives

- Describe the biochemical structure of ribonucleotides
- Describe the similarities and differences between RNA and DNA
- Describe the functions of the three main types of RNA used in protein synthesis
- Explain how RNA can serve as hereditary information

Structurally speaking, ribonucleic acid (RNA), is quite similar to DNA. However, whereas DNA molecules are typically long and double stranded, RNA molecules are much shorter and are typically single stranded. RNA molecules perform a variety of roles in the cell but are mainly involved in the process of protein synthesis (translation) and its regulation.

RNA Structure

RNA is typically single stranded and is made of ribonucleotides that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and a phosphate group. The subtle structural difference between the sugars gives DNA added stability, making DNA more suitable for storage of genetic information, whereas the relative instability of RNA makes it more suitable for its more short-term functions.

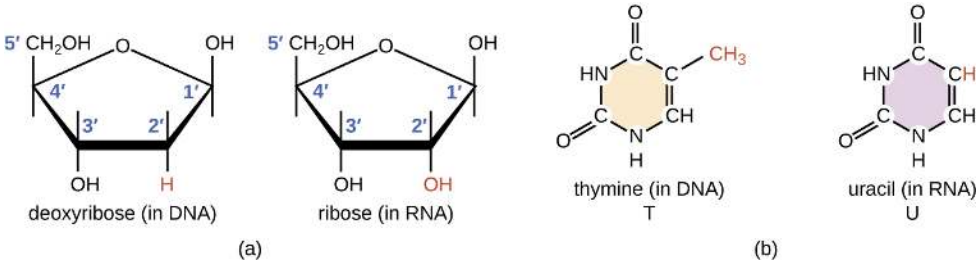


Figure 9.1.4.1: (a) Ribonucleotides contain the pentose sugar ribose instead of the deoxyribose found in deoxyribonucleotides. (b) RNA contains the pyrimidine uracil in place of thymine found in DNA.

The RNA-specific pyrimidine uracil forms a complementary base pair with adenine and is used instead of the thymine used in DNA. Even though RNA is single stranded, most types of RNA molecules show extensive intramolecular base pairing between complementary sequences within the RNA strand, creating a predictable three-dimensional structure essential for their function (Figure 9.1.4.1 and Figure 9.1.4.2).

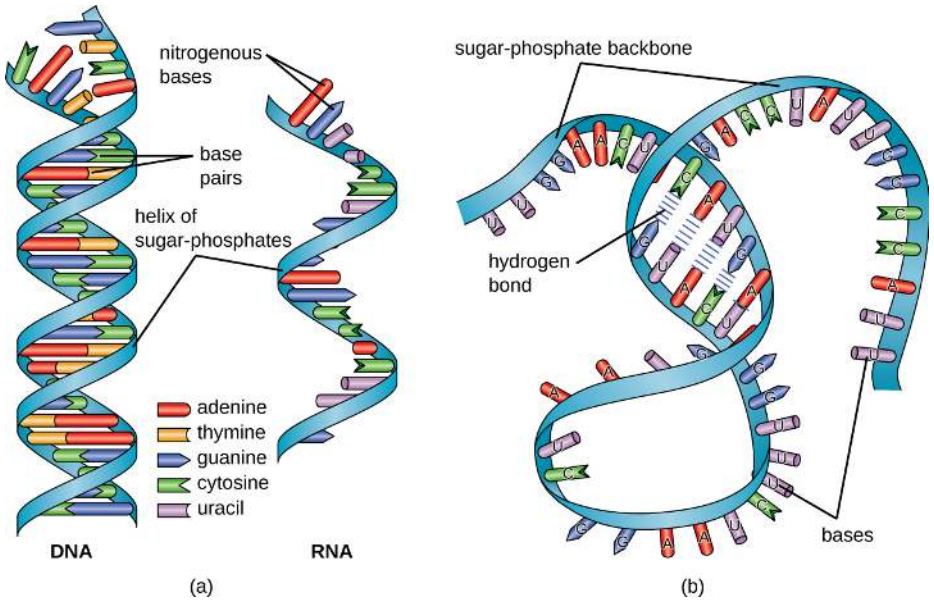


Figure 9.1.4.2: (a) DNA is typically double stranded, whereas RNA is typically single stranded. (b) Although it is single stranded, RNA can fold upon itself, with the folds stabilized by short areas of complementary base pairing within the molecule, forming a three-dimensional structure.

? Exercise 9.1.4.1

How does the structure of RNA differ from the structure of DNA?

Functions of RNA in Protein Synthesis

Cells access the information stored in DNA by creating RNA to direct the synthesis of proteins through the process of translation. Proteins within a cell have many functions, including building cellular structures and serving as enzyme catalysts for cellular chemical reactions that give cells their specific characteristics. The three main types of RNA directly involved in protein synthesis are messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA).

In 1961, French scientists François Jacob and Jacques Monod hypothesized the existence of an intermediary between DNA and its protein products, which they called messenger RNA.[1] Evidence supporting their hypothesis was gathered soon afterwards showing that information from DNA is transmitted to the ribosome for protein synthesis using mRNA. If DNA serves as the complete library of cellular information, mRNA serves as a photocopy of specific information needed at a particular point in time that serves as the instructions to make a protein.

The mRNA carries the message from the DNA, which controls all of the cellular activities in a cell. If a cell requires a certain protein to be synthesized, the gene for this product is "turned on" and the mRNA is synthesized through the process of transcription (see RNA Transcription). The mRNA then interacts with ribosomes and other cellular machinery (Figure 9.1.4.3) to direct the synthesis of the protein it encodes during the process of translation (see Protein Synthesis). mRNA is relatively unstable and short-lived in the cell, especially in prokaryotic cells, ensuring that proteins are only made when needed.

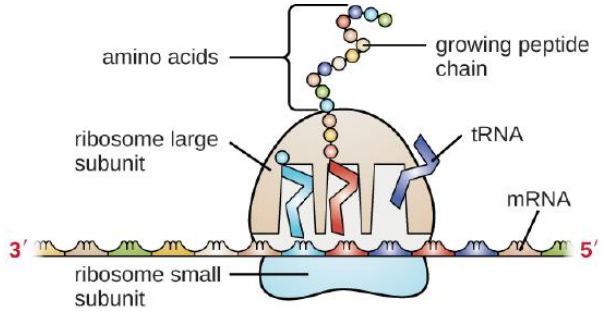


Figure 9.1.4.3: A generalized illustration of how mRNA and tRNA are used in protein synthesis within a cell.

rRNA and tRNA are stable types of RNA. In prokaryotes and eukaryotes, tRNA and rRNA are encoded in the DNA, then copied into long RNA molecules that are cut to release smaller fragments containing the individual mature RNA species. In eukaryotes, synthesis, cutting, and assembly of rRNA into ribosomes takes place in the nucleolus region of the nucleus, but these activities occur in the cytoplasm of prokaryotes. Neither of these types of RNA carries instructions to direct the synthesis of a polypeptide, but they play other important roles in protein synthesis.

Ribosomes are composed of rRNA and protein. As its name suggests, rRNA is a major constituent of ribosomes, composing up to about 60% of the ribosome by mass and providing the location where the mRNA binds. The rRNA ensures the proper alignment of the mRNA, tRNA, and the ribosomes; the rRNA of the ribosome also has an enzymatic activity (peptidyl transferase) and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis. Although rRNA had long been thought to serve primarily a structural role, its catalytic role within the ribosome was proven in 2000.[2] Scientists in the laboratories of Thomas Steitz (1940–) and Peter Moore(1939–) at Yale University were able to crystallize the ribosome structure from *Haloarcula marismortui*, a halophilic archaeon isolated from the Dead Sea. Because of the importance of this work, Steitz shared the 2009 Nobel Prize in Chemistry with other scientists who made significant contributions to the understanding of ribosome structure.

Transfer RNA is the third main type of RNA and one of the smallest, usually only 70–90 nucleotides long. It carries the correct amino acid to the site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized (Figure **9.1.4.4**). Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis (Table **9.1.4.1**).

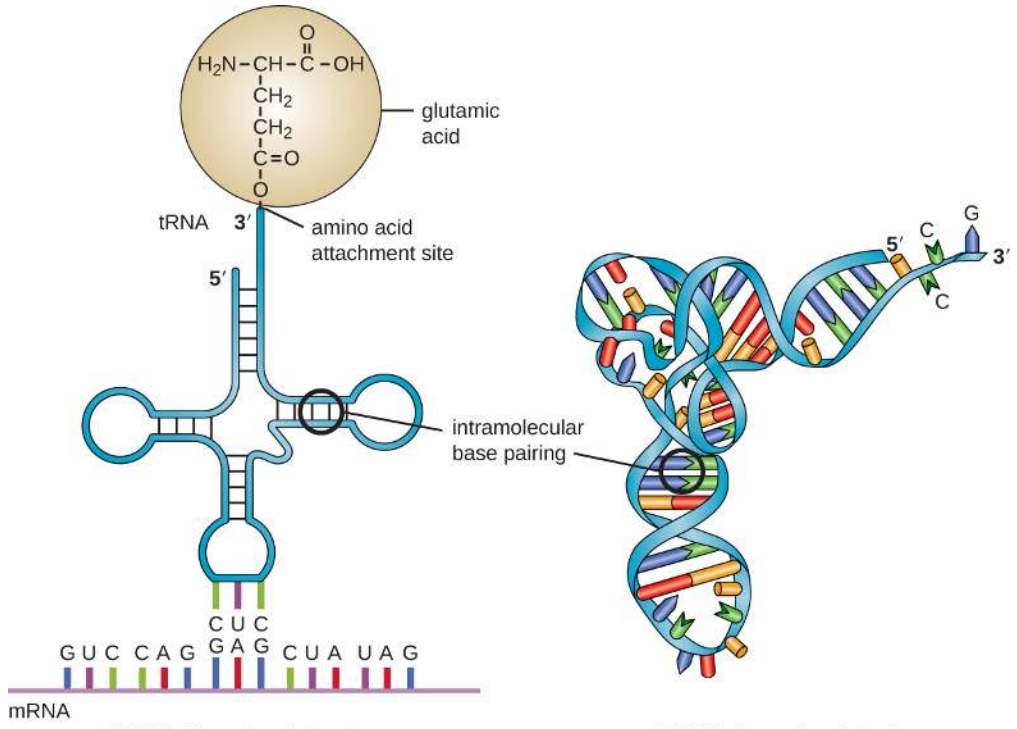


Figure **9.1.4.4**: A tRNA molecule is a single-stranded molecule that exhibits significant intracellular base pairing, giving it its characteristic three-dimensional shape.

Table **9.1.4.1**: Structure and Function of RNA

	mRNA	rRNA	tRNA
Structure	Short, unstable, single-stranded RNAcorresponding to a gene encoded within DNA	Longer, stable RNA molecules composing 60% of ribosome's mass	Short (70-90 nucleotides), stable RNA with extensive intramolecular base pairing; contains an amino acid binding site and an mRNA binding site
Function	Serves as intermediary between DNA and protein; used by ribosome to direct synthesis of protein it encodes	Ensures the proper alignment of mRNA, tRNA, and ribosome during protein synthesis; catalyzes peptide bond formation between amino acids	Carries the correct amino acid to the site of protein synthesis in the ribosome

? Exercise 9.1.4.1

What are the functions of the three major types of RNA molecules involved in protein synthesis?

RNA as Hereditary Information

Although RNA does not serve as the hereditary information in most cells, RNA does hold this function for many viruses that do not contain DNA. Thus, RNA clearly does have the additional capacity to serve as genetic information. Although RNA is typically single stranded within cells, there is significant diversity in viruses. Rhinoviruses, which cause the common cold; influenza viruses; and the Ebola virus are single-stranded RNA viruses. Rotaviruses, which cause severe gastroenteritis in children and other immunocompromised individuals, are examples of double-stranded RNA viruses. Because double-stranded RNA is uncommon in eukaryotic cells, its presence serves as an indicator of viral infection. The implications for a virus having an RNA genome instead of a DNA genome are discussed in more detail in Viruses.

Key Concepts and Summary

- **Ribonucleic acid (RNA)** is typically single stranded and contains ribose as its pentose sugar and the pyrimidine uracil instead of thymine. An RNA strand can undergo significant intramolecular base pairing to take on a three-dimensional structure.
- There are three main types of RNA, all involved in protein synthesis.
- Messenger RNA (**mRNA**) serves as the intermediary between DNA and the synthesis of protein products during translation.
- Ribosomal RNA (**rRNA**) is a type of stable RNA that is a major constituent of ribosomes. It ensures the proper alignment of the mRNA and the ribosomes during protein synthesis and catalyzes the formation of the peptide bonds between two aligned amino acids during protein synthesis.
- Transfer RNA (**tRNA**) is a small type of stable RNA that carries an amino acid to the corresponding site of protein synthesis in the ribosome. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain being synthesized.
- Although RNA is not used for long-term genetic information in cells, many viruses do use RNA as their genetic material.

Footnotes

1. 1 A. Rich. "The Era of RNA Awakening: Structural Biology of RNA in the Early Years." *Quarterly Reviews of Biophysics* 42 no. 2 (2009):117–137.
2. 2 P. Nissen et al. "The Structural Basis of Ribosome Activity in Peptide Bond Synthesis." *Science* 289 no. 5481 (2000):920–930.

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9.1.5: What Are Genes?

Learning Objectives

- Explain the two functions of the genome
- Explain the meaning of the central dogma of molecular biology
- Differentiate between genotype and phenotype and explain how environmental factors influence phenotype

Clinical Focus: Part 1

Mark is 60-year-old software engineer who suffers from type II diabetes, which he monitors and keeps under control largely through diet and exercise. One spring morning, while doing some gardening, he scraped his lower leg while walking through blackberry brambles. He continued working all day in the yard and did not bother to clean the wound and treat it with antibiotic ointment until later that evening. For the next 2 days, his leg became increasingly red, swollen, and warm to the touch. It was sore not only on the surface, but deep in the muscle. After 24 hours, Mark developed a fever and stiffness in the affected leg. Feeling increasingly weak, he called a neighbor, who drove him to the emergency department.

Exercise 9.1.5.1

1. Did Mark wait too long to seek medical attention? At what point do his signs and symptoms warrant seeking medical attention?
2. What types of infections or other conditions might be responsible for Mark's symptoms?

DNA serves two essential functions that deal with cellular information. First, DNA is the genetic material responsible for inheritance and is passed from parent to offspring for all life on earth. To preserve the integrity of this genetic information, DNA must be replicated with great accuracy, with minimal errors that introduce changes to the DNA sequence. A genome contains the full complement of DNA within a cell and is organized into smaller, discrete units called genes that are arranged on chromosomes and plasmids. The second function of DNA is to direct and regulate the construction of the proteins necessary to a cell for growth and reproduction in a particular cellular environment.

A gene is composed of DNA that is "read" or transcribed to produce an RNA molecule during the process of transcription. One major type of RNA molecule, called messenger RNA (mRNA), provides the information for the ribosome to catalyze protein synthesis in a process called translation. The processes of transcription and translation are collectively referred to as gene expression. Gene expression is the synthesis of a specific protein with a sequence of amino acids that is encoded in the gene. The flow of genetic information from DNA to RNA to protein is described by the central dogma (Figure 9.1.5.1). This central dogma of molecular biology further elucidates the mechanism behind Beadle and Tatum's "one gene-one enzyme" hypothesis (see Using Microbiology to Discover the Secrets of Life). Each of the processes of replication, transcription, and translation includes the stages of 1) initiation, 2) elongation (polymerization), and 3) termination. These stages will be described in more detail in this chapter.



Figure 9.1.5.1: The central dogma states that DNA encodes messenger RNA, which, in turn, encodes protein.

A cell's genotype is the full collection of genes it contains, whereas its phenotype is the set of observable characteristics that result from those genes. The phenotype is the product of the array of proteins being produced by the cell at a given time, which is influenced by the cell's genotype as well as interactions with the cell's environment. Genes code for proteins that have functions in the cell. Production of a specific protein encoded by an individual gene often results in a distinct phenotype for the cell compared with the phenotype without that protein. For this reason, it is also common to refer to the genotype of an individual gene and its phenotype. Although a cell's genotype remains constant, not all genes are used to direct the production of their proteins

simultaneously. Cells carefully regulate expression of their genes, only using genes to make specific proteins when those proteins are needed (Figure 9.1.5.2).

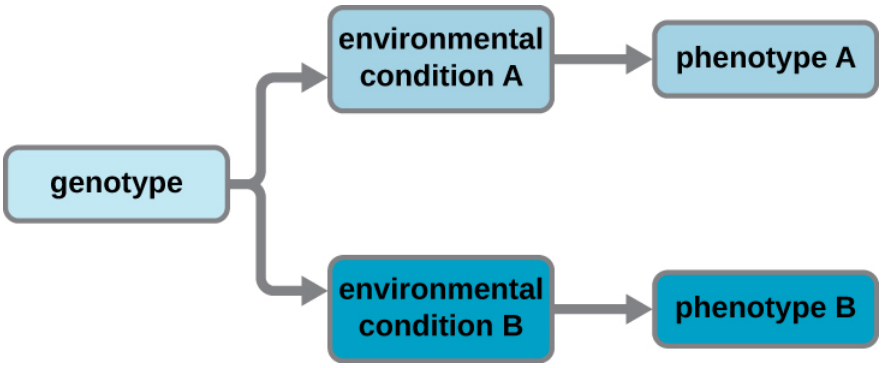


Figure 9.1.5.2: Phenotype is determined by the specific genes within a genotype that are expressed under specific conditions. Although multiple cells may have the same genotype, they may exhibit a wide range of phenotypes resulting from differences in patterns of gene expression in response to different environmental conditions.

Exercise 9.1.5.2

1. What are the two functions of DNA?
2. Distinguish between the genotype and phenotype of a cell.
3. How can cells have the same genotype but differ in their phenotype?

Use and Abuse of Genome Data

Why can some humans harbor opportunistic pathogens like *Haemophilus influenzae*, *Staphylococcus aureus*, or *Streptococcus pyogenes*, in their upper respiratory tracts but remain asymptomatic carriers, while other individuals become seriously ill when infected? There is evidence suggesting that differences in susceptibility to infection between patients may be a result, at least in part, of genetic differences between human hosts. For example, genetic differences in human leukocyte antigens (HLAs) and red blood cell antigens among hosts have been implicated in different immune responses and resulting disease progression from infection with *H. influenzae*.

Because the genetic interplay between pathogen and host may contribute to disease outcomes, understanding differences in genetic makeup between individuals may be an important clinical tool. Ecological genomics is a relatively new field that seeks to understand how the genotypes of different organisms interact with each other in nature. The field answers questions about how gene expression of one organism affects gene expression of another. Medical applications of ecological genomics will focus on how pathogens interact with specific individuals, as opposed to humans in general. Such analyses would allow medical professionals to use knowledge of an individual's genotype to apply more individualized plans for treatment and prevention of disease.

With the advent of next-generation sequencing, it is relatively easy to obtain the entire genomic sequences of pathogens; a bacterial genome can be sequenced in as little as a day. The speed and cost of sequencing the human genome has also been greatly reduced and, already, individuals can submit samples to receive extensive reports on their personal genetic traits, including ancestry and carrier status for various genetic diseases. As sequencing technologies progress further, such services will continue to become less expensive, more extensive, and quicker.

However, as this day quickly approaches, there are many ethical concerns with which society must grapple. For example, should genome sequencing be a standard practice for everybody? Should it be required by law or by employers if it will lower health-care costs? If one refuses genome sequencing, does he or she forfeit his or her right to health insurance coverage? For what purposes should the data be used? Who should oversee proper use of these data? If genome sequencing reveals predisposition to a particular disease, do insurance companies have the right to increase rates? Will employers treat an employee differently? Knowing that environmental influences also affect disease development, how should the data on the presence of a particular disease-causing allele in an individual be used ethically? The Genetic Information Nondiscrimination Act of 2008 (GINA) currently prohibits discriminatory practices based on genetic information by both health insurance

companies and employers. However, GINA does not cover life, disability, or long-term care insurance policies. Clearly, all members of society must continue to engage in conversations about these issues so that such genomic data can be used to improve health care while simultaneously protecting an individual's rights.

Key Concepts and Summary

- DNA serves two important cellular functions: It is the genetic material passed from parent to offspring and it serves as the information to direct and regulate the construction of the proteins necessary for the cell to perform all of its functions.
- The **central dogma** states that DNA organized into genes specifies the sequences of messenger RNA (mRNA), which, in turn, specifies the amino acid sequence of proteins.
- The genotype of a cell is the full collection of genes a cell contains. Not all genes are used to make proteins simultaneously. The phenotype is a cell's observable characteristics resulting from the proteins it is producing at a given time under specific environmental conditions.

Footnotes

1. 1 D.J. Edwards, K.E. Holt. "Beginner's Guide to Comparative Bacterial Genome Analysis Using Next-Generation Sequence Data." *Microbial Informatics and Experimentation* 3 no. 1 (2013):2.

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9.1.E: Biochemistry of the Genome (Exercises)

10.1: Using Microbiology to Discover the Secrets of Life

DNA was discovered and characterized long before its role in heredity was understood. Microbiologists played significant roles in demonstrating that DNA is the hereditary information found within cells. In the 1850s and 1860s, Gregor Mendel experimented with true-breeding garden peas to demonstrate the heritability of specific observable traits. In 1869, Friedrich Miescher isolated and purified a compound rich in phosphorus from the nuclei of white blood cells; he named the compound nuclein.

Multiple Choice

Frederick Griffith infected mice with a combination of dead R and live S bacterial strains. What was the outcome, and why did it occur?

A. The mice will live. Transformation was not required.
B. The mice will die. Transformation of genetic material from R to S was required.
C. The mice will live. Transformation of genetic material from S to R was required.
D. The mice will die. Transformation was not required.

Answer

D

Why was the alga *Acetabularia* a good model organism for Joachim Hämmerling to use to identify the location of genetic material?

A. It lacks a nuclear membrane.
B. It self-fertilizes.
C. It is a large, asymmetrical, single cell easy to see with the naked eye.
D. It makes a protein capsid.

Answer

C

Which of the following best describes the results from Hershey and Chase's experiment using bacterial viruses with ^{35}S-labeled proteins or ^{32}P-labeled DNA that are consistent with protein being the molecule responsible for hereditary?

A. After infection with the ^{35}S-labeled viruses and centrifugation, only the pellet would be radioactive.
B. After infection with the ^{35}S-labeled viruses and centrifugation, both the pellet and the supernatant would be radioactive.
C. After infection with the ^{32}P-labeled viruses and centrifugation, only the pellet would be radioactive.
D. After infection with the ^{32}P-labeled viruses and centrifugation, both the pellet and the supernatant would be radioactive.

Answer

A

Which method did Morgan and colleagues use to show that hereditary information was carried on chromosomes?

A. statistical predictions of the outcomes of crosses using true-breeding parents
B. correlations between microscopic observations of chromosomal movement and the characteristics of offspring
C. transformation of nonpathogenic bacteria to pathogenic bacteria
D. mutations resulting in distinct defects in metabolic enzymatic pathways

Answer

B

According to Beadle and Tatum's "one gene–one enzyme" hypothesis, which of the following enzymes will eliminate the transformation of hereditary material from pathogenic bacteria to nonpathogenic bacteria?

A. carbohydrate-degrading enzymes
B. proteinases
C. ribonucleases
D. deoxyribonucleases

Answer

D

Fill in the Blank

The element _____ is unique to nucleic acids compared with other macromolecules.

Answer

phosphorus

In the late 1800s and early 1900s, the macromolecule thought to be responsible for heredity was _____.

Answer

protein

Short Answer

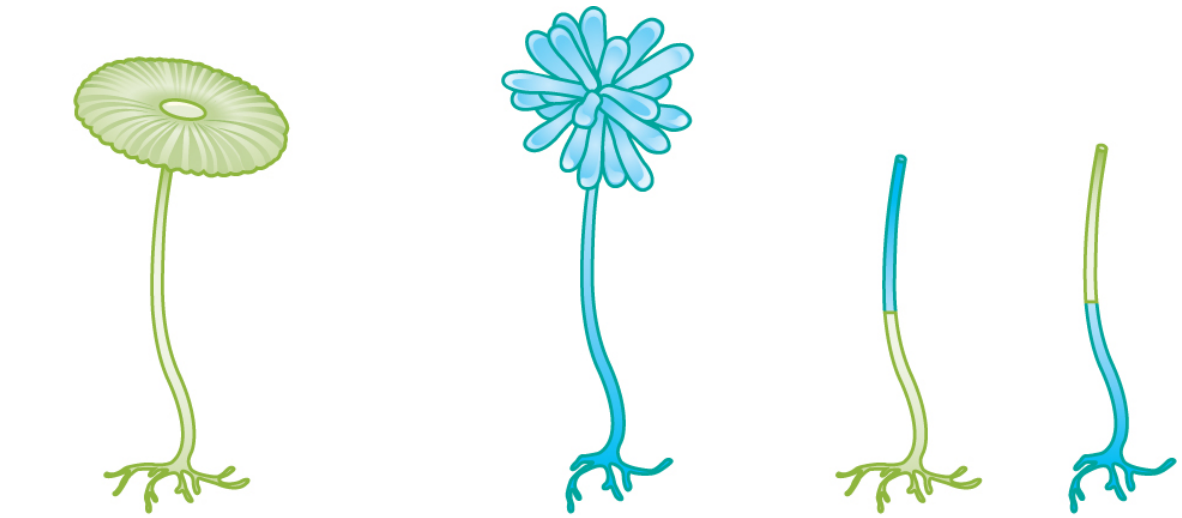
Why do bacteria and viruses make good model systems for various genetic studies?

Why was nucleic acid disregarded for so long as the molecule responsible for the transmission of hereditary information?

Bacteriophages inject their genetic material into host cells, whereas animal viruses enter host cells completely. Why was it important to use a bacteriophage in the Hershey–Chase experiment rather than an animal virus?

Critical Thinking

In the figure shown, if the nuclei were contained within the stalks of *Acetabularia*, what types of caps would you expect from the pictured grafts?



Why are Hershey and Chase credited with identifying DNA as the carrier of heredity even though DNA had been discovered many years before?

10.2: Structure and Function of DNA

Nucleic acids are composed of nucleotides, each of which contains a pentose sugar, a phosphate group, and a nitrogenous base. Deoxyribonucleotides within DNA contain deoxyribose as the pentose sugar. DNA contains the pyrimidines cytosine and thymine,

and the purines adenine and guanine. Nucleotides are linked together by phosphodiester bonds between the 5′ phosphate group of one nucleotide and the 3′ hydroxyl group of another.

Multiple Choice

Which of the following is not found within DNA?

A. thymine
B. phosphodiester bonds
C. complementary base pairing
D. amino acids

Answer

D

If 30% of the bases within a DNA molecule are adenine, what is the percentage of thymine?

A. 20%
B. 25%
C. 30%
D. 35%

Answer

C

Which of the following statements about base pairing in DNA is incorrect?

A. Purines always base pairs with pyrimidines.
B. Adenine binds to guanine.
C. Base pairs are stabilized by hydrogen bonds.
D. Base pairing occurs at the interior of the double helix.

Answer

B

If a DNA strand contains the sequence 5′-ATTCCGGATCGA-3′, which of the following is the sequence of the complementary strand of DNA?

A. 5′-TAAGGCCTAGCT-3′
B. 5′-ATTCCGGATCGA-3′
C. 3′-TAACCGGTACGT-5′
D. 5′-TCGATCCGGAAT-3′

Answer

D

During denaturation of DNA, which of the following happens?

A. Hydrogen bonds between complementary bases break.
B. Phosphodiester bonds break within the sugar-phosphate backbone.
C. Hydrogen bonds within the sugar-phosphate backbone break.
D. Phosphodiester bonds between complementary bases break.

Answer

A.

Fill in the Blank

The end of a nucleic acid strand with a free phosphate group is called the _____.

Answer

5′ end

True/False

The work of Rosalind Franklin and R.G. Gosling was important in demonstrating the helical nature of DNA.

Answer

True

The A-T base pair has more hydrogen bonding than the C-G base pair.

Answer

False

Short Answer

What is the role of phosphodiester bonds within the sugar-phosphate backbone of DNA?

What is meant by the term “antiparallel?”

Why is DNA with a high GC content more difficult to denature than that with a low GC content?

Critical Thinking

A certain DNA sample is found to have a makeup consisting of 22% thymine. Use Chargaff’s rules to fill in the percentages for the other three nitrogenous bases.

adenine	guanine	thymine	cytosine
___%	___%	22%	___%

In considering the structure of the DNA double helix, how would you expect the structure to differ if there was base pairing between two purines? Between two pyrimidines?

10.3: Structure and Function of RNA

Ribonucleic acid (RNA) is typically single stranded and contains ribose as its pentose sugar and the pyrimidine uracil instead of thymine. An RNA strand can undergo significant intramolecular base pairing to take on a three-dimensional structure. There are three main types of RNA, all involved in protein synthesis. Messenger RNA (mRNA) serves as the intermediary between DNA and the synthesis of protein products during translation.

Multiple Choice

Which of the following types of RNA codes for a protein?

A. dsRNA
B. mRNA
C. rRNA
D. tRNA

Answer

B

A nucleic acid is purified from a mixture. The molecules are relatively small, contain uracil, and most are covalently bound to an amino acid. Which of the following was purified?

A. DNA
B. mRNA
C. rRNA
D. tRNA

Answer

D

Which of the following types of RNA is known for its catalytic abilities?

A. dsRNA
B. mRNA
C. rRNA
D. tRNA

Answer

C

Ribosomes are composed of rRNA and what other component?

A. protein
B. polypeptides
C. DNA
D. mRNA

Answer

A

Which of the following may use RNA as its genome?

A. a bacterium
B. an archaeon
C. a virus
D. a eukaryote

Answer

C

Matching

Match the correct molecule with its description:

___tRNA	A. is a major component of ribosome
___rRNA	B. is a copy of the information in a gene
___mRNA	C. carries an amino acid to the ribosome

Answer

C, A, B

True/False

Ribosomes are composed mostly of RNA.

Answer

True

Double-stranded RNA is commonly found inside cells.

Answer

False

Short Answer

What are the differences between DNA nucleotides and RNA nucleotides?

How is the information stored within the base sequence of DNA used to determine a cell's properties?

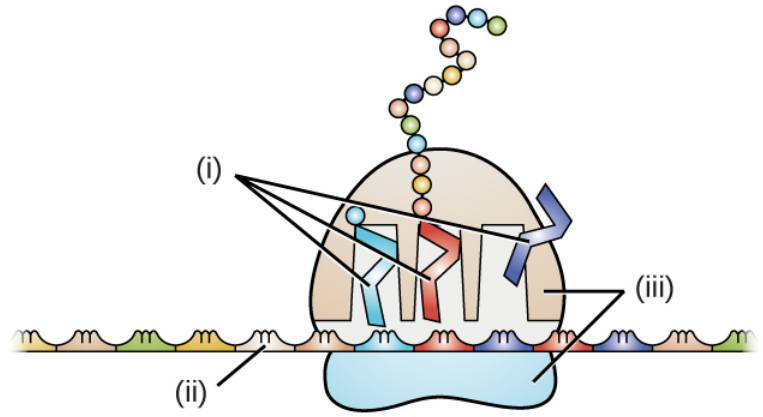
How do complementary base pairs contribute to intramolecular base pairing within an RNA molecule?

If an antisense RNA has the sequence 5′AUUCGAAUGC3′, what is the sequence of the mRNA to which it will bind? Be sure to label the 5′ and 3′ ends of the molecule you draw.

Why does double-stranded RNA (dsRNA) stimulate RNA interference?

Critical Thinking

Identify the location of mRNA, rRNA, and tRNA in the figure.



Why does it make sense that tRNA and rRNA molecules are more stable than mRNA molecules?

10.4: The Structure and Function of Cellular Genomes

The entire genetic content of a cell is its genome. Genes code for proteins, or stable RNA molecules, each of which carries out a specific function in the cell. Although the genotype that a cell possesses remains constant, expression of genes is dependent on environmental conditions. A phenotype is the observable characteristics of a cell (or organism) at a given point in time and results from the complement of genes currently being used.

Multiple Choice

Which of the following correctly describes the structure of the typical eukaryotic genome?

A. diploid
B. linear
C. singular
D. double stranded

Answer

A

Which of the following is typically found as part of the prokaryotic genome?

A. chloroplast DNA
B. linear chromosomes
C. plasmids
D. mitochondrial DNA

Answer

C

Serratia marcescens cells produce a red pigment at room temperature. The red color of the colonies is an example of which of the following?

A. genotype
B. phenotype
C. change in DNA base composition
D. adaptation to the environment

Answer

B

Which of the following genes would not likely be encoded on a plasmid?

A. genes encoding toxins that damage host tissue
B. genes encoding antibacterial resistance
C. gene encoding enzymes for glycolysis
D. genes encoding enzymes for the degradation of an unusual substrate

Answer

C

Histones are DNA binding proteins that are important for DNA packaging in which of the following?

A. double-stranded and single-stranded DNA viruses
B. archaea and bacteria
C. bacteria and eukaryotes
D. eukaryotes and archaea

Answer

D

True/False

Within an organism, phenotypes may change while genotypes remain constant.

Answer

True

Noncoding DNA has no biological purpose.

Answer

False

Fill in the Blank

Plasmids are typically transferred among members of a bacterial community by _____ gene transfer.

Answer

horizontal

Short Answer

What are some differences in chromosomal structures between prokaryotes and eukaryotes?

How do prokaryotes and eukaryotes manage to fit their lengthy DNA inside of cells? Why is this necessary?

What are some functions of noncoding DNA?

In the chromatin of eukaryotic cells, which regions of the chromosome would you expect to be more compact: the regions that contain genes being actively copied into RNA or those that contain inactive genes?

Critical Thinking

A new type of bacteriophage has been isolated and you are in charge of characterizing its genome. The base composition of the bacteriophage is A (15%), C (20%), T (35%), and G (30%). What can you conclude about the genome of the virus?

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9.2: DNA Replication

Learning Objectives

- Explain the meaning of semiconservative DNA replication
- Explain why DNA replication is bidirectional and includes both a leading and lagging strand
- Explain why Okazaki fragments are formed
- Describe the process of DNA replication and the functions of the enzymes involved
- Identify the differences between DNA replication in bacteria and eukaryotes
- Explain the process of rolling circle replication

The elucidation of the structure of the double helix by James Watson and Francis Crick in 1953 provided a hint as to how DNA is copied during the process of replication. Separating the strands of the double helix would provide two templates for the synthesis of new complementary strands, but exactly how new DNA molecules were constructed was still unclear. In one model, semiconservative replication, the two strands of the double helix separate during DNA replication, and each strand serves as a template from which the new complementary strand is copied; after replication, each double-stranded DNA includes one parental or "old" strand and one "new" strand. There were two competing models also suggested: conservative and dispersive, which are shown in Figure **9.2.1**.

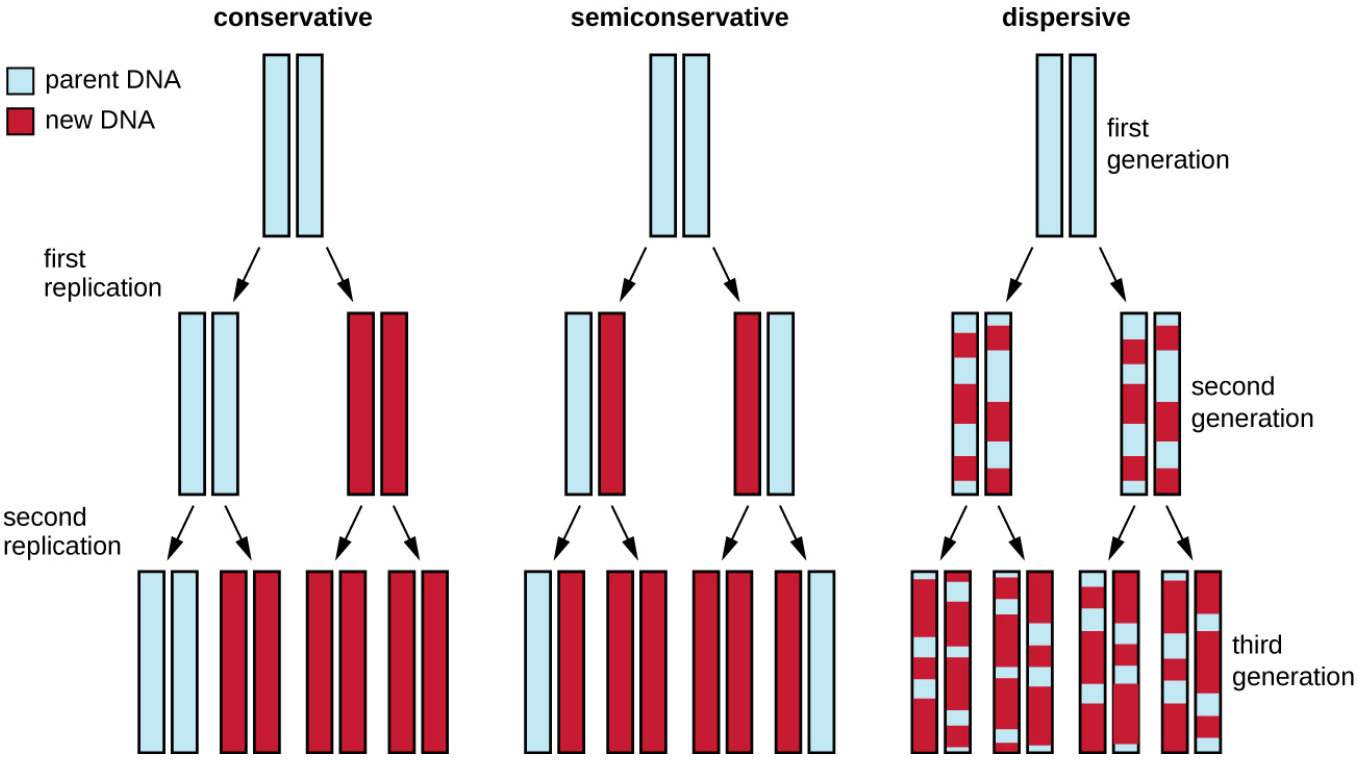


Figure **9.2.1**: There were three models suggested for DNA replication. In the conservative model, parental DNA strands (blue) remained associated in one DNA molecule while new daughter strands (red) remained associated in newly formed DNA molecules. In the semiconservative model, parental strands separated and directed the synthesis of a daughter strand, with each resulting DNA molecule being a hybrid of a parental strand and a daughter strand. In the dispersive model, all resulting DNA strands have regions of double-stranded parental DNA and regions of double-stranded daughter DNA.

Matthew Meselson (1930–) and Franklin Stahl (1929–) devised an experiment in 1958 to test which of these models correctly represents DNA replication (Figure **9.2.2**). They grew *E. coli* for several generations in a medium containing a "heavy" isotope of nitrogen (^{15}N) that was incorporated into nitrogenous bases and, eventually, into the DNA. This labeled the parental DNA. The *E. coli* culture was then shifted into a medium containing ^{14}N and allowed to grow for one generation. The cells were harvested and the DNA was isolated. The DNA was separated by ultracentrifugation, during which the DNA formed bands according to its density. DNA grown in ^{15}N would be expected to form a band at a higher density position than that grown in ^{14}N. Meselson and

Stahl noted that after one generation of growth in ^{14}N, the single band observed was intermediate in position in between DNA of cells grown exclusively in ^{15}N or ^{14}N. This suggested either a semiconservative or dispersive mode of replication. Some cells were allowed to grow for one more generation in ^{14}N and spun again. The DNA harvested from cells grown for two generations in ^{14}N formed two bands: one DNA band was at the intermediate position between ^{15}N and ^{14}N, and the other corresponded to the band of ^{14}N DNA. These results could only be explained if DNA replicates in a semiconservative manner. Therefore, the other two models were ruled out. As a result of this experiment, we now know that during DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied. The new strand will be complementary to the parental or "old" strand. The resulting DNA molecules have the same sequence and are divided equally into the two daughter cells.

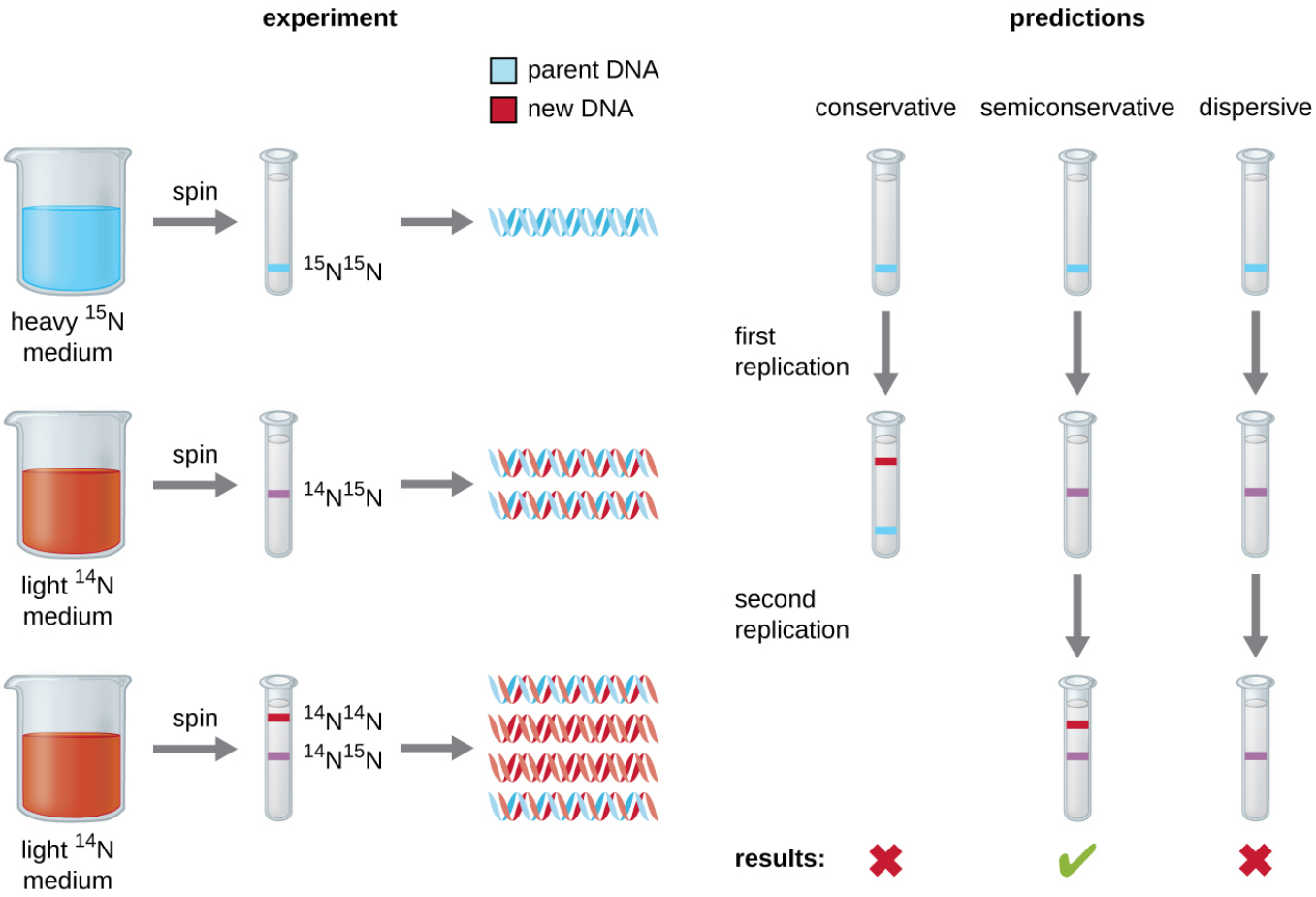


Figure **9.2.2**: Meselson and Stahl experimented with *E. coli* grown first in heavy nitrogen (^{15}N) then in ^{14}N. DNA grown in ^{15}N (blue band) was heavier than DNA grown in ^{14}N (red band), and sedimented to a lower level on ultracentrifugation. After one round of replication, the DNA sedimented halfway between the ^{15}N and ^{14}N levels (purple band), ruling out the conservative model of replication. After a second round of replication, the dispersive model of replication was ruled out. These data supported the semiconservative replication model.

? Exercise 9.2.1

What would have been the conclusion of Meselson and Stahl's experiment if, after the first generation, they had found two bands of DNA?

DNA Replication in Bacteria

DNA replication has been well studied in bacteria primarily because of the small size of the genome and the mutants that are available. *E. coli* has 4.6 million base pairs (Mbp) in a single circular chromosome and all of it is replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the circle bidirectionally (i.e., in both directions). This means that approximately 1000 nucleotides are added per second. The process is quite rapid and occurs with few errors.

DNA replication uses a large number of proteins and enzymes (Table **9.2.1**). One of the key players is the enzyme DNA polymerase, also known as DNA pol. In bacteria, three main types of DNA polymerases are known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair. DNA pol III adds deoxyribonucleotides each complementary to a nucleotide on the template strand, one by one to the 3'-OH group of the growing DNA chain. The addition of these nucleotides requires energy. This energy is present in the bonds of three phosphate groups attached to each nucleotide (a triphosphate nucleotide), similar to how energy is stored in the phosphate bonds of adenosine triphosphate (ATP) (Figure **9.2.3**). When the bond between the phosphates is broken and diphosphate is released, the energy released allows for the formation of a covalent phosphodiester bond by dehydration synthesis between the incoming nucleotide and the free 3'-OH group on the growing DNA strand.

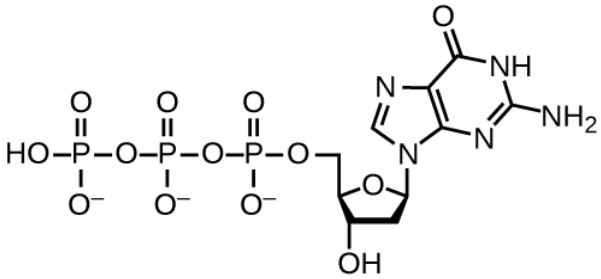


Figure **9.2.3**: This structure shows the guanosine triphosphate deoxyribonucleotide that is incorporated into a growing DNA strand by cleaving the two end phosphate groups from the molecule and transferring the energy to the sugar phosphate bond. The other three nucleotides form analogous structures.

Initiation

The initiation of replication occurs at specific nucleotide sequence called the origin of replication, where various proteins bind to begin the replication process. *E. coli* has a single origin of replication (as do most prokaryotes), called *oriC*, on its one chromosome. The origin of replication is approximately 245 base pairs long and is rich in adenine-thymine (AT) sequences.

Some of the proteins that bind to the origin of replication are important in making single-stranded regions of DNA accessible for replication. Chromosomal DNA is typically wrapped around histones (in eukaryotes and archaea) or histone-like proteins (in bacteria), and is supercoiled, or extensively wrapped and twisted on itself. This packaging makes the information in the DNA molecule inaccessible. However, enzymes called topoisomerases change the shape and supercoiling of the chromosome. For bacterial DNA replication to begin, the supercoiled chromosome is relaxed by topoisomerase II, also called DNA gyrase. An enzyme called helicase then separates the DNA strands by breaking the hydrogen bonds between the nitrogenous base pairs. Recall that AT sequences have fewer hydrogen bonds and, hence, have weaker interactions than guanine-cytosine (GC) sequences. These enzymes require ATP hydrolysis. As the DNA opens up, Y-shaped structures called replication forks are formed. Two replication forks are formed at the origin of replication, allowing for bidirectional replication and formation of a structure that looks like a bubble when viewed with a transmission electron microscope; as a result, this structure is called a replication bubble. The DNA near each replication fork is coated with single-stranded binding proteins to prevent the single-stranded DNA from rewinding into a double helix.

Once single-stranded DNA is accessible at the origin of replication, DNA replication can begin. However, DNA pol III is able to add nucleotides only in the 5' to 3' direction (a new DNA strand can be only extended in this direction). This is because DNA polymerase requires a free 3'-OH group to which it can add nucleotides by forming a covalent phosphodiester bond between the 3'-OH end and the 5' phosphate of the next nucleotide. This also means that it cannot add nucleotides if a free 3'-OH group is not available, which is the case for a single strand of DNA. The problem is solved with the help of an RNA sequence that provides the free 3'-OH end. Because this sequence allows the start of DNA synthesis, it is appropriately called the primer. The primer is five to 10 nucleotides long and complementary to the parental or template DNA. It is synthesized by RNA primase, which is an RNA polymerase. Unlike DNA polymerases, RNA polymerases do not need a free 3'-OH group to synthesize an RNA molecule. Now that the primer provides the free 3'-OH group, DNA polymerase III can now extend this RNA primer, adding DNA nucleotides one by one that are complementary to the template strand (Figure **9.2.1**).

Elongation

During elongation in DNA replication, the addition of nucleotides occurs at its maximal rate of about 1000 nucleotides per second. DNA polymerase III can only extend in the 5’ to 3’ direction, which poses a problem at the replication fork. The DNA double helix is antiparallel; that is, one strand is oriented in the 5’ to 3’ direction and the other is oriented in the 3’ to 5’ direction (see Structure and Function of DNA). During replication, one strand, which is complementary to the 3’ to 5’ parental DNA strand, is synthesized continuously toward the replication fork because polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the leading strand. The other strand, complementary to the 5’ to 3’ parental DNA, grows away from the replication fork, so the polymerase must move back toward the replication fork to begin adding bases to a new primer, again in the direction away from the replication fork. It does so until it bumps into the previously synthesized strand and then it moves back again (Figure **9.2.4**). These steps produce small DNA sequence fragments known as Okazaki fragments, each separated by RNA primer. Okazaki fragments are named after the Japanese research team and married couple Reiji and Tsuneko Okazaki, who first discovered them in 1966. The strand with the Okazaki fragments is known as the lagging strand, and its synthesis is said to be discontinuous.

The leading strand can be extended from one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3’ to 5’, and that of the leading strand 5’ to 3’. A protein called the sliding clamp holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. Beyond its role in initiation, topoisomerase also prevents the overwinding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA polymerase I, and the gaps are filled in. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme DNA ligase that catalyzes the formation of covalent phosphodiester linkage between the 3’-OH end of one DNA fragment and the 5’ phosphate end of the other fragment, stabilizing the sugar-phosphate backbone of the DNA molecule.

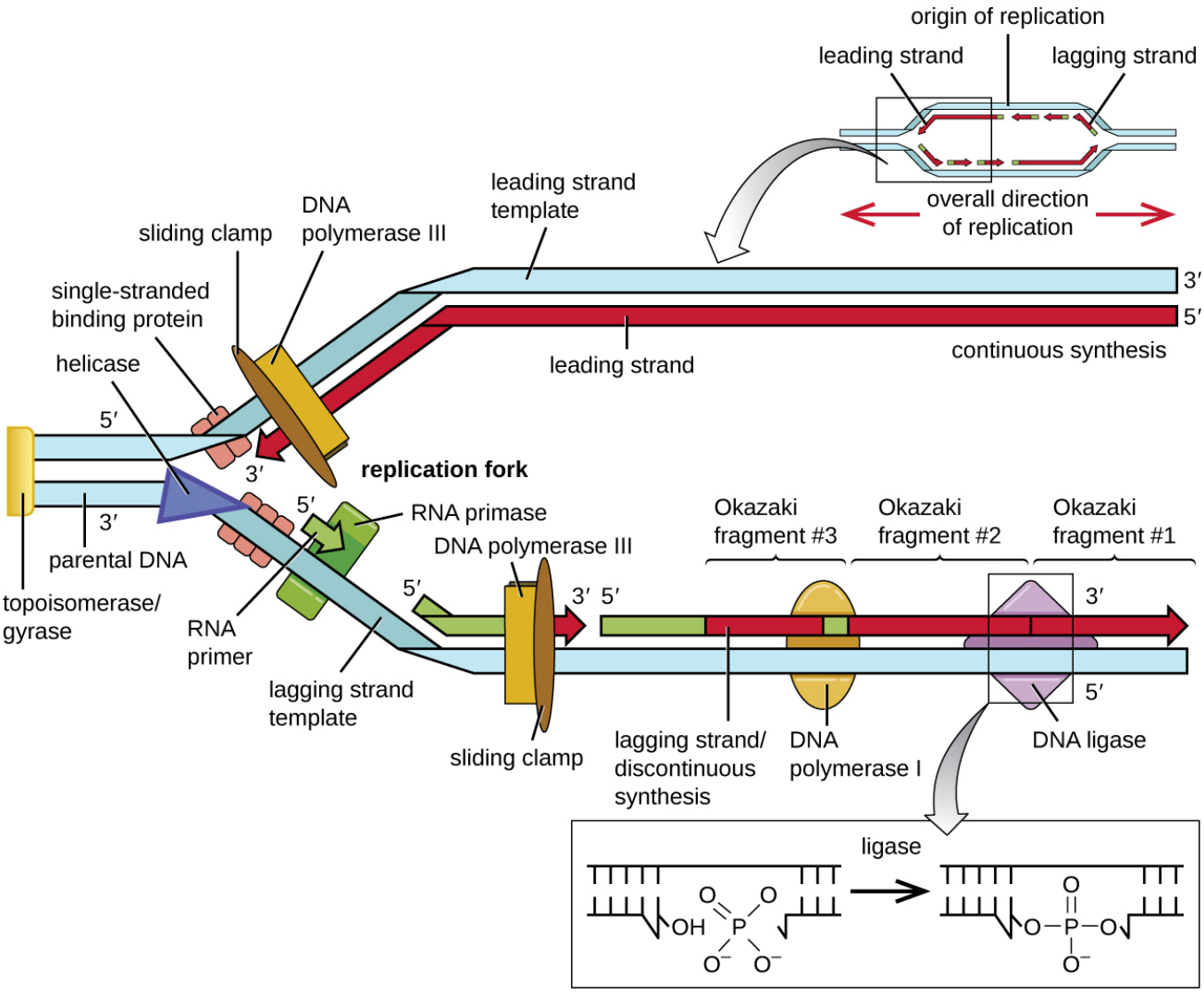


Figure **9.2.4**: At the origin of replication, topoisomerase II relaxes the supercoiled chromosome. Two replication forks are formed by the opening of the double-stranded DNA at the origin, and helicase separates the DNA strands, which are coated by single-stranded binding proteins to keep the strands separated. DNA replication occurs in both directions. An RNA primer complementary to the parental strand is synthesized by RNA primase and is elongated by DNA polymerase III through the addition of nucleotides to the 3'-OH end. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. RNA primers within the lagging strand are removed by the exonuclease activity of DNA polymerase I, and the Okazaki fragments are joined by DNA ligase.

Termination

Once the complete chromosome has been replicated, termination of DNA replication must occur. Although much is known about initiation of replication, less is known about the termination process. Following replication, the resulting complete circular genomes of prokaryotes are concatenated, meaning that the circular DNA chromosomes are interlocked and must be separated from each other. This is accomplished through the activity of bacterial topoisomerase IV, which introduces double-stranded breaks into DNA molecules, allowing them to separate from each other; the enzyme then reseals the circular chromosomes. The resolution of concatemers is an issue unique to prokaryotic DNA replication because of their circular chromosomes. Because both bacterial DNA gyrase and topoisomerase IV are distinct from their eukaryotic counterparts, these enzymes serve as targets for a class of antimicrobial drugs called quinolones.

Table **9.2.1**: The Molecular Machinery Involved in Bacterial DNA Replication

Enzyme or Factor	Function
DNA pol I	Exonuclease activity removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5’ to 3’ direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments on the lagging strand to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Single-stranded binding proteins	Bind to single-stranded DNA to prevent hydrogen bonding between DNA strands, reforming double-stranded DNA
Sliding clamp	Helps hold DNA pol III in place when nucleotides are being added
Topoisomerase II (DNA gyrase)	Relaxes supercoiled chromosome to make DNA more accessible for the initiation of replication; helps relieve the stress on DNA when unwinding, by causing breaks and then resealing the DNA
Topoisomerase IV	Introduces single-stranded break into concatenated chromosomes to release them from each other, and then reseals the DNA

? Exercise 9.2.2

1. Which enzyme breaks the hydrogen bonds holding the two strands of DNA together so that replication can occur?
2. Is it the lagging strand or the leading strand that is synthesized in the direction toward the opening of the replication fork?
3. Which enzyme is responsible for removing the RNA primers in newly replicated bacterial DNA?

DNA Replication in Eukaryotes

Eukaryotic genomes are much more complex and larger than prokaryotic genomes and are typically composed of multiple linear chromosomes (Table **9.2.2**). The human genome, for example, has 3 billion base pairs per haploid set of chromosomes, and 6 billion base pairs are inserted during replication. There are multiple origins of replication on each eukaryotic chromosome (Figure **9.2.5**); the human genome has 30,000 to 50,000 origins of replication. The rate of replication is approximately 100 nucleotides per second —10 times slower than prokaryotic replication.

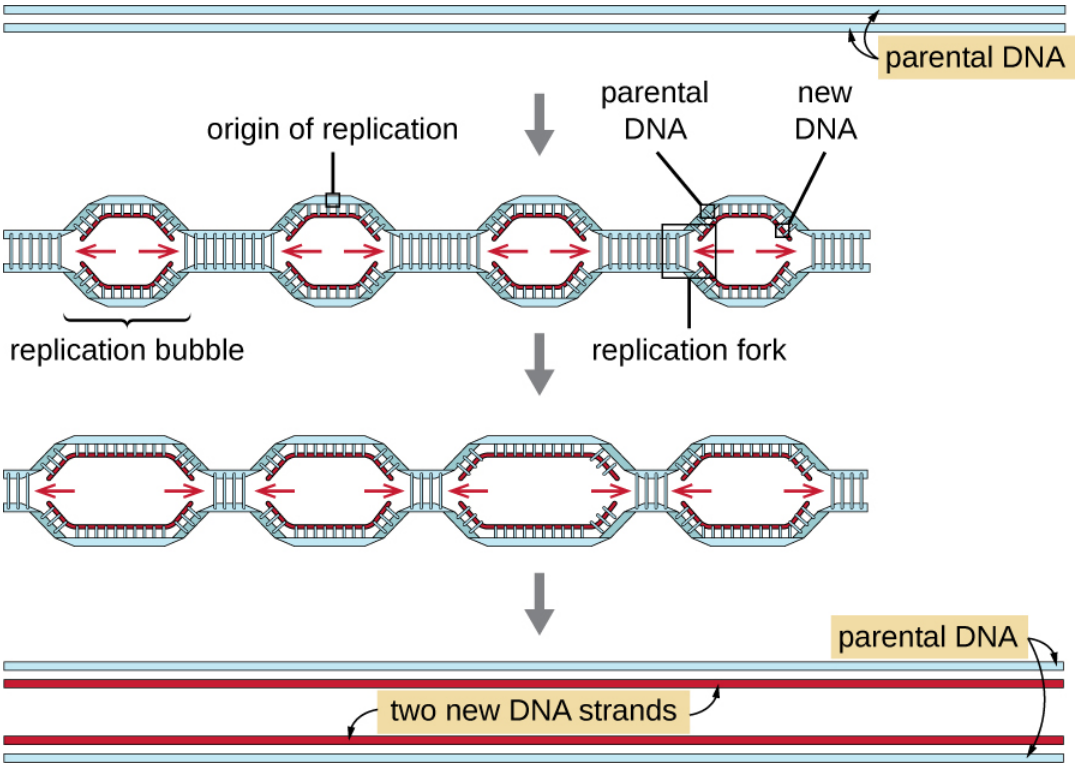


Figure **9.2.5**: Eukaryotic chromosomes are typically linear, and each contains multiple origins of replication.

The essential steps of replication in eukaryotes are the same as in prokaryotes. Before replication can start, the DNA has to be made available as a template. Eukaryotic DNA is highly supercoiled and packaged, which is facilitated by many proteins, including histones (see Structure and Function of Cellular Genomes). At the origin of replication, a prereplication complex composed of several proteins, including helicase, forms and recruits other enzymes involved in the initiation of replication, including topoisomerase to relax supercoiling, single-stranded binding protein, RNA primase, and DNA polymerase. Following initiation of replication, in a process similar to that found in prokaryotes, elongation is facilitated by eukaryotic DNA polymerases. The leading strand is continuously synthesized by the eukaryotic polymerase enzyme pol δ, while the lagging strand is synthesized by pol ε. A sliding clamp protein holds the DNA polymerase in place so that it does not fall off the DNA. The enzyme ribonuclease H (RNase H), instead of a DNA polymerase as in bacteria, removes the RNA primer, which is then replaced with DNA nucleotides. The gaps that remain are sealed by DNA ligase.

Because eukaryotic chromosomes are linear, one might expect that their replication would be more straightforward. As in prokaryotes, the eukaryotic DNA polymerase can add nucleotides only in the 5’ to 3’ direction. In the leading strand, synthesis continues until it reaches either the end of the chromosome or another replication fork progressing in the opposite direction. On the lagging strand, DNA is synthesized in short stretches, each of which is initiated by a separate primer. When the replication fork reaches the end of the linear chromosome, there is no place to make a primer for the DNA fragment to be copied at the end of the chromosome. These ends thus remain unpaired and, over time, they may get progressively shorter as cells continue to divide.

The ends of the linear chromosomes are known as telomeres and consist of noncoding repetitive sequences. The telomeres protect coding sequences from being lost as cells continue to divide. In humans, a six base-pair sequence, TTAGGG, is repeated 100 to 1000 times to form the telomere. The discovery of the enzyme telomerase (Figure **9.2.6**) clarified our understanding of how chromosome ends are maintained. Telomerase contains a catalytic part and a built-in RNA template. It attaches to the end of the chromosome, and complementary bases to the RNA template are added on the 3’ end of the DNA strand. Once the 3’ end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. In this way, the ends of the chromosomes are replicated. In humans, telomerase is typically active in germ cells and adult stem cells; it is not active in adult somatic cells and may be associated with the aging of these cells. Eukaryotic microbes including fungi and protozoans also produce telomerase to maintain chromosomal integrity. For her discovery of telomerase and its action, Elizabeth Blackburn (1948–) received the Nobel Prize for Medicine or Physiology in 2009.

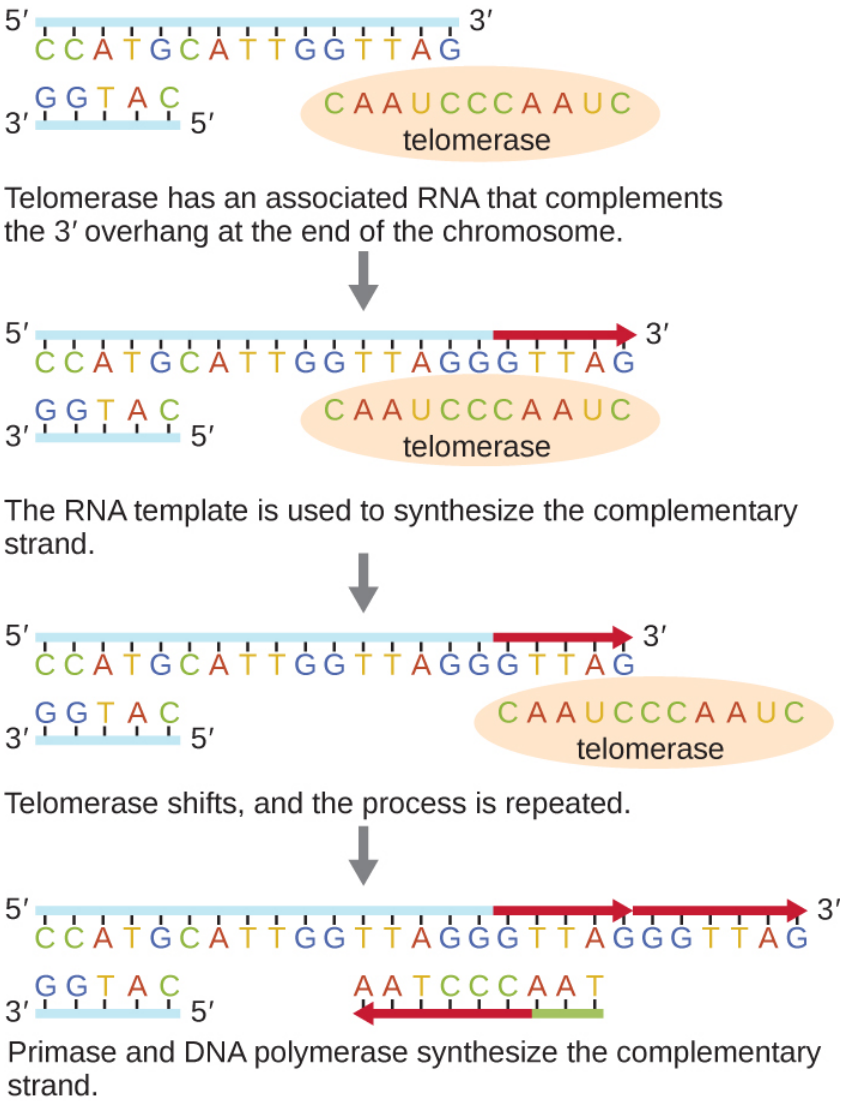


Figure **9.2.6**: In eukaryotes, the ends of the linear chromosomes are maintained by the action of the telomerase enzyme.

Table **9.2.2**: Comparison of Bacterial and Eukaryotic Replication

Property	Bacteria	Eukaryotes
Genome structure	Single circular chromosome	Multiple linear chromosomes
Number of origins per chromosome	Single	Multiple
Rate of replication	1000 nucleotides per second	100 nucleotides per second
Telomerase	Not present	Present
RNA primer removal	DNA pol I	RNase H
Strand elongation	DNA pol III	pol δ, pol ε

? Exercise 9.2.3

1. How does the origin of replication differ between eukaryotes and prokaryotes?
2. What polymerase enzymes are responsible for DNA synthesis during eukaryotic replication?
3. What is found at the ends of the chromosomes in eukaryotes and why?

DNA Replication of Extrachromosomal Elements: Plasmids and Viruses

To copy their nucleic acids, plasmids and viruses frequently use variations on the pattern of DNA replication described for prokaryote genomes. For more information on the wide range of viral replication strategies, see The Viral Life Cycle.

Rolling Circle Replication

Whereas many bacterial plasmids (see Unique Characteristics of Prokaryotic Cells) replicate by a process similar to that used to copy the bacterial chromosome, other plasmids, several bacteriophages, and some viruses of eukaryotes use rolling circle replication (Figure **9.2.7**). The circular nature of plasmids and the circularization of some viral genomes on infection make this possible. Rolling circle replication begins with the enzymatic nicking of one strand of the double-stranded circular molecule at the double-stranded origin (dso) site. In bacteria, DNA polymerase III binds to the 3'-OH group of the nicked strand and begins to unidirectionally replicate the DNA using the un-nicked strand as a template, displacing the nicked strand as it does so. Completion of DNA replication at the site of the original nick results in full displacement of the nicked strand, which may then recircularize into a single-stranded DNA molecule. RNA primase then synthesizes a primer to initiate DNA replication at the single-stranded origin (sso) site of the single-stranded DNA (ssDNA) molecule, resulting in a double-stranded DNA (dsDNA) molecule identical to the other circular DNA molecule.

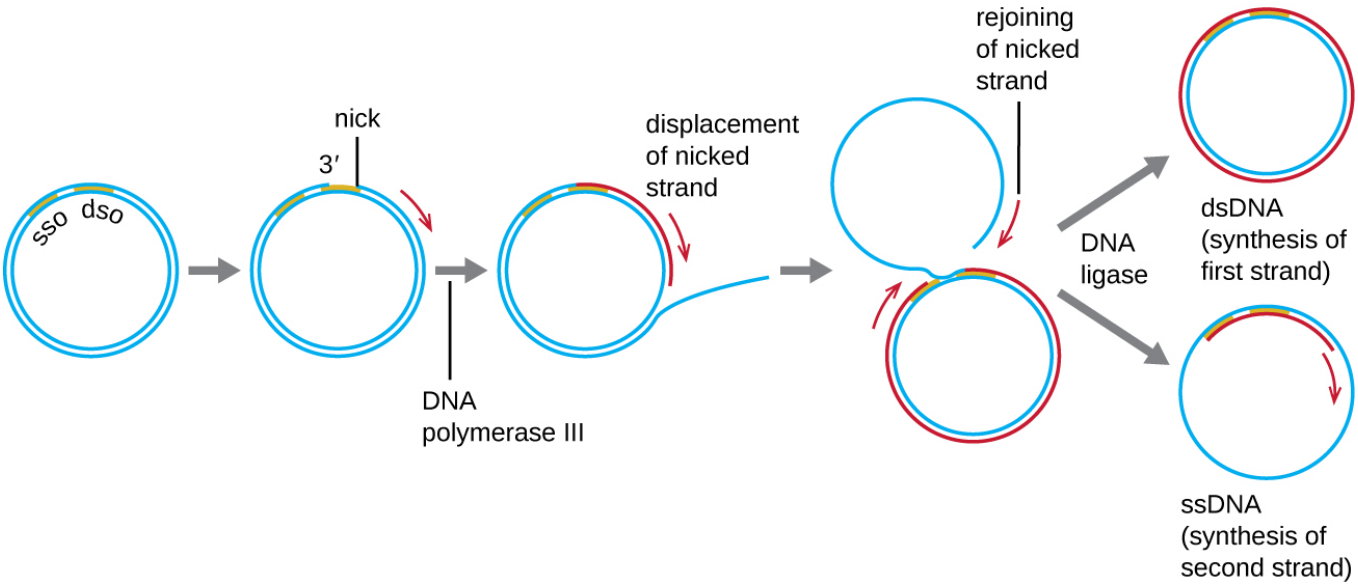


Figure **9.2.7**: The process of rolling circle replication results in the synthesis of a single new copy of the circular DNA molecule, as shown here.

? Exercise 9.2.4

Is there a lagging strand in rolling circle replication? Why or why not?

Key Concepts and Summary

- The DNA replication process is **semiconservative**, which results in two DNA molecules, each having one parental strand of DNA and one newly synthesized strand.
- In bacteria, the **initiation of replication** occurs at the **origin of replication**, where **supercoiled** DNA is unwound by **DNA gyrase**, made single-stranded by **helicase**, and bound by **single-stranded binding protein** to maintain its single-stranded state. **Primase** synthesizes a short RNA **primer**, providing a free 3'-OH group to which **DNA polymerase III** can add DNA nucleotides.
- During **elongation**, the **leading strand** of DNA is synthesized continuously from a single primer. The **lagging strand** is synthesized discontinuously in short **Okazaki fragments**, each requiring its own primer. The RNA primers are removed and replaced with DNA nucleotides by bacterial **DNA polymerase I**, and **DNA ligase** seals the gaps between these fragments.
- **Termination** of replication in bacteria involves the resolution of circular DNA concatemers by topoisomerase IV to release the two copies of the circular chromosome.

- Eukaryotes typically have multiple linear chromosomes, each with multiple origins of replication. Overall, replication in eukaryotes is similar to that in prokaryotes.
- The linear nature of eukaryotic chromosomes necessitates **telomeres** to protect genes near the end of the chromosomes. **Telomerase** extends telomeres, preventing their degradation, in some cell types.
- **Rolling circle replication** is a type of rapid unidirectional DNA synthesis of a circular DNA molecule used for the replication of some plasmids.

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9.3: RNA Transcription

Learning Objectives

- Explain how RNA is synthesized using DNA as a template
- Distinguish between transcription in prokaryotes and eukaryotes

During the process of transcription, the information encoded within the DNA sequence of one or more genes is transcribed into a strand of RNA, also called an RNA transcript. The resulting single-stranded RNA molecule, composed of ribonucleotides containing the bases adenine (A), cytosine (C), guanine (G), and uracil (U), acts as a mobile molecular copy of the original DNA sequence. Transcription in prokaryotes and in eukaryotes requires the DNA double helix to partially unwind in the region of RNA synthesis. The unwound region is called a transcription bubble. Transcription of a particular gene always proceeds from one of the two DNA strands that acts as a template, the so-called antisense strand. The RNA product is complementary to the template strand of DNA and is almost identical to the nontemplate DNA strand, or the sense strand. The only difference is that in RNA, all of the T nucleotides are replaced with U nucleotides; during RNA synthesis, U is incorporated when there is an A in the complementary antisense strand.

Transcription in Bacteria

Bacteria use the same RNA polymerase to transcribe all of their genes. Like DNA polymerase, RNA polymerase adds nucleotides one by one to the 3’-OH group of the growing nucleotide chain. One critical difference in activity between DNA polymerase and RNA polymerase is the requirement for a 3’-OH onto which to add nucleotides: DNA polymerase requires such a 3’-OH group, thus necessitating a primer, whereas RNA polymerase does not. During transcription, a ribonucleotide complementary to the DNA template strand is added to the growing RNA strand and a covalent phosphodiester bond is formed by dehydration synthesis between the new nucleotide and the last one added. In *E. coli*, RNA polymerase comprises six polypeptide subunits, five of which compose the polymerase core enzyme responsible for adding RNA nucleotides to a growing strand. The sixth subunit is known as sigma (σ). The σ factor enables RNA polymerase to bind to a specific promoter, thus allowing for the transcription of various genes. There are various σ factors that allow for transcription of various genes.

Initiation

The initiation of transcription begins at a promoter, a DNA sequence onto which the transcription machinery binds and initiates transcription. The nucleotide pair in the DNA double helix that corresponds to the site from which the first 5’ RNA nucleotide is transcribed is the initiation site. Nucleotides preceding the initiation site are designated “upstream,” whereas nucleotides following the initiation site are called “downstream” nucleotides. In most cases, promoters are located just upstream of the genes they regulate. Although promoter sequences vary among bacterial genomes, a few elements are conserved. At the –10 and –35 positions within the DNA prior to the initiation site (designated +1), there are two promoter consensus sequences, or regions that are similar across all promoters and across various bacterial species. The –10 consensus sequence, called the TATA box, is TATAAT. The –35 sequence is recognized and bound by σ.

Elongation

The elongation in transcription phase begins when the σ subunit dissociates from the polymerase, allowing the core enzyme to synthesize RNA complementary to the DNA template in a 5’ to 3’ direction at a rate of approximately 40 nucleotides per second. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it (Figure **9.3.1**).

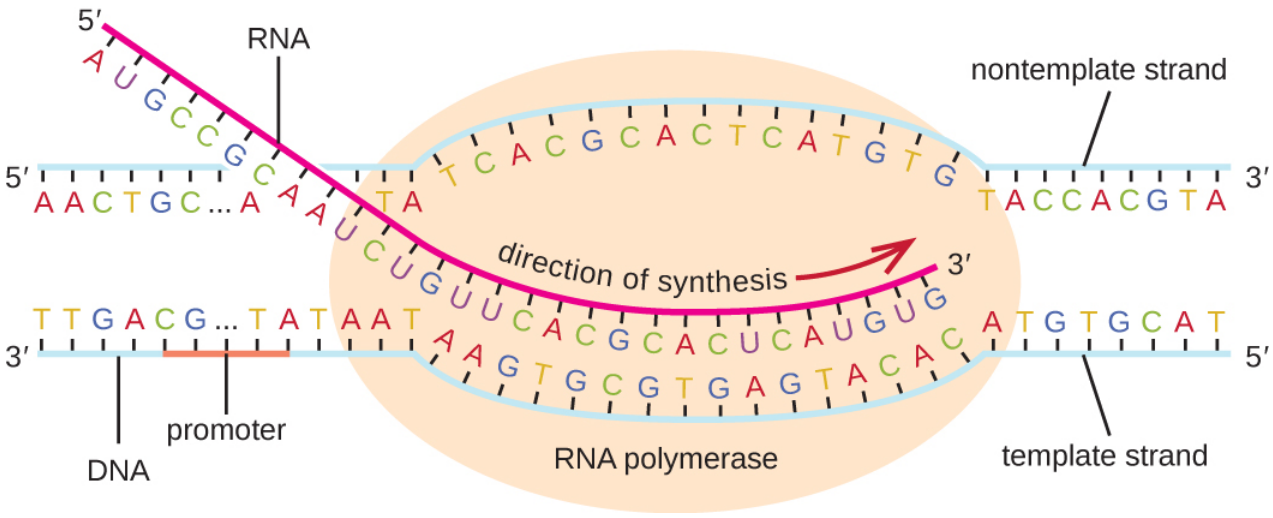


Figure **9.3.1**: During elongation, the bacterial RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to 3' direction, and unwinds and rewinds the DNA as it is read.

Termination

Once a gene is transcribed, the bacterial polymerase must dissociate from the DNA template and liberate the newly made RNA. This is referred to as termination of transcription. The DNA template includes repeated nucleotide sequences that act as termination signals, causing RNA polymerase to stall and release from the DNA template, freeing the RNA transcript.

? Exercise 9.3.1

1. Where does σ factor of RNA polymerase bind DNA to start transcription?
2. What occurs to initiate the polymerization activity of RNA polymerase?
3. Where does the signal to end transcription come from?

Transcription in Eukaryotes

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few significant differences (see Table **9.3.1**). Eukaryotes use three different polymerases, RNA polymerases I, II, and III, all structurally distinct from the bacterial RNA polymerase. Each transcribes a different subset of genes. Interestingly, archaea contain a single RNA polymerase that is more closely related to eukaryotic RNA polymerase II than to its bacterial counterpart. Eukaryotic mRNAs are also usually monocistronic, meaning that they each encode only a single polypeptide, whereas prokaryotic mRNAs of bacteria and archaea are commonly polycistronic, meaning that they encode multiple polypeptides.

The most important difference between prokaryotes and eukaryotes is the latter's membrane-bound nucleus, which influences the ease of use of RNA molecules for protein synthesis. With the genes bound in a nucleus, the eukaryotic cell must transport protein-encoding RNA molecules to the cytoplasm to be translated. Protein-encoding primary transcripts, the RNA molecules directly synthesized by RNA polymerase, must undergo several processing steps to protect these RNA molecules from degradation during the time they are transferred from the nucleus to the cytoplasm and translated into a protein. For example, eukaryotic mRNAs may last for several hours, whereas the typical prokaryotic mRNA lasts no more than 5 seconds.

The primary transcript (also called pre-mRNA) is first coated with RNA-stabilizing proteins to protect it from degradation while it is processed and exported out of the nucleus. The first type of processing begins while the primary transcript is still being synthesized; a special 7-methylguanosine nucleotide, called the 5' cap, is added to the 5' end of the growing transcript. In addition to preventing degradation, factors involved in subsequent protein synthesis recognize the cap, which helps initiate translation by ribosomes. Once elongation is complete, another processing enzyme then adds a string of approximately 200 adenine nucleotides to the 3' end, called the poly-A tail. This modification further protects the pre-mRNA from degradation and signals to cellular factors that the transcript needs to be exported to the cytoplasm.

Eukaryotic genes that encode polypeptides are composed of coding sequences called exons (*ex*-on signifies that they are *ex*pressed) and intervening sequences called introns (*int*-ron denotes their *int*ervening role). Transcribed RNA sequences corresponding to introns do not encode regions of the functional polypeptide and are removed from the pre-mRNA during processing. It is essential that all of the intron-encoded RNA sequences are completely and precisely removed from a pre-mRNA before protein synthesis so that the exon-encoded RNA sequences are properly joined together to code for a functional polypeptide. If the process errs by even a single nucleotide, the sequences of the rejoined exons would be shifted, and the resulting polypeptide would be nonfunctional. The process of removing intron-encoded RNA sequences and reconnecting those encoded by exons is called RNA splicing and is facilitated by the action of a spliceosome containing small nuclear ribonucleo proteins (snRNPs). Intron-encoded RNA sequences are removed from the pre-mRNA while it is still in the nucleus. Although they are not translated, introns appear to have various functions, including gene regulation and mRNA transport. On completion of these modifications, the mature transcript, the mRNA that encodes a polypeptide, is transported out of the nucleus, destined for the cytoplasm for translation. Introns can be spliced out differently, resulting in various exons being included or excluded from the final mRNA product. This process is known as alternative splicing. The advantage of alternative splicing is that different types of mRNA transcripts can be generated, all derived from the same DNA sequence. In recent years, it has been shown that some archaea also have the ability to splice their pre-mRNA.

Table **9.3.1**: Comparison of Transcription in Bacteria Versus Eukaryotes

Property	Bacteria	Eukaryotes
Number of polypeptides encoded per mRNA	Monocistronic or polycistronic	Exclusively monocistronic
Strand elongation	core + σ = holoenzyme	RNA polymerases I, II, or III
Addition of 5’ cap	No	Yes
Addition of 3’ poly-A tail	No	Yes
Splicing of pre-mRNA	No	Yes

Link to Learning

Visualize how mRNA splicing happens by watching the process in action in this video. See how introns are removed during RNA splicing here.

Exercise 9.3.2

1. In eukaryotic cells, how is the RNA transcript from a gene for a protein modified after it is transcribed?
2. Do exons or introns contain information for protein sequences?

Clinical Focus: Part 2

In the emergency department, a nurse told Mark that he had made a good decision to come to the hospital because his symptoms indicated an infection that had gotten out of control. Mark’s symptoms had progressed, with the area of skin affected and the amount of swelling increasing. Within the affected area, a rash had begun, blistering and small gas pockets underneath the outermost layer of skin had formed, and some of the skin was becoming gray. Based on the putrid smell of the pus draining from one of the blisters, the rapid progression of the infection, and the visual appearance of the affected skin, the physician immediately began treatment for necrotizing fasciitis. Mark’s physician ordered a culture of the fluid draining from the blister and also ordered blood work, including a white blood cell count.

Mark was admitted to the intensive care unit and began intravenous administration of a broad-spectrum antibiotic to try to minimize further spread of the infection. Despite antibiotic therapy, Mark’s condition deteriorated quickly. Mark became confused and dizzy. Within a few hours of his hospital admission, his blood pressure dropped significantly and his breathing became shallower and more rapid. Additionally, blistering increased, with the blisters intensifying in color to purplish black, and the wound itself seemed to be progressing rapidly up Mark’s leg.

Exercise 9.3.3

1. What are possible causative agents of Mark's necrotizing fasciitis?
2. What are some possible explanations for why the antibiotic treatment does not seem to be working?

Key Concepts and Summary

- During **transcription**, the information encoded in DNA is used to make RNA.
- **RNA polymerase** synthesizes RNA, using the antisense strand of the DNA as template by adding complementary RNA nucleotides to the 3' end of the growing strand.
- RNA polymerase binds to DNA at a sequence called a **promoter** during the **initiation of transcription**.
- Genes encoding proteins of related functions are frequently transcribed under the control of a single promoter in prokaryotes, resulting in the formation of a **polycistronic mRNA** molecule that encodes multiple polypeptides.
- Unlike DNA polymerase, RNA polymerase does not require a 3'-OH group to add nucleotides, so a **primer** is not needed during initiation.
- **Termination of transcription** in bacteria occurs when the RNA polymerase encounters specific DNA sequences that lead to stalling of the polymerase. This results in release of RNA polymerase from the DNA template strand, freeing the **RNA transcript**.
- Eukaryotes have three different RNA polymerases. Eukaryotes also have monocistronic mRNA, each encoding only a single polypeptide.
- Eukaryotic primary transcripts are processed in several ways, including the addition of a **5' cap** and a 3′-**poly-A tail**, as well as **splicing**, to generate a mature mRNA molecule that can be transported out of the nucleus and that is protected from degradation.

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9.3A: mRNA Processing

Eukaryotic pre-mRNA receives a 5′ cap and a 3′ poly (A) tail before introns are removed and the mRNA is considered ready for translation.

LEARNING OBJECTIVES

Outline the steps of pre-mRNA processing

Key Takeaways

Pre-mRNA Processing

The eukaryotic pre-mRNA undergoes extensive processing before it is ready to be translated. The additional steps involved in eukaryotic mRNA maturation create a molecule with a much longer half-life than a prokaryotic mRNA. Eukaryotic mRNAs last for several hours, whereas the typical *E. coli* mRNA lasts no more than five seconds.

Pre-mRNAs are first coated in RNA-stabilizing proteins; these protect the pre-mRNA from degradation while it is processed and exported out of the nucleus. The three most important steps of pre-mRNA processing are the addition of stabilizing and signaling factors at the 5′ and 3′ ends of the molecule, and the removal of intervening sequences that do not specify the appropriate amino acids. In rare cases, the mRNA transcript can be “edited” after it is transcribed.

5′ Capping

While the pre-mRNA is still being synthesized, a 7-methylguanosine cap is added to the 5′ end of the growing transcript by a 5′-to-5′ phosphate linkage. This moiety protects the nascent mRNA from degradation. In addition, initiation factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

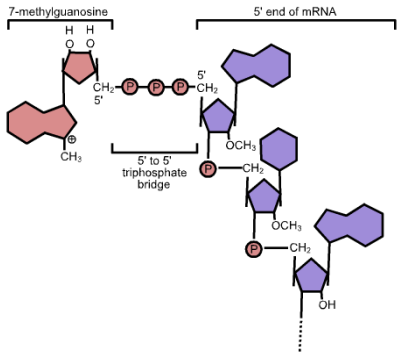


Figure: **5′ cap structure**: Capping of the pre-mRNA involves the addition of 7-methylguanosine (m^7G) to the 5′ end. The cap protects the 5′ end of the primary RNA transcript from attack by ribonucleases and is recognized by eukaryotic initiation factors involved in assembling the ribosome on the mature mRNA prior to initiating translation.

3′ Poly-A Tail

While RNA Polymerase II is still transcribing downstream of the proper end of a gene, the pre-mRNA is cleaved by an endonuclease-containing protein complex between an AAUAAA consensus sequence and a GU-rich sequence. This releases the functional pre-mRNA from the rest of the transcript, which is still attached to the RNA Polymerase. An enzyme called poly (A) polymerase (PAP) is part of the same protein complex that cleaves the pre-mRNA and it immediately adds a string of approximately 200 A nucleotides, called the poly (A) tail, to the 3′ end of the just-cleaved pre-mRNA. The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation.

image

Poly (A) Polymerase adds a 3′ poly (A) tail to the pre-mRNA.: The pre-mRNA is cleaved off the rest of the growing transcript before RNA Polymerase II has stopped transcribing. This cleavage is done by an endonuclease-containing protein complex that

binds to an AAUAAA sequence upstream of the cleavage site and to a GU-rich sequence downstream of the cut site. Immediately after the cleavage, Poly (A) Polymerase (PAP), which is also part of the protein complex, catalyzes the addition of up to 200 A nucleotides to the 3′ end of the just-cleaved pre-mRNA.

Pre-mRNA Splicing

Eukaryotic genes are composed of exons, which correspond to protein-coding sequences (*ex*-on signifies that they are *ex*pressed), and intervening sequences called introns (*int*-ron denotes their *int*ervening role), which may be involved in gene regulation, but are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins.

Discovery of Introns

The discovery of introns came as a surprise to researchers in the 1970s who expected that pre-mRNAs would specify protein sequences without further processing, as they had observed in prokaryotes. The genes of higher eukaryotes very often contain one or more introns. While these regions may correspond to regulatory sequences, the biological significance of having many introns or having very long introns in a gene is unclear. It is possible that introns slow down gene expression because it takes longer to transcribe pre-mRNAs with lots of introns. Alternatively, introns may be nonfunctional sequence remnants left over from the fusion of ancient genes throughout evolution. This is supported by the fact that separate exons often encode separate protein subunits or domains. For the most part, the sequences of introns can be mutated without ultimately affecting the protein product.

Intron Processing

All introns in a pre-mRNA must be completely and precisely removed before protein synthesis. If the process errs by even a single nucleotide, the reading frame of the rejoined exons would shift, and the resulting protein would be dysfunctional. The process of removing introns and reconnecting exons is called splicing. Introns are removed and degraded while the pre-mRNA is still in the nucleus. Splicing occurs by a sequence-specific mechanism that ensures introns will be removed and exons rejoined with the accuracy and precision of a single nucleotide. The splicing of pre-mRNAs is conducted by complexes of proteins and RNA molecules called spliceosomes.

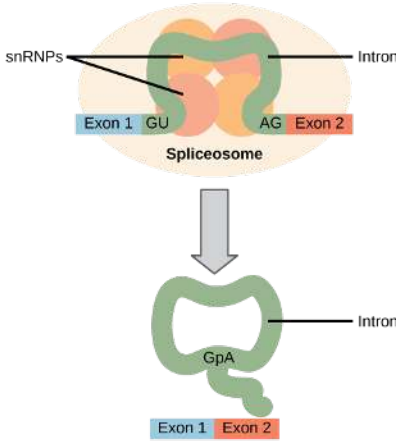


Figure: **Pre-mRNA splicing**: Pre-mRNA splicing involves the precise removal of introns from the primary RNA transcript. The splicing process is catalyzed by large complexes called spliceosomes. Each spliceosome is composed of five subunits called snRNPs. The spliceseome's actions result in the splicing together of the two exons and the release of the intron in a lariat form.

Each spliceosome is composed of five subunits called snRNPs (for small nuclear ribonucleoparticles, and pronounced "snurps".) Each snRNP is itself a complex of proteins and a special type of RNA found only in the nucleus called snRNAs (small nuclear RNAs). Spliceosomes recognize sequences at the 5′ end of the intron because introns always start with the nucleotides GU and they recognize sequences at the 3′ end of the intron because they always end with the nucleotides AG. The spliceosome cleaves the pre-mRNA's sugar phosphate backbone at the G that starts the intron and then covalently attaches that G to an internal A nucleotide within the intron. Then the spliceosme connects the 3′ end of the first exon to the 5′ end of the following exon, cleaving the 3′ end of the intron in the process. This results in the splicing together of the two exons and the release of the intron in a lariat form.

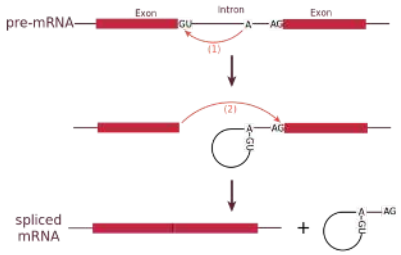


Figure: **Mechanism of pre-mRNA splicing.**: The snRNPs of the spliceosome were left out of this figure, but it shows the sites within the intron whose interactions are catalyzed by the spliceosome. Initially, the conserved G which starts an intron is cleaved from the 3′ end of the exon upstream to it and the G is covalently attached to an internal A within the intron. Then the 3′ end of the just-released exon is joined to the 5′ end of the next exon, cleaving the bond that attaches the 3′ end of the intron to its adjacent exon. This both joins the two exons and removes the intron in lariat form.

Key Points

- A 7-methylguanosine cap is added to the 5′ end of the pre-mRNA while elongation is still in progress. The 5′ cap protects the nascent mRNA from degradation and assists in ribosome binding during translation.
- A poly (A) tail is added to the 3′ end of the pre-mRNA once elongation is complete. The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation.
- Introns are removed from the pre-mRNA before the mRNA is exported to the cytoplasm.

Key Terms

- **intron**: a portion of a split gene that is included in pre-RNA transcripts but is removed during RNA processing and rapidly degraded
- **moiety**: a specific segment of a molecule
- **spliceosome**: a dynamic complex of RNA and protein subunits that removes introns from precursor mRNA

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9.4: Protein Synthesis (Translation)

Learning Objectives

- Describe the genetic code and explain why it is considered almost universal
- Explain the process of translation and the functions of the molecular machinery of translation
- Compare translation in eukaryotes and prokaryotes

The synthesis of proteins consumes more of a cell's energy than any other metabolic process. In turn, proteins account for more mass than any other macromolecule of living organisms. They perform virtually every function of a cell, serving as both functional (e.g., enzymes) and structural elements. The process of translation, or protein synthesis, the second part of gene expression, involves the decoding by a ribosome of an mRNA message into a polypeptide product.

The Genetic Code

Translation of the mRNA template converts nucleotide-based genetic information into the "language" of amino acids to create a protein product. A protein sequence consists of 20 commonly occurring amino acids. Each amino acid is defined within the mRNA by a triplet of nucleotides called a codon. The relationship between an mRNA codon and its corresponding amino acid is called the genetic code.

The three-nucleotide code means that there is a total of 64 possible combinations (4^3, with four different nucleotides possible at each of the three different positions within the codon). This number is greater than the number of amino acids and a given amino acid is encoded by more than one codon (Figure **9.4.1**). This redundancy in the genetic code is called degeneracy. Typically, whereas the first two positions in a codon are important for determining which amino acid will be incorporated into a growing polypeptide, the third position, called the wobble position, is less critical. In some cases, if the nucleotide in the third position is changed, the same amino acid is still incorporated.

Whereas 61 of the 64 possible triplets code for amino acids, three of the 64 codons do not code for an amino acid; they terminate protein synthesis, releasing the polypeptide from the translation machinery. These are called stop codon**s** or nonsense codon**s**. Another codon, AUG, also has a special function. In addition to specifying the amino acid methionine, it also typically serves as the start codon to initiate translation. The reading frame, the way nucleotides in mRNA are grouped into codons, for translation is set by the AUG start codon near the 5' end of the mRNA. Each set of three nucleotides following this start codon is a codon in the mRNA message.

The genetic code is nearly universal. With a few exceptions, virtually all species use the same genetic code for protein synthesis, which is powerful evidence that all extant life on earth shares a common origin. However, unusual amino acids such as selenocysteine and pyrrolysine have been observed in archaea and bacteria. In the case of selenocysteine, the codon used is UGA (normally a stop codon). However, UGA can encode for selenocysteine using a stem-loop structure (known as the selenocysteine insertion sequence, or SECIS element), which is found at the 3' untranslated region of the mRNA. Pyrrolysine uses a different stop codon, UAG. The incorporation of pyrrolysine requires the *pylS* gene and a unique transfer RNA (tRNA) with a CUA anticodon.

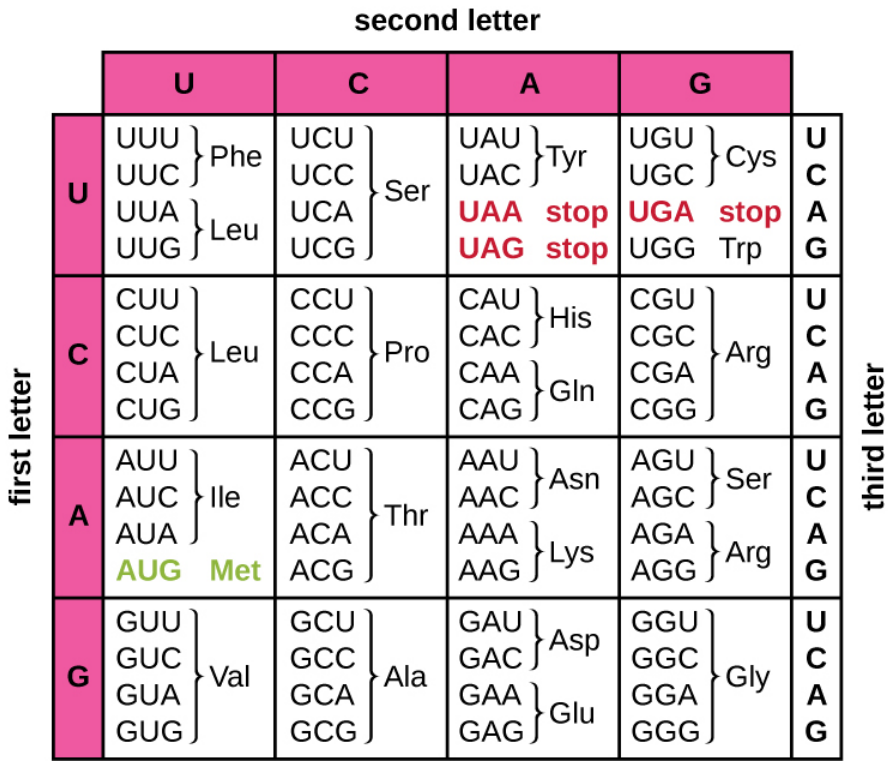


Figure 9.4.1: This figure shows the genetic code for translating each nucleotide triplet in mRNA into an amino acid or a termination signal in a nascent protein. The first letter of a codon is shown vertically on the left, the second letter of a codon is shown horizontally across the top, and the third letter of a codon is shown vertically on the right. (credit: modification of work by National Institutes of Health)

? Exercise 9.4.1

1. How many bases are in each codon?
2. What amino acid is coded for by the codon AAU?
3. What happens when a stop codon is reached?

The Protein Synthesis Machinery

In addition to the mRNA template, many molecules and macromolecules contribute to the process of translation. The composition of each component varies across taxa; for instance, ribosomes may consist of different numbers of ribosomal RNAs (rRNAs) and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors.

Ribosomes

A ribosome is a complex macromolecule composed of catalytic rRNAs (called ribozymes) and structural rRNAs, as well as many distinct polypeptides. Mature rRNAs make up approximately 50% of each ribosome. Prokaryotes have 70S ribosomes, whereas eukaryotes have 80S ribosomes in the cytoplasm and rough endoplasmic reticulum, and 70S ribosomes in mitochondria and chloroplasts. Ribosomes dissociate into large and small subunits when they are not synthesizing proteins and reassociate during the initiation of translation. In *E. coli*, the small subunit is described as 30S (which contains the 16S rRNA subunit), and the large subunit is 50S (which contains the 5S and 23S rRNA subunits), for a total of 70S (Svedberg units are not additive). Eukaryote ribosomes have a small 40S subunit (which contains the 18S rRNA subunit) and a large 60S subunit (which contains the 5S, 5.8S and 28S rRNA subunits), for a total of 80S. The small subunit is responsible for binding the mRNA template, whereas the large subunit binds tRNAs (discussed in the next subsection).

Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction: reading the mRNA from 5’ to 3’ and synthesizing the polypeptide from the N terminus to the C terminus. The complete structure containing an

mRNA with multiple associated ribosomes is called a polyribosome (or polysome). In both bacteria and archaea, before transcriptional termination occurs, each protein-encoding transcript is already being used to begin synthesis of numerous copies of the encoded polypeptide(s) because the processes of transcription and translation can occur concurrently, forming polyribosomes (Figure **9.4.2**). The reason why transcription and translation can occur simultaneously is because both of these processes occur in the same 5' to 3' direction, they both occur in the cytoplasm of the cell, and because the RNA transcript is not processed once it is transcribed. This allows a prokaryotic cell to respond to an environmental signal requiring new proteins very quickly. In contrast, in eukaryotic cells, simultaneous transcription and translation is not possible. Although polyribosomes also form in eukaryotes, they cannot do so until RNA synthesis is complete and the RNA molecule has been modified and transported out of the nucleus.

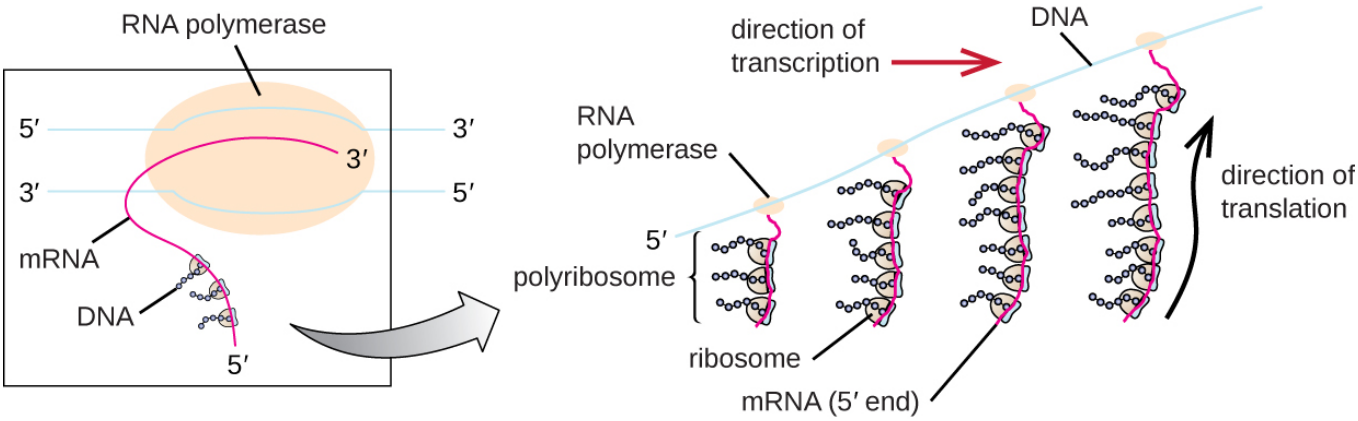


Figure **9.4.2**: In prokaryotes, multiple RNA polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

Transfer RNAs

Transfer RNAs (tRNAs) are structural RNA molecules and, depending on the species, many different types of tRNAs exist in the cytoplasm. Bacterial species typically have between 60 and 90 types. Serving as adaptors, each tRNA type binds to a specific codon on the mRNA template and adds the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually "translate" the language of RNA into the language of proteins. As the adaptor molecules of translation, it is surprising that tRNAs can fit so much specificity into such a small package. The tRNA molecule interacts with three factors: aminoacyl tRNA synthetases, ribosomes, and mRNA.

Mature tRNAs take on a three-dimensional structure when complementary bases exposed in the single-stranded RNA molecule hydrogen bond with each other (Figure **9.4.3**). This shape positions the amino-acid binding site, called the CCA amino acid binding end, which is a cytosine-cytosine-adenine sequence at the 3' end of the tRNA, and the anticodonat the other end. The anticodon is a three-nucleotide sequence that bonds with an mRNA codon through complementary base pairing.

An amino acid is added to the end of a tRNA molecule through the process of tRNA "charging," during which each tRNA molecule is linked to its correct or cognate amino acid by a group of enzymes called aminoacyl tRNA synthetases. At least one type of aminoacyl tRNA synthetase exists for each of the 20 amino acids. During this process, the amino acid is first activated by the addition of adenosine monophosphate (AMP) and then transferred to the tRNA, making it a charged tRNA, and AMP is released.

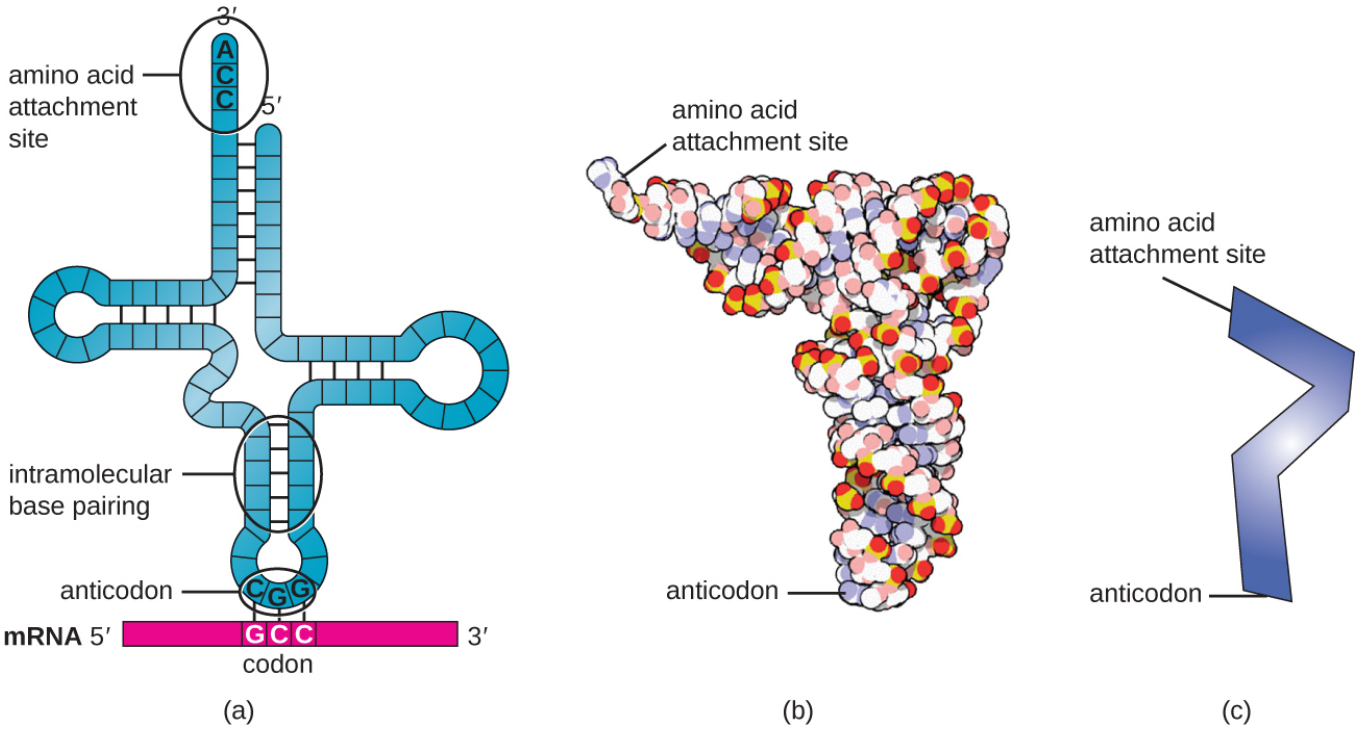


Figure **9.4.3**: (a) After folding caused by intramolecular base pairing, a tRNA molecule has one end that contains the anticodon, which interacts with the mRNA codon, and the CCA amino acid binding end. (b) A space-filling model is helpful for visualizing the three-dimensional shape of tRNA. (c) Simplified models are useful when drawing complex processes such as protein synthesis.

? Exercise 9.4.2

1. Describe the structure and composition of the prokaryotic ribosome.
2. In what direction is the mRNA template read?
3. Describe the structure and function of a tRNA.

The Mechanism of Protein Synthesis

Translation is similar in prokaryotes and eukaryotes. Here we will explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between bacterial and eukaryotic translation.

Initiation

The initiation of protein synthesis begins with the formation of an initiation complex. In *E. coli*, this complex involves the small 30S ribosome, the mRNA template, three initiation factors that help the ribosome assemble correctly, guanosine triphosphate (GTP) that acts as an energy source, and a special initiator tRNA carrying *N*-formyl-methionine(fMet-tRNA^{fMet}) (Figure **9.4.4**). The initiator tRNA interacts with the start codon AUG of the mRNA and carries a formylated methionine (fMet). Because of its involvement in initiation, fMet is inserted at the beginning (N terminus) of every polypeptide chain synthesized by *E. coli*. In *E. coli* mRNA, a leader sequence upstream of the first AUG codon, called the Shine-Dalgarno sequence (also known as the ribosomal binding site AGGAGG), interacts through complementary base pairing with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. At this point, the 50S ribosomal subunit then binds to the initiation complex, forming an intact ribosome.

In eukaryotes, initiation complex formation is similar, with the following differences:

- The initiator tRNA is a different specialized tRNA carrying methionine, called Met-tRNAi
- Instead of binding to the mRNA at the Shine-Dalgarno sequence, the eukaryotic initiation complex recognizes the 5’ cap of the eukaryotic mRNA, then tracks along the mRNA in the 5’ to 3’ direction until the AUG start codon is recognized. At this point, the 60S subunit binds to the complex of Met-tRNAi, mRNA, and the 40S subunit.

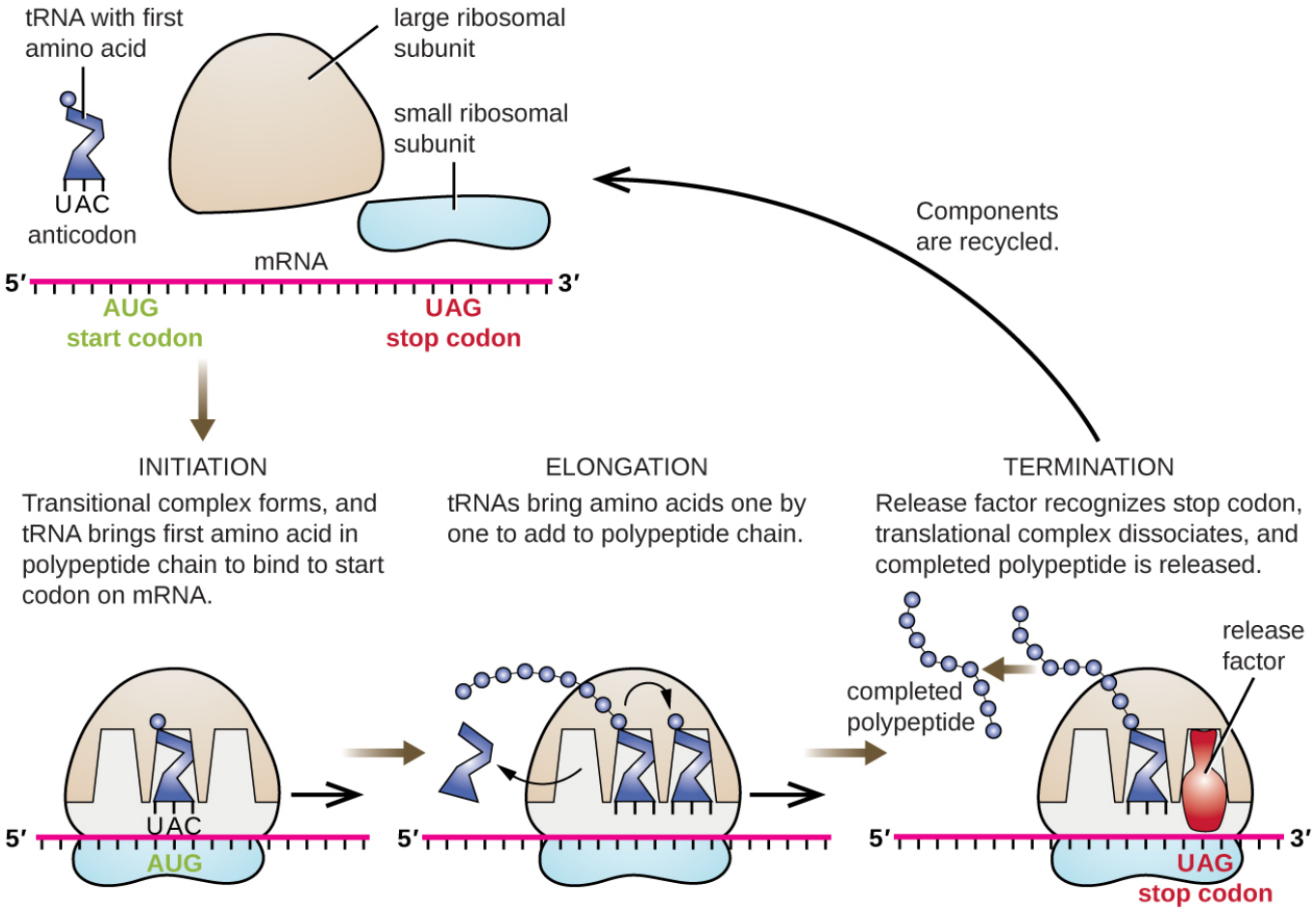


Figure **9.4.4**: Translation in bacteria begins with the formation of the initiation complex, which includes the small ribosomal subunit, the mRNA, the initiator tRNA carrying N-formyl-methionine, and initiation factors. Then the 50S subunit binds, forming an intact ribosome.

Elongation

In prokaryotes and eukaryotes, the basics of elongation of translation are the same. In *E. coli*, the binding of the 50S ribosomal subunit to produce the intact ribosome forms three functionally important ribosomal sites: The A (aminoacyl) site binds incoming charged aminoacyl tRNAs. The P (peptidyl) site binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The E (exit) site releases dissociated tRNAs so that they can be recharged with free amino acids. There is one notable exception to this assembly line of tRNAs: During initiation complex formation, bacterial fMet−tRNA^{fMet} or eukaryotic Met-tRNAi enters the P site directly without first entering the A site, providing a free A site ready to accept the tRNA corresponding to the first codon after the AUG.

Elongation proceeds with single-codon movements of the ribosome each called a translocation event. During each translocation event, the charged tRNAs enter at the A site, then shift to the P site, and then finally to the E site for removal. Ribosomal movements, or steps, are induced by conformational changes that advance the ribosome by three bases in the 3’ direction. Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA. The formation of each peptide bond is catalyzed by peptidyl transferase, an RNA-based ribozyme that is integrated into the 50S ribosomal subunit. The amino acid bound to the P-site tRNA is also linked to the growing polypeptide chain. As the ribosome steps across the mRNA, the former P-site tRNA enters the E site, detaches from the amino acid, and is expelled. Several of the steps during elongation, including binding of a charged aminoacyl tRNA to the A site and translocation, require energy derived from GTP hydrolysis, which is catalyzed by specific elongation factors. Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200 amino-acid protein can be translated in just 10 seconds.

Termination

The termination of translation occurs when a nonsense codon (UAA, UAG, or UGA) is encountered for which there is no complementary tRNA. On aligning with the A site, these nonsense codons are recognized by release factors in prokaryotes and eukaryotes that result in the P-site amino acid detaching from its tRNA, releasing the newly made polypeptide. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation init iation complex.

In summary, there are several key features that distinguish prokaryotic gene expression from that seen in eukaryotes. These are illustrated in Figure **9.4.5** and listed in Figure **9.4.6**.

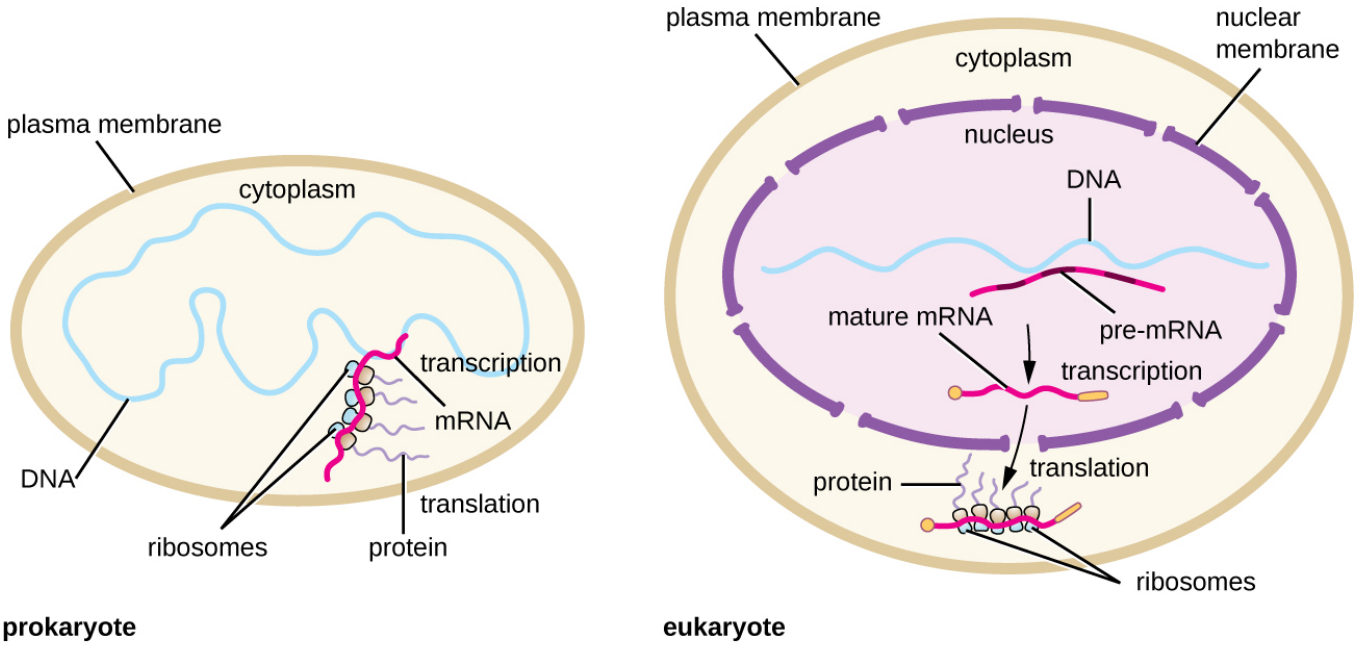


Figure **9.4.5**: (a) In prokaryotes, the processes of transcription and translation occur simultaneously in the cytoplasm, allowing for a rapid cellular response to an environmental cue. (b) In eukaryotes, transcription is localized to the nucleus and translation is localized to the cytoplasm, separating these processes and necessitating RNA processing for stability.

Comparison of Translation in Bacteria Versus Eukaryotes

Property	Bacteria	Eukaryotes
Ribosomes	70S • 30S (small subunit) with 16S rRNA subunit • 50S (large subunit) with 5S and 23S rRNA subunits	80S • 40S (small subunit) with 18S rRNA subunit • 60S (large subunit) with 5S, 5.8S, and 28S rRNA subunits
Amino acid carried by initiator tRNA	fMet	Met
Shine-Dalgarno sequence in mRNA	Present	Absent
Simultaneous transcription and translation	Yes	No

Figure **9.4.6**: Comparison of translation in bacteria versus eukaryotes

Protein Targeting, Folding, and Modification

During and after translation, polypeptides may need to be modified before they are biologically active. Post-translational modifications include:

1. removal of translated signal sequences—short tails of amino acids that aid in directing a protein to a specific cellular compartment
2. proper “folding” of the polypeptide and association of multiple polypeptide subunits, often facilitated by chaperone proteins, into a distinct three-dimensional structure
3. proteolytic processing of an inactive polypeptide to release an active protein component, and
4. various chemical modifications (e.g., phosphorylation, methylation, or glycosylation) of individual amino acids.

? Exercise 9.4.3

1. What are the components of the initiation complex for translation in prokaryotes?
2. What are two differences between initiation of prokaryotic and eukaryotic translation?
3. What occurs at each of the three active sites of the ribosome?
4. What causes termination of translation?

Key Concepts and Summary

- In **translation**, polypeptides are synthesized using mRNA sequences and cellular machinery, including tRNAs that match mRNA **codons** to specific amino acids and ribosomes composed of RNA and proteins that catalyze the reaction.
- The **genetic code** is **degenerate** in that several mRNA codons code for the same amino acids. The genetic code is almost universal among living organisms.
- Prokaryotic (70S) and cytoplasmic eukaryotic (80S) ribosomes are each composed of a large subunit and a small subunit of differing sizes between the two groups. Each subunit is composed of rRNA and protein. Organelle ribosomes in eukaryotic cells resemble prokaryotic ribosomes.
- Some 60 to 90 species of tRNA exist in bacteria. Each tRNA has a three-nucleotide **anticodon** as well as a binding site for a **cognate amino acid**. All tRNAs with a specific anticodon will carry the same amino acid.
- **Initiation** of translation occurs when the small ribosomal subunit binds with **initiation factors** and an initiator tRNA at the **start codon** of an mRNA, followed by the binding to the initiation complex of the large ribosomal subunit.
- In prokaryotic cells, the start codon codes for N-formyl-methionine carried by a special initiator tRNA. In eukaryotic cells, the start codon codes for methionine carried by a special initiator tRNA. In addition, whereas ribosomal binding of the mRNA in prokaryotes is facilitated by the Shine-Dalgarno sequence within the mRNA, eukaryotic ribosomes bind to the 5’ cap of the mRNA.
- During the **elongation** stage of translation, a **charged tRNA** binds to mRNA in the **A site** of the ribosome; a peptide bond is catalyzed between the two adjacent amino acids, breaking the bond between the first amino acid and its tRNA; the ribosome moves one codon along the mRNA; and the first tRNA is moved from the **P site** of the ribosome to the **E site** and leaves the ribosomal complex.
- **Termination** of translation occurs when the ribosome encounters a **stop codon**, which does not code for a tRNA. Release factors cause the polypeptide to be released, and the ribosomal complex dissociates.
- In prokaryotes, transcription and translation may be coupled, with translation of an mRNA molecule beginning as soon as transcription allows enough mRNA exposure for the binding of a ribosome, prior to transcription termination. Transcription and translation are not coupled in eukaryotes because transcription occurs in the nucleus, whereas translation occurs in the cytoplasm or in association with the rough endoplasmic reticulum.
- Polypeptides often require one or more **post-translational modifications** to become biologically active.

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9.5: Mutations

Learning Objectives

- Compare point mutations and frameshift mutations
- Describe the differences between missense, nonsense, and silent mutations
- Describe the differences between light and dark repair
- Explain how different mutagens act
- Explain why the Ames test can be used to detect carcinogens
- Analyze sequences of DNA and identify examples of types of mutations

A mutation is a heritable change in the DNA sequence of an organism. The resulting organism, called a mutant, may have a recognizable change in phenotype compared to the wild type, which is the phenotype most commonly observed in nature. A change in the DNA sequence is conferred to mRNA through transcription, and may lead to an altered amino acid sequence in a protein on translation. Because proteins carry out the vast majority of cellular functions, a change in amino acid sequence in a protein may lead to an altered phenotype for the cell and organism.

Effects of Mutations on DNA Sequence

There are several types of mutations that are classified according to how the DNA molecule is altered. One type, called a point mutation, affects a single base and most commonly occurs when one base is substituted or replaced by another. Mutations also result from the addition of one or more bases, known as an insertion, or the removal of one or more bases, known as a deletion.

Exercise 9.5.1

What type of a mutation occurs when a gene has two fewer nucleotides in its sequence?

Effects of Mutations on Protein Structure and Function

Point mutations may have a wide range of effects on protein function (Figure **9.5.1**). As a consequence of the degeneracy of the genetic code, a point mutation will commonly result in the same amino acid being incorporated into the resulting polypeptide despite the sequence change. This change would have no effect on the protein's structure, and is thus called a silent mutation. A missense mutation results in a different amino acid being incorporated into the resulting polypeptide. The effect of a missense mutation depends on how chemically different the new amino acid is from the wild-type amino acid. The location of the changed amino acid within the protein also is important. For example, if the changed amino acid is part of the enzyme's active site, then the effect of the missense mutation may be significant. Many missense mutations result in proteins that are still functional, at least to some degree. Sometimes the effects of missense mutations may be only apparent under certain environmental conditions; such missense mutations are called conditional mutations. Rarely, a missense mutation may be beneficial. Under the right environmental conditions, this type of mutation may give the organism that harbors it a selective advantage. Yet another type of point mutation, called a nonsense mutation, converts a codon encoding an amino acid (a sense codon) into a stop codon (a nonsense codon). Nonsense mutations result in the synthesis of proteins that are shorter than the wild type and typically not functional.

Deletions and insertions also cause various effects. Because codons are triplets of nucleotides, insertions or deletions in groups of three nucleotides may lead to the insertion or deletion of one or more amino acids and may not cause significant effects on the resulting protein's functionality. However, frameshift mutations, caused by insertions or deletions of a number of nucleotides that are not a multiple of three are extremely problematic because a shift in the reading frame results (Figure **9.5.1**). Because ribosomes read the mRNA in triplet codons, frameshift mutations can change every amino acid after the point of the mutation. The new reading frame may also include a stop codon before the end of the coding sequence. Consequently, proteins made from genes containing frameshift mutations are nearly always nonfunctional.

point mutation: substitution of a single base

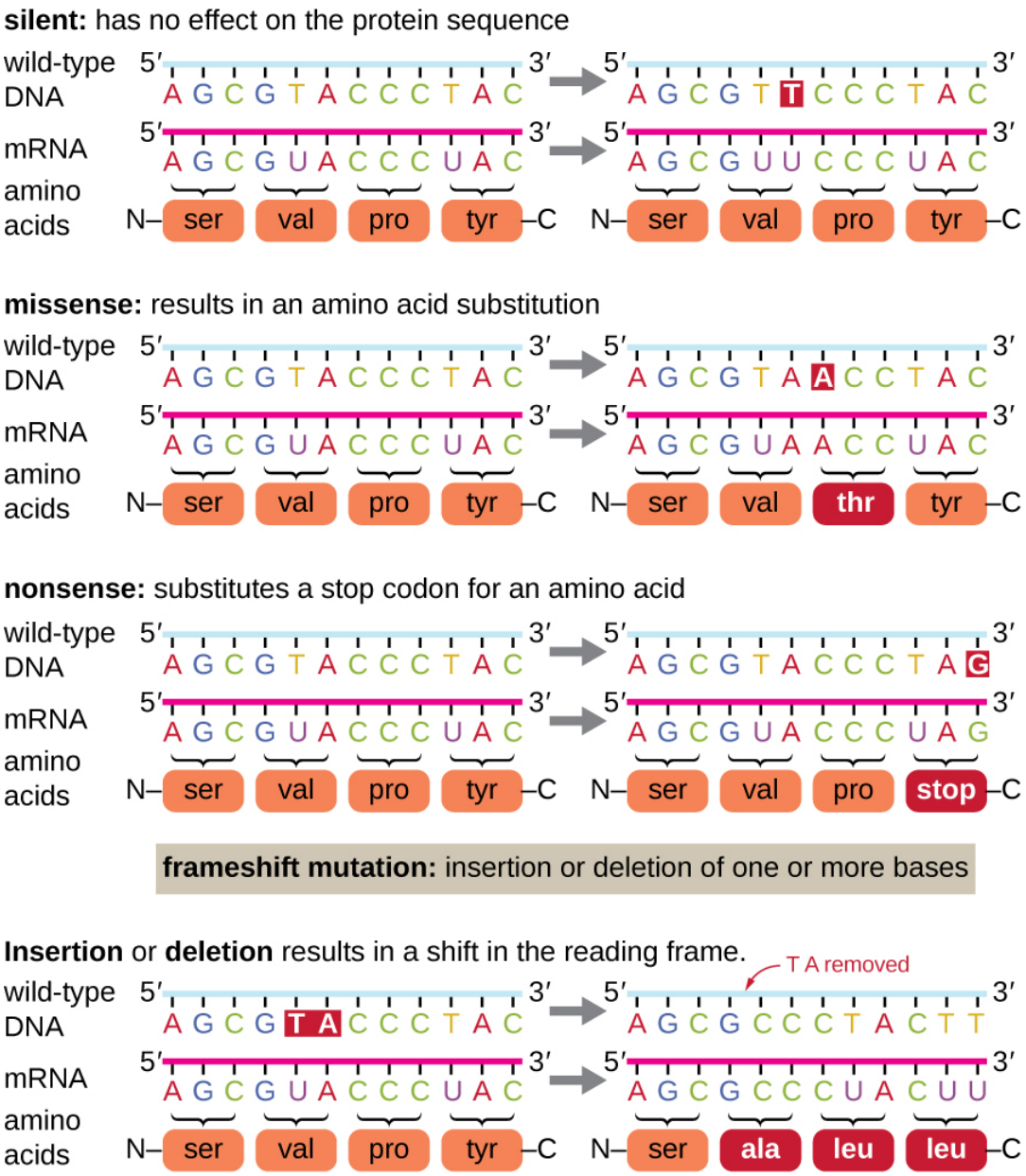


Figure **9.5.1**: Mutations can lead to changes in the protein sequence encoded by the DNA.

Exercise 9.5.2

1. What are the reasons a nucleotide change in a gene for a protein might not have any effect on the phenotype of that gene?
2. Is it possible for an insertion of three nucleotides together after the fifth nucleotide in a protein-coding gene to produce a protein that is shorter than normal? How or how not?

A Beneficial Mutation

Since the first case of infection with human immunodeficiency virus (HIV) was reported in 1981, nearly 40 million people have died from HIV infection,[1] the virus that causes acquired immune deficiency syndrome (AIDS). The virus targets helper T cells that play a key role in bridging the innate and adaptive immune response, infecting and killing cells normally involved in

the body's response to infection. There is no cure for HIV infection, but many drugs have been developed to slow or block the progression of the virus. Although individuals around the world may be infected, the highest prevalence among people 15–49 years old is in sub-Saharan Africa, where nearly one person in 20 is infected, accounting for greater than 70% of the infections worldwide[2] (Figure **9.5.2**). Unfortunately, this is also a part of the world where prevention strategies and drugs to treat the infection are the most lacking.

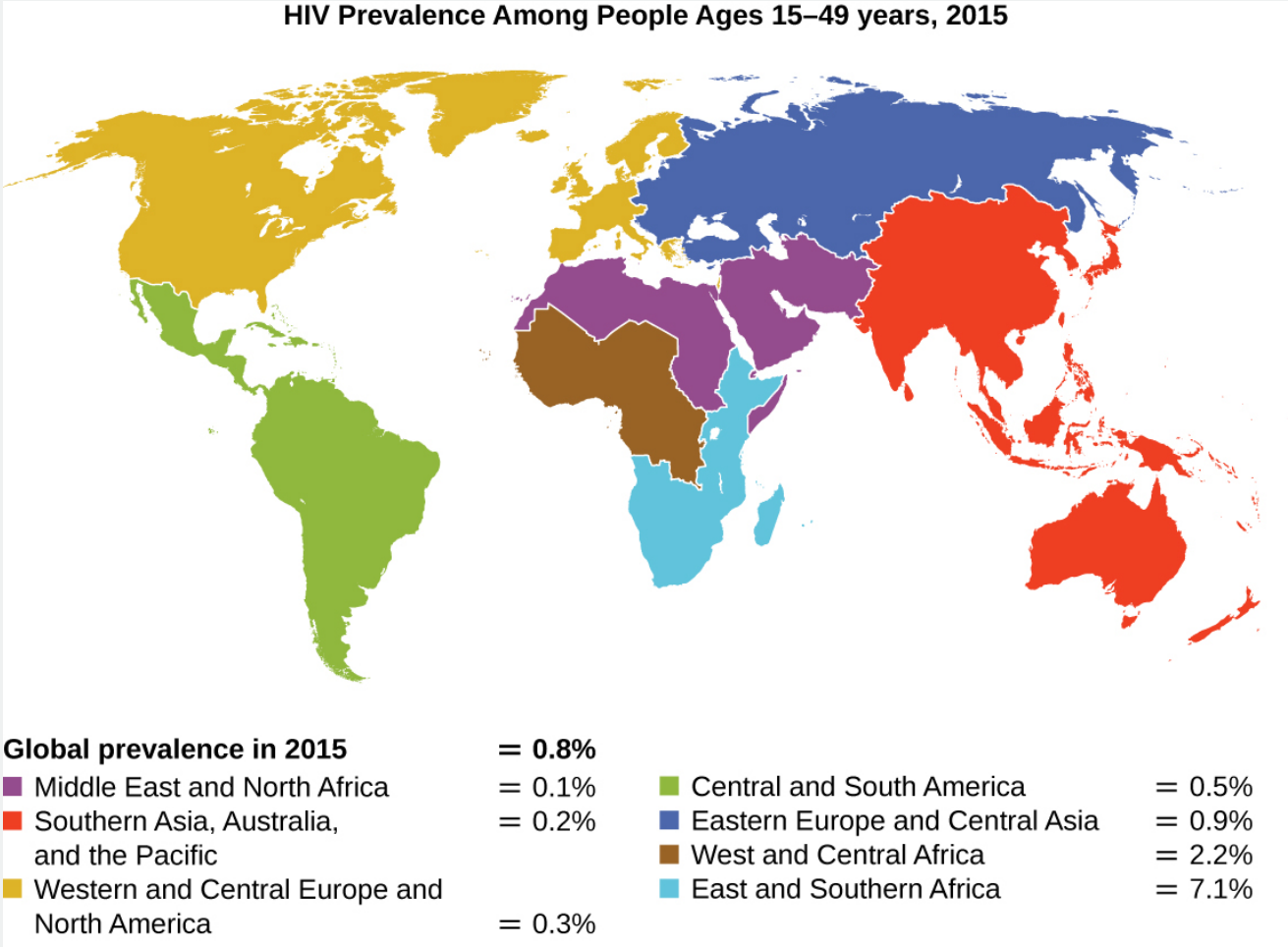


Figure **9.5.2**: HIV is highly prevalent in sub-Saharan Africa, but its prevalence is quite low in some other parts of the world.

In recent years, scientific interest has been piqued by the discovery of a few individuals from northern Europe who are resistant to HIV infection. In 1998, American geneticist Stephen J. O'Brien at the National Institutes of Health (NIH) and colleagues published the results of their genetic analysis of more than 4,000 individuals. These indicated that many individuals of Eurasian descent (up to 14% in some ethnic groups) have a deletion mutation, called CCR5-delta 32, in the gene encoding CCR5. CCR5 is a coreceptor found on the surface of T cells that is necessary for many strains of the virus to enter the host cell. The mutation leads to the production of a receptor to which HIV cannot effectively bind and thus blocks viral entry. People homozygous for this mutation have greatly reduced susceptibility to HIV infection, and those who are heterozygous have some protection from infection as well.

It is not clear why people of northern European descent, specifically, carry this mutation, but its prevalence seems to be highest in northern Europe and steadily decreases in populations as one moves south. Research indicates that the mutation has been present since before HIV appeared and may have been selected for in European populations as a result of exposure to the plague or smallpox. This mutation may protect individuals from plague (caused by the bacterium *Yersinia pestis*) and smallpox (caused by the variola virus) because this receptor may also be involved in these diseases. The age of this mutation is a matter of debate, but estimates suggest it appeared between 1875 years to 225 years ago, and may have been spread from Northern Europe through Viking invasions.

> This exciting finding has led to new avenues in HIV research, including looking for drugs to block CCR5 binding to HIV in individuals who lack the mutation. Although DNA testing to determine which individuals carry the CCR5-delta 32 mutation is possible, there are documented cases of individuals homozygous for the mutation contracting HIV. For this reason, DNA testing for the mutation is not widely recommended by public health officials so as not to encourage risky behavior in those who carry the mutation. Nevertheless, inhibiting the binding of HIV to CCR5 continues to be a valid strategy for the development of drug therapies for those infected with HIV.

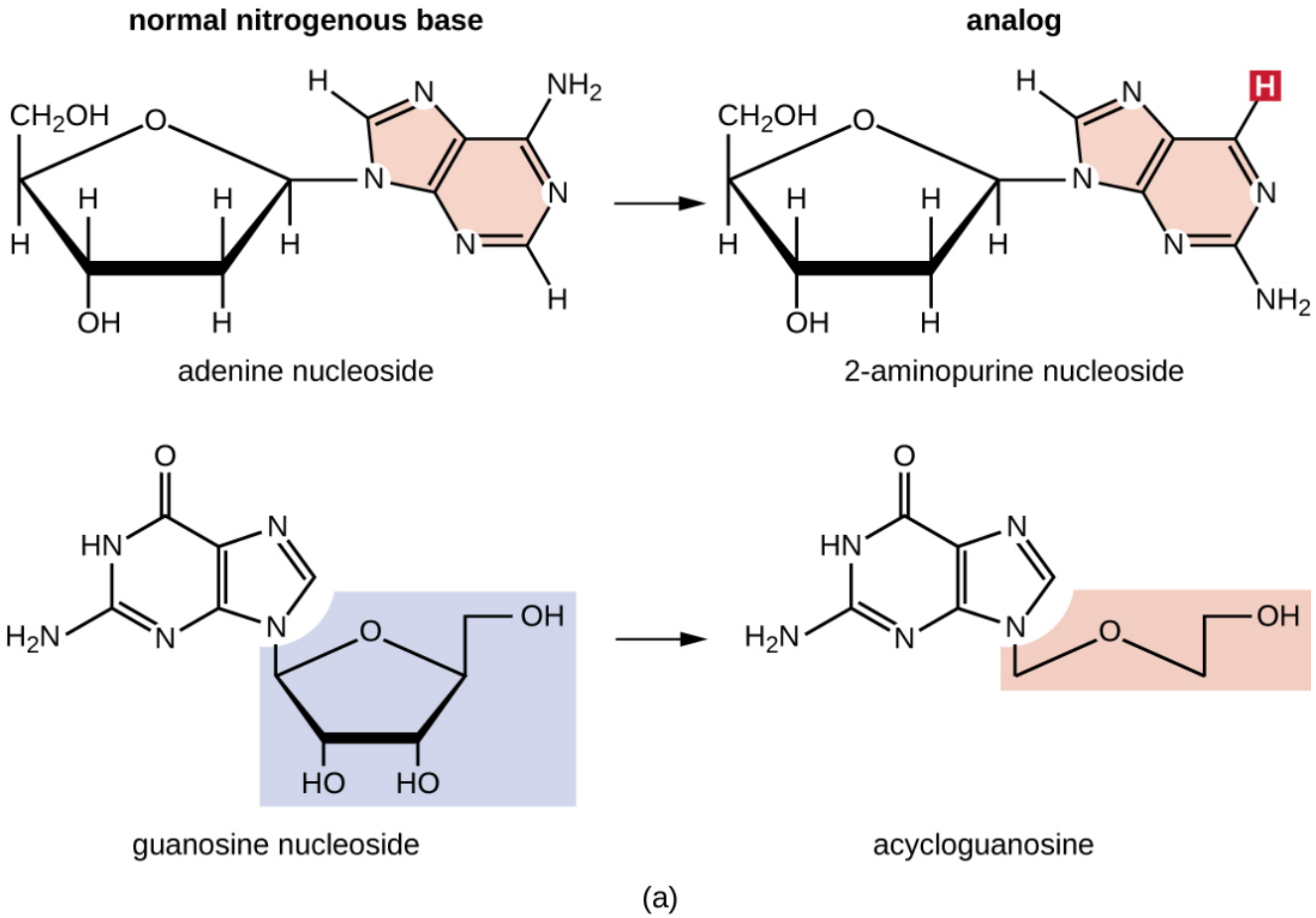
Causes of Mutations

Mistakes in the process of DNA replication can cause spontaneous mutations to occur. The error rate of DNA polymerase is one incorrect base per billion base pairs replicated. Exposure to mutagens can cause induced mutations, which are various types of chemical agents or radiation (Table **9.5.1**). Exposure to a mutagen can increase the rate of mutation more than 1000-fold. Mutagens are often also carcinogens, agents that cause cancer. However, whereas nearly all carcinogens are mutagenic, not all mutagens are necessarily carcinogens.

Chemical Mutagens

Various types of chemical mutagens interact directly with DNA either by acting as nucleoside analogs or by modifying nucleotide bases. Chemicals called nucleoside analogs are structurally similar to normal nucleotide bases and can be incorporated into DNA during replication (Figure **9.5.3**). These base analogs induce mutations because they often have different base-pairing rules than the bases they replace. Other chemical mutagens can modify normal DNA bases, resulting in different base-pairing rules. For example, nitrous acid deaminates cytosine, converting it to uracil. Uracil then pairs with adenine in a subsequent round of replication, resulting in the conversion of a GC base pair to an AT base pair. Nitrous acid also deaminates adenine to hypoxanthine, which base pairs with cytosine instead of thymine, resulting in the conversion of a TA base pair to a CG base pair.

Chemical mutagens known as intercalating agents work differently. These molecules slide between the stacked nitrogenous bases of the DNA double helix, distorting the molecule and creating atypical spacing between nucleotide base pairs (Figure **9.5.4**). As a result, during DNA replication, DNA polymerase may either skip replicating several nucleotides (creating a deletion) or insert extra nucleotides (creating an insertion). Either outcome may lead to a frameshift mutation. Combustion products like polycyclic aromatic hydrocarbons are particularly dangerous intercalating agents that can lead to mutation-caused cancers. The intercalating agents ethidium bromide and acridine orange are commonly used in the laboratory to stain DNA for visualization and are potential mutagens.



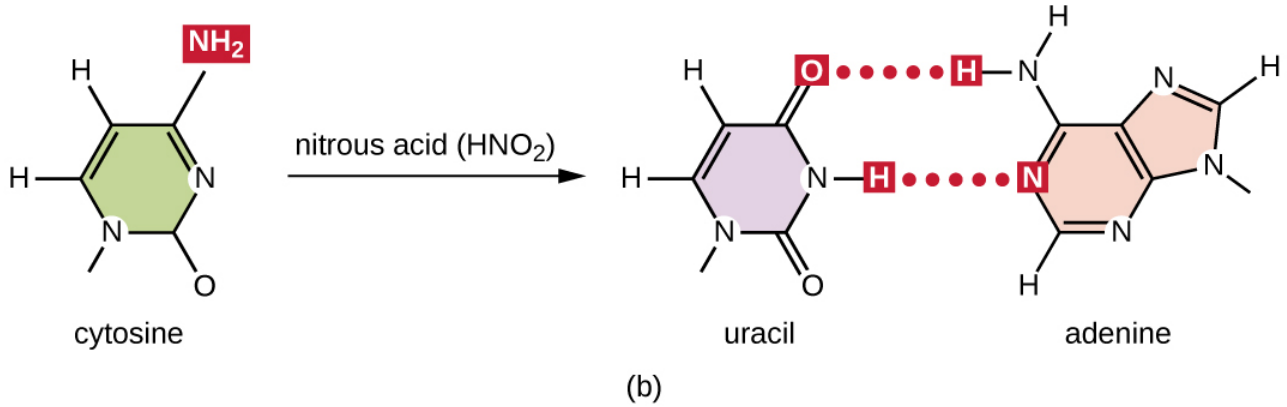


Figure **9.5.3**: (a) 2-aminopurine nucleoside (2AP) structurally is a nucleoside analog to adenine nucleoside, whereas 5-bromouracil (5BU) is a nucleoside analog to thymine nucleoside. 2AP base pairs with C, converting an AT base pair to a GC base pair after several rounds of replication. 5BU pairs with G, converting an AT base pair to a GC base pair after several rounds of replication. (b) Nitrous acid is a different type of chemical mutagen that modifies already existing nucleoside bases like C to produce U, which base pairs with A. This chemical modification, as shown here, results in converting a CG base pair to a TA base pair.

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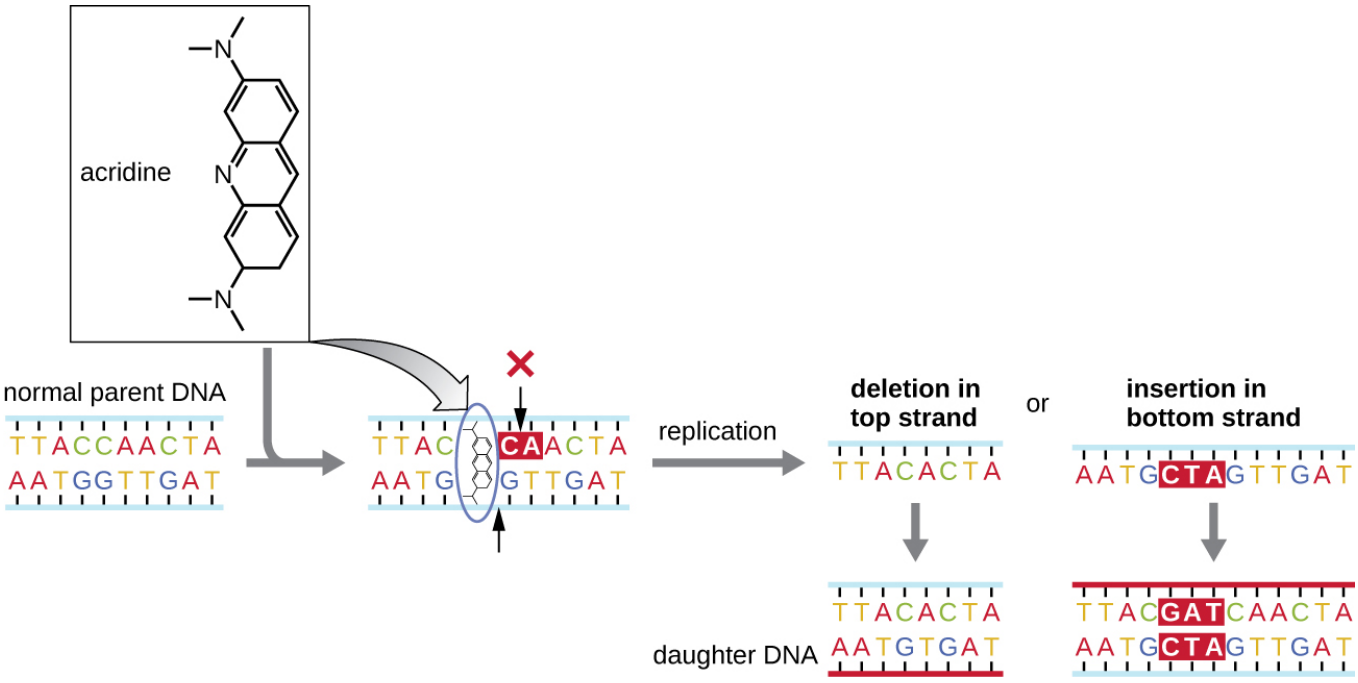
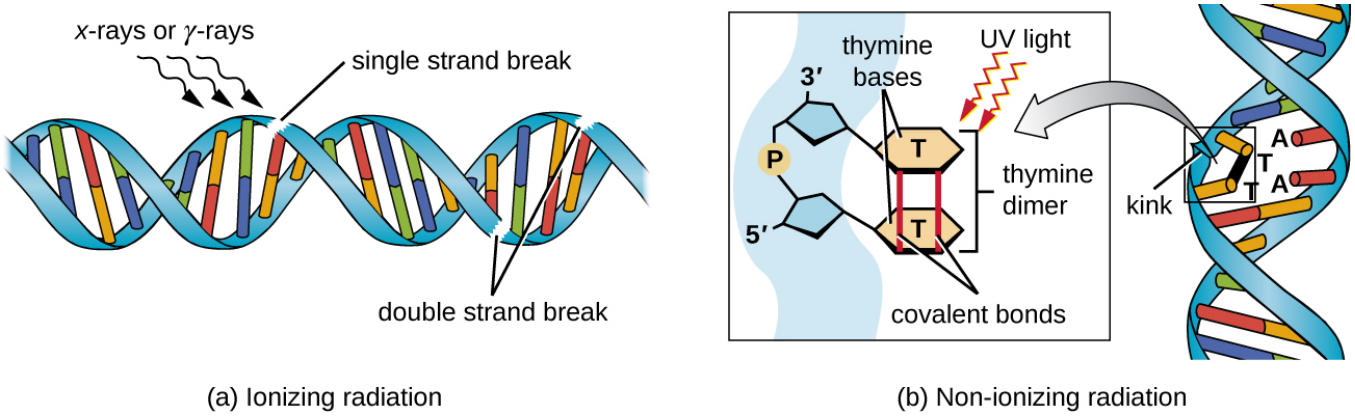


Figure **9.5.4**: Intercalating agents, such as acridine, introduce atypical spacing between base pairs, resulting in DNA polymerase introducing either a deletion or an insertion, leading to a potential frameshift mutation.

Radiation

Exposure to either ionizing or nonionizing radiation can each induce mutations in DNA, although by different mechanisms. Strong ionizing radiation like X-rays and gamma rays can cause single- and double-stranded breaks in the DNA backbone through the formation of hydroxyl radicals on radiation exposure (Figure **9.5.5**). Ionizing radiation can also modify bases; for example, the deamination of cytosine to uracil, analogous to the action of nitrous acid.[3] Ionizing radiation exposure is used to kill microbes to sterilize medical devices and foods, because of its dramatic nonspecific effect in damaging DNA, proteins, and other cellular components (see Using Physical Methods to Control Microorganisms).

Nonionizing radiation, like ultraviolet light, is not energetic enough to initiate these types of chemical changes. However, nonionizing radiation can induce dimer formation between two adjacent pyrimidine bases, commonly two thymines, within a nucleotide strand. During thymine dimer formation, the two adjacent thymines become covalently linked and, if left unrepaired, both DNA replication and transcription are stalled at this point. DNA polymerase may proceed and replicate the dimer incorrectly, potentially leading to frameshift or point mutations.



(a) Ionizing radiation (b) Non-ionizing radiation

Figure **9.5.5**: (a) Ionizing radiation may lead to the formation of single-stranded and double-stranded breaks in the sugar-phosphate backbone of DNA, as well as to the modification of bases (not shown). (b) Nonionizing radiation like ultraviolet light can lead to the formation of thymine dimers, which can stall replication and transcription and introduce frameshift or point mutations.

Table **9.5.1**: A Summary of Mutagenic Agents

Mutagenic Agents	Mode of Action	Effect on DNA	Resulting Type of Mutation
Nucleoside analogs			
2-aminopurine	Is inserted in place of A but base pairs with C	Converts AT to GC base pair	Point
5-bromouracil	Is inserted in place of T but base pairs with G	Converts AT to GC base pair	Point
Nucleotide-modifying agent			
Nitrous oxide	Deaminates C to U	Converts GC to AT base pair	Point
Intercalating agents			
Acridine orange, ethidium bromide, polycyclic aromatic hydrocarbons	Distorts double helix, creates unusual spacing between nucleotides	Introduces small deletions and insertions	Frameshift
Ionizing radiation			
X-rays, γ-rays	Forms hydroxyl radicals	Causes single- and double-strand DNA breaks	Repair mechanisms may introduce mutations
X-rays, γ-rays	Modifies bases (e.g., deaminating C to U)	Converts GC to AT base pair	Point
Nonionizing radiation			
Ultraviolet	Forms pyrimidine (usually thymine) dimers	Causes DNA replication errors	Frameshift or point

? Exercise 9.5.3

1. How does a base analog introduce a mutation?
2. How does an intercalating agent introduce a mutation?
3. What type of mutagen causes thymine dimers?

DNA Repair

The process of DNA replication is highly accurate, but mistakes can occur spontaneously or be induced by mutagens. Uncorrected mistakes can lead to serious consequences for the phenotype. Cells have developed several repair mechanisms to minimize the number of mutations that persist.

Proofreading

Most of the mistakes introduced during DNA replication are promptly corrected by most DNA polymerases through a function called proofreading. In proofreading, the DNA polymerase reads the newly added base, ensuring that it is complementary to the corresponding base in the template strand before adding the next one. If an incorrect base has been added, the enzyme makes a cut to release the wrong nucleotide and a new base is added.

Mismatch Repair

Some errors introduced during replication are corrected shortly after the replication machinery has moved. This mechanism is called mismatch repair. The enzymes involved in this mechanism recognize the incorrectly added nucleotide, excise it, and replace it with the correct base. One example is the methyl-directed mismatch repair in *E. coli*. The DNA is hemimethylated. This means that the parental strand is methylated while the newly synthesized daughter strand is not. It takes several minutes before the new strand is methylated. Proteins MutS, MutL, and MutH bind to the hemimethylated site where the incorrect nucleotide is found.

MutH cuts the nonmethylated strand (the new strand). An exonuclease removes a portion of the strand (including the incorrect nucleotide). The gap formed is then filled in by DNA pol III and ligase.

Repair of Thymine Dimers

Because the production of thymine dimers is common (many organisms cannot avoid ultraviolet light), mechanisms have evolved to repair these lesions. In nucleotide excision repair (also called dark repair), enzymes remove the pyrimidine dimer and replace it with the correct nucleotides (Figure **9.5.6**). In *E. coli*, the DNA is scanned by an enzyme complex. If a distortion in the double helix is found that was introduced by the pyrimidine dimer, the enzyme complex cuts the sugar-phosphate backbone several bases upstream and downstream of the dimer, and the segment of DNA between these two cuts is then enzymatically removed. DNA pol I replaces the missing nucleotides with the correct ones and DNA ligase seals the gap in the sugar-phosphate backbone.

The direct repair (also called light repair) of thymine dimers occurs through the process of photoreactivation in the presence of visible light. An enzyme called photolyase recognizes the distortion in the DNA helix caused by the thymine dimer and binds to the dimer. Then, in the presence of visible light, the photolyase enzyme changes conformation and breaks apart the thymine dimer, allowing the thymines to again correctly base pair with the adenines on the complementary strand. Photoreactivation appears to be present in all organisms, with the exception of placental mammals, including humans. Photoreactivation is particularly important for organisms chronically exposed to ultraviolet radiation, like plants, photosynthetic bacteria, algae, and corals, to prevent the accumulation of mutations caused by thymine dimer formation.

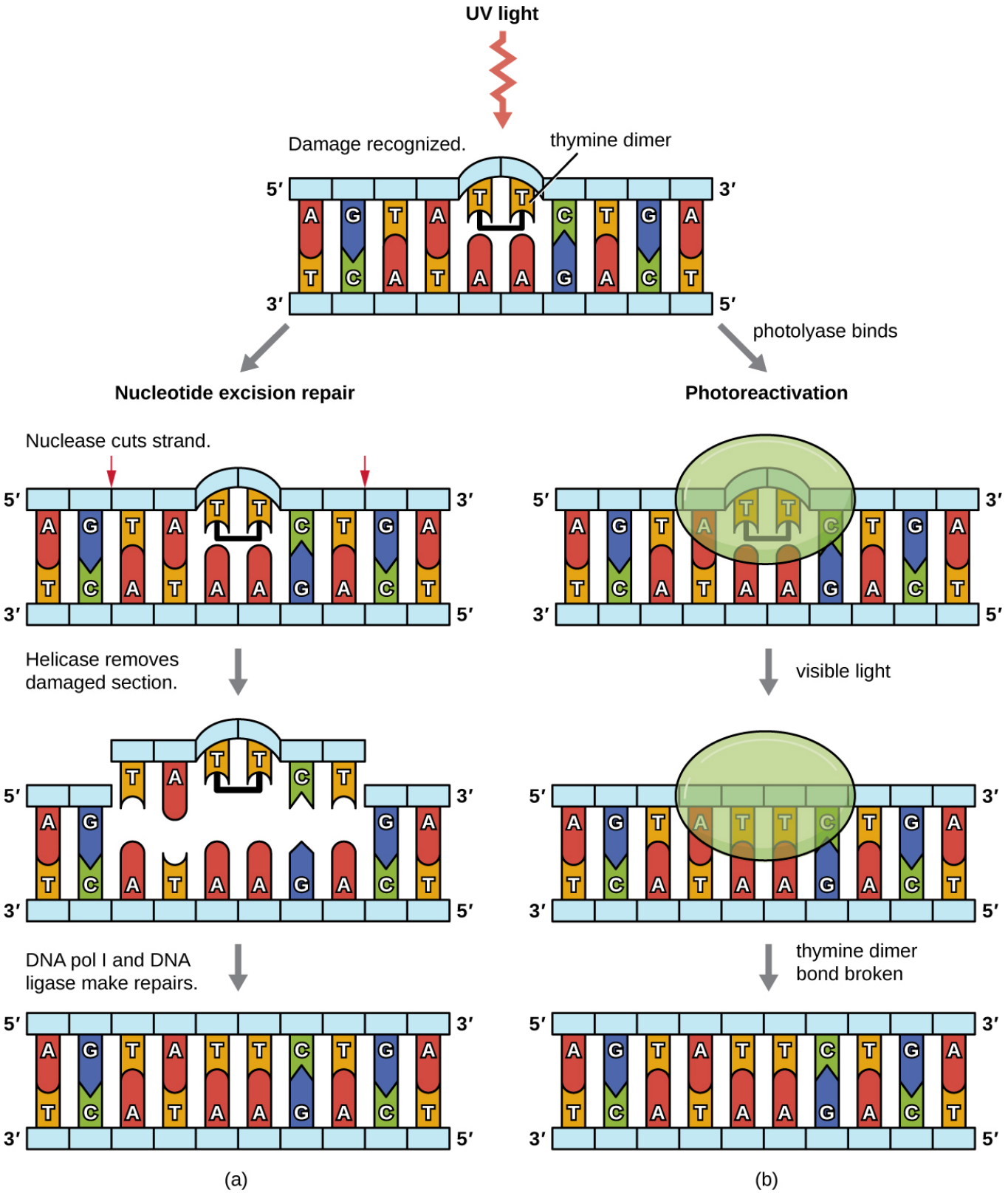


Figure **9.5.6**: Bacteria have two mechanisms for repairing thymine dimers. (a) In nucleotide excision repair, an enzyme complex recognizes the distortion in the DNA complex around the thymine dimer and cuts and removes the damaged DNA strand. The correct nucleotides are replaced by DNA pol I and the nucleotide strand is sealed by DNA ligase. (b) In photoreactivation, the enzyme photolyase binds to the thymine dimer and, in the presence of visible light, breaks apart the dimer, restoring the base pairing of the thymines with complementary adenines on the opposite DNA strand.

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? Exercise 9.5.4

1. During mismatch repair, how does the enzyme recognize which is the new and which is the old strand?
2. What type of mutation does photolyase repair?

Identifying Bacterial Mutants

One common technique used to identify bacterial mutants is called replica plating. This technique is used to detect nutritional mutants, called auxotrophs, which have a mutation in a gene encoding an enzyme in the biosynthesis pathway of a specific nutrient, such as an amino acid. As a result, whereas wild-type cells retain the ability to grow normally on a medium lacking the specific nutrient, auxotrophs are unable to grow on such a medium. During replica plating (Figure **9.5.7**), a population of bacterial cells is mutagenized and then plated as individual cells on a complex nutritionally complete plate and allowed to grow into colonies. Cells from these colonies are removed from this master plate, often using sterile velvet. This velvet, containing cells, is then pressed in the same orientation onto plates of various media. At least one plate should also be nutritionally complete to ensure that cells are being properly transferred between the plates. The other plates lack specific nutrients, allowing the researcher to discover various auxotrophic mutants unable to produce specific nutrients. Cells from the corresponding colony on the nutritionally complete plate can be used to recover the mutant for further study.

? Exercise 9.5.5

Why are cells plated on a nutritionally complete plate in addition to nutrient-deficient plates when looking for a mutant?

The Ames Test

The Ames test, developed by Bruce Ames (1928–) in the 1970s, is a method that uses bacteria for rapid, inexpensive screening of the carcinogenic potential of new chemical compounds. The test measures the mutation rate associated with exposure to the compound, which, if elevated, may indicate that exposure to this compound is associated with greater cancer risk. The Ames test uses as the test organism a strain of *Salmonella typhimurium* that is a histidine auxotroph, unable to synthesize its own histidine because of a mutation in an essential gene required for its synthesis. After exposure to a potential mutagen, these bacteria are plated onto a medium lacking histidine, and the number of mutants regaining the ability to synthesize histidine is recorded and compared with the number of such mutants that arise in the absence of the potential mutagen (Figure **9.5.8**). Chemicals that are more mutagenic will bring about more mutants with restored histidine synthesis in the Ames test. Because many chemicals are not directly mutagenic but are metabolized to mutagenic forms by liver enzymes, rat liver extract is commonly included at the start of this experiment to mimic liver metabolism. After the Ames test is conducted, compounds identified as mutagenic are further tested for their potential carcinogenic properties by using other models, including animal models like mice and rats.

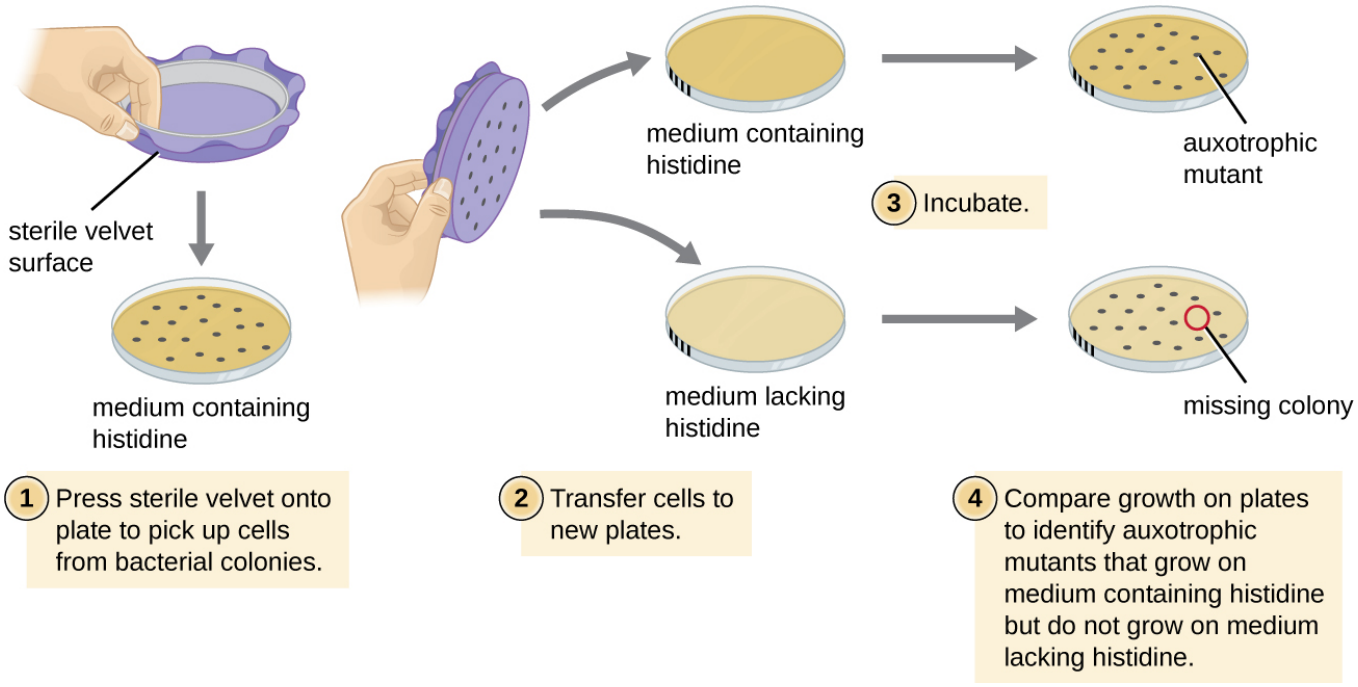


Figure 9.5.7: Identification of auxotrophic mutants, like histidine auxotrophs, is done using replica plating. After mutagenesis, colonies that grow on nutritionally complete medium but not on medium lacking histidine are identified as histidine auxotrophs.

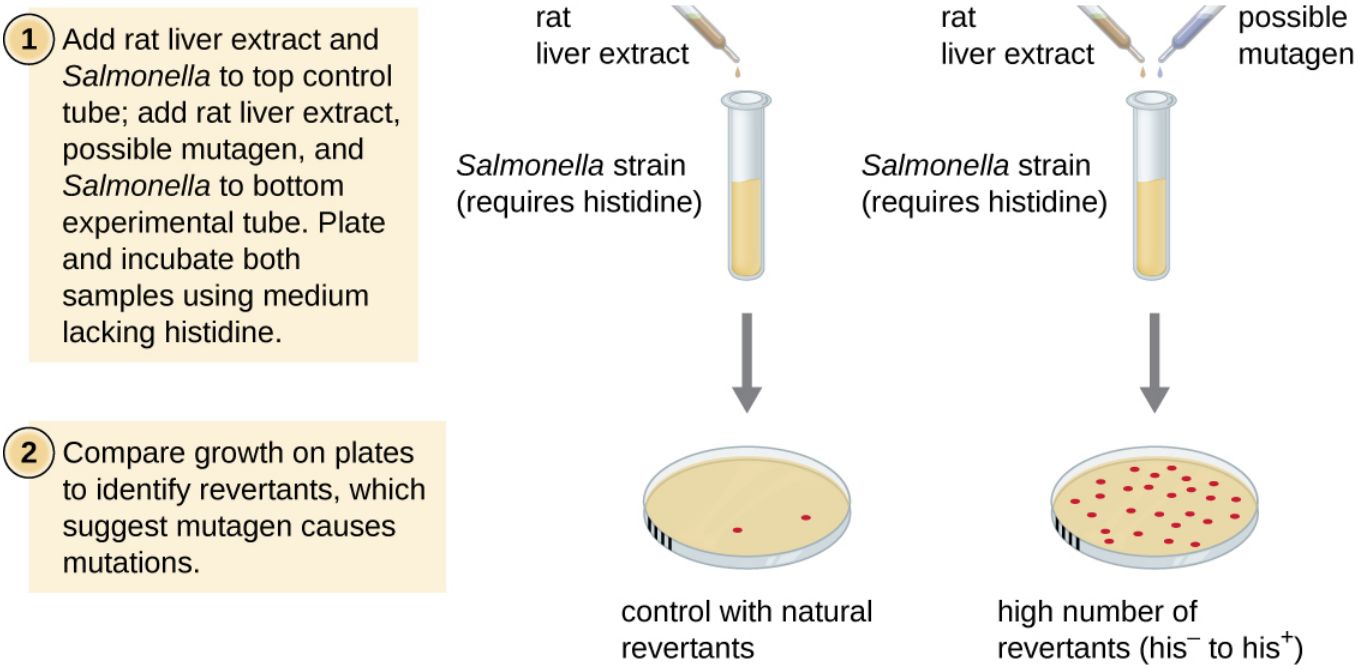


Figure 9.5.8: The Ames test is used to identify mutagenic, potentially carcinogenic chemicals. A *Salmonella* histidine auxotroph is used as the test strain, exposed to a potential mutagen/carcinogen. The number of reversion mutants capable of growing in the absence of supplied histidine is counted and compared with the number of natural reversion mutants that arise in the absence of the potential mutagen.

? Exercise 9.5.6

1. What mutation is used as an indicator of mutation rate in the Ames test?
2. Why can the Ames test work as a test for carcinogenicity?

Key Concepts and Summary

- A **mutation** is a heritable change in DNA. A mutation may lead to a change in the amino-acid sequence of a protein, possibly affecting its function.
- A **point mutation** affects a single base pair. A point mutation may cause a **silent mutation** if the mRNA codon codes for the same amino acid, a **missense mutation** if the mRNA codon codes for a different amino acid, or a **nonsense mutation** if the mRNA codon becomes a stop codon.
- Missense mutations may retain function, depending on the chemistry of the new amino acid and its location in the protein. Nonsense mutations produce truncated and frequently nonfunctional proteins.
- A **frameshift mutation** results from an insertion or deletion of a number of nucleotides that is not a multiple of three. The change in reading frame alters every amino acid after the point of the mutation and results in a nonfunctional protein.
- **Spontaneous mutations** occur through DNA replication errors, whereas **induced mutations** occur through exposure to a **mutagen**.
- Mutagenic agents are frequently carcinogenic but not always. However, nearly all carcinogens are mutagenic.
- Chemical mutagens include base analogs and chemicals that modify existing bases. In both cases, mutations are introduced after several rounds of DNA replication.
- **Ionizing radiation,** such as X-rays and γ-rays, leads to breakage of the phosphodiester backbone of DNA and can also chemically modify bases to alter their base-pairing rules.
- **Nonionizing radiation** like ultraviolet light may introduce pyrimidine (thymine) dimers, which, during DNA replication and transcription, may introduce frameshift or point mutations.
- Cells have mechanisms to repair naturally occurring mutations. DNA polymerase has proofreading activity. Mismatch repair is a process to repair incorrectly incorporated bases after DNA replication has been completed.
- Pyrimidine dimers can also be repaired. In **nucleotide excision repair (dark repair)**, enzymes recognize the distortion introduced by the pyrimidine dimer and replace the damaged strand with the correct bases, using the undamaged DNA strand as a template. Bacteria and other organisms may also use **direct repair**, in which the photolyase enzyme, in the presence of visible light, breaks apart the pyrimidines.
- Through comparison of growth on the complete plate and lack of growth on media lacking specific nutrients, specific loss-of-function mutants called **auxotrophs** can be identified.
- The **Ames test** is an inexpensive method that uses auxotrophic bacteria to measure mutagenicity of a chemical compound. Mutagenicity is an indicator of carcinogenic potential.

Footnotes

1. 1 World Health Organization. “ Global Health Observatory (GHO) Data, HIV/AIDS.” http://www.who.int/gho/hiv/en/. Accessed August 5, 2016.
2. 2 World Health Organization. “ Global Health Observatory (GHO) Data, HIV/AIDS.” http://www.who.int/gho/hiv/en/. Accessed August 5, 2016.
3. 3 K.R. Tindall et al. “Changes in DNA Base Sequence Induced by Gamma-Ray Mutagenesis of Lambda Phage and Prophage.” *Genetics* 118 no. 4 (1988):551–560.

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9.5A: DNA Repair

Most mistakes during replication are corrected by DNA polymerase during replication or by post-replication repair mechanisms.

LEARNING OBJECTIVES

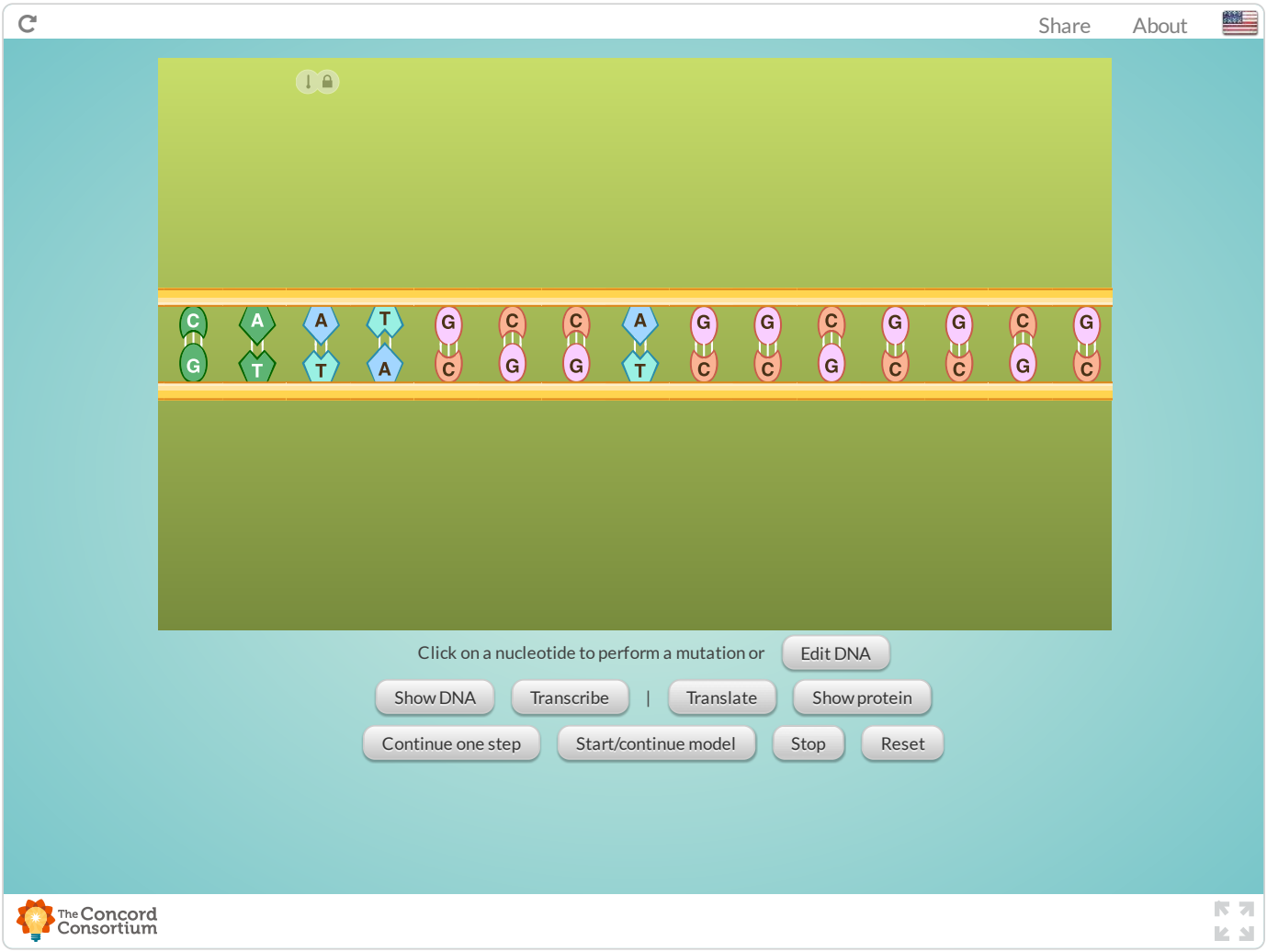
Explain how errors during replication are repaired

Key Takeaways

Errors during Replication

DNA replication is a highly accurate process, but mistakes can occasionally occur as when a DNA polymerase inserts a wrong base. Uncorrected mistakes may sometimes lead to serious consequences, such as cancer. Repair mechanisms can correct the mistakes, but in rare cases mistakes are not corrected, leading to mutations; in other cases, repair enzymes are themselves mutated or defective.

Mutations: In this interactive, you can "edit" a DNA strand and cause a mutation. Take a look at the effects!



Most of the mistakes during DNA replication are promptly corrected by DNA polymerase which proofreads the base that has just been added. In proofreading, the DNA pol reads the newly-added base before adding the next one so a correction can be made. The polymerase checks whether the newly-added base has paired correctly with the base in the template strand. If it is the correct base, the next nucleotide is added. If an incorrect base has been added, the enzyme makes a cut at the phosphodiester bond and releases the incorrect nucleotide. This is performed by the exonuclease action of DNA pol III. Once the incorrect nucleotide has been removed, a new one will be added again.

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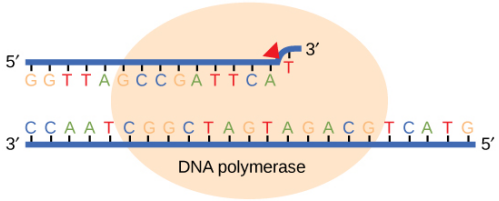


Figure: **DNA polymerase proofreading**: Proofreading by DNA polymerase corrects errors during replication.

Some errors are not corrected during replication, but are instead corrected after replication is completed; this type of repair is known as mismatch repair. The enzymes recognize the incorrectly-added nucleotide and excise it; this is then replaced by the correct base. If this remains uncorrected, it may lead to more permanent damage. How do mismatch repair enzymes recognize which of the two bases is the incorrect one? In *E. coli*, after replication, the nitrogenous base adenine acquires a methyl group; the parental DNA strand will have methyl groups, whereas the newly-synthesized strand lacks them. Thus, DNA polymerase is able to remove the incorrectly-incorporated bases from the newly-synthesized, non-methylated strand. In eukaryotes, the mechanism is not very well understood, but it is believed to involve recognition of unsealed nicks in the new strand, as well as a short-term continuing association of some of the replication proteins with the new daughter strand after replication has been completed.

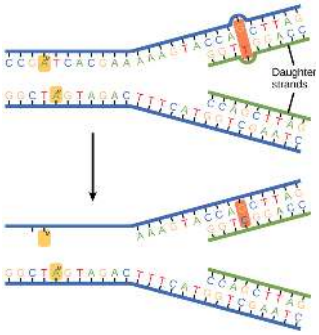


Figure: **Mismatch Repair**: In mismatch repair, the incorrectly-added base is detected after replication. The mismatch-repair proteins detect this base and remove it from the newly-synthesized strand by nuclease action. The gap is now filled with the correctly-paired base.

In another type of repair mechanism, nucleotide excision repair, enzymes replace incorrect bases by making a cut on both the 3′ and 5′ ends of the incorrect base. The segment of DNA is removed and replaced with the correctly-paired nucleotides by the action of DNA pol. Once the bases are filled in, the remaining gap is sealed with a phosphodiester linkage catalyzed by DNA ligase. This repair mechanism is often employed when UV exposure causes the formation of pyrimidine dimers.

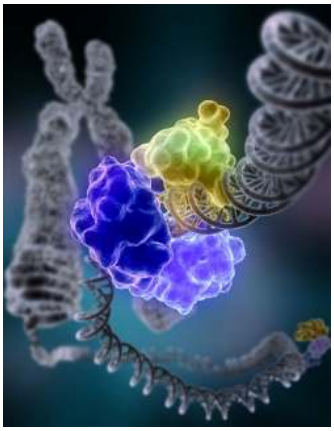


Figure: **DNA Ligase I Repairing Chromosomal Damage**: DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. A special enzyme, DNA ligase (shown here in color), encircles the double helix to repair a broken strand of DNA. DNA ligase is responsible for repairing the millions of DNA breaks generated during the normal course of a cell's life. Without molecules that can mend such breaks, cells can malfunction, die, or become cancerous. DNA ligases catalyse the crucial step of joining breaks in duplex DNA during DNA repair, replication and recombination, and require either Adenosine triphosphate (ATP) or Nicotinamide adenine dinucleotide (NAD+) as a cofactor.

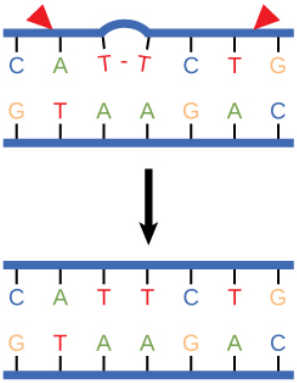


Figure: **Nucleotide Excision Repairs**: Nucleotide excision repairs thymine dimers. When exposed to UV, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced.

DNA Damage and Mutations

Errors during DNA replication are not the only reason why mutations arise in DNA. Mutations, variations in the nucleotide sequence of a genome, can also occur because of damage to DNA. Such mutations may be of two types: induced or spontaneous. Induced mutations are those that result from an exposure to chemicals, UV rays, X-rays, or some other environmental agent. Spontaneous mutations occur without any exposure to any environmental agent; they are a result of natural reactions taking place within the body.

Mutations may have a wide range of effects. Some mutations are not expressed; these are known as silent mutations. Point mutations are those mutations that affect a single base pair. The most common nucleotide mutations are substitutions, in which one base is replaced by another. These can be of two types: transitions or transversions. Transition substitution refers to a purine or pyrimidine being replaced by a base of the same kind; for example, a purine such as adenine may be replaced by the purine guanine. Transversion substitution refers to a purine being replaced by a pyrimidine or vice versa; for example, cytosine, a pyrimidine, is replaced by adenine, a purine. Mutations can also be the result of the addition of a base, known as an insertion, or the removal of a base, known as a deletion. Sometimes a piece of DNA from one chromosome may get translocated to another chromosome or to another region of the same chromosome.

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Key Points

- Mismatch repair enzymes recognize mis-incorporated bases, remove them from DNA, and replace them with the correct bases.
- In nucleotide excision repair, enzymes remove incorrect bases with a few surrounding bases, which are replaced with the correct bases with the help of a DNA polymerase and the template DNA.
- When replication mistakes are not corrected, they may result in mutations, which sometimes can have serious consequences.
- Point mutations, one base substituted for another, can be silent (no effect) or may have effects ranging from mild to severe.
- Mutations may also involve insertions (addition of a base), deletion (loss of a base), or translocation (movement of a DNA section to a new location on the same or another chromosome).

Key Terms

- **mismatch repair**: a system for recognizing and repairing some forms of DNA damage and erroneous insertion, deletion, or mis-incorporation of bases that can arise during DNA replication and recombination

- **nucleotide excision repair**: a DNA repair mechanism that corrects damage done by UV radiation, including thymine dimers and 6,4 photoproducts that cause bulky distortions in the DNA

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9.6: Gene Regulation - Operon Theory

Learning Objectives

- Compare inducible operons and repressible operons
- Describe why regulation of operons is important

Each nucleated cell in a multicellular organism contains copies of the same DNA. Similarly, all cells in two pure bacterial cultures inoculated from the same starting colony contain the same DNA, with the exception of changes that arise from spontaneous mutations. If each cell in a multicellular organism has the same DNA, then how is it that cells in different parts of the organism's body exhibit different characteristics? Similarly, how is it that the same bacterial cells within two pure cultures exposed to different environmental conditions can exhibit different phenotypes? In both cases, each genetically identical cell does not turn on, or express, the same set of genes. Only a subset of proteins in a cell at a given time is expressed.

Genomic DNA contains both structural genes, which encode products that serve as cellular structures or enzymes, and regulatory genes, which encode products that regulate gene expression. The expression of a gene is a highly regulated process. Whereas regulating gene expression in multicellular organisms allows for cellular differentiation, in single-celled organisms like prokaryotes, it primarily ensures that a cell's resources are not wasted making proteins that the cell does not need at that time.

Elucidating the mechanisms controlling gene expression is important to the understanding of human health. Malfunctions in this process in humans lead to the development of cancer and other diseases. Understanding the interaction between the gene expression of a pathogen and that of its human host is important for the understanding of a particular infectious disease. Gene regulation involves a complex web of interactions within a given cell among signals from the cell's environment, signaling molecules within the cell, and the cell's DNA. These interactions lead to the expression of some genes and the suppression of others, depending on circumstances.

Prokaryotes and eukaryotes share some similarities in their mechanisms to regulate gene expression; however, gene expression in eukaryotes is more complicated because of the temporal and spatial separation between the processes of transcription and translation. Thus, although most regulation of gene expression occurs through transcriptional control in prokaryotes, regulation of gene expression in eukaryotes occurs at the transcriptional level and post-transcriptionally (after the primary transcript has been made).

Prokaryotic Gene Regulation

In bacteria and archaea, structural proteins with related functions are usually encoded together within the genome in a block called an operon and are transcribed together under the control of a single promoter, resulting in the formation of a polycistronic transcript (Figure **9.6.1**). In this way, regulation of the transcription of all of the structural genes encoding the enzymes that catalyze the many steps in a single biochemical pathway can be controlled simultaneously, because they will either all be needed at the same time, or none will be needed. For example, in *E. coli*, all of the structural genes that encode enzymes needed to use lactose as an energy source lie next to each other in the lactose (or *lac*) operon under the control of a single promoter, the *lac* promoter. French scientists François Jacob (1920–2013) and Jacques Monod at the Pasteur Institute were the first to show the organization of bacterial genes into operons, through their studies on the *lac* operon of *E. coli*. For this work, they won the Nobel Prize in Physiology or Medicine in 1965. Although eukaryotic genes are not organized into operons, prokaryotic operons are excellent models for learning about gene regulation generally. There are some gene clusters in eukaryotes that function similar to operons. Many of the principles can be applied to eukaryotic systems and contribute to our understanding of changes in gene expression in eukaryotes that can result pathological changes such as cancer.

Each operon includes DNA sequences that influence its own transcription; these are located in a region called the regulatory region. The regulatory region includes the promoter and the region surrounding the promoter, to which transcription factors, proteins encoded by regulatory genes, can bind. Transcription factors influence the binding of RNA polymerase to the promoter and allow its progression to transcribe structural genes. A repressor is a transcription factor that suppresses transcription of a gene in response to an external stimulus by binding to a DNA sequence within the regulatory region called the operator, which is located between the RNA polymerase binding site of the promoter and the transcriptional start site of the first structural gene. Repressor binding

physically blocks RNA polymerase from transcribing structural genes. Conversely, an activator is a transcription factor that increases the transcription of a gene in response to an external stimulus by facilitating RNA polymerase binding to the promoter. An inducer, a third type of regulatory molecule, is a small molecule that either activates or represses transcription by interacting with a repressor or an activator.

In prokaryotes, there are examples of operons whose gene products are required rather consistently and whose expression, therefore, is unregulated. Such operons are constitutively expressed, meaning they are transcribed and translated continuously to provide the cell with constant intermediate levels of the protein products. Such genes encode enzymes involved in housekeeping functions required for cellular maintenance, including DNA replication, repair, and expression, as well as enzymes involved in core metabolism. In contrast, there are other prokaryotic operons that are expressed only when needed and are regulated by repressors, activators, and inducers.

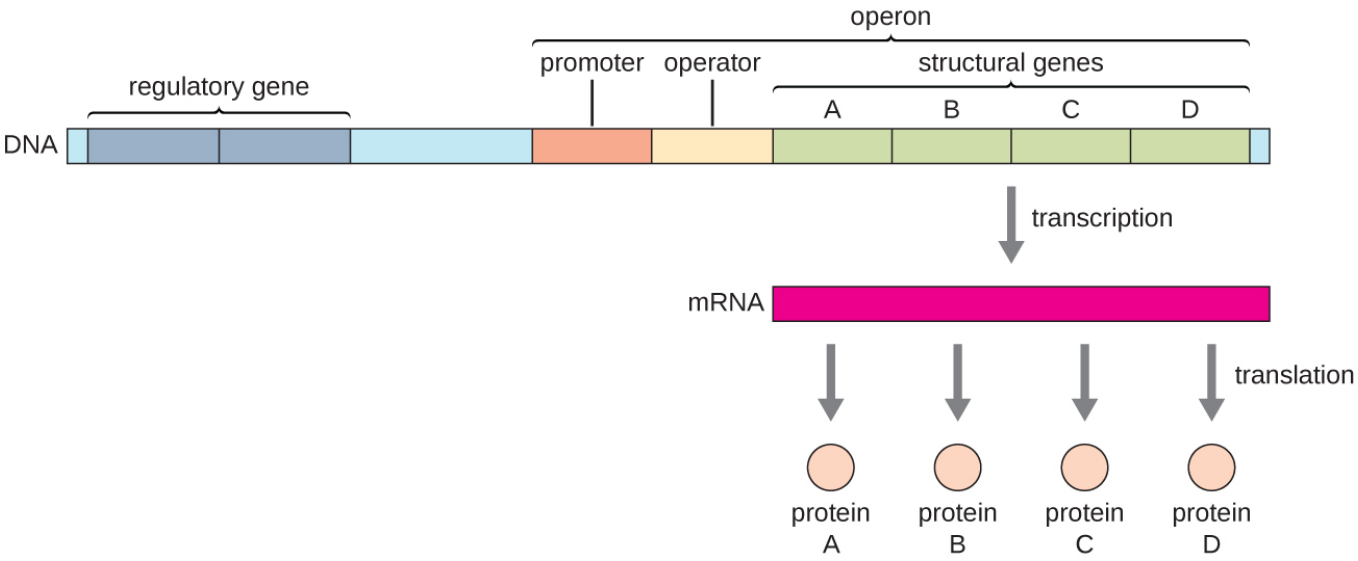


Figure **9.6.1**: In prokaryotes, structural genes of related function are often organized together on the genome and transcribed together under the control of a single promoter. The operon's regulatory region includes both the promoter and the operator. If a repressor binds to the operator, then the structural genes will not be transcribed. Alternatively, activators may bind to the regulatory region, enhancing transcription.

? Exercise 9.6.1

1. What are the parts in the DNA sequence of an operon?
2. What types of regulatory molecules are there?

Regulation by Repression

Prokaryotic operons are commonly controlled by the binding of repressors to operator regions, thereby preventing the transcription of the structural genes. Such operons are classified as either **repressible operons** or inducible operons. Repressible operons, like the tryptophan (*trp*) operon, typically contain genes encoding enzymes required for a biosynthetic pathway. As long as the product of the pathway, like tryptophan, continues to be required by the cell, a repressible operon will continue to be expressed. However, when the product of the biosynthetic pathway begins to accumulate in the cell, removing the need for the cell to continue to make more, the expression of the operon is repressed. Conversely, **inducible operons**, like the *lac* operon of *E. coli*, often contain genes encoding enzymes in a pathway involved in the metabolism of a specific substrate like lactose. These enzymes are only required when that substrate is available, thus expression of the operons is typically induced only in the presence of the substrate.

The trp Operon: A Repressible Operon

E. coli can synthesize tryptophan using enzymes that are encoded by five structural genes located next to each other in the *trp* operon (Figure **9.6.2**). When environmental tryptophan is low, the operon is turned on. This means that transcription is initiated, the genes are expressed, and tryptophan is synthesized. However, if tryptophan is present in the environment, the *trp* operon is turned off. Transcription does not occur and tryptophan is not synthesized.

When tryptophan is not present in the cell, the repressor by itself does not bind to the operator; therefore, the operon is active and tryptophan is synthesized. However, when tryptophan accumulates in the cell, two tryptophan molecules bind to the *trp* repressor molecule, which changes its shape, allowing it to bind to the *trp* operator. This binding of the active form of the *trp* repressor to the operator blocks RNA polymerase from transcribing the structural genes, stopping expression of the operon. Thus, the actual product of the biosynthetic pathway controlled by the operon regulates the expression of the operon.

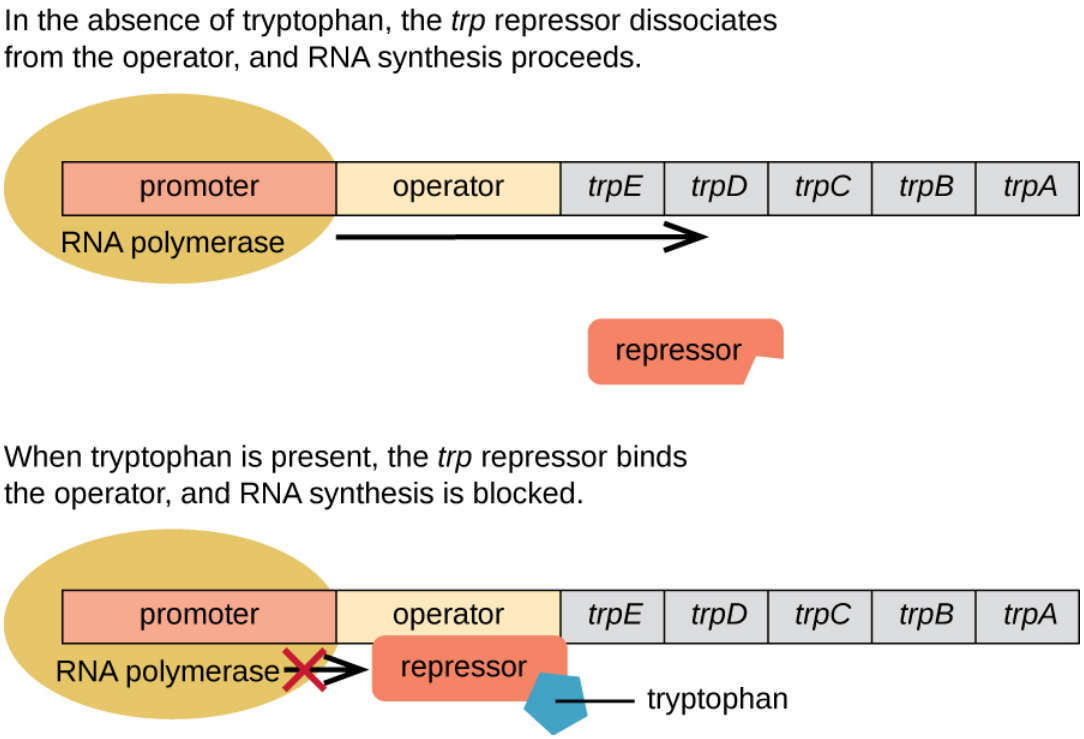


Figure **9.6.2**: The five structural genes needed to synthesize tryptophan in *E. coli* are located next to each other in the *trp* operon. When tryptophan is absent, the repressor protein does not bind to the operator, and the genes are transcribed. When tryptophan is plentiful, tryptophan binds the repressor protein at the operator sequence. This physically blocks the RNA polymerase from transcribing the tryptophan biosynthesis genes.

Link to Learning

Watch this video to learn more about the *trp* operon.

The *lac* Operon: An Inducible Operon

The *lac* operon is an example of an inducible operon that is also subject to activation in the absence of glucose (Figure **9.6.3**). The *lac* operon encodes three structural genes necessary to acquire and process the disaccharide lactose from the environment, breaking it down into the simple sugars glucose and galactose. For the *lac* operon to be expressed, lactose must be present. This makes sense for the cell because it would be energetically wasteful to create the enzymes to process lactose if lactose was not available.

In the absence of lactose, the *lac* repressor is bound to the operator region of the *lac* operon, physically preventing RNA polymerase from transcribing the structural genes. However, when lactose is present, the lactose inside the cell is converted to allolactose. Allolactose serves as an inducer molecule, binding to the repressor and changing its shape so that it is no longer able to bind to the operator DNA. Removal of the repressor in the presence of lactose allows RNA polymerase to move through the operator region and begin transcription of the *lac* structural genes.

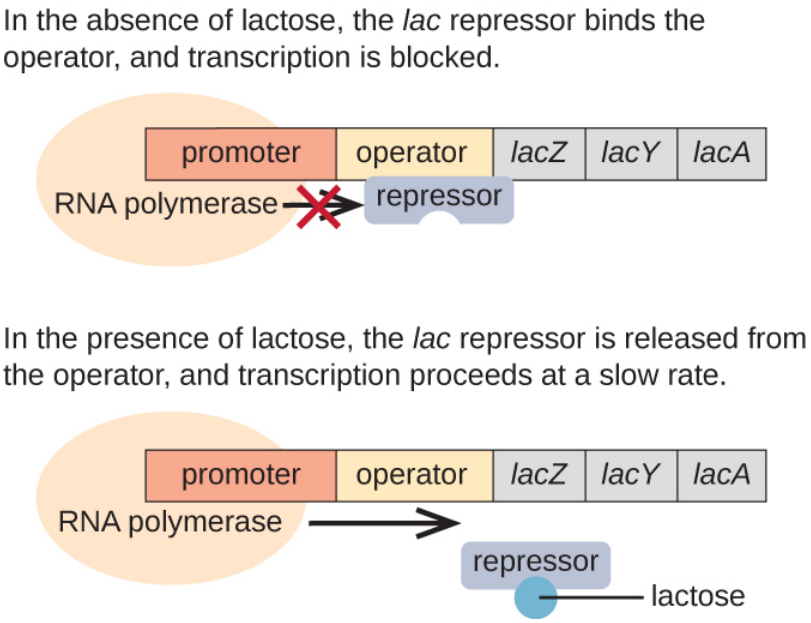


Figure **9.6.3**: The three structural genes that are needed to degrade lactose in *E. coli* are located next to each other in the *lac* operon. When lactose is absent, the repressor protein binds to the operator, physically blocking the RNA polymerase from transcribing the *lac* structural genes. When lactose is available, a lactose molecule binds the repressor protein, preventing the repressor from binding to the operator sequence, and the genes are transcribed.

The *lac* Operon: Activation by Catabolite Activator Protein

Bacteria typically have the ability to use a variety of substrates as carbon sources. However, because glucose is usually preferable to other substrates, bacteria have mechanisms to ensure that alternative substrates are only used when glucose has been depleted. Additionally, bacteria have mechanisms to ensure that the genes encoding enzymes for using alternative substrates are expressed only when the alternative substrate is available. In the 1940s, Jacques Monod was the first to demonstrate the preference for certain substrates over others through his studies of *E. coli*'s growth when cultured in the presence of two different substrates simultaneously. Such studies generated diauxic growth curves, like the one shown in Figure **9.6.4**. Although the preferred substrate glucose is used first, *E. coli* grows quickly and the enzymes for lactose metabolism are absent. However, once glucose levels are depleted, growth rates slow, inducing the expression of the enzymes needed for the metabolism of the second substrate, lactose. Notice how the growth rate in lactose is slower, as indicated by the lower steepness of the growth curve.

The ability to switch from glucose use to another substrate like lactose is a consequence of the activity of an enzyme called Enzyme IIA (EIIA). When glucose levels drop, cells produce less ATP from catabolism (see Catabolism of Carbohydrates), and EIIA becomes phosphorylated. Phosphorylated EIIA activates adenylyl cyclase, an enzyme that converts some of the remaining ATP to cyclic AMP (cAMP), a cyclic derivative of AMP and important signaling molecule involved in glucose and energy metabolism in *E. coli*. As a result, cAMP levels begin to rise in the cell (Figure **9.6.5**).

The *lac* operon also plays a role in this switch from using glucose to using lactose. When glucose is scarce, the accumulating cAMP caused by increased adenylyl cyclase activity binds to catabolite activator protein (CAP), also known as cAMP receptor protein (CRP). The complex binds to the promoter region of the *lac* operon (Figure **9.6.6**). In the regulatory regions of these operons, a CAP binding site is located upstream of the RNA polymerase binding site in the promoter. Binding of the CAP-cAMP complex to this site increases the binding ability of RNA polymerase to the promoter region to initiate the transcription of the structural genes. Thus, in the case of the *lac* operon, for transcription to occur, lactose must be present (removing the lac repressor protein) and glucose levels must be depleted (allowing binding of an activating protein). When glucose levels are high, there is catabolite repression of operons encoding enzymes for the metabolism of alternative substrates. Because of low cAMP levels under these conditions, there is an insufficient amount of the CAP-cAMP complex to activate transcription of these operons. See Table **9.6.1** for a summary of the regulation of the lac operon.

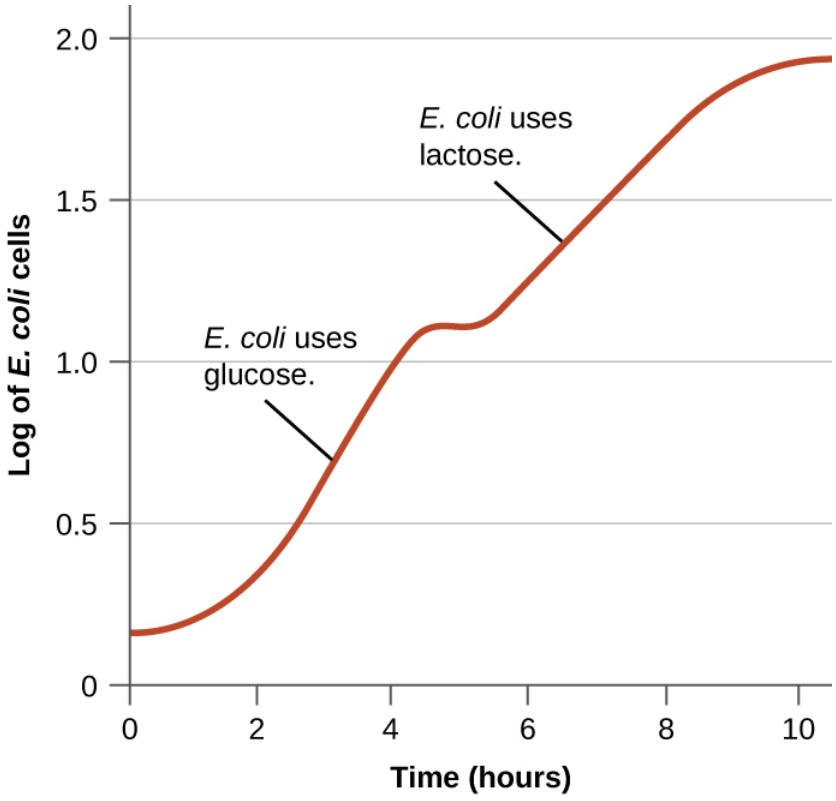


Figure 9.6.4: When grown in the presence of two substrates, *E. coli* uses the preferred substrate (in this case glucose) until it is depleted. Then, enzymes needed for the metabolism of the second substrate are expressed and growth resumes, although at a slower rate.

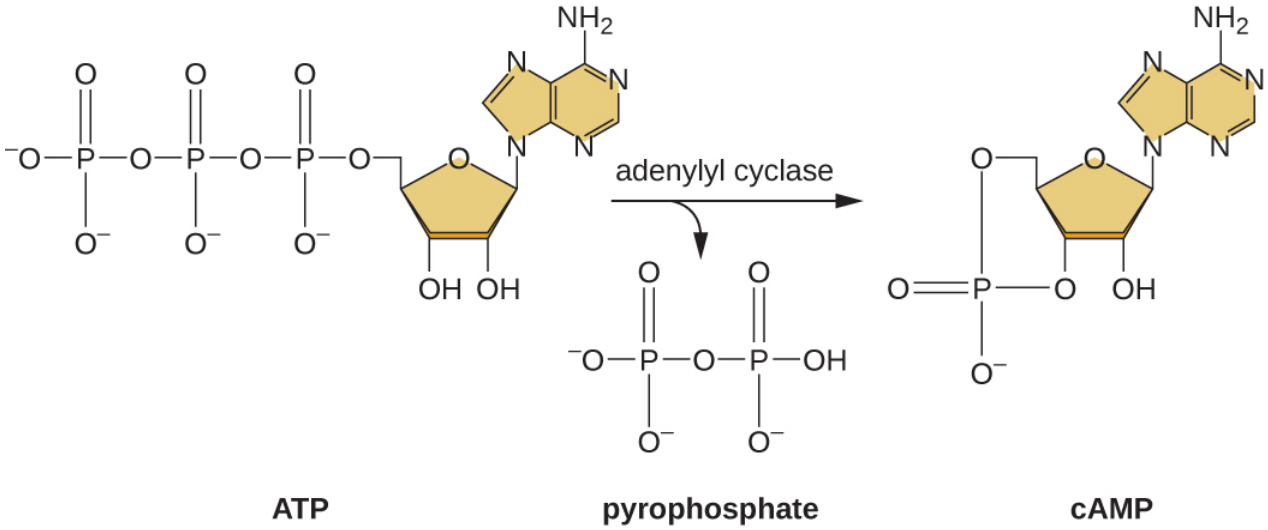


Figure 9.6.5: When ATP levels decrease due to depletion of glucose, some remaining ATP is converted to cAMP by adenylyl cyclase. Thus, increased cAMP levels signal glucose depletion.

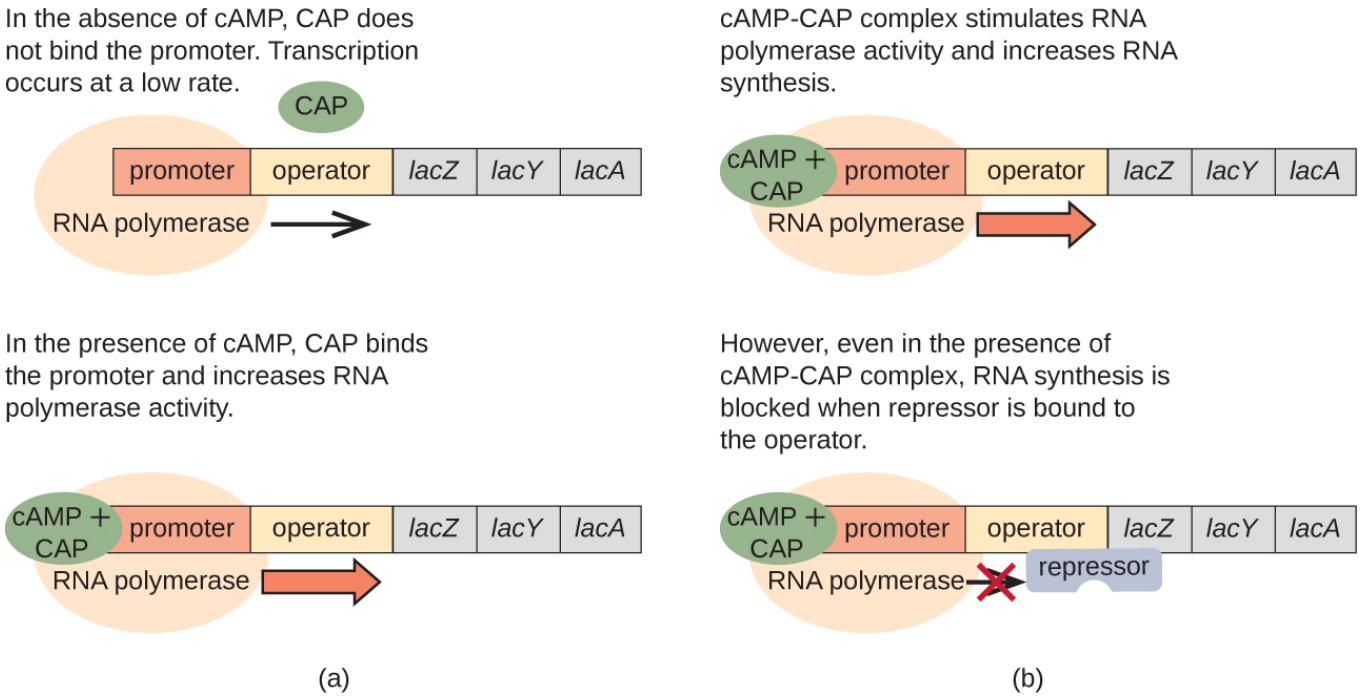


Figure **9.6.6**: (a) In the presence of cAMP, CAP binds to the promoters of operons, like the *lac* operon, that encode genes for enzymes for the use of alternate substrates. (b) For the *lac* operon to be expressed, there must be activation by cAMP-CAP as well as removal of the lac repressor from the operator.

Table **9.6.1**: Conditions Affecting Transcription of the lac Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	–	–	+	No
+	–	+	–	Some
–	+	–	+	No
–	+	+	–	Yes

Link to Learning

Watch an animated tutorial about the workings of lac operon here.

Exercise 9.6.2

1. What affects the binding of the *trp* operon repressor to the operator?
2. How and when is the behavior of the *lac* repressor protein altered?
3. In addition to being repressible, how else is the *lac* operon regulated?

Global Responses of Prokaryotes

In prokaryotes, there are also several higher levels of gene regulation that have the ability to control the transcription of many related operons simultaneously in response to an environmental signal. A group of operons all controlled simultaneously is called a regulon.

Alarmones

When sensing impending stress, prokaryotes alter the expression of a wide variety of operons to respond in coordination. They do this through the production of alarmones, which are small intracellular nucleotide derivatives. Alarmones change which genes are expressed and stimulate the expression of specific stress-response genes. The use of alarmones to alter gene expression in response

to stress appears to be important in pathogenic bacteria. On encountering host defense mechanisms and other harsh conditions during infection, many operons encoding virulence genes are upregulated in response to alarmone signaling. Knowledge of these responses is key to being able to fully understand the infection process of many pathogens and to the development of therapies to counter this process.

Alternate σ Factors

Since the σ subunit of bacterial RNA polymerase confers specificity as to which promoters should be transcribed, altering the σ factor used is another way for bacteria to quickly and globally change what regulons are transcribed at a given time. The σ factor recognizes sequences within a bacterial promoter, so different σ factors will each recognize slightly different promoter sequences. In this way, when the cell senses specific environmental conditions, it may respond by changing which σ factor it expresses, degrading the old one and producing a new one to transcribe the operons encoding genes whose products will be useful under the new environmental condition. For example, in sporulating bacteria of the genera *Bacillus* and *Clostridium* (which include many pathogens), a group of σ factors controls the expression of the many genes needed for sporulation in response to sporulation-stimulating signals.

? Exercise 9.6.3

1. What is the name given to a collection of operons that can be regulated as a group?
2. What type of stimulus would trigger the transcription of a different σ factor?

Additional Methods of Regulation in Bacteria: Attenuation and Riboswitches

Although most gene expression is regulated at the level of transcription initiation in prokaryotes, there are also mechanisms to control both the completion of transcription as well as translation concurrently. Since their discovery, these mechanisms have been shown to control the completion of transcription and translation of many prokaryotic operons. Because these mechanisms link the regulation of transcription and translation directly, they are specific to prokaryotes, because these processes are physically separated in eukaryotes.

One such regulatory system is attenuation, whereby secondary stem-loop structures formed within the 5’ end of an mRNA being transcribed determine if transcription to complete the synthesis of this mRNA will occur and if this mRNA will be used for translation. Beyond the transcriptional repression mechanism already discussed, attenuation also controls expression of the *trp* operon in *E. coli* (Figure **9.6.7**). The *trp* operon regulatory region contains a leader sequence called *trpL* between the operator and the first structural gene, which has four stretches of RNA that can base pair with each other in different combinations. When a terminator stem-loop forms, transcription terminates, releasing RNA polymerase from the mRNA. However, when an antiterminator stem-loop forms, this prevents the formation of the terminator stem-loop, so RNA polymerase can transcribe the structural genes.

A related mechanism of concurrent regulation of transcription and translation in prokaryotes is the use of a riboswitch, a small region of noncoding RNA found within the 5’ end of some prokaryotic mRNA molecules (Figure **9.6.8**). A riboswitch may bind to a small intracellular molecule to stabilize certain secondary structures of the mRNA molecule. The binding of the small molecule determines which stem-loop structure forms, thus influencing the completion of mRNA synthesis and protein synthesis.

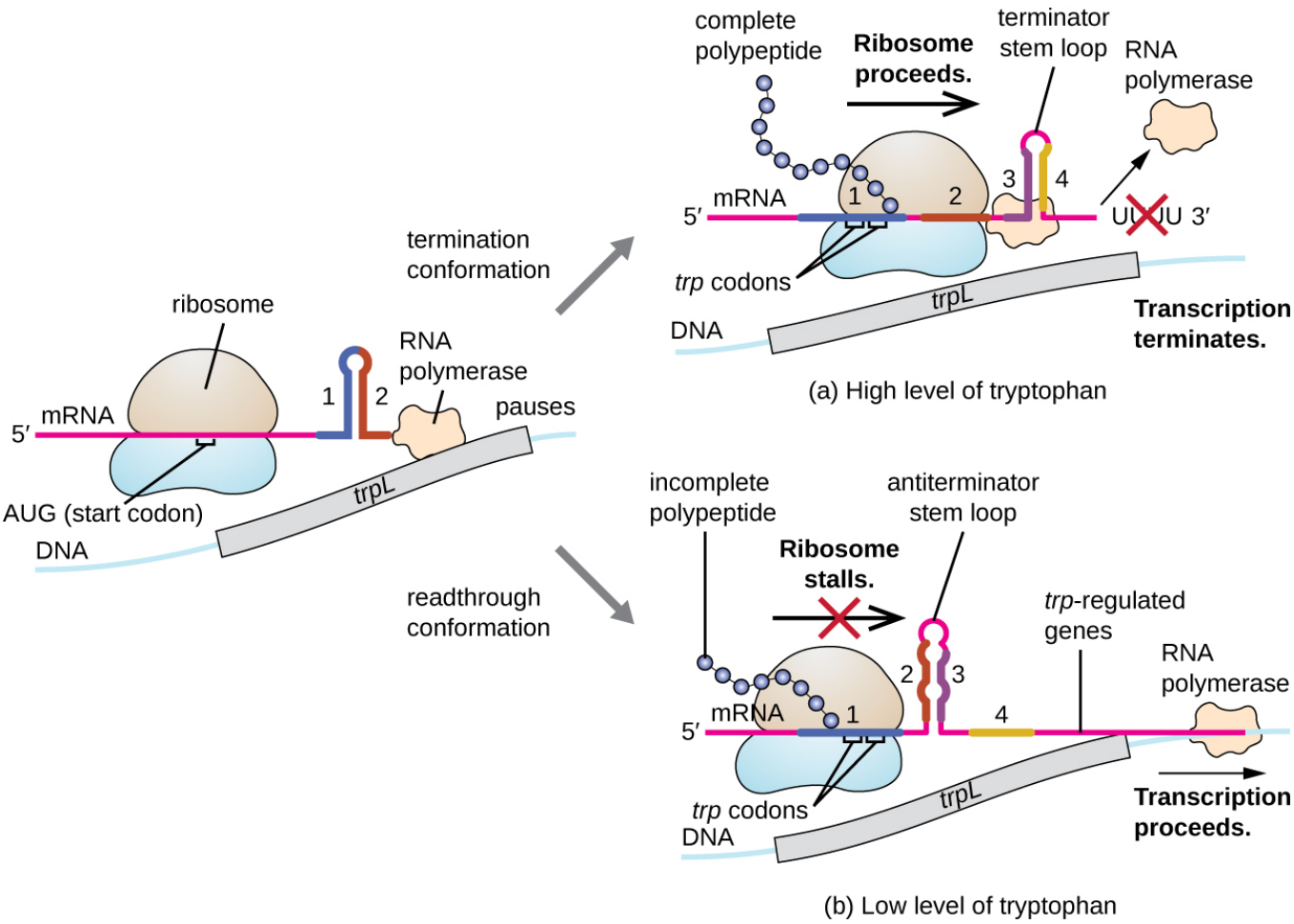


Figure **9.6.7**: When tryptophan is plentiful, translation of the short leader peptide encoded by *trpL* proceeds, the terminator loop between regions 3 and 4 forms, and transcription terminates. When tryptophan levels are depleted, translation of the short leader peptide stalls at region 1, allowing regions 2 and 3 to form an antiterminator loop, and RNA polymerase can transcribe the structural genes of the *trp* operon.

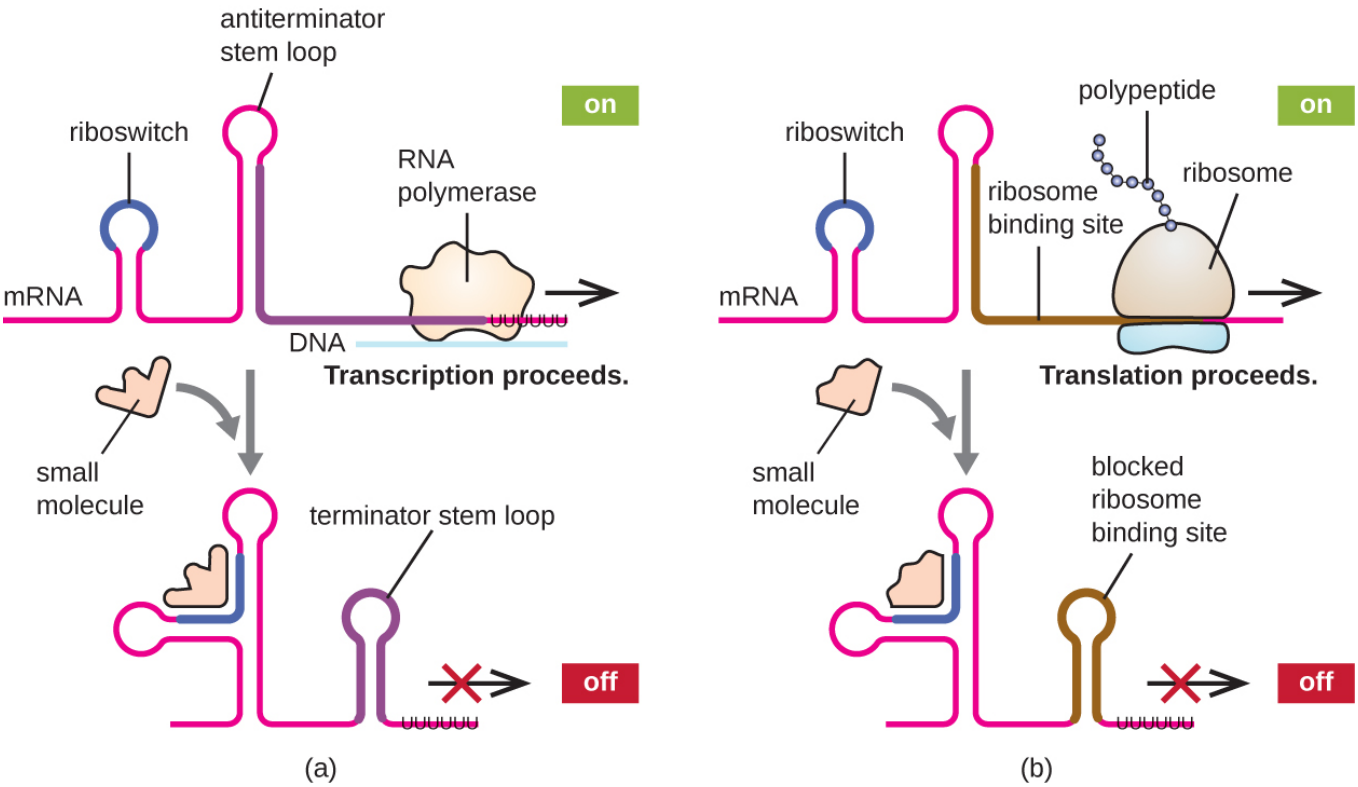


Figure **9.6.8**: Riboswitches found within prokaryotic mRNA molecules can bind to small intracellular molecules, stabilizing certain RNA structures, influencing either the completion of the synthesis of the mRNA molecule itself (left) or the protein made using that mRNA (right).

Other Factors Affecting Gene Expression in Prokaryotes and Eukaryotes

Although the focus on our discussion of transcriptional control used prokaryotic operons as examples, eukaryotic transcriptional control is similar in many ways. As in prokaryotes, eukaryotic transcription can be controlled through the binding of transcription factors including repressors and activators. Interestingly, eukaryotic transcription can be influenced by the binding of proteins to regions of DNA, called enhancers, rather far away from the gene, through DNA looping facilitated between the enhancer and the promoter (Figure **9.6.9**). Overall, regulating transcription is a highly effective way to control gene expression in both prokaryotes and eukaryotes. However, the control of gene expression in eukaryotes in response to environmental and cellular stresses can be accomplished in additional ways without the binding of transcription factors to regulatory regions.

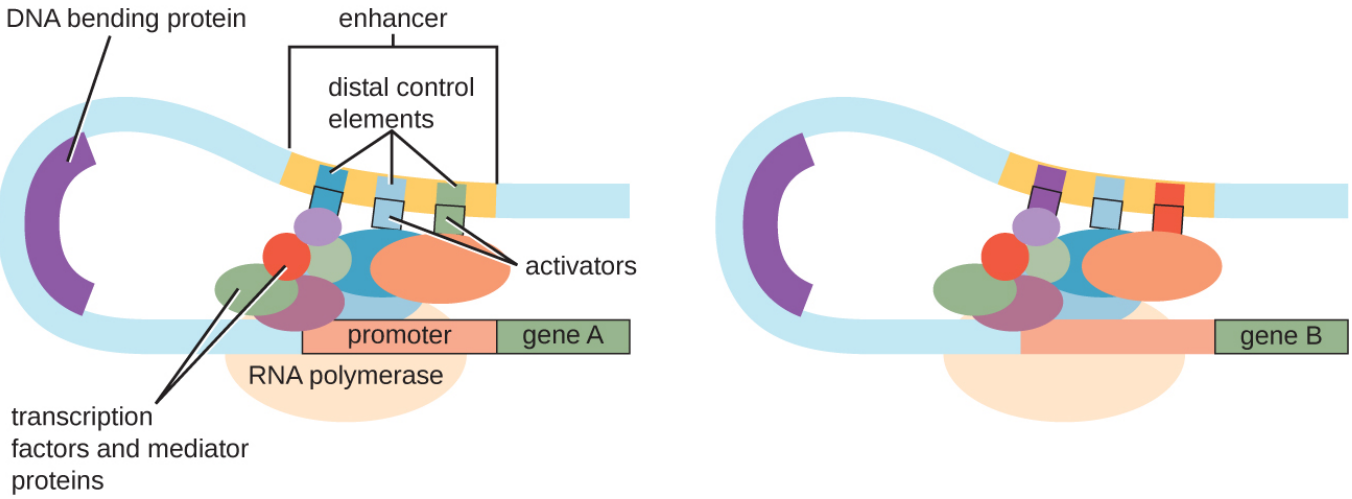


Figure **9.6.9**: In eukaryotes, an enhancer is a DNA sequence that promotes transcription. Each enhancer is made up of short DNA sequences called distal control elements. Activators bound to the distal control elements interact with mediator proteins and transcription factors. Two different genes may have the same promoter but different distal control elements, enabling differential gene expression.

DNA-Level Control

In eukaryotes, the DNA molecules or associated histones can be chemically modified in such a way as to influence transcription; this is called epigenetic regulation. Methylation of certain cytosine nucleotides in DNA in response to environmental factors has been shown to influence use of such DNA for transcription, with DNA methylation commonly correlating to lowered levels of gene expression. Additionally, in response to environmental factors, histone proteins for packaging DNA can also be chemically modified in multiple ways, including acetylation and deacetylation, influencing the packaging state of DNA and thus affecting the availability of loosely wound DNA for transcription. These chemical modifications can sometimes be maintained through multiple rounds of cell division, making at least some of these epigenetic changes heritable.

Link to Learning

This video describes how epigenetic regulation controls gene expression.

Exercise Runaway argument for \eatSpaces?

1. What stops or allows transcription to proceed when attenuation is operating?
2. What determines the state of a riboswitch?
3. Describe the function of an enhancer.
4. Describe two mechanisms of epigenetic regulation in eukaryotes.

Clinical Focus: Resolution

Although Mark survived his bout with necrotizing fasciitis, he would now have to undergo a skin-grafting surgery, followed by long-term physical therapy. Based on the amount of muscle mass he lost, it is unlikely that his leg will return to full strength, but his physical therapist is optimistic that he will regain some use of his leg.

Laboratory testing revealed the causative agent of Mark's infection was a strain of group A streptococcus (Group A strep). As required by law, Mark's case was reported to the state health department and ultimately to the Centers for Disease Control and Prevention (CDC). At the CDC, the strain of group A strep isolated from Mark was analyzed more thoroughly for methicillin resistance.

Methicillin resistance is genetically encoded and is becoming more common in group A strep through horizontal gene transfer. In necrotizing fasciitis, blood flow to the infected area is typically limited because of the action of various genetically encoded bacterial toxins. This is why there is typically little to no bleeding as a result of the incision test. Unfortunately, these bacterial

toxins limit the effectiveness of intravenous antibiotics in clearing infection from the skin and underlying tissue, meaning that antibiotic resistance alone does not explain the ineffectiveness of Mark's treatment. Nevertheless, intravenous antibiotic therapy was warranted to help minimize the possible outcome of sepsis, which is a common outcome of necrotizing fasciitis. Through genomic analysis by the CDC of the strain isolated from Mark, several of the important virulence genes were shown to be encoded on prophages, indicating that transduction is important in the horizontal gene transfer of these genes from one bacterial cell to another.

Key Concepts and Summary

- **Gene expression** is a tightly regulated process.
- Gene expression in prokaryotes is largely regulated at the point of transcription. Gene expression in eukaryotes is additionally regulated post-transcriptionally.
- Prokaryotic structural genes of related function are often organized into **operons**, all controlled by transcription from a single promoter. The regulatory region of an operon includes the promoter itself and the region surrounding the promoter to which transcription factors can bind to influence transcription.
- Although some operons are **constitutively expressed**, most are subject to regulation through the use of **transcription factors** (repressors and activators). A **repressor** binds to an **operator**, a DNA sequence within the regulatory region between the RNA polymerase binding site in the promoter and first structural gene, thereby physically blocking transcription of these operons. An **activator** binds within the regulatory region of an operon, helping RNA polymerase bind to the promoter, thereby enhancing the transcription of this operon. An **inducer**influences transcription through interacting with a repressor or activator.
- The *trp* operon is a classic example of a **repressible operon**. When tryptophan accumulates, tryptophan binds to a repressor, which then binds to the operator, preventing further transcription.
- The *lac* operon is a classic example an **inducible operon**. When lactose is present in the cell, it is converted to allolactose. Allolactose acts as an inducer, binding to the repressor and preventing the repressor from binding to the operator. This allows transcription of the structural genes.
- The *lac* operon is also subject to activation. When glucose levels are depleted, some cellular ATP is converted into cAMP, which binds to the **catabolite activator protein (CAP)**. The cAMP-CAP complex activates transcription of the *lac* operon. When glucose levels are high, its presence prevents transcription of the *lac* operon and other operons by **catabolite repression**.
- Small intracellular molecules called **alarmones** are made in response to various environmental stresses, allowing bacteria to control the transcription of a group of operons, called a regulon.
- Bacteria have the ability to change which **σ factor** of RNA polymerase they use in response to environmental conditions to quickly and globally change which regulons are transcribed.
- Prokaryotes have regulatory mechanisms, including **attenuation** and the use of **riboswitches**, to simultaneously control the completion of transcription and translation from that transcript. These mechanisms work through the formation of stem loops in the 5' end of an mRNA molecule currently being synthesized.
- There are additional points of regulation of gene expression in prokaryotes and eukaryotes. In eukaryotes, **epigenetic regulation** by chemical modification of DNA or histones, and regulation of RNA processing are two methods.

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9.7: How Asexual Prokaryotes Achieve Genetic Diversity

Learning Objectives

- Compare the processes of transformation, transduction, and conjugation
- Explain how asexual gene transfer results in prokaryotic genetic diversity
- Explain the structure and consequences for bacterial genetic diversity of transposons

Typically, when we consider genetic transfer, we think of vertical gene transfer, the transmission of genetic information from generation to generation. Vertical gene transfer is by far the main mode of transmission of genetic information in all cells. In sexually reproducing organisms, crossing-over events and independent assortment of individual chromosomes during meiosis contribute to genetic diversity in the population. Genetic diversity is also introduced during sexual reproduction, when the genetic information from two parents, each with different complements of genetic information, are combined, producing new combinations of parental genotypes in the diploid offspring. The occurrence of mutations also contributes to genetic diversity in a population. Genetic diversity of offspring is useful in changing or inconsistent environments and may be one reason for the evolutionary success of sexual reproduction.

When prokaryotes and eukaryotes reproduce asexually, they transfer a nearly identical copy of their genetic material to their offspring through vertical gene transfer. Although asexual reproduction produces more offspring more quickly, any benefits of diversity among those offspring are lost. How then do organisms whose dominant reproductive mode is asexual create genetic diversity? In prokaryotes, horizontal gene transfer (HGT), the introduction of genetic material from one organism to another organism within the same generation, is an important way to introduce genetic diversity. HGT allows even distantly related species to share genes, influencing their phenotypes. It is thought that HGT is more prevalent in prokaryotes but that only a small fraction of the prokaryotic genome may be transferred by this type of transfer at any one time. As the phenomenon is investigated more thoroughly, it may be revealed to be even more common. Many scientists believe that HGT and mutation are significant sources of genetic variation, the raw material for the process of natural selection, in prokaryotes. Although HGT is more common among evolutionarily related organisms, it may occur between any two species that live together in a natural community.

HGT in prokaryotes is known to occur by the three primary mechanisms that are illustrated in Figure **9.7.1**:

1. Transformation: naked DNA is taken up from the environment
2. Transduction: genes are transferred between cells in a virus (see The Viral Life Cycle)
3. Conjugation: use of a hollow tube called a conjugation pilus to transfer genes between cells

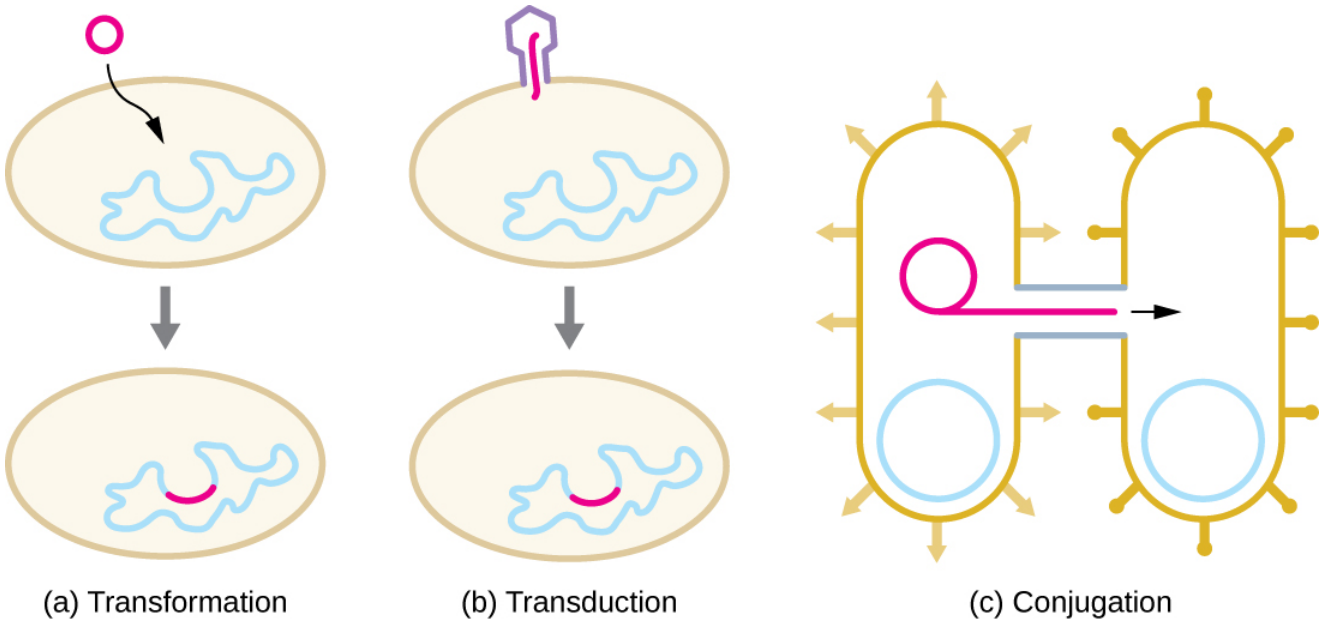


Figure **9.7.1**: There are three prokaryote-specific mechanisms leading to horizontal gene transfer in prokaryotes. a) In transformation, the cell takes up DNA directly from the environment. The DNA may remain separate as a plasmid or be incorporated into the host genome. b) In transduction, a bacteriophage injects DNA that is a hybrid of viral DNA and DNA from a previously infected bacterial cell. c) In conjugation, DNA is transferred between cells through a cytoplasmic bridge after a conjugation pilus draws the two cells close enough to form the bridge.

? Exercise 9.7.1

1. What are three ways sexual reproduction introduces genetic variation into offspring?
2. What is a benefit of asexual reproduction?
3. What are the three mechanisms of horizontal gene transfer in prokaryotes?

Transformation

Frederick Griffith was the first to demonstrate the process of transformation. In 1928, he showed that live, nonpathogenic *Streptococcus pneumoniae* bacteria could be transformed into pathogenic bacteria through exposure to a heat-killed pathogenic strain. He concluded that some sort of agent, which he called the "transforming principle," had been passed from the dead pathogenic bacteria to the live, nonpathogenic bacteria. In 1944, Oswald Avery (1877–1955), Colin MacLeod (1909–1972), and Maclyn McCarty (1911–2005) demonstrated that the transforming principle was DNA (see Using Microbiology to Discover the Secrets of Life).

In transformation, the prokaryote takes up naked DNA found in its environment and that is derived from other cells that have lysed on death and released their contents, including their genome, into the environment. Many bacteria are naturally competent, meaning that they actively bind to environmental DNA, transport it across their cell envelopes into their cytoplasm, and make it single stranded. Typically, double-stranded foreign DNA within cells is destroyed by nucleases as a defense against viral infection. However, these nucleases are usually ineffective against single-stranded DNA, so this single-stranded DNA within the cell has the opportunity to recombine into the bacterial genome. A molecule of DNA that contains fragments of DNA from different organisms is called recombinant DNA. (Recombinant DNA will be discussed in more detail in Microbes and the Tools of Genetic Engineering.) If the bacterium incorporates the new DNA into its own genome through recombination, the bacterial cell may gain new phenotypic properties. For example, if a nonpathogenic bacterium takes up DNA for a toxin gene from a pathogen and then incorporates it into its chromosome, it, too, may become pathogenic. Plasmid DNA may also be taken up by competent bacteria and confer new properties to the cell. Overall, transformation in nature is a relatively inefficient process because environmental DNA levels are low because of the activity of nucleases that are also released during cellular lysis. Additionally, genetic recombination is inefficient at incorporating new DNA sequences into the genome.

In nature, bacterial transformation is an important mechanism for the acquisition of genetic elements encoding virulence factors and antibiotic resistance. Genes encoding resistance to antimicrobial compounds have been shown to be widespread in nature, even in environments not influenced by humans. These genes, which allow microbes living in mixed communities to compete for limited resources, can be transferred within a population by transformation, as well as by the other processes of HGT. In the laboratory, we can exploit the natural process of bacterial transformation for genetic engineering to make a wide variety of medicinal products, as discussed in Microbes and the Tools of Genetic Engineering.

? Exercise 9.7.2

Why does a bacterial cell make environmental DNA brought into the cell into a single-stranded form?

Transduction

Viruses that infect bacteria (bacteriophages) may also move short pieces of chromosomal DNA from one bacterium to another in a process called transduction (see Figure 6.2.3). Recall that in generalized transduction, any piece of chromosomal DNA may be transferred to a new host cell by accidental packaging of chromosomal DNA into a phage head during phage assembly. By contrast, specialized transduction results from the imprecise excision of a lysogenic prophage from the bacterial chromosome such that it carries with it a piece of the bacterial chromosome from either side of the phage's integration site to a new host cell. As a result, the host may acquire new properties. This process is called lysogenic conversion. Of medical significance, a lysogenic phage may carry with it a virulence gene to its new host. Once inserted into the new host's chromosome, the new host may gain pathogenicity. Several pathogenic bacteria, including *Corynebacterium diphtheriae* (the causative agent of diphtheria) and *Clostridium botulinum* (the causative agent of botulism), are virulent because of the introduction of toxin-encoding genes by lysogenic bacteriophages, affirming the clinical relevance of transduction in the exchange of genes involved in infectious disease. Archaea have their own viruses that translocate genetic material from one individual to another.

? Exercise 9.7.3

1. What is the agent of transduction of prokaryotic cells?
2. In specialized transduction, where does the transducing piece of DNA come from?

The Clinical Consequences of Transduction

Paul, a 23-year-old relief worker from Atlanta, traveled to Haiti in 2011 to provide aid following the 2010 earthquake. After working there for several weeks, he suddenly began experiencing abdominal distress, including severe cramping, nausea, vomiting, and watery diarrhea. He also began to experience intense muscle cramping. At a local clinic, the physician suspected that Paul's symptoms were caused by cholera because there had been a cholera outbreak after the earthquake. Because cholera is transmitted by the fecal-oral route, breaches in sanitation infrastructure, such as often occur following natural disasters, may precipitate outbreaks. The physician confirmed the presumptive diagnosis using a cholera dipstick test. He then prescribed Paul a single dose of doxycycline, as well as oral rehydration salts, instructing him to drink significant amounts of clean water.

Cholera is caused by the gram-negative curved rod *Vibrio cholerae* (Figure **9.7.2**). Its symptoms largely result from the production of the cholera toxin (CT), which ultimately activates a chloride transporter to pump chloride ions out of the epithelial cells into the gut lumen. Water then follows the chloride ions, causing the prolific watery diarrhea characteristic of cholera. The gene encoding the cholera toxin is incorporated into the bacterial chromosome of *V. cholerae* through infection of the bacterium with the lysogenic filamentous CTX phage, which carries the CT gene and introduces it into the chromosome on integration of the prophage. Thus, pathogenic strains of *V. cholerae* result from horizontal gene transfer by specialized transduction.

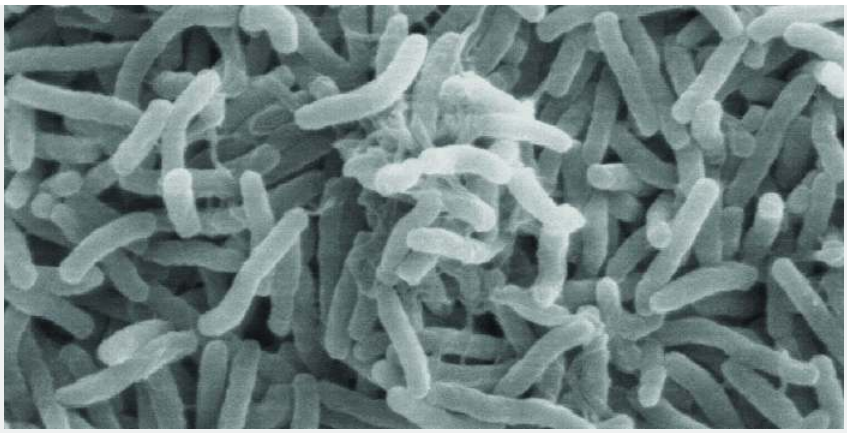


Figure **9.7.2**: A scanning electron micrograph of *Vibrio cholerae* shows its characteristic curved rod shape.

Exercise 9.7.4

1. Why are outbreaks of cholera more common as a result of a natural disaster?
2. Why is muscle cramping a common symptom of cholera? Why is treatment with oral rehydration salts so important for the treatment of cholera?
3. In areas stricken by cholera, what are some strategies that people could use to prevent disease transmission?

Conjugation

In conjugation, DNA is directly transferred from one prokaryote to another by means of a conjugation pilus, which brings the organisms into contact with one another. In *E. coli*, the genes encoding the ability to conjugate are located on a bacterial plasmid called the F plasmid, also known as the fertility factor, and the conjugation pilus is called the F pilus. The F-plasmid genes encode both the proteins composing the F pilus and those involved in rolling circle replication of the plasmid. Cells containing the F plasmid, capable of forming an F pilus, are called F^+ cells or donor cells, and those lacking an F plasmid are called F^- cells or recipient cells.

Conjugation of the F Plasmid

During typical conjugation in *E. coli*, the F pilus of an F^+ cell comes into contact with an F^- cell and retracts, bringing the two cell envelopes into contact (Figure **9.7.3**). Then a cytoplasmic bridge forms between the two cells at the site of the conjugation pilus. As rolling circle replication of the F plasmid occurs in the F^+ cell, a single-stranded copy of the F plasmid is transferred through the cytoplasmic bridge to the F^- cell, which then synthesizes the complementary strand, making it double stranded. The F^- cell now becomes an F^+ cell capable of making its own conjugation pilus. Eventually, in a mixed bacterial population containing both F^+ and F^- cells, all cells will become F^+ cells. Genes on the *E. coli* F plasmid also encode proteins preventing conjugation between F^+ cells.

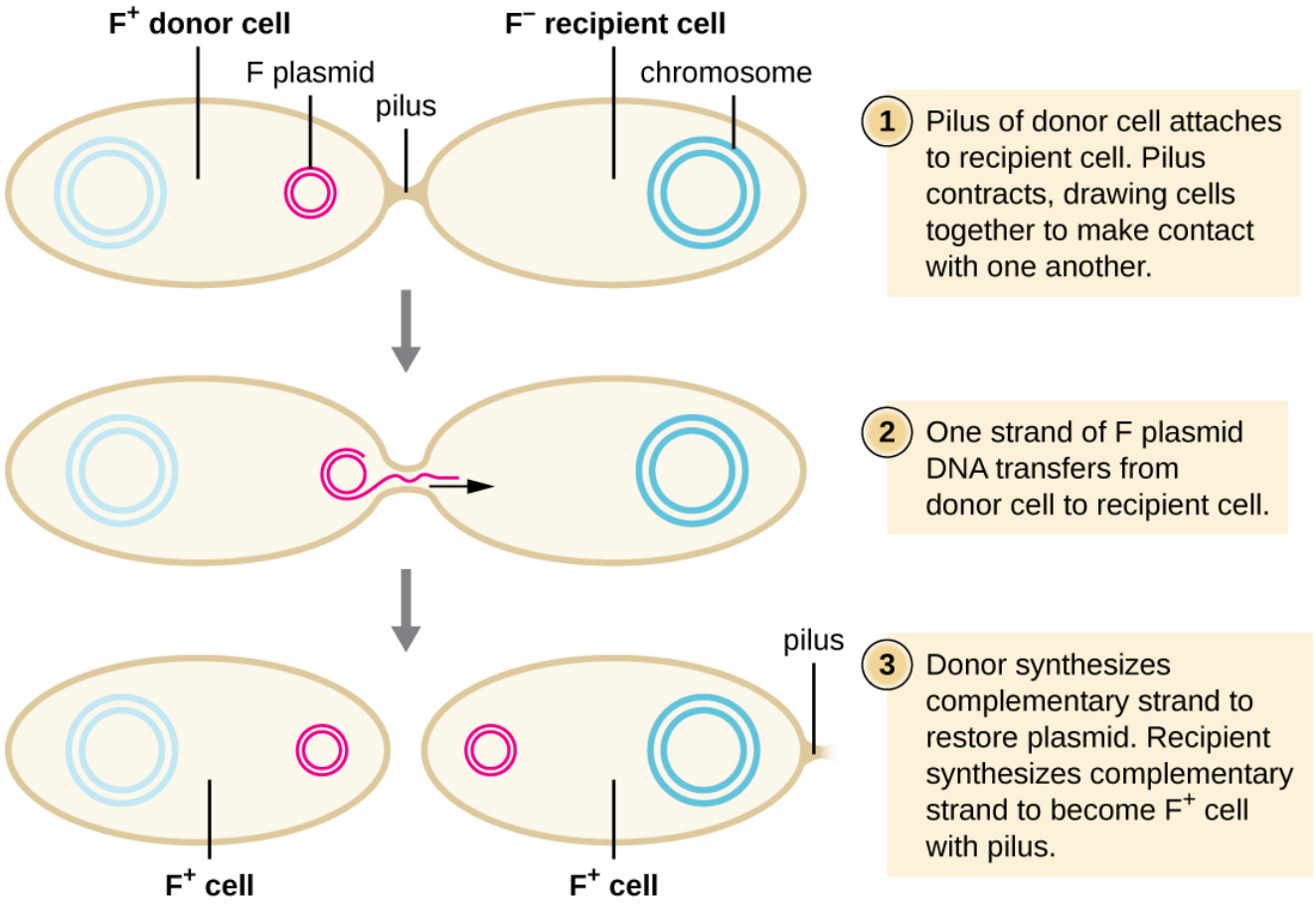


Figure **9.7.3**: Typical conjugation of the F plasmid from an F^+ cell to an F^- cell is brought about by the conjugation pilus bringing the two cells into contact. A single strand of the F plasmid is transferred to the F^- cell, which is then made double stranded.

Conjugation of F' and Hfr Cells

Although typical conjugation in *E. coli* results in the transfer of the F-plasmid DNA only, conjugation may also transfer chromosomal DNA. This is because the F plasmid occasionally integrates into the bacterial chromosome through recombination between the plasmid and the chromosome, forming an Hfr cell (Figure **9.7.4**). "Hfr" refers to the high frequency of recombination seen when recipient F^- cells receive genetic information from Hfr cells through conjugation. Similar to the imprecise excision of a prophage during specialized transduction, the integrated F plasmid may also be imprecisely excised from the chromosome, producing an F' plasmid that carries with it some chromosomal DNA adjacent to the integration site. On conjugation, this DNA is introduced to the recipient cell and may be either maintained as part of the F' plasmid or be recombined into the recipient cell's bacterial chromosome.

Hfr cells may also treat the bacterial chromosome like an enormous F plasmid and attempt to transfer a copy of it to a recipient F^- cell. Because the bacterial chromosome is so large, transfer of the entire chromosome takes a long time (Figure **9.7.5**). However, contact between bacterial cells during conjugation is transient, so it is unusual for the entire chromosome to be transferred. Host chromosomal DNA near the integration site of the F plasmid, displaced by the unidirectional process of rolling circle replication, is more likely to be transferred and recombined into a recipient cell's chromosome than host genes farther away. Thus, the relative location of bacterial genes on the Hfr cell's genome can be mapped based on when they are transferred through conjugation. As a result, prior to the age of widespread bacterial genome sequencing, distances on prokaryotic genome maps were often measured in minutes.

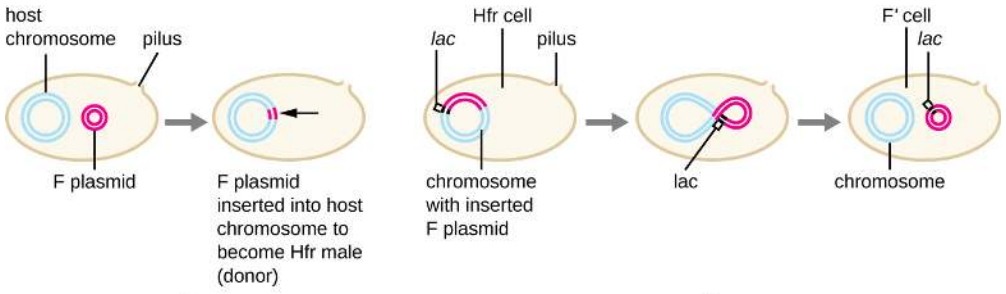


Figure **9.7.4**: (a) The F plasmid can occasionally integrate into the bacterial chromosome, producing an Hfr cell. (b) Imprecise excision of the F plasmid from the chromosome of an Hfr cell may lead to the production of an F' plasmid that carries chromosomal DNA adjacent to the integration site. This F' plasmid can be transferred to an F^- cell by conjugation.

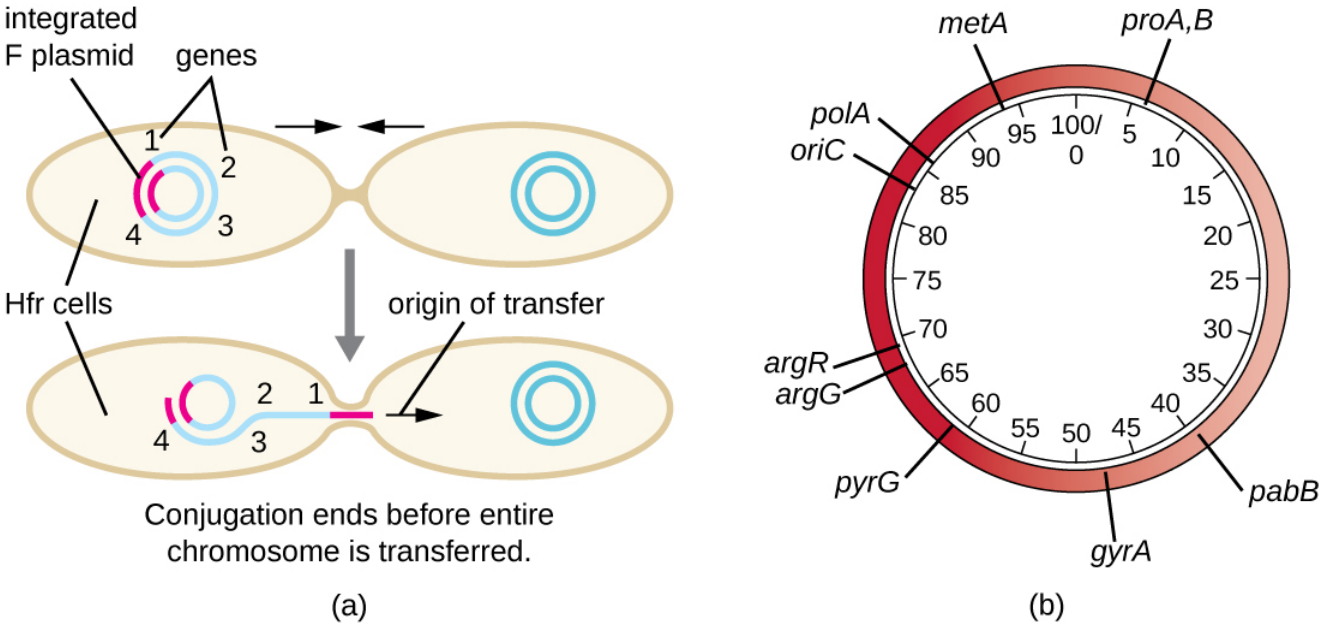


Figure **9.7.5**: (a) An Hfr cell may attempt to transfer the entire bacterial chromosome to an F^- cell, treating the chromosome like an extremely large F plasmid. However, contact between cells during conjugation is temporary. Chromosomal genes closest to the integration site (gene 1) that are first displaced during rolling circle replication will be transferred more quickly than genes far away from the integration site (gene 4). Hence, they are more likely to be recombined into the recipient F^- cell's chromosome. (b) The time it takes for a gene to be transferred, as detected by recombination into the F^- cell's chromosome, can be used to generate a map of the bacterial genome, such as this genomic map of *E. coli*. Note that it takes approximately 100 minutes for the entire genome (4.6 Mbp) of an Hfr strain of *E. coli* to be transferred by conjugation.

Consequences and Applications of Conjugation

Plasmids are an important type of extrachromosomal DNA element in bacteria and, in those cells that harbor them, are considered to be part of the bacterial genome. From a clinical perspective, plasmids often code for genes involved in virulence. For example, genes encoding proteins that make a bacterial cell resistant to a particular antibiotic are encoded on R plasmids. R plasmids, in addition to their genes for antimicrobial resistance, contain genes that control conjugation and transfer of the plasmid. R plasmids are able to transfer between cells of the same species and between cells of different species. Single R plasmids commonly contain multiple genes conferring resistance to multiple antibiotics.

Genes required for the production of various toxins and molecules important for colonization during infection may also be found encoded on plasmids. For example, verotoxin-producing strains of *E. coli* (VTEC) appear to have acquired the genes encoding the Shiga toxin from its gram-negative relative *Shigella dysenteriae* through the acquisition of a large plasmid encoding this toxin. VTEC causes severe diarrheal disease that may result in hemolytic uremic syndrome(HUS), which may be lead to kidney failure and death.

In nonclinical settings, bacterial genes that encode metabolic enzymes needed to degrade specialized atypical compounds like polycyclic aromatic hydrocarbons (PAHs) are also frequently encoded on plasmids. Additionally, certain plasmids have the ability

to move from bacterial cells to other cell types, like those of plants and animals, through mechanisms distinct from conjugation. Such mechanisms and their use in genetic engineering are covered in Modern Applications of Microbial Genetics.

? Exercise 9.7.5

1. What type of replication occurs during conjugation?
2. What occurs to produce an Hfr *E. coli* cell?
3. What types of traits are encoded on plasmids?

Transposition

Genetic elements called transposons (transposable elements), or "jumping genes," are molecules of DNA that include special inverted repeat sequences at their ends and a gene encoding the enzyme transposase (Figure 9.7.6). Transposons allow the entire sequence to independently excise from one location in a DNA molecule and integrate into the DNA elsewhere through a process called transposition. Transposons were originally discovered in maize (corn) by American geneticist Barbara McClintock (1902–1992) in the 1940s. Transposons have since been found in all types of organisms, both prokaryotes and eukaryotes. Thus, unlike the three previous mechanisms discussed, transposition is not prokaryote-specific. Most transposons are nonreplicative, meaning they move in a "cut-and-paste" fashion. Some may be replicative, however, retaining their location in the DNA while making a copy to be inserted elsewhere ("copy and paste"). Because transposons can move within a DNA molecule, from one DNA molecule to another, or even from one cell to another, they have the ability to introduce genetic diversity. Movement within the same DNA molecule can alter phenotype by inactivating or activating a gene.

Transposons may carry with them additional genes, moving these genes from one location to another with them. For example, bacterial transposons can relocate antibiotic resistance genes, moving them from chromosomes to plasmids. This mechanism has been shown to be responsible for the colocalization of multiple antibiotic resistance genes on a single R plasmid in *Shigella* strains causing bacterial dysentery. Such an R plasmid can then be easily transferred among a bacterial population through the process of conjugation.

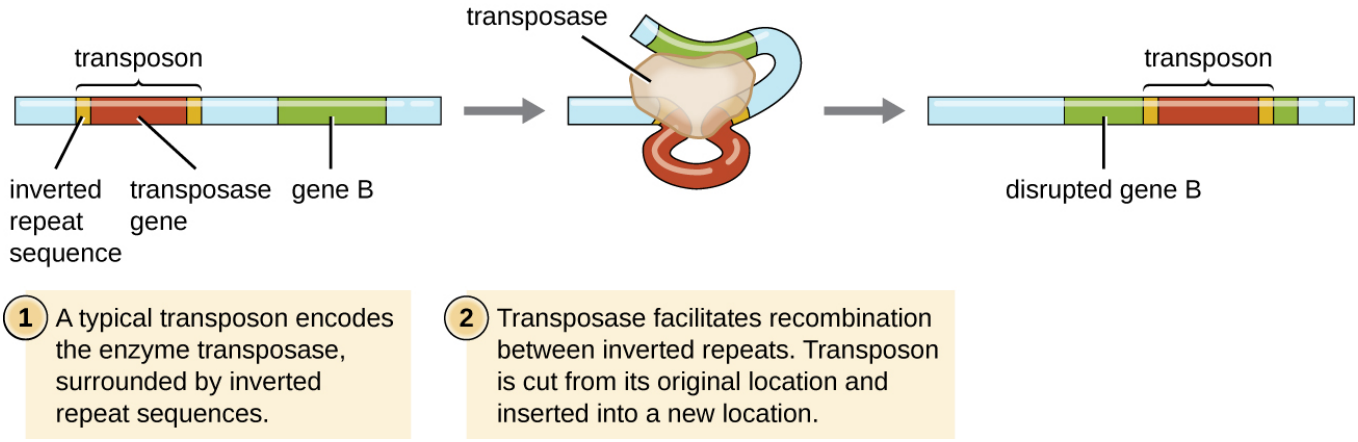


Figure 9.7.6: Transposons are segments of DNA that have the ability to move from one location to another because they code for the enzyme transposase. In this example, a nonreplicative transposon has disrupted gene B. The consequence of that the transcription of gene B may now have been interrupted.

? Exercise 9.7.6

What are two ways a transposon can affect the phenotype of a cell it moves to?

Table 9.7.1 summarizes the processes discussed in this section.

Table 9.7.1: Summary of Mechanisms of Genetic Diversity in Prokaryotes

Term	Definition
Conjugation	Transfer of DNA through direct contact using a conjugation pilus
Transduction	Mechanism of horizontal gene transfer in bacteria in which genes are transferred through viral infection
Transformation	Mechanism of horizontal gene transfer in which naked environmental DNA is taken up by a bacterial cell
Transposition	Process whereby DNA independently excises from one location in a DNA molecule and integrates elsewhere

Clinical Focus: Part 3

Despite continued antibiotic treatment, Mark's infection continued to progress rapidly. The infected region continued to expand, and he had to be put on a ventilator to help him breathe. Mark's physician ordered surgical removal of the infected tissue. Following an initial surgery, Mark's wound was monitored daily to ensure that the infection did not return, but it continued to spread.

After two additional rounds of surgery, the infection finally seemed to be contained. A few days later, Mark was removed from the ventilator and was able to breathe on his own. However, he had lost a great deal of skin and soft tissue on his lower leg.

Exercise 9.7.7

1. Why does the removal of infected tissue stem the infection?
2. What are some likely complications of this method of treatment?

Key Concepts and Summary

- **Horizontal gene transfer** is an important way for asexually reproducing organisms like prokaryotes to acquire new traits.
- There are three mechanisms of horizontal gene transfer typically used by bacteria: **transformation**, **transduction**, and **conjugation**.
- Transformation allows for competent cells to take up naked DNA, released from other cells on their death, into their cytoplasm, where it may recombine with the host genome.
- In **generalized transduction**, any piece of chromosomal DNA may be transferred by accidental packaging of the degraded host chromosome into a phage head. In **specialized transduction**, only chromosomal DNA adjacent to the integration site of a lysogenic phage may be transferred as a result of imprecise excision of the prophage.
- Conjugation is mediated by the **F plasmid,** which encodes a **conjugation pilus** that brings an F plasmid-containing **F^+ cell** into contact with an **F^- cell**.
- The rare integration of the F plasmid into the bacterial chromosome, generating an **Hfr cell**, allows for transfer of chromosomal DNA from the donor to the recipient. Additionally, imprecise excision of the F plasmid from the chromosome may generate an F' plasmid that may be transferred to a recipient by conjugation.
- Conjugation transfer of **R plasmids** is an important mechanism for the spread of antibiotic resistance in bacterial communities.
- **Transposons** are molecules of DNA with inverted repeats at their ends that also encode the enzyme transposase, allowing for their movement from one location in DNA to another. Although found in both prokaryotes and eukaryotes, transposons are clinically relevant in bacterial pathogens for the movement of virulence factors, including antibiotic resistance genes.

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9.E: Mechanisms of Microbial Genetics (Exercises)

11.1: What Are Genes?

A gene is composed of DNA that is “read” or transcribed to produce an RNA molecule during the process of transcription. One major type of RNA molecule, called messenger RNA (mRNA), provides the information for the ribosome to catalyze protein synthesis in a process called translation. The processes of transcription and translation are collectively referred to as gene expression.

Multiple Choice

DNA does all but which of the following?

A. serves as the genetic material passed from parent to offspring
B. remains constant despite changes in environmental conditions
C. provides the instructions for the synthesis of messenger RNA
D. is read by ribosomes during the process of translation

Answer

D

According to the central dogma, which of the following represents the flow of genetic information in cells?

A. protein to DNA to RNA
B. DNA to RNA to protein
C. RNA to DNA to protein
D. DNA to protein to RNA

Answer

B

True/False

Cells are always producing proteins from every gene they possess.

Answer

False

Fill in the Blank

The process of making an RNA copy of a gene is called _____.

Answer

transcription

A cell’s _____ remains constant whereas its phenotype changes in response to environmental influences.

Answer

genotype or genome

Short Answer

Can two observably different cells have the same genotype? Explain.

Critical Thinking

A pure culture of an unknown bacterium was streaked onto plates of a variety of media. You notice that the colony morphology is strikingly different on plates of minimal media with glucose compared to that seen on trypticase soy agar plates. How can you

explain these differences in colony morphology?

11.2: DNA Replication

The DNA replication process is semiconservative, which results in two DNA molecules, each having one parental strand of DNA and one newly synthesized strand. In bacteria, the initiation of replication occurs at the origin of replication, where supercoiled DNA is unwound by DNA gyrase, made single-stranded by helicase, and bound by single-stranded binding protein to maintain its single-stranded state.

Multiple Choice

Which of the following is the enzyme that replaces the RNA nucleotides in a primer with DNA nucleotides?

A. DNA polymerase III
B. DNA polymerase I
C. primase
D. helicase

Answer

B

Which of the following is not involved in the initiation of replication?

A. ligase
B. DNA gyrase
C. single-stranded binding protein
D. primase

Answer

A

Which of the following enzymes involved in DNA replication is unique to eukaryotes?

A. helicase
B. DNA polymerase
C. ligase
D. telomerase

Answer

D

Which of the following would be synthesized using 5′-CAGTTCGGA-3′ as a template?

A. 3′-AGGCTTGAC-4′
B. 3′-TCCGAACTG-5′
C. 3′-GTCAAGCCT-5′
D. 3′-CAGTTCGGA-5′

Answer

C

Fill in the Blank

The enzyme responsible for relaxing supercoiled DNA to allow for the initiation of replication is called _____.

Answer

DNA gyrase or topoisomerase II

Unidirectional replication of a circular DNA molecule like a plasmid that involves nicking one DNA strand and displacing it while synthesizing a new strand is called _____.

Answer

rolling circle replication

True/False

More primers are used in lagging strand synthesis than in leading strand synthesis.

Answer

True

Short Answer

Why is primase required for DNA replication?

What is the role of single-stranded binding protein in DNA replication?

Below is a DNA sequence. Envision that this is a section of a DNA molecule that has separated in preparation for replication, so you are only seeing one DNA strand. Construct the complementary DNA sequence (indicating 5’ and 3’ ends).

DNA sequence: 3’-T A C T G A C T G A C G A T C-5’

Critical Thinking

Review Figure 11.2.1 and Figure 11.2.2. Why was it important that Meselson and Stahl continue their experiment to at least two rounds of replication after isotopic labeling of the starting DNA with ^{15}N, instead of stopping the experiment after only one round of replication?

If deoxyribonucleotides that lack the 3’-OH groups are added during the replication process, what do you expect will occur?

11.3: RNA Transcription

During the process of transcription, the information encoded within the DNA sequence of one or more genes is transcribed into a strand of RNA, also called an RNA transcript. The resulting single-stranded RNA molecule, composed of ribonucleotides containing the bases adenine, cytosine, guanine, and uracil, acts as a mobile molecular copy of the original DNA sequence. Transcription in prokaryotes and in eukaryotes requires the DNA double helix to partially unwind in the region of RNA synthesis.

Multiple Choice

During which stage of bacterial transcription is the σ subunit of the RNA polymerase involved?

A. initiation
B. elongation
C. termination
D. splicing

Answer

A

Which of the following components is involved in the initiation of transcription?

A. primer
B. origin
C. promoter
D. start codon

Answer

C

Which of the following is not a function of the 5’ cap and 3’ poly-A tail of a mature eukaryotic mRNA molecule?

A. to facilitate splicing
B. to prevent mRNA degradation
C. to aid export of the mature transcript to the cytoplasm
D. to aid ribosome binding to the transcript

Answer

A

Mature mRNA from a eukaryote would contain each of these features except which of the following?

A. exon-encoded RNA
B. intron-encoded RNA
C. 5’ cap
D. 3’ poly-A tail

Answer

B

Fill in the Blank

A _____ mRNA is one that codes for multiple polypeptides.

Answer

polycistronic

The protein complex responsible for removing intron-encoded RNA sequences from primary transcripts in eukaryotes is called the _____.

Answer

Spliceosome

Short Answer

What is the purpose of RNA processing in eukaryotes? Why don’t prokaryotes require similar processing?

Below is a DNA sequence. Envision that this is a section of a DNA molecule that has separated in preparation for transcription, so you are only seeing the antisense strand. Construct the mRNA sequence transcribed from this template.

Antisense DNA strand: 3’-T A C T G A C T G A C G A T C-5’

Critical Thinking

Predict the effect of an alteration in the sequence of nucleotides in the –35 region of a bacterial promoter.

11.4: Protein Synthesis (Translation)

The synthesis of proteins consumes more of a cell’s energy than any other metabolic process. In turn, proteins account for more mass than any other macromolecule of living organisms. They perform virtually every function of a cell, serving as both functional (e.g., enzymes) and structural elements. The process of translation, or protein synthesis, the second part of gene expression, involves the decoding by a ribosome of an mRNA message into a polypeptide product.

Multiple Choice

Which of the following is the name of the three-base sequence in the mRNA that binds to a tRNA molecule?

A. P site
B. codon

C. anticodon
D. CCA binding site

Answer

B

Which component is the last to join the initiation complex during the initiation of translation?

A. the mRNA molecule
B. the small ribosomal subunit
C. the large ribosomal subunit
D. the initiator tRNA

Answer

C

During elongation in translation, to which ribosomal site does an incoming charged tRNA molecule bind?

A. A site
B. P site
C. E site
D. B site

Answer

A

Which of the following is the amino acid that appears at the N-terminus of all newly translated prokaryotic and eukaryotic polypeptides?

A. tryptophan
B. methionine
C. selenocysteine
D. glycine

Answer

B

When the ribosome reaches a nonsense codon, which of the following occurs?

A. a methionine is incorporated
B. the polypeptide is released
C. a peptide bond forms
D. the A site binds to a charged tRNA

Answer

B

Fill in the Blank

The third position within a codon, in which changes often result in the incorporation of the same amino acid into the growing polypeptide, is called the _____.

Answer

wobble position

The enzyme that adds an amino acid to a tRNA molecule is called _____.

Answer

aminoacyl-tRNA synthetase

True/False

Each codon within the genetic code encodes a different amino acid.

Answer

False

Short Answer

Why does translation terminate when the ribosome reaches a stop codon? What happens?

How does the process of translation differ between prokaryotes and eukaryotes?

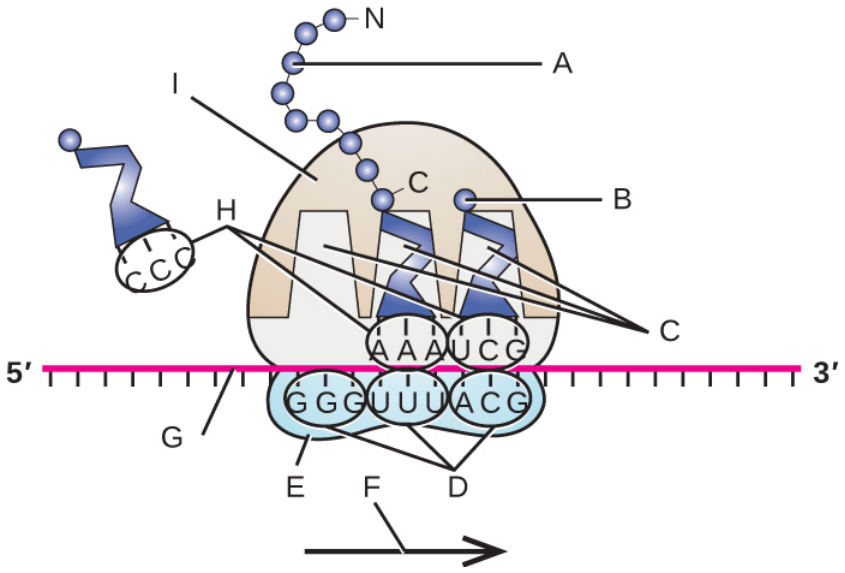
What is meant by the genetic code being nearly universal?

Below is an antisense DNA sequence. Translate the mRNA molecule synthesized using the genetic code, recording the resulting amino acid sequence, indicating the N and C termini.

Antisense DNA strand: 3’-T A C T G A C T G A C G A T C-5’

Critical Thinking

Label the following in the figure: ribosomal E, P, and A sites; mRNA; codons; anticodons; growing polypeptide; incoming amino acid; direction of translocation; small ribosomal unit; large ribosomal unit.



Prior to the elucidation of the genetic code, prominent scientists, including Francis Crick, had predicted that each mRNA codon, coding for one of the 20 amino acids, needed to be at least three nucleotides long. Why is it not possible for codons to be any shorter?

11.5: Mutations

A mutation is a heritable change in the DNA sequence of an organism. The resulting organism, called a mutant, may have a recognizable change in phenotype compared to the wild type, which is the phenotype most commonly observed in nature. A change in the DNA sequence is conferred to mRNA through transcription, and may lead to an altered amino acid sequence in a protein on translation.

Multiple Choice

Which of the following is a change in the sequence that leads to formation of a stop codon?

A. missense mutation
B. nonsense mutation
C. silent mutation
D. deletion mutation

Answer

B

The formation of pyrimidine dimers results from which of the following?

A. spontaneous errors by DNA polymerase
B. exposure to gamma radiation
C. exposure to ultraviolet radiation
D. exposure to intercalating agents

Answer

C

Which of the following is an example of a frameshift mutation?

A. a deletion of a codon
B. missense mutation
C. silent mutation
D. deletion of one nucleotide

Answer

D

Which of the following is the type of DNA repair in which thymine dimers are directly broken down by the enzyme photolyase?

A. direct repair
B. nucleotide excision repair
C. mismatch repair
D. proofreading

Answer

A

Which of the following regarding the Ames test is true?

A. It is used to identify newly formed auxotrophic mutants.
B. It is used to identify mutants with restored biosynthetic activity.
C. It is used to identify spontaneous mutants.
D. It is used to identify mutants lacking photoreactivation activity.

Answer

B

Fill in the Blank

A chemical mutagen that is structurally similar to a nucleotide but has different base-pairing rules is called a _____.

Answer

nucleoside analog

The enzyme used in light repair to split thymine dimers is called _____.

Answer

photolyase

The phenotype of an organism that is most commonly observed in nature is called the _____.

Answer

wild type

True/False

Carcinogens are typically mutagenic.

Answer

True

Short Answer

Why is it more likely that insertions or deletions will be more detrimental to a cell than point mutations?

Critical Thinking

Below are several DNA sequences that are mutated compared with the wild-type sequence: 3'-T A C T G A C T G A C G A T C-5'. Envision that each is a section of a DNA molecule that has separated in preparation for transcription, so you are only seeing the template strand. Construct the complementary DNA sequences (indicating 5' and 3' ends) for each mutated DNA sequence, then transcribe (indicating 5' and 3' ends) the template strands, and translate the mRNA molecules using the genetic code, recording the resulting amino acid sequence (indicating the N and C termini). What type of mutation is each?

Mutated DNA Template Strand #1: 3'-T A C T G T C T G A C G A T C-5' Complementary DNA sequence: mRNA sequence transcribed from template: Amino acid sequence of peptide: Type of mutation:
Mutated DNA Template Strand #2: 3'-T A C G G A C T G A C G A T C-5' Complementary DNA sequence: mRNA sequence transcribed from template: Amino acid sequence of peptide: Type of mutation:
Mutated DNA Template Strand #3: 3'-T A C T G A C T G A C T A T C-5' Complementary DNA sequence: mRNA sequence transcribed from template: Amino acid sequence of peptide: Type of mutation:
Mutated DNA Template Strand #4: 3'-T A C G A C T G A C T A T C-5' Complementary DNA sequence: mRNA sequence transcribed from template: Amino acid sequence of peptide: Type of mutation:

Why do you think the Ames test is preferable to the use of animal models to screen chemical compounds for mutagenicity?

11.6: How Asexual Prokaryotes Achieve Genetic Diversity

How do organisms whose dominant reproductive mode is asexual create genetic diversity? In prokaryotes, horizontal gene transfer (HGT), the introduction of genetic material from one organism to another organism within the same generation, is an important way to introduce genetic diversity. HGT allows even distantly related species to share genes, influencing their phenotypes.

Multiple Choice

Which is the mechanism by which improper excision of a prophage from a bacterial chromosome results in packaging of bacterial genes near the integration site into a phage head?

A. conjugation
B. generalized transduction
C. specialized transduction
D. transformation

Answer

C

Which of the following refers to the uptake of naked DNA from the surrounding environment?

A. conjugation
B. generalized transduction
C. specialized transduction
D. transformation

Answer

D

The F plasmid is involved in which of the following processes?

A. conjugation
B. transduction
C. transposition
D. transformation

Answer

A

Which of the following refers to the mechanism of horizontal gene transfer naturally responsible for the spread of antibiotic resistance genes within a bacterial population?

A. conjugation
B. generalized transduction
C. specialized transduction
D. transformation

Answer

A

Fill in the Blank

A small DNA molecule that has the ability to independently excise from one location in a larger DNA molecule and integrate into the DNA elsewhere is called a _____.

Answer

transposon or transposable element

_____ is a group of mechanisms that allow for the introduction of genetic material from one organism to another organism within the same generation.

Answer

Horizontal gene transfer

True/False

Asexually reproducing organisms lack mechanisms for generating genetic diversity within a population.

Answer

False

Short Answer

Briefly describe two ways in which chromosomal DNA from a donor cell may be transferred to a recipient cell during the process of conjugation.

Describe what happens when a nonsense mutation is introduced into the gene encoding transposase within a transposon.

11.7: Gene Regulation - Operon Theory

Genomic DNA contains both structural genes, which encode products that serve as cellular structures or enzymes, and regulatory genes, which encode products that regulate gene expression. The expression of a gene is a highly regulated process. Whereas regulating gene expression in multicellular organisms allows for cellular differentiation, in single-celled organisms like prokaryotes, it ensures that a cell's resources are not wasted making proteins that the cell does not need at that time.

Multiple Choice

An operon of genes encoding enzymes in a biosynthetic pathway is likely to be which of the following?

A. inducible
B. repressible
C. constitutive
D. monocistronic

Answer

B

An operon encoding genes that are transcribed and translated continuously to provide the cell with constant intermediate levels of the protein products is said to be which of the following?

A. repressible
B. inducible
C. constitutive
D. activated

Answer

C

Which of the following conditions leads to maximal expression of the *lac* operon?

A. lactose present, glucose absent
B. lactose present, glucose present
C. lactose absent, glucose absent
D. lactose absent, glucose present

Answer

A

Which of the following is a type of regulation of gene expression unique to eukaryotes?

A. attenuation
B. use of alternate σ factor
C. chemical modification of histones
D. alarmones

Answer

C

Fill in the Blank

The DNA sequence, to which repressors may bind, that lies between the promoter and the first structural gene is called the _____.

Answer

operator

The prevention of expression of operons encoding substrate use pathways for substrates other than glucose when glucose is present is called _____.

Answer

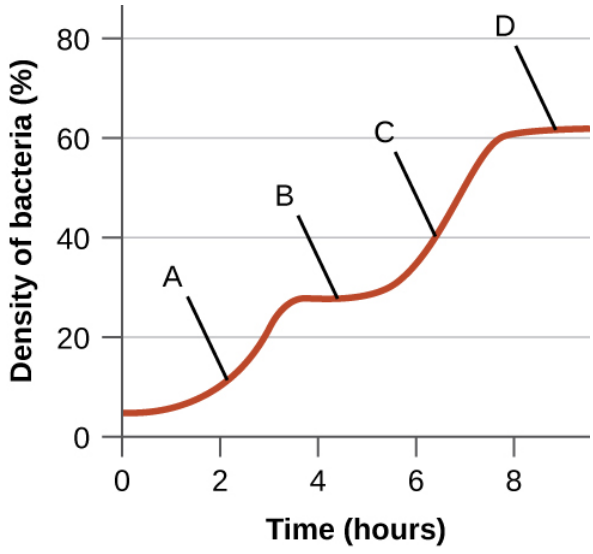
catabolite repression

Short Answer

What are two ways that bacteria can influence the transcription of multiple different operons simultaneously in response to a particular environmental condition?

Critical Thinking

The following figure is from Monod's original work on diauxic growth showing the growth of *E. coli* in the simultaneous presence of xylose and glucose as the only carbon sources. Explain what is happening at points A–D with respect to the carbon source being used for growth, and explain whether the xylose-use operon is being expressed (and why). Note that expression of the enzymes required for xylose use is regulated in a manner similar to the expression of the enzymes required for lactose use.



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CHAPTER OVERVIEW

10: Control of Microbial Growth

How clean is clean? People wash their cars and vacuum the carpets, but most would not want to eat from these surfaces. Similarly, we might eat with silverware cleaned in a dishwasher, but we could not use the same dishwasher to clean surgical instruments. As these examples illustrate, “clean” is a relative term. Car washing, vacuuming, and dishwashing all reduce the microbial load on the items treated, thus making them “cleaner.” But whether they are “clean enough” depends on their intended use. Because people do not normally eat from cars or carpets, these items do not require the same level of cleanliness that silverware does. Likewise, because silverware is not used for invasive surgery, these utensils do not require the same level of cleanliness as surgical equipment, which requires sterilization to prevent infection.

Why not play it safe and sterilize everything? Sterilizing everything we come in contact with is impractical, as well as potentially dangerous. As this chapter will demonstrate, sterilization protocols often require time- and labor-intensive treatments that may degrade the quality of the item being treated or have toxic effects on users. Therefore, the user must consider the item’s intended application when choosing a cleaning method to ensure that it is “clean enough.”

Location	Average number CFUs per 6.5 × 6.5 cm area
Door latch	256
Door lock	14
Door lock control	182
Door handle	29
Window control	4
Cruise control button	69
Steering wheel	239
Interior steering wheel	390
Radio volume knob	99
Gear shifter	115
Center console	506



Figure **10.1**: Most environments, including cars, are not sterile. A study[1] analyzed 11 locations within 18 different cars to determine the number of microbial colony-forming units (CFUs) present. The center console harbored by far the most microbes (506 CFUs), possibly because that is where drinks are placed (and often spilled). Frequently touched sites also had high concentrations. (credit “photo”: modification of work by Jeff Wilcox)

10.1: Controlling Microbial Growth

10.2: Testing the Effectiveness of Antiseptics and Disinfectants

10.3: Using Physical Methods to Control Microorganisms

10.4: Using Chemicals to Control Microorganisms

10.E: Control of Microbial Growth (Exercises)

Footnotes

1. 1 R.E. Stephenson et al. “Elucidation of Bacteria Found in Car Interiors and Strategies to Reduce the Presence of Potential Pathogens.” *Biofouling* 30 no. 3 (2014):337–346.

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10.1: Controlling Microbial Growth

Learning Objectives

- Compare disinfectants, antiseptics, and sterilants
- Describe the principles of controlling the presence of microorganisms through sterilization and disinfection
- Differentiate between microorganisms of various biological safety levels and explain methods used for handling microbes at each level

Clinical Focus: Part 1

Roberta is a 46-year-old real estate agent who recently underwent a cholecystectomy (surgery to remove painful gallstones). The surgery was performed laparoscopically with the aid of a duodenoscope, a specialized endoscope that allows surgeons to see inside the body with the aid of a tiny camera. On returning home from the hospital, Roberta developed abdominal pain and a high fever. She also experienced a burning sensation during urination and noticed blood in her urine. She notified her surgeon of these symptoms, per her postoperative instructions.

Exercise 10.1.1

What are some possible causes of Roberta's symptoms?

To prevent the spread of human disease, it is necessary to control the growth and abundance of microbes in or on various items frequently used by humans. Inanimate items, such as doorknobs, toys, or towels, which may harbor microbes and aid in disease transmission, are called **fomites**. Two factors heavily influence the level of cleanliness required for a particular fomite and, hence, the protocol chosen to achieve this level. The first factor is the application for which the item will be used. For example, invasive applications that require insertion into the human body require a much higher level of cleanliness than applications that do not. The second factor is the level of resistance to antimicrobial treatment by potential pathogens. For example, foods preserved by canning often become contaminated with the bacterium *Clostridium botulinum*, which produces the neurotoxin that causes botulism. Because *C. botulinum* can produce endospores that can survive harsh conditions, extreme temperatures and pressures must be used to eliminate the endospores. Other organisms may not require such extreme measures and can be controlled by a procedure such as washing clothes in a laundry machine.

Laboratory Biological Safety Levels

For researchers or laboratory personnel working with pathogens, the risks associated with specific pathogens determine the levels of cleanliness and control required. The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) have established four classification levels, called "biological safety levels" (BSLs). Various organizations around the world, including the World Health Organization (WHO) and the European Union (EU), use a similar classification scheme. According to the CDC, the BSL is determined by the agent's infectivity, ease of transmission, and potential disease severity, as well as the type of work being done with the agent.[1]

Each BSL requires a different level of biocontainment to prevent contamination and spread of infectious agents to laboratory personnel and, ultimately, the community. For example, the lowest BSL, BSL-1, requires the fewest precautions because it applies to situations with the lowest risk for microbial infection.

BSL-1 agents are those that generally do not cause infection in healthy human adults. These include noninfectious bacteria, such as nonpathogenic strains of *Escherichia coli* and *Bacillus subtilis*, and viruses known to infect animals other than humans, such as baculoviruses (insect viruses). Because working with BSL-1 agents poses very little risk, few precautions are necessary. Laboratory workers use standard aseptic technique and may work with these agents at an open laboratory bench or table, wearing personal protective equipment (PPE) such as a laboratory coat, goggles, and gloves, as needed. Other than a sink for handwashing and doors to separate the laboratory from the rest of the building, no additional modifications are needed.

Agents classified as BSL-2 include those that pose moderate risk to laboratory workers and the community, and are typically "indigenous," meaning that they are commonly found in that geographical area. These include bacteria such as *Staphylococcus aureus* and *Salmonella* spp., and viruses like hepatitis, mumps, and measles viruses. BSL-2 laboratories require additional precautions beyond those of BSL-1, including restricted access; required PPE, including a face shield in some circumstances; and the use of biological safety cabinets for procedures that may disperse agents through the air (called "aerosolization"). BSL-2 laboratories are equipped with self-closing doors, an eyewash station, and an autoclave, which is a specialized device for sterilizing materials with pressurized steam before use or disposal. BSL-1 laboratories may also have an autoclave.

BSL-3 agents have the potential to cause lethal infections by inhalation. These may be either indigenous or "exotic," meaning that they are derived from a foreign location, and include pathogens such as *Mycobacterium tuberculosis*, *Bacillus anthracis*, West Nile virus, and human immunodeficiency virus (HIV). Because of the serious nature of the infections caused by BSL-3 agents, laboratories working with them require restricted access. Laboratory workers are under medical surveillance, possibly receiving vaccinations for the microbes with which they work. In addition to the standard PPE already mentioned, laboratory personnel in BSL-3 laboratories must also wear a respirator and work with microbes and infectious agents in a biological safety cabinet at all times. BSL-3 laboratories require a hands-free sink, an eyewash station near the exit, and two sets of self-closing and locking doors at the entrance. These laboratories are equipped with directional airflow, meaning that clean air is pulled through the laboratory from clean areas to potentially contaminated areas. This air cannot be recirculated, so a constant supply of clean air is required.

BSL-4 agents are the most dangerous and often fatal. These microbes are typically exotic, are easily transmitted by inhalation, and cause infections for which there are no treatments or vaccinations. Examples include Ebola virus and Marburg virus, both of which cause hemorrhagic fevers, and smallpox virus. There are only a small number of laboratories in the United States and around the world appropriately equipped to work with these agents. In addition to BSL-3 precautions, laboratory workers in BSL-4 facilities must also change their clothing on entering the laboratory, shower on exiting, and decontaminate all material on exiting. While working in the laboratory, they must either wear a full-body protective suit with a designated air supply or conduct all work within a biological safety cabinet with a high-efficiency particulate air (HEPA)-filtered air supply and a doubly HEPA-filtered exhaust. If wearing a suit, the air pressure within the suit must be higher than that outside the suit, so that if a leak in the suit occurs, laboratory air that may be contaminated cannot be drawn into the suit (Figure **10.1.1**). The laboratory itself must be located either in a separate building or in an isolated portion of a building and have its own air supply and exhaust system, as well as its own decontamination system. The BSLs are summarized in Figure **10.1.2**.



Figure **10.1.1**: A protective suit like this one is an additional precaution for those who work in BSL-4 laboratories. This suit has its own air supply and maintains a positive pressure relative to the outside, so that if a leak occurs, air will flow out of the suit, not into it from the laboratory. (credit: modification of work by Centers for Disease Control and Prevention)

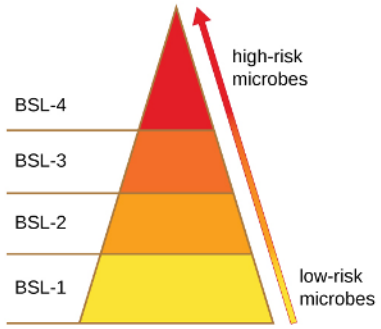
Biosafety Levels			
Biological Safety Levels	**Description**	**Examples**	**CDC Classification**
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	*Mycobacterium tuberculosis*	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	*Staphylococcus aureus*	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of *Escherichia coli*	

Figure **10.1.2**: The CDC classifies infectious agents into four biosafety levels based on potential risk to laboratory personnel and the community. Each level requires a progressively greater level of precaution. (credit "pyramid": modification of work by Centers for Disease Control and Prevention)

Link to Learning

To learn more about the four BSLs, visit the CDC's website.

Exercise 10.1.2

What are some factors used to determine the BSL necessary for working with a specific pathogen?

Sterilization

The most extreme protocols for microbial control aim to achieve sterilization: the complete removal or killing of all vegetative cells, endospores, and viruses from the targeted item or environment. Sterilization protocols are generally reserved for laboratory, medical, manufacturing, and food industry settings, where it may be imperative for certain items to be completely free of potentially infectious agents. Sterilization can be accomplished through either physical means, such as exposure to high heat, pressure, or filtration through an appropriate filter, or by chemical means. Chemicals that can be used to achieve sterilization are called sterilant**s**. Sterilants effectively kill all microbes and viruses, and, with appropriate exposure time, can also kill endospores.

For many clinical purposes, aseptic technique is necessary to prevent contamination of sterile surfaces. Aseptic technique involves a combination of protocols that collectively maintain sterility, or asepsis, thus preventing contamination of the patient with microbes and infectious agents. Failure to practice aseptic technique during many types of clinical procedures may introduce microbes to the patient's body and put the patient at risk for sepsis, a systemic inflammatory response to an infection that results in high fever, increased heart and respiratory rates, shock, and, possibly, death. Medical procedures that carry risk of contamination must be performed in a sterile field, a designated area that is kept free of all vegetative microbes, endospores, and viruses. Sterile fields are created according to protocols requiring the use of sterilized materials, such as packaging and drapings, and strict procedures for washing and application of sterilants. Other protocols are followed to maintain the sterile field while the medical procedure is being performed.

One food sterilization protocol, commercial sterilization, uses heat at a temperature low enough to preserve food quality but high enough to destroy common pathogens responsible for food poisoning, such as *C. botulinum*. Because *C. botulinum* and its endospores are commonly found in soil, they may easily contaminate crops during harvesting, and these endospores can later germinate within the anaerobic environment once foods are canned. Metal cans of food contaminated with *C. botulinum* will bulge

due to the microbe's production of gases; contaminated jars of food typically bulge at the metal lid. To eliminate the risk for *C. botulinum* contamination, commercial food-canning protocols are designed with a large margin of error. They assume an impossibly large population of endospores (10^{12} per can) and aim to reduce this population to 1 endospore per can to ensure the safety of canned foods. For example, low- and medium-acid foods are heated to 121 °C for a minimum of 2.52 minutes, which is the time it would take to reduce a population of 10^{12} endospores per can down to 1 endospore at this temperature. Even so, commercial sterilization does not eliminate the presence of all microbes; rather, it targets those pathogens that cause spoilage and foodborne diseases, while allowing many nonpathogenic organisms to survive. Therefore, "sterilization" is somewhat of a misnomer in this context, and commercial sterilization may be more accurately described as "quasi-sterilization."

? Exercise 10.1.3

What is the difference between sterilization and aseptic technique?

Link to Learning

The Association of Surgical Technologists publishes standards for aseptic technique, including creating and maintaining a sterile field.

Other Methods of Control

Sterilization protocols require procedures that are not practical, or necessary, in many settings. Various other methods are used in clinical and nonclinical settings to reduce the microbial load on items. Although the terms for these methods are often used interchangeably, there are important distinctions (Figure **10.1.3**).

The process of disinfection inactivates most microbes on the surface of a fomite by using antimicrobial chemicals or heat. Because some microbes remain, the disinfected item is not considered sterile. Ideally, disinfectants should be fast acting, stable, easy to prepare, inexpensive, and easy to use. An example of a natural disinfectant is vinegar; its acidity kills most microbes. Chemical disinfectants, such as chlorine bleach or products containing chlorine, are used to clean nonliving surfaces such as laboratory benches, clinical surfaces, and bathroom sinks. Typical disinfection does not lead to sterilization because endospores tend to survive even when all vegetative cells have been killed.

Unlike disinfectants, antiseptics are antimicrobial chemicals safe for use on living skin or tissues. Examples of antiseptics include hydrogen peroxide and isopropyl alcohol. The process of applying an antiseptic is called antisepsis. In addition to the characteristics of a good disinfectant, antiseptics must also be selectively effective against microorganisms and able to penetrate tissue deeply without causing tissue damage.

The type of protocol required to achieve the desired level of cleanliness depends on the particular item to be cleaned. For example, those used clinically are categorized as critical, semicritical, and noncritical. Critical items must be sterile because they will be used inside the body, often penetrating sterile tissues or the bloodstream; examples of critical items include surgical instruments, catheters, and intravenous fluids. Gastrointestinal endoscopes and various types of equipment for respiratory therapies are examples of semicritical items; they may contact mucous membranes or nonintact skin but do not penetrate tissues. Semicritical items do not typically need to be sterilized but do require a high level of disinfection. Items that may contact but not penetrate intact skin are noncritical items; examples are bed linens, furniture, crutches, stethoscopes, and blood pressure cuffs. These articles need to be clean but not highly disinfected.

The act of handwashing is an example of degerming, in which microbial numbers are significantly reduced by gently scrubbing living tissue, most commonly skin, with a mild chemical (e.g., soap) to avoid the transmission of pathogenic microbes. Wiping the skin with an alcohol swab at an injection site is another example of degerming. These degerming methods remove most (but not all) microbes from the skin's surface.

The term sanitization refers to the cleansing of fomites to remove enough microbes to achieve levels deemed safe for public health. For example, commercial dishwashers used in the food service industry typically use very hot water and air for washing and drying; the high temperatures kill most microbes, sanitizing the dishes. Surfaces in hospital rooms are commonly sanitized using a

chemical disinfectant to prevent disease transmission between patients. Figure **10.1.3** summarizes common protocols, definitions, applications, and agents used to control microbial growth.

Common Protocols for Control of Microbial Growth			
Protocol	**Definition**	**Common Application**	**Common Agents**
For Use on Fomites			
Disinfection	Reduces or destroys microbial load of an inanimate item through application of heat or antimicrobial chemicals	Cleaning surfaces like laboratory benches, clinical surfaces, and bathrooms	Chlorine bleach, phenols (e.g., Lysol), glutaraldehyde
Sanitization	Reduces microbial load of an inanimate item to safe public health levels through application of heat or antimicrobial chemicals	Commercial dishwashing of eating utensils, cleaning public restrooms	Detergents containing phosphates (e.g., Finish), industrial-strength cleaners containing quaternary ammonium compounds
Sterilization	Completely eliminates all vegetative cells, endospores, and viruses from an inanimate item	Preparation of surgical equipment and of needles used for injection	Pressurized steam (autoclave), chemicals, radiation
For Use on Living Tissue			
Antisepsis	Reduces microbial load on skin or tissue through application of an antimicrobial chemical	Cleaning skin broken due to injury; cleaning skin before surgery	Boric acid, isopropyl alcohol, hydrogen peroxide, iodine (betadine)
Degerming	Reduces microbial load on skin or tissue through gentle to firm scrubbing and the use of mild chemicals	Handwashing	Soap, alcohol swab

Figure **10.1.3**: Common protocols for control of microbial growth

? Exercise 10.1.4

1. What is the difference between a disinfectant and an antiseptic?
2. Which is most effective at removing microbes from a product: sanitization, degerming, or sterilization? Explain.

Clinical Focus: Part 2

Roberta's physician suspected that a bacterial infection was responsible for her sudden-onset high fever, abdominal pain, and bloody urine. Based on these symptoms, the physician diagnosed a urinary tract infection (UTI). A wide variety of bacteria may cause UTIs, which typically occur when bacteria from the lower gastrointestinal tract are introduced to the urinary tract. However, Roberta's recent gallstone surgery caused the physician to suspect that she had contracted a nosocomial (hospital-acquired) infection during her surgery. The physician took a urine sample and ordered a urine culture to check for the presence of white blood cells, red blood cells, and bacteria. The results of this test would help determine the cause of the infection. The physician also prescribed a course of the antibiotic ciprofloxacin, confident that it would clear Roberta's infection.

Exercise 10.1.5

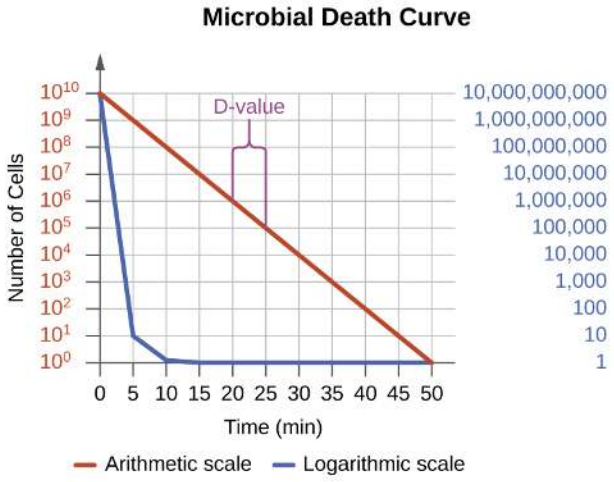
What are some possible ways that bacteria could have been introduced to Roberta's urinary tract during her surgery?

Measuring Microbial Control

Physical and chemical methods of microbial control that kill the targeted microorganism are identified by the suffix *-cide* (or *-cidal*). The prefix indicates the type of microbe or infectious agent killed by the treatment method: bactericide**s** kill bacteria, viricide**s** kill or inactivate viruses, and fungicide**s** kill fungi. Other methods do not kill organisms but, instead, stop their growth, making their population static; such methods are identified by the suffix *-stat* (or *-static*). For example, bacteriostatic treatments inhibit the growth of bacteria, whereas fungistatic treatments inhibit the growth of fungi. Factors that determine whether a particular treatment is *-cidal* or *-static* include the types of microorganisms targeted, the concentration of the chemical used, and the nature of the treatment applied.

Although *-static* treatments do not actually kill infectious agents, they are often less toxic to humans and other animals, and may also better preserve the integrity of the item treated. Such treatments are typically sufficient to keep the microbial population of an item in check. The reduced toxicity of some of these *-static* chemicals also allows them to be impregnated safely into plastics to prevent the growth of microbes on these surfaces. Such plastics are used in products such as toys for children and cutting boards for food preparation. When used to treat an infection, *-static* treatments are typically sufficient in an otherwise healthy individual, preventing the pathogen from multiplying, thus allowing the individual's immune system to clear the infection.

The degree of microbial control can be evaluated using a microbial death curve to describe the progress and effectiveness of a particular protocol. When exposed to a particular microbial control protocol, a fixed percentage of the microbes within the population will die. Because the rate of killing remains constant even when the population size varies, the percentage killed is more useful information than the absolute number of microbes killed. Death curves are often plotted as semilog plots just like microbial growth curves because the reduction in microorganisms is typically logarithmic (Figure **10.1.4**). The amount of time it takes for a specific protocol to produce a one order-of-magnitude decrease in the number of organisms, or the death of 90% of the population, is called the decimal reduction time (DRT) or D-value.



Time (min)	Number of cells
0	10^{10}
5	10^{9}
10	10^{8}
15	10^{7}
20	10^{6}
25	10^{5}
30	10^{4}
35	10^{3}
40	10^{2}
45	10^{1}
50	10^{0}

Figure **10.1.4**: Microbial death is logarithmic and easily observed using a semilog plot instead of an arithmetic one. The decimal reduction time (D-value) is the time it takes to kill 90% of the population (a 1-log decrease in the total population) when exposed to a specific microbial control protocol, as indicated by the purple bracket.

Several factors contribute to the effectiveness of a disinfecting agent or microbial control protocol. First, as demonstrated in Figure **10.1.4**, the length of time of exposure is important. Longer exposure times kill more microbes. Because microbial death of a population exposed to a specific protocol is logarithmic, it takes longer to kill a high-population load than a low-population load exposed to the same protocol. A shorter treatment time (measured in multiples of the D-value) is needed when starting with a smaller number of organisms. Effectiveness also depends on the susceptibility of the agent to that disinfecting agent or protocol. The concentration of disinfecting agent or intensity of exposure is also important. For example, higher temperatures and higher concentrations of disinfectants kill microbes more quickly and effectively. Conditions that limit contact between the agent and the targeted cells cells—for example, the presence of bodily fluids, tissue, organic debris (e.g., mud or feces), or biofilms on surfaces—increase the cleaning time or intensity of the microbial control protocol required to reach the desired level of cleanliness. All these factors must be considered when choosing the appropriate protocol to control microbial growth in a given situation.

? Exercise 10.1.6

1. What are two possible reasons for choosing a bacteriostatic treatment over a bactericidal one?
2. Name at least two factors that can compromise the effectiveness of a disinfecting agent.

Key Concepts and Summary

- Inanimate items that may harbor microbes and aid in their transmission are called **fomites**. The level of cleanliness required for a fomite depends both on the item's use and the infectious agent with which the item may be contaminated.
- The CDC and the NIH have established four **biological safety levels (BSLs)** for laboratories performing research on infectious agents. Each level is designed to protect laboratory personnel and the community. These BSLs are determined by the agent's infectivity, ease of transmission, and potential disease severity, as well as the type of work being performed with the agent.
- **Disinfection** removes potential pathogens from a fomite, whereas **antisepsis** uses antimicrobial chemicals safe enough for tissues; in both cases, microbial load is reduced, but microbes may remain unless the chemical used is strong enough to be a **sterilant**.
- The amount of cleanliness (**sterilization** versus high-level disinfection versus general cleanliness) required for items used clinically depends on whether the item will come into contact with sterile tissues (**critical item)**, mucous membranes (**semicritical item**), or intact skin (**noncritical item**).
- Medical procedures with a risk for contamination should be carried out in a **sterile field** maintained by proper **aseptic technique** to prevent **sepsis**.
- Sterilization is necessary for some medical applications as well as in the food industry, where endospores of *Clostridium botulinum* are killed through **commercial sterilization** protocols.
- Physical or chemical methods to control microbial growth that result in death of the microbe are indicated by the suffixes *-cide* or *-cidal* (e.g., as with **bactericides**, **viricides**, and **fungicides**), whereas those that inhibit microbial growth are indicated by the suffixes *-stat* or*-static* (e.g., **bacteriostatic**, **fungistatic**).
- **Microbial death curves** display the logarithmic decline of living microbes exposed to a method of microbial control. The time it takes for a protocol to yield a 1-log (90%) reduction in the microbial population is the **decimal reduction time**, or **D-value**.
- When choosing a microbial control protocol, factors to consider include the length of exposure time, the type of microbe targeted, its susceptibility to the protocol, the intensity of the treatment, the presence of organics that may interfere with the protocol, and the environmental conditions that may alter the effectiveness of the protocol.

Footnotes

1. 1 US Centers for Disease Control and Prevention. "Recognizing the Biosafety Levels." http://www.cdc.gov/training/quicklearns/biosafety/. Accessed June 7, 2016.

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10.2: Testing the Effectiveness of Antiseptics and Disinfectants

Learning Objectives

- Describe why the phenol coefficient is used
- Compare and contrast the disk-diffusion, use-dilution, and in-use methods for testing the effectiveness of antiseptics, disinfectants, and sterilants

The effectiveness of various chemical disinfectants is reflected in the terms used to describe them. Chemical disinfectants are grouped by the power of their activity, with each category reflecting the types of microbes and viruses its component disinfectants are effective against. High-level germicides have the ability to kill vegetative cells, fungi, viruses, and endospores, leading to sterilization, with extended use. Intermediate-level germicides, as their name suggests, are less effective against endospores and certain viruses, and low-level germicides kill only vegetative cells and certain enveloped viruses, and are ineffective against endospores.

However, several environmental conditions influence the potency of an antimicrobial agent and its effectiveness. For example, length of exposure is particularly important, with longer exposure increasing efficacy. Similarly, the concentration of the chemical agent is also important, with higher concentrations being more effective than lower ones. Temperature, pH, and other factors can also affect the potency of a disinfecting agent.

One method to determine the effectiveness of a chemical agent includes swabbing surfaces before and after use to confirm whether a sterile field was maintained during use. Additional tests are described in the sections that follow. These tests allow for the maintenance of appropriate disinfection protocols in clinical settings, controlling microbial growth to protect patients, health-care workers, and the community.

Phenol Coefficient

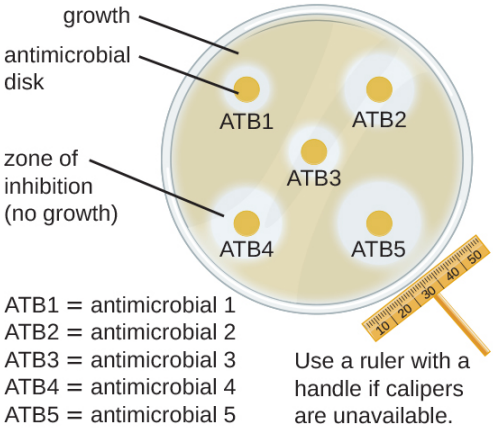
The effectiveness of a disinfectant or antiseptic can be determined in a number of ways. Historically, a chemical agent's effectiveness was often compared with that of phenol, the first chemical agent used by Joseph Lister. In 1903, British chemists Samuel Rideal (1863–1929) and J. T. Ainslie Walker (1868–1930) established a protocol to compare the effectiveness of a variety of chemicals with that of phenol, using as their test organisms *Staphylococcus aureus* (a gram-positive bacterium) and *Salmonella enterica* serovar Typhi (a gram-negative bacterium). They exposed the test bacteria to the antimicrobial chemical solutions diluted in water for 7.5 minutes. They then calculated a phenol coefficient for each chemical for each of the two bacteria tested. A phenol coefficient of 1.0 means that the chemical agent has about the same level of effectiveness as phenol. A chemical agent with a phenol coefficient of less than 1.0 is less effective than phenol. An example is formalin, with phenol coefficients of 0.3 (*S. aureus*) and 0.7 (*S. enterica* serovar Typhi). A chemical agent with a phenol coefficient greater than 1.0 is more effective than phenol, such as chloramine, with phenol coefficients of 133 and 100, respectively. Although the phenol coefficient was once a useful measure of effectiveness, it is no longer commonly used because the conditions and organisms used were arbitrarily chosen.

Exercise 10.2.1

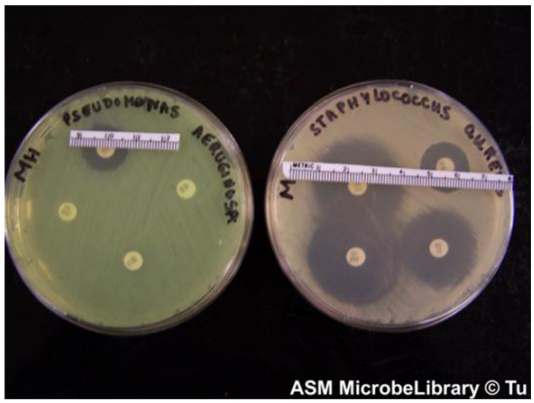
What are the differences between the three levels of disinfectant effectiveness?

Disk-Diffusion Method

The disk-diffusion method involves applying different chemicals to separate, sterile filter paper disks (Figure **10.2.1**). The disks are then placed on an agar plate that has been inoculated with the targeted bacterium and the chemicals diffuse out of the disks into the agar where the bacteria have been inoculated. As the "lawn" of bacteria grows, zones of inhibition of microbial growth are observed as clear areas around the disks. Although there are other factors that contribute to the sizes of zones of inhibition (e.g., whether the agent is water soluble and able to diffuse in the agar), larger zones typically correlate to increased inhibition effectiveness of the chemical agent. The diameter across each zone is measured in millimeters.



(a)



(b)

Figure **10.2.1**: A disk-diffusion assay is used to determine the effectiveness of chemical agents against a particular microbe. (a) A plate is inoculated with various antimicrobial discs. The zone of inhibition around each disc indicates how effective that antimicrobial is against the particular species being tested. (b) On these plates, four antimicrobial agents are tested for efficacy in killing *Pseudomonas aeruginosa* (left) and *Staphylococcus aureus* (right). These antimicrobials are much more effective at killing *S. aureus*, as indicated by the size of the zones of inhibition. (credit b: modification of work by American Society for Microbiology)

? Exercise 10.2.2

When comparing the activities of two disinfectants against the same microbe, using the disk-diffusion assay, and assuming both are water soluble and can easily diffuse in the agar, would a more effective disinfectant have a larger zone of inhibition or a smaller one?

Use-Dilution Test

Other methods are also used for measuring the effectiveness of a chemical agent in clinical settings. The use-dilution test is commonly used to determine a chemical's disinfection effectiveness on an inanimate surface. For this test, a cylinder of stainless steel is dipped in a culture of the targeted microorganism and then dried. The cylinder is then dipped in solutions of disinfectant at various concentrations for a specified amount of time. Finally, the cylinder is transferred to a new test tube containing fresh sterile medium that does not contain disinfectant, and this test tube is incubated. Bacterial survival is demonstrated by the presence of turbidity in the medium, whereas killing of the target organism on the cylinder by the disinfectant will produce no turbidity.

The Association of Official Agricultural Chemists International (AOAC), a nonprofit group that establishes many protocol standards, has determined that a minimum of 59 of 60 replicates must show no growth in such a test to achieve a passing result, and the results must be repeatable from different batches of disinfectant and when performed on different days. Disinfectant manufacturers perform use-dilution tests to validate the efficacy claims for their products, as designated by the EPA.

? Exercise 10.2.3

Is the use-dilution test performed in a clinical setting? Why?

In-Use Test

An in-use test can determine whether an actively used solution of disinfectant in a clinical setting is microbially contaminated (Figure **10.2.2**). A 1-mL sample of the used disinfectant is diluted into 9 mL of sterile broth medium that also contains a compound to inactivate the disinfectant. Ten drops, totaling approximately 0.2 mL of this mixture, are then inoculated onto each of two agar plates. One plate is incubated at 37 °C for 3 days and the other is incubated at room temperature for 7 days. The plates are monitored for growth of microbial colonies. Growth of five or more colonies on either plate suggests that viable microbial cells existed in the disinfectant solution and that it is contaminated. Such in-use tests monitor the effectiveness of disinfectants in the clinical setting.

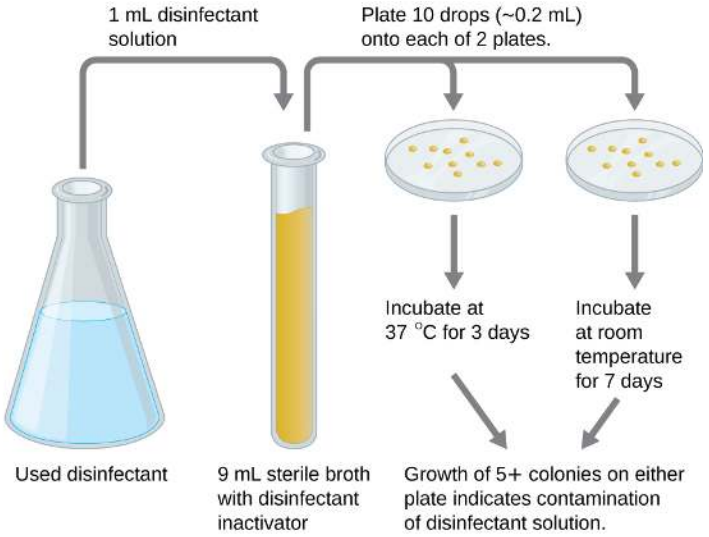


Figure **10.2.2**: Used disinfectant solutions in a clinical setting can be checked with the in-use test for contamination with microbes.

? Exercise 10.2.4

What does a positive in-use test indicate?

Clinical Focus: Resolution

Despite antibiotic treatment, Roberta's symptoms worsened. She developed pyelonephritis, a severe kidney infection, and was rehospitalized in the intensive care unit (ICU). Her condition continued to deteriorate, and she developed symptoms of septic shock. At this point, her physician ordered a culture from her urine to determine the exact cause of her infection, as well as a drug sensitivity test to determine what antibiotics would be effective against the causative bacterium. The results of this test indicated resistance to a wide range of antibiotics, including the carbapenems, a class of antibiotics that are used as the last resort for many types of bacterial infections. This was an alarming outcome, suggesting that Roberta's infection was caused by a so-called superbug: a bacterial strain that has developed resistance to the majority of commonly used antibiotics. In this case, the causative agent belonged to the carbapenem-resistant Enterobacteriaceae (CRE), a drug-resistant family of bacteria normally found in the digestive system (Figure **10.2.3**). When CRE is introduced to other body systems, as might occur through improperly cleaned surgical instruments, catheters, or endoscopes, aggressive infections can occur.

CRE infections are notoriously difficult to treat, with a 40%–50% fatality rate. To treat her kidney infection and septic shock, Roberta was treated with dialysis, intravenous fluids, and medications to maintain blood pressure and prevent blood clotting. She was also started on aggressive treatment with intravenous administration of a new drug called tigecycline, which has been successful in treating infections caused by drug-resistant bacteria.

After several weeks in the ICU, Roberta recovered from her CRE infection. However, public health officials soon noticed that Roberta's case was not isolated. Several patients who underwent similar procedures at the same hospital also developed CRE infections, some dying as a result. Ultimately, the source of the infection was traced to the duodenoscopes used in the procedures. Despite the hospital staff meticulously following manufacturer protocols for disinfection, bacteria, including CRE, remained within the instruments and were introduced to patients during procedures.



Figure **10.2.3**: CRE is an extremely drug-resistant strain of bacteria that is typically associated with nosocomial infections. (credit: Centers for Disease Control and Prevention)

Who Is Responsible?

Carbapenem-resistant Enterobacteriaceae infections due to contaminated endoscopes have become a high-profile problem in recent years. Several CRE outbreaks have been traced to endoscopes, including a case at Ronald Reagan UCLA Medical Center in early 2015 in which 179 patients may have been exposed to a contaminated endoscope. Seven of the patients developed infections, and two later died. Several lawsuits have been filed against Olympus, the manufacturer of the endoscopes. Some claim that Olympus did not obtain FDA approval for design changes that may have led to contamination, and others claim that the manufacturer knowingly withheld information from hospitals concerning defects in the endoscopes.

Lawsuits like these raise difficult-to-answer questions about liability. Invasive procedures are inherently risky, but negative outcomes can be minimized by strict adherence to established protocols. Who is responsible, however, when negative outcomes occur due to flawed protocols or faulty equipment? Can hospitals or health-care workers be held liable if they have strictly followed a flawed procedure? Should manufacturers be held liable—and perhaps be driven out of business—if their lifesaving equipment fails or is found defective? What is the government's role in ensuring that use and maintenance of medical equipment and protocols are fail-safe?

Protocols for cleaning or sterilizing medical equipment are often developed by government agencies like the FDA, and other groups, like the AOAC, a nonprofit scientific organization that establishes many protocols for standard use globally. These procedures and protocols are then adopted by medical device and equipment manufacturers. Ultimately, the end-users (hospitals and their staff) are responsible for following these procedures and can be held liable if a breach occurs and patients become ill from improperly cleaned equipment.

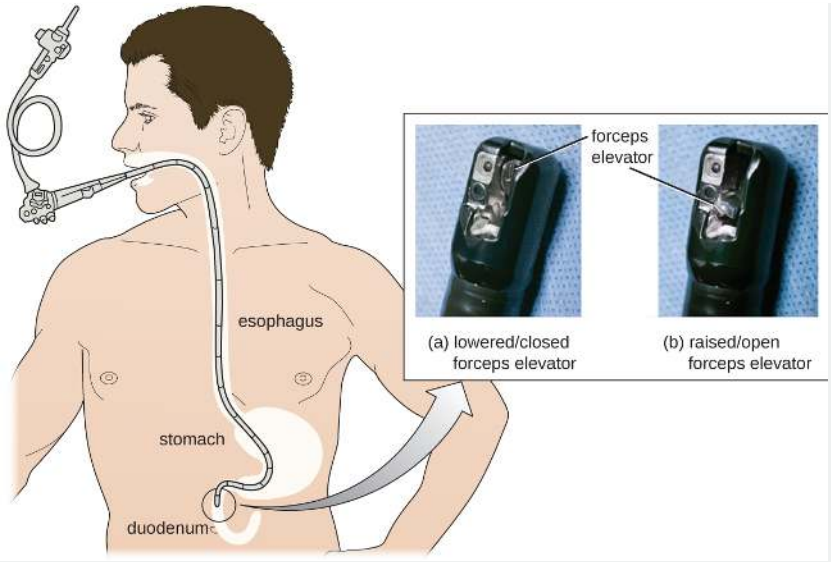


Figure **10.2.4**: The elevator mechanism in a duodenoscope contains crevices that are difficult to disinfect. Pathogens that survive disinfection protocols can be passed from one patient to another, causing serious infections. (credit "photos": modification of work by Centers for Disease Control and Prevention)

Unfortunately, protocols are not infallible, and sometimes it takes negative outcomes to reveal their flaws. In 2008, the FDA had approved a disinfection protocol for endoscopes, using glutaraldehyde (at a lower concentration when mixed with phenol), o-phthalaldehyde, hydrogen peroxide, peracetic acid, and a mix of hydrogen peroxide with peracetic acid. However, subsequent CRE outbreaks from endoscope use showed that this protocol alone was inadequate.

As a result of CRE outbreaks, hospitals, manufacturers, and the FDA are investigating solutions. Many hospitals are instituting more rigorous cleaning procedures than those mandated by the FDA. Manufacturers are looking for ways to redesign duodenoscopes to minimize hard-to-reach crevices where bacteria can escape disinfectants, and the FDA is updating its protocols. In February 2015, the FDA added new recommendations for careful hand cleaning of the duodenoscope elevator mechanism (the location where microbes are most likely to escape disinfection), and issued more careful documentation about quality control of disinfection protocols (Figure **10.2.4**).

There is no guarantee that new procedures, protocols, or equipment will completely eliminate the risk for infection associated with endoscopes. Yet these devices are used successfully in 500,000–650,000 procedures annually in the United States, many of them lifesaving. At what point do the risks outweigh the benefits of these devices, and who should be held responsible when negative outcomes occur?

Key Concepts and Summary

- Chemical disinfectants are grouped by the types of microbes and infectious agents they are effective against. **High-level germicides** kill vegetative cells, fungi, viruses, and endospores, and can ultimately lead to sterilization. **Intermediate-level germicides** cannot kill all viruses and are less effective against endospores. **Low-level germicides** kill vegetative cells and some enveloped viruses, but are ineffective against endospores.
- The effectiveness of a disinfectant is influenced by several factors, including length of exposure, concentration of disinfectant, temperature, and pH.
- Historically, the effectiveness of a chemical disinfectant was compared with that of phenol at killing *Staphylococcus aureus* and *Salmonella enterica* serovar Typhi, and a **phenol coefficient** was calculated.
- The **disk-diffusion method** is used to test the effectiveness of a chemical disinfectant against a particular microbe.
- The **use-dilution test** determines the effectiveness of a disinfectant on a surface. **In-use tests** can determine whether disinfectant solutions are being used correctly in clinical settings.

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10.3: Using Physical Methods to Control Microorganisms

Learning Objectives

- Understand and compare various physical methods of controlling microbial growth, including heating, refrigeration, freezing, high-pressure treatment, desiccation, lyophilization, irradiation, and filtration

For thousands of years, humans have used various physical methods of microbial control for food preservation. Common control methods include the application of high temperatures, radiation, filtration, and desiccation (drying), among others. Many of these methods nonspecifically kill cells by disrupting membranes, changing membrane permeability, or damaging proteins and nucleic acids by denaturation, degradation, or chemical modification. Various physical methods used for microbial control are described in this section.

Heat

Heating is one of the most common—and oldest—forms of microbial control. It is used in simple techniques like cooking and canning. Heat can kill microbes by altering their membranes and denaturing proteins. The **thermal death point (TDP)** of a microorganism is the lowest temperature at which all microbes are killed in a 10-minute exposure. Different microorganisms will respond differently to high temperatures, with some (e.g., endospore-formers such as *C. botulinum*) being more heat tolerant. A similar parameter, the **thermal death time (TDT)**, is the length of time needed to kill all microorganisms in a sample at a given temperature. These parameters are often used to describe sterilization procedures that use high heat, such as autoclaving. Boiling is one of the oldest methods of moist-heat control of microbes, and it is typically quite effective at killing vegetative cells and some viruses. However, boiling is less effective at killing endospores; some endospores are able to survive up to 20 hours of boiling. Additionally, boiling may be less effective at higher altitudes, where the boiling point of water is lower and the boiling time needed to kill microbes is therefore longer. For these reasons, boiling is not considered a useful sterilization technique in the laboratory or clinical setting.

Many different heating protocols can be used for sterilization in the laboratory or clinic, and these protocols can be broken down into two main categories: **dry-heat sterilization** and **moist-heat sterilization**. Aseptic technique in the laboratory typically involves some dry-heat sterilization protocols using direct application of high heat, such as sterilizing inoculating loops (Figure 10.3.1). Incineration at very high temperatures destroys all microorganisms. Dry heat can also be applied for relatively long periods of time (at least 2 hours) at temperatures up to 170 °C by using a dry-heat sterilizer, such as an oven. However, moist-heat sterilization is typically the more effective protocol because it penetrates cells better than dry heat does.



(a) (b)

Figure 10.3.1: (a) Sterilizing a loop, often referred to as “flaming a loop,” is a common component of aseptic technique in the microbiology laboratory and is used to incinerate any microorganisms on the loop. (b) Alternatively, a bactericinerator may be used to reduce aerosolization of microbes and remove the presence of an open flame in the laboratory. These are examples of dry-heat sterilization by the direct application of high heat capable of incineration. (credit a: modification of work by Anh-Hue Tu; credit b: modification of work by Brian Forster)

Autoclaves

Autoclaves rely on moist-heat sterilization. They are used to raise temperatures above the boiling point of water to sterilize items such as surgical equipment from vegetative cells, viruses, and especially endospores, which are known to survive boiling temperatures, without damaging the items. Charles Chamberland (1851–1908) designed the modern autoclave in 1879 while working in the laboratory of Louis Pasteur. The autoclave is still considered the most effective method of sterilization (Figure **10.3.2**). Outside laboratory and clinical settings, large industrial autoclaves called retorts allow for moist-heat sterilization on a large scale.

In general, the air in the chamber of an autoclave is removed and replaced with increasing amounts of steam trapped within the enclosed chamber, resulting in increased interior pressure and temperatures above the boiling point of water. The two main types of autoclaves differ in the way that air is removed from the chamber. In gravity displacement autoclaves, steam is introduced into the chamber from the top or sides. Air, which is heavier than steam, sinks to the bottom of the chamber, where it is forced out through a vent. Complete displacement of air is difficult, especially in larger loads, so longer cycles may be required for such loads. In prevacuum sterilizers, air is removed completely using a high-speed vacuum before introducing steam into the chamber. Because air is more completely eliminated, the steam can more easily penetrate wrapped items. Many autoclaves are capable of both gravity and prevacuum cycles, using the former for the decontamination of waste and sterilization of media and unwrapped glassware, and the latter for sterilization of packaged instruments.

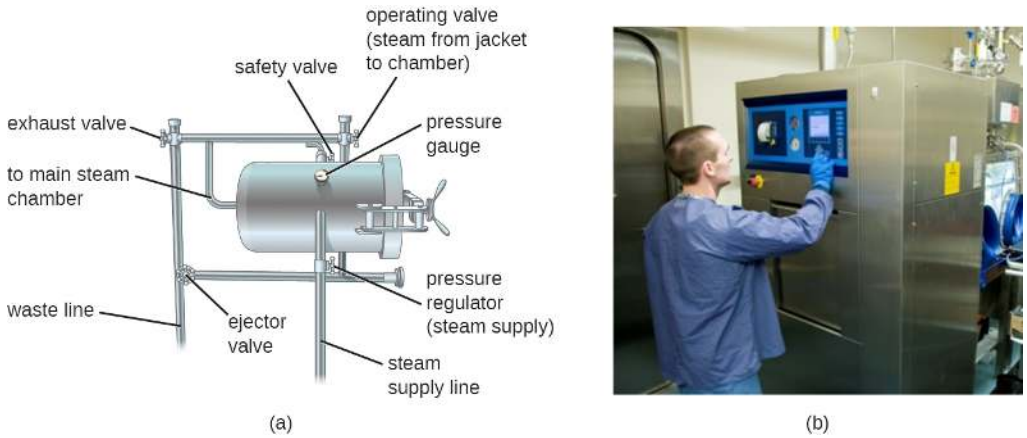


Figure **10.3.2**: (a) An autoclave is commonly used for sterilization in the laboratory and in clinical settings. By displacing the air in the chamber with increasing amounts of steam, pressure increases, and temperatures exceeding 100 °C can be achieved, allowing for complete sterilization. (b) A researcher programs an autoclave to sterilize a sample. (credit a: modification of work by Courtney Harrington; credit b: modification of work by Lackemeyer MG, Kok-Mercado Fd, Wada J, Bollinger L, Kindrachuk J, Wahl-Jensen V, Kuhn JH, Jahrling PB)

Standard operating temperatures for autoclaves are 121 °C or, in some cases, 132 °C, typically at a pressure of 15 to 20 pounds per square inch (psi). The length of exposure depends on the volume and nature of material being sterilized, but it is typically 20 minutes or more, with larger volumes requiring longer exposure times to ensure sufficient heat transfer to the materials being sterilized. The steam must directly contact the liquids or dry materials being sterilized, so containers are left loosely closed and instruments are loosely wrapped in paper or foil. The key to autoclaving is that the temperature must be high enough to kill endospores to achieve complete sterilization.

Because sterilization is so important to safe medical and laboratory protocols, quality control is essential. Autoclaves may be equipped with recorders to document the pressures and temperatures achieved during each run. Additionally, internal indicators of various types should be autoclaved along with the materials to be sterilized to ensure that the proper sterilization temperature has been reached (Figure **10.3.3**). One common type of indicator is the use of heat-sensitive autoclave tape, which has white stripes that turn black when the appropriate temperature is achieved during a successful autoclave run. This type of indicator is relatively inexpensive and can be used during every run. However, autoclave tape provides no indication of length of exposure, so it cannot be used as an indicator of sterility. Another type of indicator, a biological indicator spore test, uses either a strip of paper or a liquid suspension of the endospores of *Geobacillus stearothermophilus* to determine whether the endospores are killed by the process. The endospores of the obligate thermophilic bacterium *G. stearothermophilus* are the gold standard used for this purpose because of their extreme heat resistance. Biological spore indicators can also be used to test the effectiveness of other sterilization protocols,

including ethylene oxide, dry heat, formaldehyde, gamma radiation, and hydrogen peroxide plasma sterilization using either *G. stearothermophilus*, *Bacillus atrophaeus*, *B. subtilis*, or *B. pumilus* spores. In the case of validating autoclave function, the endospores are incubated after autoclaving to ensure no viable endospores remain. Bacterial growth subsequent to endospore germination can be monitored by biological indicator spore tests that detect acid metabolites or fluorescence produced by enzymes derived from viable *G. stearothermophilus*. A third type of autoclave indicator is the Diack tube, a glass ampule containing a temperature-sensitive pellet that melts at the proper sterilization temperature. Spore strips or Diack tubes are used periodically to ensure the autoclave is functioning properly.

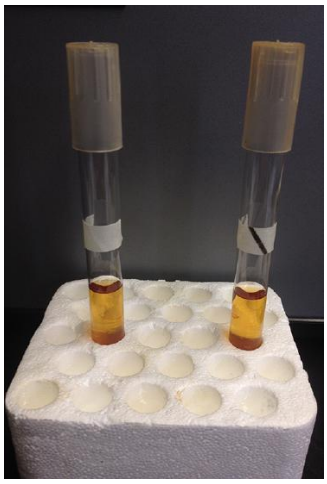


Figure **10.3.3**: The white strips on autoclave tape (left tube) turn dark during a successful autoclave run (right tube). (credit: modification of work by Brian Forster)

Pasteurization

Although complete sterilization is ideal for many medical applications, it is not always practical for other applications and may also alter the quality of the product. Boiling and autoclaving are not ideal ways to control microbial growth in many foods because these methods may ruin the consistency and other organoleptic (sensory) qualities of the food. Pasteurization is a form of microbial control for food that uses heat but does not render the food sterile. Traditional pasteurization kills pathogens and reduces the number of spoilage-causing microbes while maintaining food quality. The process of pasteurization was first developed by Louis Pasteur in the 1860s as a method for preventing the spoilage of beer and wine. Today, pasteurization is most commonly used to kill heat-sensitive pathogens in milk and other food products (e.g., apple juice and honey) (Figure **10.3.4**). However, because pasteurized food products are not sterile, they will eventually spoil.

The methods used for milk pasteurization balance the temperature and the length of time of treatment. One method, high-temperature short-time (HTST) pasteurization, exposes milk to a temperature of 72 °C for 15 seconds, which lowers bacterial numbers while preserving the quality of the milk. An alternative is ultra-high-temperature (UHT) pasteurization, in which the milk is exposed to a temperature of 138 °C for 2 or more seconds. UHT pasteurized milk can be stored for a long time in sealed containers without being refrigerated; however, the very high temperatures alter the proteins in the milk, causing slight changes in the taste and smell. Still, this method of pasteurization is advantageous in regions where access to refrigeration is limited.

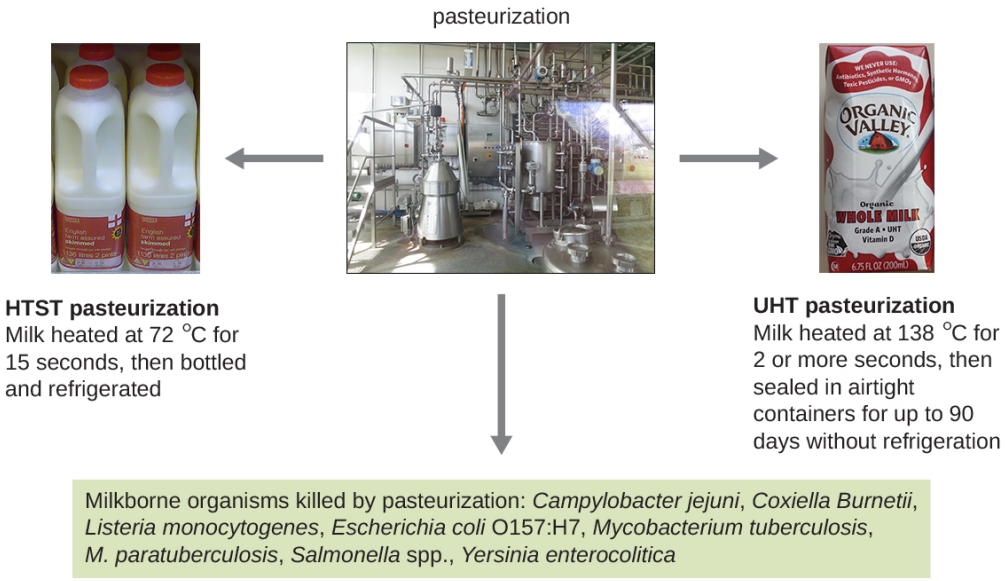


Figure **10.3.4**: Two different methods of pasteurization, HTST and UHT, are commonly used to kill pathogens associated with milk spoilage. (credit left: modification of work by Mark Hillary; credit right: modification of work by Kerry Ceszyk)

? Exercise 10.3.1

1. In an autoclave, how are temperatures above boiling achieved?
2. How would the onset of spoilage compare between HTST-pasteurized and UHT-pasteurized milk?
3. Why is boiling not used as a sterilization method in a clinical setting?

Refrigeration and Freezing

Just as high temperatures are effective for controlling microbial growth, exposing microbes to low temperatures can also be an easy and effective method of microbial control, with the exception of psychrophiles, which prefer cold temperatures (see Temperature and Microbial Growth). Refrigerators used in home kitchens or in the laboratory maintain temperatures between 0 °C and 7 °C. This temperature range inhibits microbial metabolism, slowing the growth of microorganisms significantly and helping preserve refrigerated products such as foods or medical supplies. Certain types of laboratory cultures can be preserved by refrigeration for later use.

Freezing below −2 °C may stop microbial growth and even kill susceptible organisms. According to the US Department of Agriculture (USDA), the only safe ways that frozen foods can be thawed are in the refrigerator, immersed in cold water changed every 30 minutes, or in the microwave, keeping the food at temperatures not conducive for bacterial growth.[1] In addition, halted bacterial growth can restart in thawed foods, so thawed foods should be treated like fresh perishables.

Bacterial cultures and medical specimens requiring long-term storage or transport are often frozen at ultra-low temperatures of −70 °C or lower. These ultra-low temperatures can be achieved by storing specimens on dry ice in an ultra-low freezer or in special liquid nitrogen tanks, which maintain temperatures lower than −196 °C (Figure **10.3.5**).



(a) (b)

Figure **10.3.5**: Cultures and other medical specimens can be stored for long periods at ultra-low temperatures. (a) An ultra-low freezer maintains temperatures at or below −70 °C. (b) Even lower temperatures can be achieved through freezing and storage in liquid nitrogen. (credit a: modification of work by "Expert Infantry"/Flickr; credit b: modification of work by USDA)

? Exercise 10.3.2

Does placing food in a refrigerator kill bacteria on the food?

Pressure

Exposure to high pressure kills many microbes. In the food industry, high-pressure processing (also called pascalization) is used to kill bacteria, yeast, molds, parasites, and viruses in foods while maintaining food quality and extending shelf life. The application of high pressure between 100 and 800 MPa (sea level atmospheric pressure is about 0.1 MPa) is sufficient to kill vegetative cells by protein denaturation, but endospores may survive these pressures.[23]

In clinical settings, hyperbaric oxygen therapy is sometimes used to treat infections. In this form of therapy, a patient breathes pure oxygen at a pressure higher than normal atmospheric pressure, typically between 1 and 3 atmospheres (atm). This is achieved by placing the patient in a hyperbaric chamber or by supplying the pressurized oxygen through a breathing tube. Hyperbaric oxygen therapy helps increase oxygen saturation in tissues that become hypoxic due to infection and inflammation. This increased oxygen concentration enhances the body's immune response by increasing the activities of neutrophils and macrophages, white blood cells that fight infections. Increased oxygen levels also contribute to the formation of toxic free radicals that inhibit the growth of oxygen-sensitive or anaerobic bacteria like as *Clostridium perfringens*, a common cause of gas gangrene. In *C. perfringens* infections, hyperbaric oxygen therapy can also reduce secretion of a bacterial toxin that causes tissue destruction. Hyperbaric oxygen therapy also seems to enhance the effectiveness of antibiotic treatments. Unfortunately, some rare risks include oxygen toxicity and effects on delicate tissues, such as the eyes, middle ear, and lungs, which may be damaged by the increased air pressure.

High pressure processing is not commonly used for disinfection or sterilization of fomites. Although the application of pressure and steam in an autoclave is effective for killing endospores, it is the high temperature achieved, and not the pressure directly, that results in endospore death.

A Streak of Bad Potluck

One Monday in spring 2015, an Ohio woman began to experience blurred, double vision; difficulty swallowing; and drooping eyelids. She was rushed to the emergency department of her local hospital. During the examination, she began to experience abdominal cramping, nausea, paralysis, dry mouth, weakness of facial muscles, and difficulty speaking and breathing. Based on these symptoms, the hospital's incident command center was activated, and Ohio public health officials were notified of a possible case of botulism. Meanwhile, other patients with similar symptoms began showing up at other local hospitals.

Because of the suspicion of botulism, antitoxin was shipped overnight from the CDC to these medical facilities, to be administered to the affected patients. The first patient died of respiratory failure as a result of paralysis, and about half of the remaining victims required additional hospitalization following antitoxin administration, with at least two requiring ventilators for breathing.

Public health officials investigated each of the cases and determined that all of the patients had attended the same church potluck the day before. Moreover, they traced the source of the outbreak to a potato salad made with home-canned potatoes. More than likely, the potatoes were canned using boiling water, a method that allows endospores of *Clostridium botulinum* to survive. *C. botulinum* produces botulinum toxin, a neurotoxin that is often deadly once ingested. According to the CDC, the Ohio case was the largest botulism outbreak in the United States in nearly 40 years.[4]

Killing *C. botulinum* endospores requires a minimum temperature of 116 °C (240 °F), well above the boiling point of water. This temperature can only be reached in a pressure canner, which is recommended for home canning of low-acid foods such as meat, fish, poultry, and vegetables (Figure **10.3.6**). Additionally, the CDC recommends boiling home-canned foods for about 10 minutes before consumption. Since the botulinum toxin is heat labile (meaning that it is denatured by heat), 10 minutes of boiling will render nonfunctional any botulinum toxin that the food may contain.



Figure **10.3.6**: (a) *Clostridium botulinum* is the causative agent of botulism. (b) A pressure canner is recommended for home canning because endospores of *C. botulinum* can survive temperatures above the boiling point of water. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by National Center for Home Food Preservation)

Link to Learning

To learn more about proper home-canning techniques, visit the CDC's website.

Desiccation

Drying, also known as desiccation or dehydration, is a method that has been used for millennia to preserve foods such as raisins, prunes, and jerky. It works because all cells, including microbes, require water for their metabolism and survival. Although drying controls microbial growth, it might not kill all microbes or their endospores, which may start to regrow when conditions are more favorable and water content is restored.

In some cases, foods are dried in the sun, relying on evaporation to achieve desiccation. Freeze-drying, or lyophilization, is another method of dessication in which an item is rapidly frozen ("snap-frozen") and placed under vacuum so that water is lost by sublimation. Lyophilization combines both exposure to cold temperatures and desiccation, making it quite effective for controlling microbial growth. In addition, lyophilization causes less damage to an item than conventional desiccation and better preserves the item's original qualities. Lyophilized items may be stored at room temperature if packaged appropriately to prevent moisture acquisition. Lyophilization is used for preservation in the food industry and is also used in the laboratory for the long-term storage and transportation of microbial cultures.

The water content of foods and materials, called the water activity, can be lowered without physical drying by the addition of solutes such as salts or sugars. At very high concentrations of salts or sugars, the amount of available water in microbial cells is reduced dramatically because water will be drawn from an area of low solute concentration (inside the cell) to an area of high solute concentration (outside the cell) (Figure **10.3.7**). Many microorganisms do not survive these conditions of high osmotic

pressure. Honey, for example, is 80% sucrose, an environment in which very few microorganisms are capable of growing, thereby eliminating the need for refrigeration. Salted meats and fish, like ham and cod, respectively, were critically important foods before the age of refrigeration. Fruits were preserved by adding sugar, making jams and jellies. However, certain microbes, such as molds and yeasts, tend to be more tolerant of desiccation and high osmotic pressures, and, thus, may still contaminate these types of foods.



Figure **10.3.7**: (a) The addition of a solute creates a hypertonic environment, drawing water out of cells. (b) Some foods can be dried directly, like raisins and jerky. Other foods are dried with the addition of salt, as in the case of salted fish, or sugar, as in the case of jam. (credit a: modification of work by "Bruce Blaus"/Wikimedia Commons; credit raisins: modification of work by Christian Schnettelker; credit jerky: modification of work by Larry Jacobsen; credit salted fish: modification of work by "The Photographer"/Wikimedia Commons; credit jam: modification of work by Kim Becker)

? Exercise 10.3.3

How does the addition of salt or sugar to food affect its water activity?

Radiation

Radiation in various forms, from high-energy radiation to sunlight, can be used to kill microbes or inhibit their growth. **Ionizing radiation** includes X-rays, gamma rays, and high-energy electron beams. Ionizing radiation is strong enough to pass into the cell, where it alters molecular structures and damages cell components. For example, ionizing radiation introduces double-strand breaks in DNA molecules. This may directly cause DNA mutations to occur, or mutations may be introduced when the cell attempts to repair the DNA damage. As these mutations accumulate, they eventually lead to cell death.

Both X-rays and gamma rays easily penetrate paper and plastic and can therefore be used to sterilize many packaged materials. In the laboratory, ionizing radiation is commonly used to sterilize materials that cannot be autoclaved, such as plastic Petri dishes and disposable plastic inoculating loops. For clinical use, ionizing radiation is used to sterilize gloves, intravenous tubing, and other latex and plastic items used for patient care. Ionizing radiation is also used for the sterilization of other types of delicate, heat-sensitive materials used clinically, including tissues for transplantation, pharmaceutical drugs, and medical equipment.

In Europe, gamma irradiation for food preservation is widely used, although it has been slow to catch on in the United States (see the Micro Connections box on this topic). Packaged dried spices are also often gamma-irradiated. Because of their ability to penetrate paper, plastic, thin sheets of wood and metal, and tissue, great care must be taken when using X-rays and gamma irradiation. These types of ionizing irradiation cannot penetrate thick layers of iron or lead, so these metals are commonly used to protect humans who may be potentially exposed.

Another type of radiation, nonionizing radiation, is commonly used for sterilization and uses less energy than ionizing radiation. It does not penetrate cells or packaging. Ultraviolet (UV) light is one example; it causes thymine dimers to form between adjacent thymines within a single strand of DNA (Figure 10.3.8). When DNA polymerase encounters the thymine dimer, it does not always incorporate the appropriate complementary nucleotides (two adenines), and this leads to formation of mutations that can ultimately kill microorganisms.

UV light can be used effectively by both consumers and laboratory personnel to control microbial growth. UV lamps are now commonly incorporated into water purification systems for use in homes. In addition, small portable UV lights are commonly used by campers to purify water from natural environments before drinking. Germicidal lamps are also used in surgical suites, biological

safety cabinets, and transfer hoods, typically emitting UV light at a wavelength of 260 nm. Because UV light does not penetrate surfaces and will not pass through plastics or glass, cells must be exposed directly to the light source.

Sunlight has a very broad spectrum that includes UV and visible light. In some cases, sunlight can be effective against certain bacteria because of both the formation of thymine dimers by UV light and by the production of reactive oxygen products induced in low amounts by exposure to visible light.

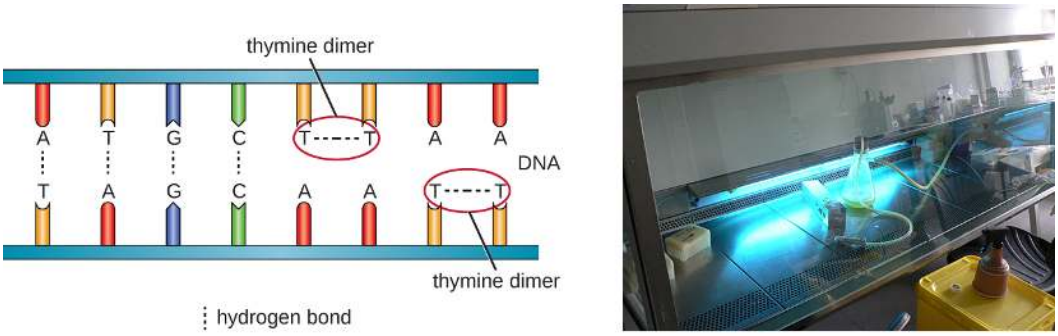


Figure **10.3.8**: (a) UV radiation causes the formation of thymine dimers in DNA, leading to lethal mutations in the exposed microbes. (b) Germicidal lamps that emit UV light are commonly used in the laboratory to sterilize equipment.

Exercise 10.3.4

1. What are two advantages of ionizing radiation as a sterilization method?
2. How does the effectiveness of ionizing radiation compare with that of nonionizing radiation?

Irradiated Food: Would You Eat That?

Of all the ways to prevent food spoilage and foodborne illness, gamma irradiation may be the most unappetizing. Although gamma irradiation is a proven method of eliminating potentially harmful microbes from food, the public has yet to buy in. Most of their concerns, however, stem from misinformation and a poor understanding of the basic principles of radiation.

The most common method of irradiation is to expose food to cobalt-60 or cesium-137 by passing it through a radiation chamber on a conveyor belt. The food does not directly contact the radioactive material and does not become radioactive itself. Thus, there is no risk for exposure to radioactive material through eating gamma-irradiated foods. Additionally, irradiated foods are not significantly altered in terms of nutritional quality, aside from the loss of certain vitamins, which is also exacerbated by extended storage. Alterations in taste or smell may occur in irradiated foods with high fat content, such as fatty meats and dairy products, but this effect can be minimized by using lower doses of radiation at colder temperatures.

In the United States, the CDC, Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) have deemed irradiation safe and effective for various types of meats, poultry, shellfish, fresh fruits and vegetables, eggs with shells, and spices and seasonings. Gamma irradiation of foods has also been approved for use in many other countries, including France, the Netherlands, Portugal, Israel, Russia, China, Thailand, Belgium, Australia, and South Africa. To help ameliorate consumer concern and assist with education efforts, irradiated foods are now clearly labeled and marked with the international irradiation symbol, called the “radura” (Figure **10.3.9**). Consumer acceptance seems to be rising, as indicated by several recent studies.



Figure 10.3.9: (a) Foods are exposed to gamma radiation by passage on a conveyor belt through a radiation chamber. (b) Gamma-irradiated foods must be clearly labeled and display the irradiation symbol, known as the "radura." (credit a, b: modification of work by U.S. Department of Agriculture)

Sonication

The use of high-frequency ultrasound waves to disrupt cell structures is called sonication. Application of ultrasound waves causes rapid changes in pressure within the intracellular liquid; this leads to cavitation, the formation of bubbles inside the cell, which can disrupt cell structures and eventually cause the cell to lyse or collapse. Sonication is useful in the laboratory for efficiently lysing cells to release their contents for further research; outside the laboratory, sonication is used for cleaning surgical instruments, lenses, and a variety of other objects such as coins, tools, and musical instruments.

Filtration

Filtration is a method of physically separating microbes from samples. Air is commonly filtered through high-efficiency particulate air (HEPA) filters (Figure 10.3.10). HEPA filters have effective pore sizes of 0.3 µm, small enough to capture bacterial cells, endospores, and many viruses, as air passes through these filters, nearly sterilizing the air on the other side of the filter. HEPA filters have a variety of applications and are used widely in clinical settings, in cars and airplanes, and even in the home. For example, they may be found in vacuum cleaners, heating and air-conditioning systems, and air purifiers.

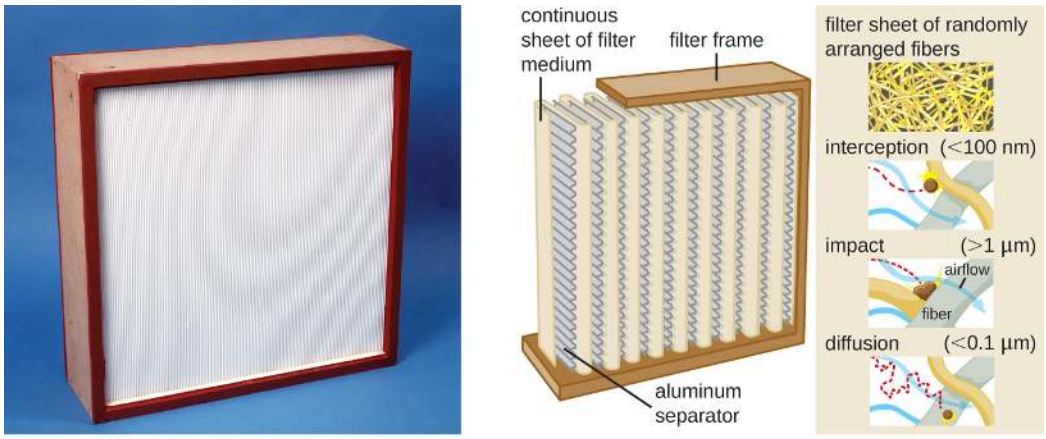


Figure 10.3.10: (a) HEPA filters like this one remove microbes, endospores, and viruses as air flows through them. (b) A schematic of a HEPA filter. (credit a: modification of work by CSIRO; credit b: modification of work by "LadyofHats"/Mariana Ruiz Villareal)

Biological Safety Cabinets

Biological safety cabinets are a good example of the use of HEPA filters. HEPA filters in biological safety cabinets (BSCs) are used to remove particulates in the air either entering the cabinet (air intake), leaving the cabinet (air exhaust), or treating both the intake and exhaust. Use of an air-intake HEPA filter prevents environmental contaminants from entering the BSC, creating a clean area for handling biological materials. Use of an air-exhaust HEPA filter prevents laboratory pathogens from contaminating the laboratory, thus maintaining a safe work area for laboratory personnel.

There are three classes of BSCs: I, II, and III. Each class is designed to provide a different level of protection for laboratory personnel and the environment; BSC II and III are also designed to protect the materials or devices in the cabinet. Table **10.3.1** summarizes the level of safety provided by each class of BSC for each BSL.

Table **10.3.1**: Biological risks and BSCs

Biological Risk Assessed	BSC Class	Protection of Personnel	Protection of Environment	Protection of Product
BSL-1, BSL-2, BSL-3	I	Yes	Yes	No
BSL-1, BSL-2, BSL-3	II	Yes	Yes	Yes
BSL-4	III; II when used in suit room with suit	Yes	Yes	Yes

Class I BSCs protect laboratory workers and the environment from a low to moderate risk for exposure to biological agents used in the laboratory. Air is drawn into the cabinet and then filtered before exiting through the building's exhaust system. Class II BSCs use directional air flow and partial barrier systems to contain infectious agents. Class III BSCs are designed for working with highly infectious agents like those used in BSL-4 laboratories. They are gas tight, and materials entering or exiting the cabinet must be passed through a double-door system, allowing the intervening space to be decontaminated between uses. All air is passed through one or two HEPA filters and an air incineration system before being exhausted directly to the outdoors (not through the building's exhaust system). Personnel can manipulate materials inside the Class III cabinet by using long rubber gloves sealed to the cabinet.

Link to Learning

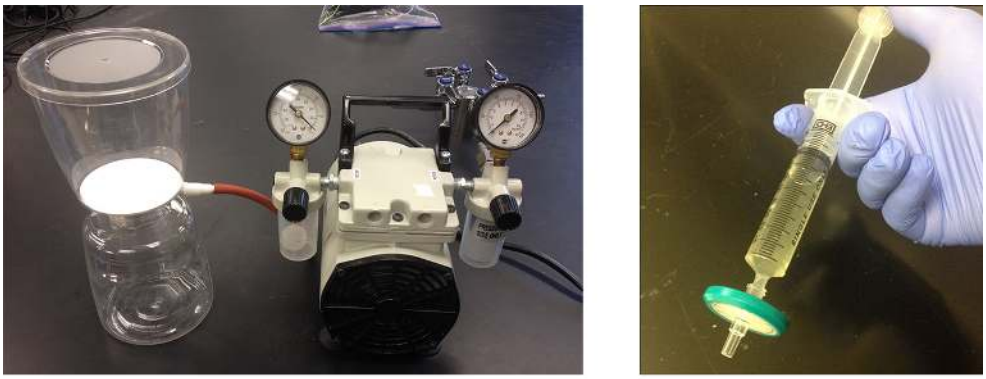
This video shows how BSCs are designed and explains how they protect personnel, the environment, and the product.

Filtration in Hospitals

HEPA filters are also commonly used in hospitals and surgical suites to prevent contamination and the spread of airborne microbes through ventilation systems. HEPA filtration systems may be designed for entire buildings or for individual rooms. For example, burn units, operating rooms, or isolation units may require special HEPA-filtration systems to remove opportunistic pathogens from the environment because patients in these rooms are particularly vulnerable to infection.

Membrane Filters

Filtration can also be used to remove microbes from liquid samples using membrane filtration. Membrane filters for liquids function similarly to HEPA filters for air. Typically, membrane filters that are used to remove bacteria have an effective pore size of 0.2 µm, smaller than the average size of a bacterium (1 µm), but filters with smaller pore sizes are available for more specific needs. Membrane filtration is useful for removing bacteria from various types of heat-sensitive solutions used in the laboratory, such as antibiotic solutions and vitamin solutions. Large volumes of culture media may also be filter sterilized rather than autoclaved to protect heat-sensitive components. Often when filtering small volumes, syringe filters are used, but vacuum filters are typically used for filtering larger volumes (Figure **10.3.11**).



(a) (b)

Figure **10.3.11**: Membrane filters come in a variety of sizes, depending on the volume of solution being filtered. (a) Larger volumes are filtered in units like these. The solution is drawn through the filter by connecting the unit to a vacuum. (b) Smaller volumes are often filtered using syringe filters, which are units that fit on the end of a syringe. In this case, the solution is pushed through by depressing the syringe's plunger. (credit a, b: modification of work by Brian Forster)

? Exercise 10.3.5

1. Would membrane filtration with a 0.2-µm filter likely remove viruses from a solution? Explain.
2. Name at least two common uses of HEPA filtration in clinical or laboratory settings.

Figure **10.3.12** and Figure **10.3.13** summarize the physical methods of control discussed in this section.

Physical Methods of Control			
Method	**Conditions**	**Mode of Action**	**Example Uses**
Heat			
Boiling	100 °C at sea level	Denatures proteins and alters membranes	Cooking, personal use, preparing certain laboratory media
Dry-heat oven	170 °C for 2 hours	Denatures proteins and alters membranes, dehydration, desiccation	Sterilization of heat-stable medical and laboratory equipment and glassware
Incineration	Exposure to flame	Destroy by burning	Flaming loop, microincinerator
Autoclave	Typical settings: 121 °C for 15 minutes at 15 pounds per square inch (psi)	Denatures proteins and alters membranes	Sterilization of microbiological media, heat-stable medical and laboratory equipment, and other heat-stable items
Pasteurization	Can vary. One type is 72 °C for 15 seconds (HTST)	Denatures proteins and alters membranes	Prevents spoilage of milk, apple juice, honey, and other ingestible liquids
Cold			
Refrigeration	0 °C to 7 °C	Inhibits metabolism (slows or arrests cell division)	Preservation of food or laboratory materials (solutions, cultures)
Freezing	Below −2 °C	Stops metabolism, may kill microbes	Long-term storage of food, laboratory cultures, or medical specimens
Pressure			
High-pressure processing	100–800 MPa	Denatures proteins and can cause cell lysis	Preservation of food
Hyberbaric oxygen therapy	Air pressure three times higher than normal	Inhibits metabolism and growth of anaerobic microbes	Treatment of certain infections (e.g., gas gangrene)
Desiccation			
Simple desiccation	Drying	Inhibits metabolism	Dried fruits, jerky
Reduce water activity	Addition of salt or water	Inhibits metabolism and can cause lysis	Salted meats and fish, honey, jams and jellies
Lyophilization	Rapid freezing under vacuum	Inhibits metabolism	Preservation of food, laboratory cultures, or reagents
Radiation			
Ionizing radiation	Exposure to X-rays or gamma rays	Alters molecular structures, introduces double-strand breaks into DNA	Sterilization of spices and heat-sensitive laboratory and medical items; used for food sterilization in Europe but not widely accepted in US
Nonionizing radiation	Exposure to ultraviolet light	Introduces thymine dimers, leading to mutations	Surface sterilization of laboratory materials, water purification

Figure **10.3.12**: Physical methods of control

Physical Methods of Control (continued)			
Method	**Conditions**	**Mode of Action**	**Example Uses**
Sonication			
Sonication	Exposure to ultrasonic waves	Cavitation (formation of empty space) disrupts cells, lysing them	Laboratory research to lyse cells; cleaning jewelry, lenses, and equipment
Filtration			
HEPA filtration	Use of high-efficiency particulate air (HEPA) filter with 0.3 μm pore size	Physically removes microbes from air	Laboratory biological safety cabinets, operating rooms, isolation units, heating and air conditioning systems, vacuum cleaners
Membrane filtration	Use of membrane filter with 0.2-μm or smaller pore size	Physically removes microbes from liquid solutions	Removal of bacteria from heat-sensitive solutions like vitamins, antibiotics, and media with heat-sensitive components

Figure **10.3.13**: Physical methods of control (continued)

Key Concepts and Summary

- Heat is a widely used and highly effective method for controlling microbial growth.
- **Dry-heat sterilization** protocols are used commonly in aseptic techniques in the laboratory. However, **moist-heat sterilization** is typically the more effective protocol because it penetrates cells better than dry heat does.
- **Pasteurization** is used to kill pathogens and reduce the number of microbes that cause food spoilage. **High-temperature, short-time pasteurization** is commonly used to pasteurize milk that will be refrigerated; **ultra-high temperature pasteurization** can be used to pasteurize milk for long-term storage without refrigeration.
- Refrigeration slows microbial growth; freezing stops growth, killing some organisms. Laboratory and medical specimens may be frozen on dry ice or at ultra-low temperatures for storage and transport.
- High-pressure processing can be used to kill microbes in food. Hyperbaric oxygen therapy to increase oxygen saturation has also been used to treat certain infections.
- **Desiccation** has long been used to preserve foods and is accelerated through the addition of salt or sugar, which decrease water activity in foods.
- **Lyophilization** combines cold exposure and desiccation for the long-term storage of foods and laboratory materials, but microbes remain and can be rehydrated.
- **Ionizing radiation**, including gamma irradiation, is an effective way to sterilize heat-sensitive and packaged materials. **Nonionizing radiation**, like ultraviolet light, is unable to penetrate surfaces but is useful for surface sterilization.
- **HEPA** filtration is commonly used in hospital ventilation systems and biological safety cabinets in laboratories to prevent transmission of airborne microbes. **Membrane filtration** is commonly used to remove bacteria from heat-sensitive solutions.

Footnotes

1. 1 US Department of Agriculture. “Freezing and Food Safety.” 2013. http://www.fsis.usda.gov/wps/portal/...afety/CT_Index. Accessed June 8, 2016.
2. 2 C. Ferstl. “High Pressure Processing: Insights on Technology and Regulatory Requirements.” Food for Thought/White Paper. Series Volume 10. Livermore, CA: The National Food Lab; July 2013.
3. 3 US Food and Drug Administration. “Kinetics of Microbial Inactivation for Alternative Food Processing Technologies: High Pressure Processing.” 2000. www.fda.gov/Food/FoodScienceR.../ucm101456.htm. Accessed July 19, 2106.
4. 4 CL McCarty et al. “Large Outbreak of Botulism Associated with a Church Potluck Meal-Ohio, 2015.” *Morbidity and Mortality Weekly Report* 64, no. 29 (2015):802–803.
5. 5 AM Johnson et al. “Consumer Acceptance of Electron-Beam Irradiated Ready-to-Eat Poultry Meats.” *Food Processing Preservation*, 28 no. 4 (2004):302–319.

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10.4: Using Chemicals to Control Microorganisms

Learning Objectives

- Understand and compare various chemicals used to control microbial growth, including their uses, advantages and disadvantages, chemical structure, and mode of action

In addition to physical methods of microbial control, chemicals are also used to control microbial growth. A wide variety of chemicals can be used as disinfectants or antiseptics. When choosing which to use, it is important to consider the type of microbe targeted; how clean the item needs to be; the disinfectant's effect on the item's integrity; its safety to animals, humans, and the environment; its expense; and its ease of use. This section describes the variety of chemicals used as disinfectants and antiseptics, including their mechanisms of action and common uses.

Phenolics

In the 1800s, scientists began experimenting with a variety of chemicals for disinfection. In the 1860s, British surgeon Joseph Lister (1827–1912) began using carbolic acid, known as phenol, as a disinfectant for the treatment of surgical wounds (see Foundations of Modern Cell Theory). In 1879, Lister's work inspired the American chemist Joseph Lawrence (1836–1909) to develop Listerine, an alcohol-based mixture of several related compounds that is still used today as an oral antiseptic. Today, carbolic acid is no longer used as a surgical disinfectant because it is a skin irritant, but the chemical compounds found in antiseptic mouthwashes and throat lozenges are called phenolics.

Chemically, phenol consists of a benzene ring with an –OH group, and phenolics are compounds that have this group as part of their chemical structure (Figure **10.4.1**). Phenolics such as thymol and eucalyptol occur naturally in plants. Other phenolics can be derived from creosote, a component of coal tar. Phenolics tend to be stable, persistent on surfaces, and less toxic than phenol. They inhibit microbial growth by denaturing proteins and disrupting membranes.

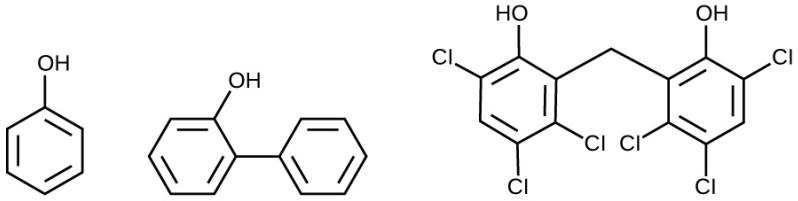


Figure **10.4.1**: Phenol and phenolic compounds have been used to control microbial growth. (a) Chemical structure of phenol, also known as carbolic acid. (b) o-Phenylphenol, a type of phenolic, has been used as a disinfectant as well as to control bacterial and fungal growth on harvested citrus fruits. (c) Hexachlorophene, another phenol, known as a bisphenol (two rings), is the active ingredient in pHisoHex.

Since Lister's time, several phenolic compounds have been used to control microbial growth. Phenolics like cresols(methylated phenols) and o-phenylphenol were active ingredients in various formulations of Lysol since its invention in 1889. o-Phenylphenol was also commonly used in agriculture to control bacterial and fungal growth on harvested crops, especially citrus fruits, but its use in the United States is now far more limited. The bisphenol hexachlorophene, a disinfectant, is the active ingredient in pHisoHex, a topical cleansing detergent widely used for handwashing in hospital settings. pHisoHex is particularly effective against gram-positive bacteria, including those causing staphylococcal and streptococcal skin infections. pHisoHex was formerly used for bathing infants, but this practice has been discontinued because it has been shown that exposure to hexachlorophene can lead to neurological problems.

Triclosan is another bisphenol compound that has seen widespread application in antibacterial products over the last several decades. Initially used in toothpastes, triclosan is now commonly used in hand soaps and is frequently impregnated into a wide variety of other products, including cutting boards, knives, shower curtains, clothing, and concrete, to make them antimicrobial. It is particularly effective against gram-positive bacteria on the skin, as well as certain gram-negative bacteria and yeasts.[1]

Triclosan: Antibacterial Overkill?

Hand soaps and other cleaning products are often marketed as “antibacterial,” suggesting that they provide a level of cleanliness superior to that of conventional soaps and cleansers. But are the antibacterial ingredients in these products really safe and effective?

About 75% of antibacterial liquid hand soaps and 30% of bar soaps contain the chemical triclosan, a phenolic, (Figure **10.4.2**).[1] Triclosan blocks an enzyme in the bacterial fatty acid-biosynthesis pathway that is not found in the comparable human pathway. Although the use of triclosan in the home increased dramatically during the 1990s, more than 40 years of research by the FDA have turned up no conclusive evidence that washing with triclosan-containing products provides increased health benefits compared with washing with traditional soap. Although some studies indicate that fewer bacteria may remain on a person’s hands after washing with triclosan-based soap, compared with traditional soap, no evidence points to any reduction in the transmission of bacteria that cause respiratory and gastrointestinal illness. In short, soaps with triclosan may remove or kill a few more germs but not enough to reduce the spread of disease.

Perhaps more disturbing, some clear risks associated with triclosan-based soaps have come to light. The widespread use of triclosan has led to an increase in triclosan-resistant bacterial strains, including those of clinical importance, such as *Salmonella enterica*; this resistance may render triclosan useless as an antibacterial in the long run.[3][4] Bacteria can easily gain resistance to triclosan through a change to a single gene encoding the targeted enzyme in the bacterial fatty acid-synthesis pathway. Other disinfectants with a less specific mode of action are much less prone to engendering resistance because it would take much more than a single genetic change.

Use of triclosan over the last several decades has also led to a buildup of the chemical in the environment. Triclosan in hand soap is directly introduced into wastewater and sewage systems as a result of the handwashing process. There, its antibacterial properties can inhibit or kill bacteria responsible for the decomposition of sewage, causing septic systems to clog and back up. Eventually, triclosan in wastewater finds its way into surface waters, streams, lakes, sediments, and soils, disrupting natural populations of bacteria that carry out important environmental functions, such as inhibiting algae. Triclosan also finds its way into the bodies of amphibians and fish, where it can act as an endocrine disruptor. Detectable levels of triclosan have also been found in various human bodily fluids, including breast milk, plasma, and urine.[5] In fact, a study conducted by the CDC found detectable levels of triclosan in the urine of 75% of 2,517 people tested in 2003–2004.[6] This finding is even more troubling given the evidence that triclosan may affect immune function in humans.[7]

In December 2013, the FDA gave soap manufacturers until 2016 to prove that antibacterial soaps provide a significant benefit over traditional soaps; if unable to do so, manufacturers will be forced to remove these products from the market.

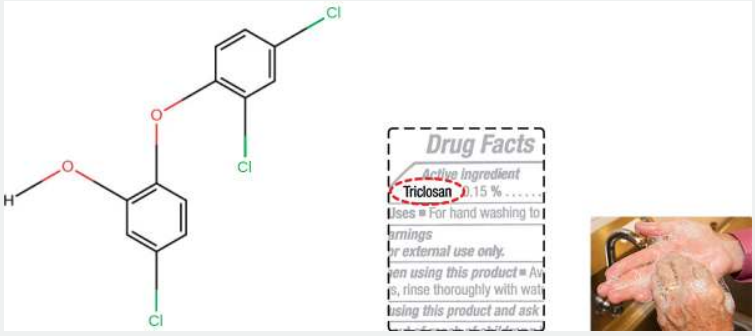


Figure **10.4.2**: Triclosan is a common ingredient in antibacterial soaps despite evidence that it poses environmental and health risks and offers no significant health benefit compared to conventional soaps. (credit b, c: modification of work by FDA)

Exercise 10.4.1

Why is triclosan more like an antibiotic than a traditional disinfectant?

Heavy Metals

Some of the first chemical disinfectants and antiseptics to be used were heavy metals. Heavy metals kill microbes by binding to proteins, thus inhibiting enzymatic activity (Figure 10.4.3). Heavy metals are oligodynamic, meaning that very small concentrations show significant antimicrobial activity. Ions of heavy metals bind to sulfur-containing amino acids strongly and bioaccumulate within cells, allowing these metals to reach high localized concentrations. This causes proteins to denature.

Heavy metals are not selectively toxic to microbial cells. They may bioaccumulate in human or animal cells, as well, and excessive concentrations can have toxic effects on humans. If too much silver accumulates in the body, for example, it can result in a condition called argyria, in which the skin turns irreversibly blue-gray. One way to reduce the potential toxicity of heavy metals is by carefully controlling the duration of exposure and concentration of the heavy metal.



(a) (b) (c) (d) (e)

Figure 10.4.3: Heavy metals denature proteins, impairing cell function and, thus, giving them strong antimicrobial properties. (a) Copper in fixtures like this door handle kills microbes that otherwise might accumulate on frequently touched surfaces. (b) Eating utensils contain small amounts of silver to inhibit microbial growth. (c) Copper commonly lines incubators to minimize contamination of cell cultures stored inside. (d) Antiseptic mouthwashes commonly contain zinc chloride. (e) This patient is suffering from argyria, an irreversible condition caused by bioaccumulation of silver in the body. (credit b: modification of work by "Shoshanah"/Flickr; credit e: modification of work by Herbert L. Fred and Hendrik A. van Dijk)

Mercury

Mercury is an example of a heavy metal that has been used for many years to control microbial growth. It was used for many centuries to treat syphilis. Mercury compounds like mercuric chloride are mainly bacteriostatic and have a very broad spectrum of activity. Various forms of mercury bind to sulfur-containing amino acids within proteins, inhibiting their functions.

In recent decades, the use of such compounds has diminished because of mercury's toxicity. It is toxic to the central nervous, digestive, and renal systems at high concentrations, and has negative environmental effects, including bioaccumulation in fish. Topical antiseptics such as mercurochrome, which contains mercury in low concentrations, and merthiolate, a tincture (a solution of mercury dissolved in alcohol) were once commonly used. However, because of concerns about using mercury compounds, these antiseptics are no longer sold in the United States.

Silver

Silver has long been used as an antiseptic. In ancient times, drinking water was stored in silver jugs.[8] Silvadene cream is commonly used to treat topical wounds and is particularly helpful in preventing infection in burn wounds. Silver nitrate drops were once routinely applied to the eyes of newborns to protect against ophthalmia neonatorum, eye infections that can occur due to exposure to pathogens in the birth canal, but antibiotic creams are more now commonly used. Silver is often combined with antibiotics, making the antibiotics thousands of times more effective.[9] Silver is also commonly incorporated into catheters and bandages, rendering them antimicrobial; however, there is evidence that heavy metals may also enhance selection for antibiotic resistance.[10]

Copper, Nickel, and Zinc

Several other heavy metals also exhibit antimicrobial activity. Copper sulfate is a common algicide used to control algal growth in swimming pools and fish tanks. The use of metallic copper to minimize microbial growth is also becoming more widespread. Copper linings in incubators help reduce contamination of cell cultures. The use of copper pots for water storage in underdeveloped

countries is being investigated as a way to combat diarrheal diseases. Copper coatings are also becoming popular for frequently handled objects such as doorknobs, cabinet hardware, and other fixtures in health-care facilities in an attempt to reduce the spread of microbes.

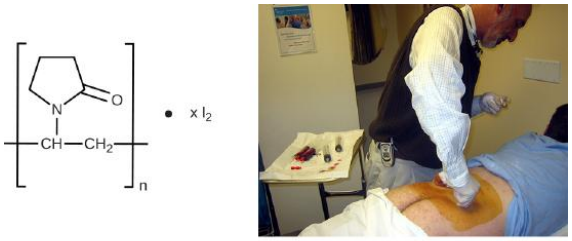
Nickel and zinc coatings are now being used in a similar way. Other forms of zinc, including zinc chloride and zinc oxide, are also used commercially. Zinc chloride is quite safe for humans and is commonly found in mouthwashes, substantially increasing their length of effectiveness. Zinc oxide is found in a variety of products, including topical antiseptic creams such as calamine lotion, diaper ointments, baby powder, and dandruff shampoos.

? Exercise 10.4.2

Why are many heavy metals both antimicrobial and toxic to humans?

Halogens

Other chemicals commonly used for disinfection are the halogens iodine, chlorine, and fluorine. Iodine works by oxidizing cellular components, including sulfur-containing amino acids, nucleotides, and fatty acids, and destabilizing the macromolecules that contain these molecules. It is often used as a topical tincture, but it may cause staining or skin irritation. An iodophor is a compound of iodine complexed with an organic molecule, thereby increasing iodine's stability and, in turn, its efficacy. One common iodophor is povidone-iodine, which includes a wetting agent that releases iodine relatively slowly. Betadine is a brand of povidone-iodine commonly used as a hand scrub by medical personnel before surgery and for topical antisepsis of a patient's skin before incision (Figure **10.4.4**).



(a) (b)

Figure **10.4.4**: (a) Betadine is a solution of the iodophor povidone-iodine. (b) It is commonly used as a topical antiseptic on a patient's skin before incision during surgery. (credit b: modification of work by Andrew Ratto)

Chlorine is another halogen commonly used for disinfection. When chlorine gas is mixed with water, it produces a strong oxidant called hypochlorous acid, which is uncharged and enters cells easily. Chlorine gas is commonly used in municipal drinking water and wastewater treatment plants, with the resulting hypochlorous acid producing the actual antimicrobial effect. Those working at water treatment facilities need to take great care to minimize personal exposure to chlorine gas. Sodium hypochlorite is the chemical component of common household bleach, and it is also used for a wide variety of disinfecting purposes. Hypochlorite salts, including sodium and calcium hypochlorites, are used to disinfect swimming pools. Chlorine gas, sodium hypochlorite, and calcium hypochlorite are also commonly used disinfectants in the food processing and restaurant industries to reduce the spread of foodborne diseases. Workers in these industries also need to take care to use these products correctly to ensure their own safety as well as the safety of consumers. A recent joint statement published by the Food and Agriculture Organization (FAO) of the United Nations and WHO indicated that none of the many beneficial uses of chlorine products in food processing to reduce the spread of foodborne illness posed risks to consumers.[11]

Another class of chlorinated compounds called chloramines are widely used as disinfectants. Chloramines are relatively stable, releasing chlorine over long periods time. Chloramines are derivatives of ammonia by substitution of one, two, or all three hydrogen atoms with chlorine atoms (Figure **10.4.5**).

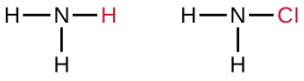


Figure **10.4.5**: Monochloroamine, one of the chloramines, is derived from ammonia by the replacement of one hydrogen atom with a chlorine atom.

Chloramines and other cholorine compounds may be used for disinfection of drinking water, and chloramine tablets are frequently used by the military for this purpose. After a natural disaster or other event that compromises the public water supply, the CDC recommends disinfecting tap water by adding small amounts of regular household bleach. Recent research suggests that sodium dichloroisocyanurate (NaDCC) may also be a good alternative for drinking water disinfection. Currently, NaDCC tablets are available for general use and for use by the military, campers, or those with emergency needs; for these uses, NaDCC is preferable to chloramine tablets. Chlorine dioxide, a gaseous agent used for fumigation and sterilization of enclosed areas, is also commonly used for the disinfection of water.

Although chlorinated compounds are relatively effective disinfectants, they have their disadvantages. Some may irritate the skin, nose, or eyes of some individuals, and they may not completely eliminate certain hardy organisms from contaminated drinking water. The fungus *Cryptosporidium*, for example, has a protective outer shell that makes it resistant to chlorinated disinfectants. Thus, boiling of drinking water in emergency situations is recommended when possible.

The halogen fluorine is also known to have antimicrobial properties that contribute to the prevention of dental caries(cavities).[12] Fluoride is the main active ingredient of toothpaste and is also commonly added to tap water to help communities maintain oral health. Chemically, fluoride can become incorporated into the hydroxyapatite of tooth enamel, making it more resistant to corrosive acids produced by the fermentation of oral microbes. Fluoride also enhances the uptake of calcium and phosphate ions in tooth enamel, promoting remineralization. In addition to strengthening enamel, fluoride also seems to be bacteriostatic. It accumulates in plaque-forming bacteria, interfering with their metabolism and reducing their production of the acids that contribute to tooth decay.

? Exercise 10.4.3

What is a benefit of a chloramine over hypochlorite for disinfecting?

Alcohols

Alcohols make up another group of chemicals commonly used as disinfectants and antiseptics. They work by rapidly denaturing proteins, which inhibits cell metabolism, and by disrupting membranes, which leads to cell lysis. Once denatured, the proteins may potentially refold if enough water is present in the solution. Alcohols are typically used at concentrations of about 70% aqueous solution and, in fact, work better in aqueous solutions than 100% alcohol solutions. This is because alcohols coagulate proteins. In higher alcohol concentrations, rapid coagulation of surface proteins prevents effective penetration of cells. The most commonly used alcohols for disinfection are ethyl alcohol(ethanol) and isopropyl alcohol (isopropanol, rubbing alcohol) (Figure **10.4.6**).

Alcohols tend to be bactericidal and fungicidal, but may also be viricidal for enveloped viruses only. Although alcohols are not sporicidal, they do inhibit the processes of sporulation and germination. Alcohols are volatile and dry quickly, but they may also cause skin irritation because they dehydrate the skin at the site of application. One common clinical use of alcohols is swabbing the skin for degerming before needle injection. Alcohols also are the active ingredients in instant hand sanitizers, which have gained popularity in recent years. The alcohol in these hand sanitizers works both by denaturing proteins and by disrupting the microbial cell membrane, but will not work effectively in the presence of visible dirt.

Last, alcohols are used to make tinctures with other antiseptics, such as the iodine tinctures discussed previously in this chapter. All in all, alcohols are inexpensive and quite effective for the disinfection of a broad range of vegetative microbes. However, one disadvantage of alcohols is their high volatility, limiting their effectiveness to immediately after application.



Figure **10.4.6**: (a) Ethyl alcohol, the intoxicating ingredient found in alcoholic drinks, is also used commonly as a disinfectant. (b) Isopropyl alcohol, also called rubbing alcohol, has a related molecular structure and is another commonly used disinfectant. (credit a photo: modification of work by D Coetzee; credit b photo: modification of work by Craig Spurrier)

? Exercise 10.4.4

1. Name at least three advantages of alcohols as disinfectants.
2. Describe several specific applications of alcohols used in disinfectant products.

Surfactants

Surface-active agents, or surfactants, are a group of chemical compounds that lower the surface tension of water. Surfactants are the major ingredients in soaps and detergents. Soaps are salts of long-chain fatty acids and have both polar and nonpolar regions, allowing them to interact with polar and nonpolar regions in other molecules (Figure **10.4.7**). They can interact with nonpolar oils and grease to create emulsions in water, loosening and lifting away dirt and microbes from surfaces and skin. Soaps do not kill or inhibit microbial growth and so are not considered antiseptics or disinfectants. However, proper use of soaps mechanically carries away microorganisms, effectively degerming a surface. Some soaps contain added bacteriostatic agents such as triclocarban or cloflucarban, compounds structurally related to triclosan, that introduce antiseptic or disinfectant properties to the soaps.

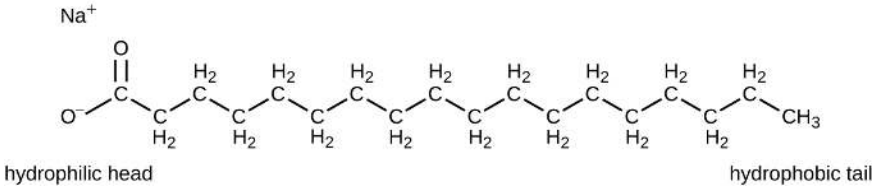
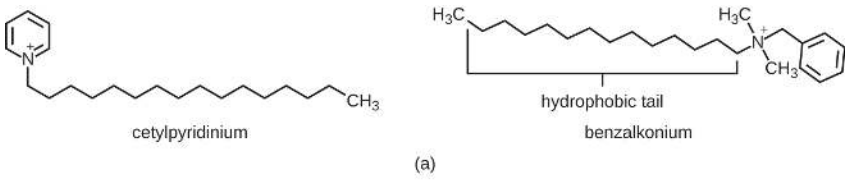


Figure **10.4.7**: Soaps are the salts (sodium salt in the illustration) of fatty acids and have the ability to emulsify lipids, fats, and oils by interacting with water through their hydrophilic heads and with the lipid at their hydrophobic tails.

Soaps, however, often form films that are difficult to rinse away, especially in hard water, which contains high concentrations of calcium and magnesium mineral salts. Detergents contain synthetic surfactant molecules with both polar and nonpolar regions that have strong cleansing activity but are more soluble, even in hard water, and, therefore, leave behind no soapy deposits. Anionic detergents, such as those used for laundry, have a negatively charged anion at one end attached to a long hydrophobic chain, whereas cationic detergents have a positively charged cation instead. Cationic detergents include an important class of disinfectants and antiseptics called the quaternary ammonium salts (quats), named for the characteristic quaternary nitrogen atom that confers the positive charge (Figure **10.4.8**). Overall, quats have properties similar to phospholipids, having hydrophilic and hydrophobic ends. As such, quats have the ability to insert into the bacterial phospholipid bilayer and disrupt membrane integrity. The cationic charge of quats appears to confer their antimicrobial properties, which are diminished when neutralized. Quats have several useful properties. They are stable, nontoxic, inexpensive, colorless, odorless, and tasteless. They tend to be bactericidal by disrupting membranes. They are also active against fungi, protozoans, and enveloped viruses, but endospores are unaffected. In clinical settings, they may be used as antiseptics or to disinfect surfaces. Mixtures of quats are also commonly found in household cleaners and disinfectants, including many current formulations of Lysol brand products, which contain benzalkonium chlorides as the active ingredients. Benzalkonium chlorides, along with the quat cetylpyrimidine chloride, are also found in products such as skin antiseptics, oral rinses, and mouthwashes.



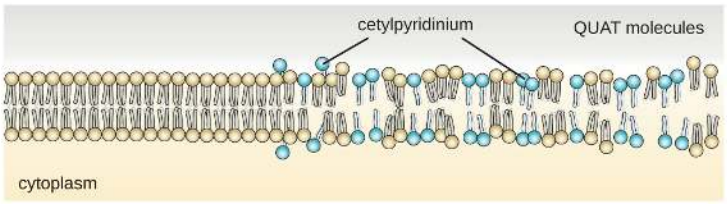


Figure **10.4.8**: (a) Two common quats are benzylalkonium chloride and cetylpyrimidine chloride. Note the hydrophobic nonpolar carbon chain at one end and the nitrogen-containing cationic component at the other end. (b) Quats are able to infiltrate the phospholipid plasma membranes of bacterial cells and disrupt their integrity, leading to death of the cell.

? Exercise 10.4.5

Why are soaps not considered disinfectants?

Handwashing the Right Way

Handwashing is critical for public health and should be emphasized in a clinical setting. For the general public, the CDC recommends handwashing before, during, and after food handling; before eating; before and after interacting with someone who is ill; before and after treating a wound; after using the toilet or changing diapers; after coughing, sneezing, or blowing the nose; after handling garbage; and after interacting with an animal, its feed, or its waste. Figure **10.4.9** illustrates the five steps of proper handwashing recommended by the CDC.

Handwashing is even more important for health-care workers, who should wash their hands thoroughly between every patient contact, after the removal of gloves, after contact with bodily fluids and potentially infectious fomites, and before and after assisting a surgeon with invasive procedures. Even with the use of proper surgical attire, including gloves, scrubbing for surgery is more involved than routine handwashing. The goal of surgical scrubbing is to reduce the normal microbiota on the skin's surface to prevent the introduction of these microbes into a patient's surgical wounds.

There is no single widely accepted protocol for surgical scrubbing. Protocols for length of time spent scrubbing may depend on the antimicrobial used; health-care workers should always check the manufacturer's recommendations. According to the Association of Surgical Technologists (AST), surgical scrubs may be performed with or without the use of brushes (Figure **10.4.9**).

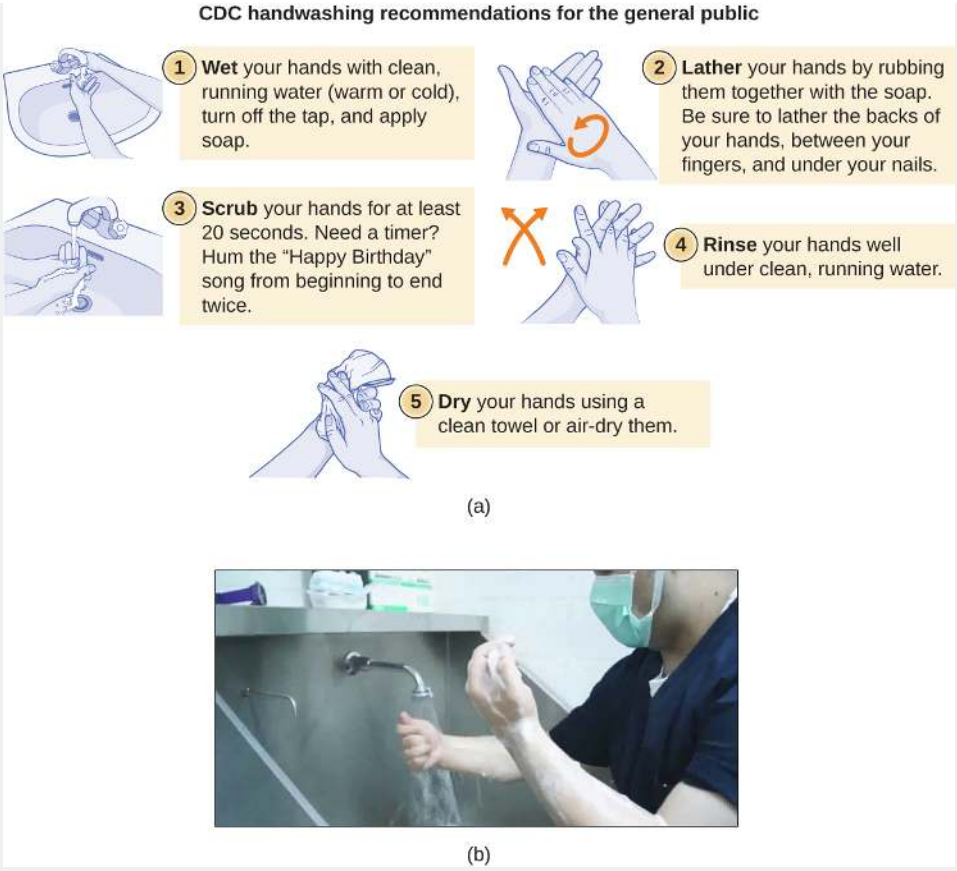


Figure 10.4.9: (a) The CDC recommends five steps as part of typical handwashing for the general public. (b) Surgical scrubbing is more extensive, requiring scrubbing starting from the fingertips, extending to the hands and forearms, and then up beyond the elbows, as shown here. (credit a: modification of work by World Health Organization)

Link to Learning

To learn more about proper handwashing, visit the CDC's website.

Bisbiguanides

Bisbiguanides were first synthesized in the 20th century and are cationic (positively charged) molecules known for their antiseptic properties (Figure 10.4.10). One important bisbiguanide antiseptic is chlorhexidine. It has broad-spectrum activity against yeasts, gram-positive bacteria, and gram-negative bacteria, with the exception of *Pseudomonas aeruginosa*, which may develop resistance on repeated exposure.[13] Chlorhexidine disrupts cell membranes and is bacteriostatic at lower concentrations or bactericidal at higher concentrations, in which it actually causes the cells' cytoplasmic contents to congeal. It also has activity against enveloped viruses. However, chlorhexidine is poorly effective against *Mycobacterium tuberculosis* and nonenveloped viruses, and it is not sporicidal. Chlorhexidine is typically used in the clinical setting as a surgical scrub and for other handwashing needs for medical personnel, as well as for topical antisepsis for patients before surgery or needle injection. It is more persistent than iodophors, providing long-lasting antimicrobial activity. Chlorhexidine solutions may also be used as oral rinses after oral procedures or to treat gingivitis. Another bisbiguanide, alexidine, is gaining popularity as a surgical scrub and an oral rinse because it acts faster than chlorhexidine.

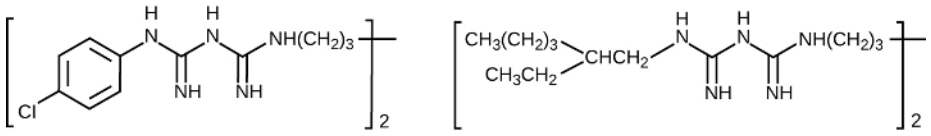


Figure **10.4.10**: The bisbiguanides chlorhexadine and alexidine are cationic antiseptic compounds commonly used as surgical scrubs.

? Exercise 10.4.6

What two effects does chlorhexidine have on bacterial cells?

Alkylating Agents

The alkylating agents are a group of strong disinfecting chemicals that act by replacing a hydrogen atom within a molecule with an alkyl group (C_nH_{2n+1}), thereby inactivating enzymes and nucleic acids (Figure **10.4.11**). The alkylating agent formaldehyde (CH_2OH) is commonly used in solution at a concentration of 37% (known as formalin) or as a gaseous disinfectant and biocide. It is a strong, broad-spectrum disinfectant and biocide that has the ability to kill bacteria, viruses, fungi, and endospores, leading to sterilization at low temperatures, which is sometimes a convenient alternative to the more labor-intensive heat sterilization methods. It also cross-links proteins and has been widely used as a chemical fixative. Because of this, it is used for the storage of tissue specimens and as an embalming fluid. It also has been used to inactivate infectious agents in vaccine preparation. Formaldehyde is very irritating to living tissues and is also carcinogenic; therefore, it is not used as an antiseptic.

Glutaraldehyde is structurally similar to formaldehyde but has two reactive aldehyde groups, allowing it to act more quickly than formaldehyde. It is commonly used as a 2% solution for sterilization and is marketed under the brand name Cidex. It is used to disinfect a variety of surfaces and surgical and medical equipment. However, similar to formaldehyde, glutaraldehyde irritates the skin and is not used as an antiseptic.

A new type of disinfectant gaining popularity for the disinfection of medical equipment is o-phthalaldehyde (OPA), which is found in some newer formulations of Cidex and similar products, replacing glutaraldehyde. o-Phthalaldehyde also has two reactive aldehyde groups, but they are linked by an aromatic bridge. o-Phthalaldehyde is thought to work similarly to glutaraldehyde and formaldehyde, but is much less irritating to skin and nasal passages, produces a minimal odor, does not require processing before use, and is more effective against mycobacteria.

Ethylene oxide is a type of alkylating agent that is used for gaseous sterilization. It is highly penetrating and can sterilize items within plastic bags such as catheters, disposable items in laboratories and clinical settings (like packaged Petri dishes), and other pieces of equipment. Ethylene oxide exposure is a form of cold sterilization, making it useful for the sterilization of heat-sensitive items. Great care needs to be taken with the use of ethylene oxide, however; it is carcinogenic, like the other alkylating agents, and is also highly explosive. With careful use and proper aeration of the products after treatment, ethylene oxide is highly effective, and ethylene oxide sterilizers are commonly found in medical settings for sterilizing packaged materials.

β-Propionolactone is an alkylating agent with a different chemical structure than the others already discussed. Like other alkylating agents, β-propionolactone binds to DNA, thereby inactivating it (Figure **10.4.11**). It is a clear liquid with a strong odor and has the ability to kill endospores. As such, it has been used in either liquid form or as a vapor for the sterilization of medical instruments and tissue grafts, and it is a common component of vaccines, used to maintain their sterility. It has also been used for the sterilization of nutrient broth, as well as blood plasma, milk, and water. It is quickly metabolized by animals and humans to lactic acid. It is also an irritant, however, and may lead to permanent damage of the eyes, kidneys, or liver. Additionally, it has been shown to be carcinogenic in animals; thus, precautions are necessary to minimize human exposure to β-propionolactone.[14]

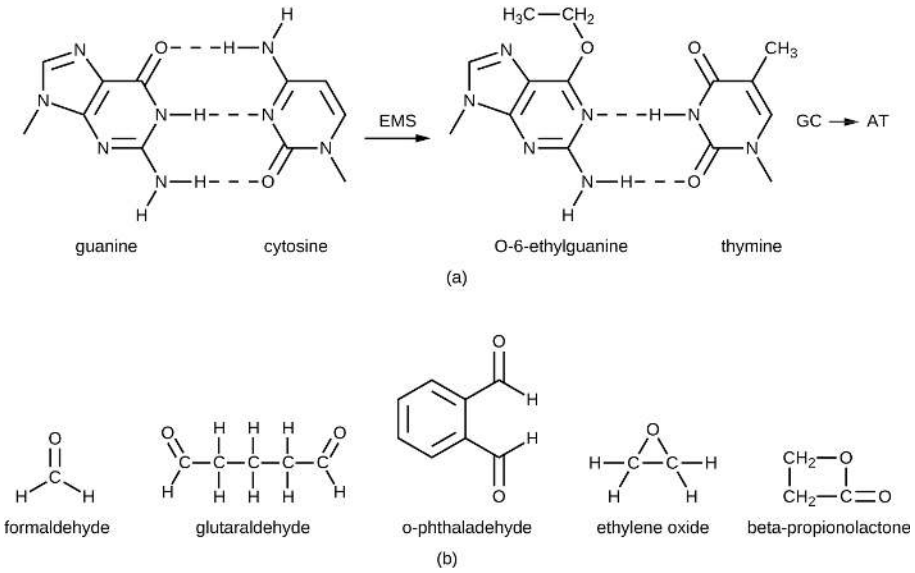


Figure **10.4.11**: (a) Alkylating agents replace hydrogen atoms with alkyl groups. Here, guanine is alkylated, resulting in its hydrogen bonding with thymine, instead of cytosine. (b) The chemical structures of several alkylating agents.

? Exercise 10.4.7

1. What chemical reaction do alkylating agents participate in?
2. Why are alkylating agents not used as antiseptics?

Diehard Prions

Prions, the acellular, misfolded proteins responsible for incurable and fatal diseases such as kuru and Creutzfeldt-Jakob disease (see Viroids, Virusoids, and Prions), are notoriously difficult to destroy. Prions are extremely resistant to heat, chemicals, and radiation. They are also extremely infectious and deadly; thus, handling and disposing of prion-infected items requires extensive training and extreme caution.

Typical methods of disinfection can reduce but not eliminate the infectivity of prions. Autoclaving is not completely effective, nor are chemicals such as phenol, alcohols, formalin, and β-propiolactone. Even when fixed in formalin, affected brain and spinal cord tissues remain infectious.

Personnel who handle contaminated specimens or equipment or work with infected patients must wear a protective coat, face protection, and cut-resistant gloves. Any contact with skin must be immediately washed with detergent and warm water without scrubbing. The skin should then be washed with 1 N NaOH or a 1:10 dilution of bleach for 1 minute. Contaminated waste must be incinerated or autoclaved in a strong basic solution, and instruments must be cleaned and soaked in a strong basic solution.

Link to Learning

For more information on the handling of animals and prion-contaminated materials, visit the guidelines published on the CDC and WHO websites.

Peroxygens

Peroxygens are strong oxidizing agents that can be used as disinfectants or antiseptics. The most widely used peroxygen is hydrogen peroxide (H_2O_2), which is often used in solution to disinfect surfaces and may also be used as a gaseous agent. Hydrogen peroxide solutions are inexpensive skin antiseptics that break down into water and oxygen gas, both of which are environmentally safe. This decomposition is accelerated in the presence of light, so hydrogen peroxide solutions typically are sold in brown or

opaque bottles. One disadvantage of using hydrogen peroxide as an antiseptic is that it also causes damage to skin that may delay healing or lead to scarring. Contact lens cleaners often include hydrogen peroxide as a disinfectant.

Hydrogen peroxide works by producing free radicals that damage cellular macromolecules. Hydrogen peroxide has broad-spectrum activity, working against gram-positive and gram-negative bacteria (with slightly greater efficacy against gram-positive bacteria), fungi, viruses, and endospores. However, bacteria that produce the oxygen-detoxifying enzymes catalase or peroxidase may have inherent tolerance to low hydrogen peroxide concentrations (Figure **10.4.12**). To kill endospores, the length of exposure or concentration of solutions of hydrogen peroxide must be increased. Gaseous hydrogen peroxide has greater efficacy and can be used as a sterilant for rooms or equipment.

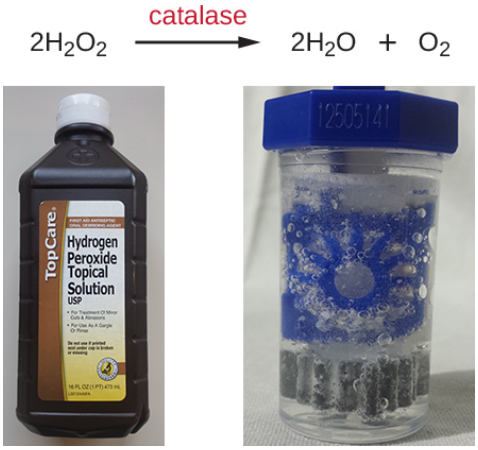


Figure **10.4.12**: Catalase enzymatically converts highly reactive hydrogen peroxide (H_2O_2) into water and oxygen. Hydrogen peroxide can be used to clean wounds. Hydrogen peroxide is used to sterilize items such as contact lenses. (credit photos: modification of work by Kerry Ceszyk)

Plasma, a hot, ionized gas, described as the fourth state of matter, is useful for sterilizing equipment because it penetrates surfaces and kills vegetative cells and endospores. Hydrogen peroxide and peracetic acid, another commonly used peroxygen, each may be introduced as a plasma. Peracetic acid can be used as a liquid or plasma sterilant insofar as it readily kills endospores, is more effective than hydrogen peroxide even at rather low concentrations, and is immune to inactivation by catalases and peroxidases. It also breaks down to environmentally innocuous compounds; in this case, acetic acid and oxygen.

Other examples of peroxygens include benzoyl peroxide and carbamide peroxide. Benzoyl peroxide is a peroxygen that used in acne medication solutions. It kills the bacterium *Propionibacterium acnes*, which is associated with acne. Carbamide peroxide, an ingredient used in toothpaste, is a peroxygen that combats oral biofilms that cause tooth discoloration and halitosis (bad breath).[15] Last, ozone gas is a peroxygen with disinfectant qualities and is used to clean air or water supplies. Overall, peroxygens are highly effective and commonly used, with no associated environmental hazard.

? Exercise 10.4.8

How do peroxides kill cells?

Supercritical Fluids

Within the last 15 years, the use of supercritical fluids, especially supercritical carbon dioxide ($scCO_2$), has gained popularity for certain sterilizing applications. When carbon dioxide is brought to approximately 10 times atmospheric pressure, it reaches a supercritical state that has physical properties between those of liquids and gases. Materials put into a chamber in which carbon dioxide is pressurized in this way can be sterilized because of the ability of $scCO_2$ to penetrate surfaces.

Supercritical carbon dioxide works by penetrating cells and forming carbonic acid, thereby lowering the cell pH considerably. This technique is effective against vegetative cells and is also used in combination with peracetic acid to kill endospores. Its efficacy can also be augmented with increased temperature or by rapid cycles of pressurization and depressurization, which more likely produce cell lysis.

Benefits of $scCO_2$ include the nonreactive, nontoxic, and nonflammable properties of carbon dioxide, and this protocol is effective at low temperatures. Unlike other methods, such as heat and irradiation, that can degrade the object being sterilized, the use of $scCO_2$ preserves the object's integrity and is commonly used for treating foods (including spices and juices) and medical devices such as endoscopes. It is also gaining popularity for disinfecting tissues such as skin, bones, tendons, and ligaments prior to transplantation. $scCO_2$ can also be used for pest control because it can kill insect eggs and larvae within products.

? Exercise 10.4.9

Why is the use of supercritical carbon dioxide gaining popularity for commercial and medical uses?

Chemical Food Preservatives

Chemical preservatives are used to inhibit microbial growth and minimize spoilage in some foods. Commonly used chemical preservatives include sorbic acid, benzoic acid, and propionic acid, and their more soluble salts potassium sorbate, sodium benzoate, and calcium propionate, all of which are used to control the growth of molds in acidic foods. Each of these preservatives is nontoxic and readily metabolized by humans. They are also flavorless, so they do not compromise the flavor of the foods they preserve.

Sorbic and benzoic acids exhibit increased efficacy as the pH decreases. Sorbic acid is thought to work by inhibiting various cellular enzymes, including those in the citric acid cycle, as well as catalases and peroxidases. It is added as a preservative in a wide variety of foods, including dairy, bread, fruit, and vegetable products. Benzoic acid is found naturally in many types of fruits and berries, spices, and fermented products. It is thought to work by decreasing intracellular pH, interfering with mechanisms such as oxidative phosphorylation and the uptake of molecules such as amino acids into cells. Foods preserved with benzoic acid or sodium benzoate include fruit juices, jams, ice creams, pastries, soft drinks, chewing gum, and pickles.

Propionic acid is thought to both inhibit enzymes and decrease intracellular pH, working similarly to benzoic acid. However, propionic acid is a more effective preservative at a higher pH than either sorbic acid or benzoic acid. Propionic acid is naturally produced by some cheeses during their ripening and is added to other types of cheese and baked goods to prevent mold contamination. It is also added to raw dough to prevent contamination by the bacterium *Bacillus mesentericus*, which causes bread to become ropy.

Other commonly used chemical preservatives include sulfur dioxide and nitrites. Sulfur dioxide prevents browning of foods and is used for the preservation of dried fruits; it has been used in winemaking since ancient times. Sulfur dioxide gas dissolves in water readily, forming sulfites. Although sulfites can be metabolized by the body, some people have sulfite allergies, including asthmatic reactions. Additionally, sulfites degrade thiamine, an important nutrient in some foods. The mode of action of sulfites is not entirely clear, but they may interfere with the disulfide bond (see Figure 7.4.5) formation in proteins, inhibiting enzymatic activity. Alternatively, they may reduce the intracellular pH of the cell, interfering with proton motive force-driven mechanisms.

Nitrites are added to processed meats to maintain color and stop the germination of *Clostridium botulinum* endospores. Nitrites are reduced to nitric oxide, which reacts with heme groups and iron-sulfur groups. When nitric oxide reacts with the heme group within the myoglobin of meats, a red product forms, giving meat its red color. Alternatively, it is thought that when nitric acid reacts with the iron-sulfur enzyme ferredoxin within bacteria, this electron transport-chain carrier is destroyed, preventing ATP synthesis. Nitrosamines, however, are carcinogenic and can be produced through exposure of nitrite-preserved meats (e.g., hot dogs, lunch meat, breakfast sausage, bacon, meat in canned soups) to heat during cooking.

Natural Chemical Food Preservatives

The discovery of natural antimicrobial substances produced by other microbes has added to the arsenal of preservatives used in food. Nisin is an antimicrobial peptide produced by the bacterium *Lactococcus lactis* and is particularly effective against gram-positive organisms. Nisin works by disrupting cell wall production, leaving cells more prone to lysis. It is used to preserve cheeses, meats, and beverages.

Natamycin is an antifungal macrolide antibiotic produced by the bacterium *Streptomyces natalensis*. It was approved by the FDA in 1982 and is used to prevent fungal growth in various types of dairy products, including cottage cheese, sliced cheese, and shredded cheese. Natamycin is also used for meat preservation in countries outside the United States.

? Exercise 10.4.10

What are the advantages and drawbacks of using sulfites and nitrites as food preservatives?

Key Concepts and Summary

- **Heavy metals**, including mercury, silver, copper, and zinc, have long been used for disinfection and preservation, although some have toxicity and environmental risks associated with them.
- **Halogens**, including chlorine, fluorine, and iodine, are also commonly used for disinfection. Chlorine compounds, including **sodium hypochlorite**, **chloramines**, and **chlorine dioxide**, are commonly used for water disinfection. Iodine, in both **tincture** and **iodophor** forms, is an effective antiseptic.
- **Alcohols**, including ethyl alcohol and isopropyl alcohol, are commonly used antiseptics that act by denaturing proteins and disrupting membranes.
- **Phenolics** are stable, long-acting disinfectants that denature proteins and disrupt membranes. They are commonly found in household cleaners, mouthwashes, and hospital disinfectants, and are also used to preserve harvested crops.
- The phenolic compound **triclosan**, found in antibacterial soaps, plastics, and textiles is technically an antibiotic because of its specific mode of action of inhibiting bacterial fatty-acid synthesis..
- **Surfactants**, including soaps and detergents, lower the surface tension of water to create emulsions that mechanically carry away microbes. Soaps are long-chain fatty acids, whereas detergents are synthetic surfactants.
- **Quaternary ammonium compounds** (**quats**) are cationic detergents that disrupt membranes. They are used in household cleaners, skin disinfectants, oral rinses, and mouthwashes.
- **Bisbiguanides** disrupt cell membranes, causing cell contents to gel. **Chlorhexidine** and **alexidine** are commonly used for surgical scrubs, for handwashing in clinical settings, and in prescription oral rinses.
- **Alkylating agents** effectively sterilize materials at low temperatures but are carcinogenic and may also irritate tissue. **Glutaraldehyde** and **o-phthalaldehyde** are used as hospital disinfectants but not as antiseptics. **Formaldehyde** is used for the storage of tissue specimens, as an embalming fluid, and in vaccine preparation to inactivate infectious agents. **Ethylene oxide** is a gas sterilant that can permeate heat-sensitive packaged materials, but it is also explosive and carcinogenic.
- **Peroxygens**, including **hydrogen peroxide**, **peracetic acid**, **benzoyl peroxide**, and ozone gas, are strong oxidizing agents that produce free radicals in cells, damaging their macromolecules. They are environmentally safe and are highly effective disinfectants and antiseptics.
- Pressurized carbon dioxide in the form of a **supercritical fluid** easily permeates packaged materials and cells, forming carbonic acid and lowering intracellular pH. Supercritical carbon dioxide is nonreactive, nontoxic, nonflammable, and effective at low temperatures for sterilization of medical devices, implants, and transplanted tissues.
- Chemical preservatives are added to a variety of foods. **Sorbic acid**, **benzoic acid**, **propionic acid**, and their more soluble salts inhibit enzymes or reduce intracellular pH.
- **Sulfites** are used in winemaking and food processing to prevent browning of foods.
- **Nitrites** are used to preserve meats and maintain color, but cooking nitrite-preserved meats may produce carcinogenic nitrosamines.
- **Nisin** and **natamycin** are naturally produced preservatives used in cheeses and meats. Nisin is effective against gram-positive bacteria and natamycin against fungi.

Footnotes

1. 1 US Food and Drug Administration. “Triclosan: What Consumers Should Know.” 2015. www.fda.gov/ForConsumers/Cons.../ucm205999.htm. Accessed June 9, 2016.
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10.E: Control of Microbial Growth (Exercises)

13.1: Controlling Microbial Growth

Inanimate items, such as doorknobs, toys, or towels, which may harbor microbes and aid in disease transmission, are called fomites. Two factors heavily influence the level of cleanliness required for a particular fomite and, hence, the protocol chosen to achieve this level. The first factor is the application for which the item will be used and the second factor is the level of resistance to antimicrobial treatment by potential pathogens.

Multiple Choice

Which of the following types of medical items requires sterilization?

A. needles
B. bed linens
C. respiratory masks
D. blood pressure cuffs

Answer

A

Which of the following is suitable for use on tissues for microbial control to prevent infection?

A. disinfectant
B. antiseptic
C. sterilant
D. water

Answer

B

Which biosafety level is appropriate for research with microbes or infectious agents that pose moderate risk to laboratory workers and the community, and are typically indigenous?

A. BSL-1
B. BSL-2
C. BSL-3
D. BSL-4

Answer

B

Which of the following best describes a microbial control protocol that inhibits the growth of molds and yeast?

A. bacteriostatic
B. fungicidal
C. bactericidal
D. fungistatic

Answer

D

The decimal reduction time refers to the amount of time it takes to which of the following?

A. reduce a microbial population by 10%
B. reduce a microbial population by 0.1%

C. reduce a microbial population by 90%
D. completely eliminate a microbial population

Answer

C

Fill in the Blank

A medical item that comes into contact with intact skin and does not penetrate sterile tissues or come into contact with mucous membranes is called a(n) _____ item.

Answer

noncritical

The goal of _____ _____ protocols is to rid canned produce of *Clostridium botulinum* endospores.

Answer

commercial sterilization

True/False

Sanitization leaves an object free of microbes.

Answer

False

Short Answer

What are some characteristics of microbes and infectious agents that would require handling in a BSL-3 laboratory?

What is the purpose of degerming? Does it completely eliminate microbes?

What are some factors that alter the effectiveness of a disinfectant?

Critical Thinking

When plotting microbial death curves, how might they look different for bactericidal versus bacteriostatic treatments?

What are the benefits of cleaning something to a level of cleanliness beyond what is required? What are some possible disadvantages of doing so?

13.2: Using Physical Methods to Control Microorganisms

For thousands of years, humans have used various physical methods of microbial control for food preservation. Common control methods include the application of high temperatures, radiation, filtration, and desiccation (drying), among others. Many of these methods nonspecifically kill cells by disrupting membranes, changing membrane permeability, or damaging proteins and nucleic acids by denaturation, degradation, or chemical modification.

Multiple Choice

Which of the following methods brings about cell lysis due to cavitation induced by rapid localized pressure changes?

A. microwaving
B. gamma irradiation
C. ultraviolet radiation
D. sonication

Answer

D

Which of the following terms is used to describe the time required to kill all of the microbes within a sample at a given temperature?

A. D-value
B. thermal death point
C. thermal death time
D. decimal reduction time

Answer

C

Which of the following microbial control methods does not actually kill microbes or inhibit their growth but instead removes them physically from samples?

A. filtration
B. desiccation
C. lyophilization
D. nonionizing radiation

Answer

A

Fill in the Blank

In an autoclave, the application of pressure to _____ is increased to allow the steam to achieve temperatures above the boiling point of water.

Answer

steam

True/False

Ionizing radiation can penetrate surfaces, but nonionizing radiation cannot.

Answer

True

Moist-heat sterilization protocols require the use of higher temperatures for longer periods of time than do dry-heat sterilization protocols do.

Answer

False

Short Answer

What is the advantage of HTST pasteurization compared with sterilization? What is an advantage of UHT treatment?

How does the addition of salt or sugar help preserve food?

Which is more effective at killing microbes: autoclaving or freezing? Explain.

Critical Thinking

In 2001, endospores of *Bacillus anthracis*, the causative agent of anthrax, were sent to government officials and news agencies via the mail. In response, the US Postal Service began to irradiate mail with UV light. Was this an effective strategy? Why or why not?

13.3: Using Chemicals to Control Microorganisms

In addition to physical methods of microbial control, chemicals are also used to control microbial growth. A wide variety of chemicals can be used as disinfectants or antiseptics. This section describes the variety of chemicals used as disinfectants and antiseptics, including their mechanisms of action and common uses.

Multiple Choice

Which of the following refers to a disinfecting chemical dissolved in alcohol?

A. iodophor
B. tincture
C. phenolic
D. peroxygen

Answer

B

Which of the following peroxygens is widely used as a household disinfectant, is inexpensive, and breaks down into water and oxygen gas?

A. hydrogen peroxide
B. peracetic acid
C. benzoyl peroxide
D. ozone

Answer

A

Which of the following chemical food preservatives is used in the wine industry but may cause asthmatic reactions in some individuals?

A. nitrites
B. sulfites
C. propionic acid
D. benzoic acid

Answer

B

Bleach is an example of which group of chemicals used for disinfection?

A. heavy metals
B. halogens
C. quats
D. bisbiguanides

Answer

B

Which chemical disinfectant works by methylating enzymes and nucleic acids and is known for being toxic and carcinogenic?

A. sorbic acid
B. triclosan
C. formaldehyde
D. hexaclorophene

Answer

C

Fill in the Blank

Doorknobs and other surfaces in clinical settings are often coated with _____, _____, or _____ to prevent the transmission of microbes.

Answer

copper, nickel, zinc

True/False

Soaps are classified as disinfectants.

Answer

False

Mercury-based compounds have fallen out of favor for use as preservatives and antiseptics.

Answer

True

Short Answer

Which solution of ethyl alcohol is more effective at inhibiting microbial growth: a 70% solution or a 100% solution? Why?

When might a gas treatment be used to control microbial growth instead of autoclaving? What are some examples?

What is the advantage of using an iodophor rather than iodine or an iodine tincture?

Critical Thinking

Looking at Figure 13.3.11 and reviewing the functional groups in Figure 7.1.5, which alkylating agent shown lacks an aldehyde group?

Do you think naturally produced antimicrobial products like nisin and natamycin should replace sorbic acid for food preservation? Why or why not?

Why is the use of skin disinfecting compounds required for surgical scrubbing and not for everyday handwashing?

13.4: Testing the Effectiveness of Antiseptics and Disinfectants

Several environmental conditions influence the potency of an antimicrobial agent and its effectiveness. For example, length of exposure is particularly important, with longer exposure increasing efficacy. Similarly, the concentration of the chemical agent is also important, with higher concentrations being more effective than lower ones. Temperature, pH, and other factors can also affect the potency of a disinfecting agent.

Multiple Choice

Which type of test is used to determine whether disinfectant solutions actively used in a clinical setting are being used correctly?

A. disk-diffusion assay
B. phenol coefficient test
C. in-use test
D. use-dilution test

Answer

C

The effectiveness of chemical disinfectants has historically been compared to that of which of the following?

A. phenol
B. ethyl alcohol
C. bleach
D. formaldehyde

Answer

A

Which of the following refers to a germicide that can kill vegetative cells and certain enveloped viruses but not endospores?

A. high-level germicide
B. intermediate-level germicide
C. low-level germicide
D. sterilant

Answer

C

Fill in the Blank

If a chemical disinfectant is more effective than phenol, then its phenol coefficient would be _____ than 1.0.

Answer

greater

If used for extended periods of time, _____ germicides may lead to sterility.

Answer

high-level

In the disk-diffusion assay, a large zone of inhibition around a disk to which a chemical disinfectant has been applied indicates _____ of the test microbe to the chemical disinfectant.

Answer

susceptibility or sensitivity

Short Answer

Why were chemical disinfectants once commonly compared with phenol?

Why is length of exposure to a chemical disinfectant important for its activity?

Critical Thinking

What are some advantages of use-dilution and in-use tests compared with the disk-diffusion assay?

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CHAPTER OVERVIEW

11: Viruses and Other Agents

11.1: Viruses - General Characteristics

11.2: The Viral Life Cycle and Host Infection Specificity

11.2.1: Medical Importance of Viruses

11.2.2: The Baltimore Virus Classification

11.2.3: Evolution of Viruses

11.3: Prevention and Treatment of Viral Infections

11.4: The Role of Viruses in Tumor Production

11.5: Culturing Viruses

11.5.1: Isolation, Culture, and Identification of Viruses

11.5.2: Tissue Culture of Animal Viruses

11.5.3: Batch Culture of Bacteriophages

11.6: Viroids and Prions

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11.1: Viruses - General Characteristics

Learning Objectives

- Describe the general characteristics of viruses as pathogens
- Describe viral genomes
- Describe the general characteristics of viral life cycles
- Differentiate among bacteriophages, plant viruses, and animal viruses
- Describe the characteristics used to identify viruses as obligate intracellular parasites

Clinical Focus: Part 1

David, a 45-year-old journalist, has just returned to the U.S. from travels in Russia, China, and Africa. He is not feeling well, so he goes to his general practitioner complaining of weakness in his arms and legs, fever, headache, noticeable agitation, and minor discomfort. He thinks it may be related to a dog bite he suffered while interviewing a Chinese farmer. He is experiencing some prickling and itching sensations at the site of the bite wound, but he tells the doctor that the dog seemed healthy and that he had not been concerned until now. The doctor ordered a culture and sensitivity test to rule out bacterial infection of the wound, and the results came back negative for any possible pathogenic bacteria.

Exercise 11.1.1

1. Based on this information, what additional tests should be performed on the patient?
2. What type of treatment should the doctor recommend?

Despite their small size, which prevented them from being seen with light microscopes, the discovery of a filterable component smaller than a bacterium that causes tobacco mosaic disease (TMD) dates back to 1892.[1] At that time, Dmitri Ivanovski, a Russian botanist, discovered the source of TMD by using a porcelain filtering device first invented by Charles Chamberland and Louis Pasteur in Paris in 1884. Porcelain Chamberland filters have a pore size of 0.1 µm, which is small enough to remove all bacteria ≥0.2 µm from any liquids passed through the device. An extract obtained from TMD-infected tobacco plants was made to determine the cause of the disease. Initially, the source of the disease was thought to be bacterial. It was surprising to everyone when Ivanovski, using a Chamberland filter, found that the cause of TMD was not removed after passing the extract through the porcelain filter. So if a bacterium was not the cause of TMD, what could be causing the disease? Ivanovski concluded the cause of TMD must be an extremely small bacterium or bacterial spore. Other scientists, including Martinus Beijerinck, continued investigating the cause of TMD. It was Beijerinck, in 1899, who eventually concluded the causative agent was not a bacterium but, instead, possibly a chemical, like a biological poison we would describe today as a toxin. As a result, the word *virus*, Latin for poison, was used to describe the cause of TMD a few years after Ivanovski's initial discovery. Even though he was not able to see the virus that caused TMD, and did not realize the cause was not a bacterium, Ivanovski is credited as the original discoverer of viruses and a founder of the field of virology.

Today, we can see viruses using electron microscopes (Figure **11.1.1**) and we know much more about them. Viruses are distinct biological entities; however, their evolutionary origin is still a matter of speculation. In terms of taxonomy, they are not included in the tree of life because they are acellular (not consisting of cells). In order to survive and reproduce, viruses must infect a cellular host, making them obligate intracellular parasites. The genome of a virus enters a host cell and directs the production of the viral components, proteins and nucleic acids, needed to form new virus particles called virions. New virions are made in the host cell by assembly of viral components. The new virions transport the viral genome to another host cell to carry out another round of infection. Table **11.1.1** summarizes the properties of viruses.

Table **11.1.1**: Properties of viruses.

Characteristics of Viruses
Infectious, acellular pathogens
Obligate intracellular parasites with host and cell-type specificity
DNA or RNA genome (never both)
Genome is surrounded by a protein capsid and, in some cases, a phospholipid membrane studded with viral glycoproteins
Lack genes for many products needed for successful reproduction, requiring exploitation of host-cell genomes to reproduce

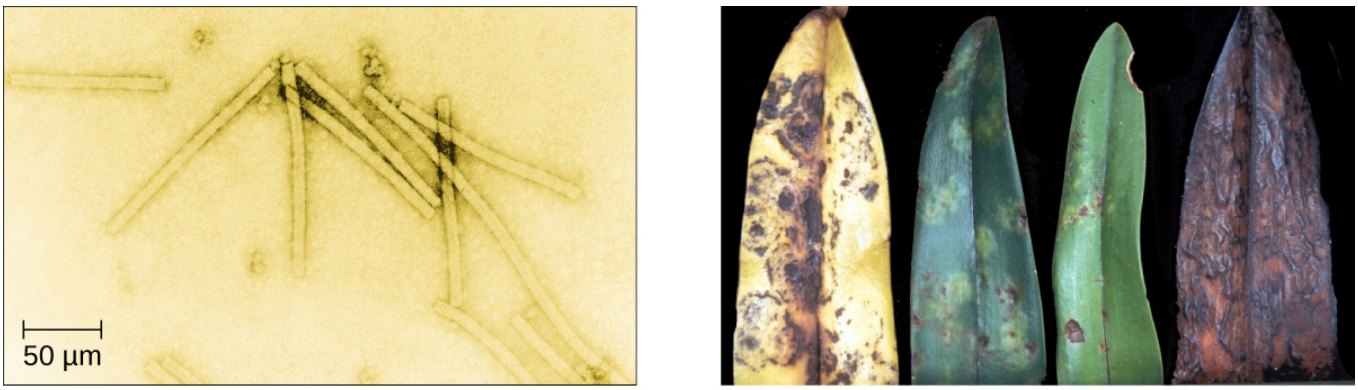


Figure **11.1.1**: (a) Tobacco mosaic virus (TMV) viewed with transmission electron microscope. (b) Plants infected with tobacco mosaic disease (TMD), caused by TMV. (credit a: modification of work by USDA Agricultural Research Service—scale-bar data from Matt Russell; credit b: modification of work by USDA Forest Service, Department of Plant Pathology Archive North Carolina State University)

? Exercise 11.1.2

Why was the first virus investigated mistaken for a toxin?

Hosts and Viral Transmission

Viruses can infect every type of host cell, including those of plants, animals, fungi, protists, bacteria, and archaea. Most viruses will only be able to infect the cells of one or a few species of organism. This is called the host range. However, having a wide host range is not common and viruses will typically only infect specific hosts and only specific cell types within those hosts. The viruses that infect bacteria are called bacteriophages, or simply phages. The word *phage* comes from the Greek word for devour. Other viruses are just identified by their host group, such as animal or plant viruses. Once a cell is infected, the effects of the virus can vary depending on the type of virus. Viruses may cause abnormal growth of the cell or cell death, alter the cell's genome, or cause little noticeable effect in the cell.

Viruses can be transmitted through direct contact, indirect contact with fomites, or through a vector: an animal that transmits a pathogen from one host to another. Arthropods such as mosquitoes, ticks, and flies, are typical vectors for viral diseases, and they may act as mechanical vectors or biological vectors. Mechanical transmission occurs when the arthropod carries a viral pathogen on the outside of its body and transmits it to a new host by physical contact. Biological transmission occurs when the arthropod carries the viral pathogen inside its body and transmits it to the new host through biting.

In humans, a wide variety of viruses are capable of causing various infections and diseases. Some of the deadliest emerging pathogens in humans are viruses, yet we have few treatments or drugs to deal with viral infections, making them difficult to eradicate.

Viruses that can be transmitted from an animal host to a human host can cause zoonoses. For example, the avian influenza virus originates in birds, but can cause disease in humans. Reverse zoonoses are caused by infection of an animal by a virus that originated in a human.

Fighting Bacteria With Viruses

The emergence of superbugs, or multidrug resistant bacteria, has become a major challenge for pharmaceutical companies and a serious health-care problem. According to a 2013 report by the US Centers for Disease Control and Prevention (CDC), more than 2 million people are infected with drug-resistant bacteria in the US annually, resulting in at least 23,000 deaths.[2] The continued use and overuse of antibiotics will likely lead to the evolution of even more drug-resistant strains.

One potential solution is the use of phage therapy, a procedure that uses bacteria-killing viruses (bacteriophages) to treat bacterial infections. Phage therapy is not a new idea. The discovery of bacteriophages dates back to the early 20th century, and phage therapy was first used in Europe in 1915 by the English bacteriologist Frederick Twort.[3] However, the subsequent discovery of penicillin and other antibiotics led to the near abandonment of this form of therapy, except in the former Soviet Union and a few countries in Eastern Europe. Interest in phage therapy outside of the countries of the former Soviet Union is only recently re-emerging because of the rise in antibiotic-resistant bacteria.[4]

Phage therapy has some advantages over antibiotics in that phages kill only one specific bacterium, whereas antibiotics kill not only the pathogen but also beneficial bacteria of the normal microbiota. Development of new antibiotics is also expensive for drug companies and for patients, especially for those who live in countries with high poverty rates.

Phages have also been used to prevent food spoilage. In 2006, the US Food and Drug Administration approved the use of a solution containing six bacteriophages that can be sprayed on lunch meats such as bologna, ham, and turkey to kill *Listeria monocytogenes*, a bacterium responsible for listeriosis, a form of food poisoning. Some consumers have concerns about the use of phages on foods, however, especially given the rising popularity of organic products. Foods that have been treated with phages must declare “bacteriophage preparation” in the list of ingredients or include a label declaring that the meat has been “treated with antimicrobial solution to reduce microorganisms.”[5]

Exercise 11.1.3

1. Why do humans not have to be concerned about the presence of bacteriophages in their food?
2. What are three ways that viruses can be transmitted between hosts?

Viral Structures

In general, virions (viral particles) are small and cannot be observed using a regular light microscope. They are much smaller than prokaryotic and eukaryotic cells; this is an adaptation allowing viruses to infect these larger cells (see Figure **11.1.2**). The size of a virion can range from 20 nm for small viruses up to 900 nm for typical, large viruses (see Figure **11.1.3**). Recent discoveries, however, have identified new giant viral species, such as *Pandoravirus salinus* and *Pithovirus sibericum*, with sizes approaching that of a bacterial cell.[6]

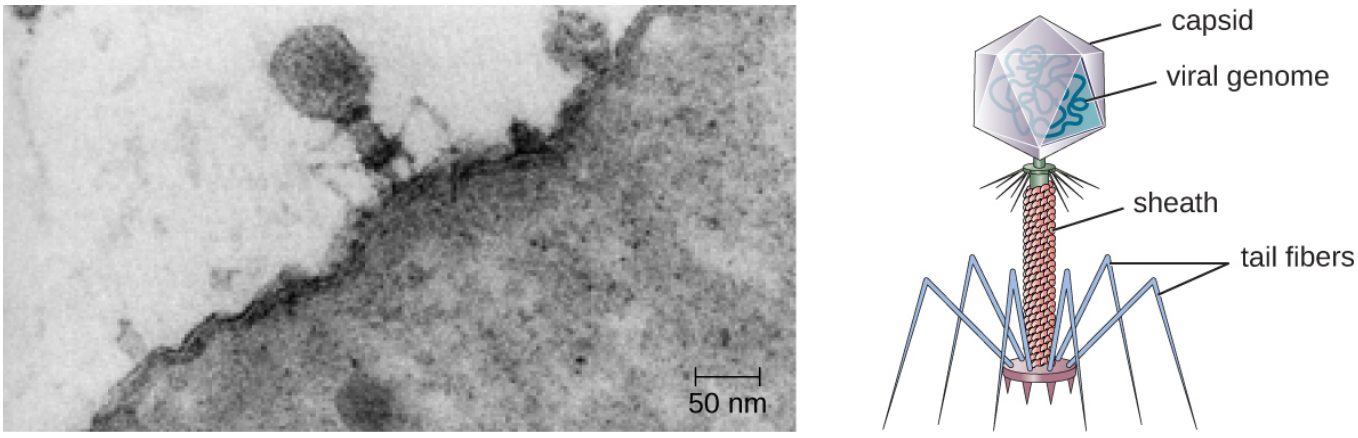


Figure **11.1.2**: (a) In this transmission electron micrograph, a bacteriophage (a virus that infects bacteria) is dwarfed by the bacterial cell it infects. (b) An illustration of the bacteriophage in the micrograph. (credit a: modification of work by U.S. Department of Energy, Office of Science, LBL, PBD)

In 1935, after the development of the electron microscope, Wendell Stanley was the first scientist to crystallize the structure of the tobacco mosaic virus and discovered that it is composed of RNA and protein. In 1943, he isolated *Influenza B virus*, which contributed to the development of an influenza (flu) vaccine. Stanley's discoveries unlocked the mystery of the nature of viruses that had been puzzling scientists for over 40 years and his contributions to the field of virology led to him being awarded the Nobel Prize in 1946.

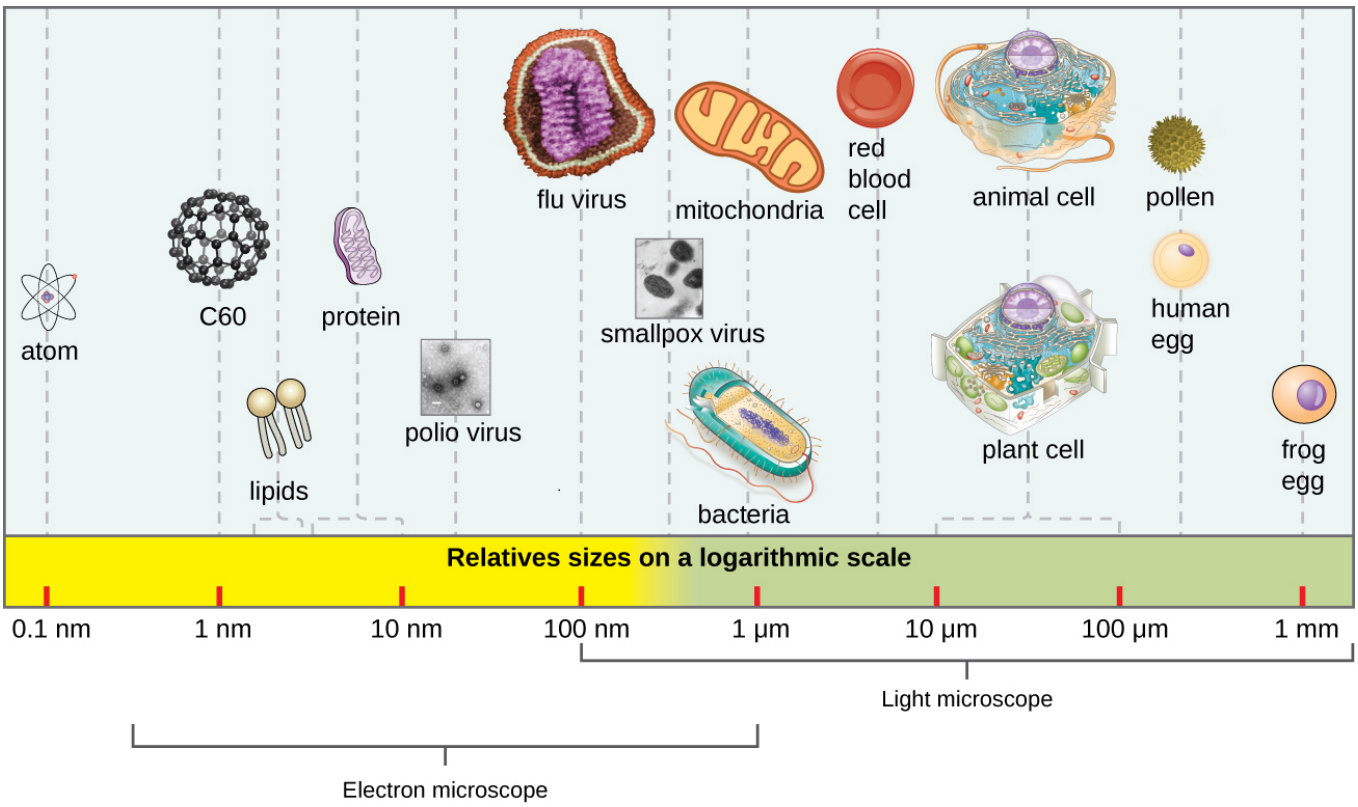


Figure **11.1.3**:The size of a virus is small relative to the size of most bacterial and eukaryotic cells and their organelles.

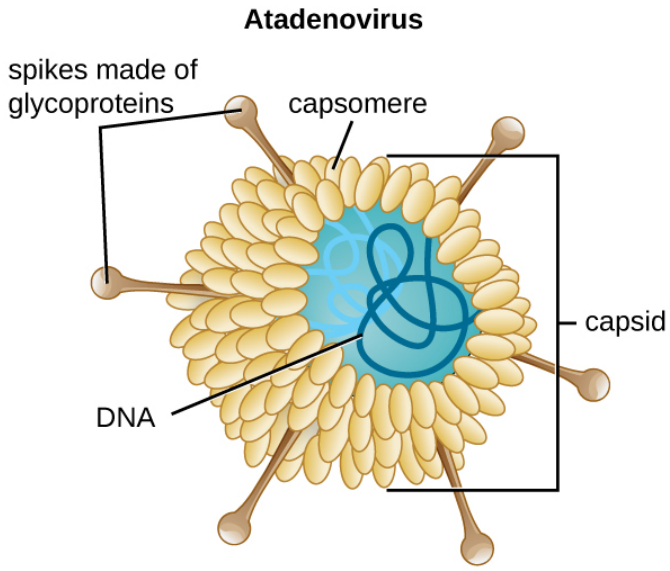
As a result of continuing research into the nature of viruses, we now know they consist of a nucleic acid (either RNA or DNA, but never both) surrounded by a protein coat called a capsid (see Figure **11.1.4**). The interior of the capsid is not filled with cytosol, as in a cell, but instead it contains the bare necessities in terms of genome and enzymes needed to direct the synthesis of new virions.

Each capsid is composed of protein subunits called capsomeres made of one or more different types of capsomere proteins that interlock to form the closely packed capsid.

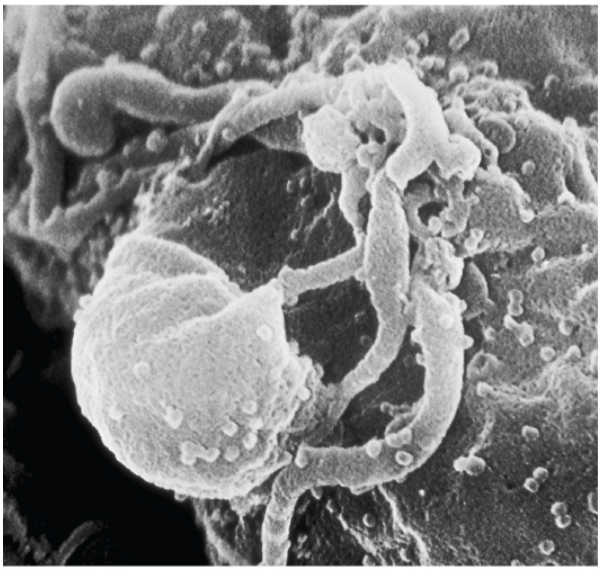
There are two categories of viruses based on general composition. Viruses formed from only a nucleic acid and capsid are called naked viruses or nonenveloped viruses. Viruses formed with a nucleic-acid packed capsid surrounded by a lipid layer are called enveloped viruses (see Figure **11.1.4**). The viral envelope is a small portion of phospholipid membrane obtained as the virion buds from a host cell. The viral envelope may either be intracellular or cytoplasmic in origin.

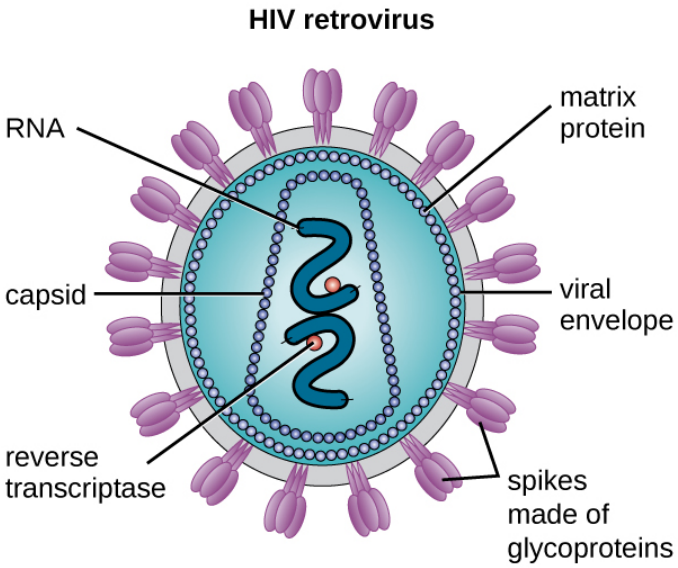
Extending outward and away from the capsid on some naked viruses and enveloped viruses are protein structures called spikes. At the tips of these spikes are structures that allow the virus to attach and enter a cell, like the influenza virus hemagglutinin spikes (H) or enzymes like the neuraminidase (N) influenza virus spikes that allow the virus to detach from the cell surface during release of new virions. Influenza viruses are often identified by their H and N spikes. For example, H1N1 influenza viruses were responsible for the pandemics in 1918 and 2009,[7] H2N2 for the pandemic in 1957, and H3N2 for the pandemic in 1968.





(a)





(b)

Figure 11.1.4: (a) The naked atadenovirus uses spikes made of glycoproteins from its capsid to bind to host cells. (b) The enveloped human immunodeficiency virus uses spikes made of glycoproteins embedded in its envelope to bind to host cells (credit a "micrograph": modification of work by NIAID; credit b "micrograph": modification of work by Centers for Disease Control and Prevention)

Viruses vary in the shape of their capsids, which can be either helical, polyhedral, or complex. A helical capsid forms the shape of tobacco mosaic virus (TMV), a naked helical virus, and Ebola virus, an enveloped helical virus. The capsid is cylindrical or rod shaped, with the genome fitting just inside the length of the capsid. Polyhedral capsids form the shapes of poliovirus and rhinovirus, and consist of a nucleic acid surrounded by a polyhedral (many-sided) capsid in the form of an icosahedron. An icosahedral capsid is a three-dimensional, 20-sided structure with 12 vertices. These capsids somewhat resemble a soccer ball. Both helical and polyhedral viruses can have envelopes. Viral shapes seen in certain types of bacteriophages, such as T4 phage, and poxviruses, like vaccinia virus, may have features of both polyhedral and helical viruses so they are described as a complex viral shape (see Figure 11.1.5). In the bacteriophage complex form, the genome is located within the polyhedral head and the sheath

connects the head to the tail fibers and tail pins that help the virus attach to receptors on the host cell's surface. Poxviruses that have complex shapes are often brick shaped, with intricate surface characteristics not seen in the other categories of capsid.

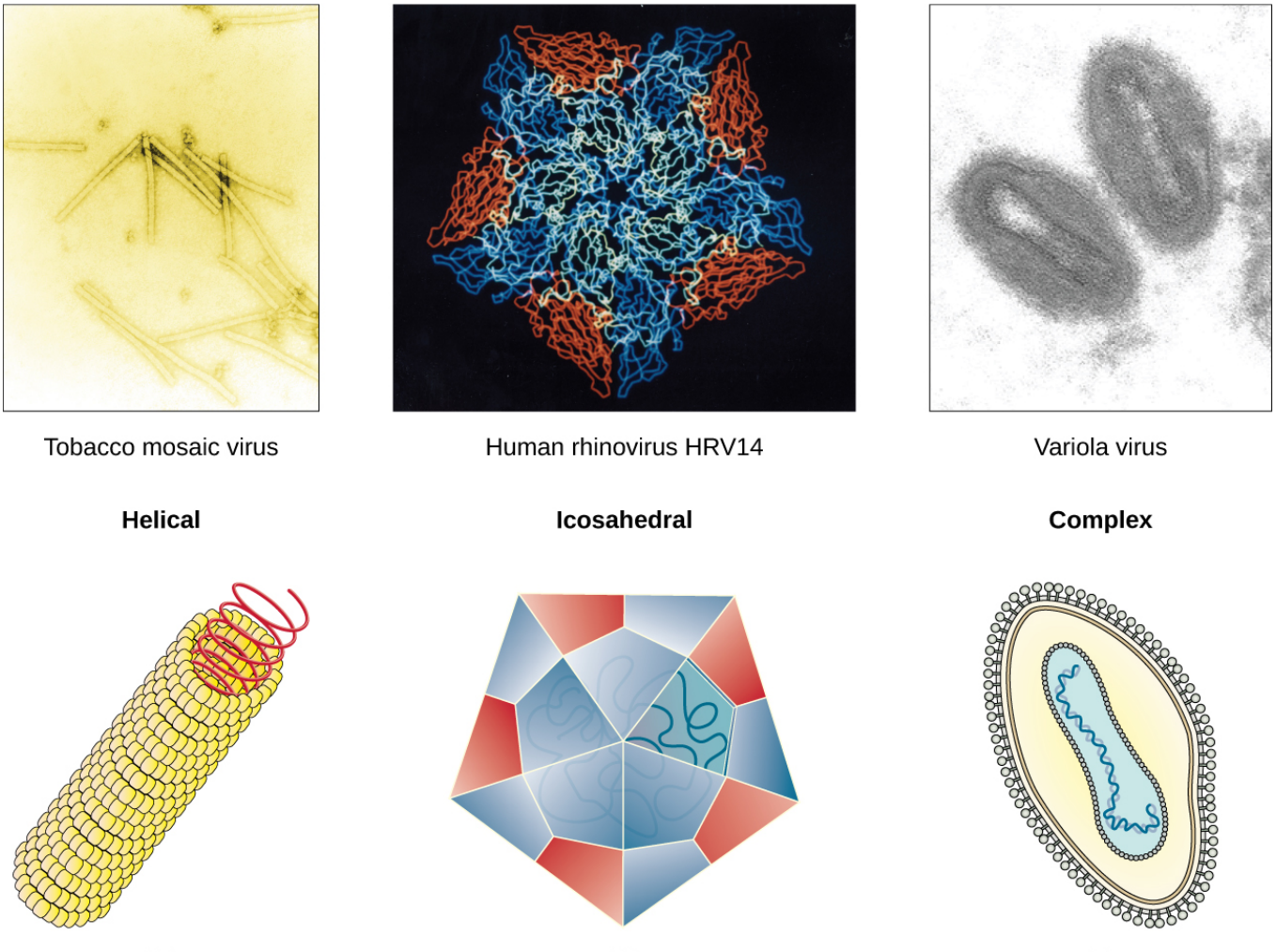


Figure **11.1.5**: Viral capsids can be (a) helical, (b) polyhedral, or (c) have a complex shape. (credit a "micrograph": modification of work by USDA ARS; credit b "micrograph": modification of work by U.S. Department of Energy)

? Exercise 11.1.4

Which types of viruses have spikes?

Clinical Focus: Part 2

David's doctor was concerned that his symptoms included prickling and itching at the site of the dog bite; these sensations could be early symptoms of rabies. Several tests are available to diagnose rabies in live patients, but no single antemortem test is adequate. The doctor decided to take samples of David's blood, saliva, and skin for testing. The skin sample was taken from the nape of the neck (posterior side of the neck near the hairline). It was about 6-mm long and contained at least 10 hair follicles, including the superficial cutaneous nerve. An immunofluorescent staining technique was used on the skin biopsy specimen to detect rabies antibodies in the cutaneous nerves at the base of the hair follicles. A test was also performed on a serum sample from David's blood to determine whether any antibodies for the rabies virus had been produced.

Meanwhile, the saliva sample was used for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis, a test that can detect the presence of viral nucleic acid (RNA). The blood tests came back positive for the presence of rabies virus antigen,

prompting David's doctor to prescribe prophylactic treatment. David is given a series of intramuscular injections of human rabies immunoglobulin along with a series of rabies vaccines.

Summary

- Viruses are generally ultramicroscopic, typically from 20 nm to 900 nm in length. Some large viruses have been found.
- **Virions** are acellular and consist of a nucleic acid, DNA or RNA, but not both, surrounded by a protein **capsid**. There may also be a phospholipid membrane surrounding the capsid.
- Viruses are obligate intracellular parasites.
- Viruses are known to infect various types of cells found in plants, animals, fungi, protists, bacteria, and archaea. Viruses typically have limited **host ranges** and infect specific cell types.
- Viruses may have **helical**, **polyhedral,** or **complex** shapes.
- Classification of viruses is based on morphology, type of nucleic acid, host range, cell specificity, and enzymes carried within the virion.
- Like other diseases, viral diseases are classified using ICD codes.

Footnotes

1. 1 H. Lecoq. "[Discovery of the First Virus, the Tobacco Mosaic Virus: 1892 or 1898?]." *Comptes Rendus de l'Academie des Sciences – Serie III – Sciences de la Vie* 324, no. 10 (2001): 929–933.
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5. 5 US Food and Drug Administration. "FDA Approval of *Listeria*-specific Bacteriophage Preparation on Ready-to-Eat (RTE) Meat and Poultry Products." www.fda.gov/food/ingredientsp.../ucm083572.htm (accessed September 22, 2015).
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11.2: The Viral Life Cycle and Host Infection Specificity

Learning Objectives

- Describe the lytic and lysogenic life cycles
- Describe the replication process of animal viruses
- Describe unique characteristics of retroviruses and latent viruses
- Discuss human viruses and their virus-host cell interactions
- Explain the process of transduction
- Describe the replication process of plant viruses

All viruses depend on cells for reproduction and metabolic processes. By themselves, viruses do not encode for all of the enzymes necessary for viral replication. But within a host cell, a virus can commandeer cellular machinery to produce more viral particles. Bacteriophages replicate only in the cytoplasm, since prokaryotic cells do not have a nucleus or organelles. In eukaryotic cells, most DNA viruses can replicate inside the nucleus, with an exception observed in the large DNA viruses, such as the poxviruses, that can replicate in the cytoplasm. RNA viruses that infect animal cells often replicate in the cytoplasm.

The Life Cycle of Viruses with Prokaryote Hosts

The life cycle of bacteriophages has been a good model for understanding how viruses affect the cells they infect, since similar processes have been observed for eukaryotic viruses, which can cause immediate death of the cell or establish a latent or chronic infection. Virulent phages typically lead to the death of the cell through cell lysis. **Temperate phages**, on the other hand, can become part of a host chromosome and are replicated with the cell genome until such time as they are induced to make newly assembled viruses, or **progeny viruses**.

The Lytic Cycle

During the lytic cycle of virulent phage, the bacteriophage takes over the cell, reproduces new phages, and destroys the cell. T-even phage is a good example of a well-characterized class of virulent phages. There are five stages in the bacteriophage lytic cycle (see Figure **11.2.1**). Attachment is the first stage in the infection process in which the phage interacts with specific bacterial surface receptors (e.g., lipopolysaccharides and OmpC protein on host surfaces). Most phages have a narrow host range and may infect one species of bacteria or one strain within a species. This unique recognition can be exploited for targeted treatment of bacterial infection by phage therapy or for phage typing to identify unique bacterial subspecies or strains. The second stage of infection is entry or penetration. This occurs through contraction of the tail sheath, which acts like a hypodermic needle to inject the viral genome through the cell wall and membrane. The phage head and remaining components remain outside the bacteria.

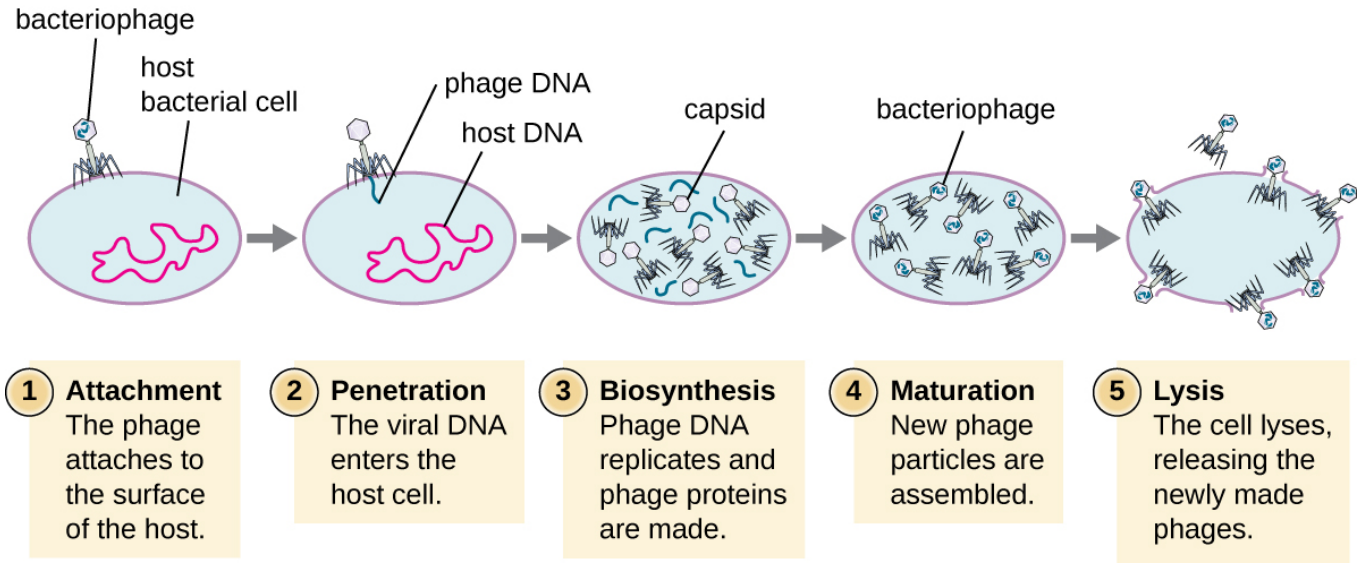


Figure **11.2.1**: A virulent phage shows only the lytic cycle pictured here. In the lytic cycle, the phage replicates and lyses the host cell.

The third stage of infection is biosynthesis of new viral components. After entering the host cell, the virus synthesizes virus-encoded endonucleases to degrade the bacterial chromosome. It then hijacks the host cell to replicate, transcribe, and translate the necessary viral components (capsomeres, sheath, base plates, tail fibers, and viral enzymes) for the assembly of new viruses. Polymerase genes are usually expressed early in the cycle, while capsid and tail proteins are expressed later. During the maturation phase, new virions are created. To liberate free phages, the bacterial cell wall is disrupted by phage proteins such as holin or lysozyme. The final stage is release. Mature viruses burst out of the host cell in a process called lysis and the progeny viruses are liberated into the environment to infect new cells.

The Lysogenic Cycle

In a lysogenic cycle, the phage genome also enters the cell through attachment and penetration. A prime example of a phage with this type of life cycle is the lambda phage. During the lysogenic cycle, instead of killing the host, the phage genome integrates into the bacterial chromosome and becomes part of the host. The integrated phage genome is called a prophage. A bacterial host with a prophage is called a lysogen. The process in which a bacterium is infected by a temperate phage is called lysogeny. It is typical of temperate phages to be latent or inactive within the cell. As the bacterium replicates its chromosome, it also replicates the phage's DNA and passes it on to new daughter cells during reproduction. The presence of the phage may alter the phenotype of the bacterium, since it can bring in extra genes (e.g., toxin genes that can increase bacterial virulence). This change in the host phenotype is called lysogenic conversion or phage conversion. Some bacteria, such as *Vibrio cholerae* and *Clostridium botulinum*, are less virulent in the absence of the prophage. The phages infecting these bacteria carry the toxin genes in their genome and enhance the virulence of the host when the toxin genes are expressed. In the case of *V. cholera*, phage encoded toxin can cause severe diarrhea; in *C. botulinum*, the toxin can cause paralysis. During lysogeny, the prophage will persist in the host chromosome until induction, which results in the excision of the viral genome from the host chromosome. After induction has occurred the temperate phage can proceed through a lytic cycle and then undergo lysogeny in a newly infected cell (see Figure **11.2.2**).

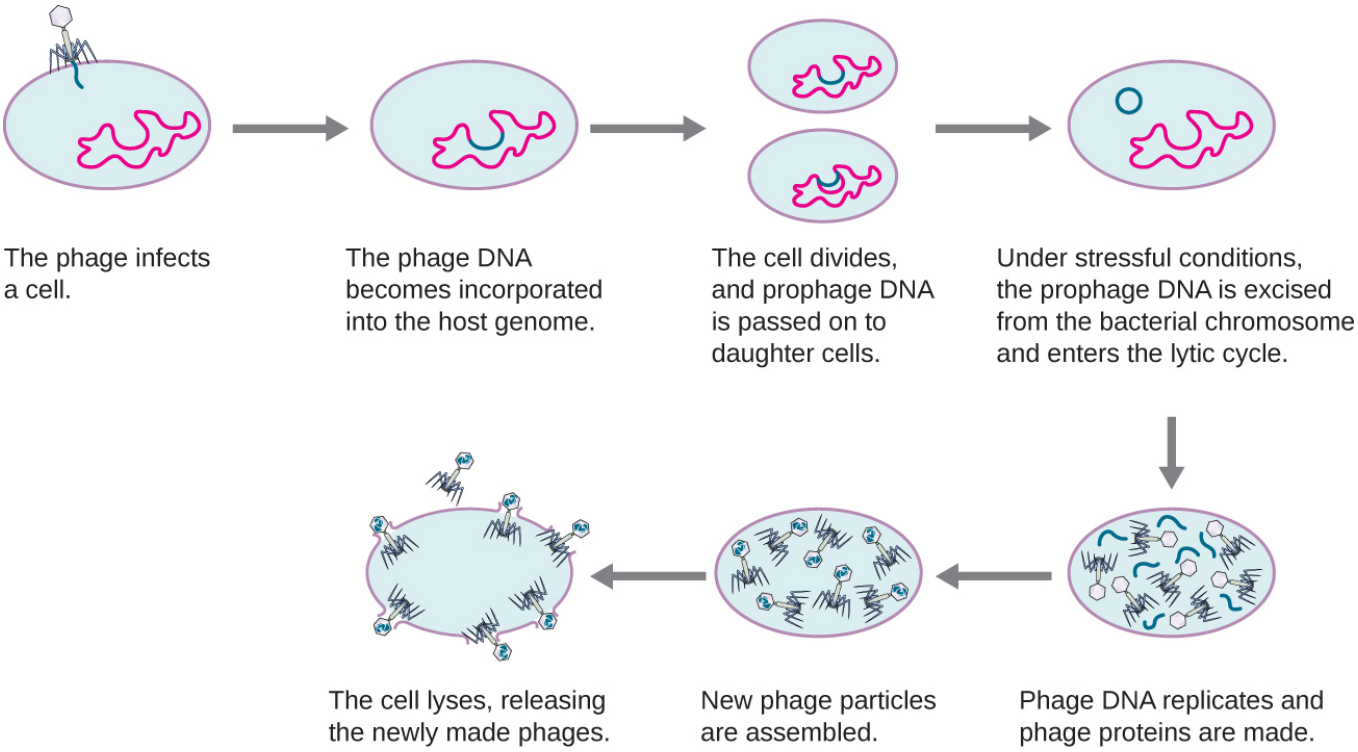


Figure **11.2.2**: A temperate bacteriophage has both lytic and lysogenic cycles. In the lysogenic cycle, phage DNA is incorporated into the host genome, forming a prophage, which is passed on to subsequent generations of cells. Environmental stressors such as starvation or exposure to toxic chemicals may cause the prophage to be excised and enter the lytic cycle.

Link to Learning

This video illustrates the stages of the lysogenic life cycle of a bacteriophage and the transition to a lytic phase.

Exercise 11.2.1

Is a latent phage undetectable in a bacterium?

Transduction

Transduction occurs when a bacteriophage transfers bacterial DNA from one bacterium to another during sequential infections. There are two types of transduction: generalized and specialized transduction. During the lytic cycle of viral replication, the virus hijacks the host cell, degrades the host chromosome, and makes more viral genomes. As it assembles and packages DNA into the phage head, packaging occasionally makes a mistake. Instead of packaging viral DNA, it takes a random piece of host DNA and inserts it into the capsid. Once released, this virion will then inject the former host's DNA into a newly infected host. The asexual transfer of genetic information can allow for DNA recombination to occur, thus providing the new host with new genes (e.g., an antibiotic-resistance gene, or a sugar-metabolizing gene). Generalized transduction occurs when a random piece of bacterial chromosomal DNA is transferred by the phage during the lytic cycle. Specialized transduction occurs at the end of the lysogenic cycle, when the prophage is excised and the bacteriophage enters the lytic cycle. Since the phage is integrated into the host genome, the prophage can replicate as part of the host. However, some conditions (e.g., ultraviolet light exposure or chemical exposure) stimulate the prophage to undergo induction, causing the phage to excise from the genome, enter the lytic cycle, and produce new phages to leave host cells. During the process of excision from the host chromosome, a phage may occasionally remove some bacterial DNA near the site of viral integration. The phage and host DNA from one end or both ends of the integration site are packaged within the capsid and are transferred to the new, infected host. Since the DNA transferred by the phage is not randomly packaged but is instead a specific piece of DNA near the site of integration, this mechanism of gene transfer is referred to as specialized transduction (see Figure **11.2.3**). The DNA can then recombine with host chromosome, giving the latter new

characteristics. Transduction seems to play an important role in the evolutionary process of bacteria, giving them a mechanism for asexual exchange of genetic information.

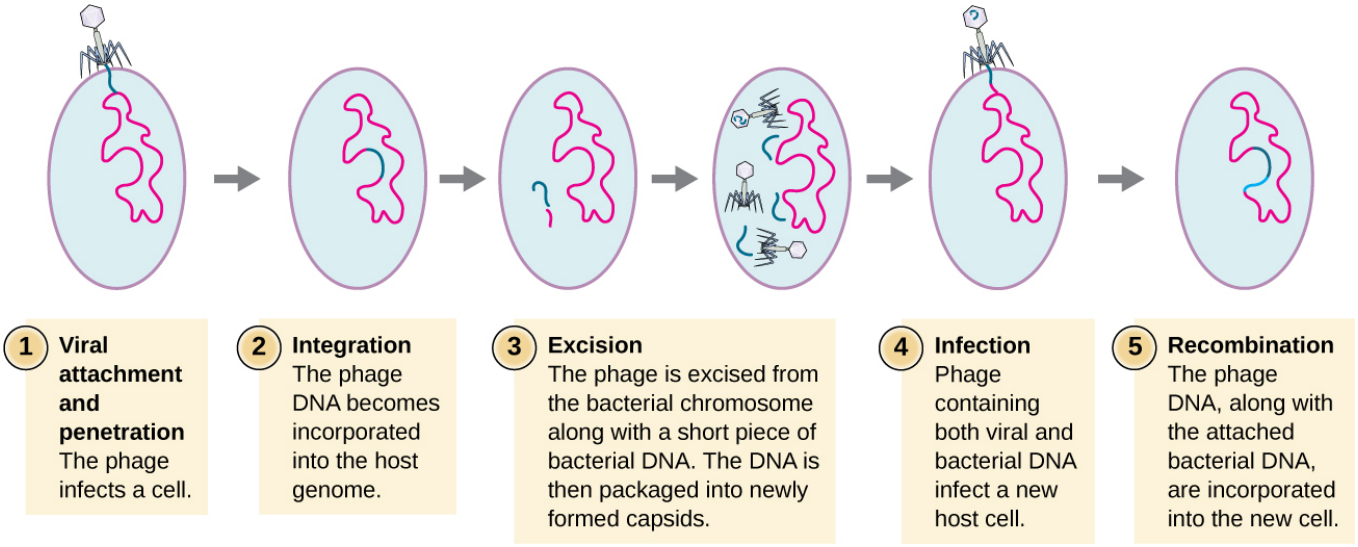


Figure **11.2.3**: This flowchart illustrates the mechanism of specialized transduction. An integrated phage excises, bringing with it a piece of the DNA adjacent to its insertion point. On reinfection of a new bacterium, the phage DNA integrates along with the genetic material acquired from the previous host.

? Exercise 11.2.2

Which phage life cycle is associated with which forms of transduction?

Life Cycle of Viruses with Animal Hosts

Lytic animal viruses follow similar infection stages to bacteriophages: attachment, penetration, biosynthesis, maturation, and release (see Figure **11.2.4**). However, the mechanisms of penetration, nucleic-acid biosynthesis, and release differ between bacterial and animal viruses. After binding to host receptors, animal viruses enter through endocytosis(engulfment by the host cell) or through membrane fusion (viral envelope with the host cell membrane). Many viruses are host specific, meaning they only infect a certain type of host; and most viruses only infect certain types of cells within tissues. This specificity is called a tissue tropism. Examples of this are demonstrated by the poliovirus, which exhibits tropism for the tissues of the brain and spinal cord, or the influenza virus, which has a primary tropism for the respiratory tract.

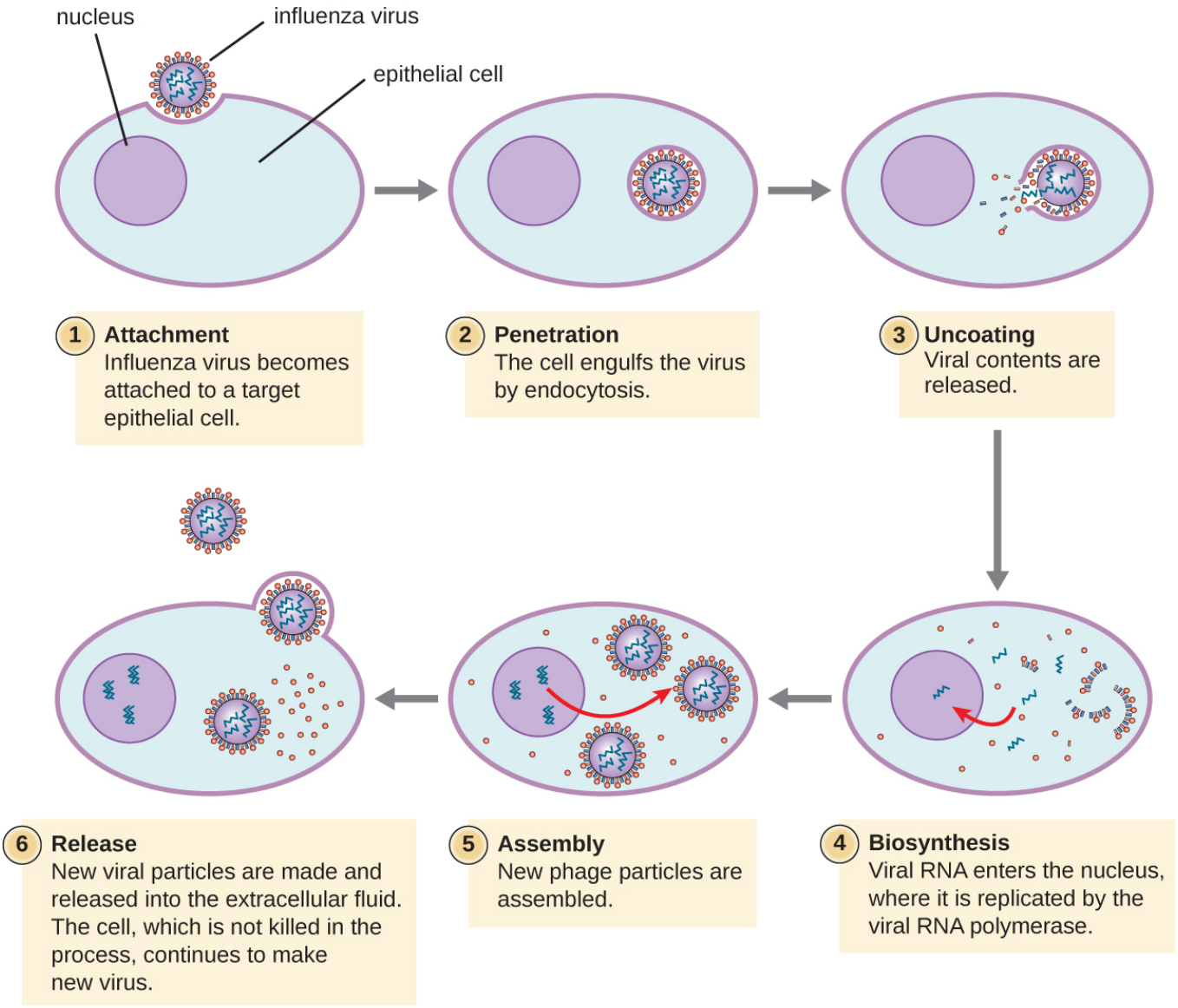


Figure 11.2.4: In influenza virus infection, viral glycoproteins attach the virus to a host epithelial cell. As a result, the virus is engulfed. Viral RNA and viral proteins are made and assembled into new virions that are released by budding.

Animal viruses do not always express their genes using the normal flow of genetic information—from DNA to RNA to protein. Some viruses have a dsDNA genome like cellular organisms and can follow the normal flow. However, others may have ssDNA, dsRNA, or ssRNA genomes. The nature of the genome determines how the genome is replicated and expressed as viral proteins. If a genome is ssDNA, host enzymes will be used to synthesize a second strand that is complementary to the genome strand, thus producing dsDNA. The dsDNA can now be replicated, transcribed, and translated similar to host DNA.

If the viral genome is RNA, a different mechanism must be used. There are three types of RNA genome: dsRNA, positive (+) single-strand (+ssRNA) or negative (−) single-strand RNA (−ssRNA). If a virus has a +ssRNA genome, it can be translated directly to make viral proteins. Viral genomic +ssRNA acts like cellular mRNA. However, if a virus contains a −ssRNA genome, the host ribosomes cannot translate it until the −ssRNA is replicated into +ssRNA by viral RNA-dependent RNA polymerase (RdRP) (see Figure 11.2.5). The RdRP is brought in by the virus and can be used to make +ssRNA from the original −ssRNA genome. The RdRP is also an important enzyme for the replication of dsRNA viruses, because it uses the negative strand of the double-stranded genome as a template to create +ssRNA. The newly synthesized +ssRNA copies can then be translated by cellular ribosomes.

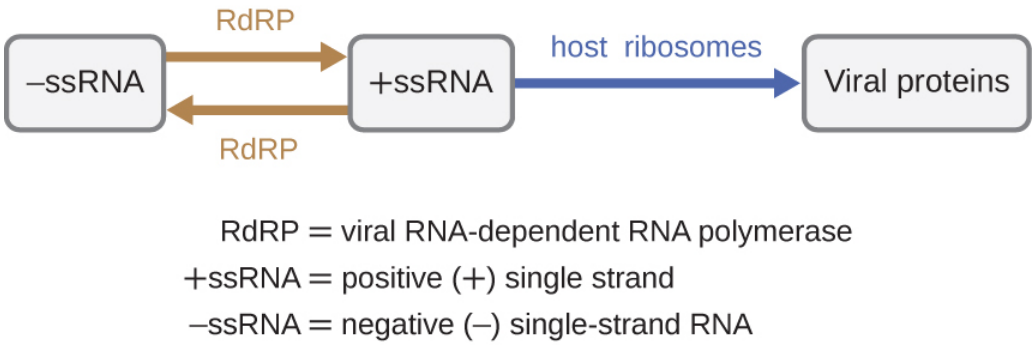


Figure **11.2.5**: RNA viruses can contain +ssRNA that can be directly read by the ribosomes to synthesize viral proteins. Viruses containing −ssRNA must first use the −ssRNA as a template for the synthesis of +ssRNA before viral proteins can be synthesized.

An alternative mechanism for viral nucleic acid synthesis is observed in the retrovirus**es**, which are +ssRNA viruses (see Figure **11.2.6**). Single-stranded RNA viruses such as HIV carry a special enzyme called reverse transcriptase within the capsid that synthesizes a complementary ssDNA (cDNA) copy using the +ssRNA genome as a template. The ssDNA is then made into dsDNA, which can integrate into the host chromosome and become a permanent part of the host. The integrated viral genome is called a provirus. The virus now can remain in the host for a long time to establish a chronic infection. The provirus stage is similar to the prophage stage in a bacterial infection during the lysogenic cycle. However, unlike prophage, the provirus does not undergo excision after splicing into the genome.

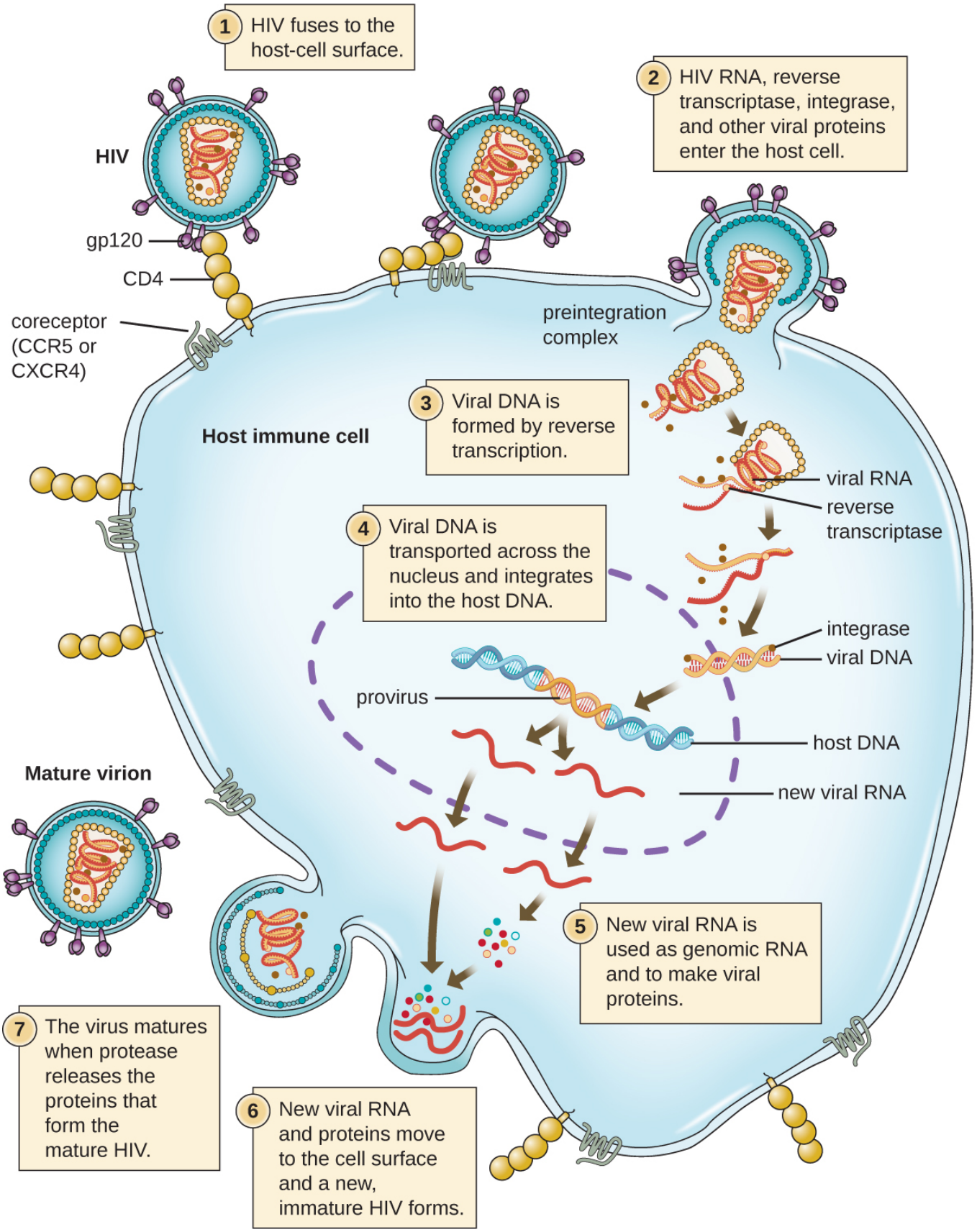


Figure 11.2.6: HIV, an enveloped, icosahedral retrovirus, attaches to a cell surface receptor of an immune cell and fuses with the cell membrane. Viral contents are released into the cell, where viral enzymes convert the single-stranded RNA genome into DNA and incorporate it into the host genome. (credit: modification of work by NIAID, NIH)

? Exercise 11.2.3

Is RNA-dependent RNA polymerase made from a viral gene or a host gene?

Persistent Infections

Persistent infection occurs when a virus is not completely cleared from the system of the host but stays in certain tissues or organs of the infected person. The virus may remain silent or undergo productive infection without seriously harming or killing the host. Mechanisms of persistent infection may involve the regulation of the viral or host gene expressions or the alteration of the host immune response. The two primary categories of persistent infections are latent infection and chronic infection. Examples of viruses that cause latent infections include herpes simplex virus (oral and genital herpes), varicella-zoster virus (chickenpox and shingles), and Epstein-Barr virus (mononucleosis). Hepatitis C virus and HIV are two examples of viruses that cause long-term chronic infections.

Latent Infection

Not all animal viruses undergo replication by the lytic cycle. There are viruses that are capable of remaining hidden or dormant inside the cell in a process called latency. These types of viruses are known as latent virus**es** and may cause latent infections. Viruses capable of latency may initially cause an acute infection before becoming dormant.

For example, the varicella-zoster virus infects many cells throughout the body and causes chickenpox, characterized by a rash of blisters covering the skin. About 10 to 12 days postinfection, the disease resolves and the virus goes dormant, living within nerve-cell ganglia for years. During this time, the virus does not kill the nerve cells or continue replicating. It is not clear why the virus stops replicating within the nerve cells and expresses few viral proteins but, in some cases, typically after many years of dormancy, the virus is reactivated and causes a new disease called shingles (Figure **11.2.7**). Whereas chickenpox affects many areas throughout the body, shingles is a nerve cell-specific disease emerging from the ganglia in which the virus was dormant.

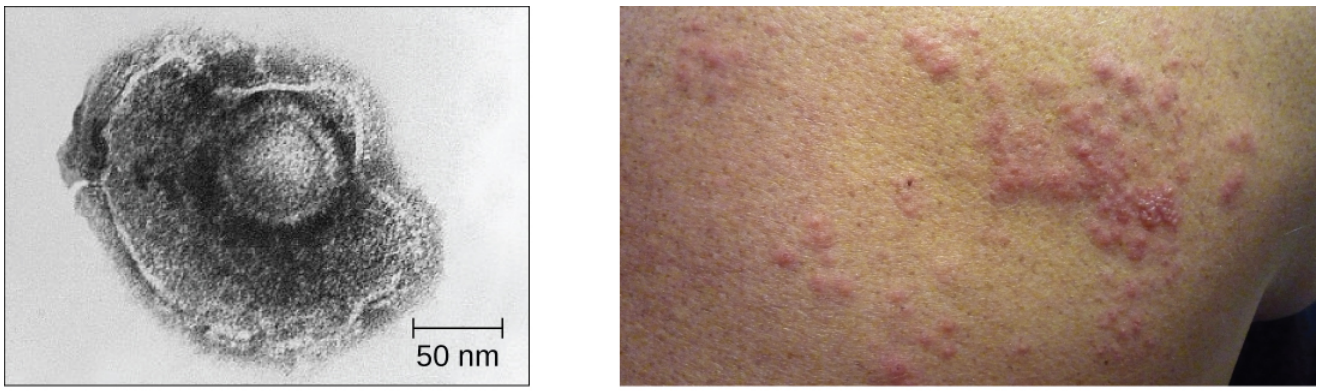


Figure **11.2.7**: (a) Varicella-zoster, the virus that causes chickenpox, has an enveloped icosahedral capsid visible in this transmission electron micrograph. Its double-stranded DNA genome becomes incorporated in the host DNA. (b) After a period of latency, the virus can reactivate in the form of shingles, usually manifesting as a painful, localized rash on one side of the body. (credit a: modification of work by Erskine Palmer and B.G. Partin—scale-bar data from Matt Russell; credit b: modification of work by Rosmarie Voegtli)

Latent viruses may remain dormant by existing as circular viral genome molecules outside of the host chromosome. Others become proviruses by integrating into the host genome. During dormancy, viruses do not cause any symptoms of disease and may be difficult to detect. A patient may be unaware that he or she is carrying the virus unless a viral diagnostic test has been performed.

Chronic Infection

A chronic infection is a disease with symptoms that are recurrent or persistent over a long time. Some viral infections can be chronic if the body is unable to eliminate the virus. HIV is an example of a virus that produces a chronic infection, often after a long period of latency. Once a person becomes infected with HIV, the virus can be detected in tissues continuously thereafter, but untreated patients often experience no symptoms for years. However, the virus maintains chronic persistence through several

mechanisms that interfere with immune function, including preventing expression of viral antigens on the surface of infected cells, altering immune cells themselves, restricting expression of viral genes, and rapidly changing viral antigens through mutation. Eventually, the damage to the immune system results in progression of the disease leading to acquired immunodeficiency syndrome (AIDS). The various mechanisms that HIV uses to avoid being cleared by the immune system are also used by other chronically infecting viruses, including the hepatitis C virus.

? Exercise 11.2.4

In what two ways can a virus manage to maintain a persistent infection?

Viral Growth Curve

Unlike the growth curve for a bacterial population, the growth curve for a virus population over its life cycle does not follow a sigmoidal curve. During the initial stage, an inoculum of virus causes infection. In the eclipse phase, viruses bind and penetrate the cells with no virions detected in the medium. The chief difference that next appears in the viral growth curve compared to a bacterial growth curve occurs when virions are released from the lysed host cell at the same time. Such an occurrence is called a burst, and the number of virions per bacterium released is described as the burst size. In a one-step multiplication curve for bacteriophage, the host cells lyse, releasing many viral particles to the medium, which leads to a very steep rise in viral titer (the number of virions per unit volume). If no viable host cells remain, the viral particles begin to degrade during the decline of the culture (see Figure **11.2.8**).

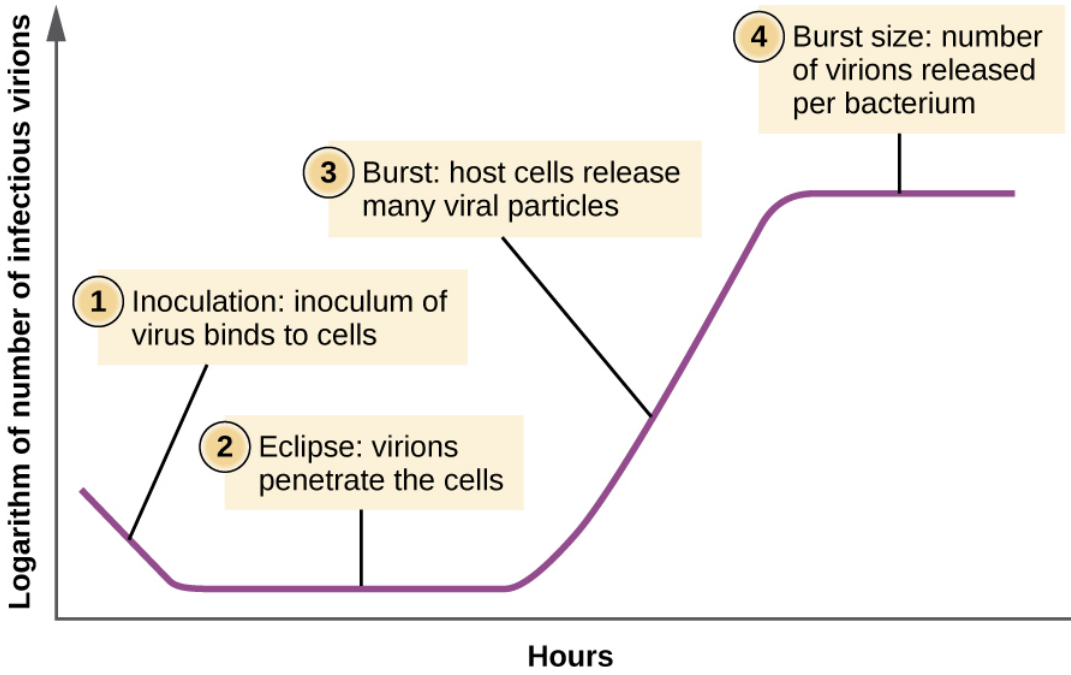


Figure **11.2.8**: The one-step multiplication curve for a bacteriophage population follows three steps: 1) inoculation, during which the virions attach to host cells; 2) eclipse, during which entry of the viral genome occurs; and 3) burst, when sufficient numbers of new virions are produced and emerge from the host cell. The burst size is the maximum number of virions produced per bacterium.

? Exercise 11.2.6

What aspect of the life cycle of a virus leads to the sudden increase in the growth curve?

Unregistered Treatments

Ebola is incurable and deadly. The outbreak in West Africa in 2014 was unprecedented, dwarfing other human Ebola epidemics in the level of mortality. Of 24,666 suspected or confirmed cases reported, 10,179 people died.

No approved treatments or vaccines for Ebola are available. While some drugs have shown potential in laboratory studies and animal models, they have not been tested in humans for safety and effectiveness. Not only are these drugs untested or unregistered but they are also in short supply.

Given the great suffering and high mortality rates, it is fair to ask whether unregistered and untested medications are better than none at all. Should such drugs be dispensed and, if so, who should receive them, in light of their extremely limited supplies? Is it ethical to treat untested drugs on patients with Ebola? On the other hand, is it ethical to withhold potentially life-saving drugs from dying patients? Or should the drugs perhaps be reserved for health-care providers working to contain the disease?

In August 2014, two infected US aid workers and a Spanish priest were treated with ZMapp, an unregistered drug that had been tested in monkeys but not in humans. The two American aid workers recovered, but the priest died. Later that month, the WHO released a report on the ethics of treating patients with the drug. Since Ebola is often fatal, the panel reasoned that it is ethical to give the unregistered drugs and unethical to withhold them for safety concerns. This situation is an example of "compassionate use" outside the well-established system of regulation and governance of therapies.

Ebola in the US

On September 24, 2014, Thomas Eric Duncan arrived at the Texas Health Presbyterian Hospital in Dallas complaining of a fever, headache, vomiting, and diarrhea—symptoms commonly observed in patients with the cold or the flu. After examination, an emergency department doctor diagnosed him with sinusitis, prescribed some antibiotics, and sent him home. Two days later, Duncan returned to the hospital by ambulance. His condition had deteriorated and additional blood tests confirmed that he has been infected with the Ebola virus.

Further investigations revealed that Duncan had just returned from Liberia, one of the countries in the midst of a severe Ebola epidemic. On September 15, nine days before he showed up at the hospital in Dallas, Duncan had helped transport an Ebola-stricken neighbor to a hospital in Liberia. The hospital continued to treat Duncan, but he died several days after being admitted.

The timeline of the Duncan case is indicative of the life cycle of the Ebola virus. The incubation time for Ebola ranges from 2 days to 21 days. Nine days passed between Duncan's exposure to the virus infection and the appearance of his symptoms. This corresponds, in part, to the eclipse period in the growth of the virus population. During the eclipse phase, Duncan would have been unable to transmit the disease to others. However, once an infected individual begins exhibiting symptoms, the disease becomes very contagious. Ebola virus is transmitted through direct contact with droplets of bodily fluids such as saliva, blood, and vomit. Duncan could conceivably have transmitted the disease to others at any time after he began having symptoms, presumably some time before his arrival at the hospital in Dallas. Once a hospital realizes a patient like Duncan is infected with Ebola virus, the patient is immediately quarantined, and public health officials initiate a back trace to identify everyone with whom a patient like Duncan might have interacted during the period in which he was showing symptoms.

Public health officials were able to track down 10 high-risk individuals (family members of Duncan) and 50 low-risk individuals to monitor them for signs of infection. None contracted the disease. However, one of the nurses charged with Duncan's care did become infected. This, along with Duncan's initial misdiagnosis, made it clear that US hospitals needed to provide additional training to medical personnel to prevent a possible Ebola outbreak in the US.

Exercise 11.2.7

1. What types of training can prepare health professionals to contain emerging epidemics like the Ebola outbreak of 2014?
2. What is the difference between a contagious pathogen and an infectious pathogen?



Figure **11.2.9**: Researchers working with Ebola virus use layers of defenses against accidental infection, including protective clothing, breathing systems, and negative air-pressure cabinets for bench work. (credit: modification of work by Randal J. Schoepp)

Link to Learning

For additional information about Ebola, please visit the CDC website.

Summary

- Many viruses target specific hosts or tissues. Some may have more than one host.
- Many viruses follow several stages to infect host cells. These stages include **attachment, penetration, uncoating, biosynthesis, maturation,** and **release**.
- Bacteriophages have a **lytic** or **lysogenic cycle**. The lytic cycle leads to the death of the host, whereas the lysogenic cycle leads to integration of phage into the host genome.
- Bacteriophages inject DNA into the host cell, whereas animal viruses enter by endocytosis or membrane fusion.
- Animal viruses can undergo **latency**, similar to lysogeny for a bacteriophage.
- The majority of plant viruses are positive-strand ssRNA and can undergo latency, chronic, or lytic infection, as observed for animal viruses.
- The growth curve of bacteriophage populations is a **one-step multiplication curve** and not a sigmoidal curve, as compared to the bacterial growth curve.
- Bacteriophages transfer genetic information between hosts using either **generalized** or **specialized transduction**.

Footnotes

1. 1 World Health Organization. “WHO Ebola Data and Statistics.” March 18, 2005. http://apps.who.int/gho/data/view.eb...150318?lang=en

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11.2.1: Medical Importance of Viruses

Learning Objectives

- Describe the fundamental characteristics of viruses

Viruses are extremely diverse and have evolved to infect nearly all life forms. Amid this diversity, viruses with similar genome organizations exhibit major conserved themes in their replication strategies. Once inside a cell, all viruses must uncoat, replicate, and transcribe their genomes, and then repackage their genomes into viral progeny that are released from cells. RNA viruses in particular must coordinate the switch between plus and minus strand synthesis and between replication and transcription while protecting their genomes from cellular nucleases. Because of the conserved nature of a virus ‘s intracellular life cycle, fundamental advances in our understanding of replication have come from viruses that infect both animal and non-animal hosts.

The devastating effects of viral diseases such as AIDs, smallpox, polio, influenza, diarrhea, and hepatitis are well known, and studies of viral pathogens are easily justified from a world health perspective. Sobering examples of emerging viral diseases have occurred. Among these are the sudden emergence of the coronavirus that causes severe acute respiratory syndrome (SARS), the continued transmission of an avian influenza virus to humans (“bird flu”), and the isolation of poliovirus vaccine -wild type recombinants that have hampered poliovirus eradication efforts. In addition, the threat of bioterrorism became a reality on U.S. soil, creating an obligation for scientists to respond with aggressive countermeasures. Vaccination remains the preferred strategy for controlling viral diseases because the intimate association of viruses with the host cellular machinery complicates the development of safe drugs. However, certain viruses have proven difficult targets for vaccines, and antiviral drugs provide the only option for controlling disease.

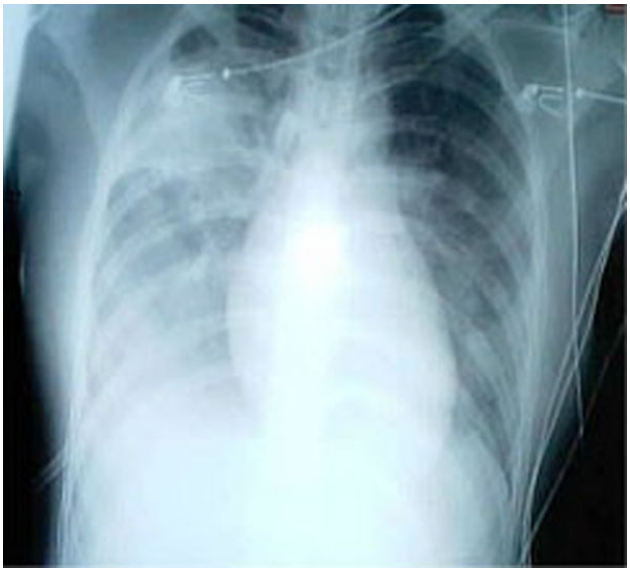


Figure: **Medical importance of SARS**: A chest x-ray showing increased opacity in both lungs, indicative of pneumonia, in a patient with SARS.

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- File:VirusBaltimoreClassification.svg - Wikipedia, the free encyclopedia. **Provided by**: Wikipedia. **Located at**: **en.Wikipedia.org/w/index.php?...ion.svg&page=1**. **License**: ***CC BY-SA: Attribution-ShareAlike***
- OpenStax College, Organizing Life on Earth. November 9, 2013. **Provided by**: OpenStax CNX. **Located at**: **http://cnx.org/content/m44588/latest/#fig-ch20_01_01**. **License**: ***CC BY: Attribution***
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Key Points

- Viruses multiply by taking control of the host cell's genetic material and regulating the synthesis and assembly of new viruses.
- Viruses are able to infect a host cell and cause acute diseases or alter its genetic material to cause chronic diseases such as cancer.
- Most viral infections can resolve in weeks but others are the cause of more serious, debilitating and sometimes fatal diseases.

Key Terms

- **vaccination**: inoculation with a vaccine in order to protect a particular disease or strain of disease.
- **eradication**: the act of plucking up by the roots; a rooting out; extirpation; utter destruction.

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11.2.2: The Baltimore Virus Classification

Learning Objectives

- List the characteristics of viruses that are useful for Baltimore classification

Virus classification is the process of naming viruses and placing them into a taxonomic system. Much like the classification systems used for cellular organisms, virus classification is the subject of ongoing debate and proposals. This is mainly due to the pseudo-living nature of viruses, which is to say they are non-living particles with some chemical characteristics similar to those of life. As such, they do not fit neatly into the established biological classification system in place for cellular organisms.

Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication. Named after David Baltimore, a Nobel Prize-winning biologist, these groups are designated by Roman numerals and discriminate viruses depending on their mode of replication and genome type. Other classifications are determined by the disease caused by the virus or its morphology, neither of which are satisfactory due to different viruses either causing the same disease or looking very similar. In addition, viral structures are often difficult to determine under the microscope.

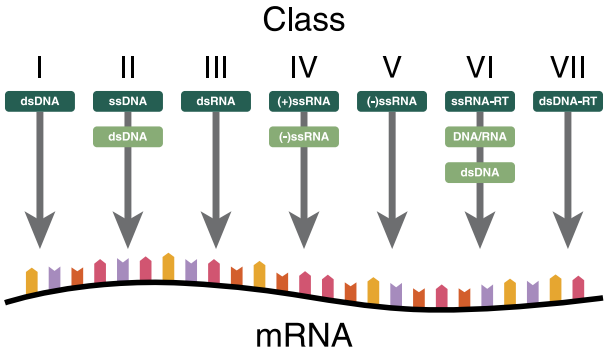


Figure: **Viral classes**: Baltimore classification of viruses

Classifying viruses according to their genome means that those in a given category will all behave in a similar fashion, offering some indication of how to proceed with further research. Viruses can be placed in one of the seven following groups:

1. I: dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)
2. II: ssDNA viruses (+)sense DNA (e.g. Parvoviruses)
3. III: dsRNA viruses (e.g. Reoviruses)
4. IV: (+)ssRNA viruses (+)sense RNA (e.g. Picornaviruses, Togaviruses)
5. V: (−)ssRNA viruses (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
6. VI: ssRNA-RT viruses (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
7. VII: dsDNA-RT viruses (e.g. Hepadnaviruses)

Key Points

- Viral genome ‘s nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication determine its class.
- Other classifications are determined by the disease caused by the virus or its morphology.
- Viruses can be placed in one of the seven groups.

Key Terms

- **genome**: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of basepairs.

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11.2.3: Evolution of Viruses

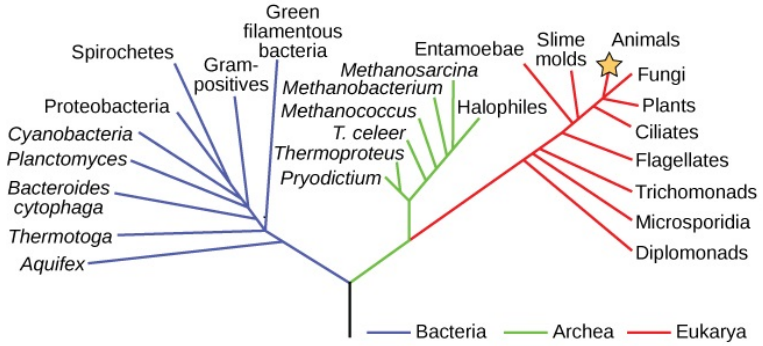
Learning Objectives

- Describe the difficulties in determining the origin of viruses

Evolution of Viruses

Although biologists have accumulated a significant amount of knowledge about how present-day viruses evolve, much less is known about how viruses originated in the first place. When exploring the evolutionary history of most organisms, scientists can look at fossil records and similar historic evidence. However, viruses do not fossilize, so researchers must conjecture by investigating how today's viruses evolve and by using biochemical and genetic information to create speculative virus histories.

While most findings agree that viruses don't have a single common ancestor, scholars have yet to find one hypothesis about virus origins that is fully accepted in the field. One possible hypothesis, called devolution or the regressive hypothesis, proposes to explain the origin of viruses by suggesting that viruses evolved from free-living cells. However, many components of how this process might have occurred are a mystery. A second hypothesis (called escapist or the progressive hypothesis) accounts for viruses having either an RNA or a DNA genome and suggests that viruses originated from RNA and DNA molecules that escaped from a host cell. A third hypothesis posits a system of self-replication similar to that of other self-replicating molecules, probably evolving alongside the cells they rely on as hosts; studies of some plant pathogens support this hypothesis.



(a) Rooted phylogenetic tree

Figure: **Common ancestor tree of life**: This phylogenetic tree of the three domains of life (Bacteria, Archaea, and Eukarya) attempts to identify when various species diverged from a common ancestor. Finding a common ancestor for viruses has proven to be far more difficult, especially since they do not fossilize.

As technology advances, scientists may develop and refine further hypotheses to explain the origin of viruses. The emerging field called virus molecular systematics attempts to do just that through comparisons of sequenced genetic material. These researchers hope to one day better understand the origin of viruses, a discovery that could lead to advances in the treatments for the ailments they produce.

Key Points

- Scientists agree that viruses don't have a single common ancestor, but have yet to agree on a single hypothesis about virus origins.
- The devolution or the regressive hypothesis suggests that viruses evolved from free-living cells.
- The escapist or the progressive hypothesis suggests that viruses originated from RNA and DNA molecules that escaped from a host cell.
- The self-replicating hypothesis posits a system of self-replication that most probably involves evolution alongside the host cells.

Key Terms

- **self-replicating**: able to generate a copy of itself
- **devolution**: degeneration (as opposed to evolution)

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11.3: Prevention and Treatment of Viral Infections

Skills to Develop

- Identify major viral illnesses that affect humans
- Compare vaccinations and anti-viral drugs as medical approaches to viruses

Viruses cause a variety of diseases in animals, including humans, ranging from the common cold to potentially fatal illnesses like meningitis (Figure **11.3.1**). These diseases can be treated by antiviral drugs or by vaccines, but some viruses, such as HIV, are capable of both avoiding the immune response and mutating to become resistant to antiviral drugs.

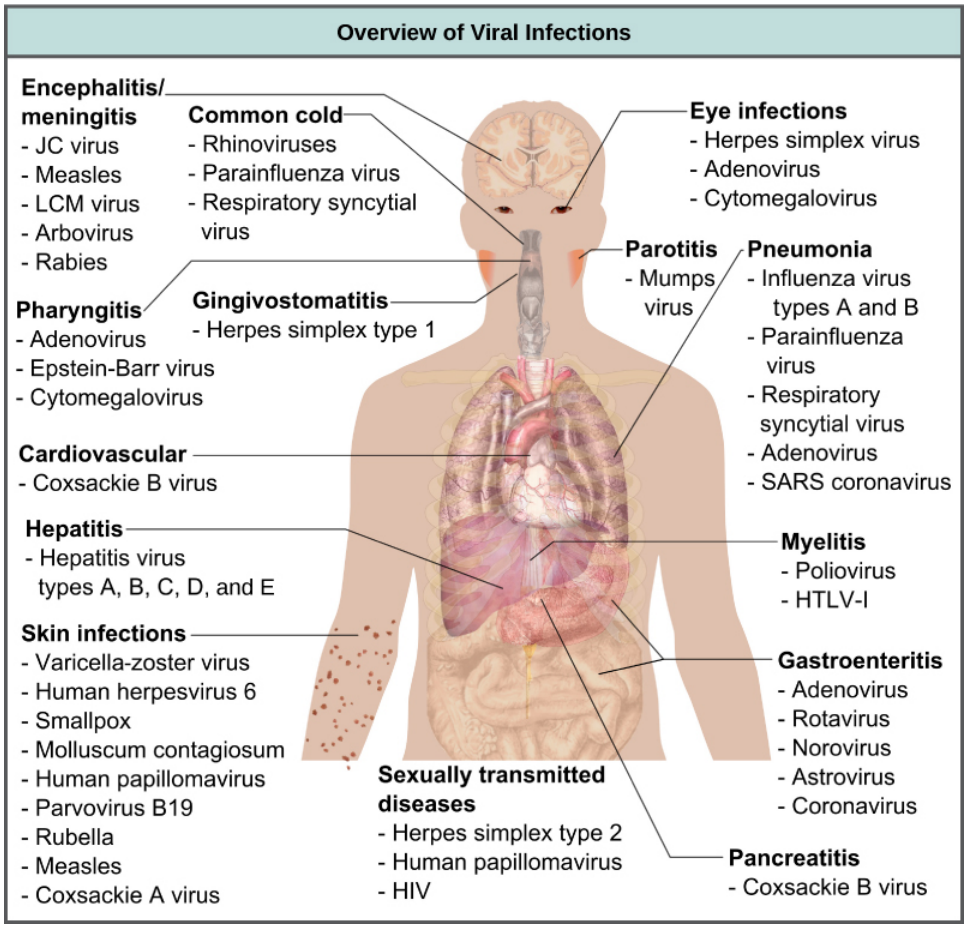


Figure **11.3.1**: Viruses can cause dozens of ailments in humans, ranging from mild illnesses to serious diseases. (credit: modification of work by Mikael Häggström)

Vaccines for Prevention

While we do have limited numbers of effective antiviral drugs, such as those used to treat HIV and influenza, the primary method of controlling viral disease is by vaccination, which is intended to prevent outbreaks by building immunity to a virus or virus family (Figure**11.3.1**). Vaccines may be prepared using live viruses, killed viruses, or molecular subunits of the virus. The killed viral vaccines and subunit viruses are both incapable of causing disease.

Live viral vaccines are designed in the laboratory to cause few symptoms in recipients while giving them protective immunity against future infections. Polio was one disease that represented a milestone in the use of vaccines. Mass immunization campaigns in the 1950s (killed vaccine) and 1960s (live vaccine) significantly reduced the incidence of the disease, which caused muscle paralysis in children and generated a great amount of fear in the general population when regional epidemics occurred. The success of the polio vaccine paved the way for the routine dispensation of childhood vaccines against measles, mumps, rubella, chickenpox, and other diseases.

The danger of using live vaccines, which are usually more effective than killed vaccines, is the low but significant danger that these viruses will revert to their disease-causing form by back mutations. Live vaccines are usually made by attenuating (weakening) the "wild-type" (disease-causing) virus by growing it in the laboratory in tissues or at temperatures different from what the virus is accustomed to in the host. Adaptations to these new cells or temperatures induce mutations in the genomes of the virus, allowing it to grow better in the laboratory while inhibiting its ability to cause disease when reintroduced into conditions found in the host. These attenuated viruses thus still cause infection, but they do not grow very well, allowing the immune response to develop in time to prevent major disease. Back mutations occur when the vaccine undergoes mutations in the host such that it readapts to the host and can again cause disease, which can then be spread to other humans in an epidemic. This type of scenario happened as recently as 2007 in Nigeria where mutations in a polio vaccine led to an epidemic of polio in that country.

Some vaccines are in continuous development because certain viruses, such as influenza and HIV, have a high mutation rate compared to other viruses and normal host cells. With influenza, mutations in the surface molecules of the virus help the organism evade the protective immunity that may have been obtained in a previous influenza season, making it necessary for individuals to get vaccinated every year. Other viruses, such as those that cause the childhood diseases measles, mumps, and rubella, mutate so infrequently that the same vaccine is used year after year.



Figure **11.3.2**: Vaccinations are designed to boost immunity to a virus to prevent infection. (credit: USACE Europe District)

Link to Learning



Watch this NOVA video to learn how microbiologists are attempting to replicate the deadly 1918 Spanish influenza virus so they can understand more about virology.

Vaccines and Anti-viral Drugs for Treatment

In some cases, vaccines can be used to treat an active viral infection. The concept behind this is that by giving the vaccine, immunity is boosted without adding more disease-causing virus. In the case of rabies, a fatal neurological disease transmitted via the saliva of rabies virus-infected animals, the progression of the disease from the time of the animal bite to the time it enters the central nervous system may be 2 weeks or longer. This is enough time to vaccinate an individual who suspects that they have been bitten by a rabid animal, and their boosted immune response is sufficient to prevent the virus from entering nervous tissue. Thus, the potentially fatal neurological consequences of the disease are averted, and the individual only has to recover from the infected bite. This approach is also being used for the treatment of Ebola, one of the fastest and most deadly viruses on earth. Transmitted by bats and great apes, this disease can cause death in 70–90 percent of infected humans within 2 weeks. Using newly developed vaccines that boost the immune response in this way, there is hope that affected individuals will be better able to control the virus, potentially saving a greater percentage of infected persons from a rapid and very painful death.

Another way of treating viral infections is the use of antiviral drugs. These drugs often have limited success in curing viral disease, but in many cases, they have been used to control and reduce symptoms for a wide variety of viral diseases. For most viruses, these drugs can inhibit the virus by blocking the actions of one or more of its proteins. It is important that the targeted proteins be encoded by viral genes and that these molecules are not present in a healthy host cell. In this way, viral growth is inhibited without damaging the host. There are large numbers of antiviral drugs available to treat infections, some specific for a particular virus and others that can affect multiple viruses.

Antivirals have been developed to treat genital herpes (herpes simplex II) and influenza. For genital herpes, drugs such as acyclovir can reduce the number and duration of episodes of active viral disease, during which patients develop viral lesions in their skin cells. As the virus remains latent in nervous tissue of the body for life, this drug is not curative but can make the symptoms of the disease more manageable. For influenza, drugs like Tamiflu (oseltamivir) (Figure **11.3.3**) can reduce the duration of “flu” symptoms by 1 or 2 days, but the drug does not prevent symptoms entirely. Tamiflu works by inhibiting an enzyme (viral neuraminidase) that allows new virions to leave their infected cells. Thus, Tamiflu inhibits the spread of virus from infected to uninfected cells. Other antiviral drugs, such as Ribavirin, have been used to treat a variety of viral infections, although its mechanism of action against certain viruses remains unclear.

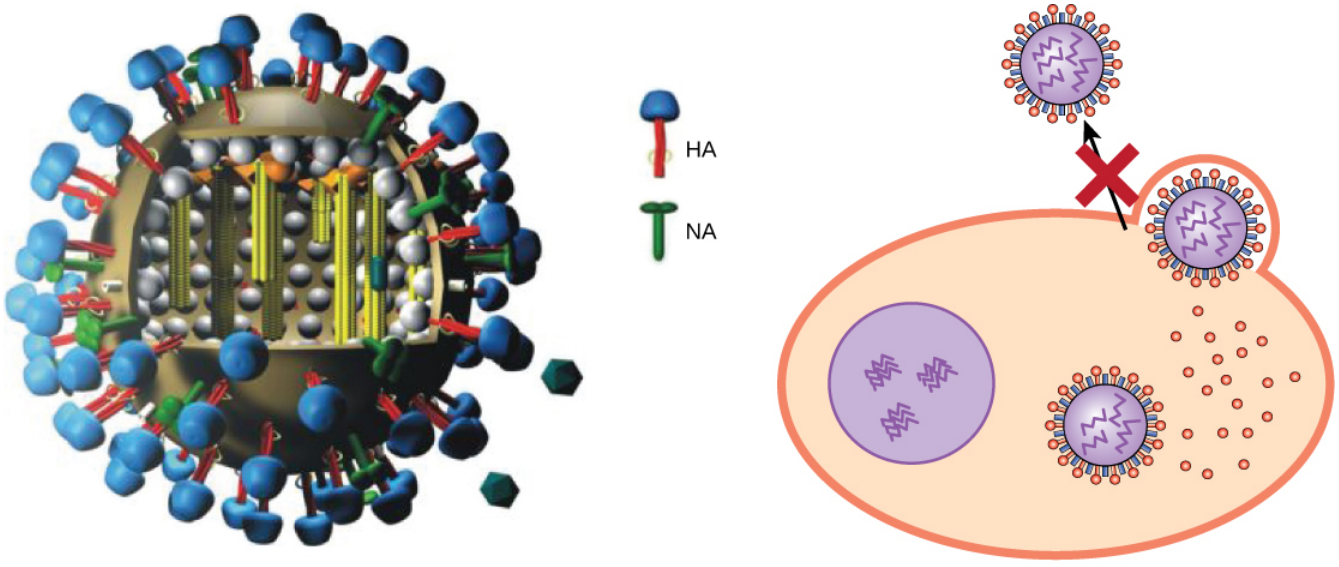


Figure **11.3.3**: (a) Tamiflu inhibits a viral enzyme called neuraminidase (NA) found in the influenza viral envelope. (b) Neuraminidase cleaves the connection between viral hemagglutinin (HA), also found in the viral envelope, and glycoproteins on the host cell surface. Inhibition of neuraminidase prevents the virus from detaching from the host cell, thereby blocking further infection. (credit a: modification of work by M. Eickmann)

By far, the most successful use of antivirals has been in the treatment of the retrovirus HIV, which causes a disease that, if untreated, is usually fatal within 10–12 years after infection. Anti-HIV drugs have been able to control viral replication to the point that individuals receiving these drugs survive for a significantly longer time than the untreated.

Anti-HIV drugs inhibit viral replication at many different phases of the HIV replicative cycle (Figure **11.3.4**). Drugs have been developed that inhibit the fusion of the HIV viral envelope with the plasma membrane of the host cell (fusion inhibitors), the conversion of its RNA genome into double-stranded DNA (reverse transcriptase inhibitors), the integration of the viral DNA into the host genome (integrase inhibitors), and the processing of viral proteins (protease inhibitors).

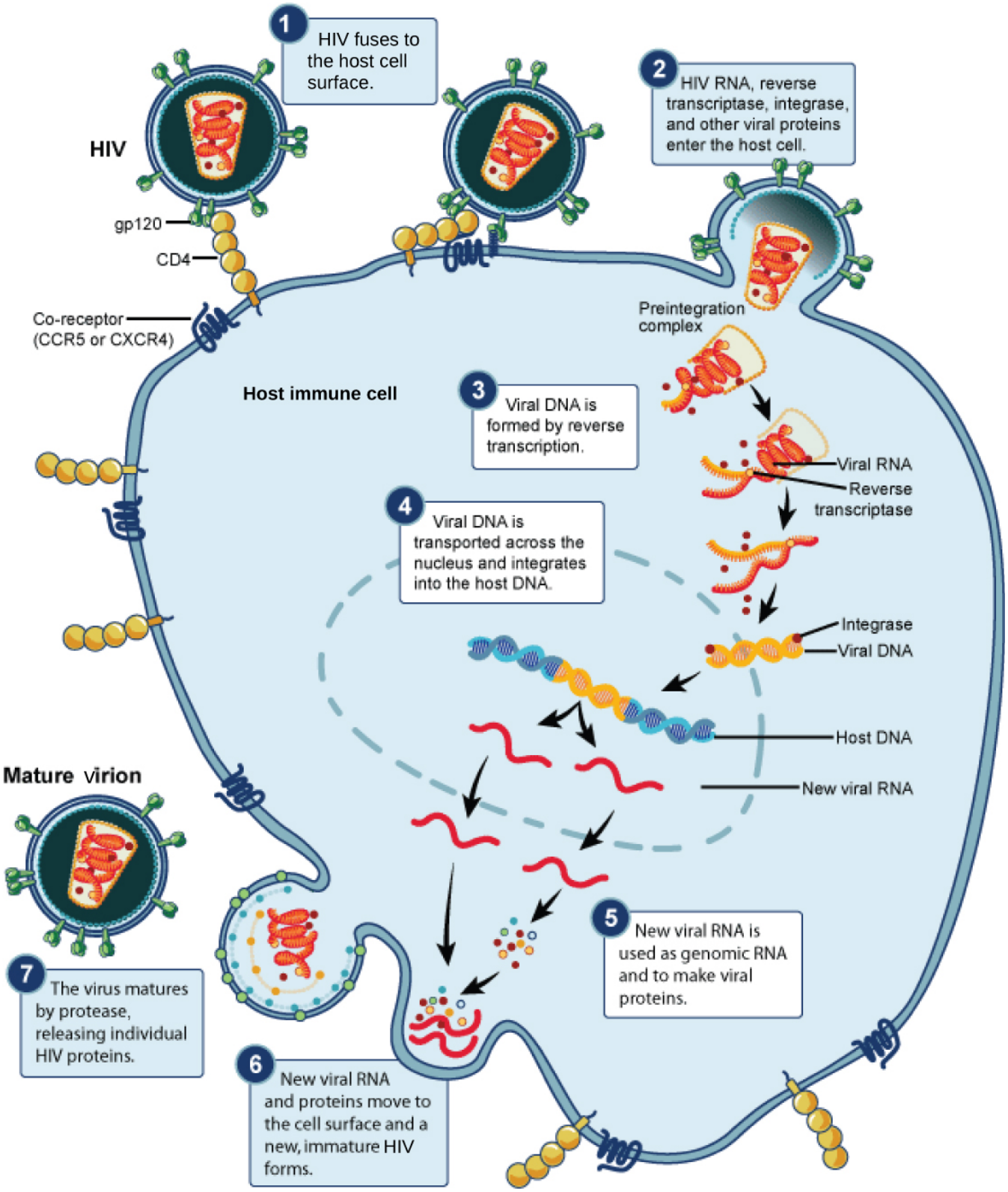


Figure **11.3.4**: HIV, an enveloped, icosahedral virus, attaches to the CD4 receptor of an immune cell and fuses with the cell membrane. Viral contents are released into the cell, where viral enzymes convert the single-stranded RNA genome into DNA and incorporate it into the host genome. (credit: NIAID, NIH)

When any of these drugs are used individually, the high mutation rate of the virus allows it to easily and rapidly develop resistance to the drug, limiting the drug's effectiveness. The breakthrough in the treatment of HIV was the development of HAART, highly active anti-retroviral therapy, which involves a mixture of different drugs, sometimes called a drug "cocktail." By attacking the

virus at different stages of its replicative cycle, it is much more difficult for the virus to develop resistance to multiple drugs at the same time. Still, even with the use of combination HAART therapy, there is concern that, over time, the virus will develop resistance to this therapy. Thus, new anti-HIV drugs are constantly being developed with the hope of continuing the battle against this highly fatal virus.

Everyday Connection: Applied Virology

The study of viruses has led to the development of a variety of new ways to treat non-viral diseases. Viruses have been used in gene therapy. Gene therapy is used to treat genetic diseases such as severe combined immunodeficiency (SCID), a heritable, recessive disease in which children are born with severely compromised immune systems. One common type of SCID is due to the lack of an enzyme, adenosine deaminase (ADA), which breaks down purine bases. To treat this disease by gene therapy, bone marrow cells are taken from a SCID patient and the ADA gene is inserted. This is where viruses come in, and their use relies on their ability to penetrate living cells and bring genes in with them. Viruses such as adenovirus, an upper respiratory human virus, are modified by the addition of the ADA gene, and the virus then transports this gene into the cell. The modified cells, now capable of making ADA, are then given back to the patients in the hope of curing them. Gene therapy using viruses as carrier of genes (viral vectors), although still experimental, holds promise for the treatment of many genetic diseases. Still, many technological problems need to be solved for this approach to be a viable method for treating genetic disease.

Another medical use for viruses relies on their specificity and ability to kill the cells they infect. Oncolytic viruses are engineered in the laboratory specifically to attack and kill cancer cells. A genetically modified adenovirus known as H101 has been used since 2005 in clinical trials in China to treat head and neck cancers. The results have been promising, with a greater short-term response rate to the combination of chemotherapy and viral therapy than to chemotherapy treatment alone. This ongoing research may herald the beginning of a new age of cancer therapy, where viruses are engineered to find and specifically kill cancer cells, regardless of where in the body they may have spread.

A third use of viruses in medicine relies on their specificity and involves using bacteriophages in the treatment of bacterial infections. Bacterial diseases have been treated with antibiotics since the 1940s. However, over time, many bacteria have developed resistance to antibiotics. A good example is methicillin-resistant *Staphylococcus aureus* (MRSA, pronounced “mersa”), an infection commonly acquired in hospitals. This bacterium is resistant to a variety of antibiotics, making it difficult to treat. The use of bacteriophages specific for such bacteria would bypass their resistance to antibiotics and specifically kill them. Although phage therapy is in use in the Republic of Georgia to treat antibiotic-resistant bacteria, its use to treat human diseases has not been approved in most countries. However, the safety of the treatment was confirmed in the United States when the U.S. Food and Drug Administration approved spraying meats with bacteriophages to destroy the food pathogen *Listeria*. As more and more antibiotic-resistant strains of bacteria evolve, the use of bacteriophages might be a potential solution to the problem, and the development of phage therapy is of much interest to researchers worldwide.

Summary

Viruses cause a variety of diseases in humans. Many of these diseases can be prevented by the use of viral vaccines, which stimulate protective immunity against the virus without causing major disease. Viral vaccines may also be used in active viral infections, boosting the ability of the immune system to control or destroy the virus. A series of antiviral drugs that target enzymes and other protein products of viral genes have been developed and used with mixed success. Combinations of anti-HIV drugs have been used to effectively control the virus, extending the lifespans of infected individuals. Viruses have many uses in medicines, such as in the treatment of genetic disorders, cancer, and bacterial infections.

Glossary

attenuation

weakening of a virus during vaccine development

back mutation

when a live virus vaccine reverts back to it disease-causing phenotype

gene therapy
treatment of genetic disease by adding genes, using viruses to carry the new genes inside the cell

oncolytic virus
virus engineered to specifically infect and kill cancer cells

phage therapy
treatment of bacterial diseases using bacteriophages specific to a particular bacterium

vaccine
weakened solution of virus components, viruses, or other agents that produce an immune response

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11.4: The Role of Viruses in Tumor Production

Describe how certain viruses may contribute to the development of tumors by altering proto-oncogenes or tumor-suppressor genes.
- Name 3 viruses that have been implicated in human cancers.

Some viruses can also play a role in converting normal host cells into tumor cells. These viruses are capable of viral transformation, that is, they transform normal cells into malignant cells. In fact, five viruses, hepatitis B virus (HBV), hepatitis C virus (HCV), human papilloma virus (HPV), Epstein-Barr virus (EBV), and human T-lymphotropic virus type I (HTLV-I) are thought to contribute to over 15% of the world's cancers. Up to 80% of these human viral-associated cancers are cervical cancer (associated with HPV) and liver cancer (associated with HBV and HCV).

The hepatitis B virus (HBV) is a DNA virus that may potentially cause chronic hepatitis in those infected. There is a strong link between chronic infection with HBV and hepatocellular carcinoma, which typically appears after 30-50 years of chronic liver damage and liver cell replacement. Chronic carriers of HBV have a 300 times greater risk of eventually developing liver cancer. Around 90% of individuals infected at birth and 10% of individuals infected as adults become chronic carriers of HBV. There are about one million chronic carriers of HBV in the US. Worldwide, HBV is responsible for 60% of all liver cancer cases.

The hepatitis C virus (HCV) is a RNA virus that may also cause chronic hepatitis in those infected. As with HBV, there is a strong link between chronic infection with HCV and liver cancer, typically appearing after 30-50 years of chronic liver damage and liver cell replacement. Around 85% of individuals infected with HCV become chronic carriers and there are approximately four million chronic carriers of HCV in the US. Worldwide, HCV is responsible for 22 % of all liver cancer cases.

The human papilloma viruses (HPV) are responsible for warts. While warts are generally considered as benign tumors, some sexually-transmitted strains of HPV (HPV-16 and 18 are definitely carcinogenic in humans; HPV-31 and 33 are probably carcinogenic), have been implicated in cervical and vulvar cancer, rectal cancer, and squamous cell carcinoma of the penis. In these tumor cells the viral DNA is usually found integrated in host cell chromosomes. In the US, HPVs are associated with 82% of the deaths due to cervical cancer each year, as well as a million precancerous lesions.

The Epstein-Barr virus (EBV), a herpes virus, normally causes benign proliferations such as infectious mononucleosis and hairy leukoplakia of the tongue. However, it can contribute to non-Hodgkin's lymphoma in AIDS patients and post-transplantation lymphoproliferative diseases, appears to be an essential factor for posterior nasopharyngeal cancer in some individuals, can be a co-factor for Burkitt's lymphoma, and contributes to smooth-muscle tumors in immunosuppressed children.

The retrovirus human T-lymphotropic virus type I (HTLV-I) can induce a rare adult T-lymphocyte leukemia-lymphoma.

The development of tumors is a multistep process depending on the accumulation of mutations altering a number of genes. The altered genes then function collectively to cause malignant growth.

Proliferation of normal cells is regulated by growth-promoting proto-oncogenes and counterbalanced by growth-restricting tumor suppressor genes. Mutations that increase the activities of proto-oncogenes to create oncogenes and/or decrease the activities of tumor suppressor genes can lead to growth of tumors. It is now known that many tumors require both activation of oncogenes from proto-oncogenes and inactivation of tumor suppressor genes for their development.

Viruses are thought to play a role in cancer development both indirectly and directly. Indirectly, the viruses may induce immunosuppression so that cancer cells are not removed by immune responses, as in the case of HIV/AIDS, or they may cause long term damage to tissues resulting in large scale cell regeneration which increases the chances of natural mutation in proto-oncogenes and tumor suppressor genes, as in the case of HBV and HCV. Directly, by

integrating into the host cell's chromosomes, some viruses may alter the normal function of the proto-oncogenes and tumor suppressor genes, as is seen with HPV and HBV.

However, most virus-associated cancers have long latency periods of several decades and only a small percentage of the people infected with the virus actually develop the cancer. This indicates other factors promoting changes in cellular genes are also involved. For example, in the case of cervical cancer and HPV, two variants of a tumor suppressor gene known as *p53* are known. One form of the *p53* gene produces a suppressor protein that is much more susceptible to degradation by an oncoprotein called E6 which is produced by carcinogenic strains of HPV.

? Name the three most common viruses associated with cancer in the US and state the cancers with which they are associated.

Summary

1. Viruses are responsible for about 15% of the world's cancers.
2. Up to 80% of these human viral-associated cancers are cervical cancer (associated with human papilloma virus or HPV) and liver cancer (associated with the hepatitis B virus or HBV and the hepatitis C virus or HCV).
3. The Epstein-Barr virus (EBV) and human T-lymphotropic virus type I (HTLV-I) also increase the risk of certain cancers.
4. The development of tumors is a multistep process depending on the accumulation of mutations altering a number of genes.
5. Most virus-associated cancers have long latency periods of several decades and only a small percentage of the people infected with the virus actually develop the cancer. This indicates other factors promoting changes in cellular genes are also involved.

Questions

Study the material in this section and then write out the answers to these questions. Do not just click on the answers and write them out. This will not test your understanding of this tutorial.

1. Describe how certain viruses may contribute to the development of tumors by altering proto-oncogenes or tumor-suppressor genes. (ans)
2. Name 3 viruses that have been implicated in human cancers.
   A. *(ans)*
   B. *(ans)*
   C. *(ans)*
3. People with chronic hepatitis B have a much higher risk of developing liver cancer. This cancer, however, usually appears after decades of chronic infection. Explain the link between HBV and liver cancer and why, if it does develop, it usually takes so long. *(ans)*
4. Multiple Choice *(ans)*

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SECTION OVERVIEW

11.5: Culturing Viruses

Topic hierarchy

11.5.1: Isolation, Culture, and Identification of Viruses

11.5.2: Tissue Culture of Animal Viruses

11.5.3: Batch Culture of Bacteriophages

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11.5.1: Isolation, Culture, and Identification of Viruses

Learning Objectives

- Discuss why viruses were originally described as filterable agents
- Describe the cultivation of viruses and specimen collection and handling
- Compare in vivo and in vitro techniques used to cultivate viruses

At the beginning of this chapter, we described how porcelain Chamberland filters with pores small enough to allow viruses to pass through were used to discover TMV. Today, porcelain filters have been replaced with membrane filters and other devices used to isolate and identify viruses.

Isolation of Viruses

Unlike bacteria, many of which can be grown on an artificial nutrient medium, viruses require a living host cell for replication. Infected host cells (eukaryotic or prokaryotic) can be cultured and grown, and then the growth medium can be harvested as a source of virus. Virions in the liquid medium can be separated from the host cells by either centrifugation or filtration. Filters can physically remove anything present in the solution that is larger than the virions; the viruses can then be collected in the filtrate (Figure 11.5.1.1).

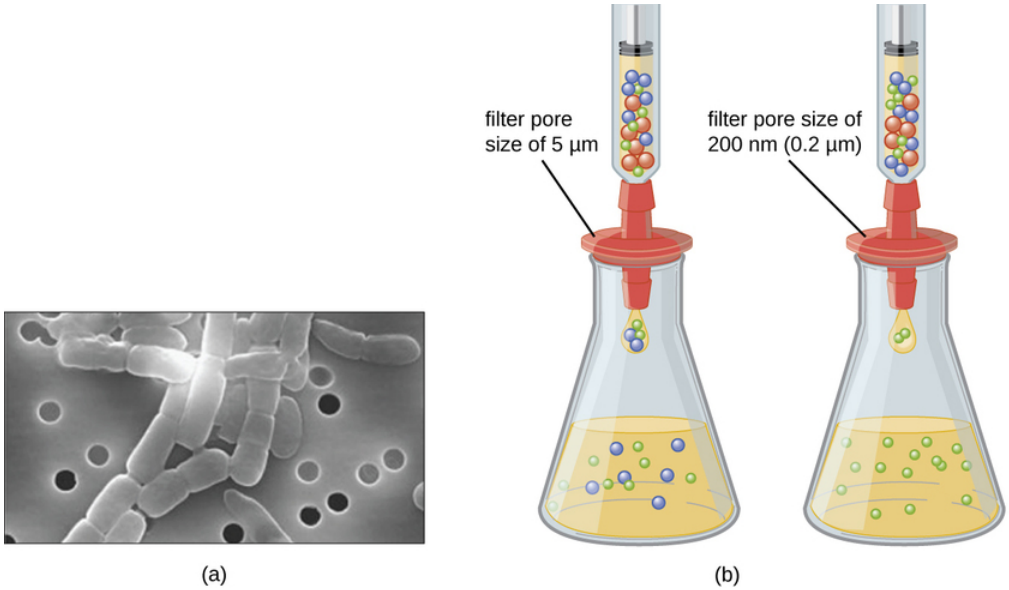


Figure 11.5.1.1: Membrane filters can be used to remove cells or viruses from a solution. (a) This scanning electron micrograph shows rod-shaped bacterial cells captured on the surface of a membrane filter. Note differences in the comparative size of the membrane pores and bacteria. Viruses will pass through this filter. (b) The size of the pores in the filter determines what is captured on the surface of the filter (animal [red] and bacteria [blue]) and removed from liquid passing through. Note the viruses (green) pass through the finer filter. (credit a: modification of work by U.S. Department of Energy)

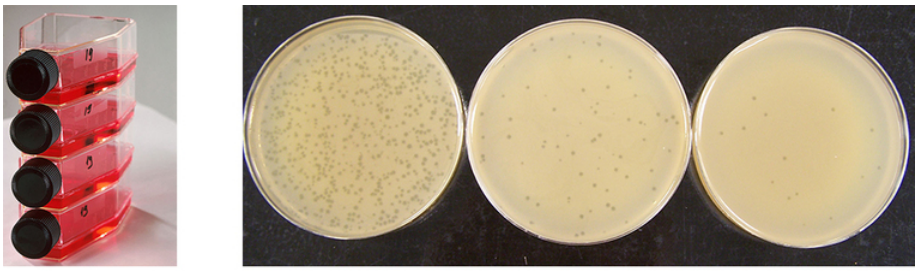
Exercise 11.5.1.1

What size filter pore is needed to collect a virus?

Cultivation of Viruses

Viruses can be grown in vivo (within a whole living organism, plant, or animal) or in vitro (outside a living organism in cells in an artificial environment, such as a test tube, cell culture flask, or agar plate). Bacteriophages can be grown in the presence of a dense layer of bacteria (also called a bacterial lawn) grown in a 0.7 % soft agar in a Petri dish or flat (horizontal) flask (Figure 11.5.1.2*a*). The agar concentration is decreased from the 1.5% usually used in culturing bacteria. The soft 0.7% agar allows the bacteriophages to easily diffuse through the medium. For lytic bacteriophages, lysing of the bacterial hosts can then be readily observed when a

clear zone called a plaque is detected (Figure **11.5.1.1*b***). As the phage kills the bacteria, many plaques are observed among the cloudy bacterial lawn.

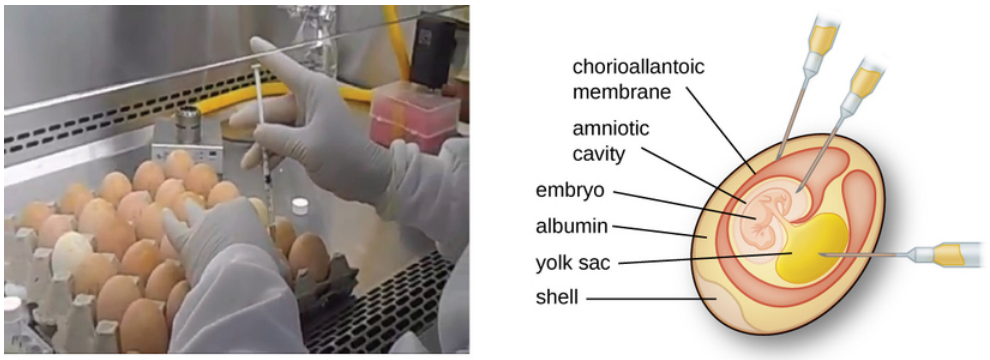


(a) (b)

Figure **11.5.1.2**: (a) Flasks like this may be used to culture human or animal cells for viral culturing. (b) These plates contain bacteriophage T4 grown on an *Escherichia coli* lawn. Clear plaques are visible where host bacterial cells have been lysed. Viral titers increase on the plates to the left. (credit a: modification of work by National Institutes of Health; credit b: modification of work by American Society for Microbiology)

Animal viruses require cells within a host animal or tissue-culture cells derived from an animal. Animal virus cultivation is important for 1) identification and diagnosis of pathogenic viruses in clinical specimens, 2) production of vaccines, and 3) basic research studies. In vivo host sources can be a developing embryo in an embryonated bird's egg (e.g., chicken, turkey) or a whole animal. For example, most of the influenza vaccine manufactured for annual flu vaccination programs is cultured in hens' eggs.

The embryo or host animal serves as an incubator for viral replication (Figure **11.5.1.3**). Location within the embryo or host animal is important. Many viruses have a tissue tropism, and must therefore be introduced into a specific site for growth. Within an embryo, target sites include the amniotic cavity, the chorioallantoic membrane, or the yolk sac. Viral infection may damage tissue membranes, producing lesions called pox; disrupt embryonic development; or cause the death of the embryo.



(a) (b)

Figure **11.5.1.3**: (a) The cells within chicken eggs are used to culture different types of viruses. (b) Viruses can be replicated in various locations within the egg, including the chorioallantoic membrane, the amniotic cavity, and the yolk sac. (credit a: modification of work by "Chung Hoang"/YouTube)

For in vitro studies, various types of cells can be used to support the growth of viruses. A primary cell culture is freshly prepared from animal organs or tissues. Cells are extracted from tissues by mechanical scraping or mincing to release cells or by an enzymatic method using trypsin or collagenase to break up tissue and release single cells into suspension. Because of anchorage-dependence requirements, primary cell cultures require a liquid culture medium in a Petri dish or tissue-culture flask so cells have a solid surface such as glass or plastic for attachment and growth. Primary cultures usually have a limited life span. When cells in a primary culture undergo mitosis and a sufficient density of cells is produced, cells come in contact with other cells. When this cell-to-cell-contact occurs, mitosis is triggered to stop. This is called contact inhibition and it prevents the density of the cells from becoming too high. To prevent contact inhibition, cells from the primary cell culture must be transferred to another vessel with fresh growth medium. This is called a secondary cell culture. Periodically, cell density must be reduced by pouring off some cells and adding fresh medium to provide space and nutrients to maintain cell growth. In contrast to primary cell cultures, continuous cell lines, usually derived from transformed cells or tumors, are often able to be subcultured many times or even grown indefinitely (in which case they are called immortal). Continuous cell lines may not exhibit anchorage dependency (they will grow in

suspension) and may have lost their contact inhibition. As a result, continuous cell lines can grow in piles or lumps resembling small tumor growths (Figure **11.5.1.4**).

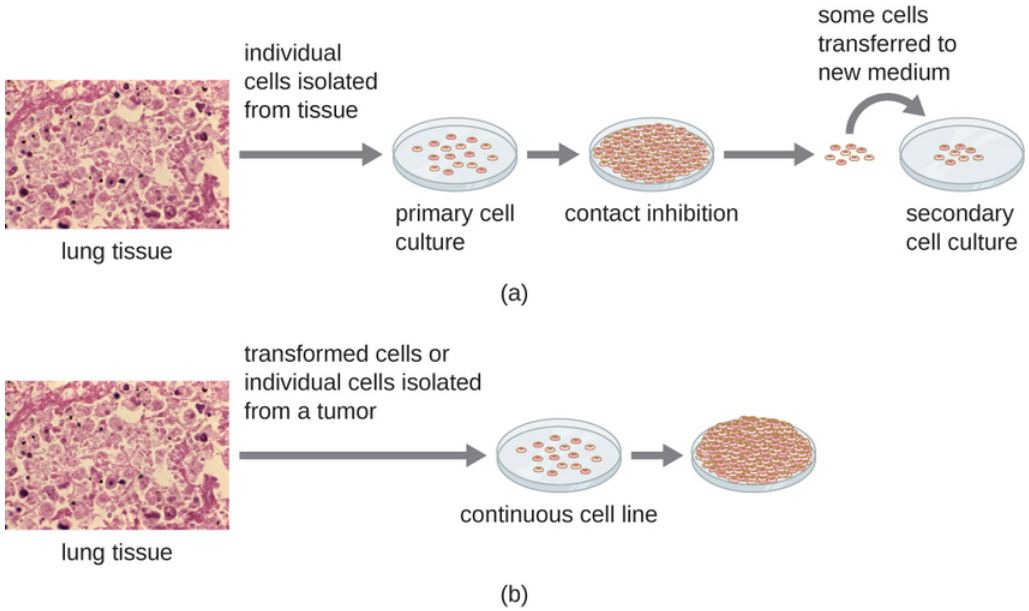


Figure **11.5.1.4**: Cells for culture are prepared by separating them from their tissue matrix. (a) Primary cell cultures grow attached to the surface of the culture container. Contact inhibition slows the growth of the cells once they become too dense and begin touching each other. At this point, growth can only be sustained by making a secondary culture. (b) Continuous cell cultures are not affected by contact inhibition. They continue to grow regardless of cell density. (credit "micrographs": modification of work by Centers for Disease Control and Prevention)

An example of an immortal cell line is the HeLa cell line, which was originally cultivated from tumor cells obtained from Henrietta Lacks, a patient who died of cervical cancer in 1951. HeLa cells were the first continuous tissue-culture cell line and were used to establish tissue culture as an important technology for research in cell biology, virology, and medicine. Prior to the discovery of HeLa cells, scientists were not able to establish tissue cultures with any reliability or stability. More than six decades later, this cell line is still alive and being used for medical research. See The Immortal Cell Line of Henrietta Lacks to read more about this important cell line and the controversial means by which it was obtained.

? Exercise 11.5.1.2

What property of cells makes periodic dilutions of primary cell cultures necessary?

The Immortal Cell Line of Henrietta Lacks

In January 1951, Henrietta Lacks, a 30-year-old African American woman from Baltimore, was diagnosed with cervical cancer at John Hopkins Hospital. We now know her cancer was caused by the human papillomavirus (HPV). Cytopathic effects of the virus altered the characteristics of her cells in a process called transformation, which gives the cells the ability to divide continuously. This ability, of course, resulted in a cancerous tumor that eventually killed Mrs. Lacks in October at age 31. Before her death, samples of her cancerous cells were taken without her knowledge or permission. The samples eventually ended up in the possession of Dr. George Gey, a biomedical researcher at Johns Hopkins University. Gey was able to grow some of the cells from Lacks's sample, creating what is known today as the immortal HeLa cell line. These cells have the ability to live and grow indefinitely and, even today, are still widely used in many areas of research.

According to Lacks's husband, neither Henrietta nor the family gave the hospital permission to collect her tissue specimen. Indeed, the family was not aware until 20 years after Lacks's death that her cells were still alive and actively being used for commercial and research purposes. Yet HeLa cells have been pivotal in numerous research discoveries related to polio, cancer, and AIDS, among other diseases. The cells have also been commercialized, although they have never themselves been

patented. Despite this, Henrietta Lacks's estate has never benefited from the use of the cells, although, in 2013, the Lacks family was given control over the publication of the genetic sequence of her cells.

This case raises several bioethical issues surrounding patients' informed consent and the right to know. At the time Lacks's tissues were taken, there were no laws or guidelines about informed consent. Does that mean she was treated fairly at the time? Certainly by today's standards, the answer would be no. Harvesting tissue or organs from a dying patient without consent is not only considered unethical but illegal, regardless of whether such an act could save other patients' lives. Is it ethical, then, for scientists to continue to use Lacks's tissues for research, even though they were obtained illegally by today's standards?

Ethical or not, Lacks's cells are widely used today for so many applications that it is impossible to list them all. Is this a case in which the ends justify the means? Would Lacks be pleased to know about her contribution to science and the millions of people who have benefited? Would she want her family to be compensated for the commercial products that have been developed using her cells? Or would she feel violated and exploited by the researchers who took part of her body without her consent? Because she was never asked, we will never know.

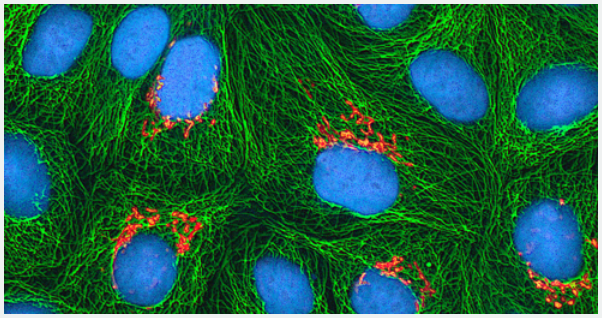


Figure 11.5.1.5: A multiphoton fluorescence image of HeLa cells in culture. Various fluorescent stains have been used to show the DNA (cyan), microtubules (green), and Golgi apparatus (orange). (credit: modification of work by National Institutes of Health)

Detection of a Virus

Regardless of the method of cultivation, once a virus has been introduced into a whole host organism, embryo, or tissue-culture cell, a sample can be prepared from the infected host, embryo, or cell line for further analysis under a brightfield, electron, or fluorescent microscope. Cytopathic effects (CPEs) are distinct observable cell abnormalities due to viral infection. CPEs can include loss of adherence to the surface of the container, changes in cell shape from flat to round, shrinkage of the nucleus, vacuoles in the cytoplasm, fusion of cytoplasmic membranes and the formation of multinucleated syncytia, inclusion bodies in the nucleus or cytoplasm, and complete cell lysis (see Figure 11.5.1.6).

Further pathological changes include viral disruption of the host genome and altering normal cells into transformed cells, which are the types of cells associated with carcinomas and sarcomas. The type or severity of the CPE depends on the type of virus involved. Figure 11.5.1.6 lists CPEs for specific viruses.

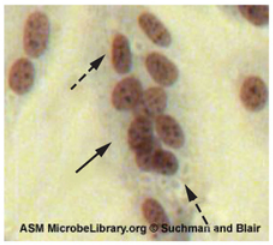
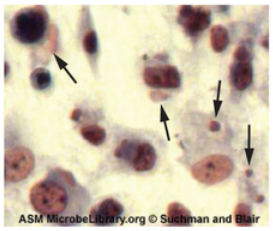
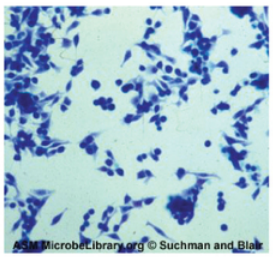
Cytopathic Effects of Specific Viruses		
Virus	**Cytopathic Effect**	**Example**
Paramyxovirus	Syncytium and faint basophilic cytoplasmic inclusion bodies	
Poxvirus	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	
Herpesvirus	Cytoplasmic stranding (arrow) and nuclear inclusion bodies (dashed arrow)	
Adenovirus	Cell enlargement, rounding, and distinctive “grape-like” clusters	

Figure **11.5.1.6**: (credit “micrographs”: modification of work by American Society for Microbiology)

Link to Learning

Watch this video to learn about the effects of viruses on cells.

Hemagglutination Assay

A serological assay is used to detect the presence of certain types of viruses in patient serum. Serum is the straw-colored liquid fraction of blood plasma from which clotting factors have been removed. Serum can be used in a direct assay called a hemagglutination assay to detect specific types of viruses in the patient’s sample. Hemagglutination is the agglutination (clumping) together of erythrocytes (red blood cells). Many viruses produce surface proteins or spikes called hemagglutinins that can bind to receptors on the membranes of erythrocytes and cause the cells to agglutinate. Hemagglutination is observable without using the microscope, but this method does not always differentiate between infectious and noninfectious viral particles, since both can agglutinate erythrocytes.

To identify a specific pathogenic virus using hemagglutination, we must use an indirect approach. Proteins called antibodies, generated by the patient's immune system to fight a specific virus, can be used to bind to components such as hemagglutinins that are uniquely associated with specific types of viruses. The binding of the antibodies with the hemagglutinins found on the virus subsequently prevent erythrocytes from directly interacting with the virus. So when erythrocytes are added to the antibody-coated viruses, there is no appearance of agglutination; agglutination has been inhibited. We call these types of indirect assays for virus-specific antibodies hemagglutination inhibition (HAI) assays. HAI can be used to detect the presence of antibodies specific to many types of viruses that may be causing or have caused an infection in a patient even months or years after infection (see Figure 11.5.1.7). This assay is described in greater detail in Agglutination Assays.

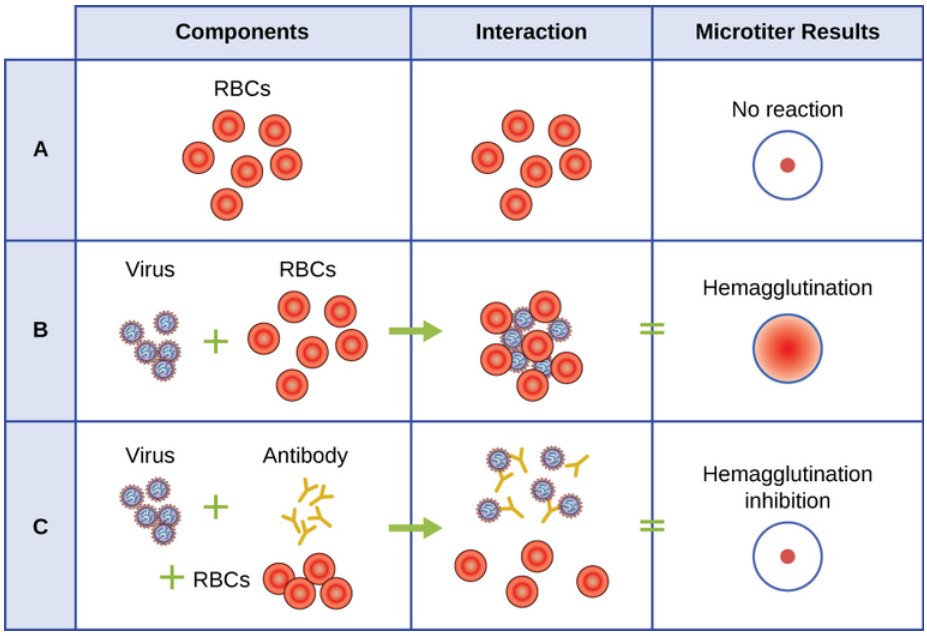


Figure 11.5.1.7: This chart shows the possible outcomes of a hemagglutination test. Row A: Erythrocytes do not bind together and will sink to the bottom of the well plate; this becomes visible as a red dot in the center of the well. Row B: Many viruses have hemagglutinins that causes agglutination of erythrocytes; the resulting hemagglutination forms a lattice structure that results in red color throughout the well. Row C: Virus-specific antibody, the viruses, and the erythrocytes are added to the well plate. The virus-specific antibodies inhibit agglutination, as can be seen as a red dot in the bottom of the well. (credit: modification of work by Centers for Disease Control and Prevention)

? Exercise 11.5.1.3

What is the outcome of a positive HIA test?

Nucleic Acid Amplification Test

Nucleic acid amplification tests (NAAT) are used in molecular biology to detect unique nucleic acid sequences of viruses in patient samples. Polymerase chain reaction (PCR) is an NAAT used to detect the presence of viral DNA in a patient's tissue or body fluid sample. PCR is a technique that amplifies (i.e., synthesizes many copies) of a viral DNA segment of interest. Using PCR, short nucleotide sequences called primers bind to specific sequences of viral DNA, enabling identification of the virus.

Reverse transcriptase-PCR (RT-PCR) is an NAAT used to detect the presence of RNA viruses. RT-PCR differs from PCR in that the enzyme reverse transcriptase (RT) is used to make a cDNA from the small amount of viral RNA in the specimen. The cDNA can then be amplified by PCR. Both PCR and RT-PCR are used to detect and confirm the presence of the viral nucleic acid in patient specimens.

HPV Scare

Michelle, a 21-year-old nursing student, came to the university clinic worried that she might have been exposed to a sexually transmitted disease (STD). Her sexual partner had recently developed several bumps on the base of his penis. He had put off

going to the doctor, but Michelle suspects they are genital warts caused by HPV. She is especially concerned because she knows that HPV not only causes warts but is a prominent cause of cervical cancer. She and her partner always use condoms for contraception, but she is not confident that this precaution will protect her from HPV.

Michelle's physician finds no physical signs of genital warts or any other STDs, but recommends that Michelle get a Pap smear along with an HPV test. The Pap smear will screen for abnormal cervical cells and the CPEs associated with HPV; the HPV test will test for the presence of the virus. If both tests are negative, Michelle can be more assured that she most likely has not become infected with HPV. However, her doctor suggests it might be wise for Michelle to get vaccinated against HPV to protect herself from possible future exposure.

Exercise 11.5.1.4

Why does Michelle's physician order two different tests instead of relying on one or the other?

Enzyme Immunoassay

Enzyme immunoassays (EIAs) rely on the ability of antibodies to detect and attach to specific biomolecules called antigens. The detecting antibody attaches to the target antigen with a high degree of specificity in what might be a complex mixture of biomolecules. Also included in this type of assay is a colorless enzyme attached to the detecting antibody. The enzyme acts as a tag on the detecting antibody and can interact with a colorless substrate, leading to the production of a colored end product. EIAs often rely on layers of antibodies to capture and react with antigens, all of which are attached to a membrane filter (see Figure **11.5.1.8**). EIAs for viral antigens are often used as preliminary screening tests. If the results are positive, further confirmation will require tests with even greater sensitivity, such as a western blot or an NAAT. EIAs are discussed in more detail in EIAs and ELISAs.

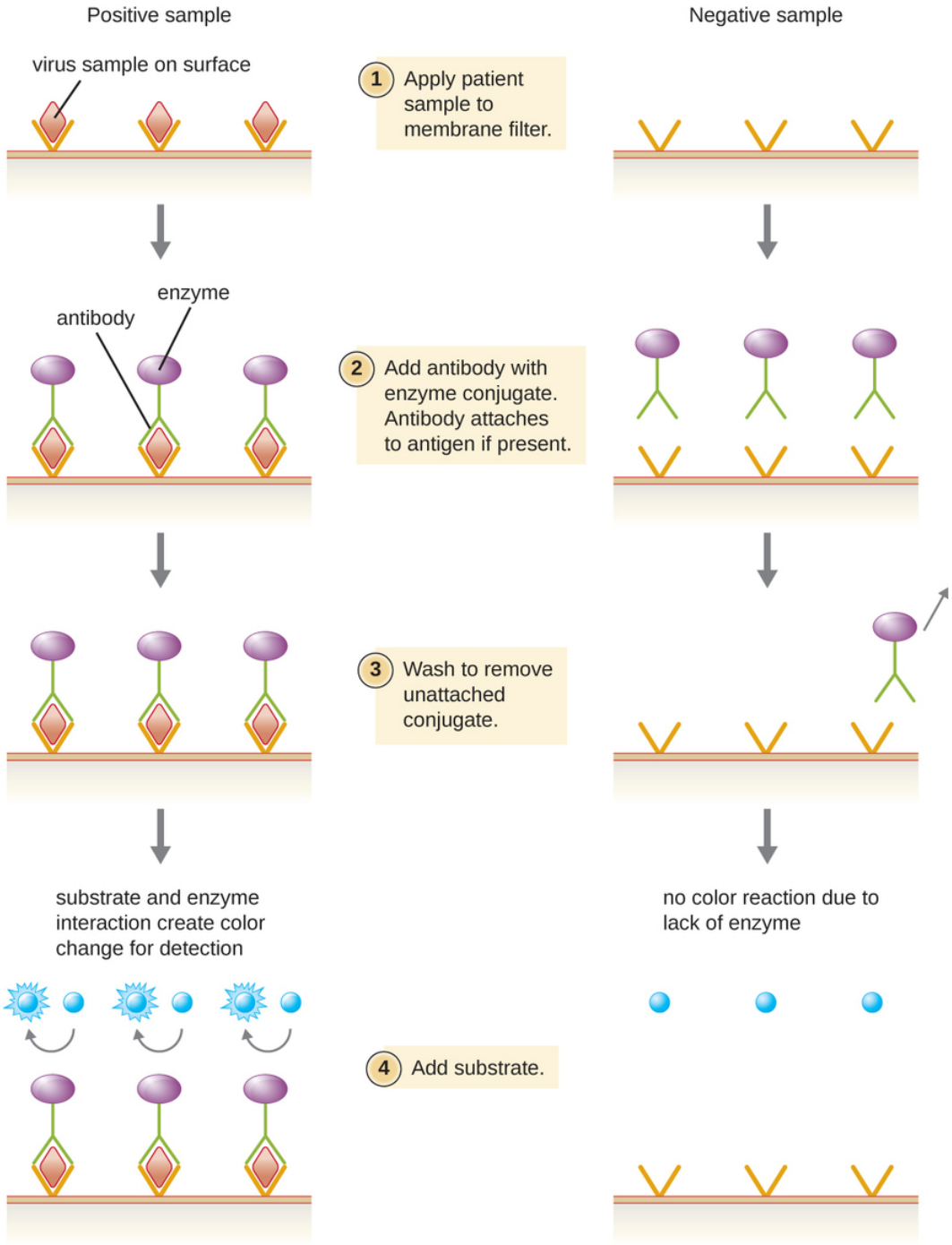


Figure **11.5.1.8**: Similar to rapid, over-the-counter pregnancy tests, EIAs for viral antigens require a few drops of diluted patient serum or plasma applied to a membrane filter. The membrane filter has been previously modified and embedded with antibody to viral antigen and internal controls. Antibody conjugate is added to the filter, with the targeted antibody attached to the antigen (in the case of a positive test). Excess conjugate is washed off the filter. Substrate is added to activate the enzyme-mediated reaction to reveal the color change of a positive test. (credit: modification of work by "Cavitri"/Wikimedia Commons)

? Exercise 11.5.1.5

What typically indicates a positive EIA test?

Clinical Focus: Part 3

Along with the RT/PCR analysis, David's saliva was also collected for viral cultivation. In general, no single diagnostic test is sufficient for antemortem diagnosis, since the results will depend on the sensitivity of the assay, the quantity of virions present at the time of testing, and the timing of the assay, since release of virions in the saliva can vary. As it turns out, the result was negative for viral cultivation from the saliva. This is not surprising to David's doctor, because one negative result is not an absolute indication of the absence of infection. It may be that the number of virions in the saliva is low at the time of sampling. It is not unusual to repeat the test at intervals to enhance the chance of detecting higher virus loads.

Exercise 11.5.1.6

Should David's doctor modify his course of treatment based on these test results?

Summary

- Viral cultivation requires the presence of some form of host cell (whole organism, embryo, or cell culture).
- Viruses can be isolated from samples by filtration.
- Viral filtrate is a rich source of released virions.
- Bacteriophages are detected by presence of clear **plaques** on bacterial lawn.
- Animal and plant viruses are detected by **cytopathic effects**, molecular techniques (PCR, RT-PCR), enzyme immunoassays, and serological assays (hemagglutination assay, hemagglutination inhibition assay).

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11.5.2: Tissue Culture of Animal Viruses

Learning Objectives

- Discover the use of, and reasons for, culturing animal viruses in host cells

Cell culture is the complex process by which cells are grown under controlled conditions, generally outside of their natural environment. In practice, the term “cell culture” now refers to the culturing of cells derived from multi-cellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi, and microbes, including viruses, bacteria, and protists. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture. Animal cell culture became a common laboratory technique in the mid-1900’s, but the concept of maintaining live cell lines separated from their original tissue source was discovered in the 19th century.

Viruses are obligate intracellular parasites that require living cells in order to replicate. Cultured cells, eggs, and laboratory animals may be used for virus isolation. Although embroyonated eggs and laboratory animals are very useful for the isolation of certain viruses, cell cultures are the sole system for virus isolation in most laboratories. The development of methods for cultivating animal cells has been essential to the progress of animal virology. To prepare cell cultures, tissue fragments are first dissociated, usually with the aid of trypsin or collagenase. The cell suspension is then placed in a flat-bottomed glass or plastic container (petri dish, a flask, a bottle, test tube) together with a suitable liquid medium. e.g. Eagle’s, and an animal serum. After a variable lag, the cells will attach and spread on the bottom of the container and then start dividing, giving rise to a primary culture. Attachment to a solid support is essential for the growth of normal cells.

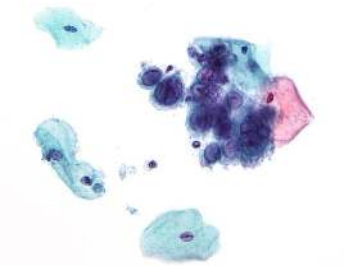


Figure: **Cytopathic effect**: Viral cytopathic effect of herpes simplex virus.

Cell cultures vary greatly in their susceptibility to different viruses. It is of utmost importance that the most sensitive cell cultures are used for a particular suspected virus. Specimens for cell culture should be transported to the laboratory as soon as possible upon being taken. Swabs should be put in a vial containing virus transport medium. Bodily fluids and tissues should be placed in a sterile container. Upon receipt, the specimen is inoculated into several different types of cell culture depending on the nature of the specimen and the clinical presentation. The maintenance media should be changed after one hour or the next morning. The inoculated tubes should be incubated at 35-37ºC in a rotating drum. Rotation is optimal for the isolation of respiratory viruses and result in an earlier appearance of the cytopathic effects (CPE) for many viruses. If stationary tubes are used, it is critical that the culture tubes be positioned so that the cell monolayer is bathed in nutrient medium.

Key Points

- Tissue culture is a useful method for cultivating clinical samples suspected of harboring a virus. This method helps with the detection, identification, and characterization of viruses in the laboratory.
- Tissue culture of animal viruses involves growing animal cells in flasks using various broth media and then infecting these cells with virus.
- Transfection can be carried out using calcium phosphate, by electroporation, or by mixing a cationic lipid with the material to produce liposomes, which fuse with the cell membrane and deposit their cargo inside.
- Cytopathic effect is a non-lytic damage that viruses cause to cells. These vary in their manifestation and damaging effect.

Key Terms

- **cell culture**: The complex process by which cells are grown under controlled conditions, generally outside of their natural environment.
- **cytopathic effect**: Refers to degenerative changes in cells, especially in tissue culture, and may be associated with the multiplication of certain viruses.

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11.5.3: Batch Culture of Bacteriophages

Learning Objectives

- Define the reasons for, and ways to batch culture bacteriophages

Strategies of Replication

Virus or phage cultures require host cells in which to multiply. For bacteriophages, cultures are grown by infecting bacterial cells. The phage can then be isolated from the resulting plaques in a lawn of bacteria on a plate.

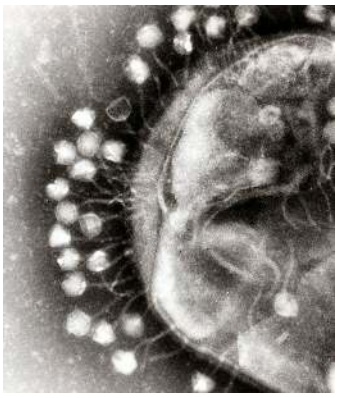


Figure: **Bacteriophages infecting a bacteria**: Virus or phage cultures require host cells in which to multiply. For bacteriophages, cultures are grown by infecting bacterial cells. The phage can then be isolated from the resulting plaques in a lawn of bacteria on a plate.

A bacteriophage is any one of a number of viruses that infect bacteria. They do this by injecting genetic material, which they carry enclosed in an outer protein capsid, into a host bacterial cell. The genetic material can be ssRNA, dsRNA, ssDNA, or dsDNA ('ss-' or 'ds-' prefix denotes single-strand or double-strand), along with either circular or linear arrangements.

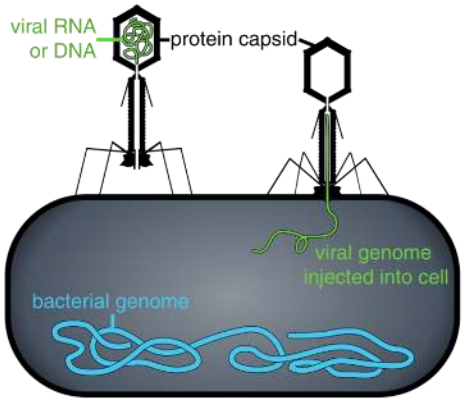


Figure: **Bacteriophage**: Diagram of how some bacteriophages infect bacterial cells.

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only those bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them. As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution within blood, lymphatic circulation, irrigation, soil water, or other environments..

Phages may be released via cell lysis, by extrusion, or, in a few cases, by budding. Lysis, by tailed phages, is achieved by an enzyme called endolysin, which attacks and breaks down the cell wall peptidoglycan. An altogether different phage type, the filamentous phages, make the host cell continually secrete new virus particles. Released virions are described as free, and, unless

defective, are capable of infecting a new bacterium. Budding is associated with certain Mycoplasma phages. In contrast to virion release, phages displaying a lysogenic cycle do not kill the host but, rather, become long-term residents as prophage.

Key Points

- A bacteriophage is a type of virus that infects bacteria. It does so by injecting genetic material – either DNA or RNA – which it carries enclosed in an outer protein capsid.
- To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella.
- Phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

Key Terms

- **bacteriophage**: A virus that specifically infects bacteria.

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11.6: Viroids and Prions

Learning Objectives

- Describe viroids and their unique characteristics
- Describe virusoids and their unique characteristics
- Describe prions and their unique characteristics

Research attempts to discover the causative agents of previously uninvestigated diseases have led to the discovery of nonliving disease agents quite different from viruses. These include particles consisting only of RNA or only of protein that, nonetheless, are able to self-propagate at the expense of a host—a key similarity to viruses that allows them to cause disease conditions. To date, these discoveries include viroids, virusoids, and the proteinaceous prions.

Viroids

In 1971, Theodor Diener, a pathologist working at the Agriculture Research Service, discovered an acellular particle that he named a viroid, meaning "virus-like." Viroids consist only of a short strand of circular RNA capable of self-replication. The first viroid discovered was found to cause potato tuber spindle disease, which causes slower sprouting and various deformities in potato plants (see Figure **11.6.1**). Like viruses, potato spindle tuber viroids (PSTVs) take control of the host machinery to replicate their RNA genome. Unlike viruses, viroids do not have a protein coat to protect their genetic information.



Figure **11.6.1**: These potatoes have been infected by the potato spindle tuber viroid (PSTV), which is typically spread when infected knives are used to cut healthy potatoes, which are then planted. (credit: Pamela Roberts, University of Florida Institute of Food and Agricultural Sciences, USDA ARS)

Viroids can result in devastating losses of commercially important agricultural food crops grown in fields and orchards. Since the discovery of PSTV, other viroids have been discovered that cause diseases in plants. Tomato planta macho viroid (TPMVd) infects tomato plants, which causes loss of chlorophyll, disfigured and brittle leaves, and very small tomatoes, resulting in loss of productivity in this field crop. Avocado sunblotch viroid (ASBVd) results in lower yields and poorer-quality fruit. ASBVd is the smallest viroid discovered thus far that infects plants. Peach latent mosaic viroid(PLMVd) can cause necrosis of flower buds and branches, and wounding of ripened fruit, which leads to fungal and bacterial growth in the fruit. PLMVd can also cause similar pathological changes in plums, nectarines, apricots, and cherries, resulting in decreased productivity in these orchards, as well. Viroids, in general, can be dispersed mechanically during crop maintenance or harvesting, vegetative reproduction, and possibly via seeds and insects, resulting in a severe drop in food availability and devastating economic consequences.

Exercise 11.6.1

What is the genome of a viroid made of?

Virusoids

A second type of pathogenic RNA that can infect commercially important agricultural crops are the virusoids, which are subviral particles best described as non–self-replicating ssRNAs. RNA replication of virusoids is similar to that of viroids but, unlike viroids, virusoids require that the cell also be infected with a specific “helper” virus. There are currently only five described types of virusoids and their associated helper viruses. The helper viruses are all from the family of Sobemoviruses. An example of a helper virus is the subterranean clover mottle virus, which has an associated virusoid packaged inside the viral capsid. Once the helper virus enters the host cell, the virusoids are released and can be found free in plant cell cytoplasm, where they possess ribozyme activity. The helper virus undergoes typical viral replication independent of the activity of the virusoid. The virusoid genomes are small, only 220 to 388 nucleotides long. A virusoid genome does not code for any proteins, but instead serves only to replicate virusoid RNA.

Virusoids belong to a larger group of infectious agents called satellite RNAs, which are similar pathogenic RNAs found in animals. Unlike the plant virusoids, satellite RNAs may encode for proteins; however, like plant virusoids, satellite RNAs must coinfect with a helper virus to replicate. One satellite RNA that infects humans and that has been described by some scientists as a virusoid is the hepatitis delta virus (HDV), which, by some reports, is also called hepatitis delta virusoid. Much larger than a plant virusoid, HDV has a circular, ssRNA genome of 1,700 nucleotides and can direct the biosynthesis of HDV-associated proteins. The HDV helper virus is the hepatitis B virus (HBV). Coinfection with HBV and HDV results in more severe pathological changes in the liver during infection, which is how HDV was first discovered.

? Exercise 11.6.2

What is the main difference between a viroid and a virusoid?

Prions

At one time, scientists believed that any infectious particle must contain DNA or RNA. Then, in 1982, Stanley Prusiner, a medical doctor studying scrapie (a fatal, degenerative disease in sheep) discovered that the disease was caused by proteinaceous infectious particles, or prions. Because proteins are acellular and do not contain DNA or RNA, Prusiner’s findings were originally met with resistance and skepticism; however, his research was eventually validated, and he received the Nobel Prize in Physiology or Medicine in 1997.

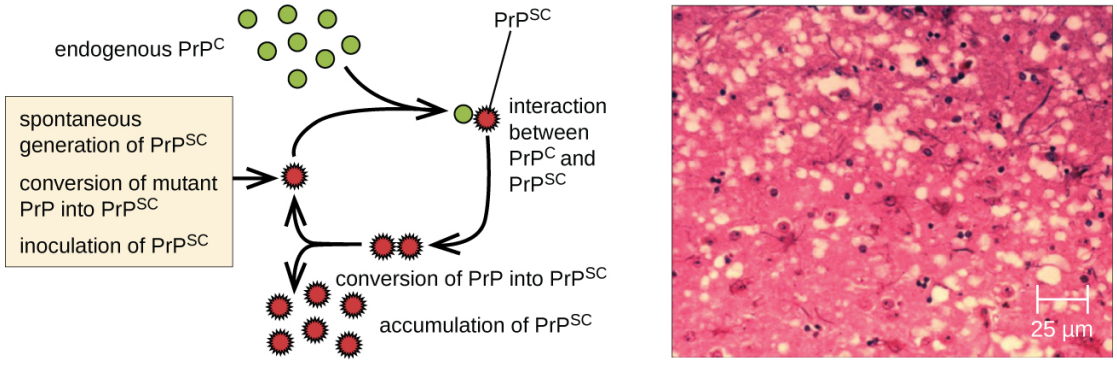


Figure **11.6.2**: Endogenous normal prion protein (PrPc) is converted into the disease-causing form (PrPsc) when it encounters this variant form of the protein. PrPsc may arise spontaneously in brain tissue, especially if a mutant form of the protein is present, or it may originate from misfolded prions consumed in food that eventually find their way into brain tissue. (credit b: modification of work by USDA)

A prion is a misfolded rogue form of a normal protein (PrPc) found in the cell. This rogue prion protein (PrPsc), which may be caused by a genetic mutation or occur spontaneously, can be infectious, stimulating other endogenous normal proteins to become misfolded, forming plaques (see Figure **11.6.2**). Today, prions are known to cause various forms of transmissible spongiform encephalopathy (TSE) in human and animals. TSE is a rare degenerative disorder that affects the brain and nervous system. The accumulation of rogue proteins causes the brain tissue to become sponge-like, killing brain cells and forming holes in the tissue, leading to brain damage, loss of motor coordination, and dementia (see Figure **11.6.3**). Infected individuals are mentally impaired

and become unable to move or speak. There is no cure, and the disease progresses rapidly, eventually leading to death within a few months or years.

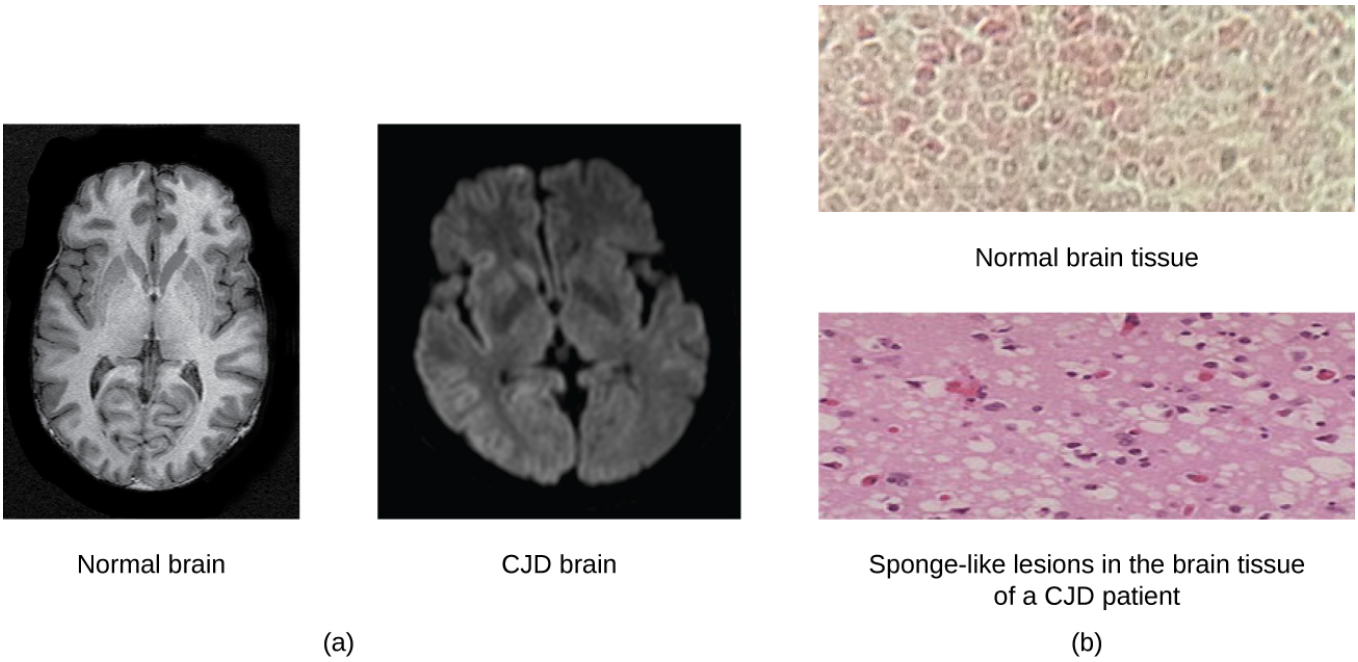


Figure **11.6.3**: Creutzfeldt-Jakob disease (CJD) is a fatal disease that causes degeneration of neural tissue. (a) These brain scans compare a normal brain to one with CJD. (b) Compared to a normal brain, the brain tissue of a CJD patient is full of sponge-like lesions, which result from abnormal formations of prion protein. (credit a (right): modification of work by Dr. Laughlin Dawes; credit b (top): modification of work by Suzanne Wakim; credit b (bottom): modification of work by Centers for Disease Control and Prevention)

TSEs in humans include kuru, fatal familial insomnia, Gerstmann-Straussler-Scheinker disease, and Creutzfeldt-Jakob disease (see Figure **11.6.3**). TSEs in animals include mad cow disease, scrapie (in sheep and goats), and chronic wasting disease (in elk and deer). TSEs can be transmitted between animals and from animals to humans by eating contaminated meat or animal feed. Transmission between humans can occur through heredity (as is often the case with GSS and CJD) or by contact with contaminated tissue, as might occur during a blood transfusion or organ transplant. There is no evidence for transmission via casual contact with an infected person. Table **11.6.1** lists TSEs that affect humans and their modes of transmission.

Table **11.6.1**: Transmissible Spongiform Encephalopathies (TSEs) in Humans

Disease	Mechanism(s) of Transmission[1]
Sporadic CJD (sCJD)	Not known; possibly by alteration of normal prior protein (PrP) to rogue form due to somatic mutation
Variant CJD (vCJD)	Eating contaminated cattle products and by secondary bloodborne transmission
Familial CJD (fCJD)	Mutation in germline PrP gene
Iatrogenic CJD (iCJD)	Contaminated neurosurgical instruments, corneal graft, gonadotrophic hormone, and, secondarily, by blood transfusion
Kuru	Eating infected meat through ritualistic cannibalism
Gerstmann-Straussler-Scheinker disease (GSS)	Mutation in germline PrP gene
Fatal familial insomnia (FFI)	Mutation in germline PrP gene

Prions are extremely difficult to destroy because they are resistant to heat, chemicals, and radiation. Even standard sterilization procedures do not ensure the destruction of these particles. Currently, there is no treatment or cure for TSE disease, and

contaminated meats or infected animals must be handled according to federal guidelines to prevent transmission.

Exercise 11.6.3

Does a prion have a genome?

Clinical Focus: Resolution

A few days later, David's doctor receives the results of the immunofluorescence test on his skin sample. The test is negative for rabies antigen. A second viral antigen test on his saliva sample also comes back negative. Despite these results, the doctor decides to continue David's current course of treatment. Given the positive RT-PCR test, it is best not to rule out a possible rabies infection.

Near the site of the bite, David receives an injection of rabies immunoglobulin, which attaches to and inactivates any rabies virus that may be present in his tissues. Over the next 14 days, he receives a series of four rabies-specific vaccinations in the arm. These vaccines activate David's immune response and help his body recognize and fight the virus. Thankfully, with treatment, David symptoms improve and he makes a full recovery.

Not all rabies cases have such a fortunate outcome. In fact, rabies is usually fatal once the patient starts to exhibit symptoms, and postbite treatments are mainly palliative (i.e., sedation and pain management).

Summary

- Other acellular agents such as **viroids**, **virusoids**, and **prions** also cause diseases. Viroids consist of small, naked ssRNAs that cause diseases in plants. Virusoids are ssRNAs that require other helper viruses to establish an infection. Prions are proteinaceous infectious particles that cause **transmissible spongiform encephalopathies**.
- Prions are extremely resistant to chemicals, heat, and radiation.
- There are no treatments for prion infection.

Footnotes

1. 1 National Institute of Neurological Disorders and Stroke. "Creutzfeldt-Jakob Disease Fact Sheet." http://www.ninds.nih.gov/disorders/cjd/detail_cjd.htm (accessed December 31, 2015).

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CHAPTER OVERVIEW

12: Antimicrobial Drugs

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi

12.1: Overview of Antimicrobial Therapy
- 12.1A: Origins of Antimicrobial Drugs
- 12.1B: Antibiotic Discovery
- 12.1C: Antibiotics and Selective Toxicity
- 12.1D: Spectrum of Antimicrobial Activity
- 12.1E: Antibiotic Classifications

12.2: Functions of Antimicrobial Drugs
- 12.2A: Inhibiting Cell Wall Synthesis
- 12.2B: Injuring the Plasma Membrane
- 12.2C: Inhibiting Nucleic Acid Synthesis
- 12.2D: Inhibiting Protein Synthesis
- 12.2E: Inhibiting Essential Metabolite Synthesis

12.3: Commonly Used Antimicrobial Drugs
- 12.3A: Synthetic Antimicrobial Drugs
- 12.3B: Naturally Occurring Antimicrobial Drugs- Antibiotics
- 12.3C: Beta-Lactam Antibiotics- Penicillins and Cephalosporins
- 12.3D: Antibiotics from Prokaryotes
- 12.3E: Antimycobacterial Antibiotics

12.4: Interactions Between Drug and Host
- 12.4A: Organ Toxicity
- 12.4B: Allergic Responses to Drugs
- 12.4C: Suppression and Alteration of Microbiota by Antimicrobials
- 12.4D: Effects of Drug Combinations

12.5: Measuring Drug Susceptibility
- 12.5A: Minimal Inhibitory Concentration (MIC)
- 12.5B: Kirby-Bauer Disk Susceptibility Test

12.6: Drug Resistance
- 12.6A: Mechanisms of Resistance
- 12.6B: Antibiotic Misuse
- 12.6C: Cost and Prevention of Resistance
- 12.6D: Biofilms, Persisters, and Antibiotic Tolerance
- 12.6E: Finding New Antimicrobial Drugs

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SECTION OVERVIEW

12.1: Overview of Antimicrobial Therapy

Topic hierarchy

12.1A: Origins of Antimicrobial Drugs

12.1B: Antibiotic Discovery

12.1C: Antibiotics and Selective Toxicity

12.1D: Spectrum of Antimicrobial Activity

12.1E: Antibiotic Classifications

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12.1A: Origins of Antimicrobial Drugs

Learning Objectives

- Recall the technical defintion of antibiotics

The history of antimicrobials begins with the observations of Pasteur and Koch, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism.

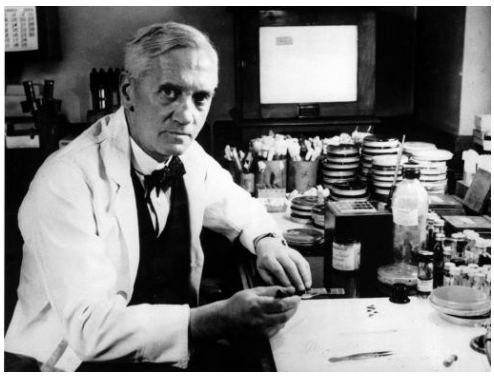


Figure: **Alexander Fleming**: In 1928 Alexander Fleming observed antibiosis against bacteria by a fungus of the genus Penicillium and postulated the effect was mediated by an antibacterial compound, penicillin, and that its antibacterial properties could be exploited for chemotherapy.

The discovery of antimicrobials like penicillin by Alexander Fleming and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

The term antibiotic was first used in 1942 by Selman Waksman and his collaborators in journal articles to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. This definition excluded substances that kill bacteria, but are not produced by microorganisms (such as gastric juices and hydrogen peroxide). It also excluded synthetic antibacterial compounds such as the sulfonamides. Many antibacterial compounds are relatively small molecules with a molecular weight of less than 2000 atomic mass units. With advances in medicinal chemistry, most of today's antibacterials chemically are semisynthetic modifications of various natural compounds.

Key Points

- Antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism.
- In today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection.
- The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world.

Key Terms

- **antimicrobial**: An agent that destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action.
- **penicillin**: Any of a group of broad-spectrum antibiotics obtained from Penicillium molds or synthesized; they have a beta-lactam structure; most are active against gram-positive bacteria and used in the treatment of various infections and diseases.

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12.1B: Antibiotic Discovery

Learning Objectives

- Describe the concept of ‘antibiosis’ and the contributions of the different scientists who discovered it

Before the early 20th century, treatments for infections were based primarily on medicinal folklore. Mixtures with antimicrobial properties that were used in treatments of infections were described over 2000 years ago. Many ancient cultures, including the ancient Egyptians and ancient Greeks, used specially selected mold and plant materials and extracts to treat infections. More recent observations made in the laboratory of antibiosis between micro- organisms led to the discovery of natural antibacterials produced by microorganisms.

Louis Pasteur observed, “if we could intervene in the antagonism observed between some bacteria, it would offer perhaps the greatest hopes for therapeutics”. The term ‘antibiosis’, meaning “against life,” was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these early antibacterial drugs. Antibiosis was first described in 1877 in bacteria when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of Bacillus anthracis. These drugs were later renamed antibiotics by Selman Waksman, an American microbiologist, in 1942.

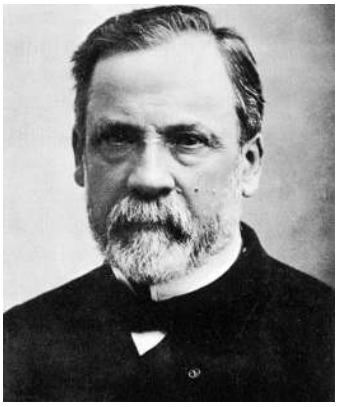


Figure: **Louis Pasteur**: Louis Pasteur was a French microbiologist and chemist best known for their experiments supporting the Germ theory of disease, and for his vaccinations, most notably the first vaccine against rabies.

John Tyndall first described antagonistic activities by fungi against bacteria in England in 1875. Synthetic antibiotic chemotherapy as a science and development of antibacterials began in Germany with Paul Ehrlich in the late 1880s. Ehrlich noted certain dyes would color human, animal, or bacterial cells, while others did not. He then proposed the idea that it might be possible to create chemicals that would act as a selective drug that would bind to and kill bacteria without harming the human host. After screening hundreds of dyes against various organisms, he discovered a medicinally useful drug, the synthetic antibacterial Salvarsan now called arsphenamine. In 1895, Vincenzo Tiberio, physician of the University of Naples discovered that a mold (Penicillium) in a water well has an antibacterial action. After this initial chemotherapeutic compound proved effective, others pursued similar lines of inquiry, but it was not until in 1928 that Alexander Fleming observed antibiosis against bacteria by a fungus of the genus Penicillium. Fleming postulated the effect was mediated by an antibacterial compound named penicillin, and that its antibacterial properties could be exploited for chemotherapy. He initially characterized some of its biological properties, but he did not pursue its further development.

Key Points

- Before the early 20th century, treatments for infections were based primarily on medicinal folklore.
- Louis Pasteur observed, “if we could intervene in the antagonism observed between some bacteria, it would offer perhaps the greatest hopes for therapeutics”.
- The term ‘antibiosis’, meaning “against life,” was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these early antibacterial drugs.

Key Terms

- **micro-organism**: A microorganism (from the Greek: μ, mikrós, “small” and ὀ, organismós, “organism”; also spelled micro-organism, micro organism or microörganism) or microbe is a microscopic organism that comprises either a single cell (unicellular), cell clusters, or multicellular relatively complex organisms.
- **chemotherapy**: Any chemical treatment intended to be therapeutic with respect to a disease state.
- **infection**: An uncontrolled growth of harmful microorganisms in a host.

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12.1C: Antibiotics and Selective Toxicity

Learning Objectives

- Describe selective toxicity

Selective Toxicity in Antibiotics

Synthetic antibiotic chemotherapy as a science and development of antibacterials began in Germany with Paul Ehrlich in the late 1880s. Ehrlich noted that certain dyes would color human, animal, or bacterial cells, while others did not. He then proposed the idea that it might be possible to create chemicals that would act as a selective drug that would bind to and kill bacteria without harming the human host. After screening hundreds of dyes against various organisms, he discovered a medicinally useful drug, the synthetic antibacterial Salvarsan now called arsphenamine.

Antibiotics are commonly classified based on their mechanism of action, chemical structure, or spectrum of activity. More specifically, narrow spectrum antibiotics target specific types of bacteria, such as Gram-negative or Gram-positive bacteria, whereas broad spectrum antibiotics affect a wide range of bacteria. Following a 40-year hiatus in discovering new classes of antibacterial compounds, three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).



Figure: **Bacterial Cultures**: In antibacterial production, microorganisms must be isolated, cultured, and tested for growth inhibition of target organisms and for their selective toxicity.

Some antibacterials have been associated with a range of adverse effects. Side-effects range from mild to very serious depending on the antibiotics used, the microbial organisms targeted, and the individual patient. Safety profiles of newer drugs are often not as well established as for those that have a long history of use. Adverse effects range from fever and nausea to major allergic reactions, including photodermatitis and anaphylaxis. Common side-effects include diarrhea, resulting from disruption of the species composition in the intestinal flora, resulting, for example, in overgrowth of pathogenic bacteria, such as *Clostridium difficile*. Antibacterials can also affect the vaginal flora, and may lead to overgrowth of yeast species of the genus *Candida* in the vulvo-vaginal area. Additional side-effects can result from interaction with other drugs, such as elevated risk of tendon damage from administration of a quinolone antibiotic with a systemic corticosteroid.

Antibacterial Production

Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics. Other antibiotics simply lack advantage over those already in use, or have no other practical applications. Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and

therapeutic activities, and the best candidates can be examined and possibly modified. A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

Key Points

- Their mechanism of action, chemical structure, or spectrum of activity are ways in which antibiotics are classified.
- Broad spectrum antibiotics affect a wide range of bacteria, while narrow spectrum antibiotics are able to target specific types.
- Antibiotics must go through a screening process, where they are isolated, cultured, and then tested for production of diffusible products that inhibit the growth of specific test organisms.
- Due to potential adverse side effects, antibiotics must also be tested for their selective toxicities.

Key Terms

- **antibacterial**: A drug having the effect of killing or inhibiting bacteria.
- **bactericidal**: An agent that kills bacteria.
- **bacteriostatic**: A drug that prevents bacterial growth and reproduction but does not necessarily kill them. When it is removed from the environment the bacteria start growing again.

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12.1D: Spectrum of Antimicrobial Activity

Learning Objectives

- Compare narrow and broad spectrum antibiotics

The range of bacteria that an antibiotic affects can be divided into narrow spectrum and broad spectrum. Narrow spectrum antibiotics act against a limited group of bacteria, either gram positive or gram negative, for example sodium fusidate only acts against staphylococcal bacteria. Broad spectrum—antibiotics act against gram positive and gram negative bacteria, for example amoxicillin.

Gram staining (or Gram's method; is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram positive bacteria. A Gram positive results in a purple/blue color while a Gram negative results in a pink/red color. The Gram stain is almost always the first step in the identification of a bacterial organism, and is the default stain performed by laboratories over a sample when no specific culture is referred. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram-variable and Gram-indeterminate groups as well.

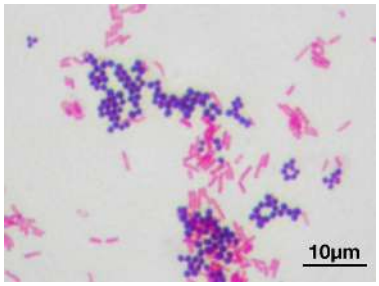


Figure: **Gram Stain**: This is a microscopic image of a Gram stain of mixed Gram-positive cocci (*Staphylococcus aureus*, purple) and Gram-negative bacilli (*Escherichia coli*, red).

A broad spectrum antibiotic acts against both Gram-positive and Gram-negative bacteria, in contrast to a narrow spectrum antibiotic, which is effective against specific families of bacteria. An example of a commonly used broad-spectrum antibiotic is ampicillin. Broad spectrum antibiotics are properly used in the following medical situations: empirically (i.e., based on the experience of the practitioner), prior to the formal identification of the causative bacteria and when there is a wide range of possible illnesses and a potentially serious illness would result if treatment is delayed. This occurs, for example, in meningitis, where the patient can become fatally ill within hours if broad-spectrum antibiotics are not initiated. Broad spectrum antibiotics are also used for drug resistant bacteria that do not respond to other, more narrow spectrum antibiotics and in the case of superinfections, where there are multiple types of bacteria causing illness, thus warranting either a broad-spectrum antibiotic or combination antibiotic therapy.

Following a 40-year hiatus in discovering new classes of antibacterial compounds, three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).

Key Points

- Broad spectrum antibiotics act against a larger group of bacteria.
- Narrow spectrum antibiotis target specific bacteria such as Gram positive or Gram negative.
- Three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).

Key Terms

- **Gram stain**: A method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative).
- **narrow spectrum antibiotic**: A type of antibiotic that targets specific types of Gram positive or Gram negative bacteria.

- **broad spectrum antibiotic**: A type of antibiotic that can affect a wide range of bacteria.

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12.1E: Antibiotic Classifications

Learning Objectives

Compare the two classes of antibiotics: bactericidal and bacteriostatic antibiotic

Antibiotics can be divided into two classes based on their mechanism of action. Bactericidal antibiotics kill bacteria; bacteriostatic antibiotics inhibit their growth or reproduction.

One way that bactericidal antibodies kill bacteria is by inhibiting cell wall synthesis. Examples include the Beta-lactam antibiotics (penicillin derivatives (penams)), cephalosporins (cephems), monobactams, and carbapenems) and vancomycin. Other ways that bactericidal antibiotics kill bacteria include inhibiting bacterial enzymes or protein translation. Other batericidal agents include daptomycin, fluoroquinolones, metronidazole, nitrofurantoin, co-trimoxazole and telithromycin. Aminoglycosidic antibiotics are usually considered bactericidal, although they may be bacteriostatic with some organisms. The MBC (minimum bactericidal concentration) is the minimum concentration of drug which can kill 99.99% of the population.

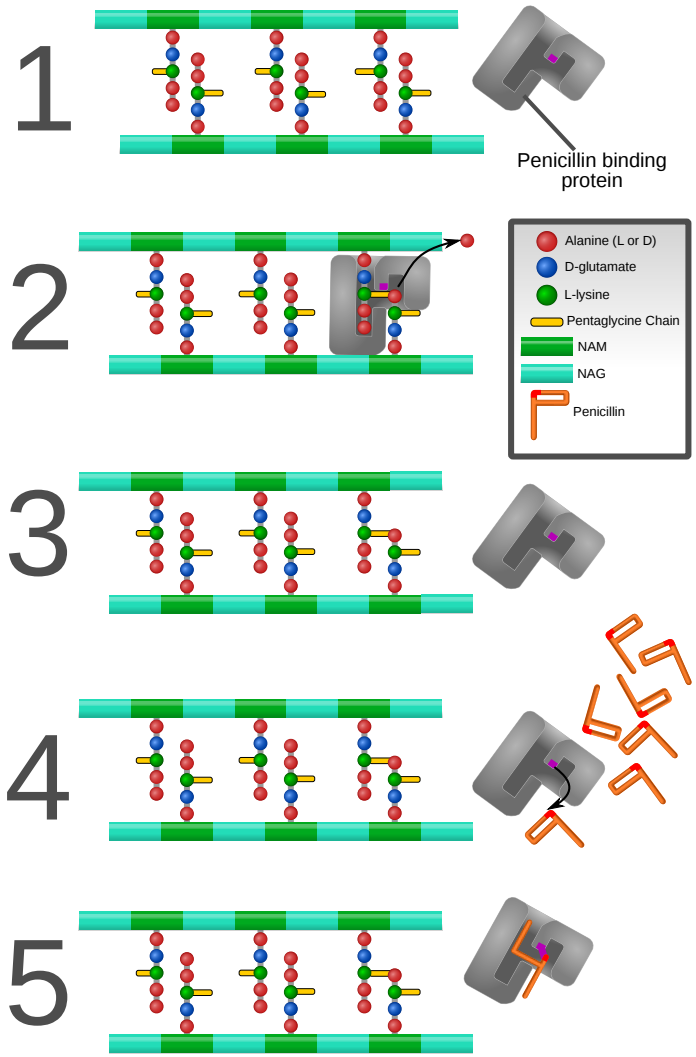


Figure: **Mechanism of penicillin inhibition**: Penicillin and most other β-lactam antibiotics act by inhibiting penicillin-binding proteins, which normally catalyze cross-linking of bacterial cell walls.

Bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism. This group includes: tetracyclines, sulfonamides, spectinomycin, trimethoprim,

chloramphenicol, macrolides and lincosamides. They must work together with the immune system to remove the microorganisms from the body. However, there is not always a precise distinction between them and bactericidal antibiotics. High concentrations of some bacteriostatic agents are also bactericidal. The MIC (minimum inhibitory concentration) is the minimum concentration of drug which can inhibit the growth of the microorganism.

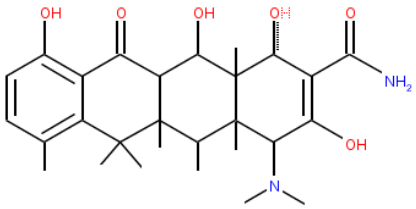


Figure: ***Structure of tetracycline****: Tetracycline antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex.*

Further categorization is based on their target specificity. "Narrow-spectrum" antibacterial antibiotics target specific types of bacteria, such as Gram-negative or Gram-positive bacteria, whereas broad-spectrum antibiotics affect a wide range of bacteria, usually both gram positive and gram negative cells. Following a 40-year hiatus in discovering new classes of antibacterial compounds, three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).

Key Points

- Bactericidal antibodies inhibit cell wall synthesis.
- Bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism.
- Bacteriostatic antibiotics must work together with the immune system to remove the microorganisms from the body.

Key Terms

- **bactericidal**: An agent that kills bacteria.
- **bacteriostatic**: A drug that prevents bacterial growth and reproduction but does not necessarily kill them. When it is removed from the environment the bacteria start growing again.

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SECTION OVERVIEW

12.2: Functions of Antimicrobial Drugs

Topic hierarchy

12.2A: Inhibiting Cell Wall Synthesis

12.2B: Injuring the Plasma Membrane

12.2C: Inhibiting Nucleic Acid Synthesis

12.2D: Inhibiting Protein Synthesis

12.2E: Inhibiting Essential Metabolite Synthesis

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12.2A: Inhibiting Cell Wall Synthesis

Learning Objectives

- Describe the two types of antimicrobial drugs that inhibit cell wall synthesis: beta-lactam and glycopeptide antibiotics

Two types of antimicrobial drugs work by inhibiting or interfering with cell wall synthesis of the target bacteria. Antibiotics commonly target bacterial cell wall formation (of which peptidoglycan is an important component) because animal cells do not have cell walls. The peptidoglycan layer is important for cell wall structural integrity, being the outermost and primary component of the wall.

The first class of antimicrobial drugs that interfere with cell wall synthesis are the β-Lactam antibiotics (beta-lactam antibiotics), consisting of all antibiotic agents that contains a β-lactam nucleus in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. β-Lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The final step in the synthesis of the peptidoglycan is facilitated by penicillin-binding proteins (PBPs). PBPs vary in their affinity for binding penicillin or other β-lactam antibiotics.

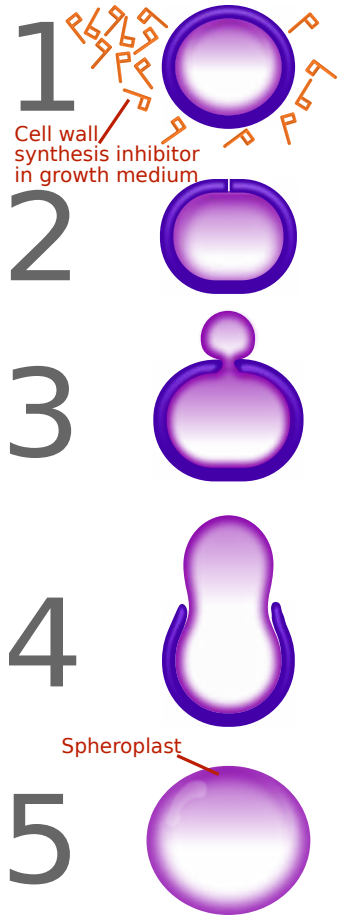


Figure: **Penicillin spheroplast generation**: Diagram depicting the failure of bacterial cell division in the presence of a cell wall synthesis inhibitor (e.g. penicillin, vancomycin).1- Penicillin (or other cell wall synthesis inhibitor) is added to the growth medium with a dividing bacterium.2- The cell begins to grow, but is unable to synthesize new cell wall to accommodate the expanding cell.3- As cellular growth continues, cytoplasm covered by plasma membrane begins to squeeze out through the gap(s) in the cell wall.4- Cell wall integrity is further violated. The cell continues to increase in size, but is unable to “pinch off” the extra cytoplasmic material into two daughter cells because the formation of a division furrow depends on the ability to synthesize new cell wall.5- The cell wall is shed entirely, forming a spheroplast, which is extremely vulnerable relative to the original cell. The loss of the cell wall also causes the cell to lose control over its shape, so even if the original bacterium were rod-shaped, the sphereoplast is generally spherical. Finally, the fact that the cell has now doubled much of its genetic and metabolic material further disrupts homeostasis, which usually leads to the cell’s death.

Bacteria often develop resistance to β-lactam antibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid.

The second class of antimicrobial drugs that interfere with cell wall synthesis are the glycopeptide antibiotics, which are composed of glycosylated cyclic or polycyclic nonribosomal peptides. Significant glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin. This class of drugs inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis. They bind to the amino acids within the cell wall preventing the addition of new units to the peptidoglycan.

Key Points

- The peptidoglycan layer is important for cell wall structural integrity, being the outermost and primary component of the wall.
- β-Lactam antibiotics are a broad class of antibiotics that includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
- β-Lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls.
- Glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin.
- Glycopeptide antibiotics inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis.

Key Terms

- **beta-lactam antibiotic**: A broad class of antibiotics that inhibit cell wall synthesis, consisting of all antibiotic agents that contains a β-lactam nucleus in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
- **Glycopeptide antibiotic**: Glycopeptide antibiotics are composed of glycosylated cyclic or polycyclic nonribosomal peptides. Significant glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin. This class of drugs inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis.
- **peptidoglycan**: A polymer of glycan and peptides found in bacterial cell walls.

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12.2B: Injuring the Plasma Membrane

Learning Objectives

- Discuss the function of the plasma membrane and how antimicrobial drugs target it

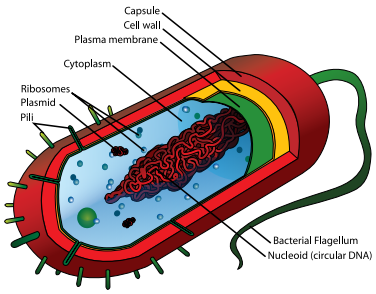


Figure: **Prokaryotic Cell**: Diagram of a typical gram-negative bacterium, with the thin cell wall sandwiched between the red outer membrane and the thin green plasma membrane.

The plasma membrane or cell membrane is a biological membrane that separates the interior of all cells from the outside environment. The plasma membrane is selectively permeable to ions and organic molecules. It controls the movement of substances in and out of cells. The membrane basically protects the cell from outside forces. It consists of the lipid bilayer with embedded proteins. Plasma membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity, and cell signaling. It serves as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton. Fungi, bacteria, and plants also have the cell wall which provides a mechanical support for the cell and precludes the passage of larger molecules. The plasma membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell and in attaching to the extracellular matrix and other cells to help group cells together to form tissues.

There are several types of antimicrobial drugs that function by disrupting or injuring the plasma membrane. One example is daptomycin, a lipopeptide which has a distinct mechanism of action, disrupting multiple aspects of bacterial cell membrane function. It appears to bind to the membrane causes rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis, which results in bacterial cell death. Another example is polymyxins antibiotics which have a general structure consisting of a cyclic peptide with a long hydrophobic tail. They disrupt the structure of the bacterial cell membrane by interacting with its phospholipids.

Key Points

- The plasma membrane or cell membrane is a biological membrane that separates the interior of all cells from the outside environment.
- Plasma membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity, and cell signaling. They serve as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton.
- Disrupting the plasma membrane causes rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis, which results in bacterial cell death.

Key Terms

- **plasma membrane**: The semipermeable membrane that surrounds the cytoplasm of a cell.
- **cell wall**: A thick, fairly rigid layer formed around individual cells of bacteria, Archaea, fungi, plants, and algae, the cell wall is external to the cell membrane and helps the cell maintain its shape and avoid damage.
- **plasma cell**: a form of lymphocyte that produces antibodies when reacted with a specific antigen; a plasmacyte

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12.2C: Inhibiting Nucleic Acid Synthesis

Learning Objectives

- State the steps where inhibitors of nucleic acid synthesis can exert their function

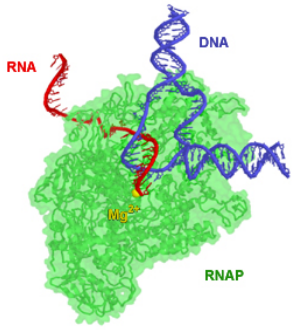


Figure: **Diagram of Transcription**: RNA Polymerase, an enzyme that produces RNA, from T. aquaticus pictured during elongation. Portions of the enzyme were made transparent so as to make the path of RNA and DNA more clear. The magnesium ion (yellow) is located at the enzyme active site.

Antimicrobial drugs can target nucleic acid (either RNA or DNA) synthesis. The antimicrobial actions of these agents are a result of differences in prokaryotic and eukaryotic enzymes involved in nucleic acid synthesis.

Prokaryotic transcription is the process in which messenger RNA transcripts of genetic material are produced for later translation into proteins. The transcription process includes the following steps: initiation, elongation and termination. Antimicrobial drugs have been developed to target each of these steps. For example, the antimicrobial rifampin binds to DNA-dependent RNA polymerase, thereby inhibiting the initiation of RNA transcription.

Other antimicrobial drugs interfere with DNA replication, the biological process that occurs in all living organisms and copies their DNA and is the basis for biological inheritance. The process starts when one double-stranded DNA molecule produces two identical copies of the molecule. In a cell, DNA replication begins at specific locations in the genome, called “origins. ” Uncoiling of DNA at the origin, and synthesis of new strands, forms a replication fork. In addition to DNA polymerase, the enzyme that synthesizes the new DNA by adding nucleotides matched to the template strand, a number of other proteins are associated with the fork and assist in the initiation and continuation of DNA synthesis. DNA replication, like all biological polymerization processes, proceeds in three enzymatically catalyzed and coordinated steps: initiation, elongation and termination. Any of the steps in the process of DNA replication can be targeted by antimicrobial drugs. For instance, quinolones inhibit DNA synthesis by interfering with the coiling of DNA strands.

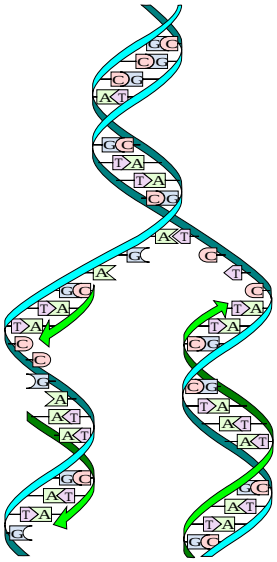


Figure: **DNA Replication**: The double helix is unwound and each strand acts as a template for the next strand. Bases are matched to synthesize the new partner strands.

Key Points

- Some antimicrobial drugs interfere with the initiation, elongation or termination of RNA transcription.
- Some antimicrobial drugs interfere with various aspects of DNA replication.
- The antimicrobial actions of these drugs are a result of differences in the prokaryotic and eukaryotic enzymes involved in nucleic acid synthesis.

Key Terms

- **transcription**: The synthesis of RNA under the direction of DNA.
- **replication**: Process by which an object, person, place or idea may be copied mimicked or reproduced.

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12.2D: Inhibiting Protein Synthesis

Learning Objectives

- Paraphrase the general mechanism of action of protein synthesis inhibitors

A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins. It usually refers to substances, such as antimicrobial drugs, that act at the ribosome level. The substances take advantage of the major differences between prokaryotic and eukaryotic ribosome structures which differ in their size, sequence, structure, and the ratio of protein to RNA. The differences in structure allow some antibiotics to kill bacteria by inhibiting their ribosomes, while leaving human ribosomes unaffected.

Translation in prokaryotes involves the assembly of the components of the translation system which are: the two ribosomal subunits (the large 50S & small 30S subunits), the mRNA to be translated, the first aminoacyl tRNA, GTP (as a source of energy), and three initiation factors that help the assembly of the initiation complex. The ribosome has three sites: the A site, the P site, and the E site (not shown in). The A site is the point of entry for the aminoacyl tRNA. The P site is where the peptidyl tRNA is formed in the ribosome. The E site which is the exit site of the now uncharged tRNA after it gives its amino acid to the growing peptide chain.

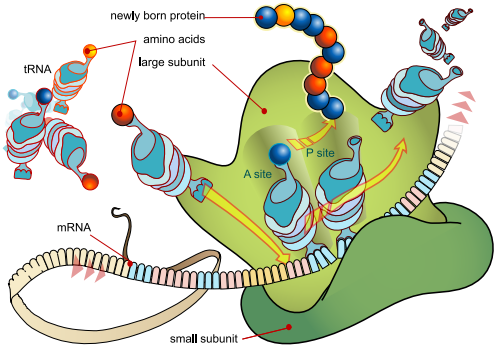


Figure: **Simplified diagram of protein synthesis**: Diagram showing how the translation of the mRNA and the synthesis of proteins is made by ribosomes.

In general, protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins like initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and ribosomal translocation), and termination. The following is a list of common antibacterial drugs and the stages which they target.

- Linezolid acts at the initiation stage, probably by preventing the formation of the initiation complex, although the mechanism is not fully understood.
- Tetracyclines and Tigecycline (a glycylcycline related to tetracyclines) block the A site on the ribosome, preventing the binding of aminoacyl tRNAs.
- Aminoglycosides, among other potential mechanisms of action, interfere with the proofreading process, causing an increased rate of error in synthesis with premature termination.
- Chloramphenicol blocks the peptidyl transfer step of elongation on the 50S ribosomal subunit in both bacteria and mitochondria.
- Macrolides, clindamycin, and aminoglycosides have evidence of inhibition of ribosomal translocation.
- Streptogramins also cause premature release of the peptide chain.

By targeting different stages of the mRNA translation, antimicrobial drugs can be changed if resistance develops to one or many of the drugs.

Key Points

- Protein synthesis inhibitors usually act at the ribosome level, taking advantage of the major differences between prokaryotic and eukaryotic ribosome structures.

- Protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins like initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and ribosomal translocation), and termination.
- By targeting different stages of the mRNA translation, antimicrobial drugs can be changed if resistance develops.

Key Terms

- **translation**: A process occurring in the ribosome, in which a strand of messenger RNA (mRNA) guides assembly of a sequence of amino acids to make a protein.

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12.2E: Inhibiting Essential Metabolite Synthesis

Learning Objectives

- Distinguish between the three main types of antimetabolite antibiotics (antifolates, pyrimidine and purine analogues)

An antimetabolite is a chemical that inhibits the use of a metabolite, a chemical that is part of normal metabolism. Such substances are often similar in structure to the metabolite that they interfere with, such as antifolates that interfere with the use of folic acid. The presence of antimetabolites can have toxic effects on cells, such as halting cell growth or cell division.

Antimetabolites are also used as antibiotics. There are three main types of antimetabolite antibiotics. The first, antifolates impair the function of folic acid leading to disruption in the production of DNA and RNA. For example, methotrexate is a folic acid analogue, and owing to structural similarity with folic acid, methotrexate binds and inhibits the enzyme dihydrofolate reductase, and thus prevents the formation of tetrahydrofolate. Because tetrahydrofolate is essential for purine and pyrimidine synthesis, its deficiency can lead to inhibited production of DNA, RNA and proteins.

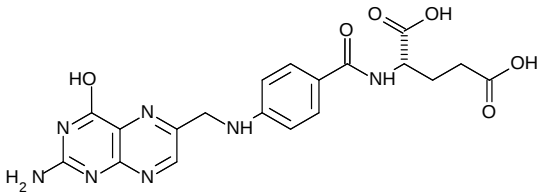


Figure: **Folic Acid Structure**: This is the chemical structure of folic acid.

The second type of antimetabolite antibiotics consist of pyrimidine analogues which mimic the structure of metabolic pyrimidines. Three nucleobases found in nucleic acids, cytosine (C), thymine (T), and uracil (U), are pyrimidine derivatives and the pyrimidine analogues disrupt their formation and consequently disrupt DNA and RNA synthesis.

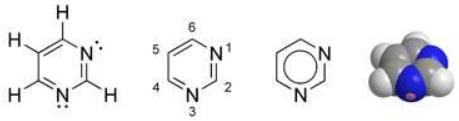


Figure: **Pyrimidine Structure**: This is the chemical structure of pyrimidine.

The purine analogues are the third type of antimetabolite antibiotics and they mimic the structure of metabolic purines. Two of the four bases in nucleic acids, adenine and guanine, are purines. Purine analogues disrupt nucleic acid production. For example, azathioprine is the main immunosuppressive cytotoxic substance that is widely used in transplants to control rejection reactions by inhibiting DNA synthesis in lymphocytes.

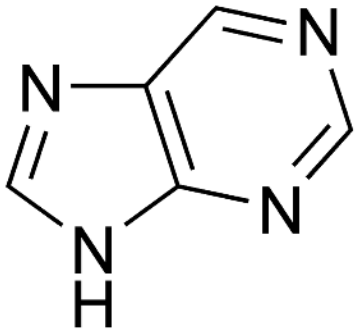


Figure: **Purine Structure**: This is the chemical structure of purine.

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Key Points

- The presence of antimetabolites can have toxic effects on cells, such as halting cell growth or cell division.
- Antimetabolites are also used as antibiotics.
- The three main types of antimetabolite antibiotics are antifolates, pyrimidine analogues and purine analogues.

Key Terms

- **antimetabolite**: Any substance that competes with or inhibits the normal metabolic process, often by acting as an analogue of an essential metabolite

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SECTION OVERVIEW

12.3: Commonly Used Antimicrobial Drugs

Topic hierarchy

12.3A: Synthetic Antimicrobial Drugs

12.3B: Naturally Occurring Antimicrobial Drugs- Antibiotics

12.3C: Beta-Lactam Antibiotics- Penicillins and Cephalosporins

12.3D: Antibiotics from Prokaryotes

12.3E: Antimycobacterial Antibiotics

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12.3A: Synthetic Antimicrobial Drugs

Learning Objectives

- Recall the synthetic antimicrobial drugs that are sulfonamide and sulphonamide based

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become resistant to the poisons and/or heavy metals.

Antimicrobial nanotechnology is a recent addition to the fight against disease-causing organisms, replacing heavy metals and toxins, and may some day be used as a viable alternative.

Infections that are acquired during a hospital visit are called "hospital acquired infections" or nosocomial infections. Similarly, when the infectious disease is picked up in the non-hospital setting, it is considered "community acquired".

Synthetic agents include: sulphonamides, cotrimoxazole, quinolones, anti-virals, anti-fungals, anti-cancer drugs, anti-malarials, anti-tuberculosis drugs, anti-leprotics, and anti-protozoals.

Sulfonamide or sulphonamide is the basis of several groups of drugs. The original antibacterial sulfonamides (sometimes called sulfa drugs or sulpha drugs) are synthetic antimicrobial agents that contain the sulfonamide group. Some sulfonamides are also devoid of antibacterial activity, e.g., the anticonvulsant sultiame. The sulfonylureas and thiazide diuretics are newer drug groups based on the antibacterial sulfonamides.

Sulfa allergies are common, and medications containing sulfonamides are prescribed carefully. It is important to make a distinction between sulfa drugs and other sulfur-containing drugs and additives, such as sulfates and sulfites, which are chemically unrelated to the sulfonamide group and do not cause the same hypersensitivity reactions seen in the sulfonamides.

In bacteria, antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthetase (DHPS), an enzyme involved in folate synthesis. As such, the microorganism will be "starved" of folate and die.

The sulfonamide chemical moiety is also present in other medications that are not antimicrobials, including thiazide diuretics (including hydrochlorothiazide, metolazone, and indapamide, among others), loop diuretics (including furosemide, bumetanide, and torsemide), sulfonylureas (including glipizide, glyburide, among others), and some COX-2 inhibitors (e.g., celecoxib), and acetazolamide.

Key Points

- The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world.
- With the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents.

- Synthetic agents include: sulphonamides, cotrimoxazole, quinolones, anti-virals, anti-fungals, anti-cancer drugs, anti-malarials, anti-tuberculosis drugs, anti-leprotics, and anti-protozoals.

Key Terms

- **antimicrobial**: An agent that destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action.
- **microorganism**: An organism that is too small to be seen by the unaided eye, especially a single-celled organism, such as a bacterium.
- **bacteria**: A type, species, or strain of bacterium.

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12.3B: Naturally Occurring Antimicrobial Drugs- Antibiotics

Learning Objectives

- Discuss the mechanism of action for protein synthesis inhibitors used as antimicrobial drugs, and recognize various naturally occuring antimicrobial drugs

An antimicrobial is a substance that kills or inhibits the growth of microorganisms bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

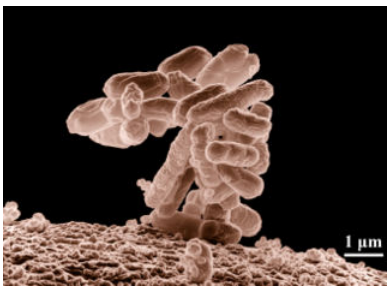


Figure: **A cluster of Escherichia coli Bacteria magnified 10,000 times.**: A cluster of Escherichia coli Bacteria magnified 10,000 times.

The discovery of antimicrobials, like penicillin and tetracycline, paved the way for better health for millions of people around the world. Before penicillin became a viable medical treatment in the early 1940's, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely. This allowed the microbe to survive, change, and become resistant to the poisons and/or heavy metals.

Antimicrobial nanotechnology is a recent addition to the fight against disease causing organisms. It replaces heavy metals and toxins and may someday be a viable alternative.

Infections that are acquired during a hospital visit are called "hospital acquired infections" or nosocomial infections. Similarly, when the infectious disease is picked up in the non-hospital setting it is considered "community acquired. "

There are mainly two classes of antimicrobial drugs: those obtained from natural sources (i.e. beta-lactam) antibiotic (such as penicillins, cephalosporins) or protein synthesis inhibitors (such as aminoglycosides, macrolides, tetracyclines, chloramphenicol, polypeptides); and synthetic agents.

A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.

A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins. While a broad interpretation of this definition could be used to describe nearly any antibiotic, in practice, it usually refers to substances that act at the ribosome level (either the ribosome itself or the translation factor), taking advantage of the major differences between prokaryotic and eukaryotic ribosome structures. Toxins such as ricin also function via protein synthesis inhibition. Ricin acts at the eukaryotic 60S.

In general, protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins, like initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and ribosomal translocation), and termination. Rifamycin inhibits prokaryotic DNA transcription into mRNA by inhibiting DNA-dependent RNA polymerase by binding its beta-subunit. Linezolid acts at the initiation stage probably by preventing the formation of the initiation complex, although the mechanism is not fully understood.

Tetracyclines and Tigecycline (a glycylcycline related to tetracyclines) block the A site on the ribosome, preventing the binding of aminoacyl tRNAs. Aminoglycosides, among other potential mechanisms of action, interfere with the proofreading process, causing increased rate of error in synthesis with premature termination. Chloramphenicol blocks the peptidyl transfer step of elongation on the 50S ribosomal subunit in both bacteria and mitochondria. Macrolides (as well as inhibiting ribosomal translocation and other potential mechanisms) bind to the 50s ribosomal subunits, inhibiting peptidyl transfer. Quinupristin/dalfopristin act synergistically, with dalfopristin, enhancing the binding of quinupristin as well as inhibiting peptidyl transfer. Quinupristin binds to a nearby site on the 50S ribosomal subunit and prevents elongation of the polypeptide. It also causes incomplete chains to be released. Macrolides, clindamycin, and aminoglycosides (with all these three having other potential mechanisms of action as well) have evidence of inhibition of ribosomal translocation. Fusidic acid prevents the turnover of elongation factor G (EF-G) from the ribosome. Macrolides and clindamycin (both also having other potential mechanisms) cause premature dissociation of the peptidyl-tRNA from the ribosome. Puromycin has a structure similar to that of the tyrosinyl aminoacyl-tRNA. Therefore, it binds to the ribosomal A site and participates in peptide bond formation, producing peptidyl-puromycin. However, it does not engage in translocation and quickly dissociates from the ribosome, causing a premature termination of polypeptide synthesis.

Key Points

- There are mainly two classes of antimicrobial drugs: those obtained from natural sources (i.e. beta-lactam antibiotic (such as penicillins, cephalosporins) or protein synthesis inhibitors (such as aminoglycosides, macrolides, tetracyclines, chloramphenicol, polypeptides); and synthetic agents.
- A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such because the nitrogen atom is attached to the β-carbon relative to the carbonyl.
- A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins.

Key Terms

- **β-lactam**: A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.
- **antimicrobial**: An agent that destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action.
- **microorganism**: An organism that is too small to be seen by the unaided eye, especially a single-celled organism, such as a bacterium.

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12.3C: Beta-Lactam Antibiotics- Penicillins and Cephalosporins

Learning Objectives

- Recognize the classes of beta-lactams and their mechanisms of action

A β-lactam (beta-lactam) ring, is a four-membered lactam. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.

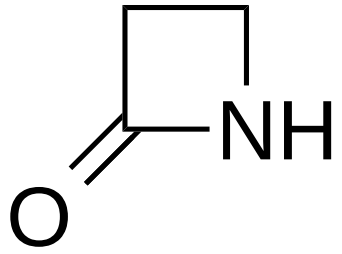


Figure: **β-Lactam**: β-Lactam ring is a four-membered lactam.

The β-lactam ring is part of the core structure of several antibiotic families, the principal ones being the penicillins, cephalosporins, carbapenems, and monobactams, which are, therefore, also called β-lactam antibiotics. Nearly all of these antibiotics work by inhibiting bacterial cell wall biosynthesis. This has a lethal effect on bacteria. Bacteria do, however, contain within their populations, in smaller quantities, bacteria that are resistant against β-lactam antibiotics. They do this by expressing the β-lactamase gene. When bacterial populations have these resistant subgroups, treatment with β-lactam can result in the resistant strain becoming more prevalent and so, more virulent.

β-Lactams are classified according to their core ring structures:

- β-Lactams fused to saturated five-membered rings;
- β-Lactams containing thiazolidine rings are named *penams*;
- β-Lactams containing pyrrolidine rings are named *carbapenams*;
- β-Lactams fused to oxazolidine rings are named *oxapenams* or *clavams*;
- β-Lactams fused to unsaturated five-membered rings;
- β-Lactams containing 2,3-dihydrothiazole rings are named *penems*;
- β-Lactams containing 2,3-dihydro-1H-pyrrole rings are named *carbapenems*;
- β-Lactams fused to unsaturated, six-membered rings;
- β-Lactams containing 3,6-dihydro-2H-1,3-thiazine rings are named *cephems*;
- β-Lactams containing 1,2,3,4-tetrahydropyridine rings are named *carbacephems*;
- β-Lactams containing 3,6-dihydro-2H-1,3-oxazine rings are named *oxacephems*; and
- β-Lactams not fused to any other ring are named *monobactams*.

Penicillin (sometimes abbreviated PCN or pen) is a group of antibiotics derived from *Penicillium fungi.* They include penicillin G, procaine penicillin, benzathine penicillin, and penicillin V. Penicillin antibiotics are historically significant because they are the first drugs that were effective against many previously serious diseases, such as syphilis, and infections caused by staphylococci and streptococci. Penicillins are still widely used today, though many types of bacteria are now resistant. All penicillins are β-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms.

The cephalosporins (sg. /ˌsɛfələˈspɔrɨn/) are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was previously known as “Cephalosporium”. Together with cephamycins, they constitute a subgroup of β-lactam antibiotics called *cephems*. Cephalosporins are indicated for the prophylaxis and treatment of infections caused by bacteria susceptible to this particular form of antibiotic. First-generation cephalosporins are active predominantly against Gram-positive bacteria, and successive generations have increased activity against Gram-negative bacteria (albeit often with reduced activity against Gram-positive organisms).

Key Points

- The principal antibiotic families of which the β-lactam ring is part of the core structure are the penicillins, cephalosporins, carbapenems, and monobactams, which are also called β-lactam antibiotics.
- A β-lactam (beta-lactam) ring is a four-membered lactam (cyclic amide). -Lactams are classified according to their core ring structures.
- The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was previously known as “Cephalosporium”.

Key Terms

- **cephalosporins**: The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was previously known as “Cephalosporium”.
- **antibiotic**: Any substance that can destroy or inhibit the growth of bacteria and similar microorganisms.
- **β-lactam**: A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.
- **β-lactam**: Any of a class of cyclic amides, that are the nitrogen analogs of lactones, formed by heating amino acids; the tautomeric enol forms are known as lactims.

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12.3D: Antibiotics from Prokaryotes

Learning Objectives

- Explain the role of Streptomyces and other prokaryotes in antibiotic production

Even though penicillin drugs, antibiotics produced by molds, were the first antibiotics successfully used to treat many serious infections, most of the naturally produced antibiotics are synthesized by bacteria. In 1939 the French microbiologist René Dubos isolated the substance tyrothricin and later showed that it was composed of two substances, gramicidin (20%) and tyrocidine (80%). These were the first antibiotics to be manufactured commercially. Gramicidin is a heterogeneous mixture of six antibiotic compounds, all of which are obtained from the soil bacterial species *Bacillus brevis* and called collectively gramicidin D.

Streptomyces is the largest antibiotic-producing genus, producing antibacterial, antifungal, and antiparasitic drugs, and also a wide range of other bioactive compounds, such as immunosuppressants. They produce over two-thirds of the clinically useful antibiotics of natural origin. The now uncommonly-used streptomycin takes its name directly from *Streptomyces*. Aminoglycosides, class of antibiotics, that are derived from bacteria of the *Streptomyces* genus are named with the suffix -mycin, whereas those that are derived from *Micromonospora* are named with the suffix -micin. However, this nomenclature system is not specific for aminoglycosides.



Figure: **Supernatant of a Streptomyces davawensis culture**: The picture shows the typical red color of the antibiotic Roseoflavin secreted by the Streptomyces cells in the culture.

Streptomycetes are characterised by a complex secondary metabolism. Almost all of the bioactive compounds produced by *Streptomyces* are initiated during the time coinciding with the aerial hyphal formation from the substrate mycelium.

Streptomycetes produce numerous antifungal compounds of medicinal importance, including nystatin (from *S. noursei*), amphotericin B (from *S. nodosus*), and natamycin (from *S. natalensis*).

Members of the *Streptomyces* genus are the source for numerous antibacterial pharmaceutical agents; among the most important of these are: Chloramphenicol (from *S. venezuelae*), Daptomycin (from *S. roseosporus*), Fosfomycin (from *S. fradiae*), Lincomycin (from *S. lincolnensis*), Neomycin (from *S. fradiae*), Puromycin (from *S. alboniger*), Streptomycin (from *S. griseus*), Tetracycline (from *S. rimosus* and *S. aureofaciens*).

Clavulanic acid (from *S. clavuligerus*) is a drug used in combination with some antibiotics (like amoxicillin) to block and/or weaken some bacterial-resistance mechanisms by irreversible beta-lactamase inhibition.

Other bacterial species produce antibiotics as well. Such an example are some *Pseudomonas* species which produce antimicrobial compounds. *P. aurantiaca* produces di-2,4-diacetylfluoroglucylmethane, a compound antibiotically active against Gram-positive organisms. Other *Pseudomonas* spp. might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide.

Key Points

- Gramicidin is one of the first antibiotics to be manufactured commercially. It is a heterogeneous mixture of six antibiotic compounds, all of which are obtained from the soil bacterial species Bacillus brevis.
- Streptomyces is the largest antibiotic-producing genus, producing antibacterial, antifungal, and antiparasitic drugs, and also a wide range of other bioactive compounds, such as immunosuppressants. They produce over two-thirds of the clinically useful antibiotics of natural origin.
- Members of the Streptomyces genus are the source for numerous antibacterial pharmaceutical agents; among the most important of these are: Chloramphenicol (from S. venezuelae), Lincomycin (from S. lincolnensis), Neomycin (from S. fradiae), Tetracycline (from S. rimosus and S. aureofaciens).
- Some Pseudomonas spp. might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide.

Key Terms

- **antibiotic**: Any substance that can destroy or inhibit the growth of bacteria and similar microorganisms.
- **beta-lactamase**: An enzyme produced by certain bacteria, responsible for their resistance to beta-lactam antibiotics such as penicillin.

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12.3E: Antimycobacterial Antibiotics

Learning Objectives

- Compare and contrast the drugs used for treatment of Mycobacterium tuberculosis and Mycobacterium leprae

Antimycobacterial antibiotics are a class of antimicrobial drugs that target mycobacterium. Mycobacterium is a genus of Actinobacteria that includes pathogens known to cause serious and infectious disease. The types of pathogens considered to be mycobacterium include Mycobacterium tuberculosis (tuberculosis) and Mycobacterium leprae (leprosy). Mycobacterium grow in a mold-like manner on the surface of liquids when cultured. Antiomycobacterial antibiotics specifically target these types of microbes.

A type of antimycobacterial antibiotic includes the class of drugs used for tuberculosis (TB) treatment. The standard "short" course treatment for TB is isoniazid, rifampicin (also known as rifampin in the United States), pyrazinamide and ethambutol for two months, then isoniazid and rifampicin alone for another four months. The patient is considered cured at six months (although there is still a relapse rate of 2 to 3%). For latent tuberculosis, the standard treatment is six to nine months of isoniazid alone.

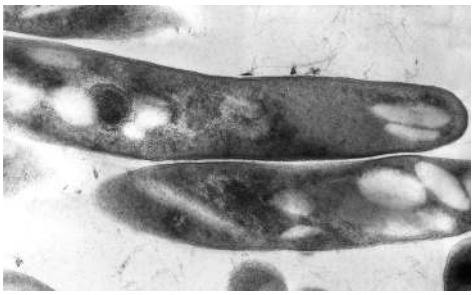


Figure: **Mycobacterium**: Mycobacterium are a class of bacteria defined by their ability to grow in a mold-like manner. Here, a TEM of Mycobacterium tuberculosis, the causative agent of tuberculosis. Antimycobacterial antibiotics target mycobacterium.

If the organism is known to be fully sensitive, then it is treated with isoniazid, rifampicin and pyrazinamide for two months, followed by isoniazid and rifampicin for four months. Ethambutol need not be used. Most regimens have an initial high-intensity phase, followed by a continuation phase (also called a consolidation phase or eradication phase) – the high-intensity phase is given first, then the continuation phase.

There are six classes of second-line drugs (SLDs) used for the treatment of TB. A drug may be classed as second-line instead of first-line for one of three possible reasons: it may be less effective than the first-line drugs (e.g., p-aminosalicylic acid); or, it may have toxic side-effects (e.g., cycloserine); or it may be unavailable in many developing countries (e.g., fluoroquinolones): aminoglycosides: e.g., amikacin (AMK), kanamycin (KM); polypeptides: e.g., capreomycin, viomycin, enviomycin; Fluoroquinolones: e.g., ciprofloxacin (CIP), levofloxacin, moxifloxacin (MXF); thioamides: e.g. ethionamide, prothionamide.

For treatment of leprosy, caused by Mycobacterium leprae, the traditional antimycobacterial drugs include promin (the first treatment introduced to fight leprosy) and dapsone (which eventually become obsolete as Mycobacterium leprae quickly evolved resistance). Modern drugs which were developed in response to the resistance was clofazimine and rifampicin. The use of multidrug therapies including dapsone, clofazimine and rifampicin were advantageous due to the low risk of antibiotic resistance. However, the use of these multidrug treatments was costly and only adopted in endemic countries when the World Health Assembly passed a resolution to eliminate leprosy in 1991.

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Key Points

- The standard “short” course treatment for TB is isoniazid, rifampicin, pyrazinamide, and ethambutol for two months, then isoniazid and rifampicin alone for another four months.
- The standard treatment for leprosy is a multidrug therapy that includes dapsone, clofazimine and rifampicin.
- Mycobacterium are defined by their ability to grow in a mold-like fashion on the surface of liquids when cultured.

Key Terms

- **tuberculosis**: Tuberculosis, MTB, or TB (short for tubercle bacillus) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually Mycobacterium tuberculosis.

- **infectious disease**: Infectious diseases, also known as transmissible diseases or communicable diseases comprise of clinically evident illness (i.e., characteristic medical signs and/or symptoms of disease) resulting from the infection, presence and growth of pathogenic biological agents in an individual host organism. In certain cases, infectious diseases may be asymptomatic for much or even all of their course in a given host. In the latter case, the disease may only be defined as a "disease" (which by definition means an illness) in hosts who secondarily become ill after contact with an asymptomatic carrier. An infection is not synonymous with an infectious disease, as some infections do not cause illness in a host.
- **leprosy**: Leprosy, also known as Hansen's disease (HD), is a chronic disease caused by the bacteria Mycobacterium leprae and Mycobacterium lepromatosis.
- **isoniazid**: a medication used in the prevention and treatment of tuberculosis, having the chemical formula C6H7N3O

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SECTION OVERVIEW

12.4: Interactions Between Drug and Host

Topic hierarchy

12.4A: Organ Toxicity

12.4B: Allergic Responses to Drugs

12.4C: Suppression and Alteration of Microbiota by Antimicrobials

12.4D: Effects of Drug Combinations

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12.4A: Organ Toxicity

Learning Objectives

- Outline the two major types of organ toxicity and their effects, recognizing additional types of toxicity

The use of antimicrobial drugs can have many unintended side-effects. The use, particularly when repeated, of many drugs can lead to an accumulation of a drug, or harmful byproducts from the metabolism of a drug, in tissues or organs. This accumulation of toxic chemicals can lead to organ damage, and in extreme cases, even organ failure and death. Two severe types of organ toxicity associated with antimicrobial drugs are nephrotoxicity and hepatotoxicity, toxicity of the kidneys and liver respectively.

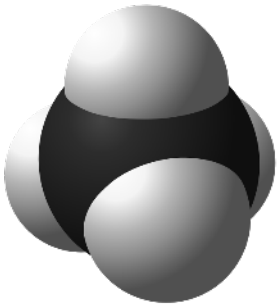


Figure: **Methane**: This space-filling model of methane shows the approximately spherical nature of this molecule.

The liver and kidneys are common organs affected by chemical toxicity. The kidneys are responsible for the filtration of the blood, so it is not surprising that deleterious agents in the blood may accumulate there. The liver is an important site for the breakdown of most metabolites in the body, and is referred to as the “metabolic clearing house” of the body. As drugs are quite often broken down in the liver, they can accumulate there and cause damage, or the byproducts of a drug’s metabolism can be toxic. In addition to direct damage to the liver by an antimicrobial drug, antimicrobial drugs can lead to the formation of dangerous toxins through the breakdown of microbes or due to interaction with other tissues in the body. These secondary toxins from drug metabolism then accumulate in the liver, potentially causing damage. Drug damage to the liver or kidneys can be particularly catastrophic as these organs are needed for the proper “cleaning” of the body. If they are compromised, this leads to further accumulation of potentially toxic metabolites further damaging organs in the body.



Figure: **Sun Poisoning**: This rash seen on a forearm is a typical reaction observed when an antibiotic causes phototoxicity.

Some of the toxic effects can be more benign, as is the case with ototoxicity, or damage to the ear. Use of some antibiotics, such as gentamicin, can cause a lose of hearing, or tinnitus (“ringing in the ears”). Ototoxicity is usually temporary with antibiotics, but permanent hearing damage, while rare, has been reported. Some antibiotics such as Tetracycline can cause phototoxicity, also known as sun poisoning; the result of which is that very short exposures to direct sunlight can cause severe skin irritation, with the appearance being quite similar to a rash or sunburn. The Tetracycline in a patient’s skin becomes toxic when exposed to sunlight, which causes an allergic reaction and leads to rash on the affected area. Broad-spectrum antibiotics in the family of fluoroquinolones can cause neurotoxicity by directly damaging neuronal receptors. This can lead to any number of psychological effects from mild cognitive dysfunction (brain fog) to more severe effects, such as hallucinations and suicidal thoughts.

While a few specific examples have been outlined here, toxic effects are not limited to the organs mentioned. In fact, almost any tissue or organ can be affected by antimicrobial drugs. However, it should be noted that the side-effects due to broad-spectrum antibiotics are actually quite rare, with organ damage being even more rare.The potential side-effects of antibiotics or other antimicrobial drugs are offset by the benefits of combating the microbial infection.

Key Points

- Antimicrobial drugs can have unintended side effects, including being toxic to organs.
- The liver and kidney are particularly susceptible to organ toxicity as they are the sites of toxin filtration and toxin metabolic breakdown.
- Almost any organ or tissue in the human body can be affected by antimicrobial toxicity.
- The toxic effects of antimicrobial drugs, while potentially harmful are very rare.

Key Terms

- **antimicrobrial drugs**: A drug administered to a patient, with the purpose of killing or slowing the growth of a microorganism, including protozoans, fungi and bacteria.
- **antibiotics**: A chemical that slows the growth of, or kills a bacteria.

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12.4B: Allergic Responses to Drugs

Learning Objectives

- Explain the physiology of an immune response responsible for an allergic reaction to drugs

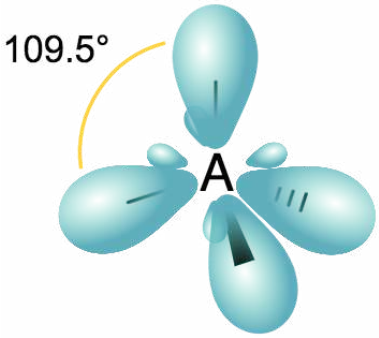


Figure: **sp3 hybridization**: In the process of sp^3 hybridization in methane, the single 2s and three 2p orbitals of carbon mix into four sp^3 hybrid orbitals, which are chemically and geometrically identical. Bond angles are 109.5°.

Virtually any substance, when exposed internally or externally to the body, can act as an allergen and illicit an immune system response, such is the case with antimicrobial drugs. While the drug acts as an allergen, the drug itself is not causing direct damage to the individual, but rather it is the response of an individual's immune system which is deleterious. An allergic reaction is the body's response to clear a foreign substance. Once the body recognizes a substance as foreign (in this case an antimicrobial drug), it starts producing antibodies, specifically immunoglobulin E (IgE) against the drug. The IgE binds directly to the drug and sets off a cascade of events, including the activation of receptors on immune system cells. This results in the production of histamines. The worst allergic reactions can be very severe and result in anaphylaxis. Anaphylaxis is an extremely severe allergic reaction caused when histamines are overproduced leading to severe contraction of bronchial muscles. In the most extreme cases, the airways close, which can lead to death. Other less severe symptoms of an allergic reaction can include, hives, angioedema (tissue swelling under the skin, often on the face), tight throat, coughing, wheezing, or watery eyes.

The exposure to a drug may not elicit an allergic reaction during the first exposure, but after the first exposure, the body creates antibodies and memory lymphocyte cells against the drug, therefore later exposures to the drug will illicit an immune response. There are many factors that can determine if an individual is sensitive to an antimicrobial drug, as with other allergens. Some factors include genetics and past exposures to other allergens, typically a person who has allergies to other things, such as various foods, is more prone to have or develop drug allergies. The most reported drug allergy is to Beta-lactam antibiotics, of which penicillin is the most well-known type, affecting 1-5% of people who take penicillin. While the most severe cases can result in anaphylaxis, most reactions are not severe. Additionally the allergic reaction may not even be due to the penicillin, as dyes and other chemicals added to antimicrobial drugs may in fact cause the allergic response instead. Taken together, recent studies show that perhaps only 1/5 people who suspect they have an allergy to penicillin do indeed have such an allergy.

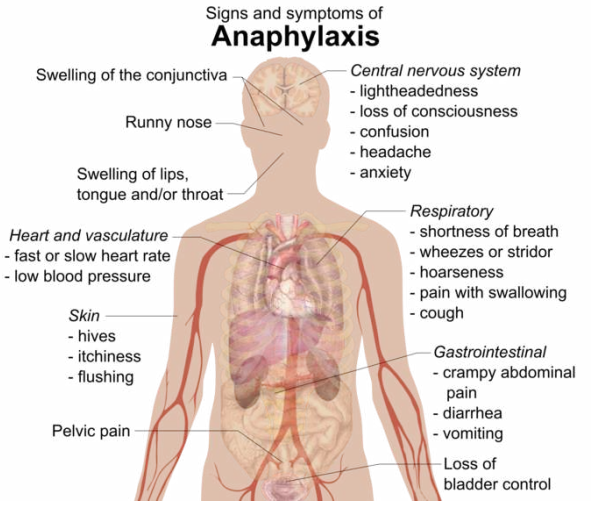


Figure: **Anaphylaxis**: A representation of the signs and symptoms of anaphylaxis that result from an allergic reaction. Signs include CNS symptoms such as confusion or lightheadedness, respiratory symptoms such as shortness of breath, gastrointestinal issues such as pain or vomiting, skin issues such as hives or itchiness, vascular symptoms such as change in heart rate or low blood pressure, and swelling of the mouth or eyes.

Key Points

- Antimicrobial drugs, can like almost any other substance, act as an allergen.
- Using a drug may not induce an allergic response the first time it is taken, however, subsequent use of the antimicrobial drugs may lead to allergic reactions.
- The most common antimicrobial drug allergy is penicillin. 1-5% of people who have taken it have suspected penicillin allergies.

Key Terms

- **allergen**: A substance, known as an antigen, which stimulates an immune response from a sensitive individual.
- **anaphylaxis**: A rapid and severe allergic reaction which can lead to death
- **Beta-lactam antibiotics**: A broad class of antibiotics of which penicillin is a member.

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12.4C: Suppression and Alteration of Microbiota by Antimicrobials

Learning Objectives

- Describe the role and function of the microbiota

The human body hosts thousands of different species of microbial organisms, known as the microbial flora or microbiota. Microbiota serve many functions in our body; most notable is the gut flora, crucial for the proper digestion of food, carbohydrate fermentation, and nutrient absorption. The gut flora in the human intestinal system has hundreds of species of microbes and over 100 trillion individual microbes; in comparison, the human body has around 10 trillion cells. Most of these microbes are bacterial and fungal. This is especially a problem when broad-spectrum antimicrobial agents are used, as antimicrobial treatments while helping to clear up pathogenic microbes from the body will often kill symbiotic bacteria. In addition, some microbial infections are due to translocation, the movement of advantageous bacteria to parts of the body where they might be harmful. An example is gut flora getting into the body's blood stream. The treatment of translocated or pathogenic bacteria may necessitate the use of antibiotics that will kill symbiotic bacteria. Antimicrobial agents which can kill beneficial gut flora can reduce the numbers of individual microbes or reduce the species of beneficial bacteria. In the case of the gut flora, this may impair the ability of a patient to properly metabolize food. If advantageous bacteria do not repopulate the intestine, this can lead to serious malnutrition problems.

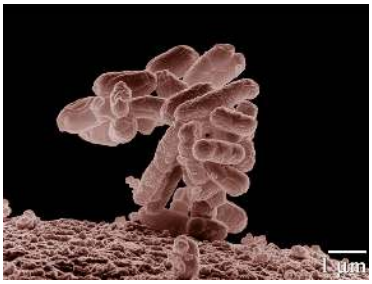


Figure: **Gut bacteria**: This is an electron micrograph, at 10,000X magnification. The oblong structures are Escherichia coli (E. coli), a symbiotic bacteria found in the human intestinal system.

In addition to serving a necessary function as gut flora due in metabolism of food, some microbiota in our bodies serve the function of keeping pathogenic microbes from inhabiting or dominating other flora at locations in our body. This is exemplified by Candida albicans, a yeast which is often found on humans. C. albicans is normally harmless, but when women take some antibiotics this can kill beneficial bacteria, specifically lactobacilli, in the vulvo-vaginal area. Without lactobacilli, C. albicans growth is not suppressed and can thus overgrow. This causes candidal vulvovaginitis, or yeast infections, a potentially painful infection of the vaginal mucous membranes by overgrown C. albicans. Yeast infections can be caused by antibiotics, as well as using aggressive topical cleaning agents such as detergents which again kill off beneficial lactobacilli allowing C. albicans to overgrow.

Fortunately there are antimicrobial agents that specifically target pathogenic bacterial species, which opposed to broad-spectrum treatments can reduce harmful effects on beneficial microbes. Sometimes the use of broad-spectrum antimicrobial agents is unavoidable; in these situations, consuming foods such as yogurt which contains beneficial bacteria can replenish the body's symbiotic microbes. In extreme cases microbes can be transplanted from a healthy individual to someone with whose symbiotic microbes have been compromised.

Key Points

- The intestinal system has many different species of microbes and huge numbers of individual microbes; we rely on these microbes for proper metabolism of food.
- The use of antimicrobial agents to slow down or kill pathogenic microbes can often kill beneficial bacteria, causing deleterious health effects.
- Our body hosts some microbes that inhibit the growth of pathogenic microbes; using antimicrobial agents can alter the flora allowing pathogenic microbes to overgrow and cause diseases.

Key Terms

- **Candidal vulvovaginitis**: Candidal vulvovaginitis or vaginal thrush, or yeast infection, is an infection of the vagina's mucous membranes by Candida albicans.
- **microbiota**: The microbial flora harbored by normal, healthy individuals.
- **pathogenic bacteria**: Bacteria which infect and cause deleterious health effects.

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12.4D: Effects of Drug Combinations

Learning Objectives

- Give examples of interactions that can render an antimicrobial ineffective

Pharmacodynamics is the field that attempts to understand the unintended effects of the use of two or more drugs. Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body or on microorganisms or parasites within or on the body. It also looks at the mechanisms of drug action and the relationship between drug concentration and effect. These changes are extraordinarily difficult to classify given the wide variety of modes of action that exist and the fact that many drugs can cause their effect through a number of different mechanisms. This wide diversity also means that, in all but the most obvious cases, it is important to investigate and understand these mechanisms. The well-founded suspicion exists that there are more unknown interactions than known ones.

Two well described interactions between antimicrobial drugs and other drugs are between antibiotics and alcohol and antibiotics and the birth control pill. Interactions between alcohol and certain antibacterials may occur, cause side-effects, and decrease effectiveness of antibacterial therapy. Potential risks of side-effects and effectiveness depend on the type of antibacterial administered. Despite the lack of a categorical contraindication, the belief that alcohol and antibacterials should never be mixed is widespread. Some antibacterials may inhibit the breakdown of alcohol, which may result in alcohol-induced vomiting, nausea, and shortness of breath. Other effects of alcohol on antibacterial activity include altered activity of the liver enzymes that break down the antibacterial compound. In addition, serum levels bacteriostatic antibacterials may be reduced by alcohol consumption, resulting in reduced efficacy and diminished pharmacotherapeutic effect.

Another well studied interaction is between antibiotics and the contraceptive pill. The majority of studies indicate that antibiotics do not interfere with contraceptive pills. In cases where antibacterials have been suggested to affect the efficiency of birth control pills may be due to an increase in the activities of hepatic liver enzymes causing increased breakdown of the pill's active ingredients. Effects on the intestinal flora, which might result in reduced absorption of estrogens in the colon, have also been suggested, but such suggestions have been inconclusive and controversial. Clinicians have recommended that extra contraceptive measures be applied during therapies using antibacterials that are suspected to interact with oral contraceptives.

Additionally, when dealing with a microbial infection, sometimes the use of two or more antibiotics can effectively combat the infection while each drug individually has little or no effect. This method is called combination therapy and is used when the nature of a microbial infection is unknown, as typified by the combination of the antibiotics ampicillin and sulbactam. The use of two antibiotics with different modes of microbial inhibition increases the chance that the treatment will combat the microbial infection. Further, tuberculosis has been treated with combination therapy for over fifty years. This is due to the phenomenon of resistance, whereby a micro-organism gains the ability to resist an antimicrobial drug, while initially the drug effectively slowed the growth of or even killed the target micro-organism. Treating tuberculosis, or other pathogenic microbes with more than one antibiotic reduces the chance that the microbe will adapt and survive the treatment, especially if the two drugs have different methods of reducing the microbe's normal functions.

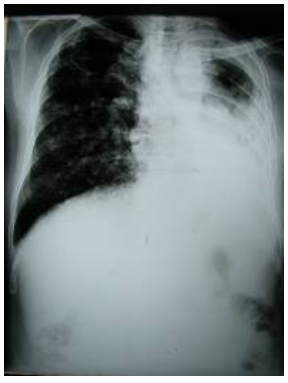


Figure: **Tuberculosis**: This x-ray of a tuberculosis patient shows the lung on the left side completely infected and the right lung partially infected (the dark areas), with tuberculosis.

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Key Points

- The interaction between one antimicrobial agent and another is very complex, along with the way they target microbes and the organism.
- While it is not certain that a drug may interact with antibiotics, it is considered wise to err on the side that there are potentially unknown and harmful interactions from mixing drugs.
- The use of more than one antimicrobial agent is an effective and widely used practice to reduce the chance that microbes will resist a treatment.

Key Terms

- **contraindication**: In medicine, a contraindication is a condition or factor that serves as a reason to withhold a certain medical treatment.
- **tuberculosis**: An infectious disease of humans and animals caused by a species of mycobacterium mainly infecting the lungs where it causes tubercles characterized by the expectoration of mucus and sputum, fever, weight loss, and chest pain, and transmitted through inhalation or ingestion of bacteria.
- **combination therapy**: Combination therapy is the use of more than one medication or other therapy. Most often, these terms refer to the simultaneous administration of two or more medications to treat a single disease.

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SECTION OVERVIEW

12.5: Measuring Drug Susceptibility

Topic hierarchy

12.5A: Minimal Inhibitory Concentration (MIC)

12.5B: Kirby-Bauer Disk Susceptibility Test

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12.5A: Minimal Inhibitory Concentration (MIC)

Learning Objectives

- Analyze data to interpret minimal inhibitory concentration values

Definition and Measurement

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation. MICs can be determined on plates of solid growth medium (called agar, shown in the "Kirby-Bauer Disk Susceptibility Test" atom) or broth dilution methods (in liquid growth media, shown in) after a pure culture is isolated. For example, to identify the MIC via broth dilution, identical doses of bacteria are cultured in wells of liquid media containing progressively lower concentrations of the drug. The minimum inhibitory concentration of the antibiotic is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth. There are also several commercial methods available to experimentally measure MIC values.



Figure: **Microbroth Dilution Method**: To identify the lowest concentration required for a given antibiotic to inhibit bacterial growth, an identical amount of bacteria is introduced into wells of liquid media containing progressively lower concentrations of the drug. (Here, the dilution series of the drug is set up from left to right: for example, well E1 might contain 100 units of drug; E2, 50 units; E3, 25 units; E4, 12.5 units; etc.). Because bacterial growth made the media in well E5 cloudy and the media in well E4 is indistinguishable from clear media, this indicates that the minimum inhibitory concentration is between the drug concentrations in wells E4 and E5. (Image courtesy of Microrao, Dept. of Microbiology, JJMMC, Davangere).

Significance and Applications

An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Because a lower MIC value indicates that less of the drug is required in order to inhibit growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents. Currently, there are a few web-based, freely accessible MIC databases. MIC scores are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Clinicians use MIC scores to choose which antibiotics to administer to patients with specific infections and to identify an effective dose of antibiotic. This is important because populations of bacteria exposed to an insufficient concentration of a particular drug or to a broad-spectrum antibiotic (one designed to inhibit many strains of bacteria) can evolve resistance to these drugs. Therefore, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains.

Key Points

- Minimum inhibitory concentration (MIC) can be determined by culturing microorganisms in liquid media or on plates of solid growth medium.
- A lower MIC value indicates that less drug is required for inhibiting growth of the organism; therefore, drugs with lower MIC scores are more effective antimicrobial agents.
- By identifying appropriate drugs and their effective concentrations, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains.

Key Terms

- **culture**: The process of growing a bacterial or other biological entity in an artificial medium.
- **minimum inhibitory concentration**: This is the lowest concentration of an antimicrobial drug that prevents visible growth of a microorganism after overnight incubation with media.
- **bacteriostatic**: A drug that prevents bacterial growth and reproduction but does not necessarily kill them. When it is removed from the environment the bacteria start growing again.

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12.5B: Kirby-Bauer Disk Susceptibility Test

Learning Objectives

- Review the procedure for the Kirby-Bauer antibiotic tes

Kirby-Bauer antibiotic testing (also called KB testing or disk diffusion antibiotic sensitivity testing) uses antibiotic-containing wafers or disks to test whether particular bacteria are susceptible to specific antibiotics. First, a pure culture of bacteria is isolated from the patient. Then, a known quantity of bacteria are grown overnight on agar (solid growth media) plates in the presence of a thin wafer that contains a known amount of a relevant antibiotic. If the bacteria are susceptible to the particular antibiotic from a wafer, an area of clear media where bacteria are not able to grow surrounds the wafer, which is known as the zone of inhibition. A larger zone of inhibition around an antibiotic-containing disk indicates that the bacteria are more sensitive to the antibiotic in the disk.

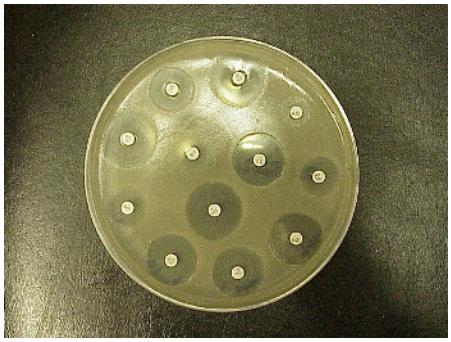


Figure: ***Kirby-Bauer test****: In Kirby–Bauer testing, discs containing antibiotics are placed on agar where bacteria are growing, and the antibiotics diffuse out into the agar. If an antibiotic stops the bacteria from growing, one can see circular areas around the wafers where bacteria have not grown.*

KB tests are performed under standardized conditions and standard-sized zones of inhibition have been established for each antibiotic. KB test results are usually reported as sensitive, intermediate, or resistant, based on the size of the zone of inhibition. If the observed zone of inhibition is greater than or equal to the size of the standard zone, the microorganism is considered to be sensitive to the antibiotic. Conversely, if the observed zone of inhibition is smaller than the standard size, the microorganism is considered to be resistant. The size of a zone of inhibition in a KB test is inversely related to the minimum inhibitory concentration (MIC), which is the amount of antibiotic required to prevent bacterial growth in an overnight culture. The MIC (in µg/ml) can be calculated from known standard-curve (linear regression) graphs based on the diameter of the observed inhibition zone diameter (in millimeters).

Clinicians can use KB test results to choose appropriate antibiotics to combat a particular infection in a patient. Administering antibiotics that specifically target the particular bacteria that are causing the infection can avoid using broad-spectrum antibiotics, which target many types of bacteria. Thus, clinical application of KB testing results can decrease the frequency with which antibiotic-resistant bacteria evolve.

Key Points

- KB tests are performed under standard conditions, so the minimum inhibitory concentration for a given antibiotic can be calculated by comparing the observed zone of inhibition ‘s size to known values.
- Clinicians use KB test results to choose antibiotics effective against the specific bacteria causing a patient’s infection. Using specifically-targeted antibiotics helps decrease the frequency of drug-resistant bacteria evolving.

Key Terms

- **Kirby-Bauer antibiotic testing**: This is a method to determine the sensitivity of microorganisms to specific antimicrobial drugs; greater drug efficacy yields larger microbe-free zones surrounding drug-containing disks after overnight growth on solid media.

- **zone of inhibition**: This is an area of media where bacteria are unable to grow, due to presence of a drug that impedes their growth.
- **minimum inhibitory concentration**: This is the lowest concentration of an antimicrobial drug that prevents visible growth of a microorganism after overnight incubation with media.

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SECTION OVERVIEW

12.6: Drug Resistance

Topic hierarchy

12.6A: Mechanisms of Resistance

12.6B: Antibiotic Misuse

12.6C: Cost and Prevention of Resistance

12.6D: Biofilms, Persisters, and Antibiotic Tolerance

12.6E: Finding New Antimicrobial Drugs

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12.6A: Mechanisms of Resistance

Learning Objectives

- Describe the mechanisms bacteria use to develop antimicrobial resistance and the factors that can lead to it

Development of microbial resistance to antimicrobial agents requires alterations in the microbe ‘s cell physiology and structure. Antimicrobial resistance is defined as the loss of susceptibility to an extent that the drug is no longer effective for clinical use against an organism. Resistance can be mediated by the environment or the microorganism itself.

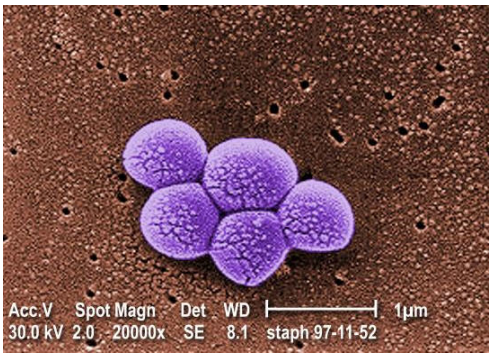


Figure: **Resistant bacterial strain**: Methicillin-resistant Staphylococcus aureus.

Environmentally-mediated antimicrobial resistance is affected by the environment’s chemical and physical properties such as pH, anaerobic conditions, cation concentrations (calcium, magnesium), and thymine-thymidine content (available metabolites and nutrients).

Microorganism-mediated antimicrobial resistance is due to genetically-encoded traits of the microorganism and can be divided into intrinsic or acquired. Intrinsic resistance is considered to be a natural and inherited property with high predictability. Once the identity of the organism is known, the aspects of its anti-microbial resistance are also recognized. On the other hand, acquired resistance results from a change in the organism’s genetic makeup. This trait is associated with only some strains of an organism’s group but not the others. It is also an unpredictable trait and necessitates the development of laboratory methods to detect it. Microorganism-mediated antimicrobial resistance is acquired by gene change or exchange such as genetic mutations, acquisition of genes from other organisms via gene transfer mechanisms, or a combination of mutational and gene transfer events. Some common pathways bacteria use to effect antimicrobial resistance include: enzymatic degradation or modification of the antimicrobial agent, decreased uptake or accumulation of the antimicrobial agent, altered antimicrobial target, circumvention of consequences of antimicrobial actions, uncoupling of antimicrobial agent-target interaction, or any combination of these mechanisms.

Key Points

- Antimicrobial resistance can be mediated by the environment or the microorganism itself.
- Environmentally-mediated antimicrobial resistance results from physical or chemical characteristics of the environment that can affect the antimicrobial agent or the microorganism.
- Microorganism-mediated antimicrobial resistance can be intrinsic or acquired.

Key Terms

- **intrinsic**: innate, inherent, inseparable from the thing itself, essential.

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12.6B: Antibiotic Misuse

Learning Objectives

- Explain the effects of antibiotic misuse

With the introduction of antibiotics into medical practice, clinically-relevant bacteria have had to adopt resistance mechanisms as part of their survival strategy. Antibiotic resistance occurs when antibiotics no longer work against disease-causing bacteria. These infections are difficult to treat and can mean longer-lasting illnesses, more doctor visits or extended hospital stays, and the need for more expensive and toxic medications. Some resistant infections can even cause death. Developing new antibiotics and other treatments to keep pace with antibiotic-resistant strains of bacteria is necessary. However, using antibiotics wisely is equally important for preventing the spread of resistant strains.

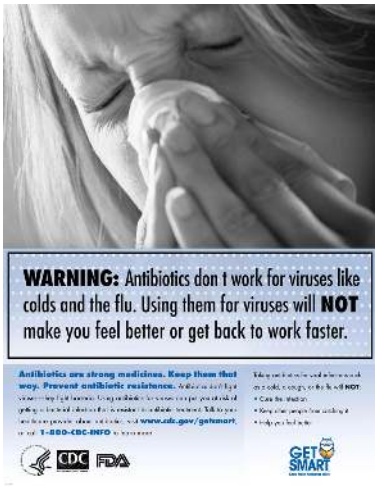


Figure: ***Figure* 12.6*B*.1**: *Antibiotic misuse. Antibiotics are not effective against viral infections. Misusing them leads to resistant bacterial strains.*

Antibiotic misuse has contributed largely to the emergence of new resistant strains. It is caused by taking an antibiotic too often for a condition it cannot treat such as viral infections and the common cold or in the wrong doses. It can also be manifested by not finishing a course of antibiotics as prescribed (stopping the antibiotic before the infection is fully cleared from the body). Overuse of antibiotics affects the bodsy's normal flora and disrupts the balance between beneficial bacteria that help digestion for example, and harmful bacteria. Excessive use of antibiotics in intensive farming units, particularly pig and poultry farms, is also seen as a growing threat. Scientists say antimicrobial resistance may be passing between animals and humans through food consumption, making the need to cut unnecessary use of antibiotics in farming even more urgent. Responsible antibiotic use in industry, and good practice for patients and physicians, are essential to keep resistant bacterial strains curable, and antibiotic treatment affordable to patients.

Key Points

- Antimicrobial resistance is a major public health concern.
- Antimicrobial resistance is brought about by antibiotic misuse, such as overuse, misuse, or interrupted treatment.
- Food industries, physicians, and patients play a role in minimizing the spread of resistance by adhering to good antibiotic practice.

Key Terms

- **course of antibiotics**: a period of continuous treatment with a drug.

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12.6C: Cost and Prevention of Resistance

Learning Objectives

- Examine the causes and effects of multidrug-resistant organisms on healthcare

Prevention and control of microbial-resistant organisms is one of the most complex management issues that health care professionals face. The clinical and financial burden to patients and health care providers is staggering. Patients who are infected with bacterial strains resistant to more than one type or class of drugs (multidrug-resistant organisms, MDRO) often have an increased risk of prolonged illness, extended hospital stay, and mortality.

The cost of care for these patients can be more than double compared to those without an MDRO infection. The alternative medication they are prescribed to overcome the infection is often substantially more costly. Multidrug resistance forces healthcare providers to use antibiotics that are more expensive or more toxic to the patient.



Figure: ***Antibiotics****: Antibiotic misuse is a major cause of the staggering healthcare costs for the treatment of resistant bacterial strains.*

When no antibiotic is effective, healthcare providers may be limited to providing supportive care rather than directly treating an infection. In a 2008 study of attributable medical costs for antibiotic resistant infections, it was estimated that infections in 188 patients from a single healthcare institution cost between $13.35 and $18.75 million dollars.

Research and development of new drugs effective against resistant bacterial strains also comes at a cost. To prevent antimicrobial resistance, the patient and the healthcare provider should discuss the appropriate medicine for the illness. Patients should follow prescription directions and should not share or take medicine that was prescribed for someone else; these virtues should be strictly practiced. Healthy lifestyle habits, including proper diet, exercise, and sleeping patterns, as well as good hygiene such as frequent hand washing, can help prevent illness. These practices, therefore, also help prevent the overuse or misuse of antibiotics and the emergence of problematic resistant strains.

Key Points

- Antimicrobial resistance to available drugs requires the development of new drugs to effectively treat resistant strains and reduce mortality from bacterial infections.
- Antimicrobial resistance can be prevented by practicing good hygiene, and being responsible with antibiotic use.
- Treating antibiotic-resistant bacterial strains is expensive for both the patient and the healthcare provider. The treatment requires extended hospital stay and costly medications.

Key Terms

- **multidrug resistance**: A condition enabling a disease-causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism.

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12.6D: Biofilms, Persisters, and Antibiotic Tolerance

Learning Objectives

- Explain the role of biofilms and persisters in multidrug tolerance, distinguishing this from multidrug resistance

Biofilms are bacteria that have formed a gated community. Biofilms are composed of an aggregate of bacterial cells and are essentially considered a multi-cellular organism. They are characterized by structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances. They live on solid surfaces (e.g., catheters,) and the extracellular material they produce protects them from external threats, such as attacks by the body's immune cells. The property of biofilms constitute a penetration barrier for most antibiotics therefore preventing the drug from reaching the microbes. It is being widely recognized that bacterial biofilms are responsible for several chronic diseases that are difficult to treat, hence hard to eradicate (e.g., cystitis, endocarditis, urinary tract infections, gingivitis, dental plaque, and other yet to be identified conditions). They differ from free-floating or planktonic bacteria that cause acute infections and are managed by antimicrobial drugs.

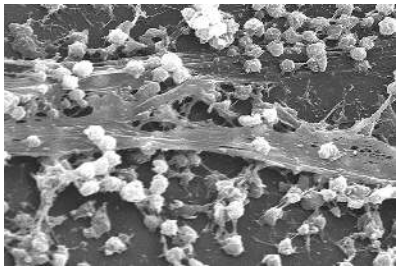


Figure: **Staphylococcus aureus biofilm**: Staphylococcus aureus forming a biofilm on a catheter.

Persisters are multidrug tolerant cells present in all bacterial populations. Bacterial populations that produce persister cells that neither grow nor die in the presence of microbicidal antibiotics are largely responsible for high levels of biofilm tolerance to antimicrobials. Persisters are not mutants, but rather phenotypic variants of the wild-type that upon inoculation produce a culture with similar levels of tolerance. Elimination of persisters remains an obstacle for the eradication of some tenacious and highly recurrent bacterial infections. Biofilms and persisters are the cause of multidrug tolerance. Multidrug tolerance differs from multidrug resistance in that it is not caused by mutant microbes but rather by microbial cells that exist in a transient, dormant state. These non-dividing cells often survive antibiotic exposure targeted to kill highly proliferating bacteria.

Key Points

- Biofilms are aggregates of microbial cells that form to avoid antimicrobial agents or attack by the immune system.
- Persisters are slow-growing, dormant microbial cells that can tolerate antibiotic treatment.
- Biofilms and persisters are responsible for chronic bacterial infections and recurrent disease.
- Antibiotic tolerance is different from antibiotic resistance but equally important as a public health burden for eradication of serious bacterial diseases.

Key Terms

- **gingivitis**: inflammation of the gums or gingivae
- **extracellular matrix**: All the connective tissues and fibers that are not part of a cell, but rather provide support.

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12.6E: Finding New Antimicrobial Drugs

Learning Objectives

- Explain the reasons for low production of new antibiotics and discuss the proposed mechanisms to evade antimicrobial resistance

Antimicrobial resistance: the problem

Antibiotics, more than any other medicines, have improved the life expectancy of mankind, however, multi-drug resistance has become common in pathogenic bacteria and multiple drugs are losing efficacy. Recent reports on the occurrence of panresistant gram-negative strains, i.e. strains resistant to every registered antibacterial drug, indicate that we are on the verge to lose the battle, taking us back to the pre-antibiotic era. There is world-wide consensus that the medical need for novel antiinfective drugs is enormous and that we are running out of time. Many achievements of modern medicine, not only treatment of infectious diseases, depend on the availability of efficacious antibiotics, still, the antibacterial development pipeline is slow and the number of new drugs reaching the market is alarmingly low. There are many reasons for this at all levels of the discovery and development process. Investments into antibiotic research and technologies is minimal; socioeconomic considerations together with regulatory hurdles have prompted pharmaceutical companies to exit the field and innovative biotech companies were confronted with problems beyond their control. Answers are needed as to where and how we can find new lead compounds with unprecedented activities?

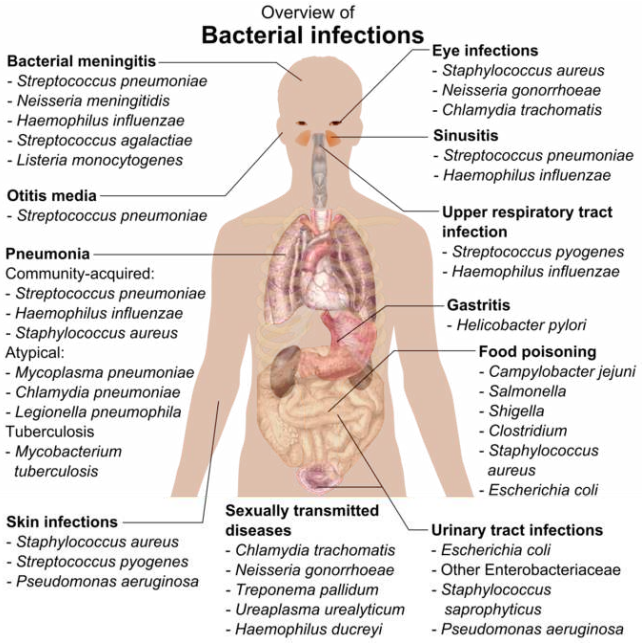


Figure: **Bacterial infections of the human body**: most bacterial species listed in this figure have developed resistance to available antibiotics necessitating new drug discovery.

Finding new antimicrobial drugs: the solution

Research on new antimicrobial compounds is geared towards innovative targets to circumvent resistance. Some of the proposed areas to investigate include: collecting and examining the list of antimicrobial resistance genes (e.g. exploring the resistome), targeting teichoic acid biosynthesis as a new method to compromise the bacterial wall integrity, producing ribosomal inhibitors to target protein synthesis, targeting outer-membrane transporters with protein epitope mimetics (e.g. mimetics of the cationic antimicrobial peptides that form part of the immune response to microbes), and developing antibody-based strategies and vaccines. The initiative to develop new antimicrobial agents is urgently needed but is a long process from invention, to development, to actual clinical application. It is also necessary to initiate a worldwide awareness on antibiotic misuse and overuse as a mean to address the root of the problem for antimicrobial resistance.

Key Points

- Finding new antimicrobial drugs requires researchers, pharmaceutical, and biotech companies to invest in new technology and discover new sources for antibiotic development.
- Proposed mechanisms to circumvent antimicrobial resistance range from exploring the list of resistance genes to antibody-based therapy and vaccines.
- Finding new candidates to target is essential but it needs to be accompanied by awareness on antibiotic misuse with the prospect to eliminate the root of the problem.

Key Terms

- **mimetic**: A substance with similar pharmacological effects to another substance.

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Index

A

A site
9.4: Protein Synthesis (Translation)
absorbance
4.4.1: The Properties of Light
acellular
1.5: Types of Cells and Microorganisms
Acidophiles
8.2.2: pH Effects on Bacterial Growth
activator
9.6: Gene Regulation - Operon Theory
active site
7.1: Introduction to Metabolism and Enzymes
active transport
3.8.1.6: Active Transport
acute disease
5.3: Infectious Diseases Characteristics and Classification
acute inflammation
6.2.6: Inflammation and Fever
adaptive immunity
6.3: Adaptive Immunity
adenine
9.1.3: Structure and Function of DNA
adenosine triphosphate
7.1: Introduction to Metabolism and Enzymes
adhesins
8.3: Bacterial Quorum Sensing
adhesion
1.6.5: Koch's Postulate
2.4.1: Biochemical Properties of Water
aerobic respiration
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
aerotolerant anaerobes
8.2.4: Oxygen Effects on Bacterial Growth
alarmones
9.6: Gene Regulation - Operon Theory
alcohols
10.4: Using Chemicals to Control Microorganisms
alexidine
10.4: Using Chemicals to Control Microorganisms
algae
1.2: The Roles of Microbes
1.5: Types of Cells and Microorganisms
alginate
8.3: Bacterial Quorum Sensing
Alkaliphiles
8.2.2: pH Effects on Bacterial Growth
Alkylating agents
10.4: Using Chemicals to Control Microorganisms
allosteric inhibitor
7.1: Introduction to Metabolism and Enzymes
allosteric inhibitors
7.1: Introduction to Metabolism and Enzymes
allosteric sites
7.1: Introduction to Metabolism and Enzymes
Ames test
9.5: Mutations
amino acid synthesis
7.7.1: Amino Acid Synthesis
amplitude
4.4.1: The Properties of Light
AMPs
6.2: Non-Adaptive Immunity
anabolic reaction
7.1.1: Factors Affecting Enzyme Activity
anabolism
7.1: Introduction to Metabolism and Enzymes
anaerobic respiration
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
Analytical epidemiology
1.6.6: Improvement in Public Health
5.6.1: Epidemiology
anergy
6.3.3.1: T Lymphocytes
anoxygenic photosynthesis
7.6.2: The Process of Photosynthesis
antibiotic
1.6.7: The Discovery of Antimicrobial Drugs
12.1B: Antibiotic Discovery
Antibiotic Misuse
12.6B: Antibiotic Misuse
Antibiotic Tolerance
12.6D: Biofilms, Persisters, and Antibiotic Tolerance
anticodon
9.4: Protein Synthesis (Translation)
antigen
6.3: Adaptive Immunity
antigenic drift
5.4: Virulence Factors
antigenic shift
5.4: Virulence Factors
antigenic variation
5.4: Virulence Factors
Antimicrobial peptides
6.2: Non-Adaptive Immunity
antiparallel
9.1.3: Structure and Function of DNA
antisepsis
10.1: Controlling Microbial Growth
apoenzyme
7.1: Introduction to Metabolism and Enzymes
7.1.1: Factors Affecting Enzyme Activity
archaea
1.5: Types of Cells and Microorganisms
aseptic technique
10.1: Controlling Microbial Growth
ATP
3.9: Eukaryote Organelles
7.1: Introduction to Metabolism and Enzymes
ATP synthase
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
attachment
11.2: The Viral Life Cycle and Host Infection Specificity
attenuation
9.6: Gene Regulation - Operon Theory
11.3: Prevention and Treatment of Viral Infections
autoimmune response
6.3: Adaptive Immunity
autotrophs
7.1: Introduction to Metabolism and Enzymes
auxotrophs
9.5: Mutations

B

B cells
6.3.2.1: B Lymphocytes and Antibodies
B lymphocytes
6.3.2.1: B Lymphocytes and Antibodies
back mutation
11.3: Prevention and Treatment of Viral Infections
bacteria
1.2: The Roles of Microbes
1.5: Types of Cells and Microorganisms
bactericides
10.1: Controlling Microbial Growth
bacteriology
1.5: Types of Cells and Microorganisms
bacteriophages
11.5.1: Isolation, Culture, and Identification of Viruses
bacteriostatic
10.1: Controlling Microbial Growth
binary fission
8.1: How Bacteria Grow
binomial nomenclature
1.3: Classification, Phylogeny, and Nomenclature
4.3: Classification and Identification
4.3.1: Kingdom Classification According to Whittaker
biodiversity
1.3: Classification, Phylogeny, and Nomenclature
biofilms
5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal
8.1: How Bacteria Grow
biological
5.5: Reservoirs of Infection and Disease Transmission
biological macromolecule
2.3.2: Synthesis of Biological Macromolecules
Biological nitrogen fixation (BNF)
8.2.3: Nitrogen Effects on Bacterial Growth
biological safety levels
10.1: Controlling Microbial Growth
biological Vector transmission
5.5: Reservoirs of Infection and Disease Transmission
biosynthesis
11.2: The Viral Life Cycle and Host Infection Specificity
Bisbiguanides
10.4: Using Chemicals to Control Microorganisms
bradykinin
6.2: Non-Adaptive Immunity

C

CAP
9.6: Gene Regulation - Operon Theory
capillary action
2.4.1: Biochemical Properties of Water

capnophile
8.2.4: Oxygen Effects on Bacterial Growth
capsid
11.1: Viruses - General Characteristics
capsule
3.5: Prokaryote Plasma Membrane
Capsule Staining
4.2: Staining Microscopic Specimens
capsules
5.4: Virulence Factors
carbon skeleton
2.5.1: Organic Molecules
carrier protein
3.8.1.4: Passive Transport - Facilitated Transport
catabolic reaction
7.1.1: Factors Affecting Enzyme Activity
catabolism
7.1: Introduction to Metabolism and Enzymes
7.5: Catabolism of Lipids and Proteins
catabolite activator protein
9.6: Gene Regulation - Operon Theory
catalase
8.2.4: Oxygen Effects on Bacterial Growth
catalysts
7.1: Introduction to Metabolism and Enzymes
cell wall
3.7: Eukaryotic External Structures
3.8.1.5: Passive Transport - Osmosis
Centers for Disease Control and Prevention
5.6: Introduction to Epidemiology
central dogma
9.1.5: What Are Genes?
central tolerance
6.3.3.1: T Lymphocytes
central vacuole
3.7: Eukaryotic External Structures
centriole
3.9: Eukaryote Organelles
centrosome
3.7: Eukaryotic External Structures
channel protein
3.8.1.4: Passive Transport - Facilitated Transport
charged tRNA
9.4: Protein Synthesis (Translation)
chemical mediators
6.2: Non-Adaptive Immunity
Chemically defined media
8.4.1: Media Used for Bacterial Growth
chemiosmosis
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
chemotrophs
7.1: Introduction to Metabolism and Enzymes
Chlorhexidine
10.4: Using Chemicals to Control Microorganisms
chlorophyll
3.7: Eukaryotic External Structures
chloroplast
3.7: Eukaryotic External Structures
cholesterol
3.8.1.1: The Plasma Membrane
chromatin
3.7: Eukaryotic External Structures
chromosome
3.7: Eukaryotic External Structures
chronic disease
5.3: Infectious Diseases Characteristics and Classification
chronic inflammation
6.2.6: Inflammation and Fever
cisternae
3.9: Eukaryote Organelles
class switching.
6.3.2.1: B Lymphocytes and Antibodies
clonal selection
6.3: Adaptive Immunity
coenzymes
7.1: Introduction to Metabolism and Enzymes
cofactor
7.1: Introduction to Metabolism and Enzymes
7.1.1: Factors Affecting Enzyme Activity
cognate amino acid
9.4: Protein Synthesis (Translation)
commensalism
5.1: Microbe and Human Symbiotic Relationships
commercial sterilization
10.1: Controlling Microbial Growth
competitive inhibitors
7.1: Introduction to Metabolism and Enzymes
complement proteins
6.2: Non-Adaptive Immunity
complement system
6.2: Non-Adaptive Immunity
6.2.2: The Complement System
complex
11.1: Viruses - General Characteristics
concentration gradient
3.8.1.3: Passive Transport - Diffusion
conjugation
3.5: Prokaryote Plasma Membrane
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
conjugation pilus
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
constitutively expressed
9.6: Gene Regulation - Operon Theory
Contact transmission
5.5: Reservoirs of Infection and Disease Transmission
contagious
5.3: Infectious Diseases Characteristics and Classification
contrast
4.4.1: The Properties of Light
critical item
10.1: Controlling Microbial Growth
culture
8.4.2: Culture Media
cyclic photophosphorylation
7.6.2: The Process of Photosynthesis
cytokine storm
6.3.3.1: T Lymphocytes
cytokines
6.2: Non-Adaptive Immunity
cytopathic effects
11.5.1: Isolation, Culture, and Identification of Viruses
cytoplasm
3.7: Eukaryotic External Structures
3.8: Eukaryotic Plasma Membrane and The Cytoplasm
cytosine
9.1.3: Structure and Function of DNA
cytosol
3.7: Eukaryotic External Structures
3.8: Eukaryotic Plasma Membrane and The Cytoplasm
cytotoxic T cells
6.3.3.1: T Lymphocytes
cytotoxic T lymphocyte
6.3: Adaptive Immunity

D

dark repair
9.5: Mutations
death
8.1: How Bacteria Grow
decimal reduction time
10.1: Controlling Microbial Growth
degenerate
9.4: Protein Synthesis (Translation)
dehydration synthesis
2.3.2: Synthesis of Biological Macromolecules
2.5.1: Organic Molecules
dendritic cell
6.3: Adaptive Immunity
dendritic cells
6.2.5: Dendritic Cells
Deoxyribonucleotides
9.1.3: Structure and Function of DNA
deoxyribose
9.1.3: Structure and Function of DNA
9.1.4: Structure and Function of RNA
Descriptive epidemiology
1.6.6: Improvement in Public Health
5.6.1: Epidemiology
Desiccation
10.3: Using Physical Methods to Control Microorganisms
differential media
8.4.1: Media Used for Bacterial Growth
8.4.4: Selective and Differential Media
diffraction
4.4.1: The Properties of Light
diffusion
3.8.1.3: Passive Transport - Diffusion
Direct Contact transmission
5.5: Reservoirs of Infection and Disease Transmission
direct viable cell count
8.1: How Bacteria Grow
disease
5.3: Infectious Diseases Characteristics and Classification
Disinfection
10.1: Controlling Microbial Growth
dispersion
4.4.1: The Properties of Light
diversity
1.3: Classification, Phylogeny, and Nomenclature
DNA
2.5.4: DNA and RNA

DNA gyrase
9.2: DNA Replication
DNA ligase
9.2: DNA Replication
DNA polymerase I
9.2: DNA Replication
DNA polymerase III
9.2: DNA Replication
domain
1.3: Classification, Phylogeny, and Nomenclature
doubling time
8.1: How Bacteria Grow

E

E site
9.4: Protein Synthesis (Translation)
edema
6.2.6: Inflammation and Fever
effector cell
6.3: Adaptive Immunity
electrochemical gradient
3.8.1.6: Active Transport
electron carriers
7.1: Introduction to Metabolism and Enzymes
electron microscopy
4.2: Staining Microscopic Specimens
electron transport system
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
elongation
9.2: DNA Replication
9.3: RNA Transcription
9.4: Protein Synthesis (Translation)
Emerging diseases
5.6.3: Global Public Health
Endemic diseases
5.6: Introduction to Epidemiology
endergonic reactions
7.1: Introduction to Metabolism and Enzymes
endocytosis
3.8.1.6: Active Transport
3.8.2: Endocytosis
endoplasmic reticulum
3.9: Eukaryote Organelles
endospore staining
4.2: Staining Microscopic Specimens
endospores
3.6: Endospores
endosymbiosis
1.6.3: What Causes Diseases?
3.10: Serial Endosymbiosis Theory (SET)
endosymbiotic theory
1.6.3: What Causes Diseases?
3.10: Serial Endosymbiosis Theory (SET)
endotoxin
5.4: Virulence Factors
enriched media
8.4.1: Media Used for Bacterial Growth
enzymes
7.1: Introduction to Metabolism and Enzymes
7.1.1: Factors Affecting Enzyme Activity
Epidemic diseases
5.6: Introduction to Epidemiology
epidemiology
5.6: Introduction to Epidemiology
epigenetic regulation
9.6: Gene Regulation - Operon Theory
epitope
6.3: Adaptive Immunity
epitopes
5.4.1.3: The Ability to Evade Adaptive Immune Defenses
erythema
6.2.6: Inflammation and Fever
ETS
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
eukarya
1.5: Types of Cells and Microorganisms
eukaryote
4.3: Classification and Identification
4.3.1: Kingdom Classification According to Whittaker
eukaryotic cell
3.2.1: Comparing Prokaryotic and Eukaryotic Cells
3.7: Eukaryotic External Structures
exergonic reaction
7.1: Introduction to Metabolism and Enzymes
exocytosis
3.8.1.6: Active Transport
exoenzymes
5.4: Virulence Factors
exotoxins
5.4: Virulence Factors
Experimental epidemiology
1.6.6: Improvement in Public Health
5.6.1: Epidemiology
exposure
1.6.5: Koch's Postulate
extracellular polymeric substance
8.1: How Bacteria Grow
Extrachromosomal DNA
9.1.1: The Structure and Function of Cellular Genomes

F

F plasmid
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
F+ cell
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
facilitated transport
3.8.1.4: Passive Transport - Facilitated Transport
Facultative anaerobes
8.2.4: Oxygen Effects on Bacterial Growth
feedback inhibition
7.1: Introduction to Metabolism and Enzymes
fermentation
7.4.2: Fermentation
Fever
6.2.6: Inflammation and Fever
filamentous temperature sensitive protein
8.5.1: Generation Time
fimbriae
3.3.3: Fimbriae and Pili
5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal
Flagella Staining
4.2: Staining Microscopic Specimens
Florence Nightingale
1.6.6: Improvement in Public Health
5.6.1: Epidemiology
Fluid Mosaic Model
3.5.1: Components and Structure - Fluid Mosaic Model
3.8.1.1: The Plasma Membrane
fluorescent
4.4.1: The Properties of Light
focal
1.6.5: Koch's Postulate
focal infection
1.6.5: Koch's Postulate
focal length
4.4.1: The Properties of Light
focal point
4.4.1: The Properties of Light
fomites
10.1: Controlling Microbial Growth
forespore
3.6: Endospores
Formaldehyde
10.4: Using Chemicals to Control Microorganisms
frameshift mutation
9.5: Mutations
frequency
4.4.1: The Properties of Light
functional groups
2.5.1: Organic Molecules
fungi
1.2: The Roles of Microbes
1.5: Types of Cells and Microorganisms
fungicides
10.1: Controlling Microbial Growth
fungistatic
10.1: Controlling Microbial Growth

G

gene expression
9.6: Gene Regulation - Operon Theory
gene therapy
11.3: Prevention and Treatment of Viral Infections
generalized
11.2: The Viral Life Cycle and Host Infection Specificity
generalized transduction
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
generation time
8.1: How Bacteria Grow
genes
9.1.5: What Are Genes?
genetic code
9.4: Protein Synthesis (Translation)
genotype
9.1.1: The Structure and Function of Cellular Genomes
germ theory of disease
1.6.3: What Causes Diseases?
Glutaraldehyde
10.4: Using Chemicals to Control Microorganisms
Glycocalyx
8.3: Bacterial Quorum Sensing
glycolipid
3.8.1.1: The Plasma Membrane

glycolysis
7.2: Catabolism of Glucose (1)- Stages 1-3 of Cellular Respiration
glycoprotein
3.8.1.1: The Plasma Membrane
Golgi apparatus
3.9: Eukaryote Organelles
Gram negative
3.4: Prokaryote Cell Wall
3.5: Prokaryote Plasma Membrane
Gram positive
3.4: Prokaryote Cell Wall
3.5: Prokaryote Plasma Membrane
Gram staining
4.2: Staining Microscopic Specimens
granulomas
6.2.6: Inflammation and Fever
granzymes
6.3.3.1: T Lymphocytes
guanine
9.1.3: Structure and Function of DNA

H

HAI
5.5: Reservoirs of Infection and Disease Transmission
haloenzyme
7.1.1: Factors Affecting Enzyme Activity
halogens
10.4: Using Chemicals to Control Microorganisms
halophiles
8.2.5: Other Environmental Effects on Bacterial Growth
halotolerant
8.2.5: Other Environmental Effects on Bacterial Growth
Handwashing
5.6.2.2: Control of Nosocomial Infections
heavy metals
10.4: Using Chemicals to Control Microorganisms
helical
11.1: Viruses - General Characteristics
helicase
9.2: DNA Replication
Helicobacter pylori
5.4.1.1: The Ability to Use Motility and Other Means to Contact Host Cells
helminth
1.5: Types of Cells and Microorganisms
Helper T cells
6.3.3.1: T Lymphocytes
helper T lymphocyte
6.3: Adaptive Immunity
HEPA
10.3: Using Physical Methods to Control Microorganisms
heterolactic fermentation
7.4.2: Fermentation
heterotrophs
7.1: Introduction to Metabolism and Enzymes
Hfr cell
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
histamine
6.2: Non-Adaptive Immunity
holoenzyme
7.1: Introduction to Metabolism and Enzymes
homolactic fermentation
7.4.2: Fermentation
horizontal gene transfer
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
host ranges
11.1: Viruses - General Characteristics
humoral immune response
6.3: Adaptive Immunity
hydrogen bond
2.4.1: Biochemical Properties of Water
hydrolysis
2.3.2: Synthesis of Biological Macromolecules
hydrophilic
3.8.1.1: The Plasma Membrane
hydrophobic
3.8.1.1: The Plasma Membrane
hyperthemophiles
8.2.1: Temperature Effects on Bacterial Growth
hypertonic
3.8.1.5: Passive Transport - Osmosis
hypotonic
3.8.1.5: Passive Transport - Osmosis

I

iatrogenic disease
5.3: Infectious Diseases Characteristics and Classification
ID50
1.6.5: Koch's Postulate
image point
4.4.1: The Properties of Light
image point (focus)
4.4.1: The Properties of Light
immune tolerance
6.3: Adaptive Immunity
immunoglobulin proteases
5.4.1.3: The Ability to Evade Adaptive Immune Defenses
immunology
1.5: Types of Cells and Microorganisms
incidence
5.6: Introduction to Epidemiology
incubation period
5.3: Infectious Diseases Characteristics and Classification
indirect Contact transmission
5.5: Reservoirs of Infection and Disease Transmission
induced fit
7.1: Introduction to Metabolism and Enzymes
induced mutations
9.5: Mutations
inducer
9.6: Gene Regulation - Operon Theory
inducible operon
9.6: Gene Regulation - Operon Theory
infection
1.6.5: Koch's Postulate
5.3: Infectious Diseases Characteristics and Classification
infectious
5.3: Infectious Diseases Characteristics and Classification
inflammation
6.2.6: Inflammation and Fever
initiation
9.4: Protein Synthesis (Translation)
initiation factors
9.4: Protein Synthesis (Translation)
initiation of replication
9.2: DNA Replication
initiation of transcription.
9.3: RNA Transcription
Injectosomes
8.3: Bacterial Quorum Sensing
integral protein
3.8.1.1: The Plasma Membrane
interference
4.4.1: The Properties of Light
interferons
6.2.3: Interferons
invasion
1.6.5: Koch's Postulate
iodophor
10.4: Using Chemicals to Control Microorganisms
ionizing radiation
9.5: Mutations
10.3: Using Physical Methods to Control Microorganisms
isomers
2.5.1: Organic Molecules
isotonic
3.8.1.5: Passive Transport - Osmosis

J

John Snow
1.6.6: Improvement in Public Health
5.6.1: Epidemiology
Joseph Lister
1.6.6: Improvement in Public Health
5.6.1: Epidemiology

K

kingdom
1.3: Classification, Phylogeny, and Nomenclature
Koch’s postulates
1.6.5: Koch's Postulate
5.3.1: Koch’s Postulates
5.6: Introduction to Epidemiology
Krebs cycle
7.2: Catabolism of Glucose (1)- Stages 1-3 of Cellular Respiration

L

lag
8.1: How Bacteria Grow
lagging strand
9.2: DNA Replication
latency
11.2: The Viral Life Cycle and Host Infection Specificity
Latent diseases
5.3: Infectious Diseases Characteristics and Classification

LD50
1.6.5: Koch's Postulate
leading strand
9.2: DNA Replication
LETHAL PLASMIDS
9.1.1: The Structure and Function of Cellular Genomes
light microscopy
4.2: Staining Microscopic Specimens
Linnaeus
4.3: Classification and Identification
4.3.1: Kingdom Classification According to Whittaker
lipases
7.5: Catabolism of Lipids and Proteins
Lipid Biosynthesis
7.7.3: Lipid Biosynthesis
lipooligosaccharide
5.4.1.3: The Ability to Evade Adaptive Immune Defenses
lithotrophs
7.1: Introduction to Metabolism and Enzymes
local
1.6.5: Koch's Postulate
local infection
1.6.5: Koch's Postulate
logarithmic
8.1: How Bacteria Grow
long attachment pili
3.3.3: Fimbriae and Pili
Louis Pasteur
1.6.2: Pasteur and Spontaneous Generation
Luria Broth
8.4.3: Complex and Synthetic Media
Lyme disease
8.4.5: Special Culture Techniques
lymph
6.3: Adaptive Immunity
lymphocytes
6.2.4: Natural Killer Cells
Lyophilization
10.3: Using Physical Methods to Control Microorganisms
lysogenic cycle
11.2: The Viral Life Cycle and Host Infection Specificity
lysogeny broth
8.4.2: Culture Media
lysosome
3.7: Eukaryotic External Structures
lytic
11.2: The Viral Life Cycle and Host Infection Specificity
lytic cycle
11.2: The Viral Life Cycle and Host Infection Specificity

M

MAC
6.2: Non-Adaptive Immunity
macromolecules
2.5.1: Organic Molecules
magnification
4.4.1: The Properties of Light
MALT
6.3: Adaptive Immunity
maturation
11.2: The Viral Life Cycle and Host Infection Specificity
maximum growth pH
8.2.2: pH Effects on Bacterial Growth
maximum permissive oxygen concentration
8.2.4: Oxygen Effects on Bacterial Growth
mechanical
5.5: Reservoirs of Infection and Disease Transmission
mechanical Vector transmission
5.5: Reservoirs of Infection and Disease Transmission
melting point
2.4.1: Biochemical Properties of Water
membrane attack complex
6.2: Non-Adaptive Immunity
membrane filtration
10.3: Using Physical Methods to Control Microorganisms
Memory B cells
6.3.2.1: B Lymphocytes and Antibodies
memory cell
6.3: Adaptive Immunity
memory T cell subtypes
6.3.3.1: T Lymphocytes
Mesophiles
8.2.1: Temperature Effects on Bacterial Growth
metabolism
7.1: Introduction to Metabolism and Enzymes
miasma theory of disease
1.6.3: What Causes Diseases?
Microaerophiles
8.2.4: Oxygen Effects on Bacterial Growth
Microbial death curves
10.1: Controlling Microbial Growth
Microbial fermentation
7.4.2: Fermentation
microbial growth
8.5: Growth Characteristics
microbiology
1.5: Types of Cells and Microorganisms
microbiome
5.2: Human Microbiota
microbiomes
1.2: The Roles of Microbes
microbiota
1.2: The Roles of Microbes
microvilli
3.8: Eukaryotic Plasma Membrane and The Cytoplasm
minimum growth pH
8.2.2: pH Effects on Bacterial Growth
minimum permissive oxygen concentration
8.2.4: Oxygen Effects on Bacterial Growth
missense mutation
9.5: Mutations
mitochondria
3.7: Eukaryotic External Structures
3.9: Eukaryote Organelles
mold
1.5: Types of Cells and Microorganisms
Molecular Koch’s postulates
1.6.5: Koch's Postulate
monomer
2.3.2: Synthesis of Biological Macromolecules
monomers
2.5.1: Organic Molecules
morbidity
5.6: Introduction to Epidemiology
morbidity rates
5.6: Introduction to Epidemiology
morphology
1.3: Classification, Phylogeny, and Nomenclature
mortality
5.6: Introduction to Epidemiology
mortality rates
5.6: Introduction to Epidemiology
MPN
8.1: How Bacteria Grow
mutagen
9.5: Mutations
mutation
9.5: Mutations
mycology
1.5: Types of Cells and Microorganisms

N

natamycin
10.4: Using Chemicals to Control Microorganisms
natural killer cells
6.2.4: Natural Killer Cells
Neisseria gonorrhoeae
3.3.3: Fimbriae and Pili
neutrophiles
8.2.2: pH Effects on Bacterial Growth
Nisin
10.4: Using Chemicals to Control Microorganisms
Nitrites
10.4: Using Chemicals to Control Microorganisms
nitrogenous base
9.1.3: Structure and Function of DNA
Noncoding DNA
9.1.1: The Structure and Function of Cellular Genomes
noncommunicable
5.3: Infectious Diseases Characteristics and Classification
noncompetitive inhibitors
7.1: Introduction to Metabolism and Enzymes
noncritical item
10.1: Controlling Microbial Growth
Noncyclic photophosphorylation
7.6.2: The Process of Photosynthesis
noninfectious
5.3: Infectious Diseases Characteristics and Classification
Nonionizing radiation
9.5: Mutations
10.3: Using Physical Methods to Control Microorganisms
nonsense mutation
9.5: Mutations

Nosocomial diseases
5.3: Infectious Diseases Characteristics and Classification
nosocomial infections
5.5: Reservoirs of Infection and Disease Transmission
5.6.2: Nosocomial Infections
5.6.2.2: Control of Nosocomial Infections
nuclear envelope
3.7: Eukaryotic External Structures
nucleic acids
9.1.3: Structure and Function of DNA
nucleolus
3.7: Eukaryotic External Structures
nucleoplasm
3.7: Eukaryotic External Structures
nucleotide excision repair
9.5: Mutations
nucleotides
9.1.3: Structure and Function of DNA
nucleus
3.7: Eukaryotic External Structures
3.9: Eukaryote Organelles
numerical aperture
4.4.1: The Properties of Light

O

obligate aerobes
8.2.4: Oxygen Effects on Bacterial Growth
obligate anaerobes
8.2.4: Oxygen Effects on Bacterial Growth
Okazaki fragments
9.2: DNA Replication
oncolytic virus
11.3: Prevention and Treatment of Viral Infections
opacity
4.4.1: The Properties of Light
operator
9.6: Gene Regulation - Operon Theory
operons
9.6: Gene Regulation - Operon Theory
opportunistic pathogens
1.6.5: Koch's Postulate
opsonization
3.3.3: Fimbriae and Pili
6.2: Non-Adaptive Immunity
Optimum oxygen concentration
8.2.4: Oxygen Effects on Bacterial Growth
organelle
3.2.1: Comparing Prokaryotic and Eukaryotic Cells
3.7: Eukaryotic External Structures
3.9: Eukaryote Organelles
organotrophs
7.1: Introduction to Metabolism and Enzymes
origin of replication
9.2: DNA Replication
osmolarity
3.8.1.5: Passive Transport - Osmosis
osmosis
3.8.1.5: Passive Transport - Osmosis
oxidative phosphorylation
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
oxygenic photosynthesis
7.6.2: The Process of Photosynthesis

P

P site
9.4: Protein Synthesis (Translation)
pandemic diseases
5.6: Introduction to Epidemiology
parasitism
5.1: Microbe and Human Symbiotic Relationships
passive transport
3.8.1.3: Passive Transport - Diffusion
Pasteurization
10.3: Using Physical Methods to Control Microorganisms
pathogen
1.5: Types of Cells and Microorganisms
Pathogenicity Islands
8.3: Bacterial Quorum Sensing
penetration
11.2: The Viral Life Cycle and Host Infection Specificity
pentose
9.1.4: Structure and Function of RNA
pentose phosphate pathway (PPP)
7.2B: Alternative to Glycolysis- the Pentose Phosphate Shunt
peptidoglycan
3.5: Prokaryote Plasma Membrane
peptidoglygan
3.4.2: Gram-Negative Wall
perforin
6.3.3.1: T Lymphocytes
period of convalescence
5.3: Infectious Diseases Characteristics and Classification
period of decline
5.3: Infectious Diseases Characteristics and Classification
period of illness
5.3: Infectious Diseases Characteristics and Classification
peripheral protein
3.8.1.1: The Plasma Membrane
peripheral tolerance
6.3.3.1: T Lymphocytes
periplasm
3.4.2: Gram-Negative Wall
Peroxidase
8.2.4: Oxygen Effects on Bacterial Growth
peroxisome
3.7: Eukaryotic External Structures
Peroxygens
10.4: Using Chemicals to Control Microorganisms
phage therapy
11.3: Prevention and Treatment of Viral Infections
phagocytosis
3.8.1.6: Active Transport
phenol coefficient
10.2: Testing the Effectiveness of Antiseptics and Disinfectants
Phenolics
10.4: Using Chemicals to Control Microorganisms
phenotype
9.1.1: The Structure and Function of Cellular Genomes
phosphodiester backbones
9.1.3: Structure and Function of DNA
phospholipases
7.5: Catabolism of Lipids and Proteins
phosphorescence
4.4.1: The Properties of Light
photosynthesis
7.6.2: The Process of Photosynthesis
photosynthetic pigments
7.6.2: The Process of Photosynthesis
photosystems
7.6.2: The Process of Photosynthesis
phototrophs
7.1: Introduction to Metabolism and Enzymes
phylogenetic tree
1.3: Classification, Phylogeny, and Nomenclature
phylogeny
1.3: Classification, Phylogeny, and Nomenclature
4.3: Classification and Identification
4.3.1: Kingdom Classification According to Whittaker
pili
3.3.3: Fimbriae and Pili
5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal
pilin
3.3.3: Fimbriae and Pili
pilus
3.5: Prokaryote Plasma Membrane
pinocytosis
3.8.1.6: Active Transport
planktonic
8.1: How Bacteria Grow
plaques
11.5.1: Isolation, Culture, and Identification of Viruses
plasma
6.2: Non-Adaptive Immunity
plasma cells
6.3.2.1: B Lymphocytes and Antibodies
plasma cell
6.3: Adaptive Immunity
plasma membrane
3.7: Eukaryotic External Structures
3.8: Eukaryotic Plasma Membrane and The Cytoplasm
3.8.1.1: The Plasma Membrane
plasmids
9.1.2: Plasmids
9.1.2A: Introduction to Plasmids
plastid
3.10: Serial Endosymbiosis Theory (SET)
point mutation
9.5: Mutations
polar molecule
2.4.1: Biochemical Properties of Water
polarity
2.4.1: Biochemical Properties of Water
polycistronic
9.3: RNA Transcription
polycistronic mRNA
9.3: RNA Transcription
polyhedral
11.1: Viruses - General Characteristics
polymer
2.3.2: Synthesis of Biological Macromolecules

polymers
2.5.1: Organic Molecules
portals of entry
1.6.5: Koch's Postulate
portals of exit
1.6.5: Koch's Postulate
pour plate
8.1: How Bacteria Grow
prevalence
5.6: Introduction to Epidemiology
primary active transport
3.8.1.6: Active Transport
primary infection
1.6.5: Koch's Postulate
Primary pathogens
1.6.5: Koch's Postulate
primase
9.2: DNA Replication
primer
9.2: DNA Replication
9.3: RNA Transcription
prions
11.6: Viroids and Prions
probiotic
5.2: Human Microbiota
prodromal period
5.3: Infectious Diseases Characteristics and Classification
prokaryote
4.3: Classification and Identification
4.3.1: Kingdom Classification According to Whittaker
prokaryotic cell
3.2.1: Comparing Prokaryotic and Eukaryotic Cells
properties of life
3.1: Characteristics of Life
Prospective epidemiology
1.6.6: Improvement in Public Health
5.6.1: Epidemiology
prostaglandins
6.2: Non-Adaptive Immunity
proteases
7.5: Catabolism of Lipids and Proteins
protein A
5.4.1.3: The Ability to Evade Adaptive Immune Defenses
protein G
5.4.1.3: The Ability to Evade Adaptive Immune Defenses
proteinaceous
11.6: Viroids and Prions
protist
1.5: Types of Cells and Microorganisms
proton motive force
7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation
Protozoa
1.2: The Roles of Microbes
protozoan
1.5: Types of Cells and Microorganisms
protozoology
1.5: Types of Cells and Microorganisms
pseudopeptidoglycan
3.5: Prokaryote Plasma Membrane
psychrophiles
8.2.1: Temperature Effects on Bacterial Growth
psychrotrophs
8.2.1: Temperature Effects on Bacterial Growth
purines
9.1.3: Structure and Function of DNA
pyrimidines
9.1.3: Structure and Function of DNA

Q

Quaternary ammonium compounds
10.4: Using Chemicals to Control Microorganisms
quats
10.4: Using Chemicals to Control Microorganisms
quorum sensing
8.1: How Bacteria Grow
8.3: Bacterial Quorum Sensing

R

R plasmids
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
reactive oxygen species
8.2.4: Oxygen Effects on Bacterial Growth
receptor
3.8.1.1: The Plasma Membrane
redox reactions
7.1: Introduction to Metabolism and Enzymes
Reemerging diseases
5.6.3: Global Public Health
Reflection
4.4.1: The Properties of Light
refraction
4.4.1: The Properties of Light
refractive index
4.4.1: The Properties of Light
regulatory T cells
6.3: Adaptive Immunity
6.3.3.1: T Lymphocytes
Release
11.2: The Viral Life Cycle and Host Infection Specificity
repressible operon
9.6: Gene Regulation - Operon Theory
repressor
9.6: Gene Regulation - Operon Theory
Reservoirs
5.5: Reservoirs of Infection and Disease Transmission
resident microbiota
1.6.5: Koch's Postulate
resolution
4.4.1: The Properties of Light
ribonucleotides
9.1.4: Structure and Function of RNA
ribose
9.1.4: Structure and Function of RNA
ribosome
3.7: Eukaryotic External Structures
ribosomes
3.9: Eukaryote Organelles
riboswitches
9.6: Gene Regulation - Operon Theory
ribozymes
9.1.4: Structure and Function of RNA
RNA
2.5.4: DNA and RNA
9.1.4: Structure and Function of RNA
RNA polymerase
9.3: RNA Transcription
RNA transcript
9.3: RNA Transcription
Robert Koch
1.6.4: Koch and Pure Culture
rolling circle replication
9.2: DNA Replication
rough endoplasmic reticulum
3.9: Eukaryote Organelles

S

secondary active transport
3.8.1.6: Active Transport
secondary infection
1.6.5: Koch's Postulate
selection
6.3: Adaptive Immunity
selective media
8.4.1: Media Used for Bacterial Growth
8.4.4: Selective and Differential Media
selectively permeable
3.8.1.2: Transport Across Membranes
semiconservative
9.2: DNA Replication
semicritical item
10.1: Controlling Microbial Growth
sepsis
10.1: Controlling Microbial Growth
serial dilutions
8.1: How Bacteria Grow
sessile
8.1: How Bacteria Grow
sex pili
3.3.3: Fimbriae and Pili
short attachment pili
3.3.3: Fimbriae and Pili
sigma factor
9.6: Gene Regulation - Operon Theory
silent mutation
9.5: Mutations
smooth endoplasmic reticulum
3.9: Eukaryote Organelles
solute
3.8.1.5: Passive Transport - Osmosis
solvent
3.8.1.5: Passive Transport - Osmosis
specialized transduction
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
11.2: The Viral Life Cycle and Host Infection Specificity
species
1.3: Classification, Phylogeny, and Nomenclature
splicing
9.3: RNA Transcription
Spontaneous mutations
9.5: Mutations

Sporadic diseases
5.6: Introduction to Epidemiology
spread plate
8.1: How Bacteria Grow
start codon
9.4: Protein Synthesis (Translation)
stationary
8.1: How Bacteria Grow
stereoisomers
2.5.1: Organic Molecules
sterilant
10.1: Controlling Microbial Growth
sterile field
10.1: Controlling Microbial Growth
Sterilization
10.1: Controlling Microbial Growth
stop codon
9.4: Protein Synthesis (Translation)
structural isomers
2.5.1: Organic Molecules
substrates
7.1: Introduction to Metabolism and Enzymes
Sulfites
10.4: Using Chemicals to Control Microorganisms
Superantigens
6.3.3.1: T Lymphocytes
supercoiled
9.2: DNA Replication
supercritical fluid
10.4: Using Chemicals to Control Microorganisms
surface tension
2.4.1: Biochemical Properties of Water
Surfactants
10.4: Using Chemicals to Control Microorganisms
symptoms
5.3: Infectious Diseases Characteristics and Classification
systemic
1.6.5: Koch's Postulate
systemic infection
1.6.5: Koch's Postulate

T

T cells
6.3.3: Cell-Mediated Immunity
taxa
1.3: Classification, Phylogeny, and Nomenclature
taxonomy
1.3: Classification, Phylogeny, and Nomenclature
4.3: Classification and Identification
4.3.1: Kingdom Classification According to Whittaker
tcr
6.3.3.1: T Lymphocytes
teichoic acid
3.5: Prokaryote Plasma Membrane
telomerase
9.2: DNA Replication
telomeres
9.2: DNA Replication
termination
9.4: Protein Synthesis (Translation)
Termination of transcription
9.3: RNA Transcription
tetracyclines
12.1E: Antibiotic Classifications
TH
6.3: Adaptive Immunity
Th1 cells
6.3.3.1: T Lymphocytes
th17 cells
6.3.3.1: T Lymphocytes
Th2 cells
6.3.3.1: T Lymphocytes
Theory of Spontaneous Generation
1.6.2: Pasteur and Spontaneous Generation
thermophiles
8.2.1: Temperature Effects on Bacterial Growth
Thymic selection
6.3.3.1: T Lymphocytes
thymine
9.1.3: Structure and Function of DNA
9.1.4: Structure and Function of RNA
tincture
10.4: Using Chemicals to Control Microorganisms
tonicity
3.8.1.5: Passive Transport - Osmosis
Toxins
5.4: Virulence Factors
transcription factors
9.6: Gene Regulation - Operon Theory
transduction
3.5: Prokaryote Plasma Membrane
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
transformation
3.5: Prokaryote Plasma Membrane
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
translation
9.4: Protein Synthesis (Translation)
transmissible spongiform encephalopathies
11.6: Viroids and Prions
transmission
1.6.5: Koch's Postulate
transmittance
4.4.1: The Properties of Light
transparency
4.4.1: The Properties of Light
transposons
9.7: How Asexual Prokaryotes Achieve Genetic Diversity
triclosan
10.4: Using Chemicals to Control Microorganisms
tubulin
3.9: Eukaryote Organelles
turgor pressure
3.8.1.5: Passive Transport - Osmosis

U

uncoating
11.2: The Viral Life Cycle and Host Infection Specificity
uracil
9.1.4: Structure and Function of RNA

V

vaccine
11.3: Prevention and Treatment of Viral Infections
vaccines
11.3: Prevention and Treatment of Viral Infections
vacuole
3.7: Eukaryotic External Structures
3.9: Eukaryote Organelles
Vector transmission
5.5: Reservoirs of Infection and Disease Transmission
Vehicle transmission
5.5: Reservoirs of Infection and Disease Transmission
vertical gene transfer
9.1.3: Structure and Function of DNA
vesicle
3.7: Eukaryotic External Structures
3.9: Eukaryote Organelles
Viral cultivation
11.5.1: Isolation, Culture, and Identification of Viruses
Viral filtrate
11.5.1: Isolation, Culture, and Identification of Viruses
viricides
10.1: Controlling Microbial Growth
virions
11.1: Viruses - General Characteristics
viroids
11.6: Viroids and Prions
virology
1.5: Types of Cells and Microorganisms
virulence
1.6.5: Koch's Postulate
virulence factors
5.4: Virulence Factors
virus
1.5: Types of Cells and Microorganisms
viruses
1.2: The Roles of Microbes
11.1: Viruses - General Characteristics
virusoids
11.6: Viroids and Prions

W

wavelength
4.4.1: The Properties of Light
WHO
5.6.3: Global Public Health
World Health Organization
5.6.3: Global Public Health

Y

yeast
1.5: Types of Cells and Microorganisms
yellow fever
8.4.5: Special Culture Techniques

Z

Z ring
8.1: How Bacteria Grow

zoonoses

5.3: Infectious Diseases Characteristics and Classification

Glossary

Sample Word 1 | Sample Definition 1

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  - Front Matter - *Undeclared*
    - TitlePage - *Undeclared*
    - InfoPage - *Undeclared*
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    - Licensing - *Undeclared*
  - 1: Introduction to Microbiology - *Undeclared*
    - 1.1: Introduction to Microbiology - *CC BY-NC-SA 4.0*
    - 1.2: The Roles of Microbes - *CC BY 4.0*
    - 1.3: Classification, Phylogeny, and Nomenclature - *CC BY-SA 4.0*
    - 1.4: Carl Woese and the Phylogenetic Tree - *CC BY-SA 4.0*
    - 1.5: Types of Cells and Microorganisms - *CC BY 4.0*
    - 1.6: History of Microbiology - *Undeclared*
      - 1.6.1: Hooke, van Leeuwenhoek, and Cohn - *CC BY-SA 4.0*
      - 1.6.2: Pasteur and Spontaneous Generation - *CC BY 4.0*
      - 1.6.3: What Causes Diseases? - *CC BY 4.0*
      - 1.6.4: Koch and Pure Culture - *CC BY-SA 4.0*
      - 1.6.5: Koch's Postulate - *CC BY 4.0*
      - 1.6.6: Improvement in Public Health - *CC BY 4.0*
      - 1.6.7: The Discovery of Antimicrobial Drugs - *CC BY-SA 4.0*
      - 1.6.E: The History (Exercises) - *CC BY 4.0*
  - 2: Chemistry of Microbiology - *Undeclared*
    - 2.1: Atomic Structure - *CC BY-SA 4.0*
      - 2.1.1: Overview of Atomic Structure - *CC BY-SA 4.0*
      - 2.1.2: Electronic Orbitals - *CC BY-SA 4.0*
    - 2.2: Chemical Bonds - *CC BY-SA 4.0*
      - 2.2.1: Ions and Ionic Bonds - *CC BY-SA 4.0*
      - 2.2.2: Covalent Bonds and Other Bonds and Interaction - *CC BY-SA 4.0*
      - 2.2.3: Hydrogen Bonding - *CC BY-SA 4.0*
    - 2.3: Chemical Reactions - *CC BY-SA 4.0*
      - 2.3.1: The Chemical Basis of Life - *CC BY-SA 4.0*
      - 2.3.2: Synthesis of Biological Macromolecules - *CC BY 4.0*
      - 2.3.3: Energy and Biochemical Reactions - *CK-12 License*
    - 2.4: Water and pH - *CC BY-SA 4.0*
      - 2.4.1: Biochemical Properties of Water - *CC BY-SA 4.0*
      - 2.4.2: pH, Buffers, Acids, and Bases - *CC BY-SA 4.0*
    - 2.5: Organic Compounds - *CC BY-SA 4.0*
      - 2.5.1: Organic Molecules - *CC BY 4.0*
      - 2.5.2: Carbohydrate Molecules - *CC BY-SA 4.0*
      - 2.5.3: Lipid Molecules - *CC BY-SA 4.0*
      - 2.5.4: DNA and RNA - *CC BY-SA 4.0*
      - 2.5.5: Amino Acids - *CC BY-SA 4.0*
      - 2.5.6: ATP- Adenosine Triphosphate - *CC BY-SA 4.0*
      - 2.5.E: Organic Molecules (Exercises) - *CC BY 4.0*
  - 3: Cell Structure and Function - *Undeclared*
    - 3.1: Characteristics of Life - *CC BY-SA 4.0*
    - 3.2: Cells and Cell Theory - *CC BY-SA 4.0*

- 3.2.1: Comparing Prokaryotic and Eukaryotic Cells - *CC BY 4.0*
- 3.3: Prokaryotic External Structures - *CC BY-SA 4.0*
  - 3.3.1: Bacteria Surface Structures - *CC BY-NC-SA 4.0*
  - 3.3.2: Flagella - *CC BY 4.0*
  - 3.3.3: Fimbriae and Pili - *CC BY 4.0*
- 3.4: Prokaryote Cell Wall - *CC BY-NC-SA 4.0*
  - 3.4.1: Antibiotics and Selective Toxicity - *CC BY-SA 4.0*
  - 3.4.2: Gram-Negative Wall - *CC BY-SA 4.0*
  - 3.4.3: Gram-Positive Cell Wall - *CC BY-SA 4.0*
  - 3.4.4: Archaea Cell Wall - *CC BY-SA 4.0*
- 3.5: Prokaryote Plasma Membrane - *CC BY 4.0*
  - 3.5.1: Components and Structure - Fluid Mosaic Model - *CC BY-SA 4.0*
- 3.6: Endospores - *CC BY-SA 4.0*
  - 3.6.1: Fungal Spores - *CC BY-NC 4.0*
- 3.7: Eukaryotic External Structures - *CC BY 4.0*
- 3.8: Eukaryotic Plasma Membrane and The Cytoplasm - *CC BY 4.0*
  - 3.8.1: The Plasma Membrane and Transport - *CC BY 4.0*
    - 3.8.1.1: The Plasma Membrane - *CC BY 4.0*
    - 3.8.1.2: Transport Across Membranes - *CC BY 4.0*
    - 3.8.1.3: Passive Transport - Diffusion - *CC BY 4.0*
    - 3.8.1.4: Passive Transport - Facilitated Transport - *CC BY 4.0*
    - 3.8.1.5: Passive Transport - Osmosis - *CC BY 4.0*
    - 3.8.1.6: Active Transport - *CC BY 4.0*
  - 3.8.2: Endocytosis - *CC BY-SA 4.0*
- 3.9: Eukaryote Organelles - *CK-12 License*
- 3.10: Serial Endosymbiosis Theory (SET) - *CC BY 4.0*

4: Microscopy, Staining, and Classification - *Undeclared*

- 4.1: Units of Measure - *CC BY-NC-SA 4.0*
- 4.2: Staining Microscopic Specimens - *CC BY 4.0*
- 4.3: Classification and Identification - *CC BY 4.0*
  - 4.3.1: Kingdom Classification According to Whittaker - *CC BY 4.0*
  - 4.3.2: Domain Classification According to Woese - *CC BY-SA 4.0*
- 4.4: Microscopy - *Undeclared*
  - 4.4.1: The Properties of Light - *CC BY 4.0*
  - 4.4.2: Types of Microscopes - *Undeclared*

5: Infectious Diseases and Epidemiology - *Undeclared*

- 5.1: Microbe and Human Symbiotic Relationships - *CC BY-SA 4.0*
- 5.2: Human Microbiota - *CC BY-NC 3.0*
  - 5.2.1: Opportunistic Pathogens - *CC BY-SA 4.0*
- 5.3: Infectious Diseases Characteristics and Classification - *CC BY 4.0*
  - 5.3.1: Koch's Postulates - *CC BY-SA 4.0*
- 5.4: Virulence Factors - *CC BY 4.0*
  - 5.4.1: Virulence Factors that Promote Colonization - *CC BY 4.0*
    - 5.4.1.1: The Ability to Use Motility and Other Means to Contact Host Cells - *CC BY 4.0*
    - 5.4.1.2: The Ability to Adhere to Host Cells and Resist Physical Removal - *CC BY 4.0*
    - 5.4.1.3: The Ability to Evade Adaptive Immune Defenses - *CC BY 4.0*
  - 5.4.2: Virulence Factors that Damage the Host - *CC BY 4.0*
- 5.5: Reservoirs of Infection and Disease Transmission - *CC BY 4.0*
- 5.6: Introduction to Epidemiology - *CC BY 4.0*
  - 5.6.1: Epidemiology - *CC BY 4.0*
    - 5.6.1.1: Descriptive Epidemiology - *CC BY-SA 4.0*
    - 5.6.1.2: Analytical Epidemiology - *CC BY-SA 4.0*
    - 5.6.1.3: Experimental Epidemiology - *CC BY-SA 4.0*
  - 5.6.2: Nosocomial Infections - *CC BY-SA 4.0*
    - 5.6.2.1: Transmission of Nosocomial Infection - *CC BY-SA 4.0*
    - 5.6.2.2: Control of Nosocomial Infections - *CC BY-SA 4.0*
  - 5.6.3: Global Public Health - *CC BY 4.0*
    - 5.6.3I: Current Epidemics - *CC BY-SA 4.0*

6: Immunology - *Undeclared*

- 6.1: Overview of the Immune System - *CC BY-SA 4.0*
  - 6.1.1: Cells and Organs of the Immune System - *CC BY-SA 4.0*
- 6.2: Non-Adaptive Immunity - *CC BY 4.0*
  - 6.2.1: Pathogen Recognition - *CC BY-SA 4.0*
    - 6.2.1.1: Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs) - *CC BY 4.0*

- 6.2.1.2: Pattern-Recognition Receptors (PRRs) - *CC BY 4.0*
- 6.2.2: The Complement System - *CC BY-SA 4.0*
- 6.2.3: Interferons - *CC BY-SA 4.0*
- 6.2.4: Natural Killer Cells - *CC BY-SA 4.0*
- 6.2.5: Dendritic Cells - *CC BY-SA 4.0*
- 6.2.6: Inflammation and Fever - *CC BY 4.0*
- 6.3: Adaptive Immunity - *CC BY 4.0*
  - 6.3.1: Antigen Epitopes - *CC BY 4.0*
  - 6.3.2: Humoral Immune Response - *CC BY-SA 4.0*
    - 6.3.2.1: B Lymphocytes and Antibodies - *CC BY 4.0*
    - 6.3.2.2: Antibody Structure - *CC BY-SA 4.0*
    - 6.3.2.3: Antibody Functions - *CC BY-SA 4.0*
  - 6.3.3: Cell-Mediated Immunity - *CC BY-SA 4.0*
    - 6.3.3.1: T Lymphocytes - *CC BY 4.0*
  - 6.3.4: Immunological Memory, Primary, and Secondary Adaptive Response - *CC BY-SA 4.0*

- 7: Microbial Metabolism - *Undeclared*
  - 7.1: Introduction to Metabolism and Enzymes - *CC BY 4.0*
    - 7.1.1: Factors Affecting Enzyme Activity - *CC BY 4.0*
  - 7.2: Catabolism of Glucose (1)- Stages 1-3 of Cellular Respiration - *CC BY 4.0*
    - 7.2A: Alternative to Glycolysis- the Entner-Doudoroff Pathway - *CC BY-SA 4.0*
    - 7.2B: Alternative to Glycolysis- the Pentose Phosphate Shunt - *CC BY-SA 4.0*
  - 7.3: Catabolism of Glucose (2)- Stage 4- Oxidative Phosphorylation - *CC BY 4.0*
  - 7.4: Catabolism of Glucose (3)- In the Absence of Oxygen - *Undeclared*
    - 7.4.1: Electron Donors and Acceptors in Anaerobic Respiration - *CC BY-SA 4.0*
    - 7.4.2: Fermentation - *CC BY 4.0*
  - 7.5: Catabolism of Lipids and Proteins - *CC BY 4.0*
  - 7.6: Anabolism of Glucose- Photosynthesis - *CC BY-SA 4.0*
    - 7.6.1: The Two Parts of Photosynthesis - *CC BY-SA 4.0*
    - 7.6.2: The Process of Photosynthesis - *CC BY 4.0*
  - 7.7: Other Anabolism - *Undeclared*
    - 7.7.1: Amino Acid Synthesis - *CC BY-SA 4.0*
    - 7.7.2: Polysaccharide Biosynthesis - *CC BY-SA 4.0*
    - 7.7.3: Lipid Biosynthesis - *CC BY-SA 4.0*
  - 7.E: Microbial Metabolism (Exercises) - *CC BY 4.0*
- 8: Microbial Growth - *CC BY 4.0*
  - 8.1: How Bacteria Grow - *CC BY 4.0*
  - 8.2: Factors that Affect Bacterial Growth - *CC BY 4.0*
    - 8.2.1: Temperature Effects on Bacterial Growth - *CC BY 4.0*
    - 8.2.2: pH Effects on Bacterial Growth - *CC BY 4.0*
    - 8.2.3: Nitrogen Effects on Bacterial Growth - *CC BY-SA 4.0*
    - 8.2.4: Oxygen Effects on Bacterial Growth - *CC BY 4.0*
    - 8.2.5: Other Environmental Effects on Bacterial Growth - *CC BY 4.0*
  - 8.3: Bacterial Quorum Sensing - *CC BY 4.0*
  - 8.4: Culturing Bacteria - *CC BY-SA 4.0*
    - 8.4.1: Media Used for Bacterial Growth - *CC BY 4.0*
    - 8.4.2: Culture Media - *CC BY-SA 4.0*
    - 8.4.3: Complex and Synthetic Media - *CC BY-SA 4.0*
    - 8.4.4: Selective and Differential Media - *CC BY-SA 4.0*
    - 8.4.5: Special Culture Techniques - *CC BY-SA 4.0*
  - 8.5: Growth Characteristics - *CC BY-NC-SA 4.0*
    - 8.5.1: Generation Time - *CC BY 4.0*
- 9: Microbial Genetics - *Undeclared*
  - 9.1: Genome, DNA, and Genes - *Undeclared*
    - 9.1.1: The Structure and Function of Cellular Genomes - *CC BY 4.0*
    - 9.1.2: Plasmids - *CC BY-SA 4.0*
      - 9.1.2A: Introduction to Plasmids - *CC BY-SA 4.0*
      - 9.1.2B: Types of Plasmids and Their Biological Significance - *CC BY-SA 4.0*
    - 9.1.3: Structure and Function of DNA - *CC BY 4.0*
    - 9.1.4: Structure and Function of RNA - *CC BY 4.0*
    - 9.1.5: What Are Genes? - *CC BY 4.0*
    - 9.1.E: Biochemistry of the Genome (Exercises) - *CC BY 4.0*
  - 9.2: DNA Replication - *CC BY 4.0*
  - 9.3: RNA Transcription - *CC BY 4.0*
    - 9.3A: mRNA Processing - *CC BY-SA 4.0*
  - 9.4: Protein Synthesis (Translation) - *CC BY 4.0*
  - 9.5: Mutations - *CC BY 4.0*
    - 9.5A: DNA Repair - *CC BY-SA 4.0*
  - 9.6: Gene Regulation - Operon Theory - *CC BY 4.0*

- 9.7: How Asexual Prokaryotes Achieve Genetic Diversity - *CC BY 4.0*
- 9.E: Mechanisms of Microbial Genetics (Exercises) - *CC BY 4.0*

- 10: Control of Microbial Growth - *CC BY 4.0*
  - 10.1: Controlling Microbial Growth - *CC BY 4.0*
  - 10.2: Testing the Effectiveness of Antiseptics and Disinfectants - *CC BY 4.0*
  - 10.3: Using Physical Methods to Control Microorganisms - *CC BY 4.0*
  - 10.4: Using Chemicals to Control Microorganisms - *CC BY 4.0*
  - 10.E: Control of Microbial Growth (Exercises) - *CC BY 4.0*
- 11: Viruses and Other Agents - *Undeclared*
  - 11.1: Viruses - General Characteristics - *CC BY 4.0*
  - 11.2: The Viral Life Cycle and Host Infection Specificity - *CC BY 4.0*
    - 11.2.1: Medical Importance of Viruses - *CC BY-SA 4.0*
    - 11.2.2: The Baltimore Virus Classification - *CC BY-SA 4.0*
    - 11.2.3: Evolution of Viruses - *CC BY-SA 4.0*
  - 11.3: Prevention and Treatment of Viral Infections - *CC BY 4.0*
  - 11.4: The Role of Viruses in Tumor Production - *CC BY 4.0*
  - 11.5: Culturing Viruses - *CC BY-SA 4.0*
    - 11.5.1: Isolation, Culture, and Identification of Viruses - *CC BY 4.0*
    - 11.5.2: Tissue Culture of Animal Viruses - *CC BY-SA 4.0*
    - 11.5.3: Batch Culture of Bacteriophages - *CC BY-SA 4.0*
  - 11.6: Viroids and Prions - *CC BY 4.0*
- 12: Antimicrobial Drugs - *CC BY-SA 4.0*
  - 12.1: Overview of Antimicrobial Therapy - *CC BY-SA 4.0*
    - 12.1A: Origins of Antimicrobial Drugs - *CC BY-SA 4.0*
    - 12.1B: Antibiotic Discovery - *CC BY-SA 4.0*
    - 12.1C: Antibiotics and Selective Toxicity - *CC BY-SA 4.0*
    - 12.1D: Spectrum of Antimicrobial Activity - *CC BY-SA 4.0*
    - 12.1E: Antibiotic Classifications - *CC BY-SA 4.0*
  - 12.2: Functions of Antimicrobial Drugs - *CC BY-SA 4.0*
    - 12.2A: Inhibiting Cell Wall Synthesis - *CC BY-SA 4.0*
    - 12.2B: Injuring the Plasma Membrane - *CC BY-SA 4.0*
    - 12.2C: Inhibiting Nucleic Acid Synthesis - *CC BY-SA 4.0*
    - 12.2D: Inhibiting Protein Synthesis - *CC BY-SA 4.0*
    - 12.2E: Inhibiting Essential Metabolite Synthesis - *CC BY-SA 4.0*
  - 12.3: Commonly Used Antimicrobial Drugs - *CC BY-SA 4.0*
    - 12.3A: Synthetic Antimicrobial Drugs - *CC BY-SA 4.0*
    - 12.3B: Naturally Occurring Antimicrobial Drugs-Antibiotics - *CC BY-SA 4.0*
    - 12.3C: Beta-Lactam Antibiotics- Penicillins and Cephalosporins - *CC BY-SA 4.0*
    - 12.3D: Antibiotics from Prokaryotes - *CC BY-SA 4.0*
    - 12.3E: Antimycobacterial Antibiotics - *CC BY-SA 4.0*
  - 12.4: Interactions Between Drug and Host - *CC BY-SA 4.0*
    - 12.4A: Organ Toxicity - *CC BY-SA 4.0*
    - 12.4B: Allergic Responses to Drugs - *CC BY-SA 4.0*
    - 12.4C: Suppression and Alteration of Microbiota by Antimicrobials - *CC BY-SA 4.0*
    - 12.4D: Effects of Drug Combinations - *CC BY-SA 4.0*
  - 12.5: Measuring Drug Susceptibility - *CC BY-SA 4.0*
    - 12.5A: Minimal Inhibitory Concentration (MIC) - *CC BY-SA 4.0*
    - 12.5B: Kirby-Bauer Disk Susceptibility Test - *CC BY-SA 4.0*
  - 12.6: Drug Resistance - *CC BY-SA 4.0*
    - 12.6A: Mechanisms of Resistance - *CC BY-SA 4.0*
    - 12.6B: Antibiotic Misuse - *CC BY-SA 4.0*
    - 12.6C: Cost and Prevention of Resistance - *CC BY-SA 4.0*
    - 12.6D: Biofilms, Persisters, and Antibiotic Tolerance - *CC BY-SA 4.0*
    - 12.6E: Finding New Antimicrobial Drugs - *CC BY-SA 4.0*
- Back Matter - *Undeclared*
  - Index - *Undeclared*
  - Glossary - *Undeclared*

- Detailed Licensing - *Undeclared*