

INTRODUCTION TO MICROBIOLOGY OER



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Introduction to Microbiology

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About the Authors

About the Book

1: INTRODUCTION TO MICROBIOLOGY

From boiling thermal hot springs to deep beneath the Antarctic ice, microorganisms can be found almost everywhere on earth in great quantities. Microorganisms (or microbes, as they are also called) are small organisms. Most are so small that they cannot be seen without a microscope.

Most microorganisms are harmless to humans and, in fact, many are helpful. They play fundamental roles in ecosystems everywhere on earth, forming the backbone of many food webs. People use them to make biofuels, medicines, and even foods. Without microbes, there would be no bread, cheese, or beer. Our bodies are filled with microbes, and our skin alone is home to trillions of them¹. Some of them we can't live without; others cause diseases that can make us sick or even kill us.



Figure 1.1: A veterinarian gets ready to clean a sea turtle covered in oil following the Deepwater Horizon oil spill in the Gulf of Mexico in 2010. After the spill, the population of a naturally occurring oil-eating marine bacterium called *Alcanivorax borkumensis* skyrocketed, helping to get rid of the oil. Scientists are working on ways to genetically engineer this bacterium to be more efficient in cleaning up future spills. (credit: modification of work by NOAA's National Ocean Service)

Although much more is known today about microbial life than ever before, the vast majority of this invisible world remains unexplored. Microbiologists continue to identify new ways that microbes benefit and threaten humans.

1.1: Introduction to Microbiology

1.2: What Our Ancestors Knew

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1.4: Types of Microorganisms

1.E: An Invisible World (Exercises)

Footnotes

1. J. Hulcr et al. "A Jungle in There: Bacteria in Belly Buttons are Highly Diverse, but Predictable." *PLoS ONE* 7 no. 11 (2012): e47712. doi:10.1371/journal.pone.0047712.

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1.1: Introduction to Microbiology

Learning Objectives

- State three harmful effects and four beneficial effects associated with the activities of microorganisms.
- Define microbiota and microbiome.
- Briefly describe two different beneficial things the human microbiome does for the normal function of our body.
- State several diseases associated with a change in our "normal" microbiota.
- 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.

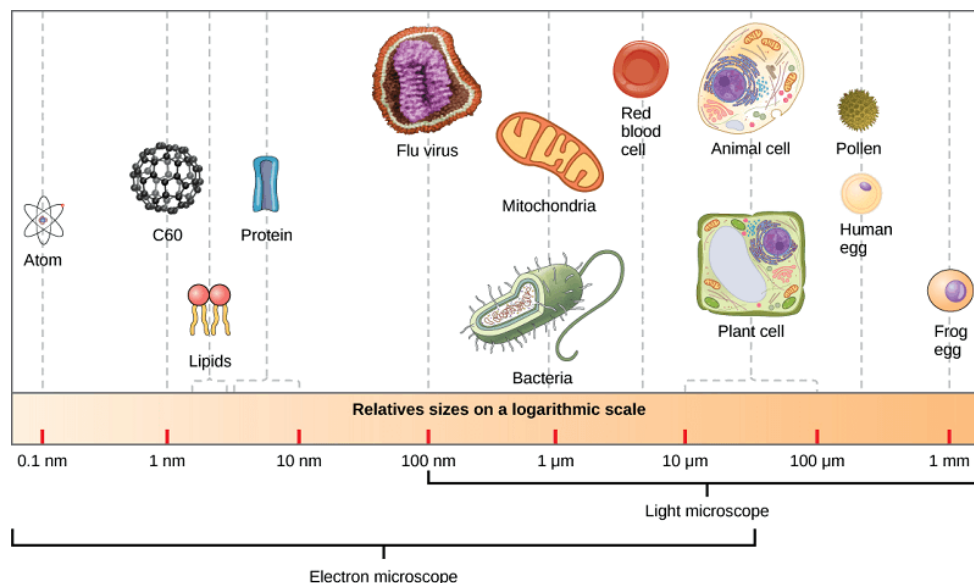


Figure 1.1.1: The size of a virus is very small relative to the size of cells and organelles.

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 learning 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 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 we 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 of 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 as 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, microorganisms 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 would 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 that 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 of 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.

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Case Study Preview: An Unknown Encephalopathy

When 41-year-old Cora arrives at the hospital with a high fever, confusion, and a stiff neck, doctors suspect meningitis - a potentially life-threatening infection. But when routine tests rule out bacteria, viruses, and fungi, her symptoms worsen and the mystery deepens. What kind of microbe is behind her rapid decline?

This real-world case guides you through diagnostic tools like lumbar punctures and Gram stains, and introduces prions - rare, acellular infectious agents that defy conventional treatment.

Follow Cora's story to explore the limits of modern microbiology - and discover how a single misfolded protein can change everything.

[Chapter 1 Case Study - An Unknown Encephalopathy.](#)

Key Concepts and 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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1.2: What Our Ancestors Knew

Learning Objectives

- Describe how our ancestors improved food with the use of invisible microbes
- Describe how the causes of sickness and disease were explained in ancient times, prior to the invention of the microscope
- Describe key historical events associated with the birth of microbiology

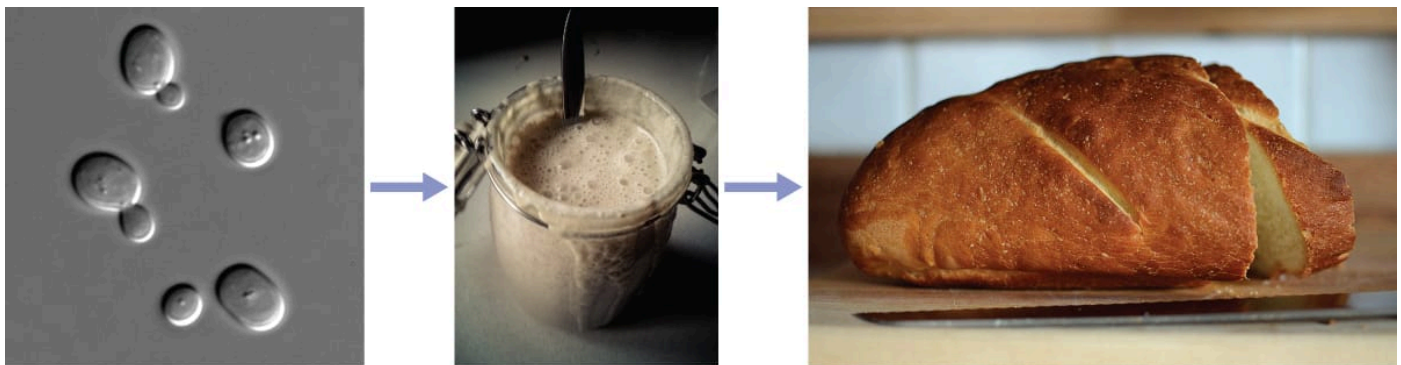
Most people today, even those who know very little about microbiology, are familiar with the concept of microbes, or “germs,” and their role in human health. Schoolchildren learn about bacteria, viruses, and other microorganisms, and many even view specimens under a microscope. But a few hundred years ago, before the invention of the microscope, the existence of many types of microbes was impossible to prove. By definition, **microorganisms**, or **microbes**, are very small organisms; many types of microbes are too small to see without a microscope, although some parasites and fungi are visible to the naked eye.

Humans have been living with—and using—microorganisms for much longer than they have been able to see them. Historical evidence suggests that humans have had some notion of microbial life since prehistoric times and have used that knowledge to develop foods as well as prevent and treat disease. In this section, we will explore some of the historical applications of microbiology as well as the early beginnings of microbiology as a science.

Fermented Foods and Beverages

People across the world have enjoyed fermented foods and beverages like beer, wine, bread, yogurt, cheese, and pickled vegetables for all of recorded history. Discoveries from several archeological sites suggest that even prehistoric people took advantage of fermentation to preserve and enhance the taste of food. Archaeologists studying pottery jars from a Neolithic village in China found that people were making a fermented beverage from rice, honey, and fruit as early as 7000 BC.²

Production of these foods and beverages requires microbial fermentation, a process that uses bacteria, mold, or yeast to convert sugars (carbohydrates) to alcohol, gases, and organic acids (Figure 1.2.2). While it is likely that people first learned about fermentation by accident—perhaps by drinking old milk that had curdled or old grape juice that had fermented—they later learned to harness the power of fermentation to make products like bread, cheese, and wine.



Yeast fermentation yields ethanol and CO₂.

Figure 1.2.2: A microscopic view of *Saccharomyces cerevisiae*, the yeast responsible for making bread rise (left). Yeast is a microorganism. Its cells metabolize the carbohydrates in flour (middle) and produce carbon dioxide, which causes the bread to rise (right). (credit middle: modification of work by Janus Sandsgaard; credit right: modification of work by “MDreibelbis”/Flickr)

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The Iceman Treateth

Prehistoric humans had a very limited understanding of the causes of disease, and various cultures developed different beliefs and explanations. While many believed that illness was punishment for angering the gods or was simply the result of fate, archaeological evidence suggests that prehistoric people attempted to treat illnesses and infections. One example of this is Ötzi the Iceman, a 5300-year-old mummy found frozen in the ice of the Ötztal Alps on the Austrian-Italian border in 1991. Because Ötzi was so well preserved by the ice, researchers discovered that he was infected with the eggs of the parasite *Trichuris trichiura*, which may have caused him to have abdominal pain and anemia. Researchers also found evidence of *Borrelia burgdorferi*, a bacterium that causes Lyme disease.³ Some researchers think Ötzi may have been trying to treat his infections with the woody fruit of the *Piptoporus betulinus* fungus, which was discovered tied to his belongings.⁴ This fungus has both laxative and antibiotic properties. Ötzi was also covered in tattoos that were made by cutting incisions into his skin, filling them with herbs, and then burning the herbs.⁵ There is speculation that this may have been another attempt to treat his health ailments.

Early Notions of Disease, Contagion, and Containment

Several ancient civilizations appear to have had some understanding that disease could be transmitted by things they could not see. This is especially evident in historical attempts to contain the spread of disease. For example, the Bible refers to the practice of quarantining people with leprosy and other diseases, suggesting that people understood that diseases could be communicable. Ironically, while leprosy is communicable, it is also a disease that progresses slowly. This means that people were likely quarantined after they had already spread the disease to others.

The ancient Greeks attributed disease to bad air, *mal'aria*, which they called "miasmatic odors." They developed hygiene practices that built on this idea. The Romans also believed in the miasma hypothesis and created a complex sanitation infrastructure to deal with sewage. In Rome, they built aqueducts, which brought fresh water into the city, and a giant sewer, the *Cloaca Maxima*, which carried waste away and into the river Tiber (Figure 1.2.3). Some researchers believe that this infrastructure helped protect the Romans from epidemics of waterborne illnesses.

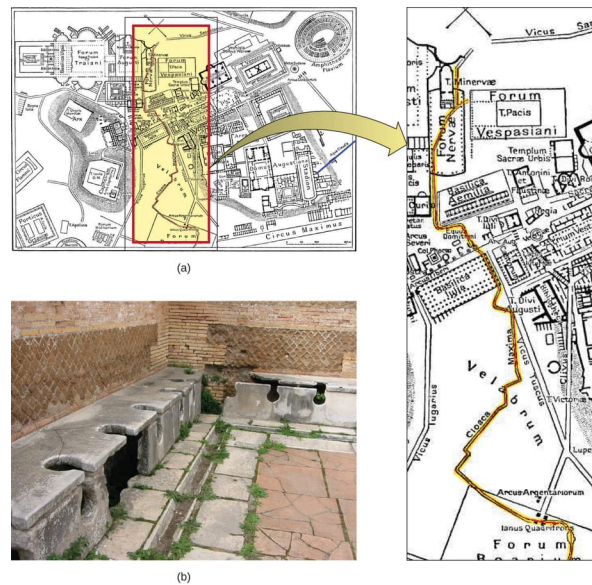


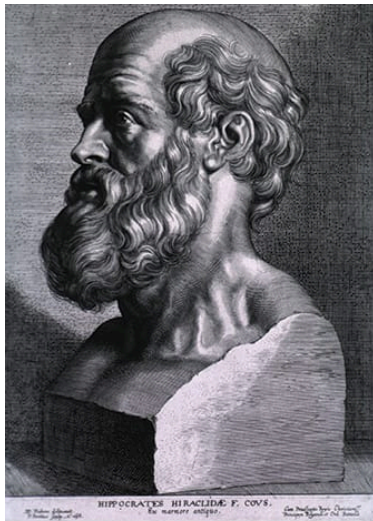
Figure 1.2.3: (a) The *Cloaca Maxima*, or “Greatest Sewer” (shown in red), ran through ancient Rome. It was an engineering marvel that carried waste away from the city and into the river Tiber. (b) These ancient latrines emptied into the *Cloaca Maxima*.

Even before the invention of the microscope, some doctors, philosophers, and scientists made great strides in understanding the invisible forces—what we now know as microbes—that can cause infection, disease, and death.

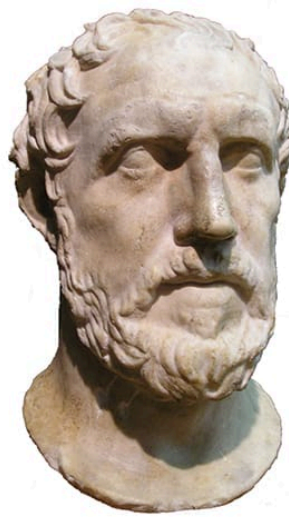
The Greek physician Hippocrates (460–370 BC) is considered the “father of Western medicine” (Figure 1.2.4a). Unlike many of his ancestors and contemporaries, he dismissed the idea that disease was caused by supernatural forces. Instead, he posited that diseases had natural causes from within patients or their environments. Hippocrates and his heirs are believed to have written the *Hippocratic Corpus*, a collection of texts that make up some of the oldest surviving medical books.⁶ Hippocrates is also often credited as the author of the Hippocratic Oath, taken by new physicians to pledge their dedication to diagnosing and treating patients without causing harm.

While Hippocrates is considered the father of Western medicine, the Greek philosopher and historian Thucydides (460–395 BC) is considered the father of scientific history because he advocated for evidence-based analysis of cause-and-effect reasoning (Figure 1.2.4b). Among his most important contributions are his observations regarding the Athenian plague that killed one-third of the population of Athens between 430 and 410 BC. Having survived the epidemic himself, Thucydides made the important observation that survivors did not get re-infected with the disease, even when taking care of actively sick people.⁷ This observation shows an early understanding of the concept of immunity.

Marcus Terentius Varro (116–27 BC) was a prolific Roman writer who was one of the first people to propose the concept that things we cannot see (what we now call microorganisms) can cause disease (Figure 1.2.4c). In *Res Rusticae* (*On Farming*), published in 36 BC, he said that “precautions must also be taken in neighborhood swamps . . . because certain minute creatures [*animalia minuta*] grow there which cannot be seen by the eye, which float in the air and enter the body through the mouth and nose and there cause serious diseases.”⁸



(a)



(b)



(c)

Figure 1.2.4: (a) Hippocrates, the “father of Western medicine,” believed that diseases had natural, not supernatural, causes. (b) The historian Thucydides observed that survivors of the Athenian plague were subsequently immune to the infection. (c) Marcus Terentius Varro proposed that disease could be caused by “certain minute creatures . . . which cannot be seen by the eye.” (credit c: modification of work by Alessandro Antonelli)

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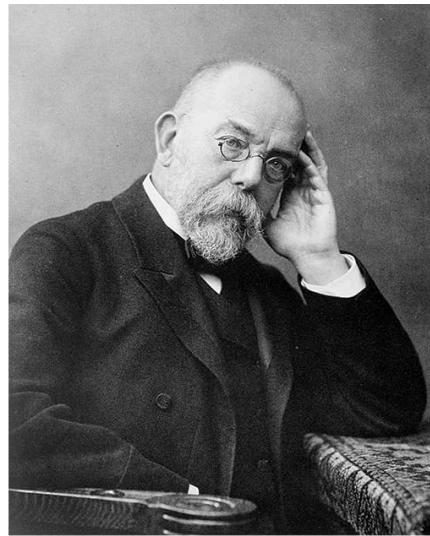
The Birth of Microbiology

While the ancients may have suspected the existence of invisible “minute creatures,” it wasn’t until the invention of the microscope that their existence was definitively confirmed. While it is unclear who exactly invented the microscope, a Dutch cloth merchant named Antonie van Leeuwenhoek (1632–1723) was the first to develop a lens powerful enough to view microbes. In 1675, using a simple but powerful microscope, Leeuwenhoek was able to observe single-celled organisms, which he described as “animalcules” or “wee little beasties,” swimming in a drop of rain water. From his drawings of these little organisms, we now know he was looking at bacteria and protists. (We will explore Leeuwenhoek’s contributions to microscopy further in [Chapter 2: How We See the Invisible World](#).)

Nearly 200 years after van Leeuwenhoek got his first glimpse of microbes, the “Golden Age of Microbiology” spawned a host of new discoveries between 1857 and 1914. Two famous microbiologists, Louis Pasteur and Robert Koch, were especially active in advancing our understanding of the unseen world of microbes (Figure 1.2.5). Pasteur, a French chemist, showed that individual microbial strains had unique properties and demonstrated that fermentation is caused by microorganisms. He also invented pasteurization, a process used to kill microorganisms responsible for spoilage, and developed vaccines for the treatment of diseases, including rabies, in animals and humans. Koch, a German physician, was the first to demonstrate the connection between a single, isolated microbe and a known human disease. For example, he discovered the bacteria that cause anthrax (*Bacillus anthracis*), cholera (*Vibrio cholera*), and tuberculosis (*Mycobacterium tuberculosis*).⁹ We will discuss these famous microbiologists, and others, in later chapters.



(a)



(b)

Figure 1.2.5: (a) Louis Pasteur (1822–1895) is credited with numerous innovations that advanced the fields of microbiology and immunology. (b) Robert Koch (1843–1910) identified the specific microbes that cause anthrax, cholera, and tuberculosis.

As microbiology has developed, it has allowed the broader discipline of biology to grow and flourish in previously unimagined ways. Much of what we know about human cells comes from our understanding of microbes, and many of the tools we use today to study cells and their genetics derive from work with microbes.

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Microbiology Toolbox

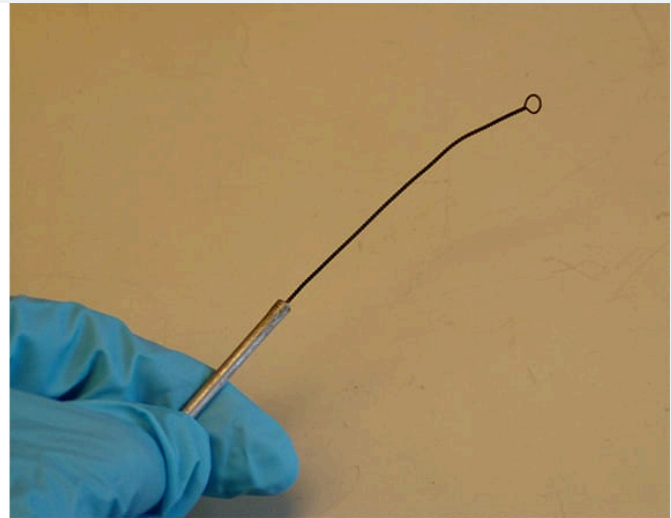
Because individual microbes are generally too small to be seen with the naked eye, the science of microbiology is dependent on technology that can artificially enhance the capacity of our natural senses of perception. Early microbiologists like Pasteur and Koch had fewer tools at their disposal than are found in modern laboratories, making their discoveries and innovations that much more impressive. Later chapters of this text will explore many applications of technology in depth, but for now, here is a brief overview of some of the fundamental tools of the microbiology lab.

- Microscopes produce magnified images of microorganisms, human cells and tissues, and many other types of specimens too small to be observed with the naked eye.
- Stains and dyes are used to add color to microbes so they can be better observed under a microscope. Some dyes can be used on living microbes, whereas others require that the specimens be fixed with chemicals or heat before staining. Some stains only work on certain types of microbes because of differences in their cellular chemical composition.
- Growth media are used to grow microorganisms in a lab setting. Some media are liquids; others are more solid or gel-like. A growth medium provides nutrients, including water, various salts, a source of carbon (like glucose), and a source of nitrogen and amino acids (like yeast extract) so microorganisms can grow and reproduce. Ingredients in a growth medium can be modified to grow unique types of microorganisms.
- A Petri dish is a flat-lidded dish that is typically 10–11 centimeters (cm) in diameter and 1–1.5 cm high. Petri dishes made out of either plastic or glass are used to hold growth media (Figure 1.2.6).
- Test tubes are cylindrical plastic or glass tubes with rounded bottoms and open tops. They can be used to grow microbes in broth, or semisolid or solid growth media.

- A Bunsen burner is a metal apparatus that creates a flame that can be used to sterilize pieces of equipment. A rubber tube carries gas (fuel) to the burner. In many labs, Bunsen burners are being phased out in favor of infrared microincinerators, which serve a similar purpose without the safety risks of an open flame.
- An inoculation loop is a handheld tool that ends in a small wire loop (Figure 1.2.6). The loop can be used to streak microorganisms on agar in a Petri dish or to transfer them from one test tube to another. Before each use, the inoculation loop must be sterilized so cultures do not become contaminated.



(a)



(b)

Figure 1.2.6: (a) This Petri dish filled with agar has been streaked with *Legionella*, the bacterium responsible for causing Legionnaire's disease. (b) An inoculation loop like this one can be used to streak bacteria on agar in a Petri dish. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Jeffrey M. Vinocur)

Key Concepts and Summary

- Microorganisms (or microbes) are living organisms that are generally too small to be seen without a microscope.
- Throughout history, humans have used microbes to make fermented foods such as beer, bread, cheese, and wine.
- Long before the invention of the microscope, some people theorized that infection and disease were spread by living things that were too small to be seen. They also correctly intuited certain principles regarding the spread of disease and immunity.
- Antonie van Leeuwenhoek, using a microscope, was the first to actually describe observations of bacteria, in 1675.
- During the Golden Age of Microbiology (1857–1914), microbiologists, including Louis Pasteur and Robert Koch, discovered many new connections between the fields of microbiology and medicine.

Footnotes

- 1 Rebecca Buxton. "Examination of Gram Stains of Spinal Fluid—Bacterial Meningitis." *American Society for Microbiology*. 2007. www.microbelibrary.org/librar...ial-meningitis
- 2 P.E. McGovern et al. "Fermented Beverages of Pre- and Proto-Historic China." *Proceedings of the National Academy of Sciences of the United States of America* 1 no. 51 (2004):17593–17598. doi:10.1073/pnas.0407921102.
- 3 A. Keller et al. "New Insights into the Tyrolean Iceman's Origin and Phenotype as Inferred by Whole-Genome Sequencing." *Nature Communications*, 3 (2012): 698. doi:10.1038/ncomms1701.
- 4 L. Capasso. "5300 Years Ago, the Ice Man Used Natural Laxatives and Antibiotics." *The Lancet*, 352 (1998) 9143: 1864. doi: 10.1016/s0140-6736(05)79939-6.
- 5 L. Capasso, L. "5300 Years Ago, the Ice Man Used Natural Laxatives and Antibiotics." *The Lancet*, 352 no. 9143 (1998): 1864. doi: 10.1016/s0140-6736(05)79939-6.
- 6 G. Pappas et al. "Insights Into Infectious Disease in the Era of Hippocrates." *International Journal of Infectious Diseases* 12 (2008) 4:347–350. doi: <http://dx.doi.org/10.1016/j.ijid.2007.11.003>.
- 7 Thucydides. *The History of the Peloponnesian War. The Second Book*. 431 BC. Translated by Richard Crawley. <http://classics.mit.edu/Thucydides/p...2.second.html>.
- 8 Plinio Prioreschi. *A History of Medicine: Roman Medicine*. Lewiston, NY: Edwin Mellen Press, 1998: p. 215.

9. [9](#) S.M. Blevins and M.S. Bronze. “Robert Koch and the ‘Golden Age’ of Bacteriology.” *International Journal of Infectious Diseases*. 14 no. 9 (2010): e744–e751. doi:10.1016/j.ijid.2009.12.003.

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1.3: Taxonomy and Phylogenies

Learning Objectives

- 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 1.3.1).



Figure 1.3.1: 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.

YouTube Video

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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, phylogenetic 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 1.3.2). He later proposed a fourth kingdom, Monera, for unicellular organisms whose cells lack nuclei, like bacteria.

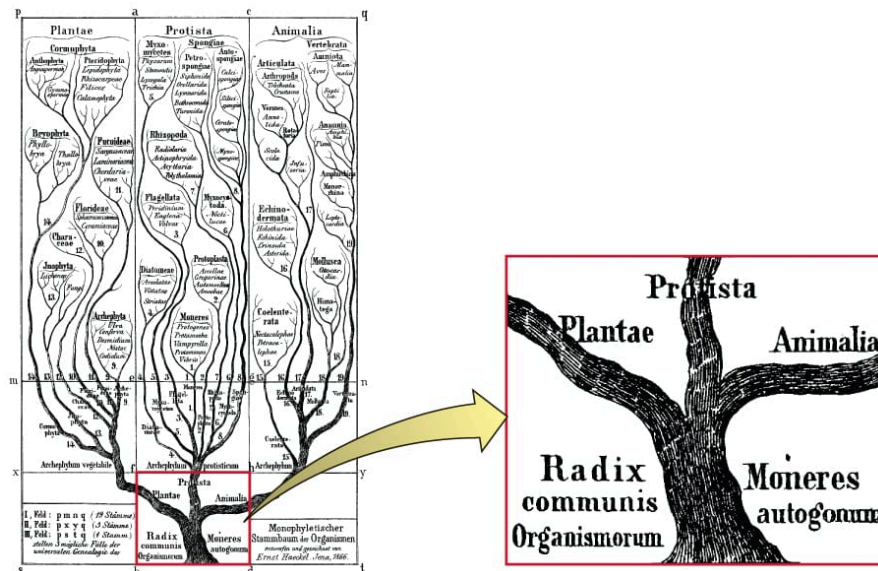


Figure 1.3.2: Ernst Haeckel's rendering of the tree of life, from his 1866 book *General Morphology of Organisms*, contained three kingdoms: Plantae, Protista, and Animalia. He later added a fourth kingdom, Monera, for unicellular organisms lacking a nucleus.

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 1.3.3 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.

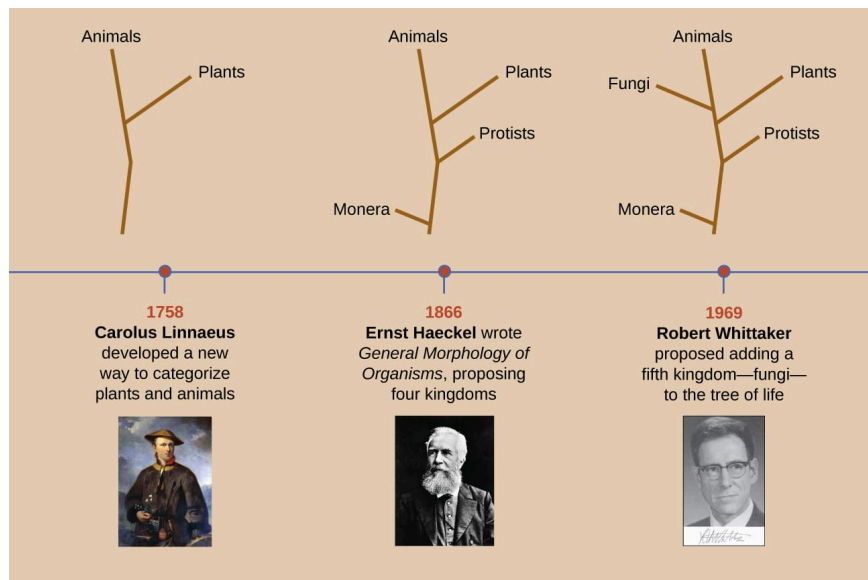


Figure 1.3.3: This timeline shows how the shape of the tree of life has changed over the centuries. Even today, the taxonomy of living organisms is continually being reevaluated and refined with advances in technology.

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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 archaeobacteria (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 1.3.4). 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.

Phylogenetic Tree of Life

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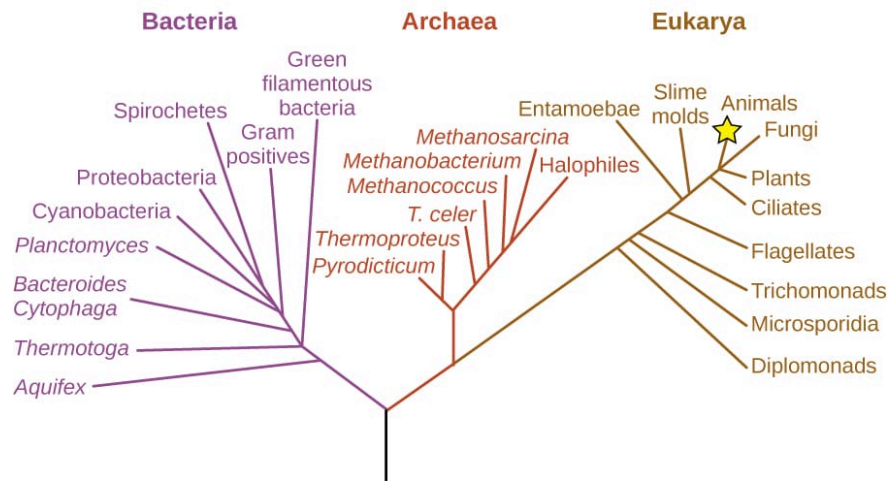


Figure 1.3.4: Scientists continue to use analysis of RNA, DNA, and proteins to determine how organisms are related. One interesting, and complicating, discovery is that of horizontal gene transfer—when a gene of one species is absorbed into another organism's genome. Horizontal gene transfer is especially 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.”

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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 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.

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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.

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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.

Key Concepts and 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.

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1.4: Types of 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 1.4.1). See Table 1.4.1 for units of length used in microbiology.

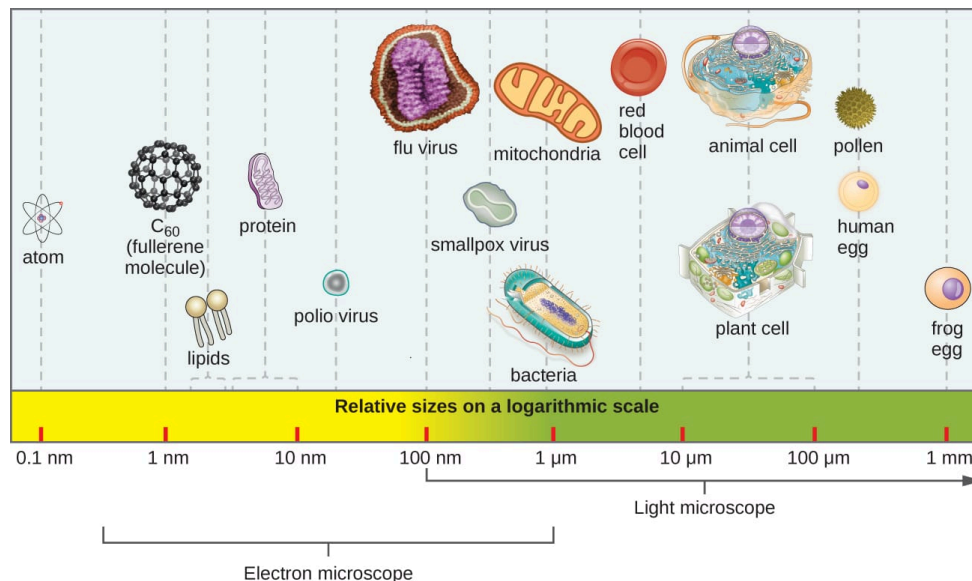


Figure 1.4.1: The relative sizes of various microscopic and nonmicroscopic objects. Note that a typical virus measures about 100 nm, 10 times smaller than a typical bacterium (~1 μm), which is at least 10 times smaller than a typical plant or animal cell (~10–100 μm). An object must measure about 100 μm to be visible without a microscope.

Table 1.4.1: 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

Interactive Element

A great resource for getting a sense of scale is the interactive [Learn Genetics Cell Size and Scale](#) website developed by the University of Utah.

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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 1.4.2 shows examples of these shapes.

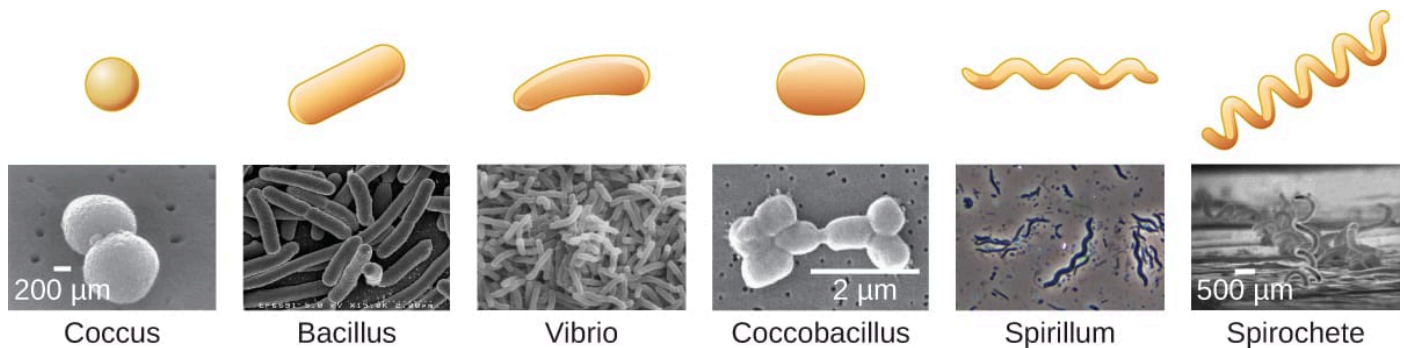


Figure 1.4.2: Common bacterial shapes. Note how coccobacillus is a combination of spherical (coccus) and rod-shaped (bacillus). (credit "Coccus": modification of work by Janice Haney Carr, Dr. Richard Facklam, Centers for Disease Control and Prevention; credit "Bacillus": modification of work by "Elapied"/Wikimedia 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 1.4.3). Some archaea live in the human body, but none have been shown to be human pathogens.



Figure 1.4.3: 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.

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 1.4.4). 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.

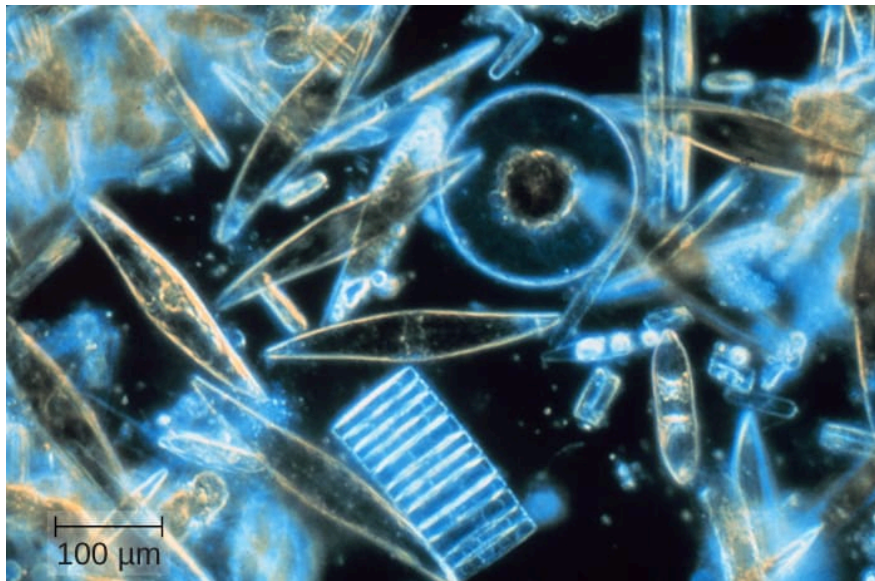


Figure 1.4.4: Assorted diatoms, a kind of algae, live in annual sea ice in McMurdo Sound, Antarctica. Diatoms range in size from 2 μm to 200 μm and are visualized here using light microscopy. (credit: National Oceanic and Atmospheric Administration)

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 1.4.5).



Figure 1.4.5: 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 1.4.6).

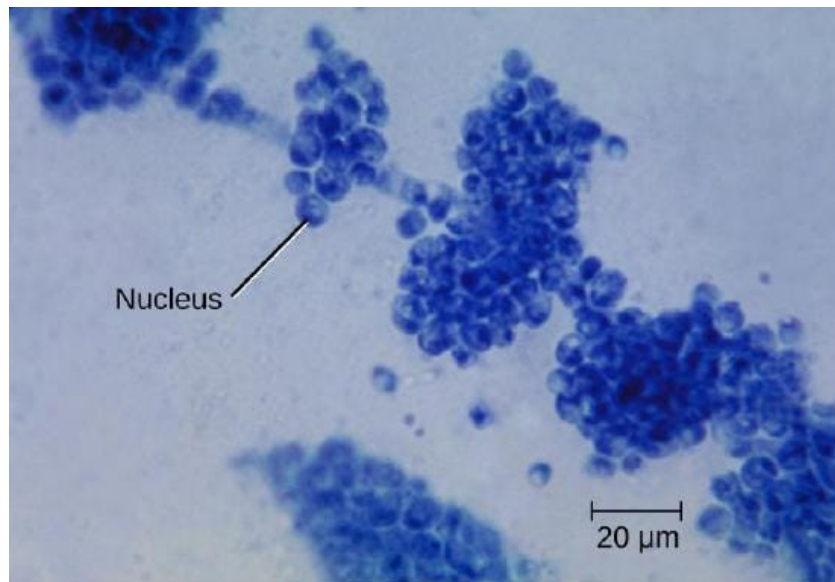


Figure 1.4.5: *Candida albicans* is a unicellular fungus, or yeast. It is the causative agent of vaginal yeast infections as well as oral thrush, a yeast infection of the mouth that commonly afflicts infants. *C. albicans* has a morphology similar to that of coccus bacteria; however, yeast is a eukaryotic 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 1.4.6). 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.

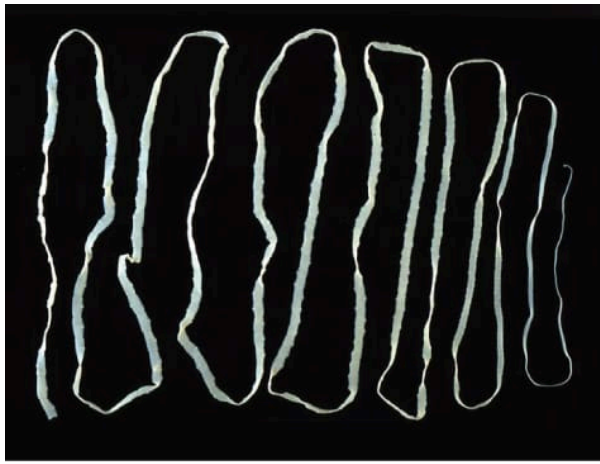


Figure 1.4.6: Large colonies of microscopic fungi can often be observed with the naked eye, as seen on the surface of these moldy oranges.

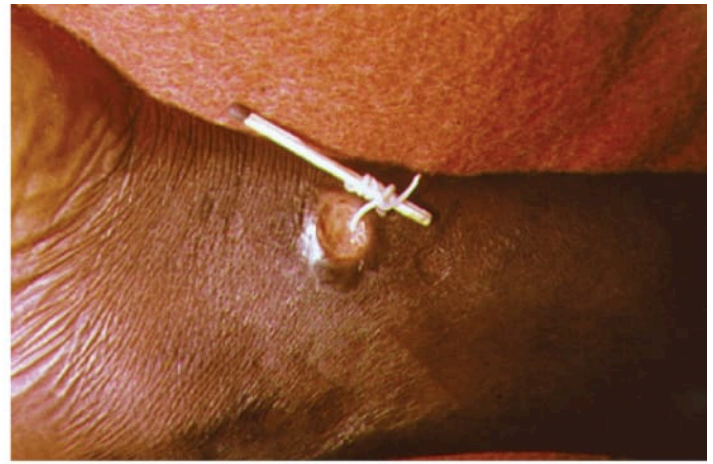
Helminths

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 1.4.7). 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)

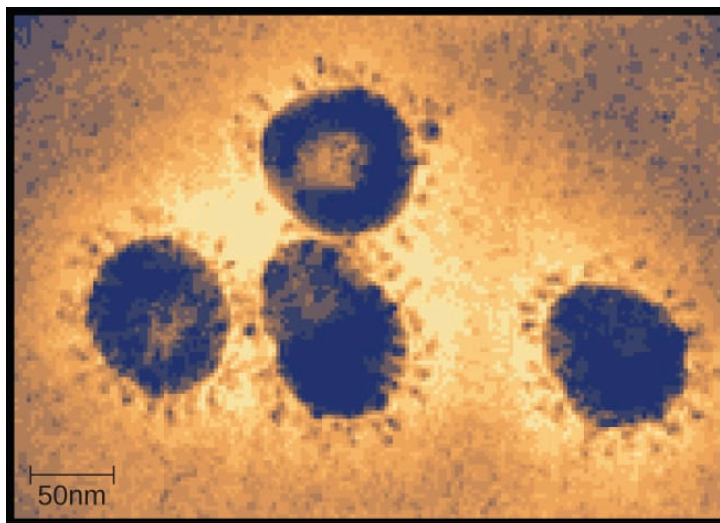


(b)

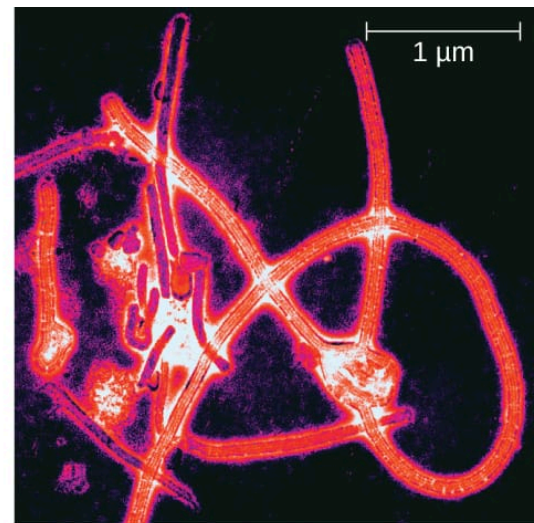
Figure 1.4.7: The beef tapeworm, *Taenia saginata*, infects both cattle and humans. *T. saginata* eggs are microscopic (around 50 μm), but adult worms like the one shown here can reach 4–10 m, taking up residence in the digestive system. (b) An adult guinea worm, *Dracunculus medinensis*, is removed 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 1.4.8). However, many viruses do not cause disease.



(a)



(b)

Figure 1.4.8: (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)

Query 1.4.1

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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 1.4.9). **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 1.4.9: 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.

Key Concepts and 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 C. Greenaway "Dracunculiasis (Guinea Worm Disease)." *Canadian Medical Association Journal* 170 no. 4 (2004):495–500.
- 2 World Health Organization. "Dracunculiasis (Guinea-Worm Disease)." *WHO*. 2015. <http://www.who.int/mediacentre/factsheets/fs359/en/>. Accessed October 2, 2015.
- 3 Kara Rogers. "Guatemala Syphilis Experiment: American Medical Research Project". *Encyclopaedia Britannica*. [www.britannica.com/event/Guat...lis-experiment](http://www.britannica.com/event/Guatemala-Syphilis-Experiment). Accessed June 24, 2015.
- 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/guatemala...ry?id=14414902>. Accessed June 24, 2015.
- 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 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 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 National Institute of Neurological Disorders and Stroke. "Creutzfeldt-Jakob Disease Fact Sheet." *NIH*. 2015. <http://www.ninds.nih.gov/disorders/c...htm#288133058>.
- 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.

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1.E: An Invisible World (Exercises)

1.1: What Our Ancestors Knew

Microorganisms (or microbes) are living organisms that are generally too small to be seen without a microscope. Throughout history, humans have used microbes to make fermented foods such as beer, bread, cheese, and wine. Long before the invention of the microscope, some people theorized that infection and disease were spread by living things that were too small to be seen. They also correctly intuited certain principles regarding the spread of disease and immunity.

Multiple Choice

Which of the following foods is NOT made by fermentation?

- A. beer
- B. bread
- C. cheese
- D. orange juice

Answer

D

Who is considered the “father of Western medicine”?

- A. Marcus Terentius Varro
- B. Thucydides
- C. Antonie van Leeuwenhoek
- D. Hippocrates

Answer

D

Who was the first to observe “animalcules” under the microscope?

- A. Antonie van Leeuwenhoek
- B. Ötzi the Iceman
- C. Marcus Terentius Varro
- D. Robert Koch

Answer

A

Who proposed that swamps might harbor tiny, disease-causing animals too small to see?

- A. Thucydides
- B. Marcus Terentius Varro
- C. Hippocrates
- D. Louis Pasteur

Answer

B

Fill in the Blank

Thucydides is known as the father of _____.

Answer

scientific history

Researchers think that Ötzi the Iceman may have been infected with ____ disease.

Answer

Lyme

The process by which microbes turn grape juice into wine is called _____.

Answer

fermentation

Short Answer

What did Thucydides learn by observing the Athenian plague?

Why was the invention of the microscope important for microbiology?

What are some ways people use microbes?

Critical Thinking

Explain how the discovery of fermented foods likely benefited our ancestors.

What evidence would you use to support this statement: Ancient people thought that disease was transmitted by things they could not see.

1.2: A Systematic Approach

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.

Multiple Choice

Which of the following was NOT a kingdom in Linnaeus's taxonomy?

- A. animal
- B. mineral
- C. protist
- D. plant

Answer

C

Which of the following is a correct usage of binomial nomenclature?

- A. Homo Sapiens
- B. *homo sapiens*
- C. *Homo sapiens*
- D. *Homo Sapiens*

Answer

C

Which scientist proposed adding a kingdom for protists?

- A. Carolus Linnaeus
- B. Carl Woese
- C. Robert Whittaker
- D. Ernst Haeckel

Answer

D

Which of the following is NOT a domain in Woese and Fox's phylogenetic tree?

- A. Plantae
- B. Bacteria
- C. Archaea
- D. Eukarya

Answer

A

Which of the following is the standard resource for identifying bacteria?

- A. *Systema Naturae*
- B. *Bergey's Manual of Determinative Bacteriology*
- C. Woese and Fox's phylogenetic tree
- D. Haeckel's *General Morphology of Organisms*

Answer

B

Short Answer

What is a phylogenetic tree?

Which of the five kingdoms in Whittaker's phylogenetic tree are prokaryotic, and which are eukaryotic?

What molecule did Woese and Fox use to construct their phylogenetic tree?

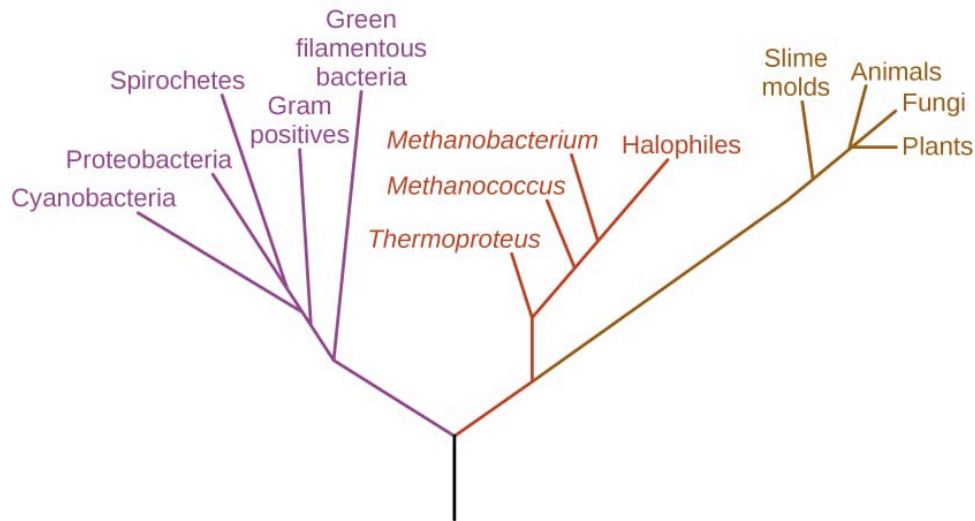
Name some techniques that can be used to identify and differentiate species of bacteria.

Critical Thinking

Why is using binomial nomenclature more useful than using common names?

Label the three Domains found on modern phylogenetic trees.

Phylogenetic Tree of Life



1.3: Types of Microorganisms

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.

Multiple Choice

Which of the following types of microorganisms is photosynthetic?

- A. yeast
- B. virus
- C. helminth
- D. alga

Answer

D

Which of the following is a prokaryotic microorganism?

- A. helminth
- B. protozoan
- C. cyanobacterium
- D. mold

Answer

C

Which of the following is acellular?

- A. virus
- B. bacterium
- C. fungus
- D. protozoan

Answer

A

Which of the following is a type of fungal microorganism?

- A. bacterium
- B. protozoan
- C. alga
- D. yeast

Answer

D

Which of the following is not a subfield of microbiology?

- A. bacteriology
- B. botany
- C. clinical microbiology
- D. virology

Answer

B

Fill in the Blank

A _____ is a disease-causing microorganism.

Answer

pathogen

Multicellular parasitic worms studied by microbiologists are called _____.

Answer

helminths

The study of viruses is _____.

Answer

virology

The cells of prokaryotic organisms lack a _____.

Answer

nucleus

Short Answer

Describe the differences between bacteria and archaea.

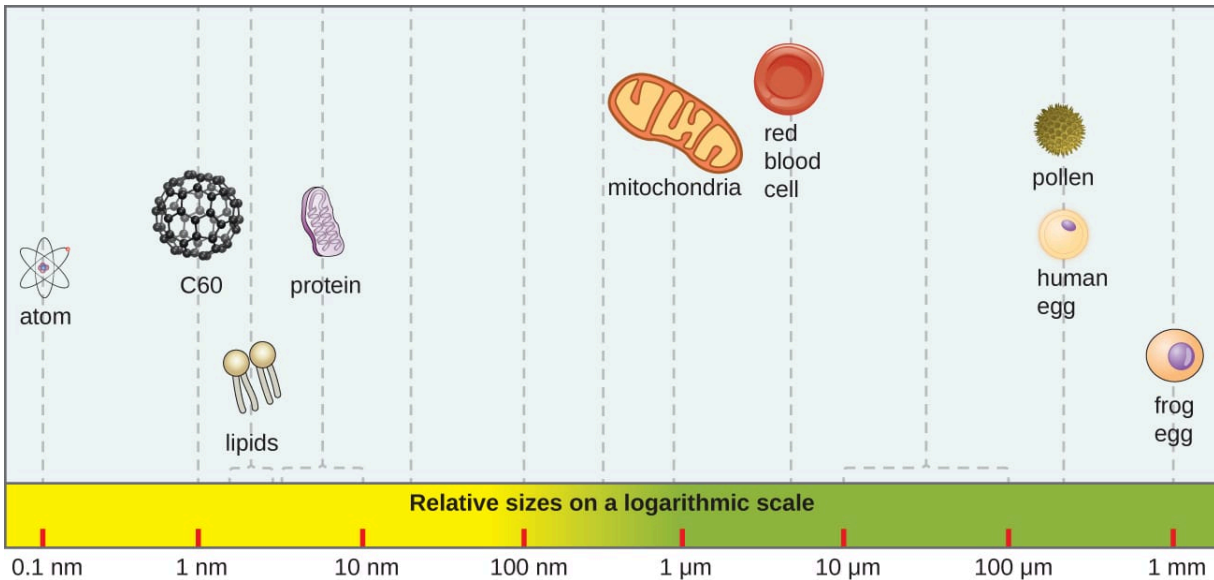
Name three structures that various protozoa use for locomotion.

Describe the actual and relative sizes of a virus, a bacterium, and a plant or animal cell.

Critical Thinking

Contrast the behavior of a virus outside versus inside a cell.

Where would a virus, bacterium, animal cell, and a prion belong on this chart?



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2: MICROSCOPES

When we look at a rainbow, its colors span the full spectrum of light that the human eye can detect and differentiate. Each hue represents a different frequency of visible light, processed by our eyes and brains and rendered as red, orange, yellow, green, or one of the many other familiar colors that have always been a part of the human experience. But only recently have humans developed an understanding of the properties of light that allow us to see images in color.

Over the past several centuries, we have learned to manipulate light to peer into previously invisible worlds—those too small or too far away to be seen by the naked eye. Through a microscope, we can examine microbial cells and colonies, using various techniques to manipulate color, size, and contrast in ways that help us identify species and diagnose disease.

Figure 2.1 illustrates how we can apply the properties of light to visualize and magnify images; but these stunning micrographs are just two examples of the numerous types of images we are now able to produce with different microscopic technologies. This chapter explores how various types of microscopes manipulate light in order to provide a window into the world of microorganisms. By understanding how various kinds of microscopes work, we can produce highly detailed images of microbes that can be useful for both research and clinical applications.

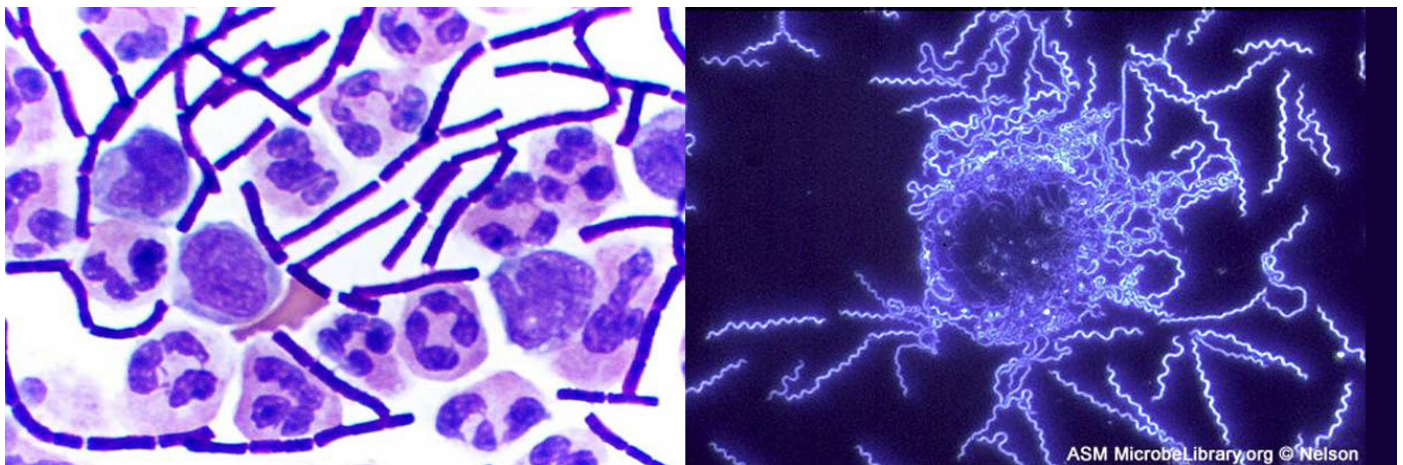


Figure 2.1: Different types of microscopy are used to visualize different structures. Brightfield microscopy (left) renders a darker image on a lighter background, producing a clear image of these *Bacillus anthracis* cells in cerebrospinal fluid (the rod-shaped bacterial cells are surrounded by larger white blood cells). Darkfield microscopy (right) increases contrast, rendering a brighter image on a darker background, as demonstrated by this image of the bacterium *Borrelia burgdorferi*, which causes Lyme disease. (credit left: modification of work by Centers for Disease Control and Prevention; credit right: modification of work by American Society for Microbiology)

2.1: The Properties of Light

2.2: Peering into the Invisible World

2.3: Instruments of Microscopy- Brightfield Microscopes

2.4: Darkfield, Phase-Contrast and DIC

2.5: Fluorescence, Confocal and Two-Photon Microscopes

2.6: Electron Microscopy

2.7: Sample Preparation for Microscopic Staining

2.8: Differential Staining

2.E: How We See the Invisible World (Exercises)

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2.1: 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

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 2.1.1).

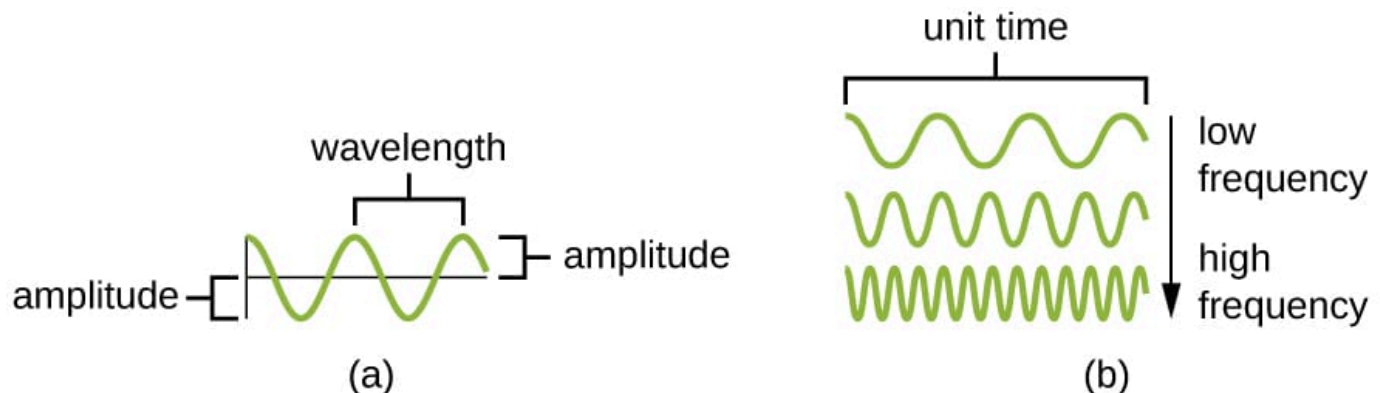
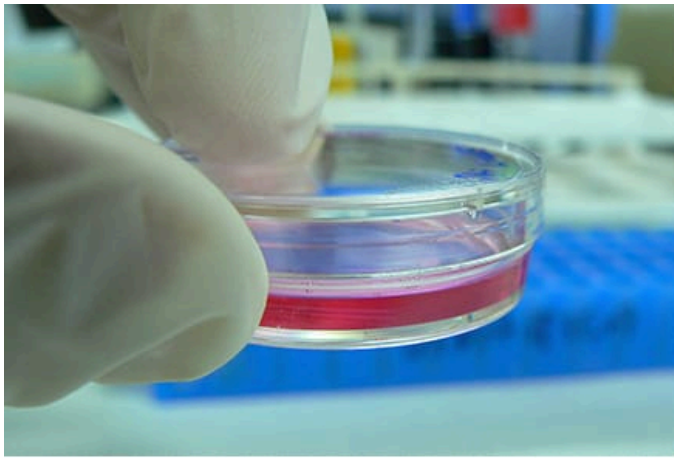


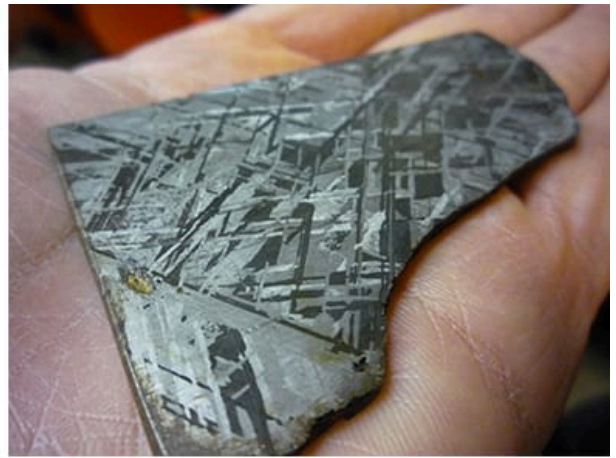
Figure 2.1.1: (a) The amplitude is the height of a wave, whereas the wavelength is the distance between one peak and the next. (b) These waves have different frequencies, or rates of vibration. The wave at the top has the lowest frequency, since it has the fewest peaks per unit time. The wave at the 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. **Absorbance** occurs 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 2.1.2 illustrates the difference between transparency and opacity.



(a)



(b)

Figure 2.1.2: (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.

Query 2.1.1

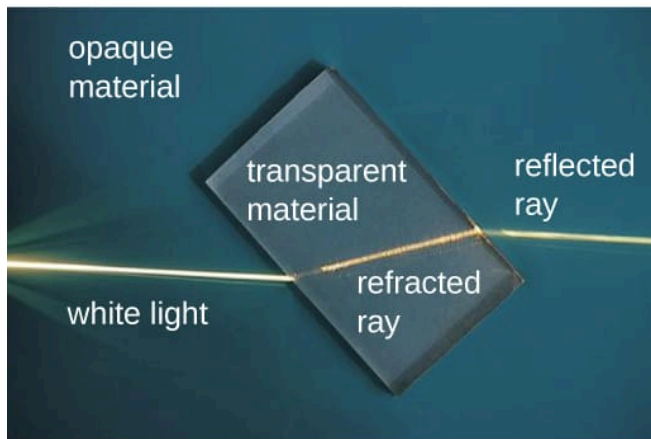
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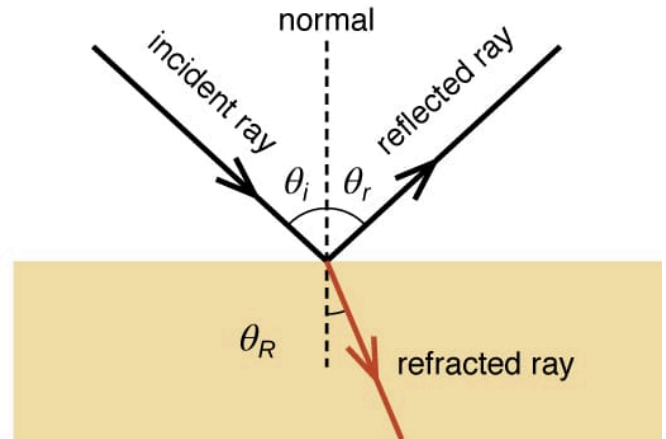


Lenses and Refraction

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 2.1.3). 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 2.1.3: (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 2.1.4).



Figure 2.1.4: 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 2.1.5). 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 2.1.5 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.

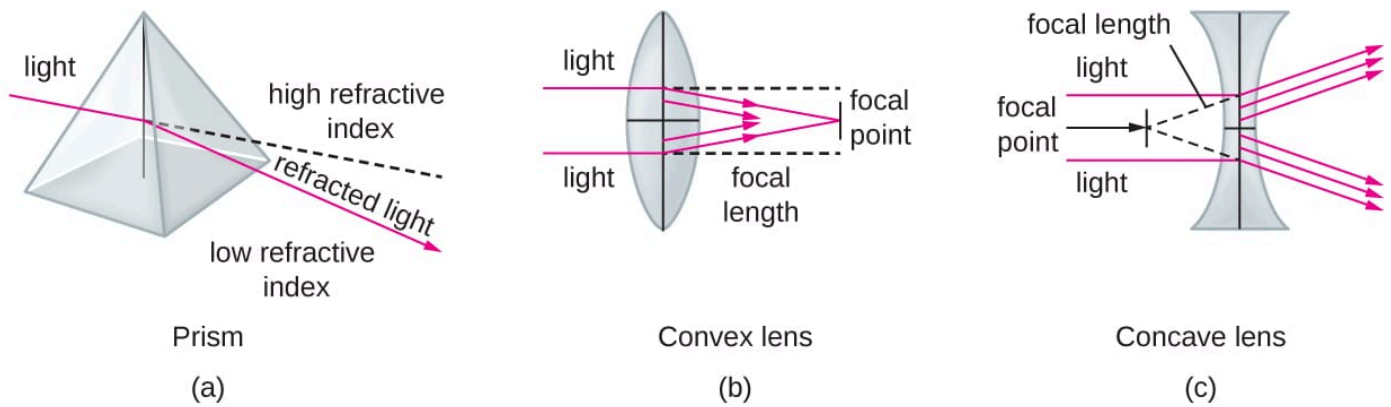


Figure 2.1.5: (a) A lens is like a collection of prisms, such as the one shown here. (b) When light passes through a convex lens, it is refracted toward a focal point on the other side of the lens. The focal length is the distance to the focal point. (c) Light passing through a concave lens is refracted 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.

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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 2.1.6).

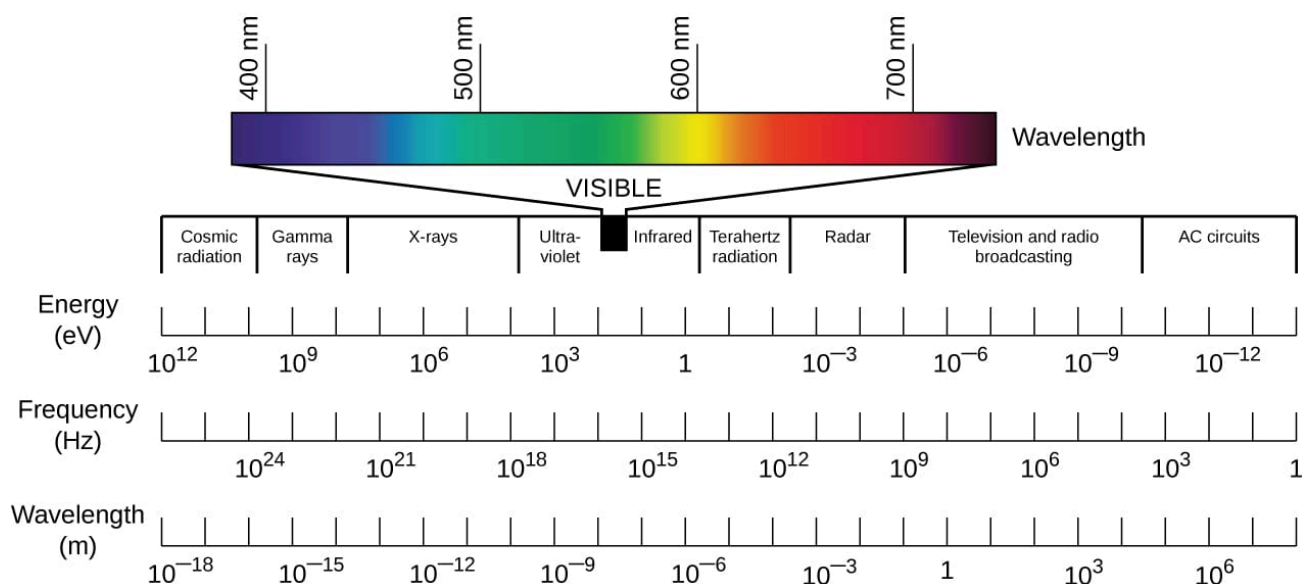


Figure 2.1.6: The electromagnetic spectrum ranges from high-frequency gamma rays to low-frequency radio waves. Visible light is the relatively small range of electromagnetic frequencies that can be sensed by the human eye. On the electromagnetic spectrum, visible light falls between ultraviolet and infrared 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 2.1.6). 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 2.1.7). 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.

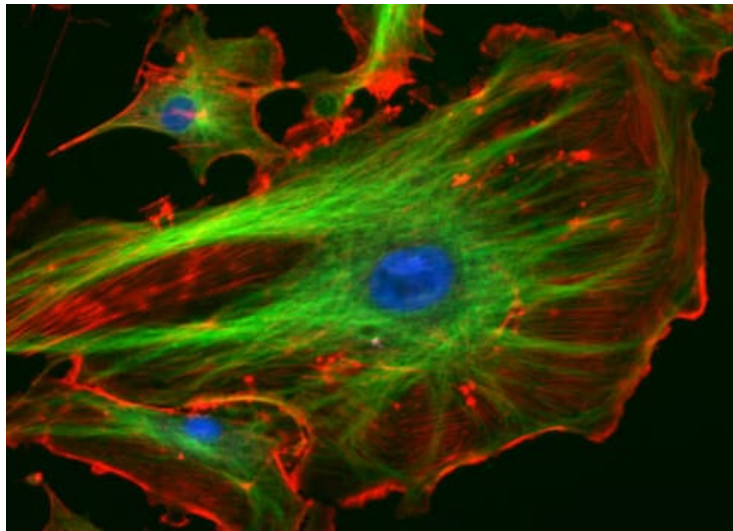


Figure 2.1.7: The fluorescent dyes absorbed by these bovine pulmonary artery endothelial cells emit brilliant colors when excited by ultraviolet light under a fluorescence microscope. Various cell structures absorb different dyes. The nuclei are stained blue with 4',6-diamidino-2-phenylindole (DAPI); microtubules are marked green by an antibody bound to FITC; and actin filaments are labeled red with phalloidin bound to tetramethylrhodamine (TRITC).

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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 $\times$ 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](#)).

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Interactive Element

Case Study Preview: “A Slam Dunk... Infection?”

What seemed like a minor basketball injury turns into a serious skin infection for 17-year-old camp counselor Cindy. As the wound swells and oozes pus, a simple microscope becomes the key to diagnosis. Students follow the journey from wet mount to Gram stain as lab techs uncover clusters of gram-positive cocci - a classic case of *Staphylococcus aureus*.

This case introduces microscopy and staining techniques in action, showing how identifying bacterial shape, arrangement, and Gram reaction can guide treatment—and why finishing your antibiotics really matters.

Can you figure out what's infecting Cindy before it spreads?

[Chapter 2 Case Study - A Slam Dunk...Infection?](#)

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.

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2.2: Peering into the Invisible World

Learning Objectives

- Describe historical developments and individual contributions that led to the invention and development of the microscope
- Compare and contrast the features of simple and compound microscopes

Some of the fundamental characteristics and functions of microscopes can be understood in the context of the history of their use. Italian scholar Girolamo Fracastoro is regarded as the first person to formally postulate that disease was spread by tiny invisible *seminaria*, or “seeds of the contagion.” In his book *De Contagione* (1546), he proposed that these seeds could attach themselves to certain objects (which he called *fomes* [cloth]) that supported their transfer from person to person. However, since the technology for seeing such tiny objects did not yet exist, the existence of the *seminaria* remained hypothetical for a little over a century—an invisible world waiting to be revealed.

Early Microscopes

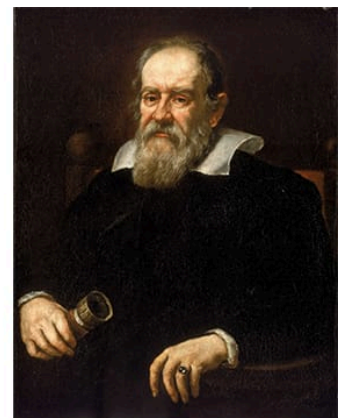
Antonie van Leeuwenhoek, sometimes hailed as “the Father of Microbiology,” is typically credited as the first person to have created microscopes powerful enough to view microbes (Figure 2.2.1). Born in the city of Delft in the Dutch Republic, van Leeuwenhoek began his career selling fabrics. However, he later became interested in lens making (perhaps to look at threads) and his innovative techniques produced microscopes that allowed him to observe microorganisms as no one had before. In 1674, he described his observations of single-celled organisms, whose existence was previously unknown, in a series of letters to the Royal Society of London. His report was initially met with skepticism, but his claims were soon verified and he became something of a celebrity in the scientific community.



(a)



(b)



(c)

Figure 2.2.1: (a) Antonie van Leeuwenhoek (1632–1723) is credited as being the first person to observe microbes, including bacteria, which he called “animalcules” and “wee little beasties.” (b) Even though van Leeuwenhoek’s microscopes were simple microscopes (as seen in this replica), they were more powerful and provided better resolution than the compound microscopes of his day. (credit b: modification of work by “Wellcome Images”/Wikimedia Commons) (c) Though more famous for developing the telescope, Galileo Galilei (1564–1642) was also one of the pioneers of microscopy.

While van Leeuwenhoek is credited with the discovery of microorganisms, others before him had contributed to the development of the microscope. These included eyeglass makers in the Netherlands in the late 1500s, as well as the Italian astronomer Galileo Galilei, who used a **compound microscope** to examine insect parts (Figure 2.2.1). Whereas van Leeuwenhoek used a **simple microscope**, in which light is passed through just one lens, Galileo’s compound microscope was more sophisticated, passing light through two sets of lenses.

Van Leeuwenhoek’s contemporary, the Englishman **Robert Hooke** (1635–1703), also made important contributions to microscopy,

publishing in his book *Micrographia* (1665) many observations using compound microscopes. Viewing a thin sample of cork through his microscope, he was the first to observe the structures that we now know as cells (Figure 2.2.2). Hooke described these structures as resembling “Honey-comb,” and as “small Boxes or Bladders of Air,” noting that each “Cavern, Bubble, or Cell” is distinct from the others (in Latin, “cell” literally means “small room”). They likely appeared to Hooke to be filled with air because the cork cells were dead, with only the rigid cell walls providing the structure.

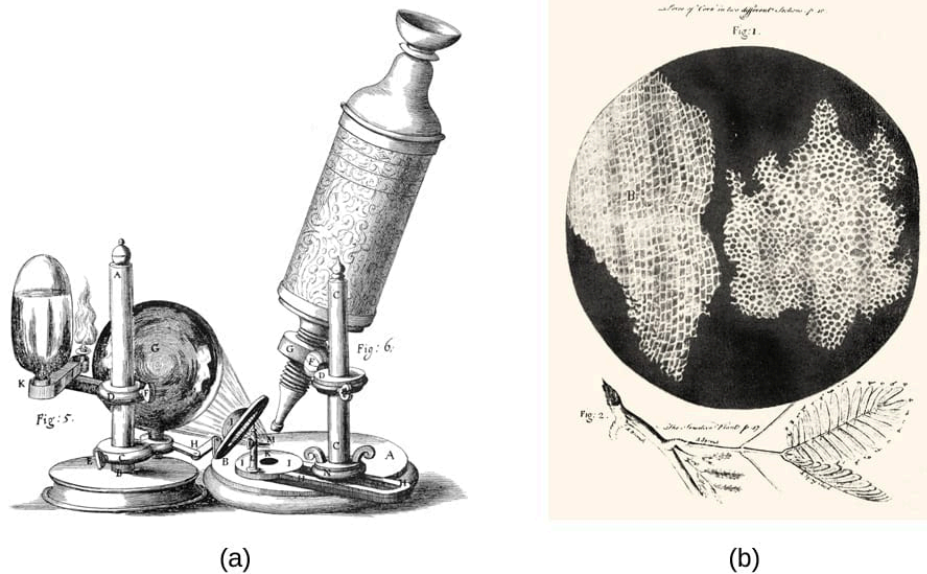


Figure 2.2.2: Robert Hooke used his (a) compound microscope to view (b) cork cells. Both of these engravings are from his seminal work *Micrographia*, published in 1665.

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Video: Seeing the Invisible: van Leeuwenhoek's first glimpses of the microbial world

In 1674, Antonie van Leeuwenhoek looked at a drop of lake water through his homemade microscope and discovered an invisible world that no one knew existed. His work inspired countless microbiology researchers, including HHMI investigator Bonnie Bassler, one of the narrators of this animated feature. Leeuwenhoek was a haberdasher and city official in Delft, The Netherlands. He started making simple microscopes and using them to observe the world around him. He was the first to discover bacteria, protists, sperm cells, blood cells, rotifers, and much more.

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Who Invented the Microscope?

While Antonie van Leeuwenhoek and Robert Hooke generally receive much of the credit for early advances in microscopy, neither can claim to be the inventor of the microscope. Some argue that this designation should belong to Hans and Zaccharias Janssen, Dutch spectacle-makers who may have invented the telescope, the simple microscope, and the compound microscope during the late 1500s or early 1600s (Figure 2.2.3). Unfortunately, little is known for sure about the Janssens, not even the exact dates of their births and deaths. The Janssens were secretive about their work and never published. It is also possible that the Janssens did not invent anything at all; their neighbor, Hans Lippershey, also developed microscopes and telescopes during the same time frame, and he is often credited with inventing the telescope. The historical records from the time are as fuzzy and imprecise as the images viewed through those early lenses, and any archived records have been lost over the centuries.



Figure 2.2.3: Zaccharias Janssen, along with his father Hans, may have invented the telescope, the simple microscope, and the compound microscope during the late 1500s or early 1600s. The historical evidence is inconclusive.

By contrast, van Leeuwenhoek and Hooke can thank ample documentation of their work for their respective legacies. Like Janssen, van Leeuwenhoek began his work in obscurity, leaving behind few records. However, his friend, the prominent physician Reinier de Graaf, wrote a letter to the editor of the *Philosophical Transactions of the Royal Society of London* calling attention to van Leeuwenhoek's powerful microscopes. From 1673 onward, van Leeuwenhoek began regularly submitting letters to the Royal Society detailing his observations. In 1674, his report describing single-celled organisms produced controversy in the scientific community, but his observations were soon confirmed when the society sent a delegation to investigate his findings. He subsequently enjoyed considerable celebrity, at one point even entertaining a visit by the czar of Russia.

Similarly, Robert Hooke had his observations using microscopes published by the Royal Society in a book called *Micrographia* in 1665. The book became a bestseller and greatly increased interest in microscopy throughout much of Europe.

Key Concepts and Summary

- Antonie van Leeuwenhoek is credited with the first observation of microbes, including protists and bacteria, with simple microscopes that he made.
- Robert Hooke was the first to describe what we now call cells.
- Simple microscopes have a single lens, while compound microscopes have multiple lenses.

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2.3: Instruments of Microscopy- Brightfield Microscopes

Learning Objectives

- Identify and describe the parts of a brightfield microscope
- Calculate total magnification for a compound microscope
- Describe the distinguishing features and typical uses for various types of light microscopes, electron microscopes, and scanning probe microscopes

The early pioneers of microscopy opened a window into the invisible world of microorganisms. But microscopy continued to advance in the centuries that followed. In 1830, Joseph Jackson Lister created an essentially modern light microscope. The 20th century saw the development of microscopes that leveraged nonvisible light, such as fluorescence microscopy, which uses an ultraviolet light source, and electron microscopy, which uses short-wavelength electron beams. These advances led to major improvements in magnification, resolution, and contrast. By comparison, the relatively rudimentary microscopes of van Leeuwenhoek and his contemporaries were far less powerful than even the most basic microscopes in use today. In this section, we will survey the broad range of modern microscopic technology and common applications for each type of microscope.

Light Microscopy

Many types of microscopes fall under the category of light microscopes, which use light to visualize images. Examples of light microscopes include brightfield microscopes, darkfield microscopes, phase-contrast microscopes, differential interference contrast microscopes, fluorescence microscopes, confocal scanning laser microscopes, and two-photon microscopes. These various types of light microscopes can be used to complement each other in diagnostics and research.

Brightfield Microscopes

The **brightfield microscope**, perhaps the most commonly used type of microscope, is a compound microscope with two or more lenses that produce a dark image on a bright background. Some brightfield microscopes are **monocular** (having a single eyepiece), though most newer brightfield microscopes are **binocular** (having two eyepieces), like the one shown in Figure 2.3.1; in either case, each eyepiece contains a lens called an **ocular lens**. The ocular lenses typically magnify images 10 times (10 $\times$). At the other end of the body tube are a set of **objective lenses** on a rotating nosepiece. The magnification of these objective lenses typically ranges from 4 $\times$ to 100 $\times$, with the magnification for each lens designated on the metal casing of the lens. The ocular and objective lenses work together to create a magnified image. The **total magnification** is the product of the ocular magnification times the objective magnification:

$$\text{ocular magnification} \times \text{objective magnification}$$

For example, if a 40 $\times$ objective lens is selected and the ocular lens is 10 $\times$, the total magnification would be

$$(40\times)(10\times) = 400\times$$

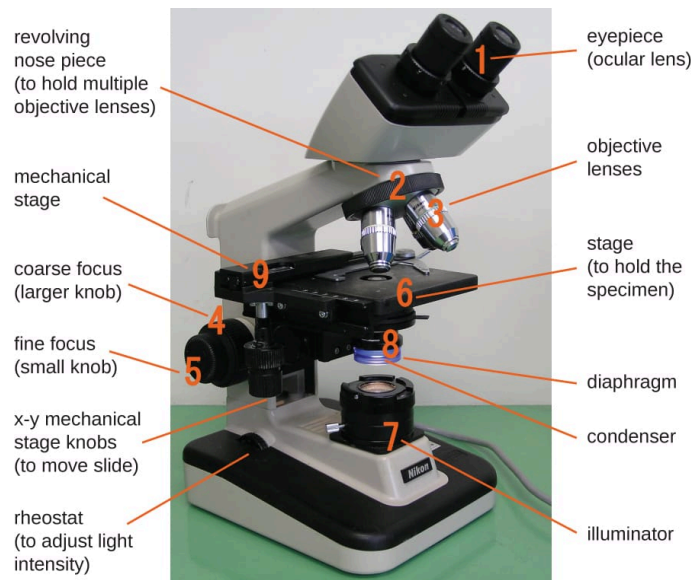


Figure 2.3.1: Components of a typical brightfield microscope.

Components of a typical brightfield microscope.

The item being viewed is called a **specimen**. The specimen is placed on a glass slide, which is then clipped into place on the **stage** (a platform) of the microscope. Once the slide is secured, the specimen on the slide is positioned over the light using the **x-y mechanical stage knobs**. These knobs move the slide on the surface of the stage, but do not raise or lower the stage. Once the specimen is centered over the light, the stage position can be raised or lowered to focus the image. The **coarse focusing knob** is used for large-scale movements with 4 $\times$ and 10 $\times$ objective lenses; the **fine focusing knob** is used for small-scale movements, especially with 40 $\times$ or 100 $\times$ objective lenses.

When images are magnified, they become dimmer because there is less light per unit area of image. Highly magnified images produced by microscopes, therefore, require intense lighting. In a brightfield microscope, this light is provided by an **illuminator**, which is typically a high-intensity bulb below the stage. Light from the illuminator passes up through **condenser lens** (located below the stage), which focuses all of the light rays on the specimen to maximize illumination. The position of the condenser can be optimized using the attached condenser focus knob; once the optimal distance is established, the condenser should not be moved to adjust the brightness. If less-than-maximal light levels are needed, the amount of light striking the specimen can be easily adjusted by opening or closing a **diaphragm** between the condenser and the specimen. In some cases, brightness can also be adjusted using the **rheostat**, a dimmer switch that controls the intensity of the illuminator.

A brightfield microscope creates an image by directing light from the illuminator at the specimen; this light is differentially transmitted, absorbed, reflected, or refracted by different structures. Different colors can behave differently as they interact with **chromophores** (pigments that absorb and reflect particular wavelengths of light) in parts of the specimen. Often, chromophores are artificially added to the specimen using stains, which serve to increase contrast and resolution. In general, structures in the specimen will appear darker, to various extents, than the bright background, creating maximally sharp images at magnifications up to about 1000 $\times$. Further magnification would create a larger image, but without increased resolution. This allows us to see objects as small as bacteria, which are visible at about 400 $\times$ or so, but not smaller objects such as viruses.

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At very high magnifications, resolution may be compromised when light passes through the small amount of air between the specimen and the lens. This is due to the large difference between the refractive indices of air and glass; the air scatters the light rays before they can be focused by the lens. To solve this problem, a drop of oil can be used to fill the space between the specimen and an **oil immersion lens**, a special lens designed to be used with immersion oils. Since the oil has a refractive index very similar to that of glass, it increases the maximum angle at which light leaving the specimen can strike the lens. This increases the light collected and, thus, the resolution of the image (Figure 2.3.2). A variety of oils can be used for different types of light.

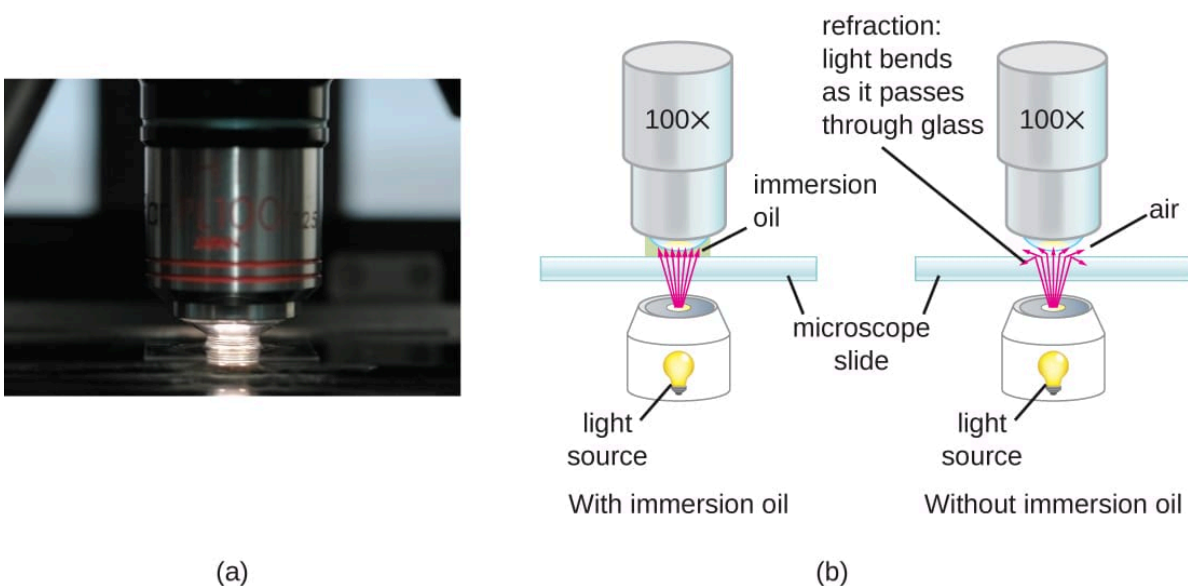


Figure 2.3.2: (a) Oil immersion lenses like this one are used to improve resolution. (b) Because immersion oil and glass have very similar refractive indices, there is a minimal amount of refraction before the light reaches the lens. Without immersion oil, light scatters as it passes through the air above the slide, degrading the resolution of the image.

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Microscope Maintenance: Best Practices

Even a very powerful microscope cannot deliver high-resolution images if it is not properly cleaned and maintained. Since lenses are carefully designed and manufactured to refract light with a high degree of precision, even a slightly dirty or scratched lens will refract light in unintended ways, degrading the image of the specimen. In addition, microscopes are rather delicate instruments, and great care must be taken to avoid damaging parts and surfaces. Among other things, proper care of a microscope includes the following:

- cleaning the lenses with lens paper
- not allowing lenses to contact the slide (e.g., by rapidly changing the focus)
- protecting the bulb (if there is one) from breakage
- not pushing an objective into a slide
- not using the coarse focusing knob when using the 40 $\times$ or greater objective lenses
- only using immersion oil with a specialized oil objective, usually the 100 $\times$ objective
- cleaning oil from immersion lenses after using the microscope
- cleaning any oil accidentally transferred from other lenses
- covering the microscope or placing it in a cabinet when not in use

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Key Concepts and Summary

- Numerous types of microscopes use various technologies to generate micrographs. Most are useful for a particular type of specimen or application.
- Light microscopy uses lenses to focus light on a specimen to produce an image. Commonly used light microscopes include brightfield, darkfield, phase-contrast, differential interference contrast, fluorescence, confocal, and two-photon microscopes.

Footnotes

1. ¹ “JEM-ARM200F Transmission Electron Microscope,” *JEOL USA Inc*, www.jeolusa.com/PRODUCTS/Tran...specifications. Accessed 8/28/2015.

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2.4: Darkfield, Phase-Contrast and DIC

Learning Objectives

- Compare and contrast darkfield, phase-contrast and DIC microscopy
- Identify which type of microscopy is most appropriate for visualizing specific types of specimen

Darkfield Microscopy

A **darkfield microscope** is a brightfield microscope that has a small but significant modification to the condenser. A small, opaque disk (about 1 cm in diameter) is placed between the illuminator and the condenser lens. This opaque light stop, as the disk is called, blocks most of the light from the illuminator as it passes through the condenser on its way to the objective lens, producing a hollow cone of light that is focused on the specimen. The only light that reaches the objective is light that has been refracted or reflected by structures in the specimen. The resulting image typically shows bright objects on a dark background (Figure 2.4.3)

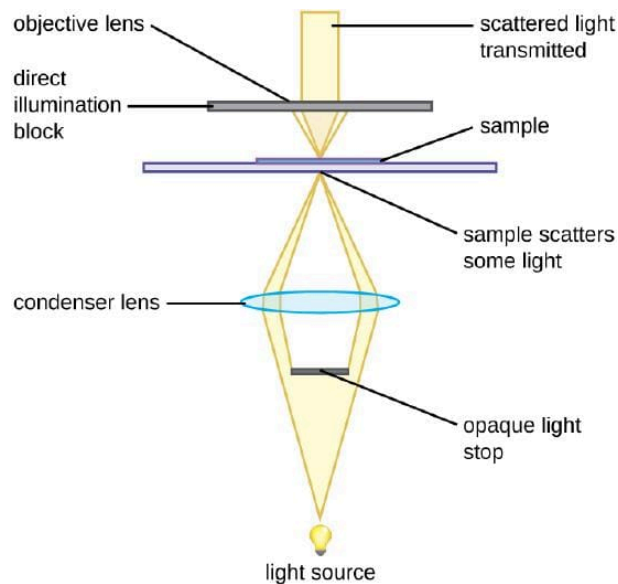


Figure 2.4.3: An opaque light stop inserted into a brightfield microscope is used to produce a darkfield image. The light stop blocks light traveling directly from the illuminator to the objective lens, allowing only light reflected or refracted off the specimen to reach the eye.

An opaque light stop inserted into a brightfield microscope is used to produce a darkfield image. The light stop blocks light traveling directly from the illuminator to the objective lens, allowing only light reflected or refracted off the specimen to reach the eye.

Darkfield microscopy can often create high-contrast, high-resolution images of specimens without the use of stains, which is particularly useful for viewing live specimens that might be killed or otherwise compromised by the stains. For example, thin spirochetes like *Treponema pallidum*, the causative agent of syphilis, can be best viewed using a darkfield microscope (Figure 2.4.4).



Figure 2.4.4: Use of a darkfield microscope allows us to view living, unstained samples of the spirochete *Treponema pallidum*. Similar to a photographic negative, the spirochetes appear bright against a dark background. (credit: Centers for Disease Control and Prevention)

Use of a darkfield microscope allows us to view living, unstained samples of the spirochete *Treponema pallidum*. Similar to a photographic negative, the spirochetes appear bright against a dark background. (credit: Centers for Disease Control and Prevention/C.W. Hubbard)

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Phase-Contrast Microscopes

Phase-contrast microscopes use refraction and interference caused by structures in a specimen to create high-contrast, high-resolution images without staining. It is the oldest and simplest type of microscope that creates an image by altering the wavelengths of light rays passing through the specimen. To create altered wavelength paths, an annular stop is used in the condenser. The annular stop produces a hollow cone of light that is focused on the specimen before reaching the objective lens. The objective contains a phase plate containing a phase ring. As a result, light traveling directly from the illuminator passes through the phase ring while light refracted or reflected by the specimen passes through the plate. This causes waves traveling through the ring to be about one-half of a wavelength out of phase with those passing through the plate. Because waves have peaks and troughs, they can add together (if in phase together) or cancel each other out (if out of phase). When the wavelengths are out of phase, wave troughs will cancel out wave peaks, which is called destructive interference. Structures that refract light then appear dark against a bright background of only unrefracted light. More generally, structures that differ in features such as refractive index will differ in levels of darkness (Figure 2.4.5).

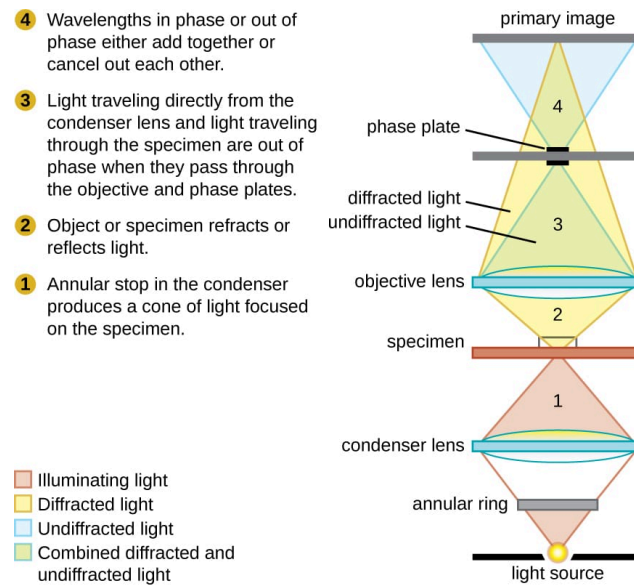


Figure 2.4.5: This diagram of a phase-contrast microscope illustrates phase differences between light passing through the object and background. These differences are produced by passing the rays through different parts of a phase plate. The light rays are superimposed in the image plane, producing contrast due to their interference.

This diagram of a phase-contrast microscope illustrates phase differences between light passing through the object and background. These differences are produced by passing the rays through different parts of a phase plate. The light rays are superimposed in the image plane, producing contrast due to their interference.

Because it increases contrast without requiring stains, phase-contrast microscopy is often used to observe live specimens. Certain structures, such as organelles in eukaryotic cells and endospores in prokaryotic cells, are especially well visualized with phase-contrast microscopy (Figure 2.4.6).

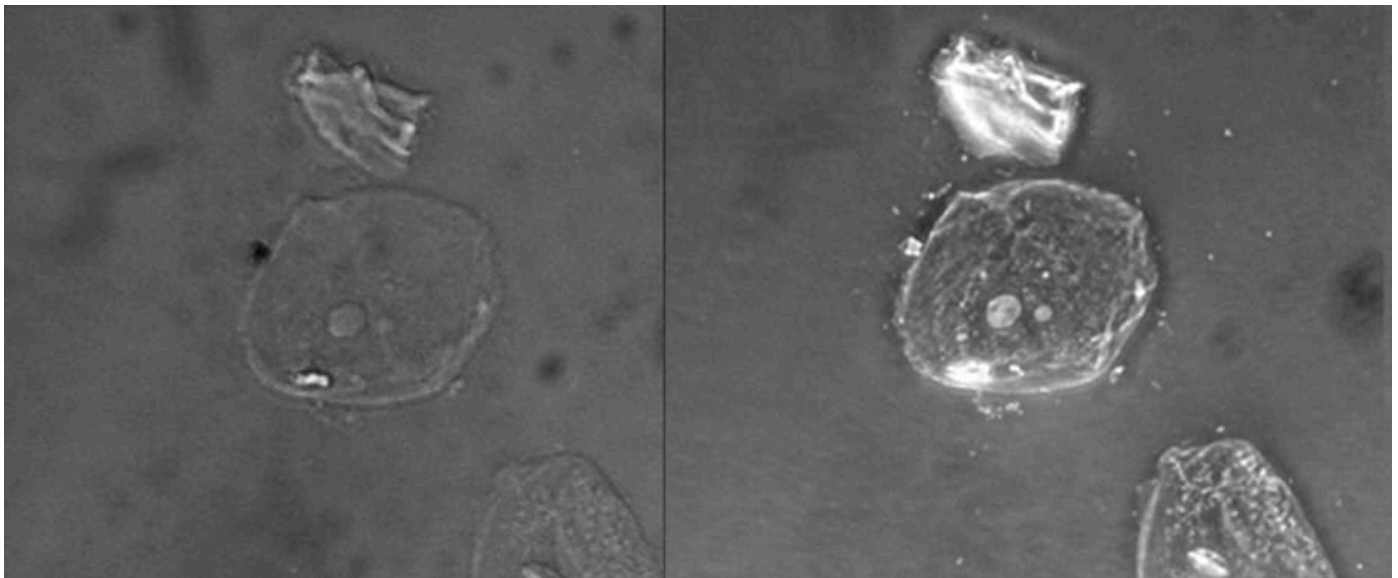


Figure 2.4.6: This figure compares a brightfield image (left) with a phase-contrast image (right) of the same unstained simple squamous epithelial cells. The cells are in the center and bottom right of each photograph (the irregular item above the cells is acellular debris). Notice that the unstained cells in the brightfield image are almost invisible against the background, whereas the cells in the phase-contrast image appear to glow against the background, revealing far more detail.

This figure compares a brightfield image (left) with a phase-contrast image (right) of the same unstained simple squamous epithelial cells. The cells are in the center and bottom right of each photograph (the irregular item above the cells is acellular debris). Notice that the unstained cells in the brightfield image are almost invisible against the background, whereas the cells in the phase-contrast image appear to glow against the background, revealing far more detail. (credit: "Clearly kefir"/Wikimedia)

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Differential Interference Contrast Microscopes

Differential interference contrast (DIC) microscopes (also known as Nomarski optics) are similar to phase-contrast microscopes in that they use interference patterns to enhance contrast between different features of a specimen. In a DIC microscope, two beams of light are created in which the direction of wave movement (polarization) differs. Once the beams pass through either the specimen or specimen-free space, they are recombined and effects of the specimens cause differences in the interference patterns generated by the combining of the beams. This results in high-contrast images of living organisms with a three-dimensional appearance. These microscopes are especially useful in distinguishing structures within live, unstained specimens. (Figure 2.4.7).



Figure 2.4.7: A DIC image of *Fonsecaea pedrosoi* grown on modified Leonian's agar. This fungus causes chromoblastomycosis, a chronic skin infection common in tropical and subtropical climates.

A DIC image of *Fonsecaea pedrosoi* grown on modified Leonian's agar. This fungus causes chromoblastomycosis, a chronic skin infection common in tropical and subtropical climates.

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Key Concepts and Summary

- Light microscopy uses lenses to focus light on a specimen to produce an image. Commonly used light microscopes include brightfield, darkfield, phase-contrast, differential interference contrast, fluorescence, confocal, and two-photon microscopes.

Footnotes

1. ¹ “JEM-ARM200F Transmission Electron Microscope,” *JEOL USA Inc*, www.jeolusa.com/PRODUCTS/Tran...specifications. Accessed 8/28/2015.

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2.5: Fluorescence, Confocal and Two-Photon Microscopes

Learning Objectives

- Explain the principle of fluorescence microscopy including the roles of excitation and emission light
- Interpret fluorescence images to evaluate the advantages of confocal and two-photon microscopy for visualizing structures in thick or live biological samples

Fluorescence Microscopes

A **fluorescence microscope** uses fluorescent chromophores called **fluorochromes**, which are capable of absorbing energy from a light source and then emitting this energy as visible light. Fluorochromes include naturally fluorescent substances (such as chlorophylls) as well as fluorescent stains that are added to the specimen to create contrast. Dyes such as Texas red and FITC are examples of fluorochromes. Other examples include the nucleic acid dyes 4',6'-diamidino-2-phenylindole (DAPI) and acridine orange.

The microscope transmits an excitation light, generally a form of EMR with a short wavelength, such as ultraviolet or blue light, toward the specimen; the chromophores absorb the excitation light and emit visible light with longer wavelengths. The excitation light is then filtered out (in part because ultraviolet light is harmful to the eyes) so that only visible light passes through the ocular lens. This produces an image of the specimen in bright colors against a dark background.

Fluorescence microscopes are especially useful in clinical microbiology. They can be used to identify pathogens, to find particular species within an environment, or to find the locations of particular molecules and structures within a cell. Approaches have also been developed to distinguish living from dead cells using fluorescence microscopy based upon whether they take up particular fluorochromes. Sometimes, multiple fluorochromes are used on the same specimen to show different structures or features.

One of the most important applications of fluorescence microscopy is a technique called **immunofluorescence**, which is used to identify certain disease-causing microbes by observing whether antibodies bind to them. (Antibodies are protein molecules produced by the immune system that attach to specific pathogens to kill or inhibit them.) There are two approaches to this technique: direct immunofluorescence assay (DFA) and indirect immunofluorescence assay (IFA). In DFA, specific antibodies (e.g., those that target the rabies virus) are stained with a fluorochrome. If the specimen contains the targeted pathogen, one can observe the antibodies binding to the pathogen under the fluorescent microscope. This is called a primary antibody stain because the stained antibodies attach directly to the pathogen.

In IFA, secondary antibodies are stained with a fluorochrome rather than primary antibodies. Secondary antibodies do not attach directly to the pathogen, but they do bind to primary antibodies. When the unstained primary antibodies bind to the pathogen, the fluorescent secondary antibodies can be observed binding to the primary antibodies. Thus, the secondary antibodies are attached indirectly to the pathogen. Since multiple secondary antibodies can often attach to a primary antibody, IFA increases the number of fluorescent antibodies attached to the specimen, making it easier visualize features in the specimen (Figure 2.5.8).

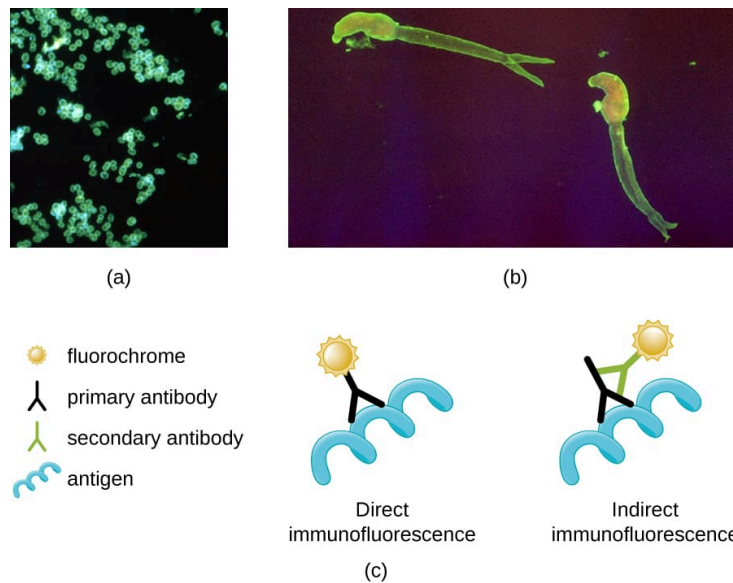


Figure 2.5.8: (a) A direct immunofluorescent stain is used to visualize *Neisseria gonorrhoeae*, the bacterium that causes gonorrhea. (b) An indirect immunofluorescent stain is used to visualize larvae of *Schistosoma mansoni*, a parasitic worm that causes schistosomiasis, an intestinal disease common in the tropics. (c) In direct immunofluorescence, the stain is absorbed by a primary antibody, which binds to the antigen. In indirect immunofluorescence, the stain is absorbed by a secondary antibody, which binds to a primary antibody, which, in turn, binds to the antigen. (credit a: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by Centers for Disease Control and Prevention)

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Confocal Microscopes

Whereas other forms of light microscopy create an image that is maximally focused at a single distance from the observer (the depth, or z-plane), a **confocal microscope** uses a laser to scan multiple z-planes successively. This produces numerous two-dimensional, high-resolution images at various depths, which can be constructed into a three-dimensional image by a computer. As with fluorescence microscopes, fluorescent stains are generally used to increase contrast and resolution. Image clarity is further enhanced by a narrow aperture that eliminates any light that is not from the z-plane. Confocal microscopes are thus very useful for examining thick specimens such as biofilms, which can be examined alive and unfixed (Figure 2.5.9).

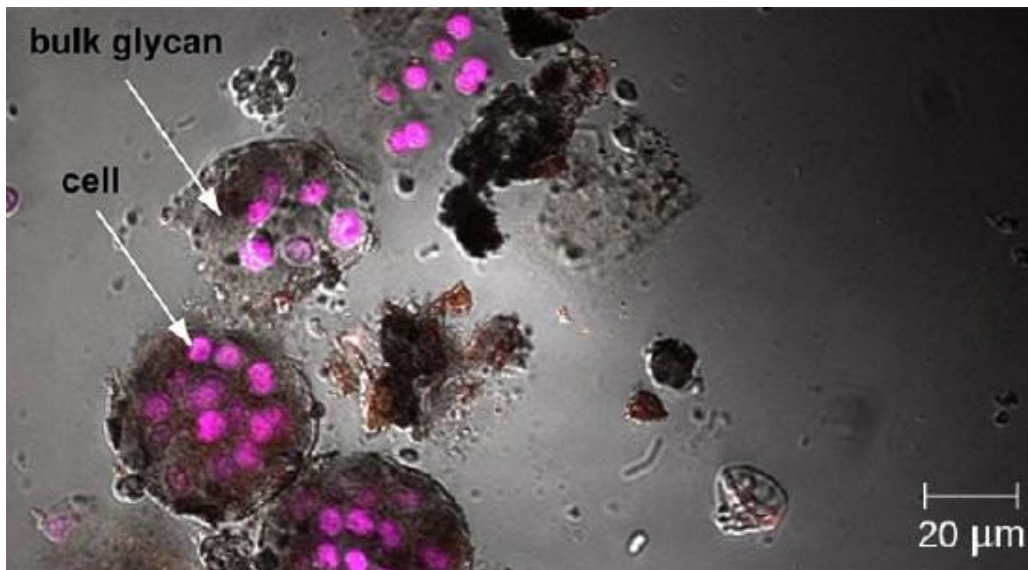


Figure 2.5.9: Confocal microscopy can be used to visualize structures such as this roof-dwelling cyanobacterium biofilm. (credit: modification of work by American Society for Microbiology).

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Two-Photon Microscopes

While the original fluorescent and confocal microscopes allowed better visualization of unique features in specimens, there were still problems that prevented optimum visualization. The effective sensitivity of fluorescence microscopy when viewing thick specimens was generally limited by out-of-focus flare, which resulted in poor resolution. This limitation was greatly reduced in the confocal microscope through the use of a confocal pinhole to reject out-of-focus background fluorescence with thin ($<1\ \mu\text{m}$), unblurred optical sections. However, even the confocal microscopes lacked the resolution needed for viewing thick tissue samples. These problems were resolved with the development of the **two-photon microscope**, which uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to visualize specimens. The low energy associated with the long-wavelength light means that two photons must strike a location at the same time to excite the fluorochrome. The low energy of the excitation light is less damaging to cells, and the long wavelength of the excitation light more easily penetrates deep into thick specimens. This makes the two-photon microscope useful for examining living cells within intact tissues—brain slices, embryos, whole organs, and even entire animals.

Currently, use of two-photon microscopes is limited to advanced clinical and research laboratories because of the high costs of the instruments. A single two-photon microscope typically costs between \$300,000 and \$500,000, and the lasers used to excite the dyes used on specimens are also very expensive. However, as technology improves, two-photon microscopes may become more readily available in clinical settings.

Key Concepts and Summary

- Light microscopy uses lenses to focus light on a specimen to produce an image. Commonly used light microscopes include brightfield, darkfield, phase-contrast, differential interference contrast, fluorescence, confocal, and two-photon microscopes.

Footnotes

1. [1](#) “JEM-ARM200F Transmission Electron Microscope,” *JEOL USA Inc*, www.jeolusa.com/PRODUCTS/Tran...specifications. Accessed 8/28/2015.

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2.6: Electron Microscopy

Learning Objectives

- Identify the advantages and limitations of EM compared to light microscopy
- Distinguish between TEM and SEM in terms of image production, resolution and types of samples analyzed

Electron Microscopy

The maximum theoretical resolution of images created by light microscopes is ultimately limited by the wavelengths of visible light. Most light microscopes can only magnify $1000\times$, and a few can magnify up to $1500\times$, but this does not begin to approach the magnifying power of an **electron microscope (EM)**, which uses short-wavelength electron beams rather than light to increase magnification and resolution.

Electrons, like electromagnetic radiation, can behave as waves, but with wavelengths of 0.005 nm , they can produce much better resolution than visible light. An EM can produce a sharp image that is magnified up to $100,000\times$. Thus, EMs can resolve subcellular structures as well as some molecular structures (e.g., single strands of DNA); however, electron microscopy cannot be used on living material because of the methods needed to prepare the specimens.

There are two basic types of EM: the **transmission electron microscope (TEM)** and the **scanning electron microscope (SEM)**(Figure 2.6.10). The TEM is somewhat analogous to the brightfield light microscope in terms of the way it functions. However, it uses an electron beam from above the specimen that is focused using a magnetic lens (rather than a glass lens) and projected through the specimen onto a detector. Electrons pass through the specimen, and then the detector captures the image (Figure 2.6.11).



(a)



(b)

Figure 2.6.10: (a) A transmission electron microscope (TEM). (b) A scanning electron microscope (SEM). (credit a: modification of work by "Deshi"/Wikimedia Commons; credit b: modification of work by "ZEISS Microscopy"/Flickr)

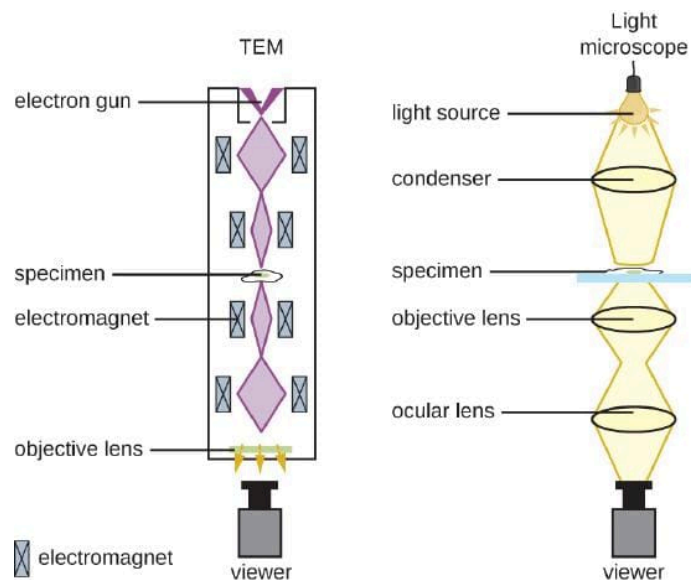


Figure 2.6.11: Electron microscopes use magnets to focus electron beams similarly to the way that light microscopes use lenses to focus light.

For electrons to pass through the specimen in a TEM, the specimen must be extremely thin (20–100 nm thick). The image is produced because of varying opacity in various parts of the specimen. This opacity can be enhanced by staining the specimen with materials such as heavy metals, which are electron dense. TEM requires that the beam and specimen be in a vacuum and that the specimen be very thin and dehydrated. The specific steps needed to prepare a specimen for observation under an EM are discussed in detail in the next section.

SEMs form images of surfaces of specimens, usually from electrons that are knocked off of specimens by a beam of electrons. This can create highly detailed images with a three-dimensional appearance that are displayed on a monitor (Figure 2.6.12). Typically, specimens are dried and prepared with fixatives that reduce artifacts, such as shriveling, that can be produced by drying, before being sputter-coated with a thin layer of metal such as gold. Whereas transmission electron microscopy requires very thin sections and allows one to see internal structures such as organelles and the interior of membranes, scanning electron microscopy can be used to view the surfaces of larger objects (such as a pollen grain) as well as the surfaces of very small samples (Figure 2.6.13). Some EMs can magnify an image up to 2,000,000¹.

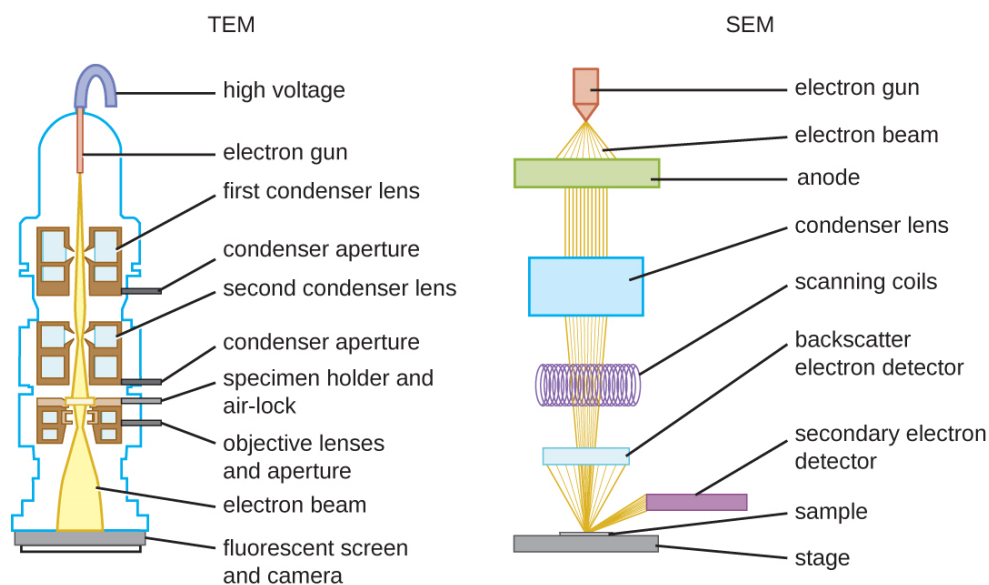
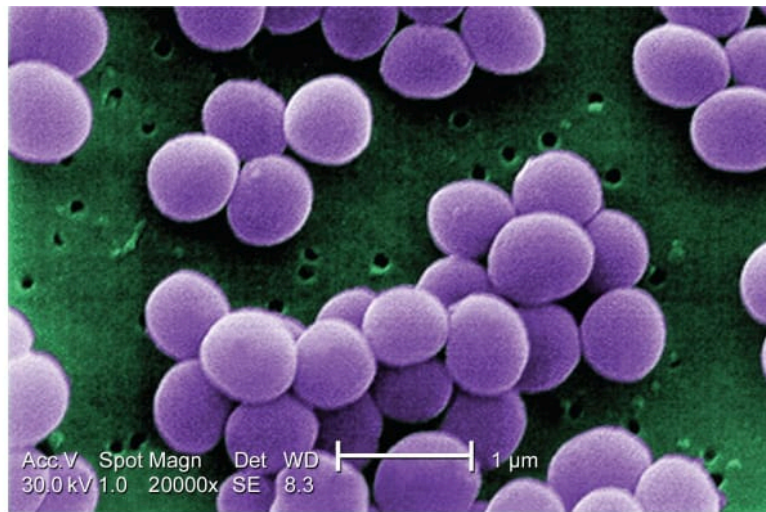


Figure 2.6.12: These schematic illustrations compare the components of transmission electron microscopes and scanning electron microscopes.



(a)



(b)

Figure 2.6.13: (a) This TEM image of cells in a biofilm shows well-defined internal structures of the cells because of varying levels of opacity in the specimen. (b) This color-enhanced SEM image of the bacterium *Staphylococcus aureus* illustrates the ability of scanning electron microscopy to render three-dimensional images of the surface structure of cells. (credit a: modification of work by American Society for Microbiology; credit b: modification of work by Centers for Disease Control and Prevention)

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Using Microscopy to Study Biofilms

A biofilm is a complex community of one or more microorganism species, typically forming as a slimy coating attached to a surface because of the production of an extrapolymeric substance (EPS) that attaches to a surface or at the interface between surfaces (e.g., between air and water). In nature, biofilms are abundant and frequently occupy complex niches within ecosystems (Figure 2.6.14). In medicine, biofilms can coat medical devices and exist within the body. Because they possess unique characteristics, such as increased resistance against the immune system and to antimicrobial drugs, biofilms are of particular interest to microbiologists and clinicians alike.

Because biofilms are thick, they cannot be observed very well using light microscopy; slicing a biofilm to create a thinner specimen might kill or disturb the microbial community. Confocal microscopy provides clearer images of biofilms because it can focus on one z-plane at a time and produce a three-dimensional image of a thick specimen. Fluorescent dyes can be helpful in identifying cells within the matrix. Additionally, techniques such as immunofluorescence and fluorescence in situ hybridization (FISH), in which fluorescent probes are used to bind to DNA, can be used.

Electron microscopy can be used to observe biofilms, but only after dehydrating the specimen, which produces undesirable artifacts and distorts the specimen. In addition to these approaches, it is possible to follow water currents through the shapes (such as cones and mushrooms) of biofilms, using video of the movement of fluorescently coated beads (Figure 2.6.15).

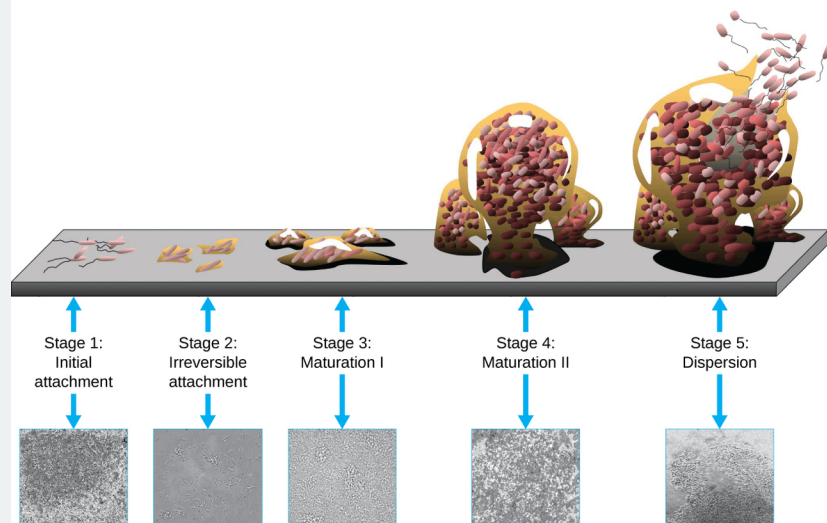


Diagram showing five stages of biofilm development of *Pseudomonas aeruginosa*. All photomicrographs are shown to same scale.

Figure 2.6.14: A biofilm forms when planktonic (free-floating) bacteria of one or more species adhere to a surface, produce slime, and form a colony. (credit: Public Library of Science).

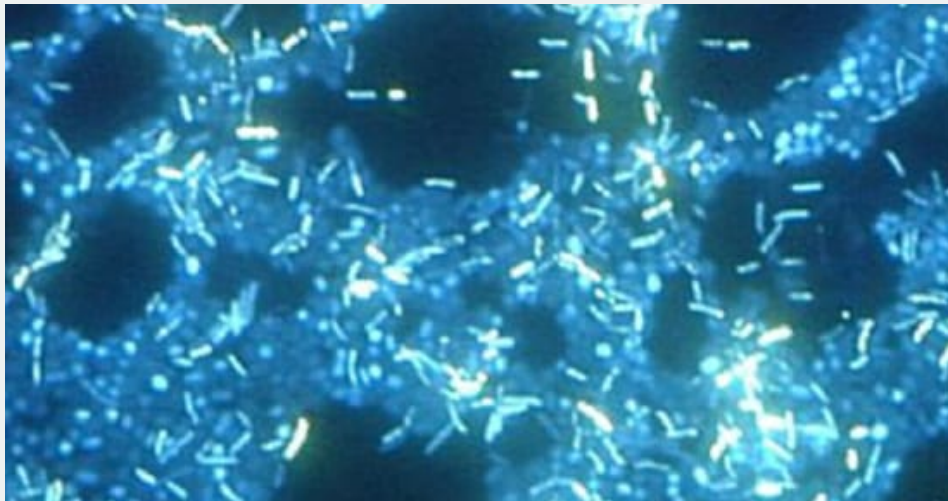


Figure 2.6.15: In this image, multiple species of bacteria grow in a biofilm on stainless steel (stained with DAPI for epifluorescence microscopy). (credit: Ricardo Murga, Rodney Donlan).

Scanning Probe Microscopy

A **scanning probe microscope** does not use light or electrons, but rather very sharp probes that are passed over the surface of the specimen and interact with it directly. This produces information that can be assembled into images with magnifications up to 100,000,000 $\times$. Such large magnifications can be used to observe individual atoms on surfaces. To date, these techniques have been used primarily for research rather than for diagnostics.

There are two types of scanning probe microscope: the **scanning tunneling microscope (STM)** and the **atomic force microscope (AFM)**. An STM uses a probe that is passed just above the specimen as a constant voltage bias creates the potential for an electric current between the probe and the specimen. This current occurs via quantum tunneling of electrons between the probe and the specimen, and the intensity of the current is dependent upon the distance between the probe and the specimen. The probe is moved horizontally above the surface and the intensity of the current is measured. Scanning tunneling microscopy can effectively map the structure of surfaces at a resolution at which individual atoms can be detected.

Similar to an STM, AFMs have a thin probe that is passed just above the specimen. However, rather than measuring variations in the current at a constant height above the specimen, an AFM establishes a constant current and measures variations in the height of the probe tip as it passes over the specimen. As the probe tip is passed over the specimen, forces between the atoms (van

der Waals forces, capillary forces, chemical bonding, electrostatic forces, and others) cause it to move up and down. Deflection of the probe tip is determined and measured using Hooke's law of elasticity, and this information is used to construct images of the surface of the specimen with resolution at the atomic level (Figure 2.6.16).

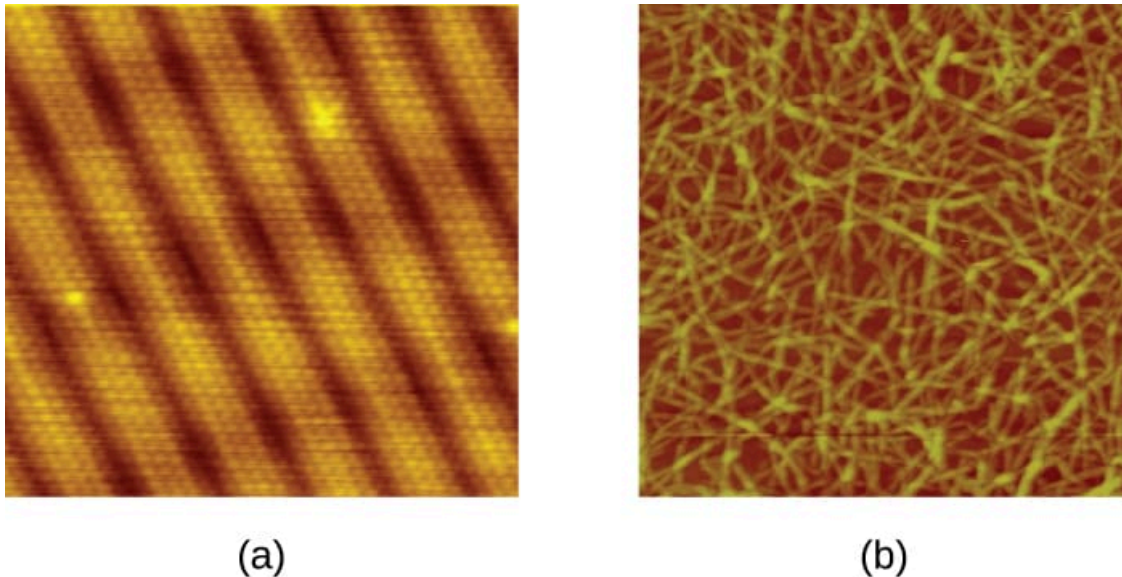


Figure 2.6.16: STMs and AFMs allow us to view images at the atomic level. (a) This STM image of a pure gold surface shows individual atoms of gold arranged in columns. (b) This AFM image shows long, strand-like molecules of nanocellulose, a laboratory-created substance derived from plant fibers. (credit a: modification of work by “Erwinrossen”/Wikimedia Commons).

LIGHT MICROSCOPES Magnification: up to about 1000× Use visible or ultraviolet light to produce an image.		
Microscope Type	Key Uses	Sample Images
Brightfield	Commonly used in a wide variety of laboratory applications as the standard microscope; produces an image on a bright background. Example: <i>Bacillus</i> sp. showing endospores.	
Darkfield	Increases contrast without staining by producing a bright image on a darker background; especially useful for viewing live specimens. Example: <i>Borrelia burgdorferi</i>	
Phase contrast	Uses refraction and interference caused by structures in the specimen to create high-contrast, high-resolution images without staining, making it useful for viewing live specimens, and structures such as endospores and organelles. Example: <i>Pseudomonas</i> sp.	
Differential interference contrast (DIC)	Uses interference patterns to enhance contrast between different features of a specimen to produce high-contrast images of living organisms with a three-dimensional appearance, making it especially useful in distinguishing structures within live, unstained specimens; images viewed reveal detailed structures within cells. Example: <i>Escherichia coli</i> O157:H7	
Fluorescence	Uses fluorescent stains to produce an image; can be used to identify pathogens, to find particular species, to distinguish living from dead cells, or to find locations of particular molecules within a cell; also used for immunofluorescence. Example: <i>P. putida</i> stained with fluorescent dyes to visualize the capsule.	
Confocal	Uses a laser to scan multiple z-planes successively, producing numerous two-dimensional, high-resolution images at various depths that can be constructed into a three-dimensional image by a computer, making this useful for examining thick specimens such as biofilms. Example: <i>Escherichia coli</i> stained with acridine orange dye to show the nucleoid regions of the cells.	
Two-photon	Uses a scanning technique, fluorochromes, and long-wavelength light (such as infrared) to penetrate deep into thick specimens such as biofilms. Example: Mouse intestine cells stained with fluorescent dye.	

Figure 2.6.17: (credit “Brightfield”: modification of work by American Society for Microbiology; credit “Darkfield”: modification of work by American Society for Microbiology; credit “Phase contrast”: modification of work by American Society for Microbiology; credit “DIC”: modification of work by American Society for Microbiology; credit “Fluorescence”: modification of work by American Society for Microbiology; credit “Confocal”: modification of work by American Society for Microbiology; credit “Two-photon”: modification of work by Alberto Diaspro, Paolo Bianchini, Giuseppe Vicidomini, Mario Faretta, Paola Ramoino, Cesare Usai).

ELECTRON MICROSCOPES Magnification: 20–100,000× or more Use electron beams focused with magnets to produce an image.		
Microscope Type	Key Uses	Sample Images
Transmission (TEM)	Uses electron beams that pass through a specimen to visualize small images; useful to observe small, thin specimens such as tissue sections and subcellular structures. Example: <i>Ebola virus</i>	
Scanning (SEM)	Uses electron beams to visualize surfaces; useful to observe the three-dimensional surface details of specimens. Example: <i>Campylobacter jejuni</i>	

Figure 2.6.18: (credit “TEM”: modification of work by American Society for Microbiology; credit “SEM”: modification of work by American Society for Microbiology)

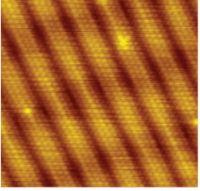
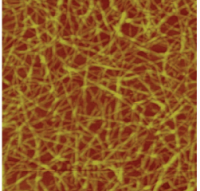
SCANNING PROBE MICROSCOPES Magnification: 100–100,000,000× or more Use very short probes that are passed over the surface of the specimen and interact with it directly.		
Microscope Type	Key Uses	Sample Images
Scanning tunneling (STM)	Uses a probe passed horizontally at a constant distance just above the specimen while the intensity of the current is measured; can map the structure of surfaces at the atomic level; works best on conducting materials but can also be used to examine organic materials such as DNA, if fixed on a surface. Example: Image of surface reconstruction on a clean gold [Au(100)] surface, as visualized using scanning tunneling microscopy.	
Atomic force (AFM)	Can be used in several ways, including using a laser focused on a cantilever to measure the bending of the tip or a probe passed above the specimen while the height needed to maintain a constant current is measured; useful to observe specimens at the atomic level and can be more easily used with nonconducting samples. Example: AFM height image of carboxymethylated nanocellulose adsorbed on a silica surface.	

Figure 2.6.19: Microscopy techniques for scanning probe microscopes.

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Key Concepts and Summary

- Electron microscopy focuses electrons on the specimen using magnets, producing much greater magnification than light microscopy. The transmission electron microscope (TEM) and scanning electron microscope (SEM) are two common forms.
- Scanning probe microscopy produces images of even greater magnification by measuring feedback from sharp probes

that interact with the specimen. Probe microscopes include the scanning tunneling microscope (STM) and the atomic force microscope (AFM).

Footnotes

- 1 [“JEM-ARM200F Transmission Electron Microscope,” JEOL USA Inc, www.jeolusa.com/PRODUCTS/Tran...specifications.](http://www.jeolusa.com/PRODUCTS/Transmission%20Electron%20Microscope) Accessed 8/28/2015.

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2.7: Sample Preparation for Microscopic Staining

Learning Objectives

- Differentiate between wet mount and fixation in terms of microscopic sample preparation
- Describe the unique features of commonly used stains
- Identify appropriate dyes for positive vs negative stains
- Differentiate between simple and differential stains

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.

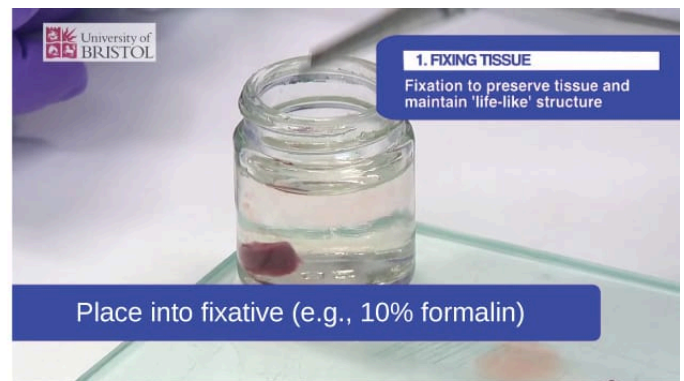
To heat-fix a sample, a thin layer of the specimen is spread on the slide (called a smear), and the slide is then briefly heated over a heat source (Figure 2.7.1). Chemical fixatives are often preferable to heat for tissue specimens. Chemical agents such as acetic acid, ethanol, methanol, formaldehyde (formalin), and glutaraldehyde can denature proteins, stop biochemical reactions, and stabilize cell structures in tissue samples (Figure 2.7.1).



(a)



(b)



(c)

Figure 2.7.1: (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)

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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**.

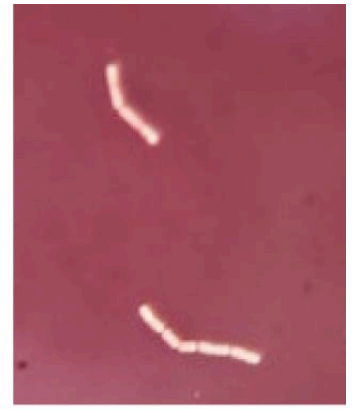
Dyes are selected for staining based on the chemical properties of the dye and the specimen being observed, which determine how the dye will interact with the specimen. In most cases, it is preferable to use a **positive stain**, a dye that will be absorbed by the cells or organisms being observed, adding 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 2.7.2).



(a)



(b)



(c)

Figure 2.7.2: (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)

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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 2.7.10 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 staining** distinguishes 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 2.7.11 provides more detail on these differential staining techniques.

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Key Concepts and Summary

- Samples must be properly prepared for microscopy. This may involve **staining**, **fixation**, and/or cutting **thin sections**.

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2.8: Differential Staining

Learning Objectives

- Explain the principles behind Gram, endospore, acid-fast, negative capsule, and flagella staining
- Describe the procedures and reagents of these differential stains
- Interpret the clinical applications of these staining methods

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 2.8.3.

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.

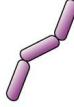
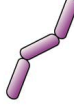
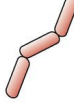
Gram stain process			
Gram staining steps	Cell effects	Gram-positive	Gram-negative
Step 1 Crystal violet <i>primary stain added to specimen smear.</i>	Stains cells purple or blue.		
Step 2 Iodine <i>mordant makes dye less soluble so it adheres to cell walls.</i>	Cells remain purple or blue.		
Step 3 Alcohol <i>decolorizer washes away stain from gram-negative cell walls.</i>	Gram-positive cells remain purple or blue. Gram-negative cells are colorless.		
Step 4 Safranin <i>counterstain allows dye adherence to gram-negative cells.</i>	Gram-positive cells remain purple or blue. Gram-negative cells appear pink or red.		

Figure 2.8.1: 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 2.8.4). 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 2.8.4). 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.

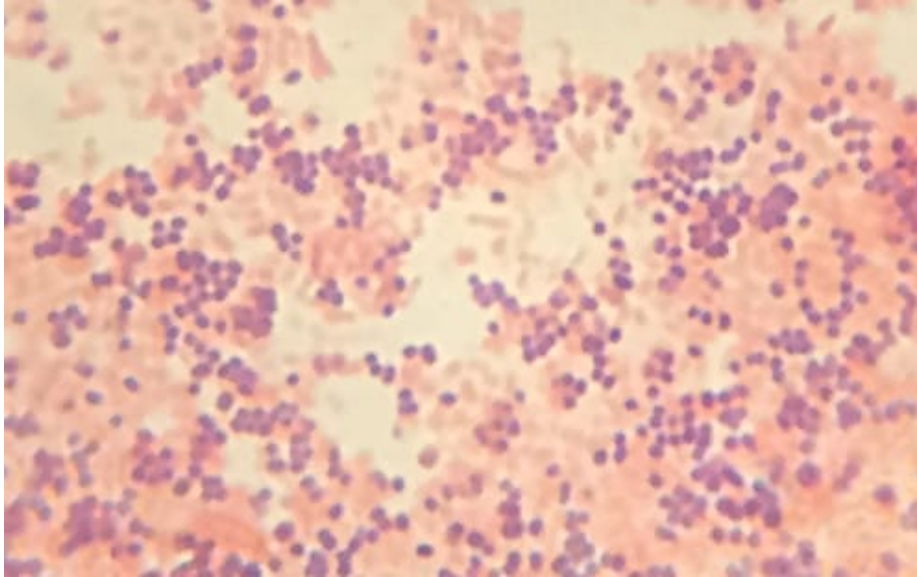


Figure 2.8.4: In this specimen, the gram-positive bacterium *Staphylococcus aureus* retains crystal violet dye even after the decolorizing agent is added. Gram-negative *Escherichia coli*, the most common Gram stain quality-control bacterium, is decolorized, and is only visible after the addition of the pink counterstain safranin. (credit: modification of work by Nina Parker)

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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 carbol-fuchsin-based methods is whether heat is used during the primary staining process. The Ziehl-Neelsen method uses heat to infuse the carbol-fuchsin 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 2.8.6).

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 2.8.6). 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.

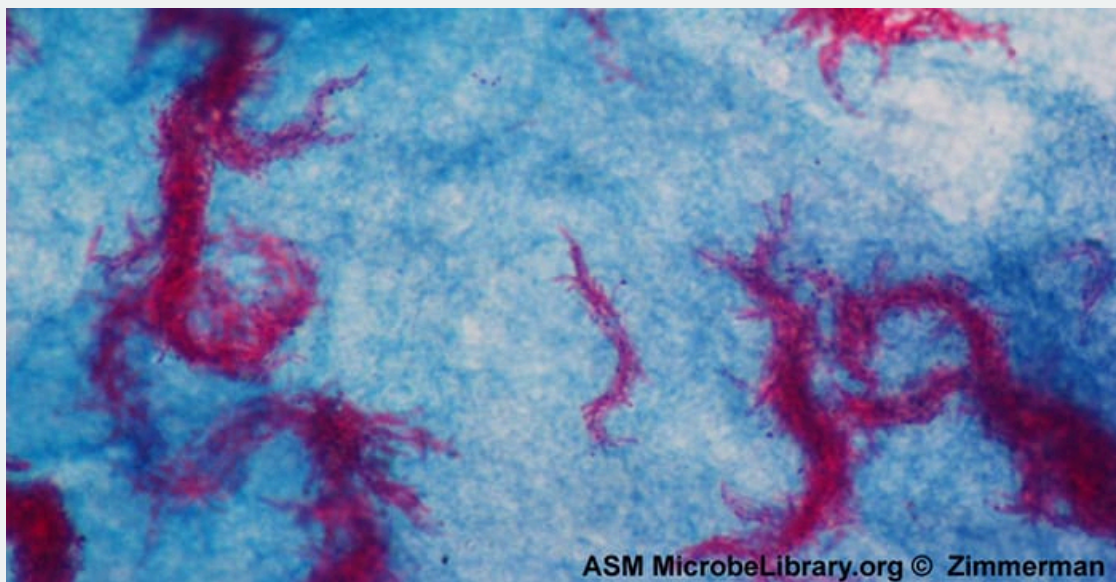


Figure 2.8.6: Ziehl-Neelsen staining has rendered these *Mycobacterium tuberculosis* cells red and the surrounding growth indicator medium blue. (credit: modification of work by American Society for Microbiology)

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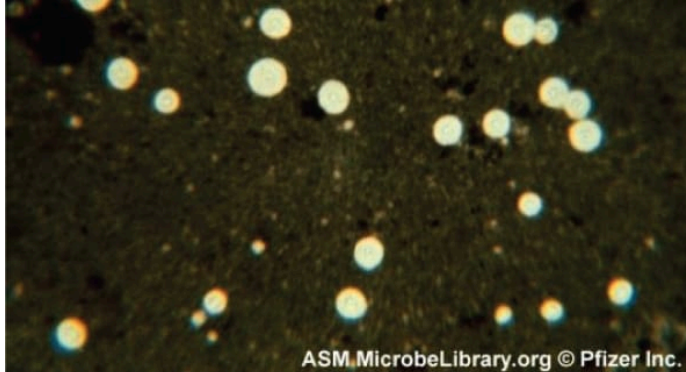


Capsule Staining

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 2.8.7). 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 2.8.7: (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)

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 2.8.8).

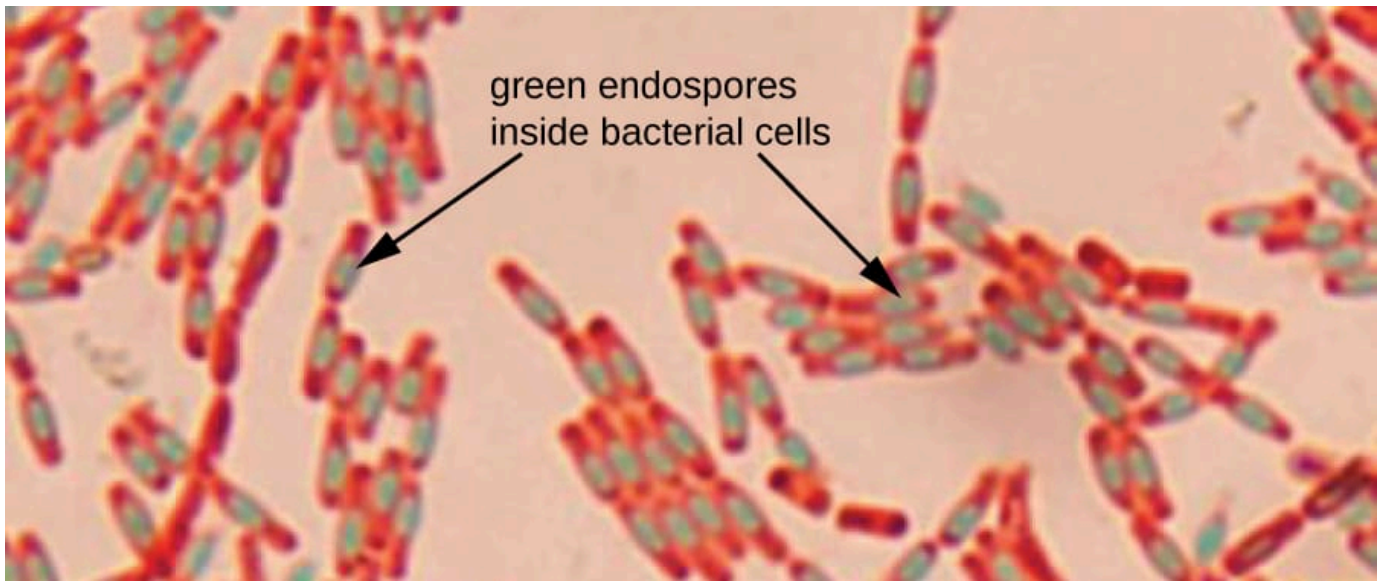


Figure 2.8.8: A stained preparation of *Bacillus subtilis* showing endospores as green and the vegetative cells as pink. (credit: modification of work by American Society for Microbiology)

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.”

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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 2.8.9).

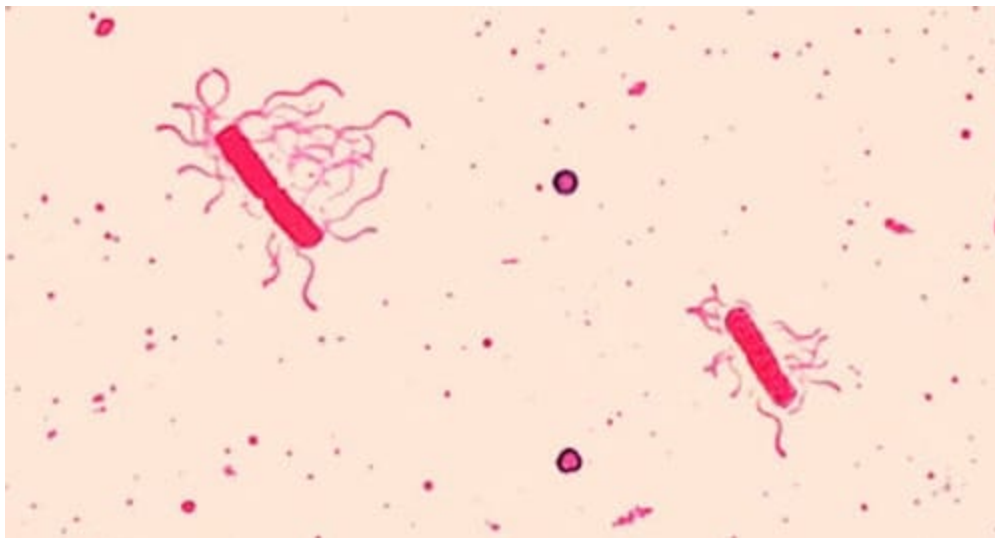


Figure 2.8.9: A flagella stain of *Bacillus cereus*, a common cause of foodborne illness, reveals that the cells have numerous flagella, used for locomotion. (credit: modification of work by Centers for Disease Control and Prevention)

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.

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SIMPLE STAINS				
Stain Type	Specific Dyes	Purpose	Outcome	Sample Images
<i>Basic stains</i>	Methylene blue, crystal violet, malachite green, basic fuchsin, carbolfuchsin, safranin	Stain negatively charged molecules and structures, such as nucleic acids and proteins	Positive stain	
<i>Acidic stains</i>	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.	
<i>Negative stains</i>	India ink, nigrosin	Stains background, not specimen	Dark background with light specimen	

Figure 2.8.10: (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)

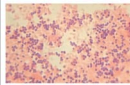
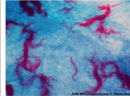
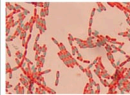
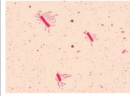
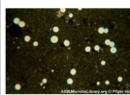
DIFFERENTIAL STAINS				
Stain Type	Specific Dyes	Purpose	Outcome	Sample Images
<i>Gram stain</i>	Uses crystal violet, Gram's iodine, ethanol (decolorizer), and safranin	Used to distinguish cells by cell-wall type (gram-positive, gram-negative)	Gram-positive cells stain purple/violet. Gram-negative cells stain pink.	
<i>Acid-fast stain</i>	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 <i>M. tuberculosis</i> , from non-acid-fast cells	Acid-fast bacteria are red; non-acid-fast cells are blue.	
<i>Endospore stain</i>	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.	
<i>Flagella stain</i>	Flagella are coated with a tannic acid or potassium alum mordant, then stained using either pararosaniline or basic fuchsin.	Used to view and study flagella in bacteria that have them.	Flagella are visible if present.	
<i>Capsule stain</i>	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 2.8.11: (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)

Key Concepts and Summary

- A variety of staining techniques can be used with light microscopy, including **Gram staining**, **acid-fast staining**, **capsule staining**, **endospore staining**, and **flagella staining**.

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2.E: How We See the Invisible World (Exercises)

2.1: The Properties of Light

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.

Multiple Choice

Which of the following has the highest energy?

- A. light with a long wavelength
- B. light with an intermediate wavelength
- C. light with a short wavelength
- D. It is impossible to tell from the information given.

Answer

C

You place a specimen under the microscope and notice that parts of the specimen begin to emit light immediately. These materials can be described as _____.

- A. fluorescent
- B. phosphorescent
- C. transparent
- D. opaque

Answer

A

Fill in the Blank

When you see light bend as it moves from air into water, you are observing _____.

Answer

refraction

Short Answer

Explain how a prism separates white light into different colors.

Critical Thinking

In [Figure 2.1.6](#), which of the following has the lowest energy?

- A. visible light
- B. X-rays
- C. ultraviolet rays

D. infrared rays

2.2: Peering into the Invisible World

Italian scholar Girolamo Fracastoro is regarded as the first person to formally postulate that disease was spread by tiny invisible seminaria. He proposed that these seeds could attach themselves to certain objects that supported their transfer from person to person. However, since the technology for seeing such tiny objects did not yet exist, the existence of the seminaria remained hypothetical for a little over a century—an invisible world waiting to be revealed.

Short Answer

Why is Antonie van Leeuwenhoek's work much better known than that of Zaccharias Janssen?

Why did the cork cells observed by Robert Hooke appear to be empty, as opposed to being full of other structures?

Multiple Choice

Who was the first to describe "cells" in dead cork tissue?

- A. Hans Janssen
- B. Zaccharias Janssen
- C. Antonie van Leeuwenhoek
- D. Robert Hooke

Answer

D

Who is the probable inventor of the compound microscope?

- A. Girolamo Fracastoro
- B. Zaccharias Janssen
- C. Antonie van Leeuwenhoek
- D. Robert Hooke

Answer

B

Fill in the Blank

A microscope that uses multiple lenses is called a _____ microscope.

Answer

compound

2.3: Instruments of Microscopy

The 20th century saw the development of microscopes that leveraged nonvisible light, such as fluorescence microscopy, which uses an ultraviolet light source, and electron microscopy, which uses short-wavelength electron beams. These advances led to major improvements in magnification, resolution, and contrast. In this section, we survey the broad range of modern microscopic technology and common applications for each type of microscope.

Multiple Choice

Which would be the best choice for viewing internal structures of a living protist such as a *Paramecium*?

- A. a brightfield microscope with a stain
- B. a brightfield microscope without a stain
- C. a darkfield microscope
- D. a transmission electron microscope

Answer

C

Which type of microscope is especially useful for viewing thick structures such as biofilms?

- A. a transmission electron microscope
- B. a scanning electron microscopes
- C. a phase-contrast microscope
- D. a confocal scanning laser microscope
- E. an atomic force microscope

Answer

D

Which type of microscope would be the best choice for viewing very small surface structures of a cell?

- A. a transmission electron microscope
- B. a scanning electron microscope
- C. a brightfield microscope
- D. a darkfield microscope
- E. a phase-contrast microscope

Answer

B

What type of microscope uses an annular stop?

- A. a transmission electron microscope
- B. a scanning electron microscope
- C. a brightfield microscope
- D. a darkfield microscope
- E. a phase-contrast microscope

Answer

E

What type of microscope uses a cone of light so that light only hits the specimen indirectly, producing a darker image on a brighter background?

- A. a transmission electron microscope
- B. a scanning electron microscope
- C. a brightfield microscope
- D. a darkfield microscope
- E. a phase-contrast microscope

Answer

D

Fill in the Blank

Chromophores that absorb and then emit light are called _____.

Answer

fluorochromes

In a(n) _____ microscope, a probe located just above the specimen moves up and down in response to forces between the atoms and the tip of the probe.

Answer

atomic force microscope

What is the total magnification of a specimen that is being viewed with a standard ocular lens and a 40 $\times$ objective lens?

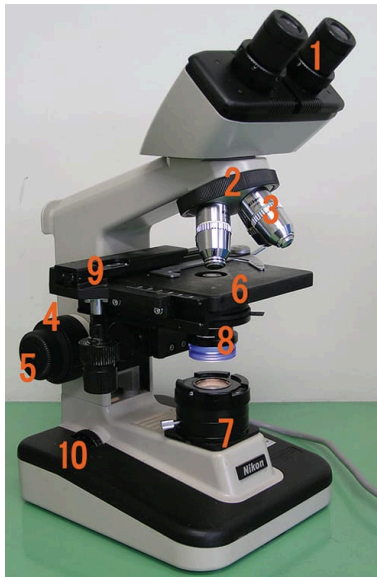
Answer

400 $\times$

Short Answer

What is the function of the condenser in a brightfield microscope?

Label each component of the brightfield microscope.



Critical Thinking

When focusing a light microscope, why is it best to adjust the focus using the coarse focusing knob before using the fine focusing knob?

You need to identify structures within a cell using a microscope. However, the image appears very blurry even though you have a high magnification. What are some things that you could try to improve the resolution of the image? Describe the most basic

factors that affect resolution when you first put the slide onto the stage; then consider more specific factors that could affect resolution for 40× and 100× lenses.

2.4: Staining Microscopic Specimens

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 focus on the most clinically relevant techniques developed to identify specific microbes, cellular structures, DNA sequences, or indicators of infection in tissue samples, under the microscope.

Multiple Choice

What mordant is used in Gram staining?

- A. crystal violet
- B. safranin
- C. acid-alcohol
- D. iodine

Answer

D

What is one difference between specimen preparation for a transmission electron microscope (TEM) and preparation for a scanning electron microscope (SEM)?

- A. Only the TEM specimen requires sputter coating.
- B. Only the SEM specimen requires sputter-coating.
- C. Only the TEM specimen must be dehydrated.
- D. Only the SEM specimen must be dehydrated.

Answer

B

Fill in the Blank

Ziehl-Neelsen staining, a type of _____ staining, is diagnostic for *Mycobacterium tuberculosis*.

Answer

acid-fast

The _____ is used to differentiate bacterial cells based on the components of their cell walls.

Answer

Gram stain

Short Answer

How could you identify whether a particular bacterial sample contained specimens with mycolic acid-rich cell walls?

Critical Thinking

You use the Gram staining procedure to stain an L-form bacterium (a bacterium that lacks a cell wall). What color will the

bacterium be after the staining procedure is finished?

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3: MACROMOLECULES

The earth is estimated to be 4.6 billion years old, but for the first 2 billion years, the atmosphere lacked oxygen, without which the earth could not support life as we know it. One hypothesis about how life emerged on earth involves the concept of a “primordial soup.” This idea proposes that life began in a body of water when metals and gases from the atmosphere combined with a source of energy, such as lightning or ultraviolet light, to form the carbon compounds that are the chemical building blocks of life. In 1952, Stanley Miller (1930–2007), a graduate student at the University of Chicago, and his professor Harold Urey (1893–1981), set out to confirm this hypothesis in a now-famous experiment. Miller and Urey combined what they believed to be the major components of the earth’s early atmosphere—water (H_2O), methane (CH_4), hydrogen (H_2), and ammonia (NH_3)—and sealed them in a sterile flask. Next, they heated the flask to produce water vapor and passed electric sparks through the mixture to mimic lightning in the atmosphere (Figure 3.1). When they analyzed the contents of the flask a week later, they found amino acids, the structural units of proteins—molecules essential to the function of all organisms.

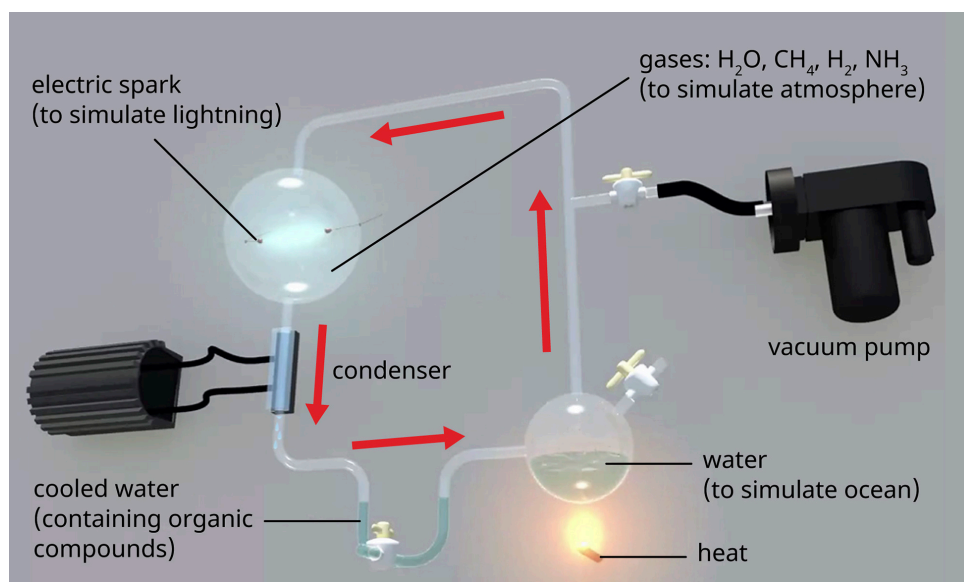


Figure 3.1: Scientist Stanley Miller and Harold Urey demonstrated that organic compounds may have originated naturally from inorganic matter. The Miller-Urey experiment illustrated here simulated the effects of lightning on chemical compounds found in the earth’s early atmosphere. The resulting reactions yielded amino acids, the chemical building blocks of proteins. (credit: modification of work by Courtney Harrington)

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3.1: Organic Molecules

3.2: Isomerism

3.3: Functional Groups and Dehydration Reaction

3.4: Carbohydrates - Monosaccharides and Disaccharides

3.5: Carbohydrates - Polysaccharides

3.6: Lipids

3.7: Proteins

3.8: Using Biochemistry to Identify Microorganisms

3.E: Microbial Biochemistry (Exercises)

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3.1: Organic Molecules

Learning Objectives

- Identify common elements and structures found in organic molecules
- Distinguish between macronutrients and micronutrients (trace elements)
- Differentiate inorganic from 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, 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 3.1.1). 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.

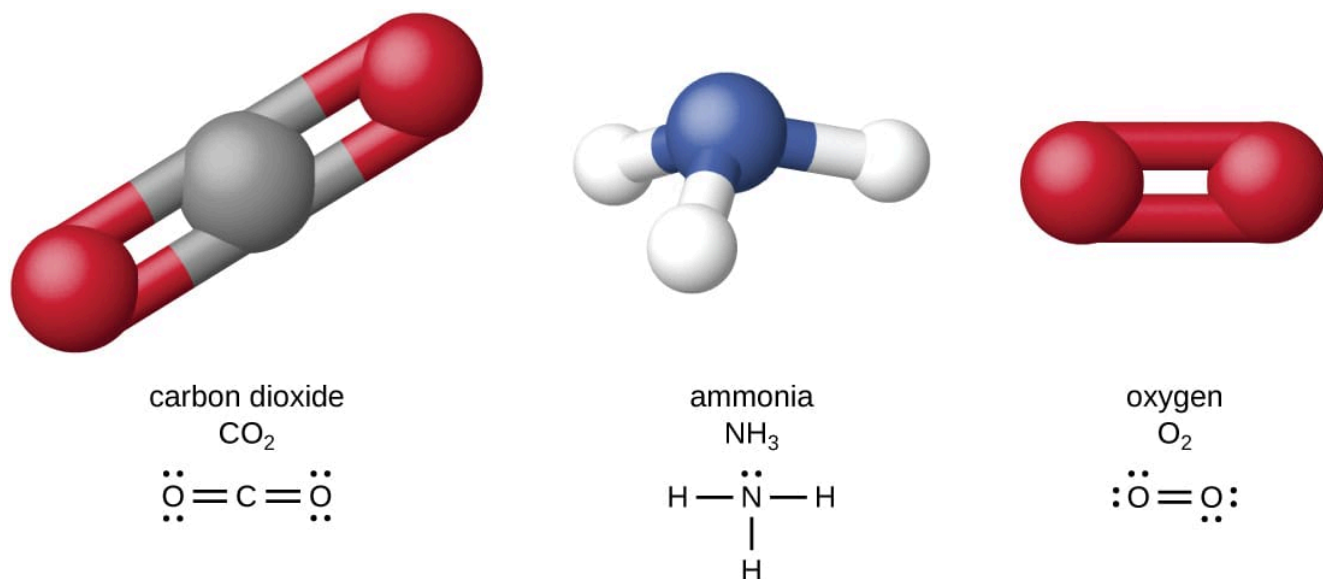


Figure 3.1.1: Some common molecules include carbon dioxide, ammonia, and oxygen, which consist of combinations of oxygen atoms (red spheres), carbon atoms (gray spheres), hydrogen atoms (white spheres), or nitrogen atoms (blue spheres).

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.

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Interactive Element

Case Study Preview: “Hot Tub Trouble”

When 16-year-old Penny shows up with an itchy rash, her doctor chalks it up to sunscreen allergies and prescribes a hydrocortisone cream. But when the rash gets worse - bumpy, blistered, and full of pus - Penny reveals she recently lounged in a hot tub. That changes everything.

In this case, you'll explore how a common water-loving bacterium, *Pseudomonas aeruginosa*, can turn a relaxing soak into an opportunistic infection. Learn how it's identified in the lab, why it resists many antibiotics, and how it hides in biofilms - making treatment tricky and prevention critical.

Think it's just a rash? Dive deeper. There's more brewing beneath the surface.

[Chapter 7 Case Study - Hot Tub Trouble](#)

Key Concepts and Summary

- The most abundant elements in cells are hydrogen, carbon, oxygen, nitrogen, phosphorus, and sulfur.

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3.2: Isomerism

Learning Objectives

- Explain the concept of isomerism
- Distinguish between structural isomers and enantiomers

Organic Molecules

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 3.2.1).

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.

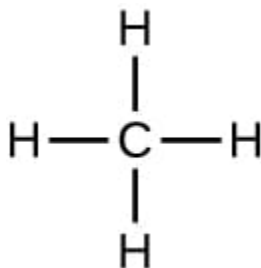


Figure 3.2.1: A carbon atom can bond with up to four other atoms. The simplest organic molecule is methane (CH₄), depicted here.

Isomerism

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, C₆H₁₂O₆, but we can see from Figure 3.2.2 that the atoms are bonded together differently.

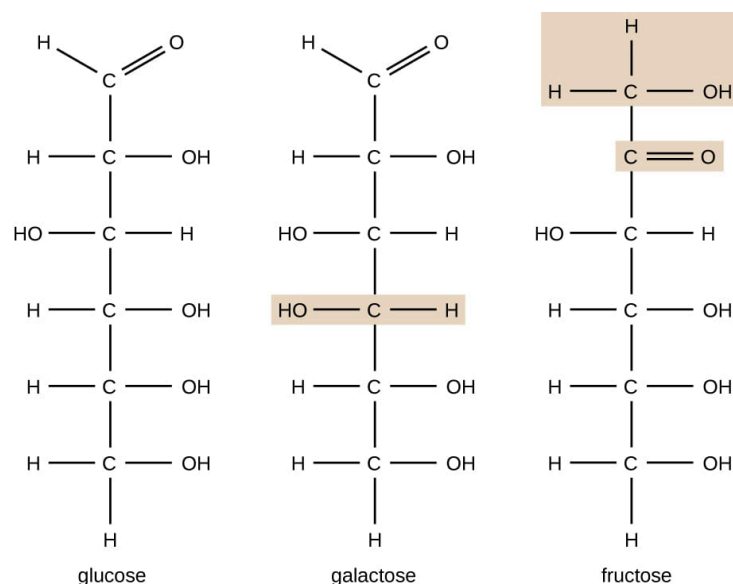


Figure 3.2.2: Glucose, galactose, and fructose have the same chemical formula ($C_6H_{12}O_6$), but these structural isomers differ in their physical and chemical properties.

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 3.2.3).

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 (*dextromethorphan*, a cough suppressant), whereas the other acts as an analgesic (*levomethorphan*, a drug similar in effect to codeine).

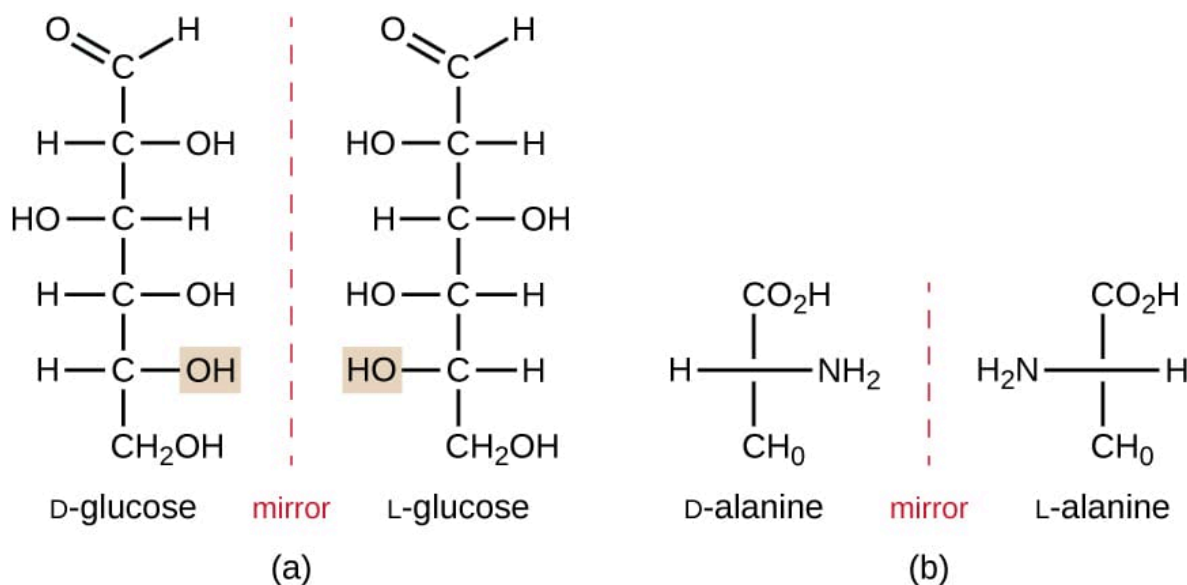


Figure 3.2.3: Enantiomers are stereoisomers that exhibit chirality. Their chemical structures are nonsuperimposable mirror images of each other. (a) D-glucose and L-glucose are monosaccharides that are enantiomers. (b) The enantiomers D-alanine and L-alanine are enantiomers found in bacterial cell walls 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.

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Key Concepts and Summary

- 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.

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3.3: Functional Groups and Dehydration Reaction

Learning Objectives

- Identifying examples of functional groups
- Describe the roles of functional groups in synthesizing polymers

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 3.3.1. 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.

Name	Functional Group	Compounds
Aldehyde	$\text{R}-\text{C}(=\text{O})-\text{H}$	Carbohydrates
Amide	$\text{R}-\text{C}(=\text{O})-\text{N}-\text{R}'$	Proteins
Amino	$\text{R}-\text{NH}_2$	Amino acids, proteins
Carbonyl	$\text{R}-\text{C}(=\text{O})-\text{R}'$	Ketones, aldehydes, carboxylic acids, amides
Carboxylic acid	$\text{R}-\text{C}(=\text{O})-\text{OH}$	Amino acids, proteins, fatty acids
Ester	$\text{R}-\text{C}(=\text{O})-\text{O}-\text{R}'$	Lipids, nucleic acids
Ether	$\text{R}-\text{O}-\text{R}'$	Disaccharides, polysaccharides, lipids
Hydroxyl	$\text{R}-\text{OH}$	Alcohols, monosaccharides, amino acids, nucleic acids
Ketone	$\text{R}-\text{C}(=\text{O})-\text{R}'$	Carbohydrates
Methyl	$\text{R}-\text{CH}_3$	Methylated compounds such as methyl alcohols and methyl esters
Phosphate	$\text{R}-\text{PO}_3\text{H}_2$	Nucleic acids, phospholipids, ATP
Sulphydryl	$\text{R}-\text{SH}$	Amino acids, proteins

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

Figure 3.3.1: Functional groups.

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Macromolecule Synthesis: Dehydration Reaction

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:

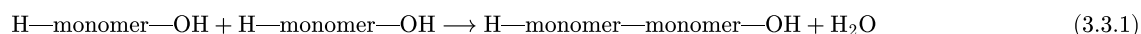


Figure 3.3.2 shows dehydration synthesis of glucose binding together to form maltose and a water molecule. Table 3.3.1 summarizes macromolecules and some of their functions.

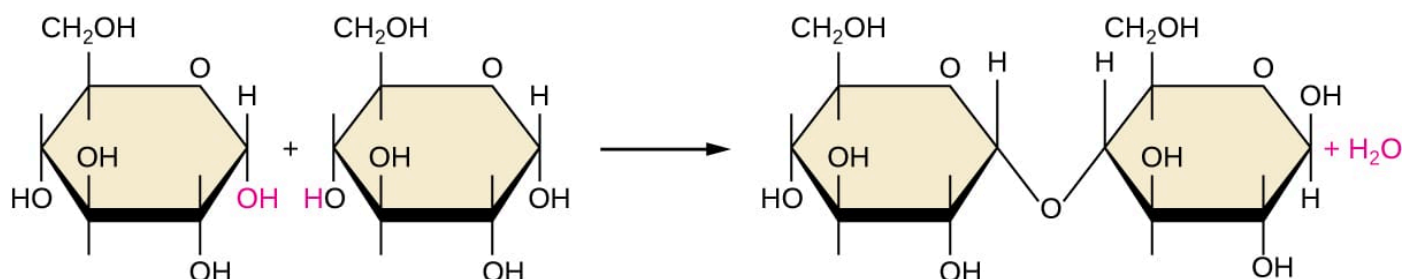


Figure 3.3.2: In this dehydration synthesis reaction, two molecules of glucose are linked together to form maltose. In the process, a water molecule is formed.

Table 3.3.1: 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

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Key Concepts and Summary

- **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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3.4: Carbohydrates - Monosaccharides and Disaccharides

Learning Objectives

- Give examples of monosaccharides and polysaccharides
- Describe the function of the simple sugars within a cell

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 $(\text{CH}_2\text{O})_n$, 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.” Although all carbohydrates contain carbon, hydrogen, and oxygen, there are some that also contain nitrogen, phosphorus, and/or sulfur. Carbohydrates have myriad different functions. They are abundant in terrestrial ecosystems, many forms of which we use as food sources. These molecules are also vital parts of macromolecular structures that store and transmit genetic information (i.e., DNA and RNA). They are the basis of biological polymers that impart strength to various structural components of organisms (e.g., cellulose and chitin), and they are the primary source of energy storage in the form of starch and glycogen.

Monosaccharides: The Sweet Ones

In biochemistry, carbohydrates are often called **saccharides**, from the Greek *sakcharon*, meaning sugar, although not all the saccharides are sweet. The simplest carbohydrates are called **monosaccharides**, or simple sugars. They are the building blocks (monomers) for the synthesis of polymers or complex carbohydrates, as will be discussed further in this section. Monosaccharides are classified based on the number of carbons in the molecule. General categories are identified using a prefix that indicates the number of carbons and the suffix *-ose*, which indicates a saccharide; for example, triose (three carbons), tetrose (four carbons), pentose (five carbons), and hexose (six carbons) (Figure 3.4.1). The hexose D-glucose is the most abundant monosaccharide in nature. Other very common and abundant hexose monosaccharides are galactose, used to make the disaccharide milk sugar lactose, and the fruit sugar fructose.

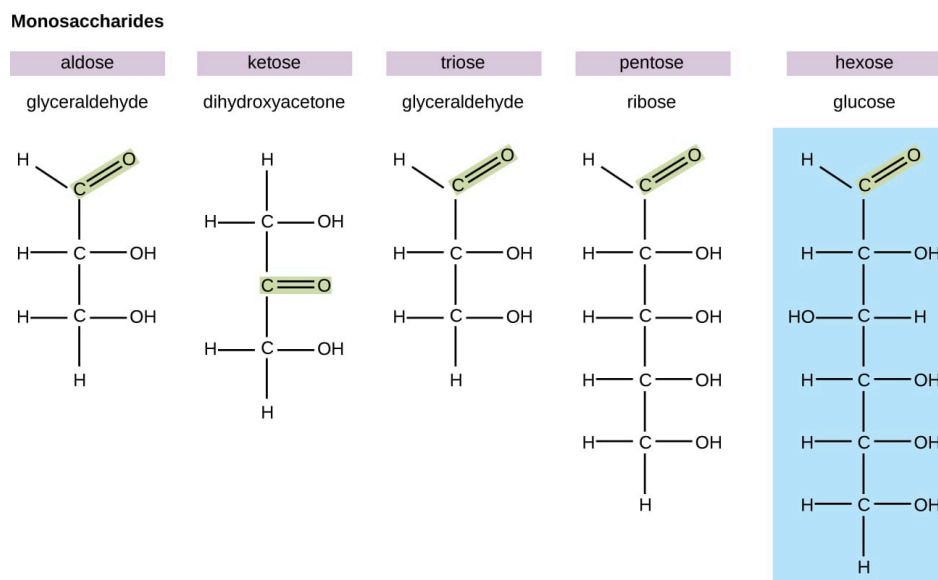


Figure 3.4.1: Monosaccharides are classified based on the position of the carbonyl group and the number of carbons in the backbone.

Monosaccharides of four or more carbon atoms are typically more stable when they adopt cyclic, or ring, structures. These ring structures result from a chemical reaction between functional groups on opposite ends of the sugar's flexible carbon chain, namely the carbonyl group and a relatively distant hydroxyl group. Glucose, for example, forms a six-membered ring (Figure 3.4.2).

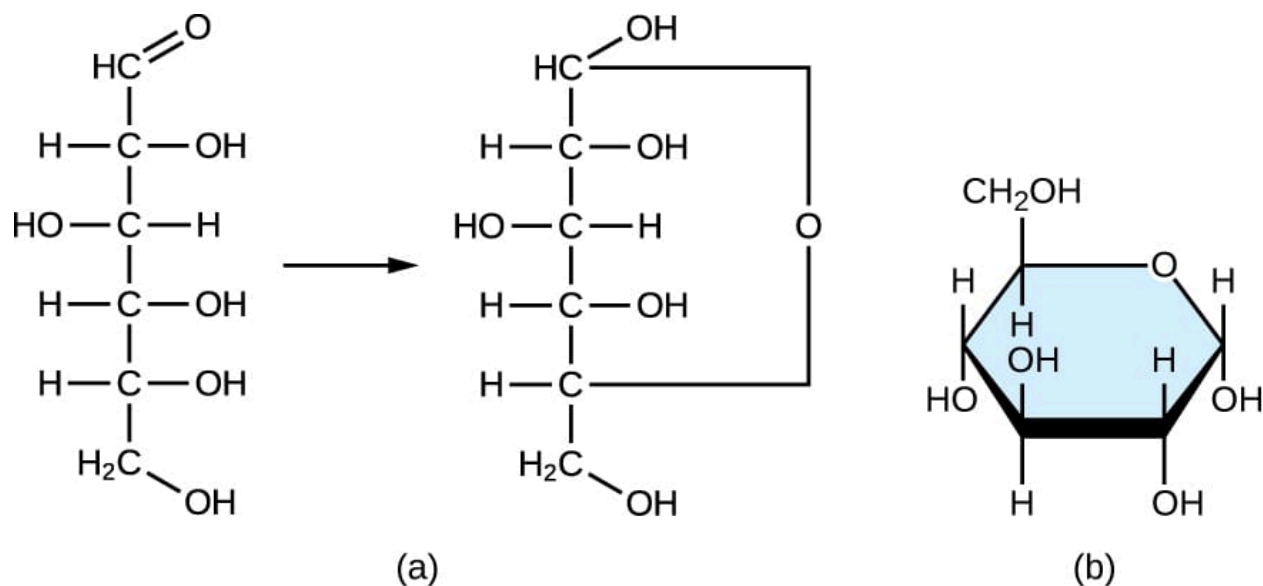
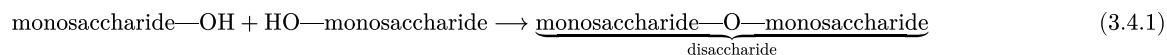


Figure 3.4.2: (a) A linear monosaccharide (glucose in this case) forms a cyclic structure. (b) This illustration shows a more realistic depiction of the cyclic monosaccharide structure. Note in these cyclic structural diagrams, the carbon atoms composing the ring are not explicitly shown.

Disaccharides

Two monosaccharide molecules may chemically bond to form a **disaccharide**. The name given to the covalent bond between the two monosaccharides is a glycosidic bond. **Glycosidic bonds** form between hydroxyl groups of the two saccharide molecules, an example of the dehydration synthesis described in the previous section of this chapter:



Common disaccharides are the grain sugar maltose, made of two glucose molecules; the milk sugar lactose, made of a galactose and a glucose molecule; and the table sugar sucrose, made of a glucose and a fructose molecule (Figure 3.4.3).

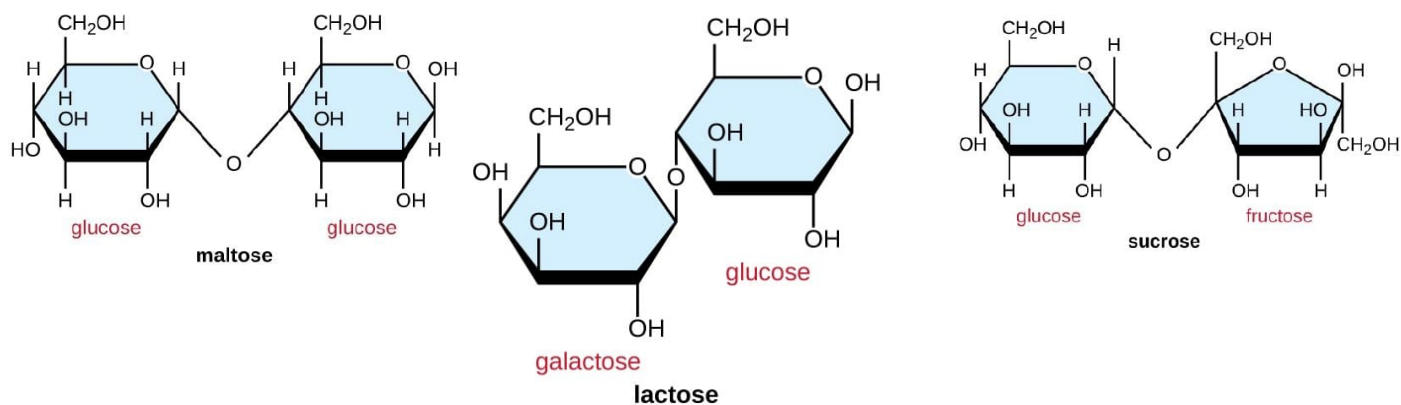


Figure 3.4.3: Common disaccharides include maltose, lactose, and sucrose.

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Key Concepts and Summary

- **Carbohydrates**, the most abundant biomolecules on earth, are widely used by organisms for structural and energy-storage purposes.
- Carbohydrates include individual sugar molecules (**monosaccharides**) as well as two or more molecules chemically linked by **glycosidic bonds**. **Monosaccharides** are classified based on the number of carbons in the molecule as trioses (3 C), tetroses (4 C), pentoses (5 C), and hexoses (6 C). They are the building blocks for the synthesis of polymers or complex carbohydrates.
- **Disaccharides** such as sucrose, lactose, and maltose are molecules composed of two monosaccharides linked together by a glycosidic bond.

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3.5: Carbohydrates - Polysaccharides

Learning Objectives

- Describe the structures of starch, glycogen and cellulose
- Describe the functions of these polysaccharides

Polysaccharides

Polysaccharides, also called glycans, are large polymers composed of hundreds of monosaccharide monomers. Unlike mono- and disaccharides, polysaccharides are not sweet and, in general, they are not soluble in water. Like disaccharides, the monomeric units of polysaccharides are linked together by glycosidic bonds.

Polysaccharides are very diverse in their structure. Three of the most biologically important polysaccharides — **starch**, **glycogen**, and **cellulose** — are all composed of repetitive glucose units, although they differ in their structure (Figure 3.5.1). Cellulose consists of a linear chain of glucose molecules and is a common structural component of cell walls in plants and other organisms. Glycogen and starch are branched polymers; glycogen is the primary energy-storage molecule in animals and bacteria, whereas plants primarily store energy in starch. The orientation of the glycosidic linkages in these three polymers is different as well and, as a consequence, linear and branched macromolecules have different properties.

Modified glucose molecules can be fundamental components of other structural polysaccharides. Examples of these types of structural polysaccharides are N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) found in bacterial cell wall peptidoglycan. Polymers of NAG form chitin, which is found in fungal cell walls and in the exoskeleton of insects.

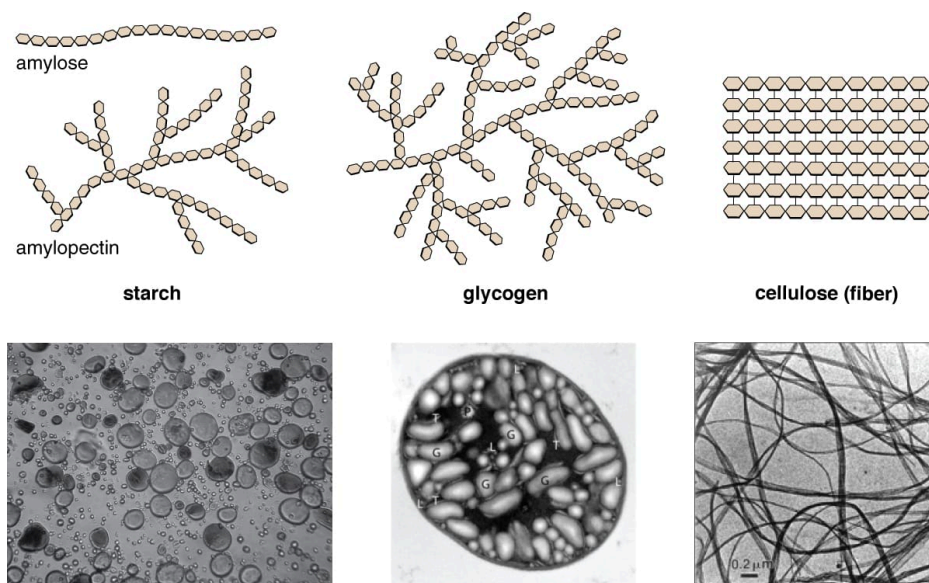


Figure 3.5.1: Starch, glycogen, and cellulose are three of the most important polysaccharides. In the top row, hexagons represent individual glucose molecules. Micrographs (bottom row) show wheat starch granules stained with iodine (left), glycogen granules (G) inside the cell of a cyanobacterium (middle), and bacterial cellulose fibers (right). (credit "iodine granules": modification of work by Kiselov Yuri; credit "glycogen granules": modification of work by Stöckel J, Elvitigala TR, Liberton M, Pakrasi HB; credit "cellulose": modification of work by American Society for Microbiology)

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Key Concepts and Summary

- **Polysaccharides**, or **glycans**, are polymers composed of hundreds of monosaccharide monomers linked together by glycosidic bonds. The energy-storage polymers **starch** and **glycogen** are examples of polysaccharides and are all composed of branched chains of glucose molecules.
- The polysaccharide **cellulose** is a common structural component of the cell walls of organisms. Other structural polysaccharides, such as N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM), incorporate modified glucose molecules and are used in the construction of peptidoglycan or chitin.

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3.6: Lipids

Learning Objectives

- Describe the chemical composition of lipids
- Describe the unique characteristics and diverse structures of lipids
- Compare and contrast triacylglycerides (triglycerides) and phospholipids
- Describe how phospholipids are used to construct biological membranes

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.

Fatty Acids and Triacylglycerides

The fatty acids are lipids that contain long-chain hydrocarbons terminated with a carboxylic acid functional group. Because of the long hydrocarbon chain, fatty acids are hydrophobic ("water fearing") or nonpolar. Fatty acids with hydrocarbon chains that contain only single bonds are called saturated fatty acids because they have the greatest number of hydrogen atoms possible and are, therefore, "saturated" with hydrogen. Fatty acids with hydrocarbon chains containing at least one double bond are called unsaturated fatty acids because they have fewer hydrogen atoms. Saturated fatty acids have a straight, flexible carbon backbone, whereas unsaturated fatty acids have "kinks" in their carbon skeleton because each double bond causes a rigid bend of the carbon skeleton. These differences in saturated versus unsaturated fatty acid structure result in different properties for the corresponding lipids in which the fatty acids are incorporated. For example, lipids containing saturated fatty acids are solids at room temperature, whereas lipids containing unsaturated fatty acids are liquids.

A triacylglycerol, or triglyceride, is formed when three fatty acids are chemically linked to a glycerol molecule (Figure 3.6.1). Triglycerides are the primary components of adipose tissue (body fat), and are major constituents of sebum (skin oils). They play an important metabolic role, serving as efficient energy-storage molecules that can provide more than double the caloric content of both carbohydrates and proteins.

Three fatty acid chains are bound to glycerol by dehydration synthesis.

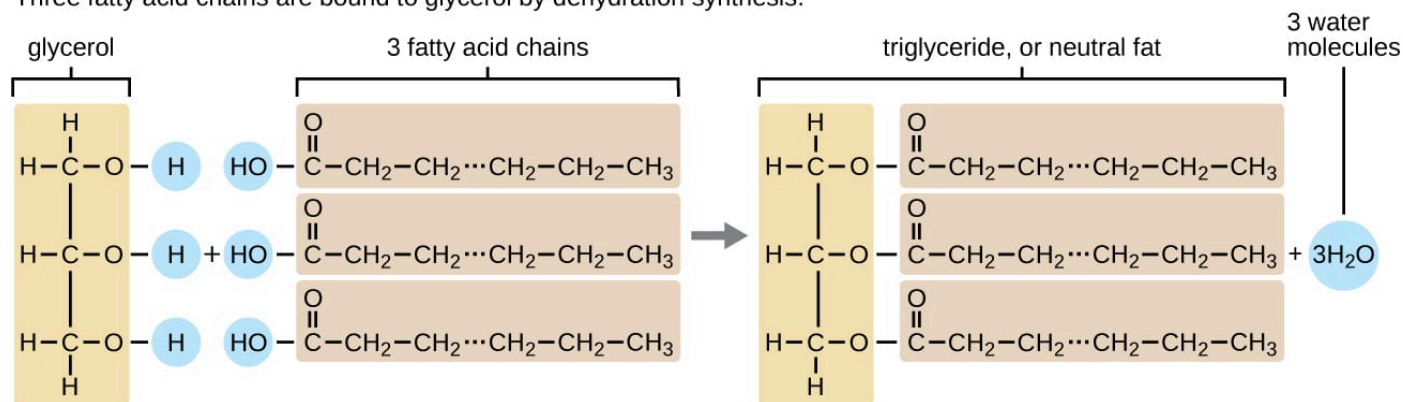


Figure 3.6.1: Triglycerides are composed of a glycerol molecule attached to three fatty acids by a dehydration synthesis reaction.

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Phospholipids and Biological Membranes

Triglycerides are classified as simple lipids because they are formed from just two types of compounds: glycerol and fatty acids. In contrast, complex lipids contain at least one additional component, for example, a phosphate group (phospholipids) or a carbohydrate moiety (glycolipids). Figure 3.6.2 depicts a typical phospholipid composed of two fatty acids linked to glycerol (a diglyceride). The two fatty acid carbon chains may be both saturated, both unsaturated, or one of each. Instead of another fatty acid molecule (as for triglycerides), the third binding position on the glycerol molecule is occupied by a modified phosphate group.

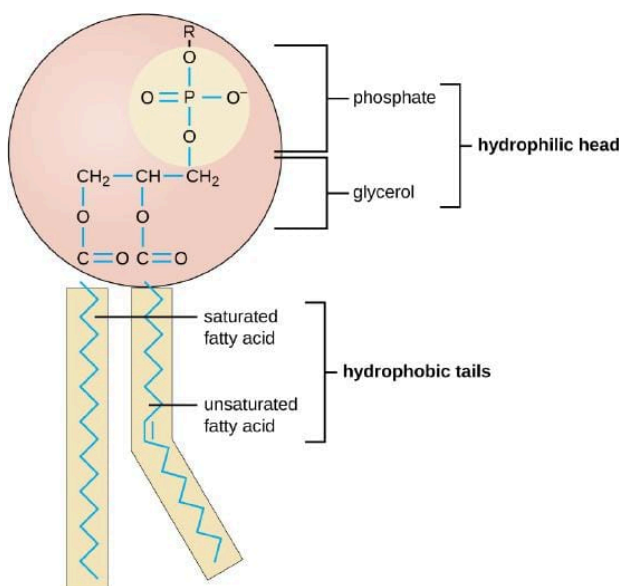


Figure 3.6.2: This illustration shows a phospholipid with two different fatty acids, one saturated and one unsaturated, bonded to the glycerol molecule. The unsaturated fatty acid has a slight kink in its structure due to the double bond.

The molecular structure of lipids results in unique behavior in aqueous environments. Figure 3.6.1 depicts the structure of a triglyceride. Because all three substituents on the glycerol backbone are long hydrocarbon chains, these compounds are nonpolar and not significantly attracted to polar water molecules—they are hydrophobic. Conversely, phospholipids such as the one shown in Figure 3.6.2 have a negatively charged phosphate group. Because the phosphate is charged, it is capable of strong attraction to water molecules and thus is hydrophilic, or “water loving.” The hydrophilic portion of the phospholipid is often referred to as a polar “head,” and the long hydrocarbon chains as nonpolar “tails.” A molecule presenting a hydrophobic portion and a hydrophilic moiety is said to be amphipathic. Notice the “R” designation within the hydrophilic head depicted in Figure 3.6.2, indicating that a polar head group can be more complex than a simple phosphate moiety. Glycolipids are examples in which carbohydrates are bonded to the lipids’ head groups.

The amphipathic nature of phospholipids enables them to form uniquely functional structures in aqueous environments. As mentioned, the polar heads of these molecules are strongly attracted to water molecules, and the nonpolar tails are not. Because of their considerable lengths, these tails are, in fact, strongly attracted to one another. As a result, energetically stable, large-scale assemblies of phospholipid molecules are formed in which the hydrophobic tails congregate within enclosed regions, shielded from contact with water by the polar heads (Figure 3.6.3). The simplest of these structures are micelles, spherical assemblies containing a hydrophobic interior of phospholipid tails and an outer surface of polar head groups. Larger and more

complex structures are created from lipid-bilayer sheets, or unit membranes, which are large, two-dimensional assemblies of phospholipids congregated tail to tail. The cell membranes of nearly all organisms are made from lipid-bilayer sheets, as are the membranes of many intracellular components. These sheets may also form lipid-bilayer spheres that are the structural basis of vesicles and liposomes, subcellular components that play a role in numerous physiological functions.

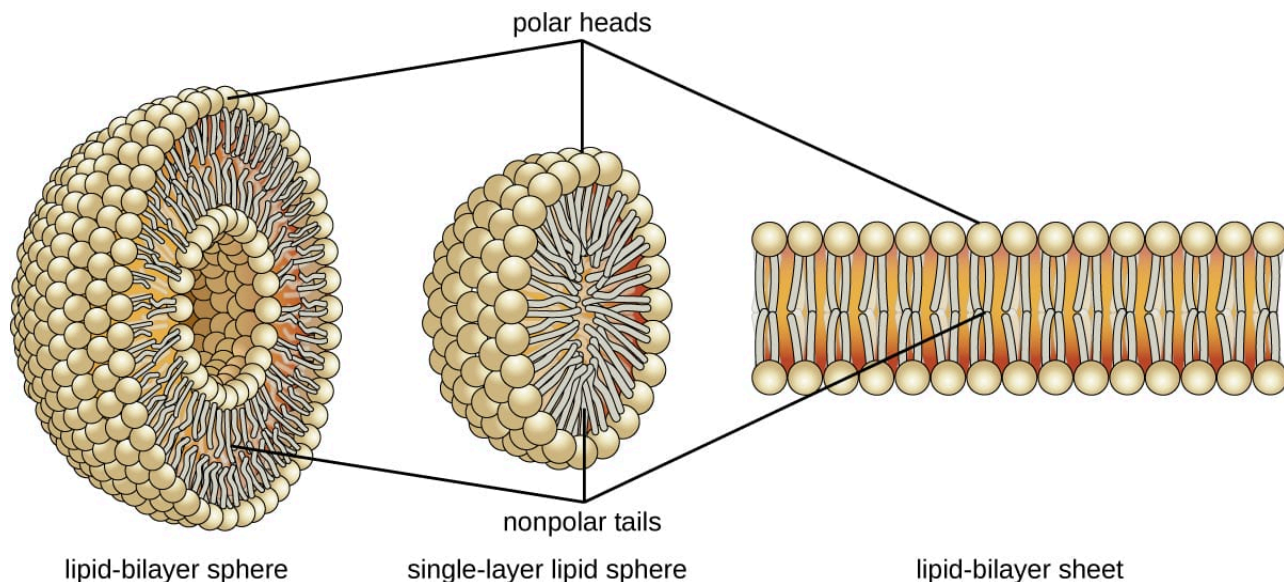


Figure 3.6.3: Phospholipids tend to arrange themselves in aqueous solution forming liposomes, micelles, or lipid bilayer sheets. (credit: modification of work by Mariana Ruiz Villarreal)

Isoprenoids and Sterols

The isoprenoids are branched lipids, also referred to as terpenoids, that are formed by chemical modifications of the isoprene molecule (Figure 3.6.4). These lipids play a wide variety of physiological roles in plants and animals, with many technological uses as pharmaceuticals (capsaicin), pigments (e.g., orange beta carotene, xanthophylls), and fragrances (e.g., menthol, camphor, limonene [lemon fragrance], and pinene [pine fragrance]). Long-chain isoprenoids are also found in hydrophobic oils and waxes. Waxes are typically water resistant and hard at room temperature, but they soften when heated and liquefy if warmed adequately. In humans, the main wax production occurs within the sebaceous glands of hair follicles in the skin, resulting in a secreted material called sebum, which consists mainly of triacylglycerol, wax esters, and the hydrocarbon squalene. There are many bacteria in the microbiota on the skin that feed on these lipids. One of the most prominent bacteria that feed on lipids is *Propionibacterium acnes*, which uses the skin's lipids to generate short-chain fatty acids and is involved in the production of acne.

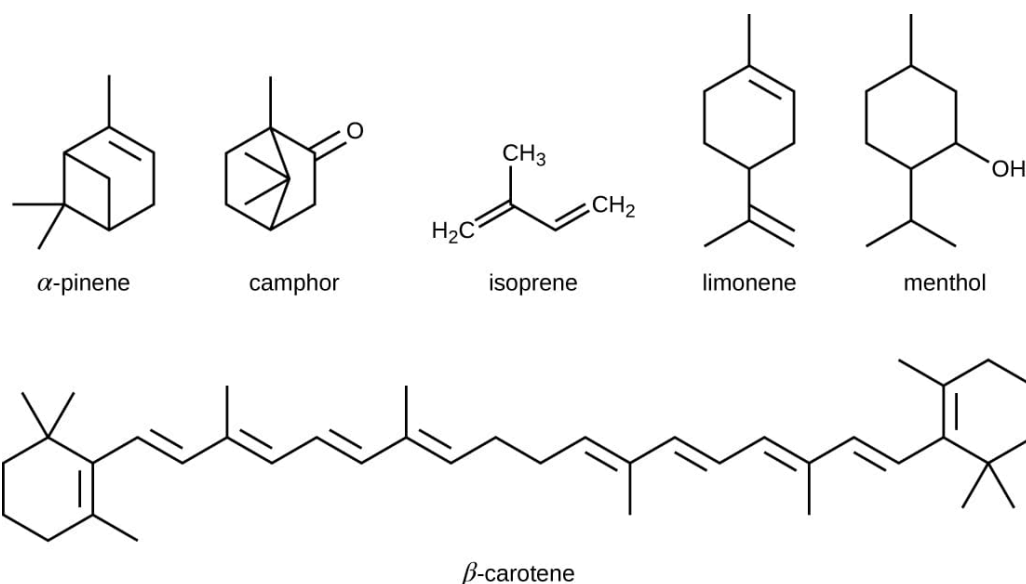


Figure 3.6.4: Five-carbon isoprene molecules are chemically modified in various ways to yield isoprenoids.

Another type of lipids are steroids, complex, ringed structures that are found in cell membranes; some function as hormones. The most common types of steroids are sterols, which are steroids containing an OH group. These are mainly hydrophobic molecules, but also have hydrophilic hydroxyl groups. The most common sterol found in animal tissues is cholesterol. Its structure consists of four rings with a double bond in one of the rings, and a hydroxyl group at the sterol-defining position. The function of cholesterol is to strengthen cell membranes in eukaryotes and in bacteria without cell walls, such as *Mycoplasma*. Prokaryotes generally do not produce cholesterol, although bacteria produce similar compounds called hopanoids, which are also multiringed structures that strengthen bacterial membranes (Figure 3.6.5). Fungi and some protozoa produce a similar compound called ergosterol, which strengthens the cell membranes of these organisms.

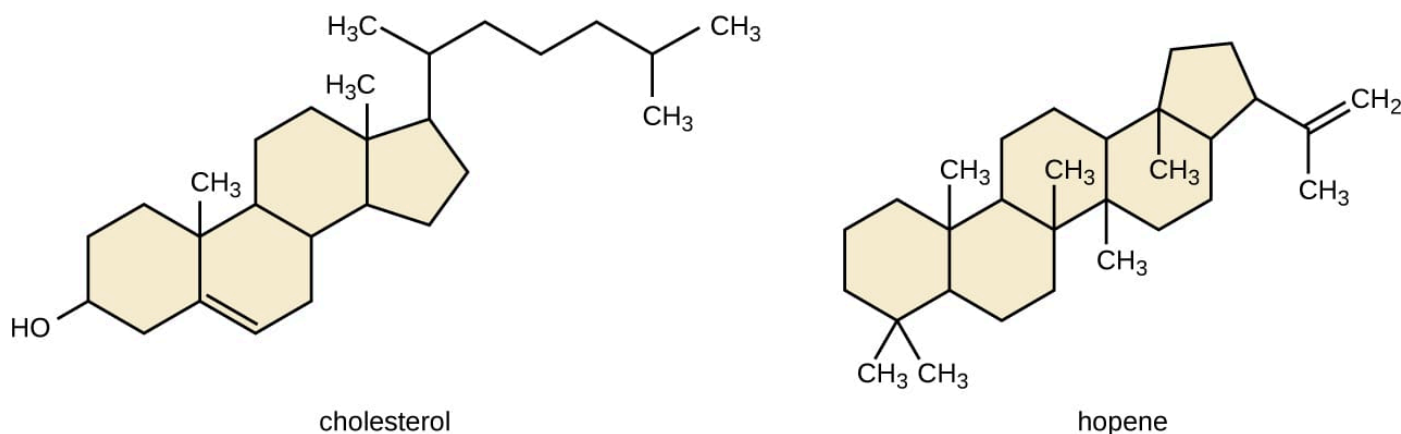


Figure 3.6.5: Cholesterol and hopene (a hopanoid compound) are molecules that reinforce the structure of the cell membranes in eukaryotes and prokaryotes, respectively.

Link to Learning: Liposomes

This [video](#) provides additional information about phospholipids and liposomes.

Key Concepts and Summary

- Lipids** are composed mainly of carbon and hydrogen, but they can also contain oxygen, nitrogen, sulfur, and phosphorous. They provide nutrients for organisms, store carbon and energy, play structural roles in membranes, and function as hormones, pharmaceuticals, fragrances, and pigments.

- Fatty acids are long-chain hydrocarbons with a carboxylic acid functional group. Their relatively long nonpolar hydrocarbon chains make them **hydrophobic**. Fatty acids with no double bonds are **saturated**; those with double bonds are **unsaturated**.
- Fatty acids chemically bond to glycerol to form structurally essential lipids such as **triglycerides** and **phospholipids**. Triglycerides comprise three fatty acids bonded to glycerol, yielding a hydrophobic molecule. Phospholipids contain both hydrophobic hydrocarbon chains and polar head groups, making them **amphipathic** and capable of forming uniquely functional large scale structures.
- Biological membranes are large-scale structures based on phospholipid bilayers that provide hydrophilic exterior and interior surfaces suitable for aqueous environments, separated by an intervening hydrophobic layer. These bilayers are the structural basis for cell membranes in most organisms, as well as subcellular components such as vesicles.
- **Isoprenoids** are lipids derived from isoprene molecules that have many physiological roles and a variety of commercial applications.
- A wax is a long-chain isoprenoid that is typically water resistant; an example of a wax-containing substance is sebum, produced by sebaceous glands in the skin. **Steroids** are lipids with complex, ringed structures that function as structural components of cell membranes and as hormones. **Sterols** are a subclass of steroids containing a hydroxyl group at a specific location on one of the molecule's rings; one example is cholesterol.
- Bacteria produce hopanoids, structurally similar to cholesterol, to strengthen bacterial membranes. Fungi and protozoa produce a strengthening agent called ergosterol.

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3.7: Proteins

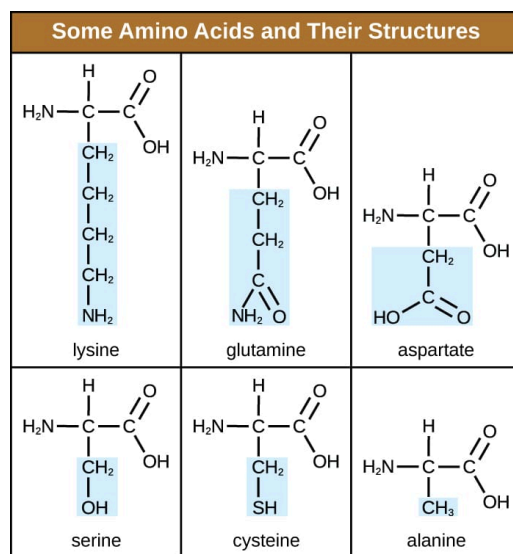
Learning Objectives

- Describe the fundamental structure of an amino acid
- Describe the chemical structures of proteins
- Summarize the unique characteristics of proteins

At the beginning of this chapter, a famous experiment was described in which scientists synthesized amino acids under conditions simulating those present on earth long before the evolution of life as we know it. These compounds 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.

Amino Acids and Peptide Bonds

An amino acid is an organic molecule in which a hydrogen atom, a carboxyl group ($-\text{COOH}$), and an amino group ($-\text{NH}_2$) are all bonded to the same carbon atom, the so-called α carbon. The fourth group bonded to the α carbon varies among the different amino acids and is called a residue or a side chain, represented in structural formulas by the letter R . A residue is a monomer that results when two or more amino acids combine and remove water molecules. The primary structure of a protein, a peptide chain, is made of amino acid residues. The unique characteristics of the functional groups and R groups allow these components of the amino acids to form hydrogen, ionic, and disulfide bonds, along with polar/nonpolar interactions needed to form secondary, tertiary, and quaternary protein structures. These groups are composed primarily of carbon, hydrogen, oxygen, nitrogen, and sulfur, in the form of hydrocarbons, acids, amides, alcohols, and amines. A few examples illustrating these possibilities are provided in Figure 3.7.1.



*Blue shading indicates R group.

Figure 3.7.1: Amino acids

Amino acids may chemically bond together by reaction of the carboxylic acid group of one molecule with the amine group of another. This reaction forms a peptide bond and a water molecule and is another example of dehydration synthesis (Figure 3.7.2). Molecules formed by chemically linking relatively modest numbers of amino acids (approximately 50 or fewer) are called peptides, and prefixes are often used to specify these numbers: dipeptides (two amino acids), tripeptides (three amino acids), and so forth. More generally, the approximate number of amino acids is designated: oligopeptides are formed by joining up to approximately 20 amino acids, whereas polypeptides are synthesized from up to approximately 50 amino acids. When the number of amino

acids linked together becomes very large, or when multiple polypeptides are used as building subunits, the macromolecules that result are called proteins. The continuously variable length (the number of monomers) of these biopolymers, along with the variety of possible *R* groups on each amino acid, allows for a nearly unlimited diversity in the types of proteins that may be formed.

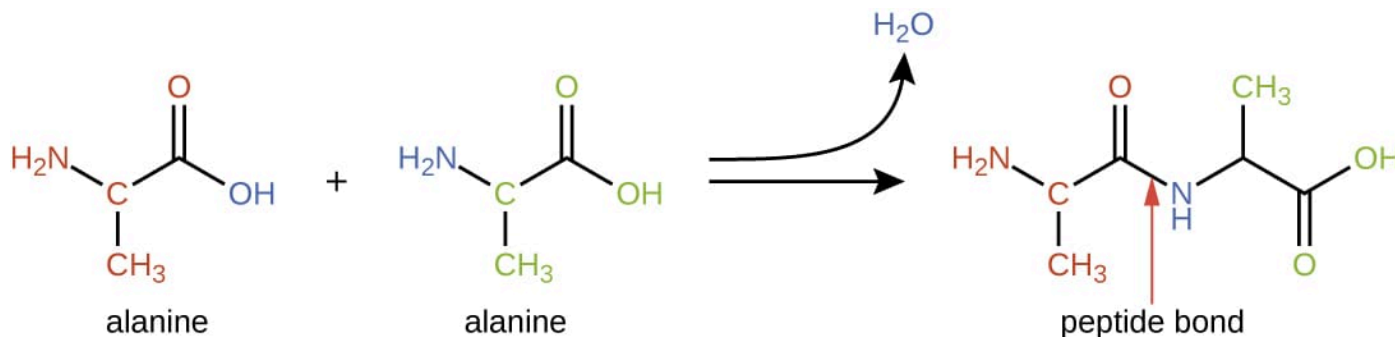


Figure 3.7.2: Peptide bond formation is a dehydration synthesis reaction. The carboxyl group of the first amino acid (alanine) is linked to the amino group of the incoming second amino acid (alanine). In the process, a molecule of water is released.

Query 3.7.1

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Protein Structure

The size (length) and specific amino acid sequence of a protein are major determinants of its shape, and the shape of a protein is critical to its function. For example, in the process of biological nitrogen fixation (see [Biogeochemical Cycles](#)), soil microorganisms collectively known as rhizobia symbiotically interact with roots of legume plants such as soybeans, peanuts, or beans to form a novel structure called a nodule on the plant roots. The plant then produces a carrier protein called leghemoglobin, a protein that carries nitrogen or oxygen. Leghemoglobin binds with a very high affinity to its substrate oxygen at a specific region of the protein where the shape and amino acid sequence are appropriate (the active site). If the shape or chemical environment of the active site is altered, even slightly, the substrate may not be able to bind as strongly, or it may not bind at all. Thus, for the protein to be fully active, it must have the appropriate shape for its function.

Protein structure is categorized in terms of four levels: primary, secondary, tertiary, and quaternary. The primary structure is simply the sequence of amino acids that make up the polypeptide chain. Figure 3.7.3 depicts the primary structure of a protein.

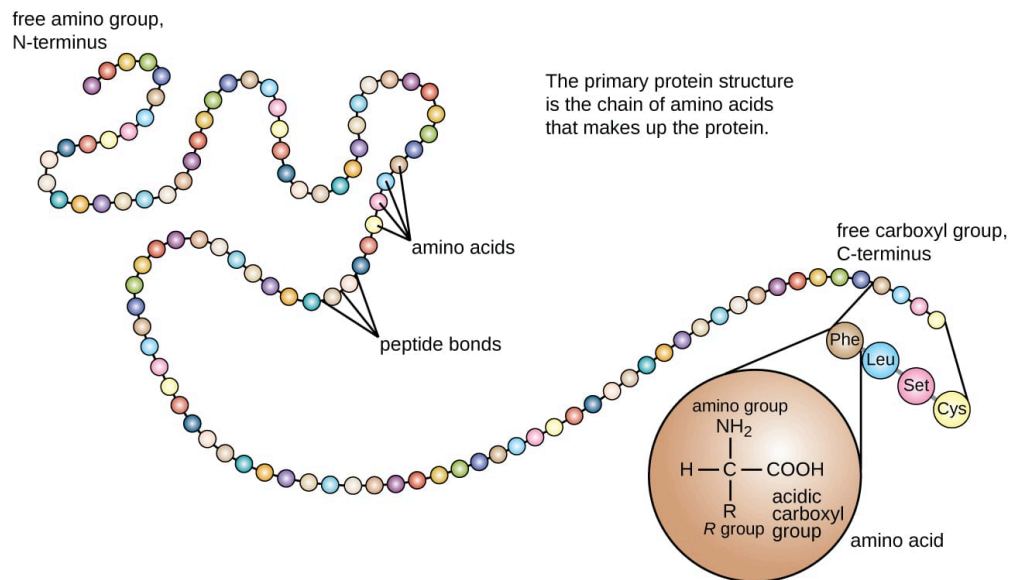


Figure 3.7.3: The primary structure of a protein is the sequence of amino acids. (credit: modification of work by National Human Genome Research Institute)

The chain of amino acids that defines a protein's primary structure is not rigid, but instead is flexible because of the nature of the bonds that hold the amino acids together. When the chain is sufficiently long, hydrogen bonding may occur between amine and carbonyl functional groups within the peptide backbone (excluding the *R* side group), resulting in localized folding of the polypeptide chain into helices and sheets. These shapes constitute a protein's secondary structure. The most common secondary structures are the α -helix and β -pleated sheet. In the α -helix structure, the helix is held by hydrogen bonds between the oxygen atom in a carbonyl group of one amino acid and the hydrogen atom of the amino group that is just four amino acid units farther along the chain. In the β -pleated sheet, the pleats are formed by similar hydrogen bonds between continuous sequences of carbonyl and amino groups that are further separated on the backbone of the polypeptide chain (Figure 3.7.4).

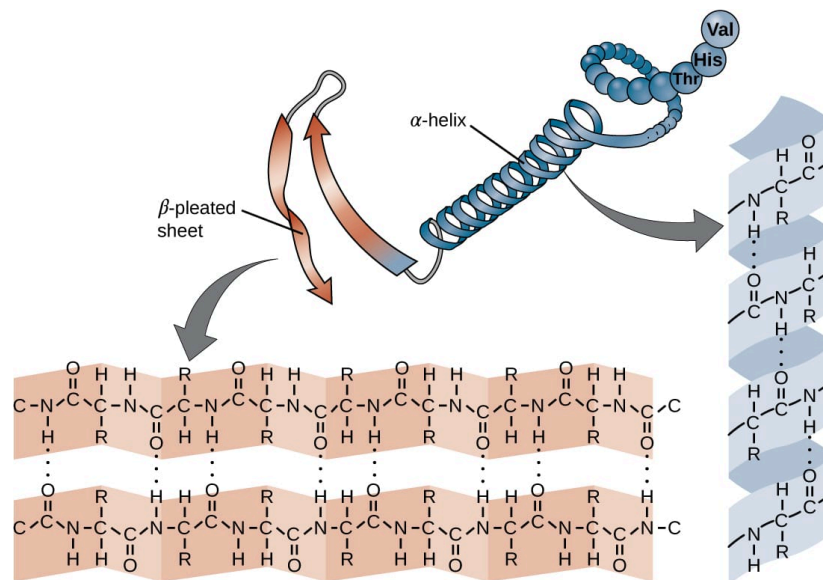


Figure 3.7.4: The secondary structure of a protein may be an α -helix or a β -pleated sheet, or both.

The next level of protein organization is the tertiary structure, which is the large-scale three-dimensional shape of a single polypeptide chain. Tertiary structure is determined by interactions between amino acid residues that are far apart in the chain. A variety of interactions give rise to protein tertiary structure, such as disulfide bridges, which are bonds between the sulfhydryl ($-SH$) functional groups on amino acid side groups; hydrogen bonds; ionic bonds; and hydrophobic interactions between nonpolar side chains. All these interactions, weak and strong, combine to determine the final three-dimensional shape of the protein and its function (Figure 3.7.5).

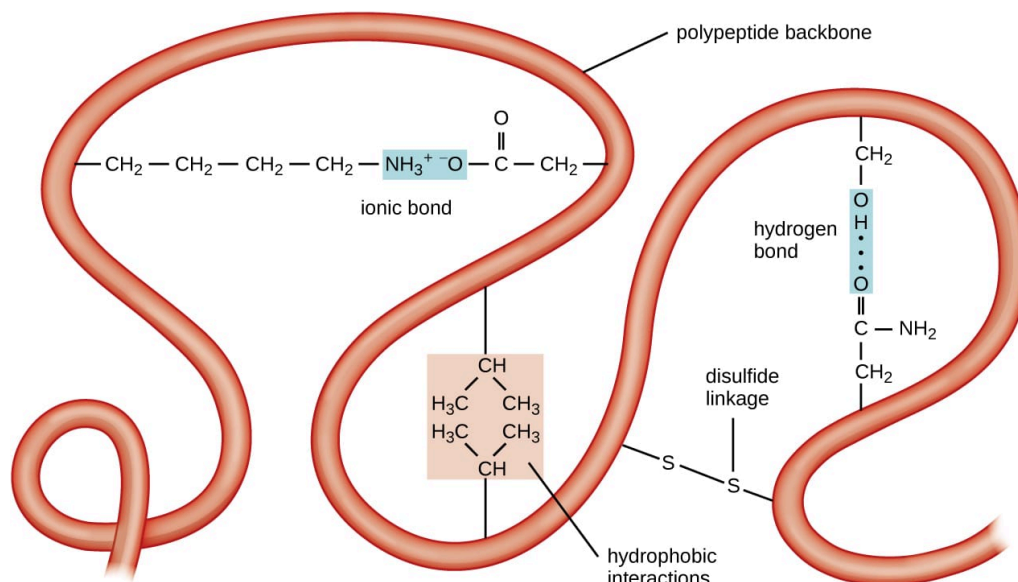


Figure 3.7.5: The tertiary structure of proteins is determined by a variety of attractive forces, including hydrophobic interactions, ionic bonding, hydrogen bonding, and disulfide linkages.

The process by which a polypeptide chain assumes a large-scale, three-dimensional shape is called protein folding. Folded proteins that are fully functional in their normal biological role are said to possess a native structure. When a protein loses its three-dimensional shape, it may no longer be functional. These unfolded proteins are denatured. Denaturation implies the loss of the secondary structure and tertiary structure (and, if present, the quaternary structure) without the loss of the primary structure.

Some proteins are assemblies of several separate polypeptides, also known as protein subunits. These proteins function adequately only when all subunits are present and appropriately configured. The interactions that hold these subunits together constitute the quaternary structure of the protein. The overall quaternary structure is stabilized by relatively weak interactions. Hemoglobin, for example, has a quaternary structure of four globular protein subunits: two α and two β polypeptides, each one containing an iron-based heme (Figure 3.7.6).

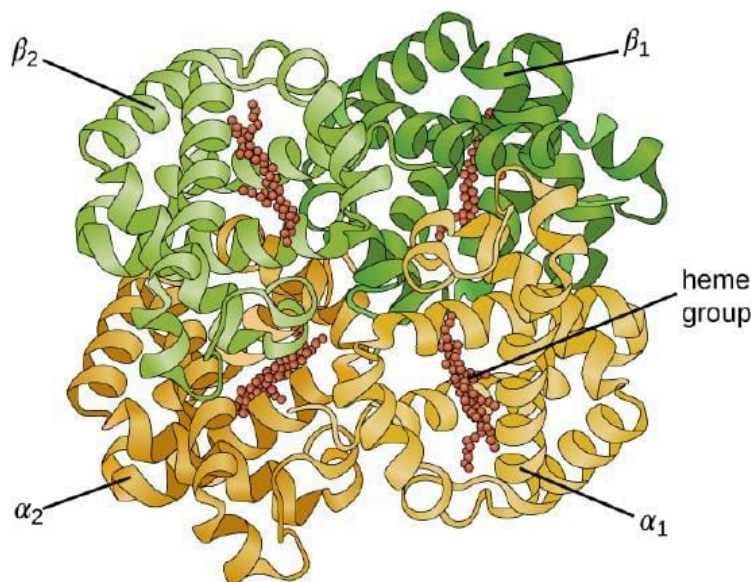


Figure 3.7.6: A hemoglobin molecule has two α and two β polypeptides together with four heme groups.

Another important class of proteins is the conjugated proteins that have a nonprotein portion. If the conjugated protein has a carbohydrate attached, it is called a glycoprotein. If it has a lipid attached, it is called a lipoprotein. These proteins are important components of membranes. Figure 3.7.7 summarizes the four levels of protein structure.

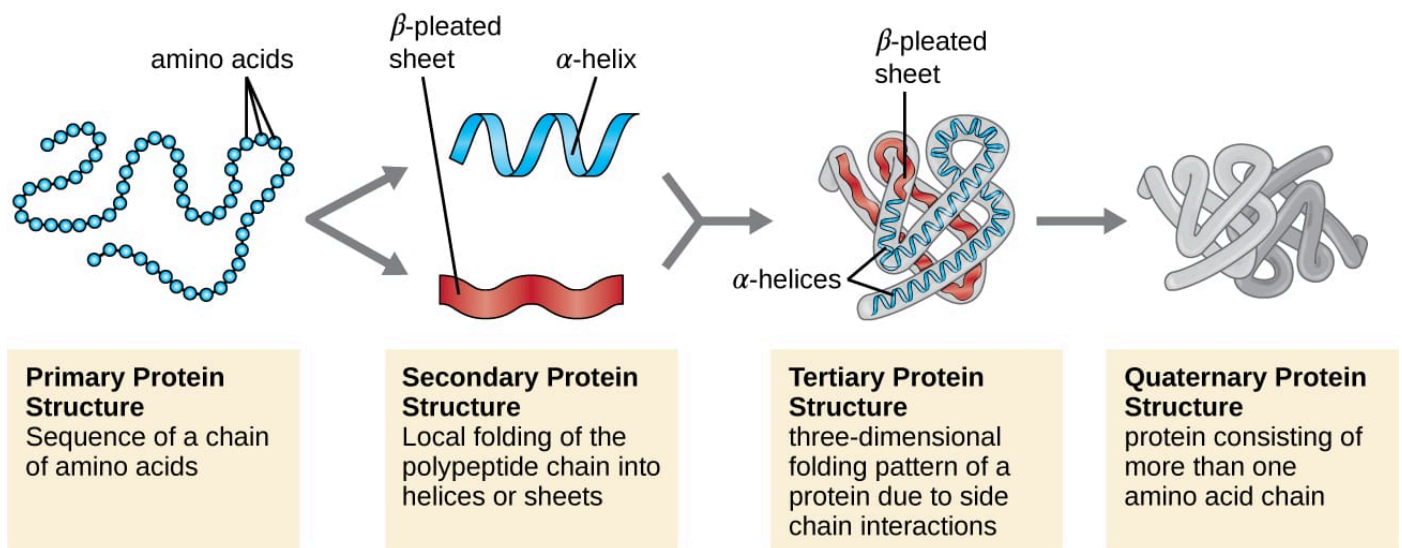


Figure 3.7.7: Protein structure has four levels of organization. (credit: modification of work by National Human Genome Research Institute)

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Primary Structure, Dysfunctional Proteins, and Cystic Fibrosis

Proteins associated with biological membranes are classified as extrinsic or intrinsic. Extrinsic proteins, also called peripheral proteins, are loosely associated with one side of the membrane. Intrinsic proteins, or integral proteins, are embedded in the membrane and often function as part of transport systems as transmembrane proteins. Cystic fibrosis (CF) is a human genetic disorder caused by a change in the transmembrane protein. It affects mostly the lungs but may also affect the pancreas, liver, kidneys, and intestine. CF is caused by a loss of the amino acid phenylalanine in a cystic fibrosis transmembrane protein (CFTR). The loss of one amino acid changes the primary structure of a protein that normally helps transport salt and water in and out of cells (Figure 3.7.8).

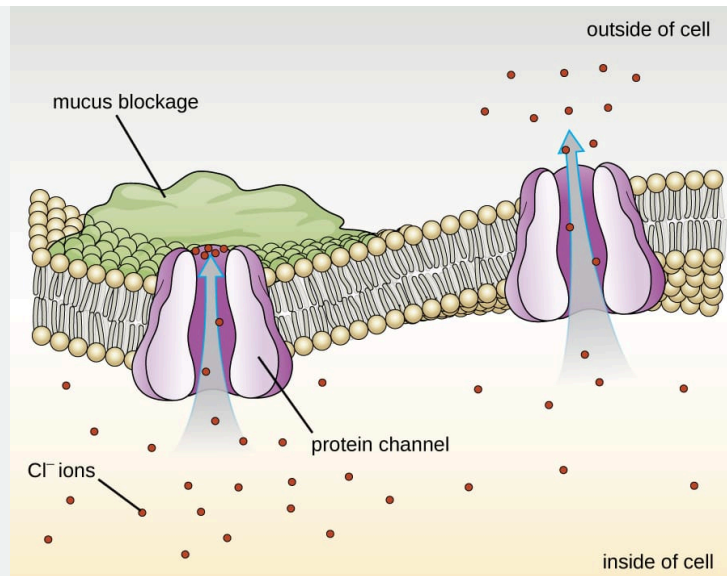
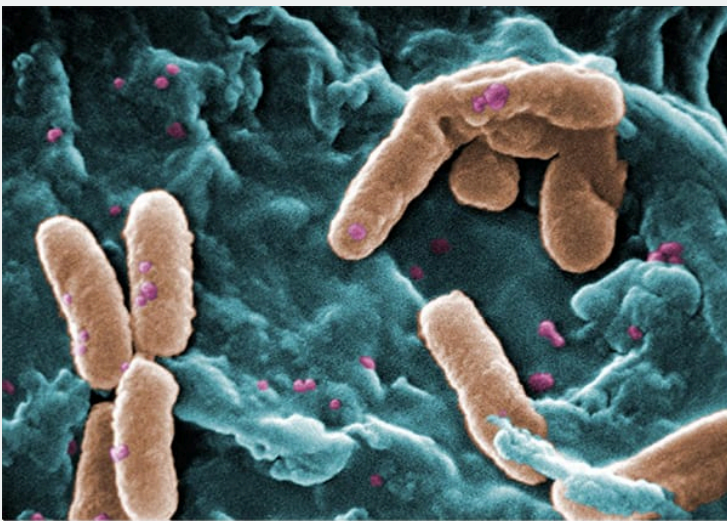


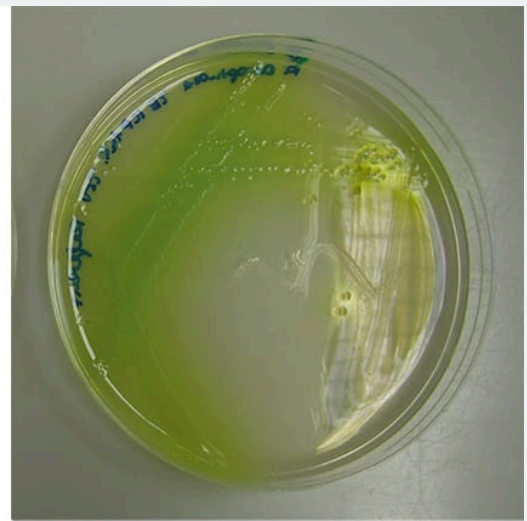
Figure 3.7.8: The normal CFTR protein is a channel protein that helps salt (sodium chloride) move in and out of cells.

The change in the primary structure prevents the protein from functioning properly, which causes the body to produce unusually thick mucus that clogs the lungs and leads to the accumulation of sticky mucus. The mucus obstructs the pancreas and stops natural enzymes from helping the body break down food and absorb vital nutrients.

In the lungs of individuals with cystic fibrosis, the altered mucus provides an environment where bacteria can thrive. This colonization leads to the formation of biofilms in the small airways of the lungs. The most common pathogens found in the lungs of patients with cystic fibrosis are *Pseudomonas aeruginosa* (Figure 3.7.9) and *Burkholderia cepacia*. *Pseudomonas* differentiates within the biofilm in the lung and forms large colonies, called “mucoid” *Pseudomonas*. The colonies have a unique pigmentation that shows up in laboratory tests (Figure 3.7.9) and provides physicians with the first clue that the patient has CF (such colonies are rare in healthy individuals).



(a)



(b)

Figure 3.7.9: (a) A scanning electron micrograph shows the opportunistic bacterium *Pseudomonas aeruginosa*. (b) Pigment-producing *P. aeruginosa* on cetrimide agar shows the green pigment called pyocyanin. (credit a: modification of work by the Centers for Disease Control and Prevention)

Link to Learning

For more information about cystic fibrosis, visit the [Cystic Fibrosis Foundation](#) website.

Key Concepts and Summary

- Amino acids are small molecules essential to all life. Each has an α carbon to which a hydrogen atom, carboxyl group, and amine group are bonded. The fourth bonded group, represented by R , varies in chemical composition, size, polarity, and charge among different amino acids, providing variation in properties.
- **Peptides** are polymers formed by the linkage of amino acids via dehydration synthesis. The bonds between the linked amino acids are called **peptide bonds**. The number of amino acids linked together may vary from a few to many.
- **Proteins** are polymers formed by the linkage of a very large number of amino acids. They perform many important functions in a cell, serving as nutrients and enzymes; storage molecules for carbon, nitrogen, and energy; and structural components.
- The structure of a protein is a critical determinant of its function and is described by a graduated classification: **primary**, **secondary**, **tertiary**, and **quaternary**. The **native structure** of a protein may be disrupted by **denaturation**, resulting in loss of its higher-order structure and its biological function.
- Some proteins are formed by several separate protein subunits, the interaction of these subunits composing the **quaternary structure** of the protein complex.
- **Conjugated proteins** have a nonpolypeptide portion that can be a carbohydrate (forming a **glycoprotein**) or a lipid fraction (forming a **lipoprotein**). These proteins are important components of membranes.

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3.8: Using Biochemistry to Identify Microorganisms

Learning Objectives

- Describe examples of biosynthesis products within a cell that can be detected to identify bacteria
- Explain the principles and workflow of MALDI-TOF mass spectrometry
- Interpret how lipid profile can be used to identify bacteria by FAME analysis

Using Biochemistry to Identify Microorganisms

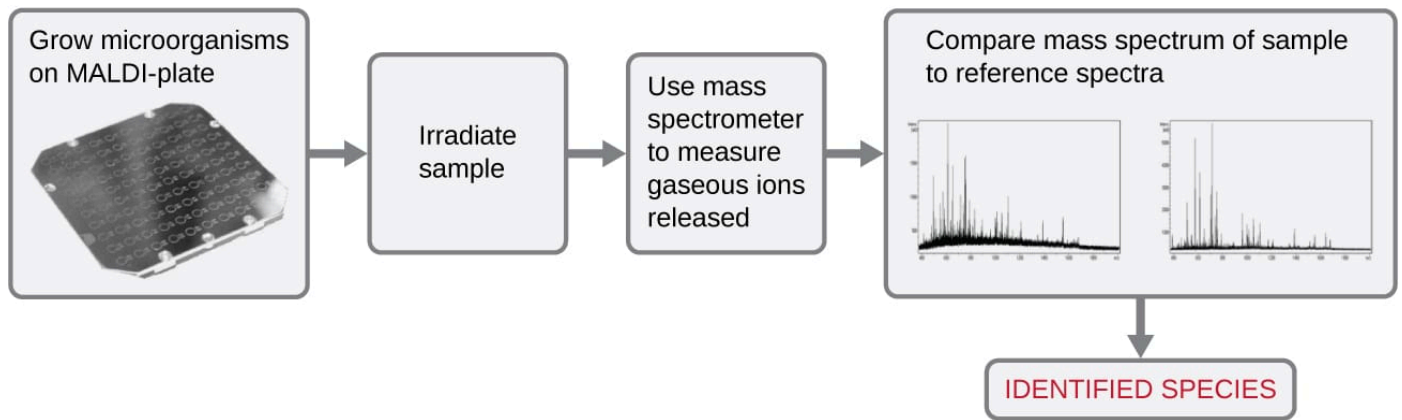
Accurate identification of bacterial isolates is essential in a clinical microbiology laboratory because the results often inform decisions about treatment that directly affect patient outcomes. For example, cases of food poisoning require accurate identification of the causative agent so that physicians can prescribe appropriate treatment. Likewise, it is important to accurately identify the causative pathogen during an outbreak of disease so that appropriate strategies can be employed to contain the epidemic.

There are many ways to detect, characterize, and identify microorganisms. Some methods rely on phenotypic biochemical characteristics, while others use genotypic identification. The biochemical characteristics of a bacterium provide many traits that are useful for classification and identification. Analyzing the nutritional and metabolic capabilities of the bacterial isolate is a common approach for determining the genus and the species of the bacterium. Some of the most important metabolic pathways that bacteria use to survive will be discussed in [Microbial Metabolism](#). In this section, we will discuss a few methods that use biochemical characteristics to identify microorganisms.

Some microorganisms store certain compounds as granules within their cytoplasm, and the contents of these granules can be used for identification purposes. For example, poly- β -hydroxybutyrate (PHB) is a carbon- and energy-storage compound found in some nonfluorescent bacteria of the genus *Pseudomonas*. Different species within this genus can be classified by the presence or the absence of PHB and fluorescent pigments. The human pathogen *P. aeruginosa* and the plant pathogen *P. syringae* are two examples of fluorescent *Pseudomonas* species that do not accumulate PHB granules.

Other systems rely on biochemical characteristics to identify microorganisms by their biochemical reactions, such as carbon utilization and other metabolic tests. In small laboratory settings or in teaching laboratories, those assays are carried out using a limited number of test tubes. However, more modern systems, such as the one developed by Biolog, Inc., are based on panels of biochemical reactions performed simultaneously and analyzed by software. Biolog's system identifies cells based on their ability to metabolize certain biochemicals and on their physiological properties, including pH and chemical sensitivity. It uses all major classes of biochemicals in its analysis. Identifications can be performed manually or with the semi- or fully automated instruments.

Another automated system identifies microorganisms by determining the specimen's mass spectrum and then comparing it to a database that contains known mass spectra for thousands of microorganisms. This method is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and uses disposable MALDI plates on which the microorganism is mixed with a specialized matrix reagent (Figure 3.8.1). The sample/reagent mixture is irradiated with a high-intensity pulsed ultraviolet laser, resulting in the ejection of gaseous ions generated from the various chemical constituents of the microorganism. These gaseous ions are collected and accelerated through the mass spectrometer, with ions traveling at a velocity determined by their mass-to-charge ratio (m/z), thus, reaching the detector at different times. A plot of detector signal versus m/z yields a mass spectrum for the organism that is uniquely related to its biochemical composition. Comparison of the mass spectrum to a library of reference spectra obtained from identical analyses of known microorganisms permits identification of the unknown microbe.



Microbes can also be identified by measuring their unique lipid profiles. As we have learned, fatty acids of lipids can vary in chain length, presence or absence of double bonds, and number of double bonds, hydroxyl groups, branches, and rings. To identify a microbe by its lipid composition, the fatty acids present in their membranes are analyzed. A common biochemical analysis used for this purpose is a technique used in clinical, public health, and food laboratories. It relies on detecting unique differences in fatty acids and is called fatty acid methyl ester (FAME) analysis. In a FAME analysis, fatty acids are extracted from the membranes of microorganisms, chemically altered to form volatile methyl esters, and analyzed by gas chromatography (GC). The resulting GC chromatogram is compared with reference chromatograms in a database containing data for thousands of bacterial isolates to identify the unknown microorganism (Figure 3.8.2).

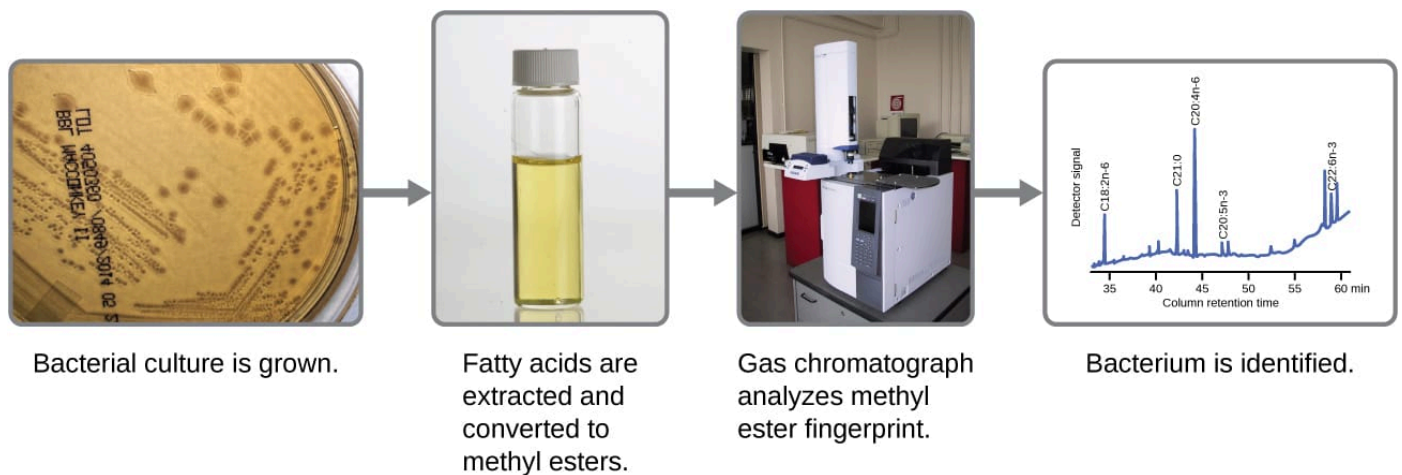


Figure 3.8.2: Fatty acid methyl ester (FAME) analysis in bacterial identification results in a chromatogram unique to each bacterium. Each peak in the gas chromatogram corresponds to a particular fatty acid methyl ester and its height is proportional to the amount present in the cell. (credit “culture”: modification of work by the Centers for Disease Control and Prevention; credit “graph”: modification of work by Zhang P. and Liu P.)

A related method for microorganism identification is called phospholipid-derived fatty acids (PLFA) analysis. Membranes are mostly composed of phospholipids, which can be saponified (hydrolyzed with alkali) to release the fatty acids. The resulting fatty acid mixture is then subjected to FAME analysis, and the measured lipid profiles can be compared with those of known microorganisms to identify the unknown microorganism.

Bacterial identification can also be based on the proteins produced under specific growth conditions within the human body. These types of identification procedures are called proteomic analysis. To perform proteomic analysis, proteins from the pathogen are first separated by high-pressure liquid chromatography (HPLC), and the collected fractions are then digested to yield smaller peptide fragments. These peptides are identified by mass spectrometry and compared with those of known microorganisms to identify the unknown microorganism in the original specimen.

Microorganisms can also be identified by the carbohydrates attached to proteins (glycoproteins) in the plasma membrane or cell

wall. Antibodies and other carbohydrate-binding proteins can attach to specific carbohydrates on cell surfaces, causing the cells to clump together. Serological tests (e.g., the Lancefield groups tests, which are used for identification of *Streptococcus* species) are performed to detect the unique carbohydrates located on the surface of the cell.

Query 3.8.1

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Key Concepts and Summary

- Accurate identification of bacteria is essential in a clinical laboratory for diagnostic and management of epidemics, pandemics, and food poisoning caused by bacterial outbreaks.
- The phenotypic identification of microorganisms involves using observable traits, including profiles of structural components such as lipids, biosynthetic products such as sugars or amino acids, or storage compounds such as poly- β -hydroxybutyrate.
- An unknown microbe may be identified from the unique mass spectrum produced when it is analyzed by **matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF)**.
- Microbes can be identified by determining their lipid compositions, using **fatty acid methyl esters (FAME)** or **phospholipid-derived fatty acids (PLFA) analysis**.
- **Proteomic analysis**, the study of all accumulated proteins of an organism, can also be used for bacterial identification.
- Glycoproteins in the plasma membrane or cell wall structures can bind to lectins or antibodies and can be used for identification.

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3.E: Microbial Biochemistry (Exercises)

3.1: 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.

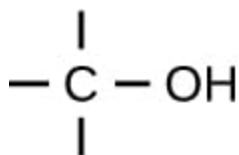
Answer

False

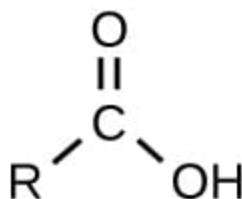
Short Answer

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

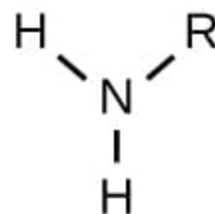
Identify the functional group in each of the depicted structural formulas.



A



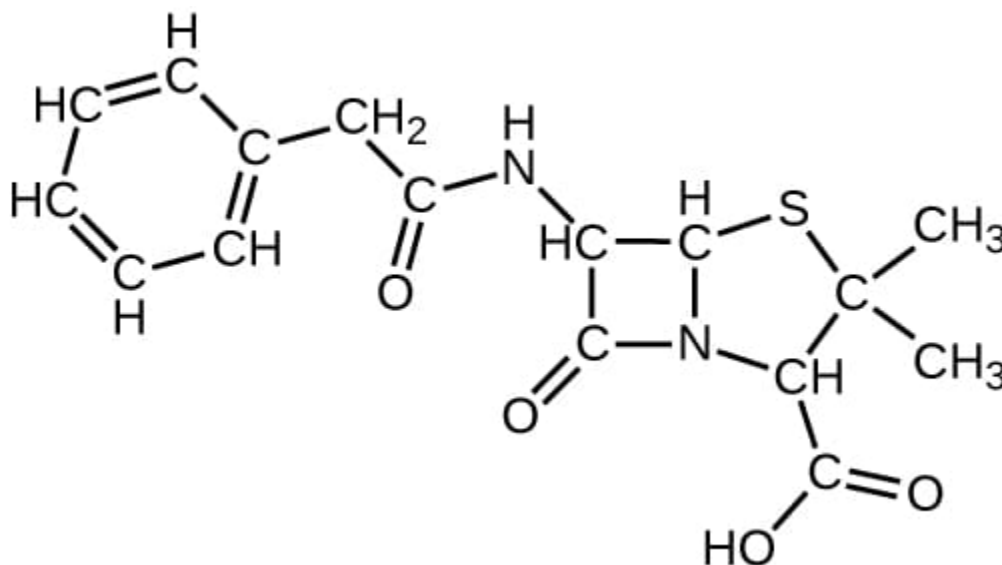
B



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).



3.2: 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 $(\text{CH}_2\text{O})_n$, 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

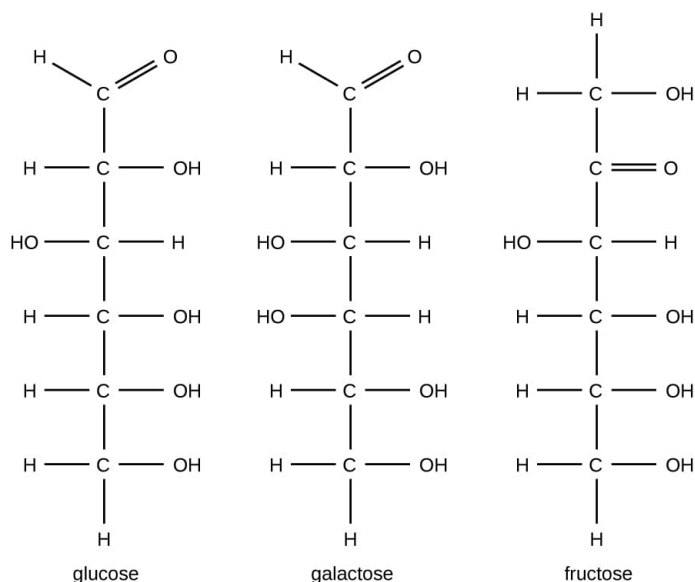
C, D, A, B

Short Answer

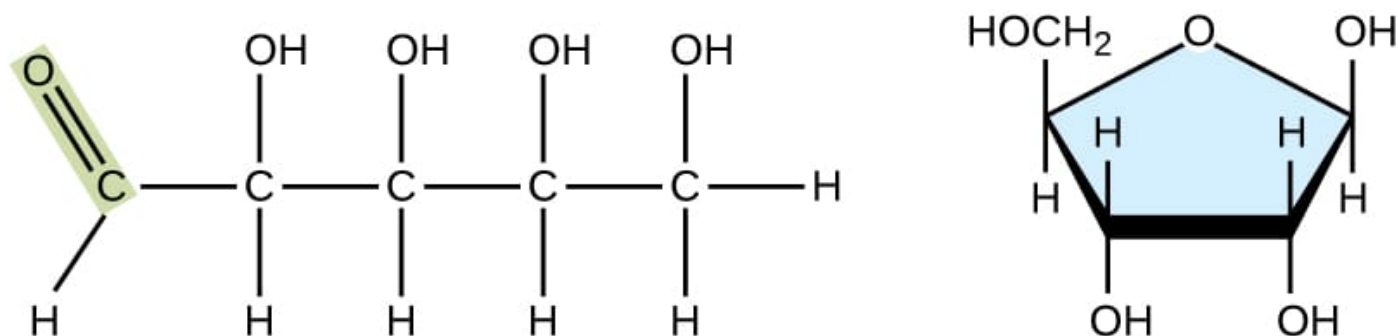
What are monosaccharides, disaccharides, and polysaccharides?

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.

3.3: 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

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?

3.4: 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.

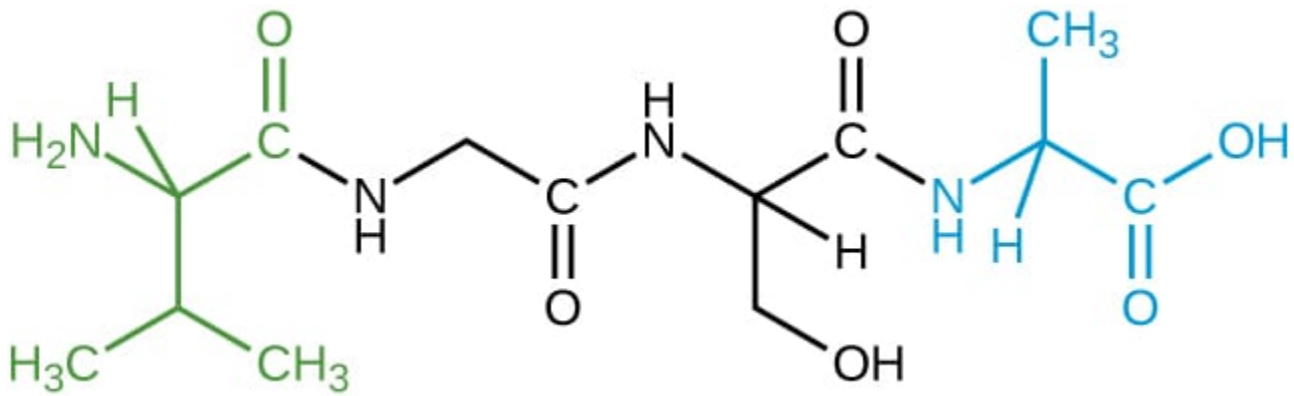
Answer

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.



3.5: 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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4: PROKARYOTIC CELL

Life takes many forms, from giant redwood trees towering hundreds of feet in the air to the tiniest known microbes, which measure only a few billionths of a meter. Humans have long pondered life's origins and debated the defining characteristics of life, but our understanding of these concepts has changed radically since the invention of the microscope. In the 17th century, observations of microscopic life led to the development of the cell theory: the idea that the fundamental unit of life is the cell, that all organisms contain at least one cell, and that cells only come from other cells.

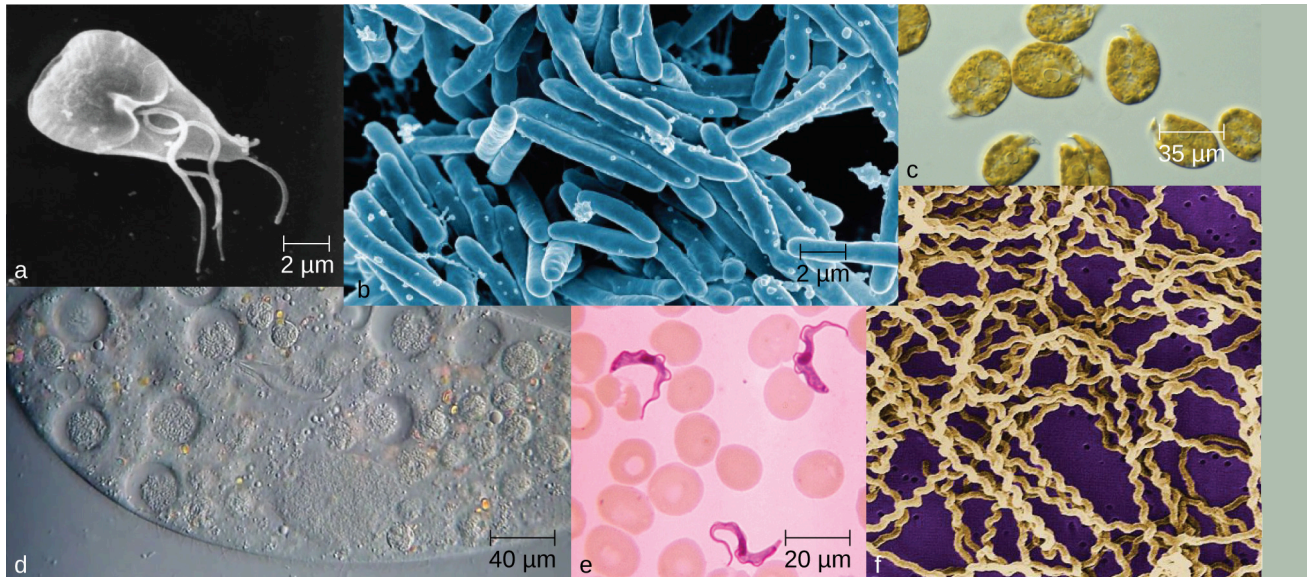


Figure 4.1: Microorganisms vary visually in their size and shape, as can be observed microscopically; but they also vary in invisible ways, such as in their metabolic capabilities. (credit a, e, f: modification of work by Centers for Disease Control and Prevention; credit b: modification of work by NIAID; credit c: modification of work by CSIRO; credit d: modification of work by "Microscopic World"/YouTube)

Despite sharing certain characteristics, cells may vary significantly. The two main types of cells are prokaryotic cells (lacking a nucleus) and eukaryotic cells (containing a well-organized, membrane-bound nucleus). Each type of cell exhibits remarkable variety in structure, function, and metabolic activity. This chapter will focus on the historical discoveries that have shaped our current understanding of microbes, including their origins and their role in human disease. We will then explore the distinguishing structures found in prokaryotic and eukaryotic cells.

4.1: Spontaneous Generation

4.2: Foundations of Modern Cell Theory

4.3: The Germ Theory of Disease

4.4: Internal Structures of Prokaryotic Cells I

4.5: Internal Structures of Prokaryotic Cells II

4.6: Cell Membrane and Transport Mechanisms

4.7: External Structures of Prokaryotic Cells

4.E: Prokaryotic Cell (Exercises)

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4.1: 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

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 4.1.1). 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.

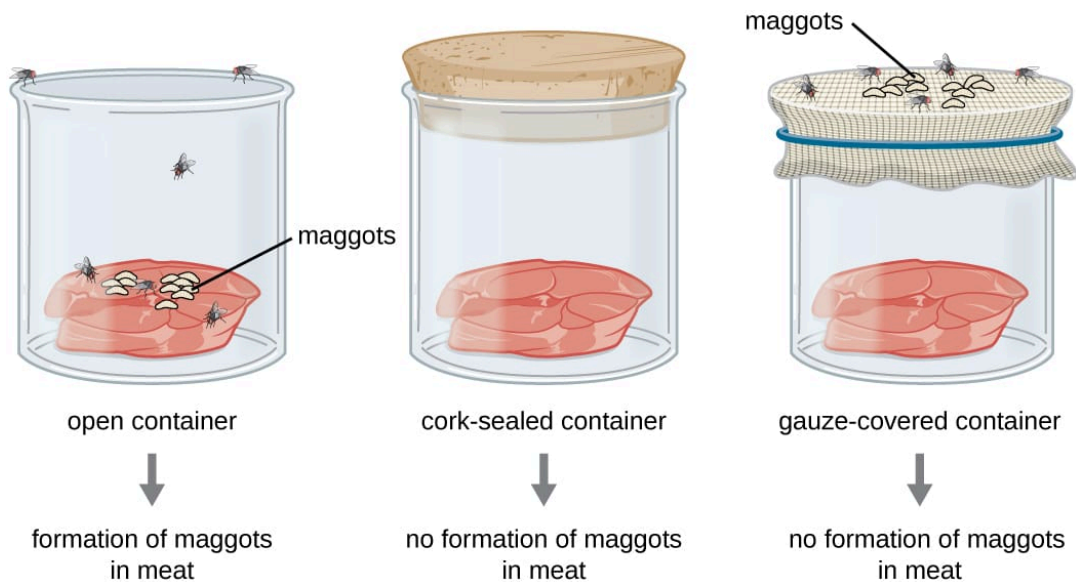


Figure 4.1.1: Francesco Redi's experimental setup consisted of an open container, a container sealed with a cork top, and a container covered in mesh that let in air but not flies. Maggots only appeared on the meat in the open container. However, maggots were also found on the gauze of the gauze-covered 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.

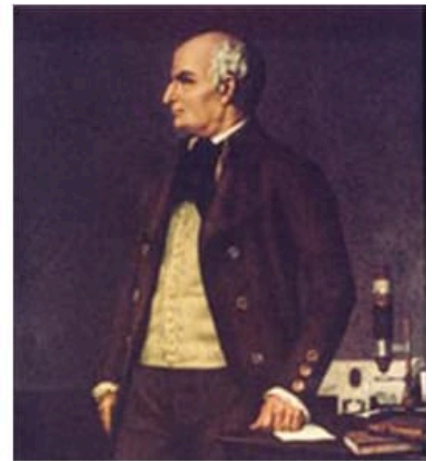
Lazzaro Spallanzani (1729–1799) did not agree with Needham's conclusions, however, and performed hundreds of carefully executed experiments using heated broth.³ As in Needham's experiment, broth in sealed jars and unsealed jars was infused with plant and animal matter. Spallanzani's results contradicted 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 4.1.2).



(a)



(b)



(c)

Figure 4.1.2: (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.

Query 4.1.1

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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 4.1.3). 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.

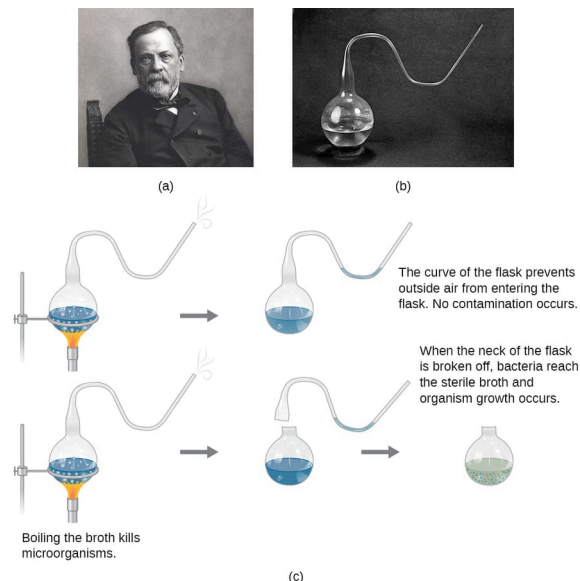


Figure 4.1.3: (a) French scientist Louis Pasteur, who definitively refuted the long-disputed theory of spontaneous generation. (b) The unique swan-neck feature of the flasks used in Pasteur’s experiment allowed air to enter the flask but prevented the entry of bacterial and fungal spores. (c) Pasteur’s 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)

Query 4.1.1

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Interactive Element

Case Study Preview: “Respiratory Distress”

What happens when antibiotics don't work - and not because of resistance? Nineteen-year-old Barbara comes down with a persistent cough, fatigue, and fever after what seemed like a simple cold. When standard antibiotics fail to treat her pneumonia, it's up to you to help figure out why.

Follow Barbara's case as you explore how doctors use tools like chest X-rays, sputum samples, and Gram stains to narrow down the cause - and learn how the absence of a bacterial cell wall can throw a wrench in treatment plans.

Ready to solve the mystery? Understanding cell structure could be the key to saving the day.

[Chapter 3 Case Study - Respiratory Distress](#)

Key Concepts and 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 K. Zwier. “Aristotle on Spontaneous Generation.” www.sju.edu/int/academics/cas...R.%20Zwier.pdf
- 2 E. Capanna. “Lazzaro Spallanzani: At the Roots of Modern Biology.” *Journal of Experimental Zoology* 285 no. 3 (1999):178–196.
- 3 R. Mancini, M. Nigro, G. Ippolito. “Lazzaro Spallanzani and His Refutation of the Theory of Spontaneous Generation.” *Le Infezioni in Medicina* 15 no. 3 (2007):199–206.
- 4 R. Vallery-Radot. *The Life of Pasteur*, trans. R.L. Devonshire. New York: McClure, Phillips and Co, 1902, 1:142.

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4.2: Foundations of Modern Cell Theory

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 4.2.1). At the time, Hooke was not aware that the cork cells were long dead and, therefore, lacked the internal structures found within living cells.

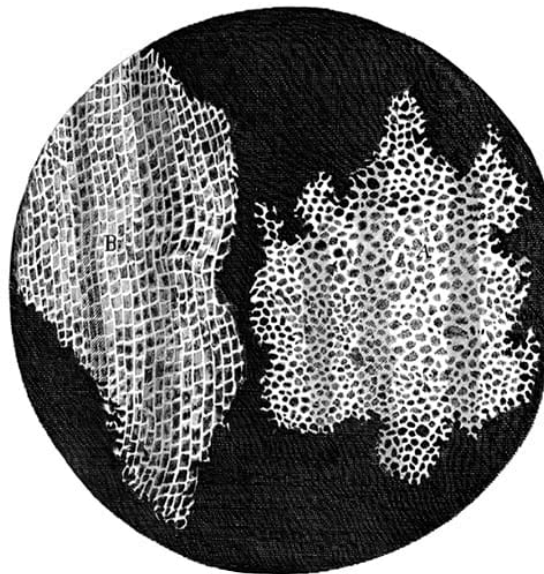


Figure 4.2.1: Robert Hooke (1635–1703) was the first to describe cells based upon his microscopic observations of cork. This illustration was published in his work *Micrographia*.

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.

Query 4.2.1

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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 4.2.2: (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}

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Endosymbiotic Theory

Video: Endosymbiotic Theory

Explore the endosymbiotic theory with the Amoeba Sisters! This theory explains the development of the eukaryote cell from prokaryote cell symbiosis. Scientific theories are also briefly defined.

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As scientists were making progress toward understanding the role of cells in plant and animal tissues, others were examining the structures within the cells themselves. In 1831, Scottish botanist Robert Brown (1773–1858) was the first to describe observations of nuclei, which he observed in plant cells. Then, in the early 1880s, German botanist Andreas Schimper (1856–1901) was the first to describe the chloroplasts of plant cells, identifying their role in starch formation during photosynthesis and noting that they divided independent of the nucleus.

Based upon the chloroplasts' ability to reproduce independently, Russian botanist Konstantin Mereschkowski (1855–1921) suggested in 1905 that chloroplasts may have originated from ancestral photosynthetic bacteria living symbiotically inside a eukaryotic cell. He proposed a similar origin for the nucleus of plant cells. This was the first articulation of the endosymbiotic hypothesis, and would explain how eukaryotic cells evolved from ancestral bacteria.

Mereschkowski's endosymbiotic hypothesis was furthered by American anatomist Ivan Wallin (1883–1969), who began to experimentally examine the similarities between mitochondria, chloroplasts, and bacteria—in other words, to put the endosymbiotic hypothesis to the test using objective investigation. Wallin published a series of papers in the 1920s supporting the endosymbiotic hypothesis, including a 1926 publication co-authored with Mereschkowski. Wallin claimed he could culture mitochondria outside of their eukaryotic host cells. Many scientists dismissed his cultures of mitochondria as resulting from bacterial contamination. Modern genome sequencing work supports the dissenting scientists by showing that much of the genome of mitochondria had been transferred to the host cell's nucleus, preventing the mitochondria from being able to live on their own.^{6 7}

Wallin's ideas regarding the endosymbiotic hypothesis were largely ignored for the next 50 years because scientists were unaware that these organelles contained their own DNA. However, with the discovery of mitochondrial and chloroplast DNA in the 1960s, the endosymbiotic hypothesis was resurrected. **Lynn Margulis** (1938–2011), an American geneticist, published her ideas regarding the endosymbiotic hypothesis of the origins of mitochondria and chloroplasts in 1967.⁸ In the decade leading up to her publication, advances in microscopy had allowed scientists to differentiate prokaryotic cells from eukaryotic cells. In her publication, Margulis reviewed the literature and argued that the eukaryotic organelles such as mitochondria and chloroplasts are of prokaryotic origin. She presented a growing body of microscopic, genetic, molecular biology, fossil, and geological data to support her claims.

Again, this hypothesis was not initially popular, but mounting genetic evidence due to the advent of DNA sequencing supported the endosymbiotic theory, which is now defined as the theory that mitochondria and chloroplasts arose as a result of prokaryotic cells establishing a symbiotic relationship within a eukaryotic host (Figure 4.2.3). With Margulis' initial endosymbiotic theory gaining wide acceptance, she expanded on the theory in her 1981 book *Symbiosis in Cell Evolution*. In it, she explains how endosymbiosis is a major driving factor in the evolution of organisms. More recent genetic sequencing and phylogenetic analysis show that mitochondrial DNA and chloroplast DNA are highly related to their bacterial counterparts, both in DNA sequence and chromosome structure. However, mitochondrial DNA and chloroplast DNA are reduced compared with nuclear DNA because many of the genes have moved from the organelles into the host cell's nucleus. Additionally, mitochondrial and chloroplast ribosomes are structurally similar to bacterial ribosomes, rather than to the eukaryotic ribosomes of their hosts. Last, the binary fission of these organelles strongly resembles the binary fission of bacteria, as compared with mitosis performed by eukaryotic cells. Since Margulis' original proposal, scientists have observed several examples of bacterial endosymbionts in modern-day eukaryotic cells. Examples include the endosymbiotic bacteria found within the guts of certain insects, such as cockroaches,⁹ and photosynthetic bacteria-like organelles found in protists.¹⁰

The Endosymbiotic Theory

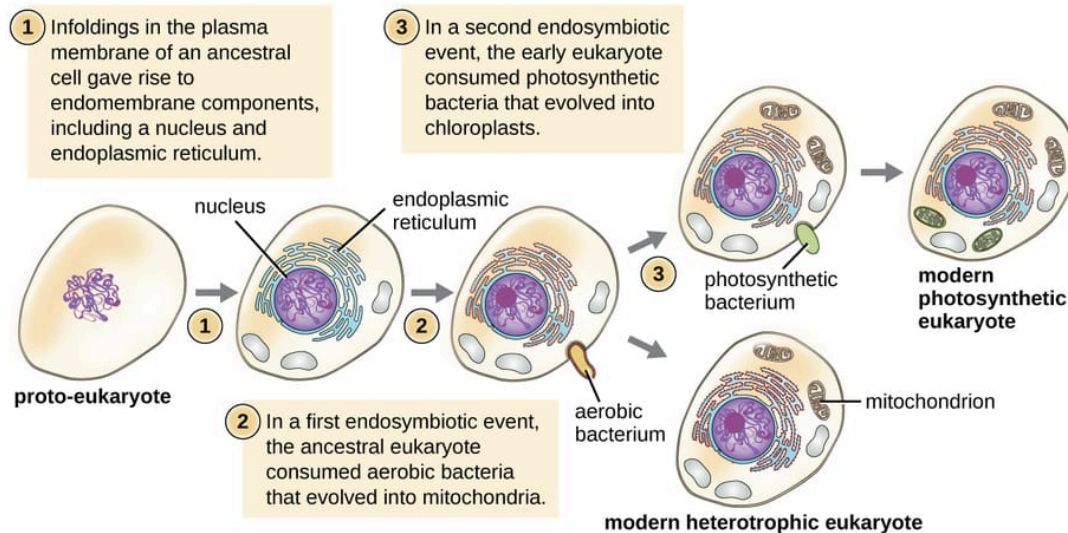


Figure 4.2.3: According to the endosymbiotic theory, mitochondria and chloroplasts are each derived from the uptake of bacteria. These bacteria established a symbiotic relationship with their host cell that eventually led to the bacteria evolving into mitochondria and chloroplasts.

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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.
- 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 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 M. Schultz. "Rudolph Virchow." *Emerging Infectious Diseases* 14 no. 9 (2008):1480-1481.
- 2 B. Kisch. "Forgotten Leaders in Modern Medicine, Valentin, Gouby, Remak, Auerbach." *Transactions of the American Philosophical Society* 44 (1954):139-317.
- 3 H. Harris. *The Birth of the Cell*. New Haven, CT: Yale University Press, 2000:133.
- 4 C. Webster (ed.). *Biology, Medicine and Society 1840-1940*. Cambridge, UK: Cambridge University Press, 1981:118-119.
- 5 C. Zuchora-Walske. *Key Discoveries in Life Science*. Minneapolis, MN: Lerner Publishing, 2015:12-13.
- 6 T. Embley, W. Martin. "Eukaryotic Evolution, Changes, and Challenges." *Nature* Vol. 440 (2006):623-630.
- 7 O.G. Berg, C.G. Kurland. "Why Mitochondrial Genes Are Most Often Found in Nuclei." *Molecular Biology and Evolution* 17 no. 6 (2000):951-961.
- 8 L. Sagan. "On the Origin of Mitosing Cells." *Journal of Theoretical Biology* 14 no. 3 (1967):225-274.
- 9 A.E. Douglas. "The Microbial Dimension in Insect Nutritional Ecology." *Functional Ecology* 23 (2009):38-47.
- 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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4.3: The Germ Theory of Disease

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

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 4.3.4) 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.

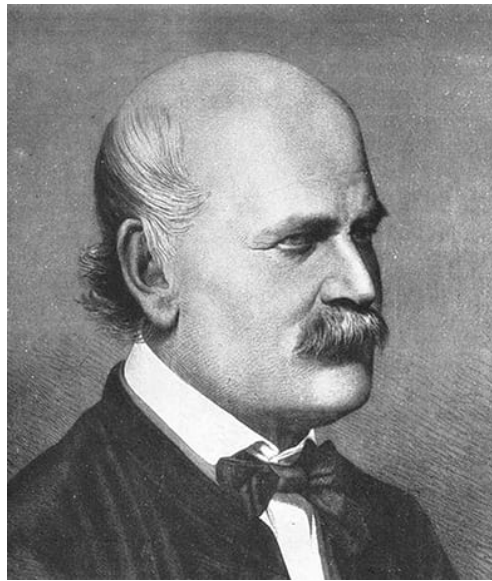


Figure 4.3.4: Ignaz Semmelweis (1818–1865) was a proponent of the importance of handwashing to prevent transfer of disease between patients by physicians.

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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.

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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 Lister would 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 4.3.5) 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 4.3.5) 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](#).

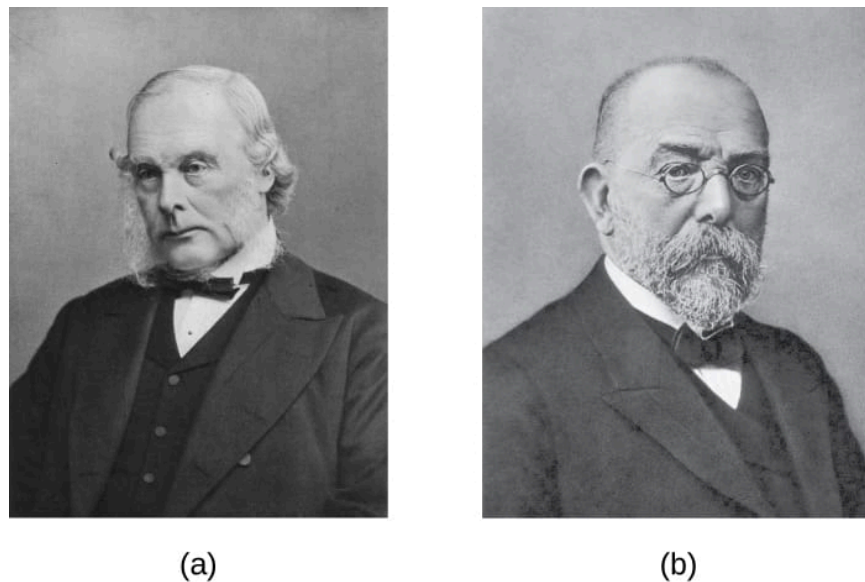


Figure 4.3.5: (a) Joseph Lister developed procedures for the proper care of surgical wounds and the sterilization of surgical equipment. (b) Robert Koch established a protocol to determine the cause of infectious disease. Both scientists contributed significantly to the acceptance of the germ theory of disease.

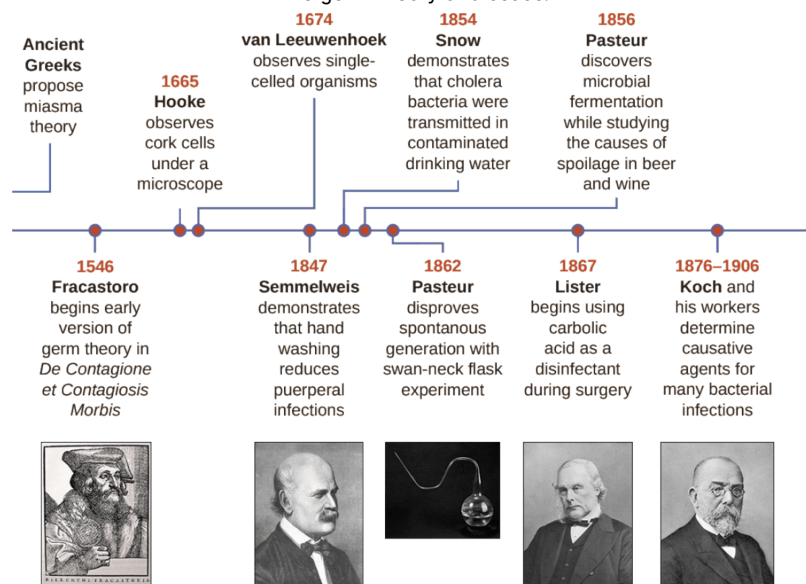


Figure 4.3.7: (credit "swan-neck flask": modification of work by Wellcome Images)

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Key Concepts and Summary

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Footnotes

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- 8 L. Sagan. "On the Origin of Mitosing Cells." *Journal of Theoretical Biology* 14 no. 3 (1967):225–274.
- 9 A.E. Douglas. "The Microbial Dimension in Insect Nutritional Ecology." *Functional Ecology* 23 (2009):38–47.
- 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.
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4.4: Internal Structures of Prokaryotic Cells I

Learning Objectives

- Explain the distinguishing characteristics of prokaryotic cells
- Describe common cell morphologies and cellular arrangements typical of prokaryotic cells and explain how cells maintain their morphology
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Compare the distinguishing characteristics of bacterial and archaeal cells

Cell theory states that the cell is the fundamental unit of life. However, cells vary significantly in size, shape, structure, and function. At the simplest level of construction, all cells possess a few fundamental components. These include **cytoplasm** (a gel-like substance composed of water and dissolved chemicals needed for growth), which is contained within a **plasma membrane** (also called a cell membrane or cytoplasmic membrane); one or more **chromosomes**, which contain the genetic blueprints of the cell; and **ribosomes**, organelles used for the production of proteins.

Beyond these basic components, cells can vary greatly between organisms, and even within the same multicellular organism. The two largest categories of cells—prokaryotic cells and eukaryotic cells—are defined by major differences in several cell structures. Prokaryotic cells lack a nucleus surrounded by a complex nuclear membrane and generally have a single, circular chromosome located in a nucleoid. Eukaryotic cells have a nucleus surrounded by a complex nuclear membrane that contains multiple, rod-shaped chromosomes.¹

All plant cells and animal cells are eukaryotic. Some microorganisms are composed of prokaryotic cells, whereas others are composed of eukaryotic cells. Prokaryotic microorganisms are classified within the domains Archaea and Bacteria, whereas eukaryotic organisms are classified within the domain Eukarya.

The structures inside a cell are analogous to the organs inside a human body, with unique structures suited to specific functions. Some of the structures found in prokaryotic cells are similar to those found in some eukaryotic cells; others are unique to prokaryotes. Although there are some exceptions, eukaryotic cells tend to be larger than prokaryotic cells. The comparatively larger size of eukaryotic cells dictates the need to compartmentalize various chemical processes within different areas of the cell, using complex membrane-bound organelles. In contrast, prokaryotic cells generally lack membrane-bound organelles; however, they often contain inclusions that compartmentalize their cytoplasm. Figure 4.4.1 illustrates structures typically associated with prokaryotic cells. These structures are described in more detail in the next section.

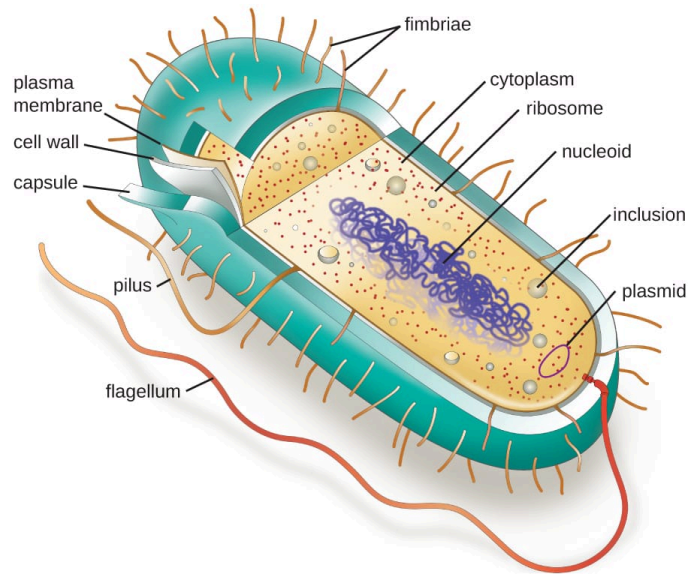


Figure 4.4.1: A typical prokaryotic cell contains a cell membrane, chromosomal DNA that is concentrated in a nucleoid, ribosomes, and a cell wall. Some prokaryotic cells may also possess flagella, pili, fimbriae, and capsules.

Common Cell Morphologies and Arrangements

Individual cells of a particular prokaryotic organism are typically similar in shape, or **cell morphology**. Although thousands of prokaryotic organisms have been identified, only a handful of cell morphologies are commonly seen microscopically. Figure 4.4.2 names and illustrates cell morphologies commonly found in prokaryotic cells. In addition to cellular shape, prokaryotic cells of the same species may group together in certain distinctive arrangements depending on the plane of cell division. Some common arrangements are shown in Figure 4.4.3.

Common Prokaryotic Cell Shapes			
Name	Description	Illustration	Image
Coccus (pl. cocci)	Round		
Bacillus (pl. bacilli)	Rod		
Vibrio (pl. vibrios)	Curved rod		
Coccobacillus (pl. coccobacilli)	Short rod		
Spirillum (pl. spirilla)	Spiral		
Spirochete (pl. spirochetes)	Long, loose, helical spiral		

Figure 4.4.2: (credit "Coccus" micrograph: modification of work by Janice Haney Carr, Centers for Disease Control and Prevention; credit "Coccobacillus" micrograph: modification of work by Janice Carr, Centers for Disease Control and Prevention; credit "Spirochete" micrograph: modification of work by Centers for Disease Control and Prevention)

Common Prokaryotic Cell Arrangements		
Name	Description	Illustration
Coccus (pl. cocci)	Single coccus	
Diplococcus (pl. diplococci)	Pair of two cocci	
Tetrad (pl. tetrads)	Grouping of four cells arranged in a square	
Streptococcus (pl. streptococci)	Chain of cocci	
Staphylococcus (pl. staphylococci)	Cluster of cocci	
Bacillus (pl. bacilli)	Single rod	
Streptobacillus (pl. streptobacilli)	Chain of rods	

Figure 4.4.3: Common cell arrangements.

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Osmotic Pressure

In most prokaryotic cells, morphology is maintained by the **cell wall** in combination with **cytoskeletal elements**. The cell wall is a structure found in most prokaryotes and some eukaryotes; it envelopes the cell membrane, protecting the cell from changes in **osmotic pressure** (Figure 4.4.4). Osmotic pressure occurs because of differences in the concentration of solutes on opposing sides of a semipermeable membrane. Water is able to pass through a semipermeable membrane, but solutes (dissolved molecules like salts, sugars, and other compounds) cannot. When the concentration of solutes is greater on one side of the membrane, water diffuses across the membrane from the side with the lower concentration (more water) to the side with the higher concentration (less water) until the concentrations on both sides become equal. This diffusion of water is called **osmosis**, and it can cause extreme osmotic pressure on a cell when its external environment changes.

The external environment of a cell can be described as an isotonic, hypertonic, or hypotonic medium. In an **isotonic medium**, the solute concentrations inside and outside the cell are approximately equal, so there is no net movement of water across the cell membrane. In a **hypertonic medium**, the solute concentration outside the cell exceeds that inside the cell, so water diffuses out of the cell and into the external medium. In a **hypotonic medium**, the solute concentration inside the cell exceeds that outside of the cell, so water will move by osmosis into the cell. This causes the cell to swell and potentially lyse, or burst.

The degree to which a particular cell is able to withstand changes in osmotic pressure is called **tonicity**. Cells that have a cell wall are better able to withstand subtle changes in osmotic pressure and maintain their shape. In hypertonic environments, cells that lack a cell wall can become dehydrated, causing **crenation**, or shriveling of the cell; the plasma membrane contracts and appears scalloped or notched (Figure 4.4.4). By contrast, cells that possess a cell wall undergo **plasmolysis** rather than crenation. In plasmolysis, the plasma membrane contracts and detaches from the cell wall, and there is a decrease in interior volume, but the cell wall remains intact, thus allowing the cell to maintain some shape and integrity for a period of time (Figure 4.4.5). Likewise, cells that lack a cell wall are more prone to lysis in hypotonic environments. The presence of a cell wall allows the cell to maintain its shape and integrity for a longer time before lysing (Figure 4.4.5).

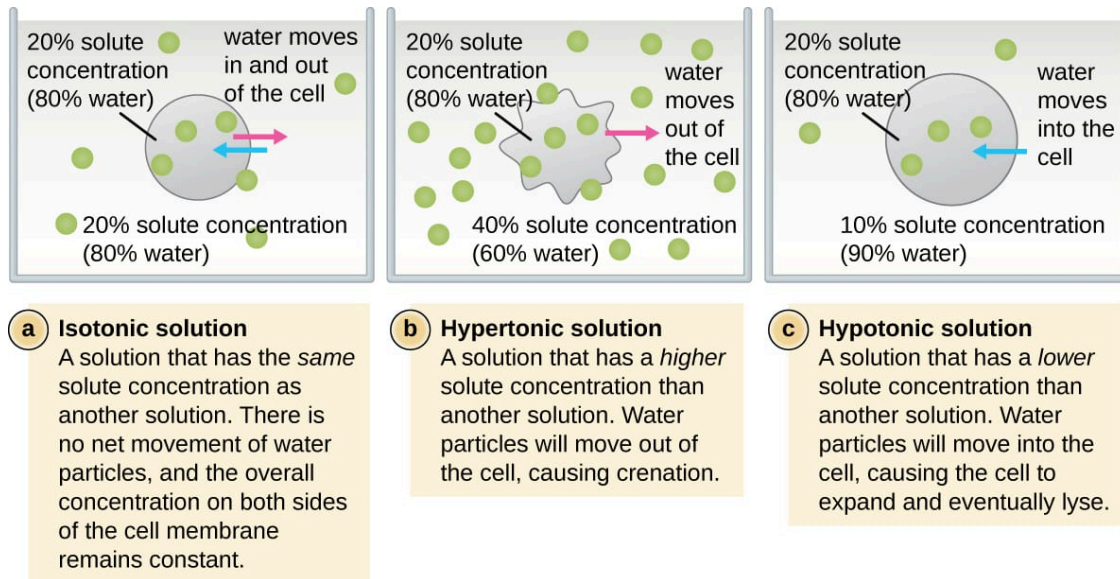


Figure 4.4.4: In cells that lack a cell wall, changes in osmotic pressure can lead to crenation in hypertonic environments or cell lysis in hypotonic environments.

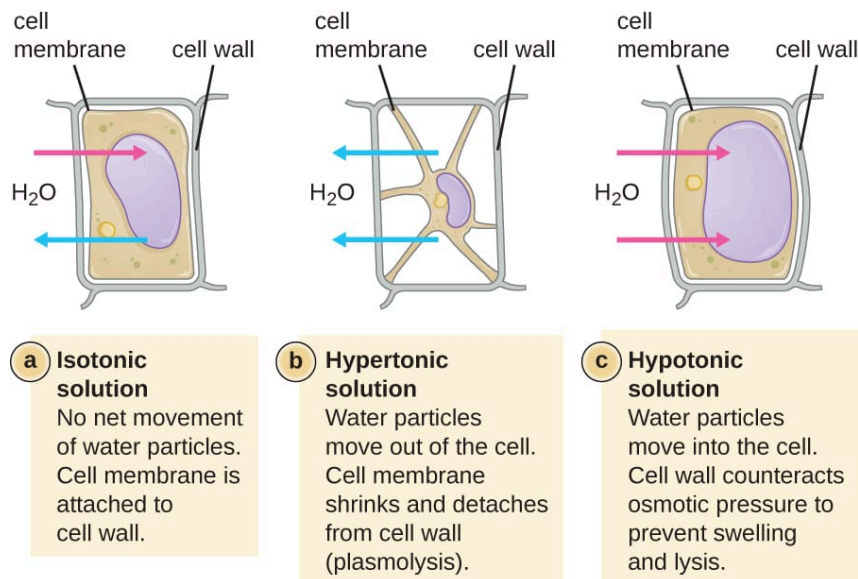


Figure 4.4.5: In prokaryotic cells, the cell wall provides some protection against changes in osmotic pressure, allowing it to maintain its shape longer. The cell membrane is typically attached to the cell wall in an isotonic medium (left). In a hypertonic medium, the cell membrane detaches from the cell wall and contracts (plasmolysis) as water leaves the cell. In a hypotonic medium (right), the cell wall prevents the cell membrane from expanding to the point of bursting, although lysis will eventually occur if too much water is absorbed.

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Key Concepts and Summary

- 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.

Footnotes

- 1 Y.-H.M. Chan, W.F. Marshall. "Scaling Properties of Cell and Organelle Size." *Organogenesis* 6 no. 2 (2010):88–96.
- 2 F. Rothfuss, M Bender, R Conrad. "Survival and Activity of Bacteria in a Deep, Aged Lake Sediment (Lake Constance)." *Microbial Ecology* 33 no. 1 (1997):69–77.
- 3 R. Sinclair et al. "Persistence of Category A Select Agents in the Environment." *Applied and Environmental Microbiology* 74 no. 3 (2008):555–563.
- 4 T.J. Silhavy, D. Kahne, S. Walker. "The Bacterial Cell Envelope." *Cold Spring Harbor Perspectives in Biology* 2 no. 5 (2010):a000414.
- 5 B. Zuber et al. "Granular Layer in the Periplasmic Space of Gram-Positive Bacteria and Fine Structures of *Enterococcus gallinarum* and *Streptococcus gordonii* Septa Revealed by Cryo-Electron Microscopy of Vitreous Sections." *Journal of Bacteriology* 188 no. 18 (2006):6652–6660.
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- 7 J.A. Garnetta et al. "Structural Insights Into the Biogenesis and Biofilm Formation by the *Escherichia coli* Common Pilus." *Proceedings of the National Academy of Sciences of the United States of America* 109 no. 10 (2012):3950–3955.
- 8 T. Proft, E.N. Baker. "Pili in Gram-Negative and Gram-Positive Bacteria—Structure, Assembly and Their Role in Disease." *Cellular and Molecular Life Sciences* 66 (2009):613.

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4.5: Internal Structures of Prokaryotic Cells II

Learning Objectives

- Explain the distinguishing characteristics of prokaryotic cells
- Describe common cell morphologies and cellular arrangements typical of prokaryotic cells and explain how cells maintain their morphology
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Compare the distinguishing characteristics of bacterial and archaeal cells

The Nucleoid

All cellular life has a DNA genome organized into one or more chromosomes. Prokaryotic chromosomes are typically circular, haploid (unpaired), and not bound by a complex nuclear membrane. Prokaryotic DNA and DNA-associated proteins are concentrated within the **nucleoid** region of the cell (Figure 4.5.6). In general, prokaryotic DNA interacts with **nucleoid-associated proteins (NAPs)** that assist in the organization and packaging of the chromosome. In bacteria, NAPs function similar to histones, which are the DNA-organizing proteins found in eukaryotic cells. In archaea, the nucleoid is organized by either NAPs or histone-like DNA organizing proteins.

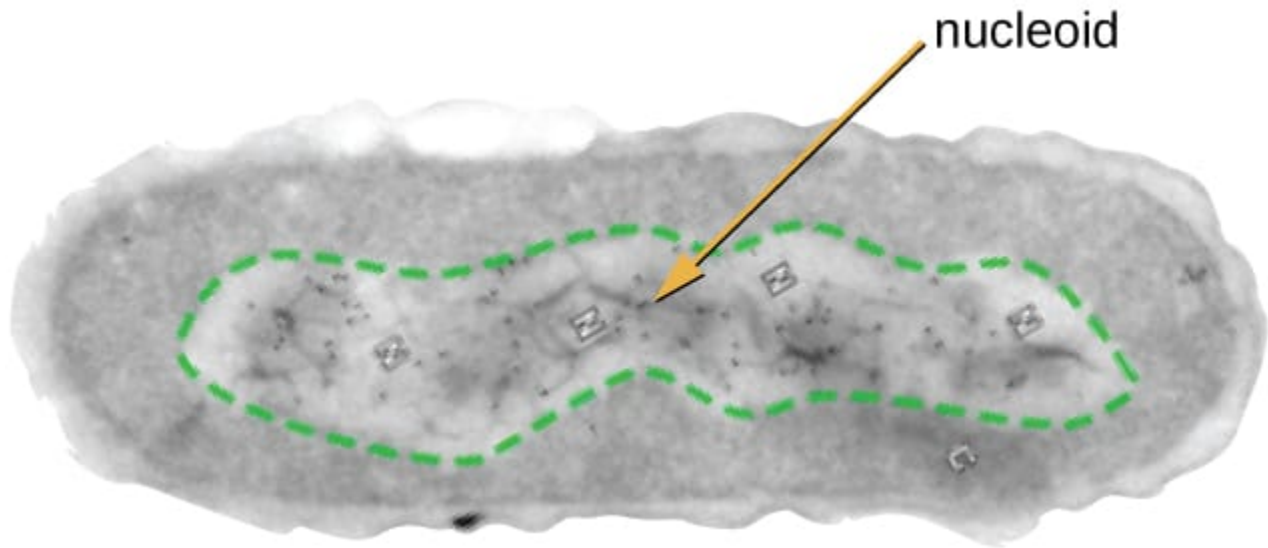


Figure 4.5.6: The nucleoid region (the area enclosed by the green dashed line) is a condensed area of DNA found within prokaryotic cells. Because of the density of the area, it does not readily stain and appears lighter in color when viewed with a transmission electron microscope.

Plasmids

Prokaryotic cells may also contain extrachromosomal DNA, or DNA that is not part of the chromosome. This extrachromosomal DNA is found in **plasmids**, which are small, circular, double-stranded DNA molecules. Cells that have plasmids often have hundreds of them within a single cell. Plasmids are more commonly found in bacteria; however, plasmids have been found in archaea and eukaryotic organisms. Plasmids often carry genes that confer advantageous traits such as antibiotic resistance; thus, they are important to the survival of the organism. We will discuss plasmids in more detail in [Mechanisms of Microbial Genetics](#).

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Ribosomes

All cellular life synthesizes proteins, and organisms in all three domains of life possess ribosomes, structures responsible protein synthesis. However, ribosomes in each of the three domains are structurally different. Ribosomes, themselves, are constructed from proteins, along with ribosomal RNA (rRNA). Prokaryotic ribosomes are found in the cytoplasm. They are called **70S ribosomes** because they have a size of 70S (Figure 4.5.7), whereas eukaryotic cytoplasmic ribosomes have a size of 80S. (The S stands for Svedberg unit, a measure of sedimentation in an ultracentrifuge, which is based on size, shape, and surface qualities of the structure being analyzed). Although they are the same size, bacterial and archaeal ribosomes have different proteins and rRNA molecules, and the archaeal versions are more similar to their eukaryotic counterparts than to those found in bacteria.

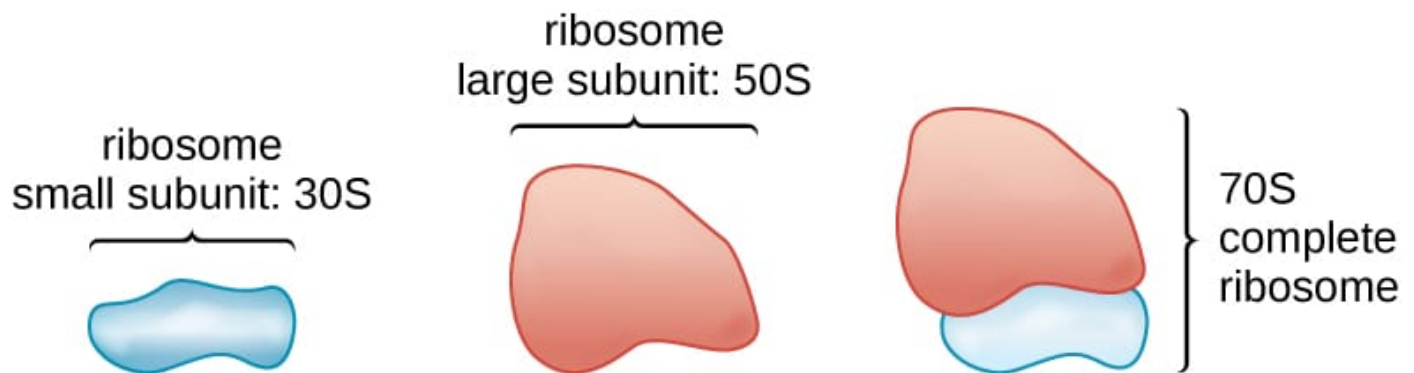


Figure 4.5.7: Prokaryotic ribosomes (70S) are composed of two subunits: the 30S (small subunit) and the 50S (large subunit), each of which are composed of protein and rRNA components.

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Inclusions

As single-celled organisms living in unstable environments, some prokaryotic cells have the ability to store excess nutrients within cytoplasmic structures called **inclusions**. Storing nutrients in a polymerized form is advantageous because it reduces the buildup of osmotic pressure that occurs as a cell accumulates solutes. Various types of inclusions store glycogen and starches, which contain carbon that cells can access for energy. **Volutin** granules, also called **metachromatic granules** because of their staining characteristics, are inclusions that store polymerized inorganic phosphate that can be used in metabolism and assist in the formation of biofilms. Microbes known to contain volutin granules include the archaea *Methanosarcina*, the bacterium *Corynebacterium diphtheriae*, and the unicellular eukaryotic alga *Chlamydomonas*. Sulfur granules, another type of inclusion, are found in sulfur bacteria of the genus *Thiobacillus*; these granules store elemental sulfur, which the bacteria use for metabolism.

Occasionally, certain types of inclusions are surrounded by a phospholipid monolayer embedded with protein. **Polyhydroxybutyrate (PHB)**, which can be produced by species of *Bacillus* and *Pseudomonas*, is an example of an inclusion that displays this type of monolayer structure. Industrially, PHB has also been used as a source of biodegradable polymers for bioplastics. Several different types of inclusions are shown in Figure 4.5.8.

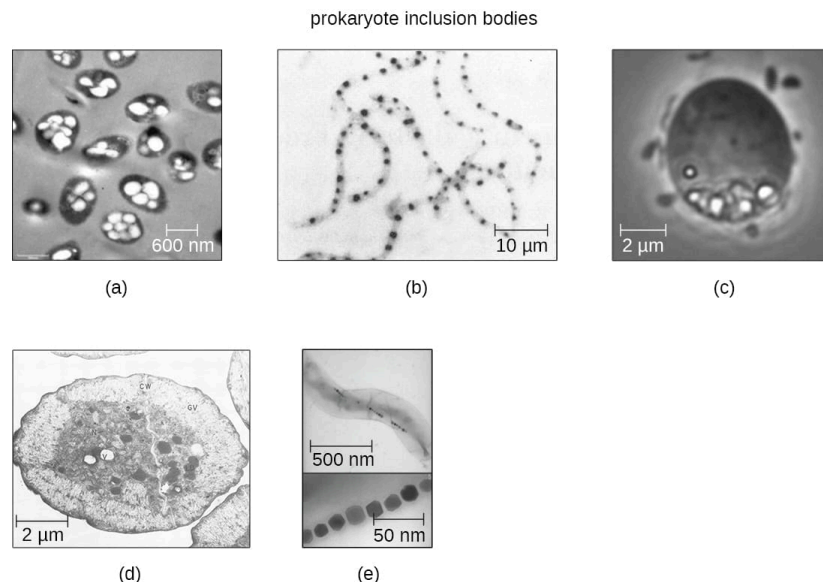


Figure 4.5.8: Prokaryotic cells may have various types of inclusions. (a) A transmission electron micrograph of polyhydroxybutyrate lipid droplets. (b) A light micrograph of volutin granules. (c) A phase-contrast micrograph of sulfur granules. (d) A transmission electron micrograph of magnetosomes. (e) A transmission electron micrograph of gas vacuoles. (credit b, c, d: modification of work by American Society for Microbiology)

Some prokaryotic cells have other types of inclusions that serve purposes other than nutrient storage. For example, some prokaryotic cells produce gas vacuoles, accumulations of small, protein-lined vesicles of gas. These gas vacuoles allow the prokaryotic cells that synthesize them to alter their buoyancy so that they can adjust their location in the water column. Magnetotactic bacteria, such as *Magnetospirillum magnetotacticum*, contain **magnetosomes**, which are inclusions of magnetic iron oxide or iron sulfide surrounded by a lipid layer. These allow cells to align along a magnetic field, aiding their movement (Figure 4.5.8). Cyanobacteria such as *Anabaena cylindrica* and bacteria such as *Halothiobacillus neapolitanus* produce **carboxysome** inclusions. Carboxysomes are composed of outer shells of thousands of protein subunits. Their interior is filled with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and carbonic anhydrase. Both of these compounds are used for carbon metabolism. Some prokaryotic cells also possess carboxysomes that sequester functionally related enzymes in one location. These structures are considered proto-organelles because they compartmentalize important compounds or chemical reactions, much like many eukaryotic organelles.

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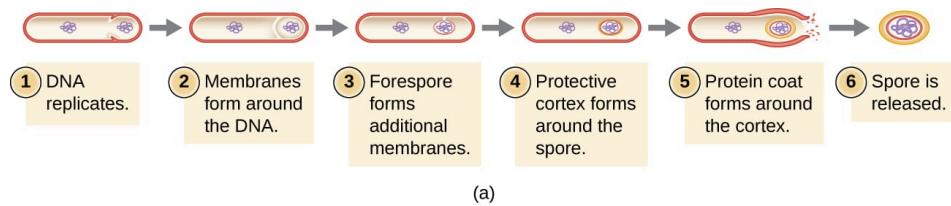
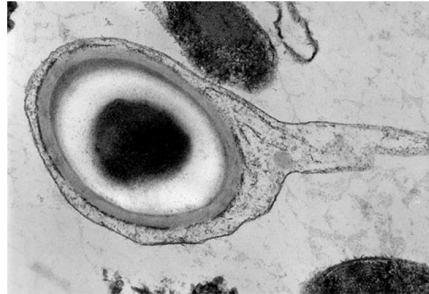
Endospores

Bacterial cells are generally observed as **vegetative cells**, but some genera of bacteria have the ability to form **endospores**, structures that essentially protect the bacterial genome in a dormant state when environmental conditions are unfavorable. Endospores (not to be confused with the reproductive spores formed by fungi) allow some bacterial cells to survive long periods without food or water, as well as exposure to chemicals, extreme temperatures, and even radiation. Table 4.5.1 compares the characteristics of vegetative cells and endospores.

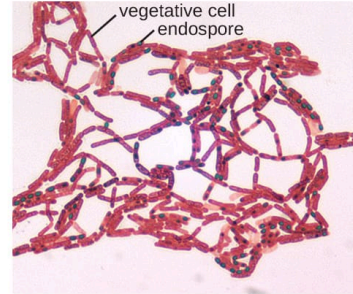
Table 4.5.1: Characteristics of Vegetative Cells versus Endospores

Vegetative Cells	Endospores
Sensitive to extreme temperatures and radiation	Resistant to extreme temperatures and radiation
Gram-positive	Do not absorb Gram stain, only special endospore stains (see Staining Microscopic Specimens)
Normal water content and enzymatic activity	Dehydrated; no metabolic activity
Capable of active growth and metabolism	Dormant; no growth or metabolic activity

The process by which vegetative cells transform into endospores is called **sporulation**, and it generally begins when nutrients become depleted or environmental conditions become otherwise unfavorable (Figure 4.5.9). The process begins with the formation of a septum in the vegetative bacterial cell. The septum divides the cell asymmetrically, separating a DNA forespore from the mother cell. The forespore, which will form the core of the endospore, is essentially a copy of the cell's chromosomes, and is separated from the mother cell by a second membrane. A cortex gradually forms around the forespore by laying down layers of calcium and dipicolinic acid between membranes. A protein spore coat then forms around the cortex while the DNA of the mother cell disintegrates. Further maturation of the endospore occurs with the formation of an outermost exosporium. The endospore is released upon disintegration of the mother cell, completing sporulation.



(b)



(c)

Figure 4.5.9: (a) Sporulation begins following asymmetric cell division. The forespore becomes surrounded by a double layer of membrane, a cortex, and a protein spore coat, before being released as a mature endospore upon disintegration of the mother cell. (b) An electron micrograph of a *Carboxydotherrnus hydrogenofomans* endospore. (c) These *Bacillus* spp. cells are undergoing sporulation. The endospores have been visualized using Malachite Green spore stain. (credit b: modification of work by Jonathan Eisen)

Endospores of certain species have been shown to persist in a dormant state for extended periods of time, up to thousands of years.² However, when living conditions improve, endospores undergo germination, reentering a vegetative state. After germination, the cell becomes metabolically active again and is able to carry out all of its normal functions, including growth and cell division.

Not all bacteria have the ability to form endospores; however, there are a number of clinically significant endospore-forming gram-positive bacteria of the genera *Bacillus* and *Clostridium*. These include *B. anthracis*, the causative agent of anthrax, which produces endospores capable of survive for many decades³; *C. tetani* (causes tetanus); *C. difficile* (causes pseudomembranous colitis); *C. perfringens* (causes gas gangrene); and *C. botulinum* (causes botulism). Pathogens such as these are particularly difficult to combat because their endospores are so hard to kill. Special sterilization methods for endospore-forming bacteria are discussed in [Control of Microbial Growth](#).

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Key Concepts and Summary

- 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.
- Outside of the nucleoid, prokaryotic cells may contain extrachromosomal DNA in **plasmids**.
- Prokaryotic **ribosomes** that are found in the cytoplasm have a size of 70S.
- Some prokaryotic cells have **inclusions** that store nutrients or chemicals for other uses.
- Some prokaryotic cells are able to form **endospores** through **sporulation** to survive in a dormant state when conditions are unfavorable. Endospores can **germinate**, transforming back into **vegetative cells** when conditions improve.
- In prokaryotic cells, the **cell envelope** includes a **plasma membrane** and usually a cell wall.
- Bacterial membranes are composed of phospholipids with integral or peripheral proteins. The fatty acid components of these phospholipids are ester-linked and are often used to identify specific types of bacteria. The proteins serve a variety of functions, including transport, cell-to-cell communication, and sensing environmental conditions. Archaeal membranes are distinct in that they are composed of fatty acids that are ether-linked to phospholipids.
- Some molecules can move across the bacterial membrane by simple diffusion, but most large molecules must be actively transported through membrane structures using cellular energy.
- Prokaryotic cell walls may be composed of **peptidoglycan** (bacteria) or **pseudopeptidoglycan** (archaea).
- Gram-positive bacterial cells are characterized by a thick **peptidoglycan** layer, whereas gram-negative bacterial cells are characterized by a thin peptidoglycan layer surrounded by an outer membrane.
- Some prokaryotic cells produce **glycocalyx** coatings, such as **capsules** and **slime layers**, that aid in attachment to surfaces and/or evasion of the host immune system.
- Some prokaryotic cells have **fimbriae** or **pili**, filamentous appendages that aid in attachment to surfaces. Pili are also used in the transfer of genetic material between cells.
- Some prokaryotic cells use one or more **flagella** to move through water. **Peritrichous** bacteria, which have numerous flagella, use **runs** and **tumbles** to move purposefully in the direction of a chemical attractant.

Footnotes

- 1 Y.-H.M. Chan, W.F. Marshall. "Scaling Properties of Cell and Organelle Size." *Organogenesis* 6 no. 2 (2010):88–96.
- 2 F. Rothfuss, M Bender, R Conrad. "Survival and Activity of Bacteria in a Deep, Aged Lake Sediment (Lake Constance)." *Microbial Ecology* 33 no. 1 (1997):69–77.
- 3 R. Sinclair et al. "Persistence of Category A Select Agents in the Environment." *Applied and Environmental Microbiology* 74 no. 3 (2008):555–563.
- 4 T.J. Silhavy, D. Kahne, S. Walker. "The Bacterial Cell Envelope." *Cold Spring Harbor Perspectives in Biology* 2 no. 5 (2010):a000414.
- 5 B. Zuber et al. "Granular Layer in the Periplasmic Space of Gram-Positive Bacteria and Fine Structures of *Enterococcus gallinarum* and *Streptococcus gordonii* Septa Revealed by Cryo-Electron Microscopy of Vitreous Sections." *Journal of Bacteriology* 188 no. 18 (2006):6652–6660
- 6 L. Gana, S. Chena, G.J. Jensena. "Molecular Organization of Gram-Negative Peptidoglycan." *Proceedings of the National Academy of Sciences of the United States of America* 105 no. 48 (2008):18953–18957.
- 7 J.A. Garnetta et al. "Structural Insights Into the Biogenesis and Biofilm Formation by the *Escherichia coli* Common Pilus." *Proceedings of the National Academy of Sciences of the United States of America* 109 no. 10 (2012):3950–3955.
- 8 T. Proft, E.N. Baker. "Pili in Gram-Negative and Gram-Positive Bacteria—Structure, Assembly and Their Role in Disease."

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4.6: Cell Membrane and Transport Mechanisms

Learning Objectives

- Explain the distinguishing characteristics of prokaryotic cells
- Describe common cell morphologies and cellular arrangements typical of prokaryotic cells and explain how cells maintain their morphology
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Compare the distinguishing characteristics of bacterial and archaeal cells

Plasma Membrane

Structures that enclose the cytoplasm and internal structures of the cell are known collectively as the **cell envelope**. In prokaryotic cells, the structures of the cell envelope vary depending on the type of cell and organism. Most (but not all) prokaryotic cells have a cell wall, but the makeup of this cell wall varies. All cells (prokaryotic and eukaryotic) have a **plasma membrane** (also called **cytoplasmic membrane** or **cell membrane**) that exhibits selective permeability, allowing some molecules to enter or leave the cell while restricting the passage of others.

The structure of the plasma membrane is often described in terms of the **fluid mosaic model**, which refers to the ability of membrane components to move fluidly within the plane of the membrane, as well as the mosaic-like composition of the components, which include a diverse array of lipid and protein components (Figure 4.6.10). The plasma membrane structure of most bacterial and eukaryotic cell types is a bilayer composed mainly of phospholipids formed with ester linkages and proteins. These phospholipids and proteins have the ability to move laterally within the plane of the membranes as well as between the two phospholipid layers.

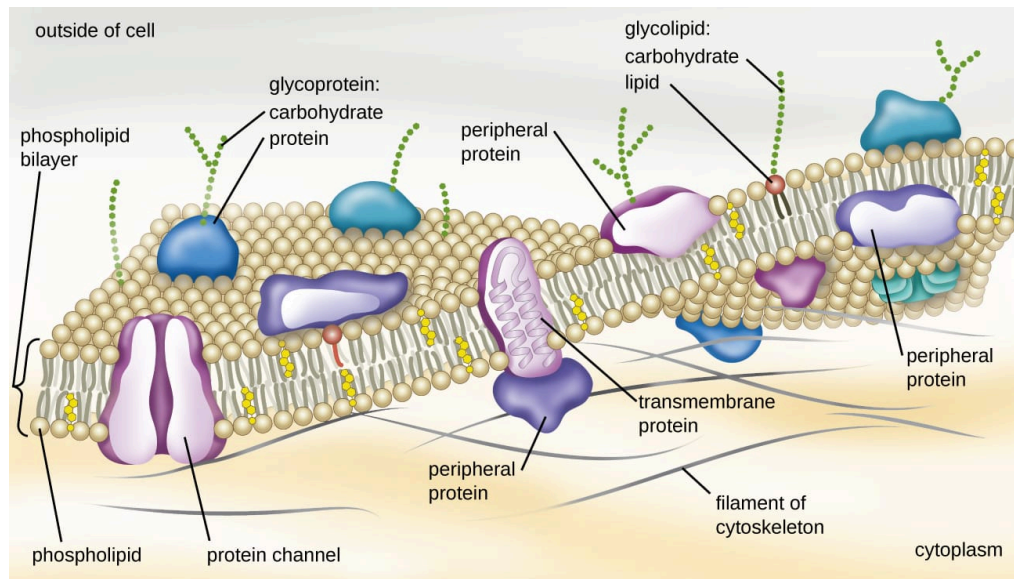


Figure 4.6.10: The bacterial plasma membrane is a phospholipid bilayer with a variety of embedded proteins that perform various functions for the cell. Note the presence of glycoproteins and glycolipids, whose carbohydrate components extend out from the surface of the cell. The abundance and arrangement of these proteins and lipids can vary greatly between species.

Archaeal membranes are fundamentally different from bacterial and eukaryotic membranes in a few significant ways. First, archaeal membrane phospholipids are formed with ether linkages, in contrast to the ester linkages found in bacterial or eukaryotic cell membranes. Second, archaeal phospholipids have branched chains, whereas those of bacterial and eukaryotic cells are straight chained. Finally, although some archaeal membranes can be formed of bilayers like those found in bacteria and eukaryotes, other archaeal plasma membranes are lipid monolayers.

Proteins on the cell's surface are important for a variety of functions, including cell-to-cell communication, and sensing environmental conditions and pathogenic virulence factors. Membrane proteins and phospholipids may have carbohydrates (sugars) associated with them and are called glycoproteins or glycolipids, respectively. These glycoprotein and glycolipid complexes extend out from the surface of the cell, allowing the cell to interact with the external environment (Figure 4.6.10). Glycoproteins and glycolipids in the plasma membrane can vary considerably in chemical composition among archaea, bacteria, and eukaryotes, allowing scientists to use them to characterize unique species.

Plasma membranes from different cells types also contain unique phospholipids, which contain fatty acids. As described in [Using Biochemistry to Identify Microorganisms](#), phospholipid-derived fatty acid analysis (PLFA) profiles can be used to identify unique types of cells based on differences in fatty acids. Archaea, bacteria, and eukaryotes each have a unique PFLA profile.

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Membrane Transport Mechanisms

One of the most important functions of the plasma membrane is to control the transport of molecules into and out of the cell. Internal conditions must be maintained within a certain range despite any changes in the external environment. The transport of substances across the plasma membrane allows cells to do so.

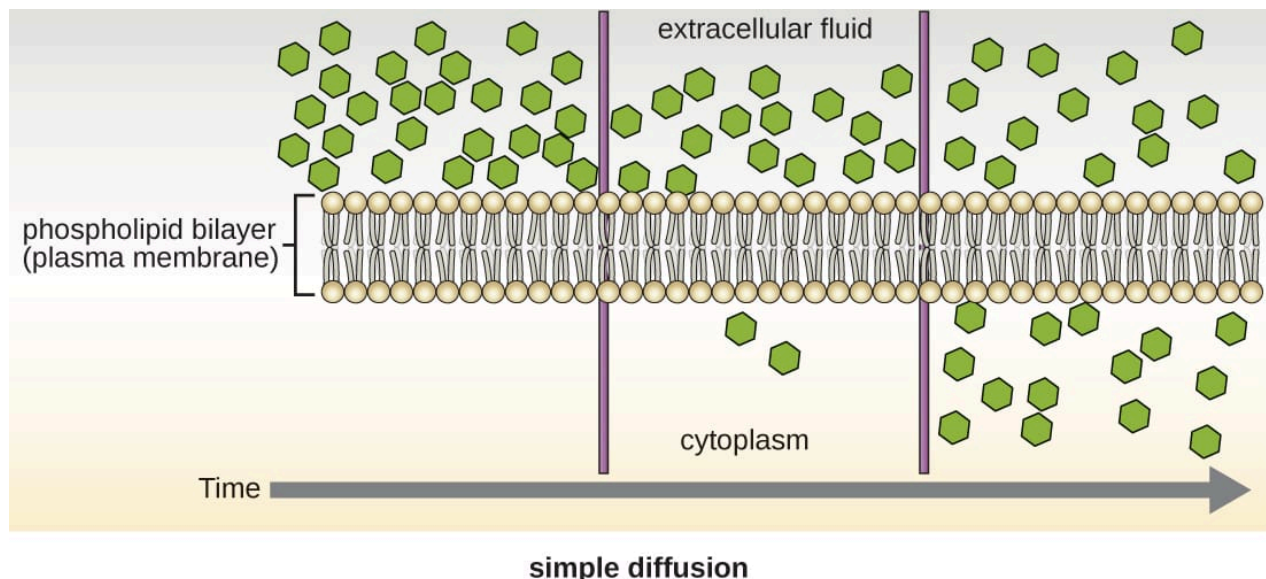


Figure 4.6.11: Simple diffusion down a concentration gradient directly across the phospholipid bilayer. (credit: modification of work by Mariana Ruiz Villareal)

Cells use various modes of transport across the plasma membrane. For example, molecules moving from a higher concentration to a lower concentration with the concentration gradient are transported by **simple diffusion**, also known as **passive transport** (Figure 4.6.11). Some small molecules, like carbon dioxide, may cross the membrane bilayer directly by simple diffusion. However, charged molecules, as well as large molecules, need the help of carriers or channels in the membrane. These structures ferry molecules across the membrane, a process known as **facilitated diffusion** (Figure 4.6.12).

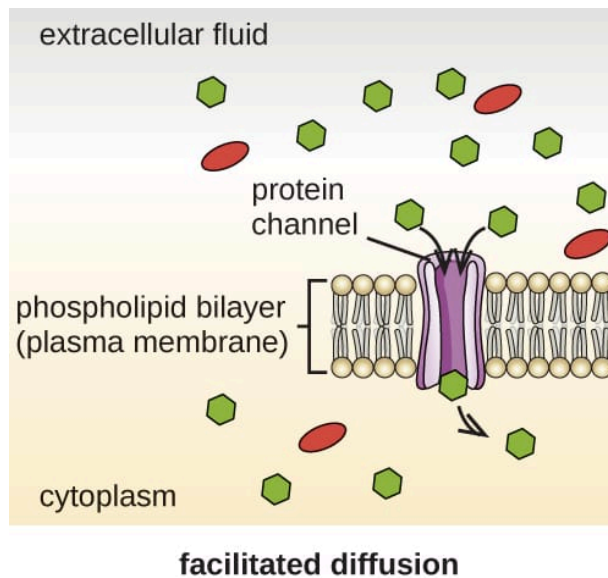


Figure 4.6.12: Facilitated diffusion down a concentration gradient through a membrane protein. (credit: modification of work by Mariana Ruiz Villareal)

Active transport occurs when cells move molecules across their membrane *against* concentration gradients (Figure 4.6.13). A major difference between passive and active transport is that active transport requires adenosine triphosphate (ATP) or other forms of energy to move molecules “uphill.” Therefore, active transport structures are often called “pumps.”

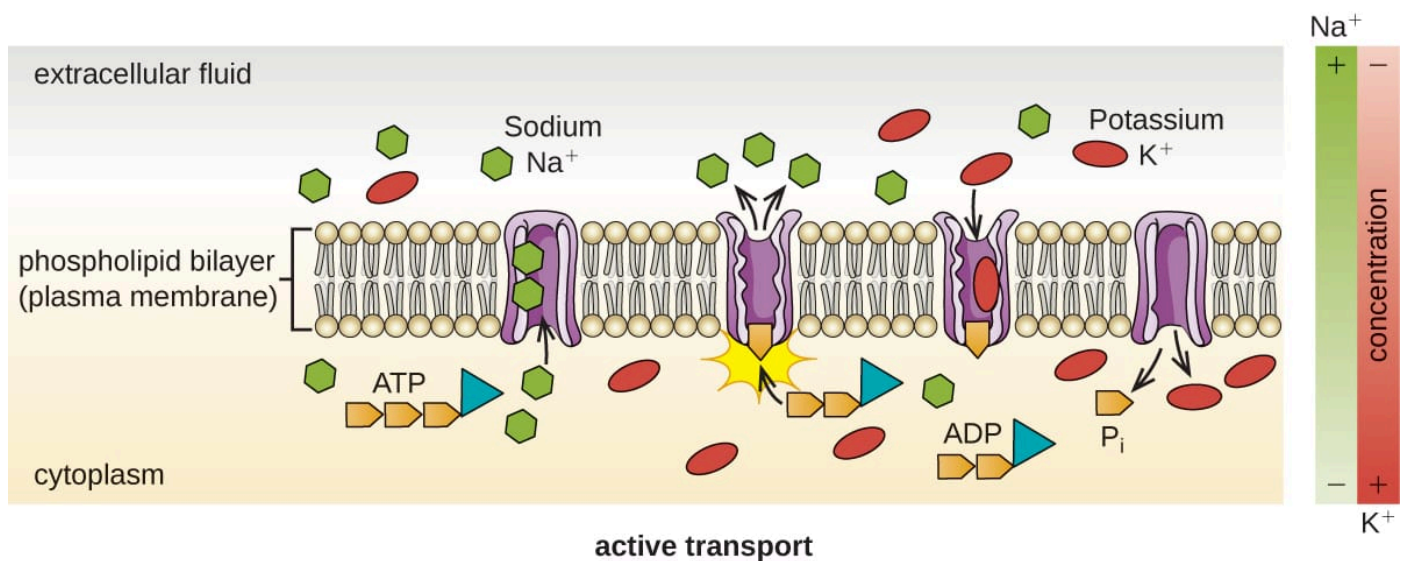


Figure 4.6.13: Active transport against a concentration gradient via a membrane pump that requires energy. (credit: modification of work by Mariana Ruiz Villareal)

Group translocation also transports substances into bacterial cells. In this case, as a molecule moves into a cell against its concentration gradient, it is chemically modified so that it does not require transport against an unfavorable concentration gradient. A common example of this is the bacterial phosphotransferase system, a series of carriers that phosphorylates (i.e., adds phosphate ions to) glucose or other sugars upon entry into cells. Since the phosphorylation of sugars is required during the early stages of sugar metabolism, the phosphotransferase system is considered to be an energy neutral system.

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Photosynthetic Membrane Structures

Some prokaryotic cells, namely cyanobacteria and photosynthetic bacteria, have membrane structures that enable them to perform photosynthesis. These structures consist of an infolding of the plasma membrane that encloses photosynthetic pigments such as green chlorophylls and bacteriochlorophylls. In cyanobacteria, these membrane structures are called thylakoids; in photosynthetic bacteria, they are called chromatophores, lamellae, or chlorosomes.

Key Concepts and Summary

- In prokaryotic cells, the **cell envelope** includes a **plasma membrane** and usually a cell wall.
- Bacterial membranes are composed of phospholipids with integral or peripheral proteins. The fatty acid components of these phospholipids are ester-linked and are often used to identify specific types of bacteria. The proteins serve a variety of functions, including transport, cell-to-cell communication, and sensing environmental conditions. Archaeal membranes are distinct in that they are composed of fatty acids that are ether-linked to phospholipids.
- Some molecules can move across the bacterial membrane by simple diffusion, but most large molecules must be actively transported through membrane structures using cellular energy.

Footnotes

- 1 Y.-H.M. Chan, W.F. Marshall. "Scaling Properties of Cell and Organelle Size." *Organogenesis* 6 no. 2 (2010):88–96.
- 2 F. Rothfuss, M Bender, R Conrad. "Survival and Activity of Bacteria in a Deep, Aged Lake Sediment (Lake Constance)." *Microbial Ecology* 33 no. 1 (1997):69–77.
- 3 R. Sinclair et al. "Persistence of Category A Select Agents in the Environment." *Applied and Environmental Microbiology* 74 no. 3 (2008):555–563.
- 4 T.J. Silhavy, D. Kahne, S. Walker. "The Bacterial Cell Envelope." *Cold Spring Harbor Perspectives in Biology* 2 no. 5 (2010):a000414.
- 5 B. Zuber et al. "Granular Layer in the Periplasmic Space of Gram-Positive Bacteria and Fine Structures of *Enterococcus gallinarum* and *Streptococcus gordonii* Septa Revealed by Cryo-Electron Microscopy of Vitreous Sections." *Journal of Bacteriology* 188 no. 18 (2006):6652–6660.
- 6 L. Gana, S. Chena, G.J. Jensena. "Molecular Organization of Gram-Negative Peptidoglycan." *Proceedings of the National Academy of Sciences of the United States of America* 105 no. 48 (2008):18953–18957.
- 7 J.A. Garnetta et al. "Structural Insights Into the Biogenesis and Biofilm Formation by the *Escherichia coli* Common Pilus." *Proceedings of the National Academy of Sciences of the United States of America* 109 no. 10 (2012):3950–3955.
- 8 T. Proft, E.N. Baker. "Pili in Gram-Negative and Gram-Positive Bacteria—Structure, Assembly and Their Role in Disease." *Cellular and Molecular Life Sciences* 66 (2009):613.

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4.7: External Structures of Prokaryotic Cells

Learning Objectives

- Explain the distinguishing characteristics of prokaryotic cells
- Describe common cell morphologies and cellular arrangements typical of prokaryotic cells and explain how cells maintain their morphology
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Compare the distinguishing characteristics of bacterial and archaeal cells

Cell Wall

The primary function of the cell wall is to protect the cell from harsh conditions in the outside environment. When present, there are notable similarities and differences among the cell walls of archaea, bacteria, and eukaryotes.

The major component of bacterial cell walls is called **peptidoglycan** (or murein); it is only found in bacteria. Structurally, peptidoglycan resembles a layer of meshwork or fabric (Figure 4.7.14). Each layer is composed of long chains of alternating molecules of **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**. The structure of the long chains has significant two-dimensional tensile strength due to the formation of peptide bridges that connect NAG and NAM within each peptidoglycan layer. In gram-negative bacteria, tetrapeptide chains extending from each NAM unit are directly cross-linked, whereas in gram-positive bacteria, these tetrapeptide chains are linked by pentaglycine cross-bridges. Peptidoglycan subunits are made inside of the bacterial cell and then exported and assembled in layers, giving the cell its shape.

Since peptidoglycan is unique to bacteria, many antibiotic drugs are designed to interfere with peptidoglycan synthesis, weakening the cell wall and making bacterial cells more susceptible to the effects of osmotic pressure (see [Mechanisms of Antibacterial Drugs](#)). In addition, certain cells of the human immune system are able “recognize” bacterial pathogens by detecting peptidoglycan on the surface of a bacterial cell; these cells then engulf and destroy the bacterial cell, using enzymes such as lysozyme, which breaks down and digests the peptidoglycan in their cell walls (see [Pathogen Recognition and Phagocytosis](#)).

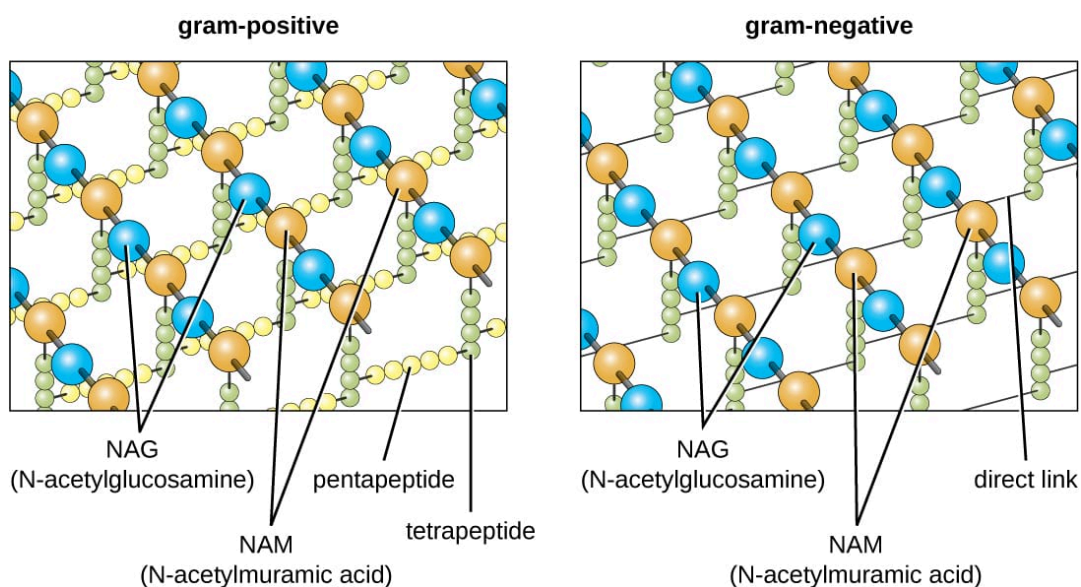


Figure 4.7.14: Peptidoglycan is composed of polymers of alternating NAM and NAG subunits, which are cross-linked by peptide bridges linking NAM subunits from various glycan chains. This provides the cell wall with tensile strength in two dimensions.

The **Gram staining** protocol (see [Staining Microscopic Specimens](#)) is used to differentiate two common types of cell wall structures (Figure 4.7.15). **Gram-positive cells** have a cell wall consisting of many layers of peptidoglycan totaling 30–100 nm

in thickness. These peptidoglycan layers are commonly embedded with teichoic acids (TAs), carbohydrate chains that extend through and beyond the peptidoglycan layer.⁴ TA is thought to stabilize peptidoglycan by increasing its rigidity. TA also plays a role in the ability of pathogenic gram-positive bacteria such as *Streptococcus* to bind to certain proteins on the surface of host cells, enhancing their ability to cause infection. In addition to peptidoglycan and TAs, bacteria of the family Mycobacteriaceae have an external layer of waxy **mycolic acids** in their cell wall; as described in [Staining Microscopic Specimens](#), these bacteria are referred to as acid-fast, since acid-fast stains must be used to penetrate the mycolic acid layer for purposes of microscopy (Figure 4.7.16).

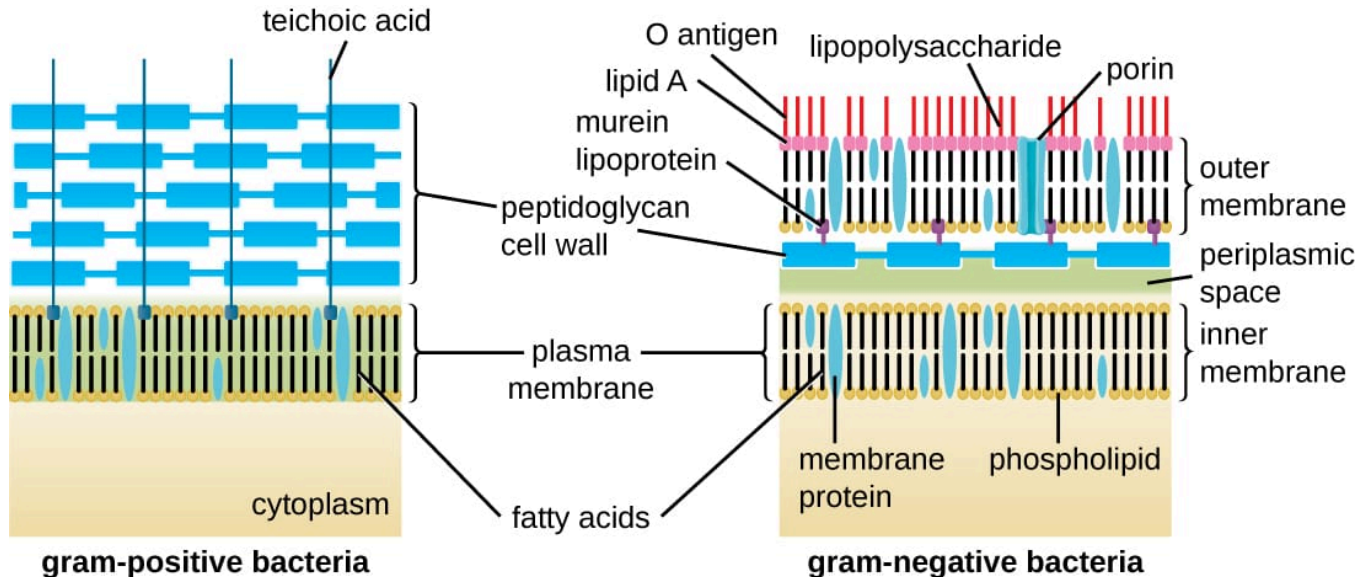


Figure 4.7.15: Bacteria contain two common cell wall structural types. Gram-positive cell walls are structurally simple, containing a thick layer of peptidoglycan with embedded teichoic acid external to the plasma membrane.⁵ Gram-negative cell walls are structurally more complex, containing three layers: the inner membrane, a thin layer of peptidoglycan, and an outer membrane containing lipopolysaccharide. (credit: modification of work by "Franciscosp2"/Wikimedia Commons)

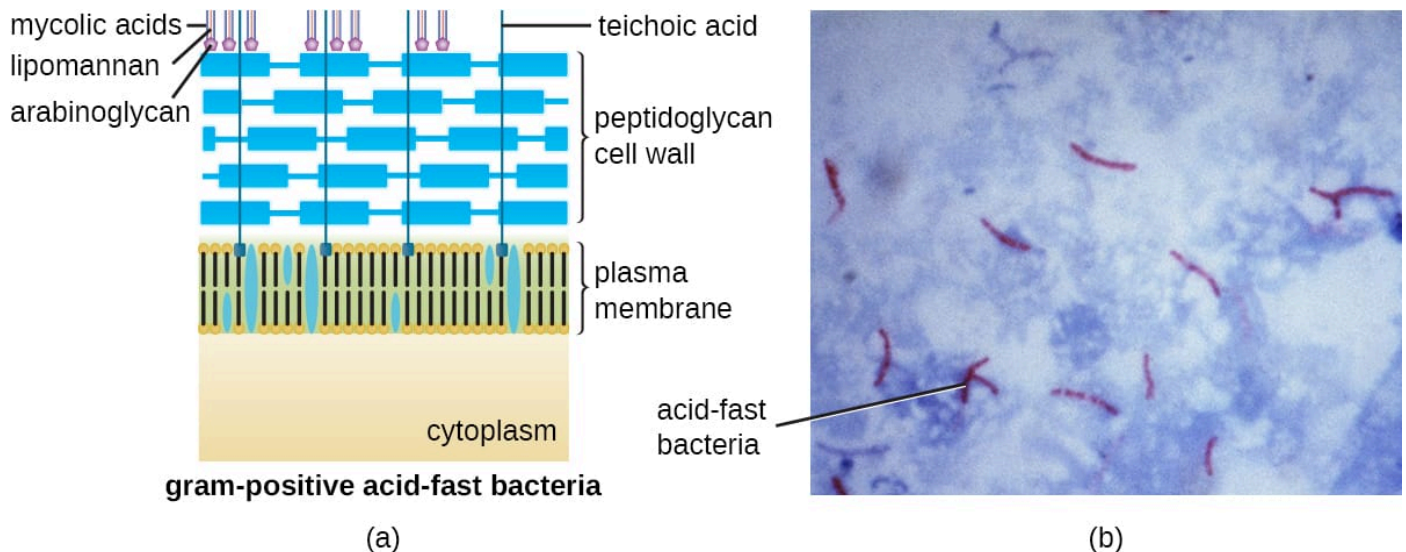


Figure 4.7.16: (a) Some gram-positive bacteria, including members of the Mycobacteriaceae, produce waxy mycolic acids found exterior to their structurally-distinct peptidoglycan. (b) The acid-fast staining protocol detects the presence of cell walls that are rich in mycolic acid. Acid-fast cells are stained red by carbolfuchsin. (credit a: modification of work by "Franciscosp2"/Wikimedia Commons; credit b: modification of work by Centers for Disease Control and Prevention)

Gram-negative cells have a much thinner layer of peptidoglycan (no more than about 4 nm thick⁶) than gram-positive cells, and the overall structure of their cell envelope is more complex. In gram-negative cells, a gel-like matrix occupies the **periplasmic space** between the cell wall and the plasma membrane, and there is a second lipid bilayer called the **outer membrane**, which is external to the peptidoglycan layer (Figure 4.7.15). This outer membrane is attached to the peptidoglycan by murein lipoprotein.

The outer leaflet of the outer membrane contains the molecule **lipopolysaccharide (LPS)**, which functions as an endotoxin in infections involving gram-negative bacteria, contributing to symptoms such as fever, hemorrhaging, and septic shock. Each LPS molecule is composed of Lipid A, a core polysaccharide, and an O side chain that is composed of sugar-like molecules that comprise the external face of the LPS (Figure 4.7.17). The composition of the O side chain varies between different species and strains of bacteria. Parts of the O side chain called antigens can be detected using serological or immunological tests to identify specific pathogenic strains like *Escherichia coli* O157:H7, a deadly strain of bacteria that causes bloody diarrhea and kidney failure.

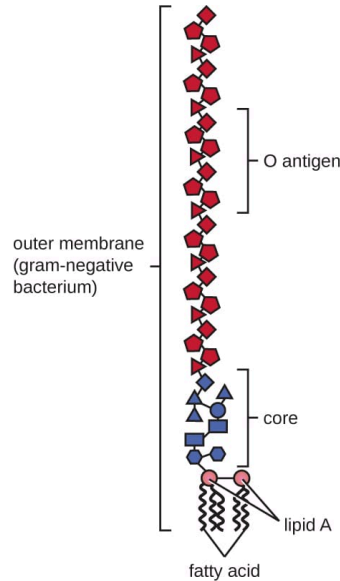


Figure 4.7.17: The outer membrane of a gram-negative bacterial cell contains lipopolysaccharide (LPS), a toxin composed of Lipid A embedded in the outer membrane, a core polysaccharide, and the O side chain.

Archaeal cell wall structure differs from that of bacteria in several significant ways. First, archaeal cell walls do not contain peptidoglycan; instead, they contain a similar polymer called pseudopeptidoglycan (pseudomurein) in which NAM is replaced with a different subunit. Other archaea may have a layer of glycoproteins or polysaccharides that serves as the cell wall instead of pseudopeptidoglycan. Last, as is the case with some bacterial species, there are a few archaea that appear to lack cell walls entirely.

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Glycocalyxes and S-Layers

Although most prokaryotic cells have cell walls, some may have additional cell envelope structures exterior to the cell wall, such as glycocalyxes and S-layers. A **glycocalyx** is a sugar coat, of which there are two important types: capsules and slime layers. A **capsule** is an organized layer located outside of the cell wall and usually composed of polysaccharides or proteins (Figure 4.7.18). A **slime layer** is a less tightly organized layer that is only loosely attached to the cell wall and can be more easily washed off. Slime layers may be composed of polysaccharides, glycoproteins, or glycolipids.

Glycocalyxes allows cells to adhere to surfaces, aiding in the formation of biofilms (colonies of microbes that form in layers on surfaces). In nature, most microbes live in mixed communities within biofilms, partly because the biofilm affords them some level of protection. Biofilms generally hold water like a sponge, preventing desiccation. They also protect cells from predation and

hinder the action of antibiotics and disinfectants. All of these properties are advantageous to the microbes living in a biofilm, but they present challenges in a clinical setting, where the goal is often to eliminate microbes.

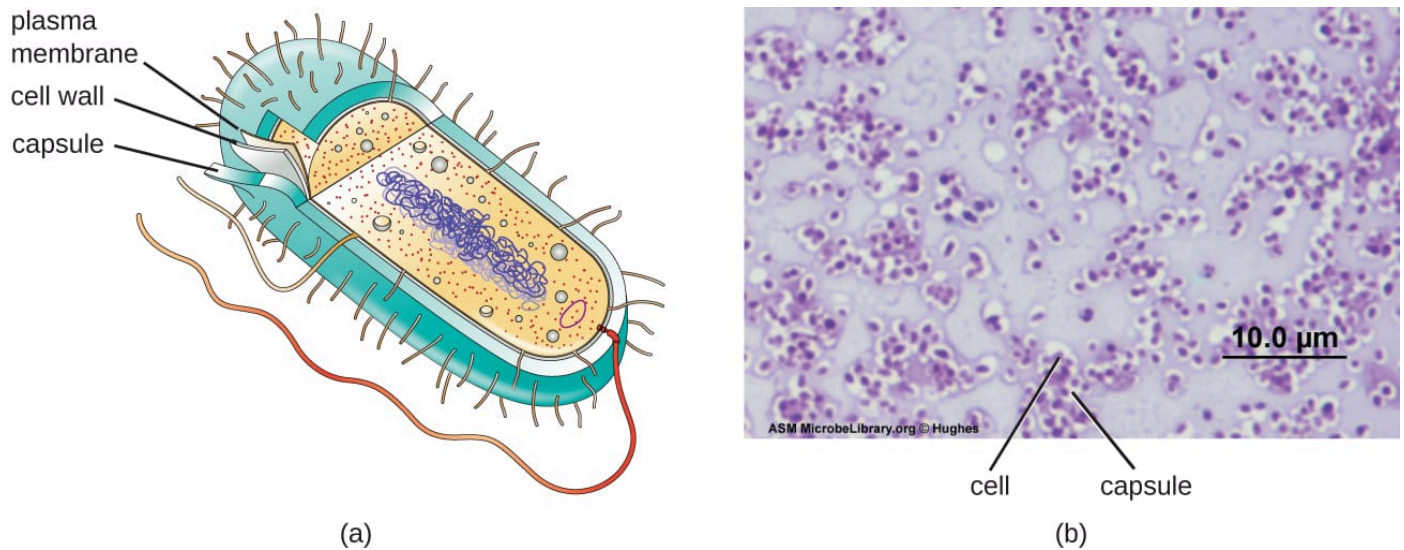


Figure 4.7.18: (a) Capsules are a type of glycocalyx composed of an organized layer of polysaccharides. (b) A capsule stain of *Pseudomonas aeruginosa*, a bacterial pathogen capable of causing many different types of infections in humans. (credit b: modification of work by American Society for Microbiology)

The ability to produce a capsule can contribute to a microbe's pathogenicity (ability to cause disease) because the capsule can make it more difficult for phagocytic cells (such as white blood cells) to engulf and kill the microorganism. *Streptococcus pneumoniae*, for example, produces a capsule that is well known to aid in this bacterium's pathogenicity. As explained in [Staining Microscopic Specimens](#), capsules are difficult to stain for microscopy; negative staining techniques are typically used.

An **S-layer** is another type of cell envelope structure; it is composed of a mixture of structural proteins and glycoproteins. In bacteria, S-layers are found outside the cell wall, but in some archaea, the S-layer serves as the cell wall. The exact function of S-layers is not entirely understood, and they are difficult to study; but available evidence suggests that they may play a variety of functions in different prokaryotic cells, such as helping the cell withstand osmotic pressure and, for certain pathogens, interacting with the host immune system.

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Filamentous Appendages

Many bacterial cells have protein appendages embedded within their cell envelopes that extend outward, allowing interaction with the environment. These appendages can attach to other surfaces, transfer DNA, or provide movement. Filamentous appendages include fimbriae, pili, and flagella.

Fimbriae and Pili

Fimbriae and pili are structurally similar and, because differentiation between the two is problematic, these terms are often used interchangeably.^{7 8} The term **fimbriae** commonly refers to short bristle-like proteins projecting from the cell surface by the hundreds. Fimbriae enable a cell to attach to surfaces and to other cells. For pathogenic bacteria, adherence to host cells is important for colonization, infectivity, and virulence. Adherence to surfaces is also important in biofilm formation.

The term **pili** (singular: pilus) commonly refers to longer, less numerous protein appendages that aid in attachment to surfaces (Figure 4.7.19). A specific type of pilus, called the **F pilus** or **sex pilus**, is important in the transfer of DNA between bacterial cells, which occurs between members of the same generation when two cells physically transfer or exchange parts of their respective genomes (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)).

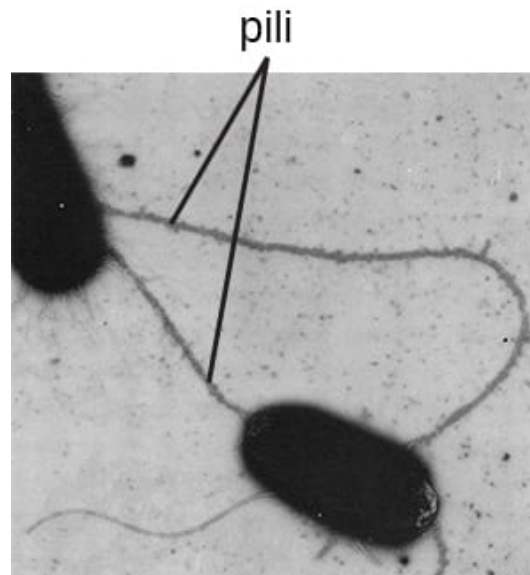


Figure 4.7.19: Bacteria may produce two different types of protein appendages that aid in surface attachment. Fimbriae typically are more numerous and shorter, whereas pili (shown here) are longer and less numerous per cell. (credit: modification of work by American Society for Microbiology)

Group A Strep

Before the structure and function of the various components of the bacterial cell envelope were well understood, scientists were already using cell envelope characteristics to classify bacteria. In 1933, Rebecca Lancefield proposed a method for serotyping various β -hemolytic strains of *Streptococcus* species using an agglutination assay, a technique using the clumping of bacteria to detect specific cell-surface antigens. In doing so, Lancefield discovered that one group of *S. pyogenes*, found in Group A, was associated with a variety of human diseases. She determined that various strains of Group A strep could be distinguished from each other based on variations in specific cell surface proteins that she named M proteins.

Today, more than 80 different strains of Group A strep have been identified based on M proteins. Various strains of Group A strep are associated with a wide variety of human infections, including streptococcal pharyngitis (strep throat), impetigo, toxic shock syndrome, scarlet fever, rheumatic fever, and necrotizing fasciitis. The M protein is an important virulence factor for Group A strep, helping these strains evade the immune system. Changes in M proteins appear to alter the infectivity of a particular strain of Group A strep.

Flagella

Flagella are structures used by cells to move in aqueous environments. Bacterial flagella act like propellers. They are stiff spiral filaments composed of flagellin protein subunits that extend outward from the cell and spin in solution. The **basal body** is the motor for the flagellum and is embedded in the plasma membrane (Figure 4.7.20). A hook region connects the basal body to the filament. Gram-positive and gram-negative bacteria have different basal body configurations due to differences in cell wall structure.

Different types of motile bacteria exhibit different arrangements of flagella (Figure 4.7.21). A bacterium with a singular flagellum, typically located at one end of the cell (polar), is said to have a **monotrichous** flagellum. An example of a monotrichously flagellated bacterial pathogen is *Vibrio cholerae*, the gram-negative bacterium that causes cholera. Cells with **amphitrichous** flagella have a flagellum or tufts of flagella at each end. An example is *Spirillum minor*, the cause of spirillary (Asian) rat-bite fever or sodoku. Cells with **lophotrichous** flagella have a tuft at one end of the cell. The gram-negative bacillus *Pseudomonas aeruginosa*, an opportunistic pathogen known for causing many infections, including “swimmer’s ear” and burn wound infections, has lophotrichous flagella. Flagella that cover the entire surface of a bacterial cell are called **peritrichous** flagella. The gram-negative bacterium *E. coli* shows a peritrichous arrangement of flagella.

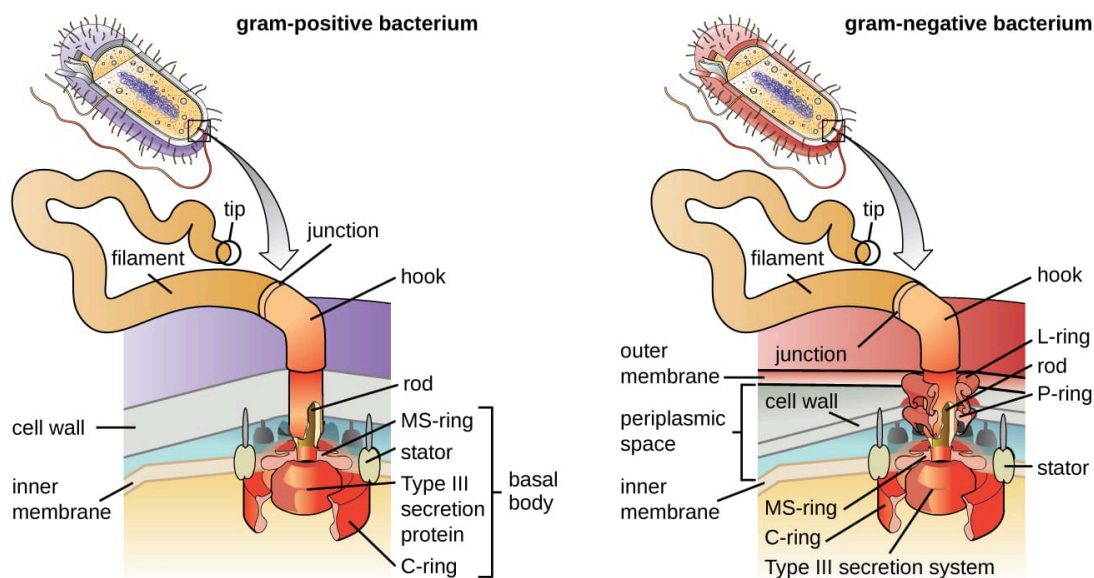


Figure 4.7.20: The basic structure of a bacterial flagellum consists of a basal body, hook, and filament. The basal body composition and arrangement differ between gram-positive and gram-negative bacteria. (credit: modification of work by “LadyofHats”/Mariana Ruiz Villareal)

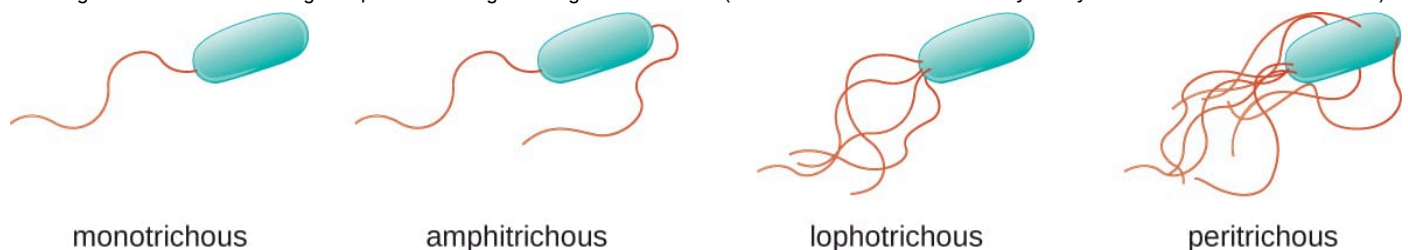


Figure 4.7.21: Flagellated bacteria may exhibit multiple arrangements of their flagella. Common arrangements include monotrichous, amphitrichous, lophotrichous, or peritrichous.

Directional movement depends on the configuration of the flagella. Bacteria can move in response to a variety of environmental signals, including light (**phototaxis**), magnetic fields (**magnetotaxis**) using magnetosomes, and, most commonly, chemical gradients (**chemotaxis**). Purposeful movement toward a chemical attractant, like a food source, or away from a repellent, like a poisonous chemical, is achieved by increasing the length of runs and decreasing the length of **tumbles**. When running, flagella rotate in a counterclockwise direction, allowing the bacterial cell to move forward. In a peritrichous bacterium, the flagella are all bundled together in a very streamlined way (Figure 4.7.22), allowing for efficient movement. When tumbling, flagella are splayed out while rotating in a clockwise direction, creating a looping motion and preventing meaningful forward movement but reorienting the cell toward the direction of the attractant. When an attractant exists, runs and tumbles still occur; however, the length of runs is longer, while the length of the tumbles is reduced, allowing overall movement toward the higher concentration of

the attractant. When no chemical gradient exists, the lengths of runs and tumbles are more equal, and overall movement is more random (Figure 4.7.23).

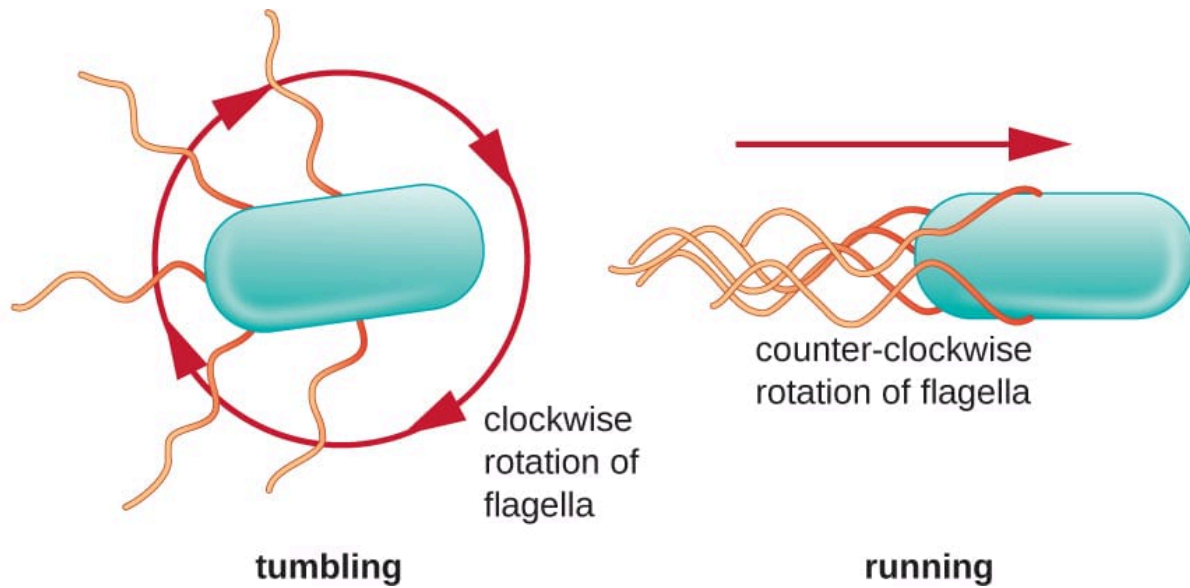


Figure 4.7.22: Bacteria achieve directional movement by changing the rotation of their flagella. In a cell with peritrichous flagella, the flagella bundle when they rotate in a counterclockwise direction, resulting in a run. However, when the flagella rotate in a clockwise direction, the flagella are no longer bundled, resulting in tumbles.

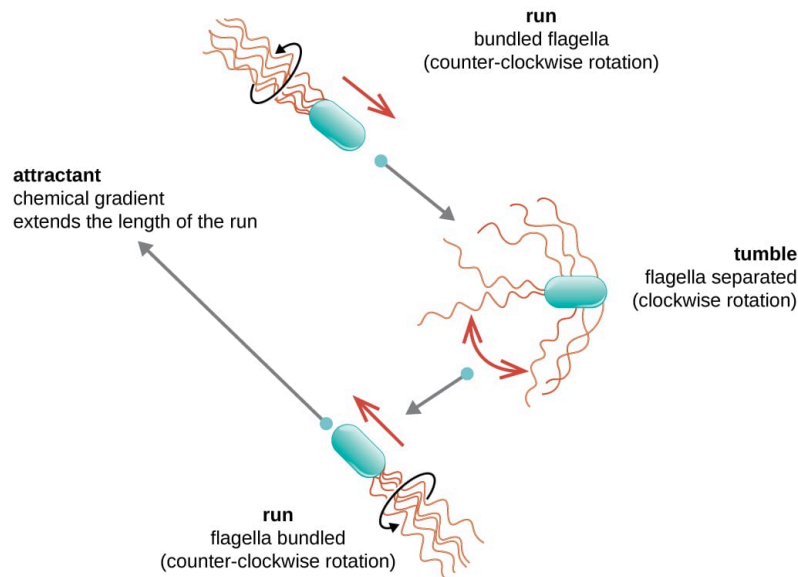


Figure 4.7.23: Without a chemical gradient, flagellar rotation cycles between counterclockwise (run) and clockwise (tumble) with no overall directional movement. However, when a chemical gradient of an attractant exists, the length of runs is extended, while the length of tumbles is decreased. This leads to chemotaxis: an overall directional movement toward the higher concentration of the attractant.

Query 4.7.1

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Key Concepts and Summary

- 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.
- Outside of the nucleoid, prokaryotic cells may contain extrachromosomal DNA in **plasmids**.
- Prokaryotic **ribosomes** that are found in the cytoplasm have a size of 70S.
- Some prokaryotic cells have **inclusions** that store nutrients or chemicals for other uses.
- Some prokaryotic cells are able to form **endospores** through **sporulation** to survive in a dormant state when conditions are unfavorable. Endospores can **germinate**, transforming back into **vegetative cells** when conditions improve.
- In prokaryotic cells, the **cell envelope** includes a **plasma membrane** and usually a cell wall.
- Bacterial membranes are composed of phospholipids with integral or peripheral proteins. The fatty acid components of these phospholipids are ester-linked and are often used to identify specific types of bacteria. The proteins serve a variety of functions, including transport, cell-to-cell communication, and sensing environmental conditions. Archaeal membranes are distinct in that they are composed of fatty acids that are ether-linked to phospholipids.
- Some molecules can move across the bacterial membrane by simple diffusion, but most large molecules must be actively transported through membrane structures using cellular energy.
- Prokaryotic cell walls may be composed of **peptidoglycan** (bacteria) or **pseudopeptidoglycan** (archaea).
- Gram-positive bacterial cells are characterized by a thick **peptidoglycan** layer, whereas gram-negative bacterial cells are characterized by a thin peptidoglycan layer surrounded by an outer membrane.
- Some prokaryotic cells produce **glycocalyx** coatings, such as **capsules** and **slime layers**, that aid in attachment to surfaces and/or evasion of the host immune system.
- Some prokaryotic cells have **fimbriae** or **pili**, filamentous appendages that aid in attachment to surfaces. Pili are also used in the transfer of genetic material between cells.
- Some prokaryotic cells use one or more **flagella** to move through water. **Peritrichous** bacteria, which have numerous flagella, use **runs** and **tumbles** to move purposefully in the direction of a chemical attractant.

Footnotes

- 1 Y.-H.M. Chan, W.F. Marshall. "Scaling Properties of Cell and Organelle Size." *Organogenesis* 6 no. 2 (2010):88–96.
- 2 F. Rothfuss, M Bender, R Conrad. "Survival and Activity of Bacteria in a Deep, Aged Lake Sediment (Lake Constance)." *Microbial Ecology* 33 no. 1 (1997):69–77.
- 3 R. Sinclair et al. "Persistence of Category A Select Agents in the Environment." *Applied and Environmental Microbiology* 74 no. 3 (2008):555–563.
- 4 T.J. Silhavy, D. Kahne, S. Walker. "The Bacterial Cell Envelope." *Cold Spring Harbor Perspectives in Biology* 2 no. 5 (2010):a000414.
- 5 B. Zuber et al. "Granular Layer in the Periplasmic Space of Gram-Positive Bacteria and Fine Structures of *Enterococcus gallinarum* and *Streptococcus gordonii* Septa Revealed by Cryo-Electron Microscopy of Vitreous Sections." *Journal of Bacteriology* 188 no. 18 (2006):6652–6660
- 6 L. Gana, S. Chena, G.J. Jensena. "Molecular Organization of Gram-Negative Peptidoglycan." *Proceedings of the National Academy of Sciences of the United States of America* 105 no. 48 (2008):18953–18957.
- 7 J.A. Garnetta et al. "Structural Insights Into the Biogenesis and Biofilm Formation by the *Escherichia coli* Common Pilus." *Proceedings of the National Academy of Sciences of the United States of America* 109 no. 10 (2012):3950–3955.
- 8 T. Proft, E.N. Baker. "Pili in Gram-Negative and Gram-Positive Bacteria—Structure, Assembly and Their Role in Disease."

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4.E: Prokaryotic Cell (Exercises)

4.1: 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?

4.2: 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?

4.3: 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

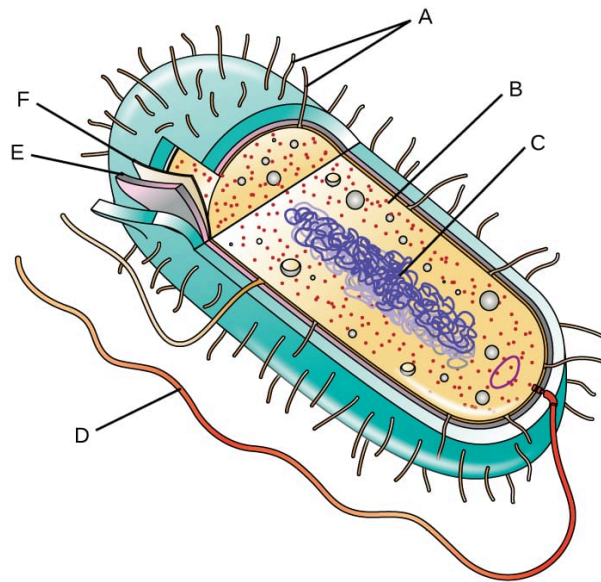
volutin (or metachromatic granule)

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

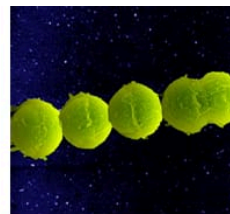
Which of the following slides is a good example of staphylococci?



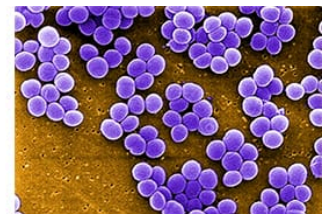
A



B



C



D

(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 botulinum*. Why might it be difficult to kill this bacterium in contaminated food?

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5: EUKARYOTIC CELL

All living organisms are made of cells, yet not all cells are the same. Among the most complex and diverse are *eukaryotic cells*, which form the building blocks of animals, plants, fungi, and protists. Unlike prokaryotic cells, eukaryotic cells contain a membrane-bound nucleus that houses their genetic material, as well as a range of specialized internal structures, or organelles, that perform distinct functions. These compartments allow eukaryotic cells to carry out intricate processes such as energy production, intracellular transport, and coordinated responses to environmental signals.

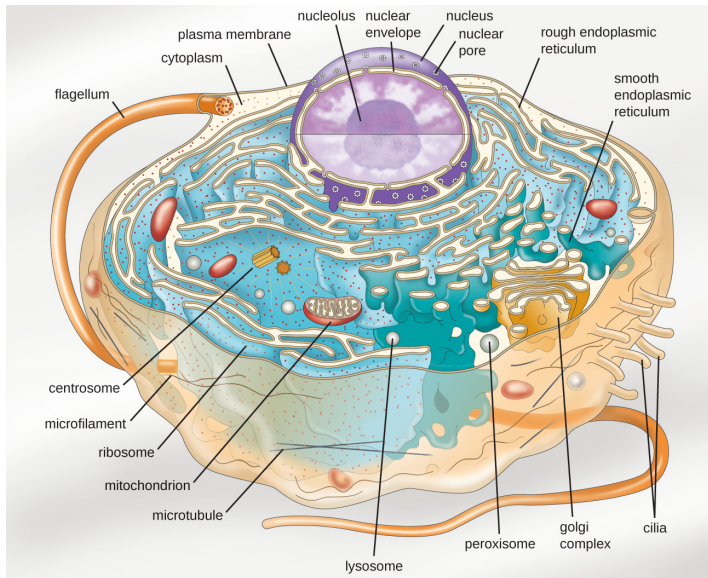


Figure 5.1: An illustration of a generalized, single-celled eukaryotic organism. Note that cells of eukaryotic organisms vary greatly in terms of structure and function, and a particular cell may not have all of the structures shown here.

Eukaryotic cells are incredibly varied in size, shape, and complexity—from single-celled organisms that swim through aquatic environments to multicellular organisms with billions of cells working in concert. (credit a, d: modification of work by CDC; credit b: modification of work by University of Edinburgh; credit c, e: modification of work by NIH)

In this chapter, we will explore the key features of eukaryotic cells, including their structure, function, and evolutionary origin. We will examine how compartmentalization contributes to cellular specialization, and how eukaryotic microbes both support ecosystems and impact human health.

5.1: Nucleus and the Endomembrane System

5.2: Cytoskeleton

5.3: Mitochondria and Chloroplasts

5.4: Cell Boundary and External Structures of Eukaryotic Cells

5.E: Eukaryotic Cell (Exercises)

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5.1: Nucleus and the Endomembrane System

Learning Objectives

- Explain the distinguishing characteristics of eukaryotic cells
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Identify and describe structures and organelles unique to eukaryotic cells
- Compare and contrast similar structures found in prokaryotic and eukaryotic cells

Eukaryotic organisms include protozoans, algae, fungi, plants, and animals. Some eukaryotic cells are independent, single-celled microorganisms, whereas others are part of multicellular organisms. The cells of eukaryotic organisms have several distinguishing characteristics. Above all, eukaryotic cells are defined by the presence of a **nucleus** surrounded by a complex nuclear membrane. Also, eukaryotic cells are characterized by the presence of **membrane-bound organelles** in the cytoplasm. Organelles such as mitochondria, the endoplasmic reticulum (ER), Golgi apparatus, lysosomes, and peroxisomes are held in place by the **cytoskeleton**, an internal network that supports transport of intracellular components and helps maintain cell shape (Figure 5.1.1). The genome of eukaryotic cells is packaged in multiple, rod-shaped chromosomes as opposed to the single, circular-shaped chromosome that characterizes most prokaryotic cells. Table 5.1.1 compares the characteristics of eukaryotic cell structures with those of bacteria and archaea.

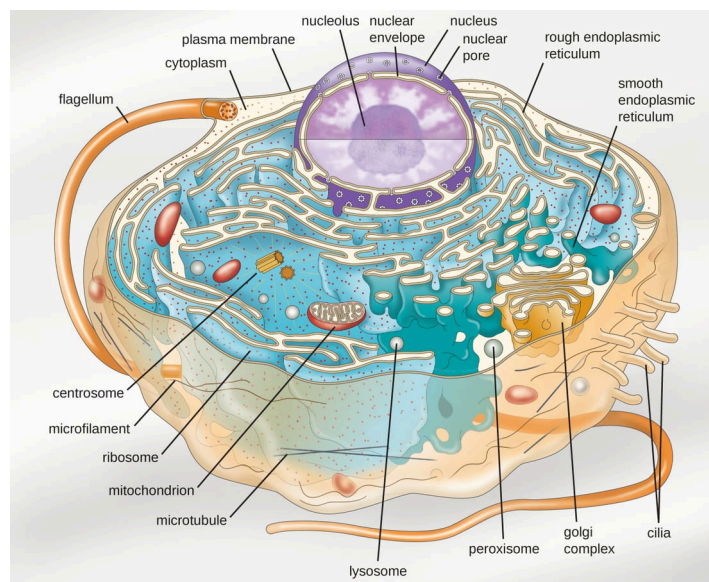


Figure 5.1.1: An illustration of a generalized, single-celled eukaryotic organism. Note that cells of eukaryotic organisms vary greatly in terms of structure and function, and a particular cell may not have all of the structures shown here.

Table 5.1.1: Summary of Cell Structures.

Cell Structure	Prokaryotes		Eukaryotes
	Bacteria	Archaea	
Size	~0.5–1 μM	~0.5–1 μM	~5–20 μM
Surface area-to-volume ratio	High	High	Low
Nucleus	No	No	Yes

Cell Structure	Prokaryotes		
	Bacteria	Archaea	Eukaryotes
Genome characteristics	<ul style="list-style-type: none"> • Single chromosome • Circular • Haploid • Lacks histones 	<ul style="list-style-type: none"> • Single chromosome • Circular • Haploid • Contains histones 	<ul style="list-style-type: none"> • Multiple chromosomes • Linear • Haploid or diploid • Contains histones
Cell division	Binary fission	Binary fission	Mitosis, meiosis
Membrane lipid composition	<ul style="list-style-type: none"> • Ester-linked • Straight-chain fatty acids • Bilayer 	<ul style="list-style-type: none"> • Ether-linked • Branched isoprenoids • Bilayer or monolayer 	<ul style="list-style-type: none"> • Ester-linked • Straight-chain fatty acids • Sterols • Bilayer
Cell wall composition	<ul style="list-style-type: none"> • Peptidoglycan, or • None 	<ul style="list-style-type: none"> • Pseudopeptidoglycan, or • Glycopeptide, or • Polysaccharide, or • Protein (S-layer), or • None 	<ul style="list-style-type: none"> • Cellulose (plants, some algae) • Chitin (molluscs, insects, crustaceans, and fungi) • Silica (some algae) • Most others lack cell walls
Motility structures	Rigid spiral flagella composed of flagellin	Rigid spiral flagella composed of archaeal flagellins	Flexible flagella and cilia composed of microtubules
Membrane-bound organelles	No	No	Yes
Endomembrane system	No	No	Yes (ER, Golgi, lysosomes)
Ribosomes	70S	70S	<ul style="list-style-type: none"> • 80S in cytoplasm and rough ER • 70S in mitochondria, chloroplasts

Query 5.1.1

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Cell Morphologies

Eukaryotic cells display a wide variety of different cell morphologies. Possible shapes include spheroid, ovoid, cuboidal, cylindrical, flat, lenticular, fusiform, discoidal, crescent, ring stellate, and polygonal (Figure 5.1.2). Some eukaryotic cells are irregular in shape, and some are capable of changing shape. The shape of a particular type of eukaryotic cell may be influenced by factors such as its primary function, the organization of its cytoskeleton, the viscosity of its cytoplasm, the rigidity of its cell membrane or cell wall (if it has one), and the physical pressure exerted on it by the surrounding environment and/or adjoining cells.

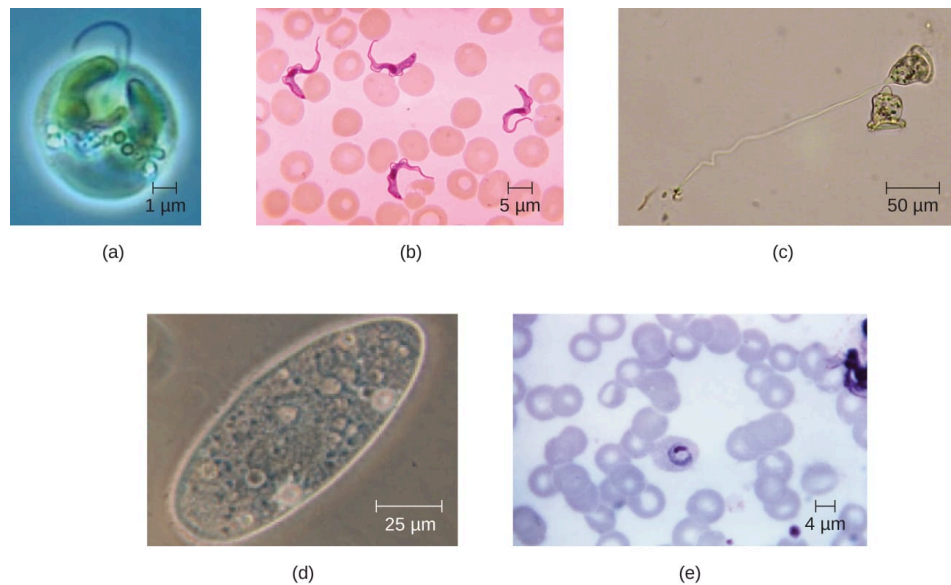


Figure 5.1.2: Eukaryotic cells come in a variety of cell shapes. (a) Spheroid *Chromulina* alga. (b) Fusiform shaped *Trypanosoma*. (c) Bell-shaped *Vorticella*. (d) Ovoid *Paramecium*. (e) Ring-shaped *Plasmodium ovale*. (credit a: modification of work by NOAA; credit b, e: modification of work by Centers for Disease Control and Prevention).

Nucleus

Unlike prokaryotic cells, in which DNA is loosely contained in the nucleoid region, eukaryotic cells possess a **nucleus**, which is surrounded by a complex nuclear membrane that houses the DNA genome (Figure 5.1.3). By containing the cell's DNA, the nucleus ultimately controls all activities of the cell and also serves an essential role in reproduction and heredity. Eukaryotic cells typically have their DNA organized into multiple linear chromosomes. The DNA within the nucleus is highly organized and condensed to fit inside the nucleus, which is accomplished by wrapping the DNA around proteins called histones.

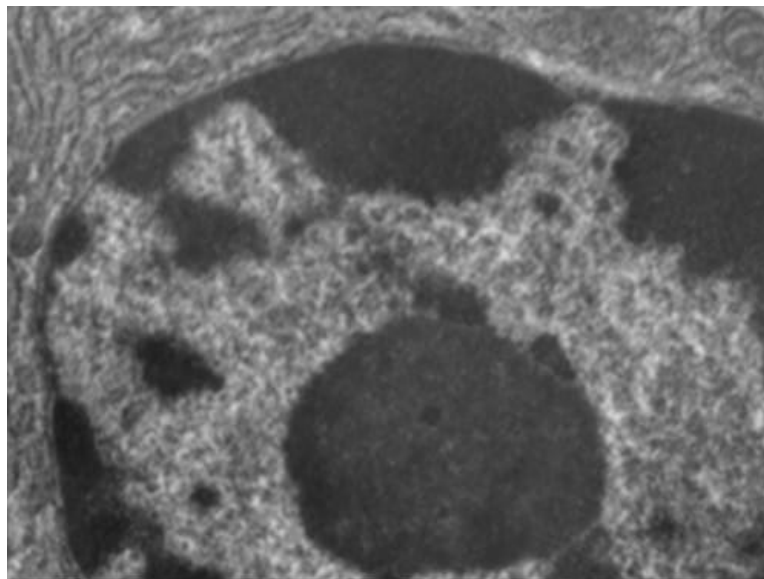


Figure 5.1.3: Eukaryotic cells have a well-defined nucleus. The nucleus of this mammalian lung cell is the large, dark, oval-shaped structure in the lower half of the image.

Although most eukaryotic cells have only one nucleus, exceptions exist. For example, protozoans of the genus *Paramecium* typically have two complete nuclei: a small nucleus that is used for reproduction (micronucleus) and a large nucleus that directs cellular metabolism (macronucleus). Additionally, some fungi transiently form cells with two nuclei, called heterokaryotic cells, during sexual reproduction. Cells whose nuclei divide, but whose cytoplasm does not, are called coenocytes.

The nucleus is bound by a complex **nuclear membrane**, often called the **nuclear envelope**, that consists of two distinct lipid bilayers that are contiguous with each other (Figure 5.1.4). Despite these connections between the inner and outer membranes, each membrane contains unique lipids and proteins on its inner and outer surfaces. The nuclear envelope contains nuclear pores, which are large, rosette-shaped protein complexes that control the movement of materials into and out of the nucleus. The overall shape of the nucleus is determined by the **nuclear lamina**, a meshwork of intermediate filaments found just inside the nuclear envelope membranes. Outside the nucleus, additional intermediate filaments form a looser mesh and serve to anchor the nucleus in position within the cell.

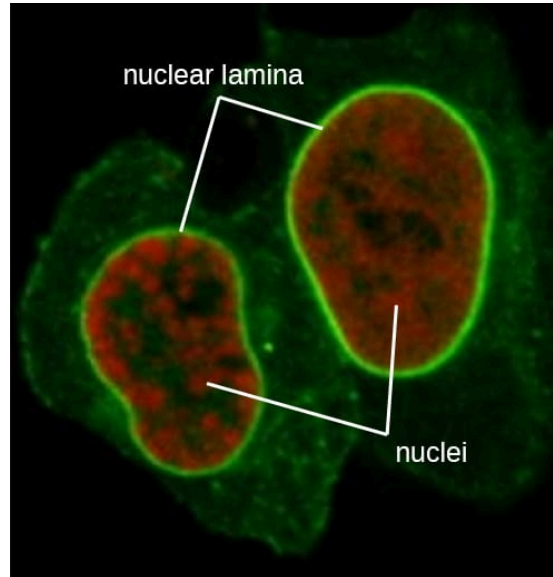


Figure 5.1.4: In this fluorescent microscope image, all the intermediate filaments have been stained with a bright green fluorescent stain. The nuclear lamina is the intense bright green ring around the faint red nuclei.

Nucleolus

The **nucleolus** is a dense region within the nucleus where ribosomal RNA (rRNA) biosynthesis occurs. In addition, the nucleolus is also the site where assembly of ribosomes begins. Preribosomal complexes are assembled from rRNA and proteins in the nucleolus; they are then transported out to the cytoplasm, where ribosome assembly is completed (Figure 5.1.5).

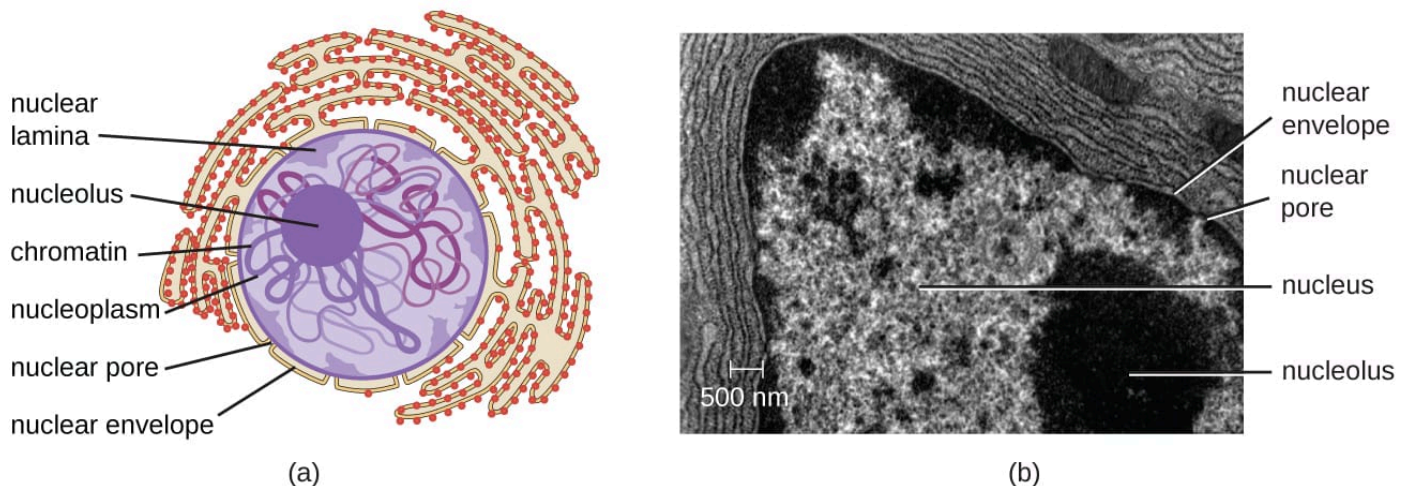


Figure 5.1.5: (a) The nucleolus is the dark, dense area within the nucleus. It is the site of rRNA synthesis and preribosomal assembly. (b) Electron micrograph showing the nucleolus.

Query 5.1.1

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Ribosomes

Ribosomes found in eukaryotic organelles such as mitochondria or chloroplasts have 70S ribosomes—the same size as prokaryotic ribosomes. However, nonorganelle-associated ribosomes in eukaryotic cells are **80S ribosomes**, composed of a 40S small subunit and a 60S large subunit. In terms of size and composition, this makes them distinct from the ribosomes of prokaryotic cells.

The two types of nonorganelle-associated eukaryotic ribosomes are defined by their location in the cell: **free ribosomes** and **membrane-bound ribosomes**. Free ribosomes are found in the cytoplasm and serve to synthesize water-soluble proteins; membrane-bound ribosomes are found attached to the rough endoplasmic reticulum and make proteins for insertion into the cell membrane or proteins destined for export from the cell.

The differences between eukaryotic and prokaryotic ribosomes are clinically relevant because certain antibiotic drugs are designed to target one or the other. For example, cycloheximide targets eukaryotic action, whereas chloramphenicol targets prokaryotic ribosomes.¹ Since human cells are eukaryotic, they generally are not harmed by antibiotics that destroy the prokaryotic ribosomes in bacteria. However, sometimes negative side effects may occur because mitochondria in human cells contain prokaryotic ribosomes.

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Endomembrane System

The **endomembrane system**, unique to eukaryotic cells, is a series of membranous tubules, sacs, and flattened disks that synthesize many cell components and move materials around within the cell (Figure 5.1.6). Because of their larger cell size, eukaryotic cells require this system to transport materials that cannot be dispersed by diffusion alone. The endomembrane system comprises several organelles and connections between them, including the endoplasmic reticulum, Golgi apparatus, lysosomes, and vesicles.

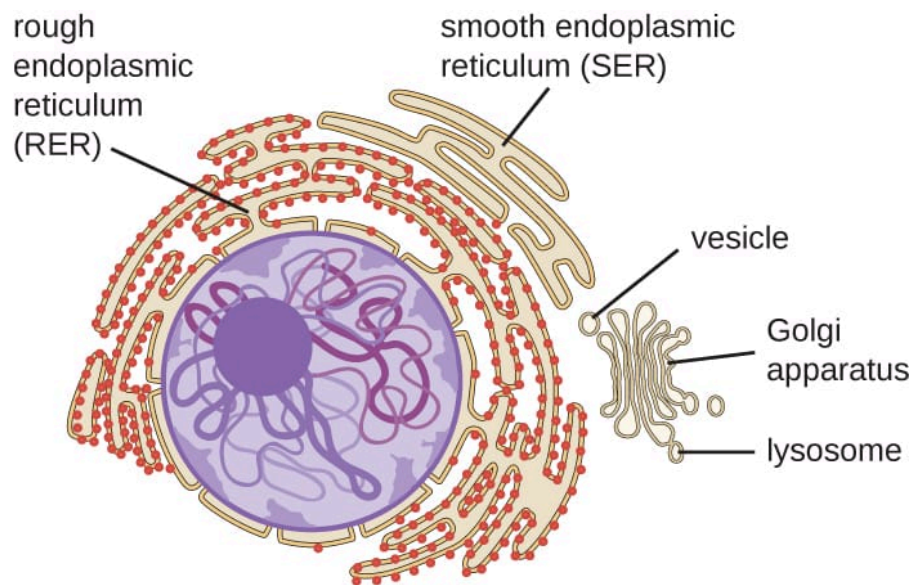


Figure 5.1.6: The endomembrane system is composed of a series of membranous intracellular structures that facilitate movement of materials throughout the cell and to the cell membrane.

Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** is an interconnected array of tubules and **cisternae** (flattened sacs) with a single lipid bilayer (Figure 5.1.7). The spaces inside of the cisternae are called **lumen** of the ER. There are two types of ER, **rough endoplasmic reticulum (RER)** and **smooth endoplasmic reticulum (SER)**. These two different types of ER are sites for the synthesis of distinctly different types of molecules. RER is studded with ribosomes bound on the cytoplasmic side of the membrane. These ribosomes make proteins destined for the plasma membrane (Figure). Following synthesis, these proteins are inserted into the membrane of the RER. Small sacs of the RER containing these newly synthesized proteins then bud off as **transport vesicles** and move either to the Golgi apparatus for further processing, directly to the plasma membrane, to the membrane of another organelle, or out of the cell. Transport vesicles are single-lipid, bilayer, membranous spheres with hollow interiors that carry molecules. SER does not have ribosomes and, therefore, appears “smooth.” It is involved in biosynthesis of lipids, carbohydrate metabolism, and detoxification of toxic compounds within the cell.

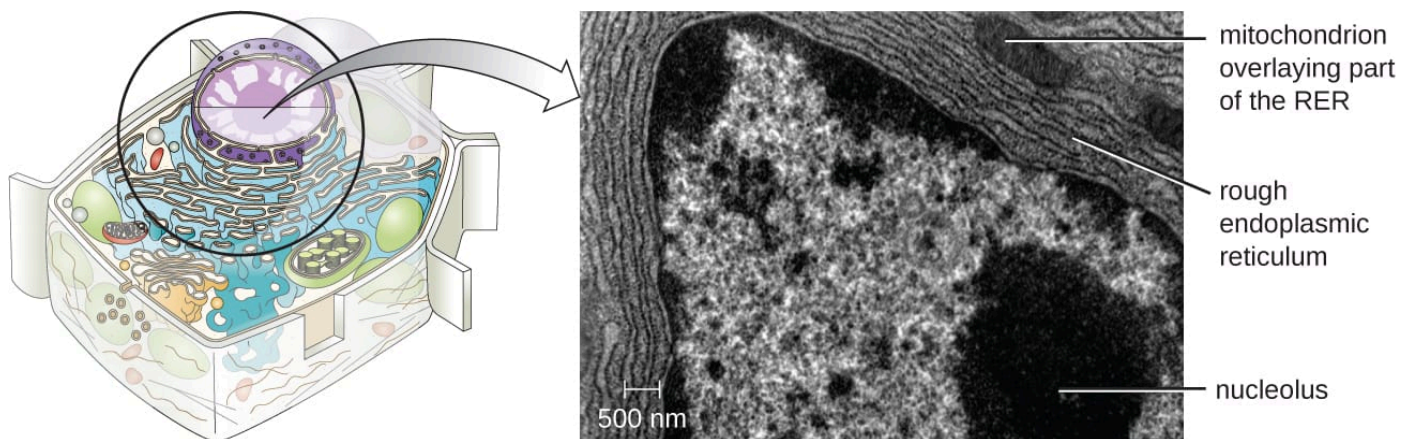


Figure 5.1.7: The rough endoplasmic reticulum is studded with ribosomes for the synthesis of membrane proteins (which give it its rough appearance).

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Golgi Apparatus

The **Golgi apparatus** was discovered within the endomembrane system in 1898 by Italian scientist Camillo Golgi (1843–1926), who developed a novel staining technique that showed stacked membrane structures within the cells of *Plasmodium*, the causative agent of malaria. The Golgi apparatus is composed of a series of membranous disks called dictyosomes, each having a single lipid bilayer, that are stacked together (Figure 5.1.8).

Enzymes in the Golgi apparatus modify lipids and proteins transported from the ER to the Golgi, often adding carbohydrate components to them, producing glycolipids, glycoproteins, or proteoglycans. Glycolipids and glycoproteins are often inserted into the plasma membrane and are important for signal recognition by other cells or infectious particles. Different types of cells can be distinguished from one another by the structure and arrangement of the glycolipids and glycoproteins contained in their plasma membranes. These glycolipids and glycoproteins commonly also serve as cell surface receptors.

Transport vesicles leaving the ER fuse with a Golgi apparatus on its receiving, or *cis*, face. The proteins are processed within the Golgi apparatus, and then additional transport vesicles containing the modified proteins and lipids pinch off from the Golgi apparatus on its outgoing, or *trans*, face. These outgoing vesicles move to and fuse with the plasma membrane or the membrane of other organelles.

Exocytosis is the process by which **secretory vesicles** (spherical membranous sacs) release their contents to the cell's exterior (Figure 5.1.8). All cells have constitutive secretory pathways in which secretory vesicles transport soluble proteins that are released from the cell continually (constitutively). Certain specialized cells also have regulated secretory pathways, which are used to store soluble proteins in secretory vesicles. Regulated secretion involves substances that are only released in response to certain events or signals. For example, certain cells of the human immune system (e.g., mast cells) secrete histamine in response to the presence of foreign objects or pathogens in the body. Histamine is a compound that triggers various mechanisms used by the immune system to eliminate pathogens.

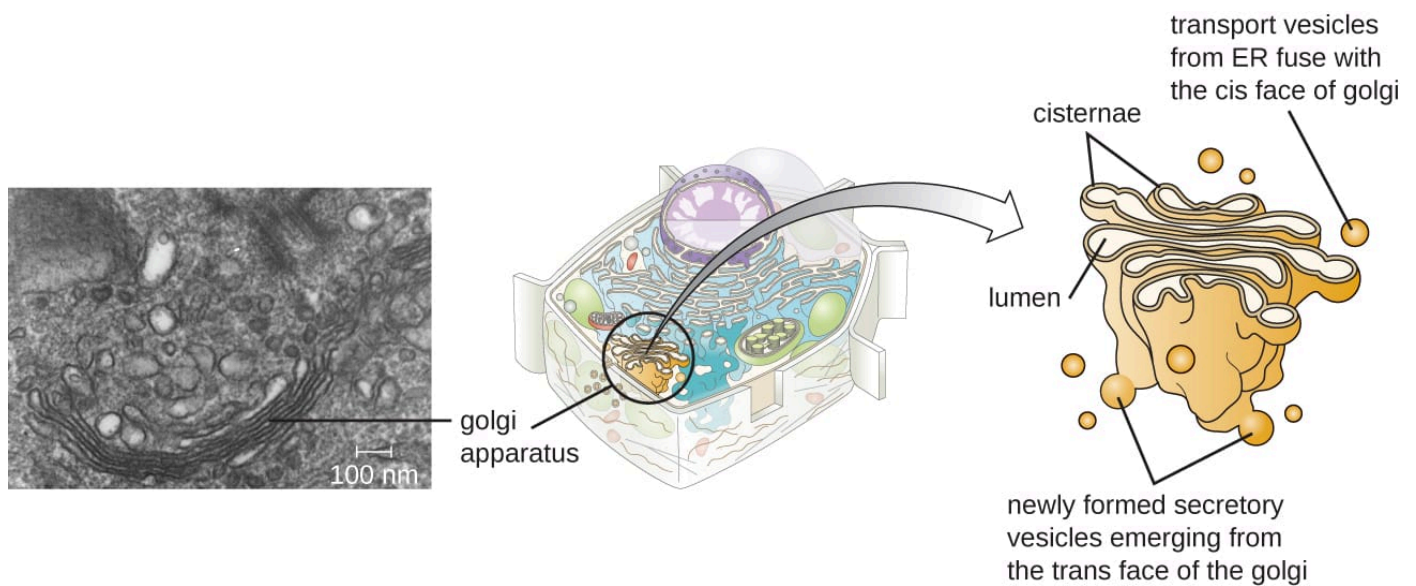


Figure 5.1.8: A transmission electron micrograph (left) of a Golgi apparatus in a white blood cell. The illustration (right) shows the cup-shaped, stacked disks and several transport vesicles. The Golgi apparatus modifies lipids and proteins, producing glycolipids and glycoproteins, respectively, which are commonly inserted into the plasma membrane.

Lysosomes

In the 1960s, Belgian scientist Christian de Duve (1917–2013) discovered **lysosomes**, membrane-bound organelles of the endomembrane system that contain digestive enzymes. Certain types of eukaryotic cells use lysosomes to break down various particles, such as food, damaged organelles or cellular debris, microorganisms, or immune complexes. Compartmentalization of the digestive enzymes within the lysosome allows the cell to efficiently digest matter without harming the cytoplasmic components of the cell.

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Peroxisomes

Christian de Duve is also credited with the discovery of **peroxisomes**, membrane-bound organelles that are not part of the endomembrane system (Figure 5.1.9). Peroxisomes form independently in the cytoplasm from the synthesis of peroxin proteins by free ribosomes and the incorporation of these peroxin proteins into existing peroxisomes. Growing peroxisomes then divide by a process similar to binary fission.

Peroxisomes were first named for their ability to produce hydrogen peroxide, a highly reactive molecule that helps to break down molecules such as uric acid, amino acids, and fatty acids. Peroxisomes also possess the enzyme catalase, which can degrade hydrogen peroxide. Along with the SER, peroxisomes also play a role in lipid biosynthesis. Like lysosomes, the compartmentalization of these degradative molecules within an organelle helps protect the cytoplasmic contents from unwanted damage.

The peroxisomes of certain organisms are specialized to meet their particular functional needs. For example, glyoxysomes are modified peroxisomes of yeasts and plant cells that perform several metabolic functions, including the production of sugar molecules. Similarly, glycosomes are modified peroxisomes made by certain trypanosomes, the pathogenic protozoans that cause Chagas disease and African sleeping sickness.

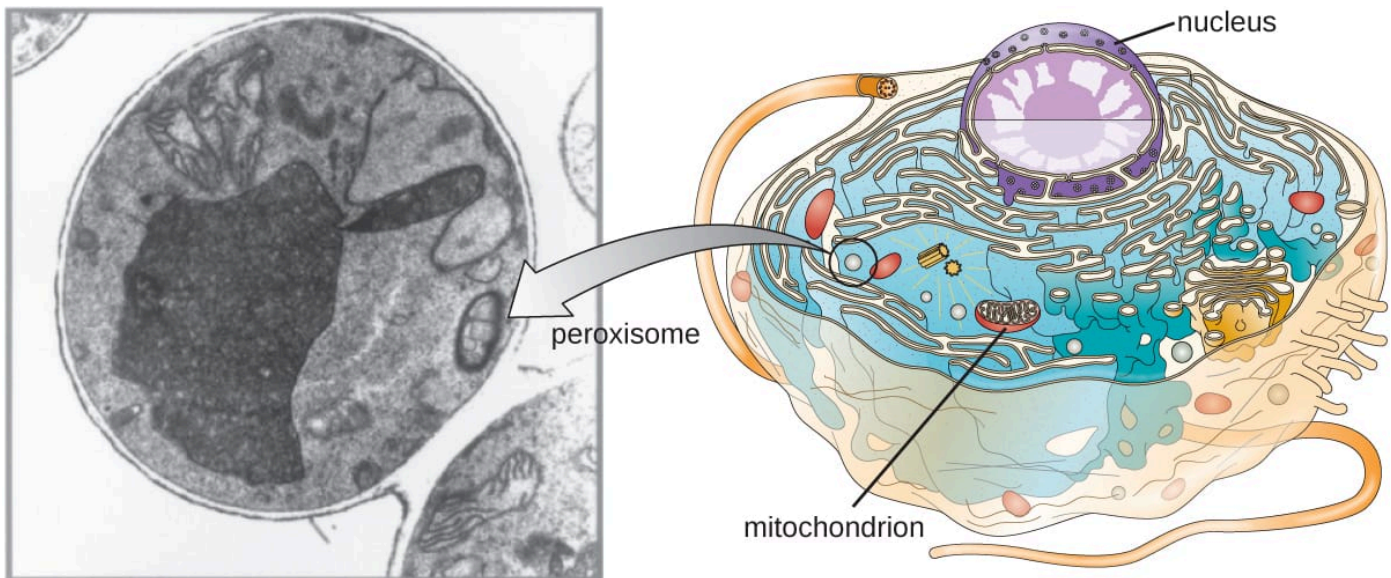


Figure 5.1.9: A transmission electron micrograph (left) of a cell containing a peroxisome. The illustration (right) shows the location of peroxisomes in a cell. These eukaryotic structures play a role in lipid biosynthesis and breaking down various molecules. They may also have other specialized functions depending on the cell type. (credit "micrograph": modification of work by American Society for Microbiology).

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- 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**, located in the nucleus of eukaryotic cells, is the site of ribosomal synthesis and the first stages of ribosome assembly.
- Eukaryotic cells contain **80S ribosomes** in the rough endoplasmic reticulum (**membrane bound-ribosomes**) and cytoplasm (**free ribosomes**). They contain 70s ribosomes in mitochondria and chloroplasts.
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- The **smooth endoplasmic reticulum** plays a role in lipid biosynthesis, carbohydrate metabolism, and detoxification of toxic compounds. The **rough endoplasmic reticulum** contains membrane-bound 80S ribosomes that synthesize proteins destined for the cell membrane
- The **Golgi apparatus** processes proteins and lipids, typically through the addition of sugar molecules, producing glycoproteins or glycolipids, components of the plasma membrane that are used in cell-to-cell communication.
- Lysosomes** contain digestive enzymes that break down small particles ingested by **endocytosis**, large particles or cells ingested by **phagocytosis**, and damaged intracellular components.
- The **cytoskeleton**, composed of **microfilaments**, **intermediate filaments**, and **microtubules**, provides structural support in eukaryotic cells and serves as a network for transport of intracellular materials.
- Centrosomes** are microtubule-organizing centers important in the formation of the mitotic spindle in mitosis.
- Mitochondria** are the site of cellular respiration. They have two membranes: an outer membrane and an inner membrane

with cristae. The mitochondrial matrix, within the inner membrane, contains the mitochondrial DNA, 70S ribosomes, and metabolic enzymes.

- The plasma membrane of eukaryotic cells is structurally similar to that found in prokaryotic cells, and membrane components move according to the fluid mosaic model. However, eukaryotic membranes contain sterols, which alter membrane fluidity, as well as glycoproteins and glycolipids, which help the cell recognize other cells and infectious particles.
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Footnotes

- 1 A.E. Barnhill, M.T. Brewer, S.A. Carlson. "Adverse Effects of Antimicrobials via Predictable or Idiosyncratic Inhibition of Host Mitochondrial Components." *Antimicrobial Agents and Chemotherapy* 56 no. 8 (2012):4046–4051.
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- 5 N. Yarlett, J.H.P. Hackstein. "Hydrogenosomes: One Organelle, Multiple Origins." *BioScience* 55 no. 8 (2005):657–658.
- 6 M. Dudzick. "Protists." OpenStax CNX. November 27, 2013. <http://cnx.org/contents/f7048bb6-e46...ef291cf7049c@1>

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5.2: Cytoskeleton

Learning Objectives

- Explain the distinguishing characteristics of eukaryotic cells
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Identify and describe structures and organelles unique to eukaryotic cells
- Compare and contrast similar structures found in prokaryotic and eukaryotic cells

Cytoskeleton

Eukaryotic cells have an internal cytoskeleton made of **microfilaments**, **intermediate filaments**, and **microtubules**. This matrix of fibers and tubes provides structural support as well as a network over which materials can be transported within the cell and on which organelles can be anchored (Figure 5.2.10). For example, the process of exocytosis involves the movement of a vesicle via the cytoskeletal network to the plasma membrane, where it can release its contents.

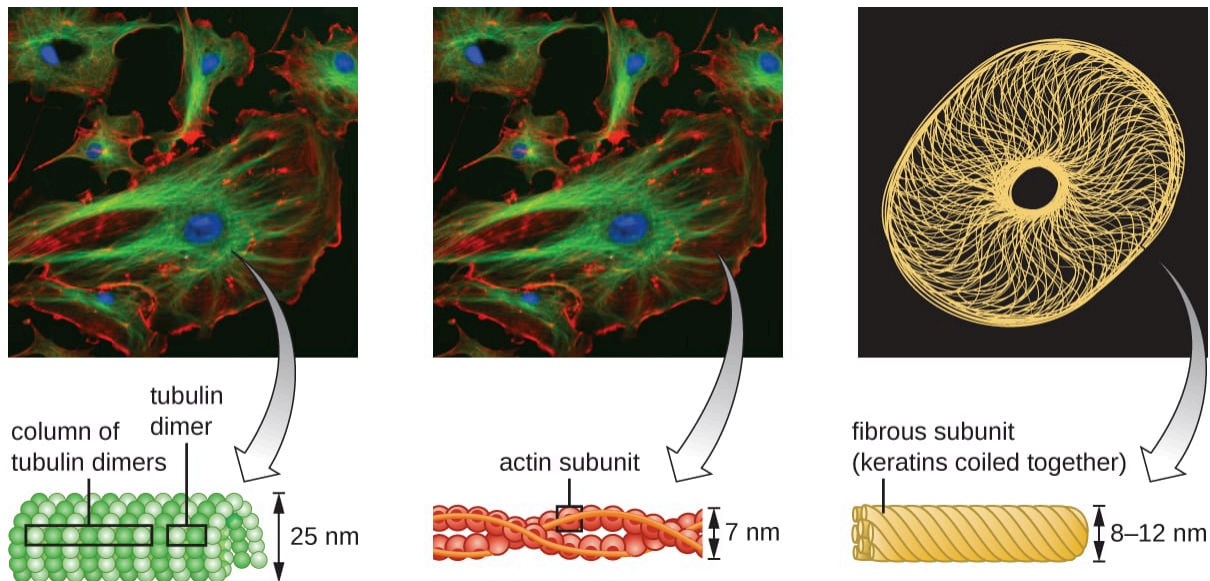


Figure 5.2.10: The cytoskeleton is a network of microfilaments, intermediate filaments, and microtubules found throughout the cytoplasm of a eukaryotic cell. In these fluorescently labeled animal cells, the microtubules are green, the actin microfilaments are red, the nucleus is blue, and keratin (a type of intermediate filament) is yellow.

Microfilaments

Microfilaments are composed of two intertwined strands of actin, each composed of **actin** monomers forming filamentous cables 6 nm in diameter² (Figure 5.2.11). The actin filaments work together with motor proteins, like myosin, to effect muscle contraction in animals or the amoeboid movement of some eukaryotic microbes. In amoeboid organisms, actin can be found in two forms: a stiffer, polymerized, gel form and a more fluid, unpolymerized soluble form. Actin in the gel form creates stability in the ectoplasm, the gel-like area of cytoplasm just inside the plasma membrane of amoeboid protozoans.

Temporary extensions of the cytoplasmic membrane called **pseudopodia** (meaning “false feet”) are produced through the forward flow of soluble actin filaments into the pseudopodia, followed by the gel-sol cycling of the actin filaments, resulting in cell motility. Once the cytoplasm extends outward, forming a pseudopodium, the remaining cytoplasm flows up to join the leading edge, thereby creating forward locomotion. Beyond amoeboid movement, microfilaments are also involved in a variety of other processes in eukaryotic cells, including cytoplasmic streaming (the movement or circulation of cytoplasm within the cell), cleavage

furrow formation during cell division, and muscle movement in animals (Figure 5.2.11). These functions are the result of the dynamic nature of microfilaments, which can polymerize and depolymerize relatively easily in response to cellular signals, and their interactions with molecular motors in different types of eukaryotic cells.

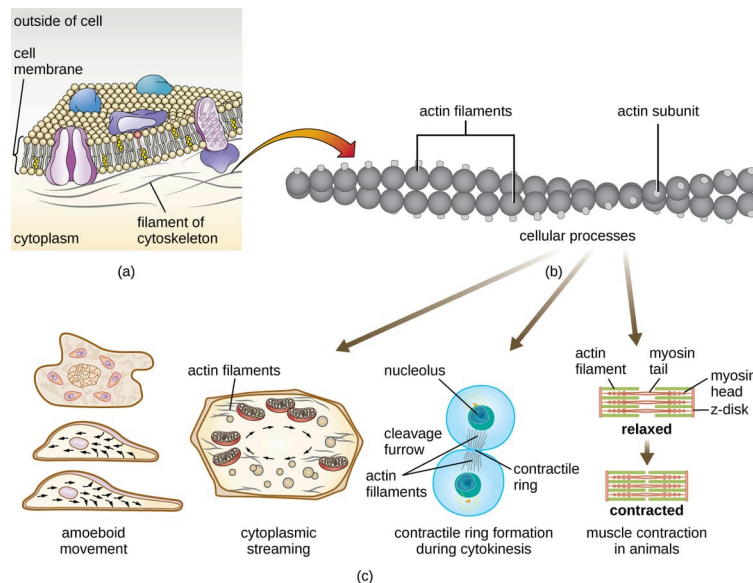


Figure 5.2.11: (a) A microfilament is composed of a pair of actin filaments. (b) Each actin filament is a string of polymerized actin monomers. (c) The dynamic nature of actin, due to its polymerization and depolymerization and its association with myosin, allows microfilaments to be involved in a variety of cellular processes, including amoeboid movement, cytoplasmic streaming, contractile ring formation during cell division, and muscle contraction in animals.

Intermediate Filaments

Intermediate filaments (Figure 5.2.12) are a diverse group of cytoskeletal filaments that act as cables within the cell. They are termed “intermediate” because their 10-nm diameter is thicker than that of actin but thinner than that of microtubules.³ They are composed of several strands of polymerized subunits that, in turn, are made up of a wide variety of monomers. Intermediate filaments tend to be more permanent in the cell and maintain the position of the nucleus. They also form the nuclear lamina (lining or layer) just inside the nuclear envelope. Additionally, intermediate filaments play a role in anchoring cells together in animal tissues. The intermediate filament protein desmin is found in desmosomes, the protein structures that join muscle cells together and help them resist external physical forces. The intermediate filament protein keratin is a structural protein found in hair, skin, and nails.

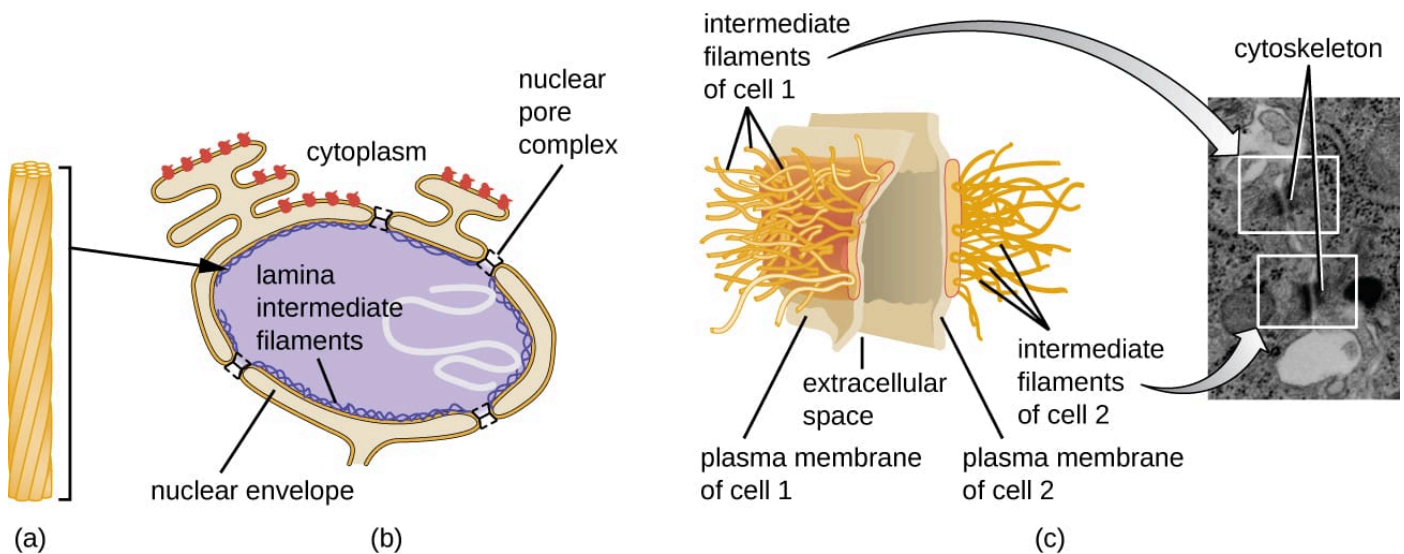


Figure 5.2.12: (a) Intermediate filaments are composed of multiple strands of polymerized subunits. They are more permanent than other cytoskeletal structures and serve a variety of functions. (b) Intermediate filaments form much of the nuclear lamina. (c) Intermediate filaments form the desmosomes between cells in some animal tissues. (credit c "illustration": modification of work by Mariana Ruiz Villareal)

Microtubules

Microtubules (Figure 5.2.13) are a third type of cytoskeletal fiber composed of tubulin dimers (α tubulin and β tubulin). These form hollow tubes 23 nm in diameter that are used as girders within the cytoskeleton.⁴ Like microfilaments, microtubules are dynamic and have the ability to rapidly assemble and disassemble. Microtubules also work with motor proteins (such as dynein and kinesin) to move organelles and vesicles around within the cytoplasm. Additionally, microtubules are the main components of eukaryotic flagella and cilia, composing both the filament and the basal body components (Figure 5.2.20).

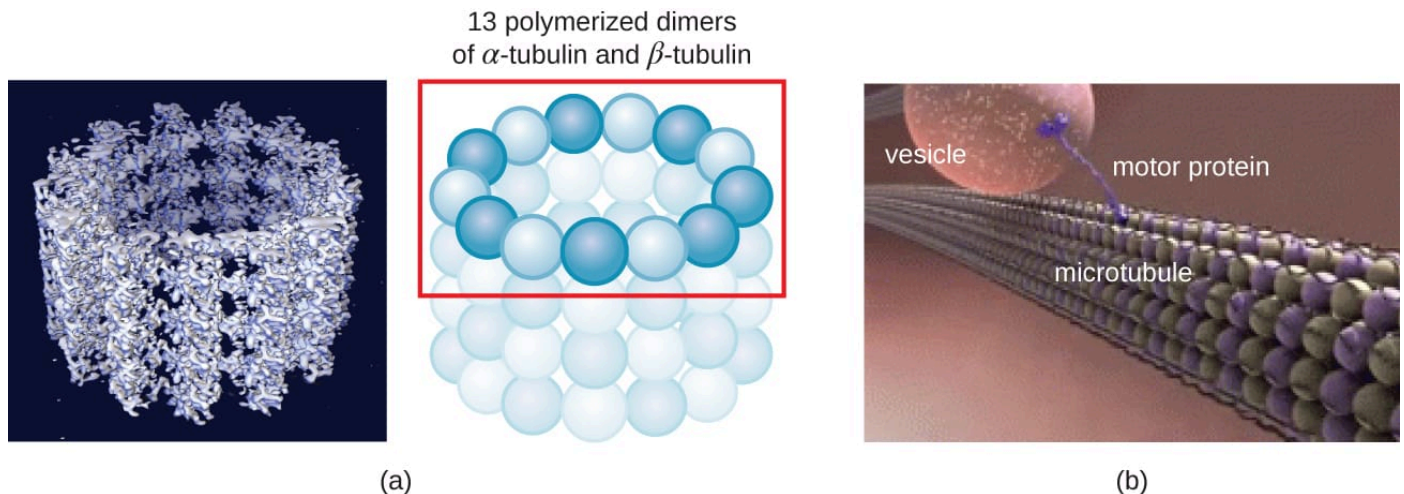


Figure 5.2.13: (a) Microtubules are hollow structures composed of polymerized tubulin dimers. (b) They are involved in several cellular processes, including the movement of organelles throughout the cytoplasm. Motor proteins carry organelles along microtubule tracks that crisscross the entire cell. (credit b: modification of work by National Institute on Aging)

In addition, microtubules are involved in cell division, forming the mitotic spindle that serves to separate chromosomes during mitosis and meiosis. The mitotic spindle is produced by two centrosomes, which are essentially microtubule-organizing centers, at opposite ends of the cell. Each centrosome is composed of a pair of centrioles positioned at right angles to each other, and each centriole is an array of nine parallel microtubules arranged in triplets (Figure 5.2.14).

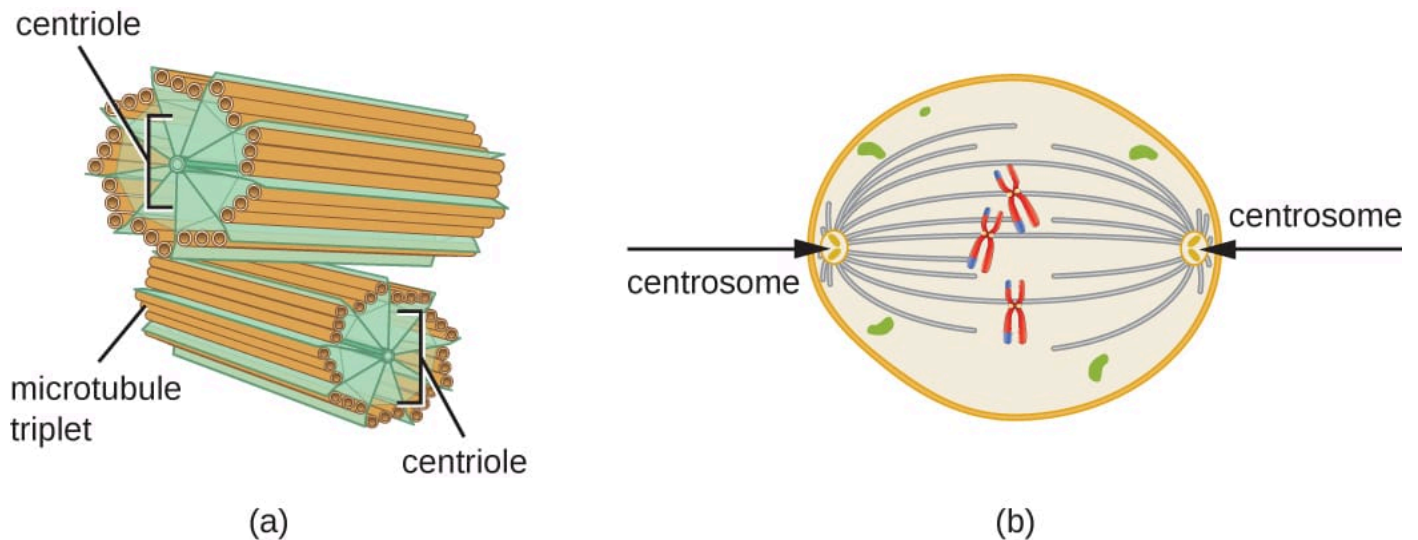


Figure 5.2.14: (a) A centrosome is composed of two centrioles positioned at right angles to each other. Each centriole is composed of nine triplets of microtubules held together by accessory proteins. (b) In animal cells, the centrosomes (arrows) serve as microtubule-organizing centers of the mitotic spindle during mitosis.

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5.3: Mitochondria and Chloroplasts

Learning Objectives

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Mitochondria

The large, complex organelles in which aerobic cellular respiration occurs in eukaryotic cells are called **mitochondria** (Figure 5.3.15). The term “mitochondrion” was first coined by German microbiologist Carl Benda in 1898 and was later connected with the process of respiration by Otto Warburg in 1913. Scientists during the 1960s discovered that mitochondria have their own genome and 70S ribosomes. The mitochondrial genome was found to be bacterial, when it was sequenced in 1976. These findings ultimately supported the endosymbiotic theory proposed by Lynn Margulis, which states that mitochondria originally arose through an endosymbiotic event in which a bacterium capable of aerobic cellular respiration was taken up by phagocytosis into a host cell and remained as a viable intracellular component.

Each mitochondrion has two lipid membranes. The **outer membrane** is a remnant of the original host cell’s membrane structures. The **inner membrane** was derived from the bacterial plasma membrane. The electron transport chain for aerobic respiration uses integral proteins embedded in the inner membrane. The **mitochondrial matrix**, corresponding to the location of the original bacterium’s cytoplasm, is the current location of many metabolic enzymes. It also contains mitochondrial DNA and 70S ribosomes. Invaginations of the inner membrane, called **cristae**, evolved to increase surface area for the location of biochemical reactions. The folding patterns of the cristae differ among various types of eukaryotic cells and are used to distinguish different eukaryotic organisms from each other.

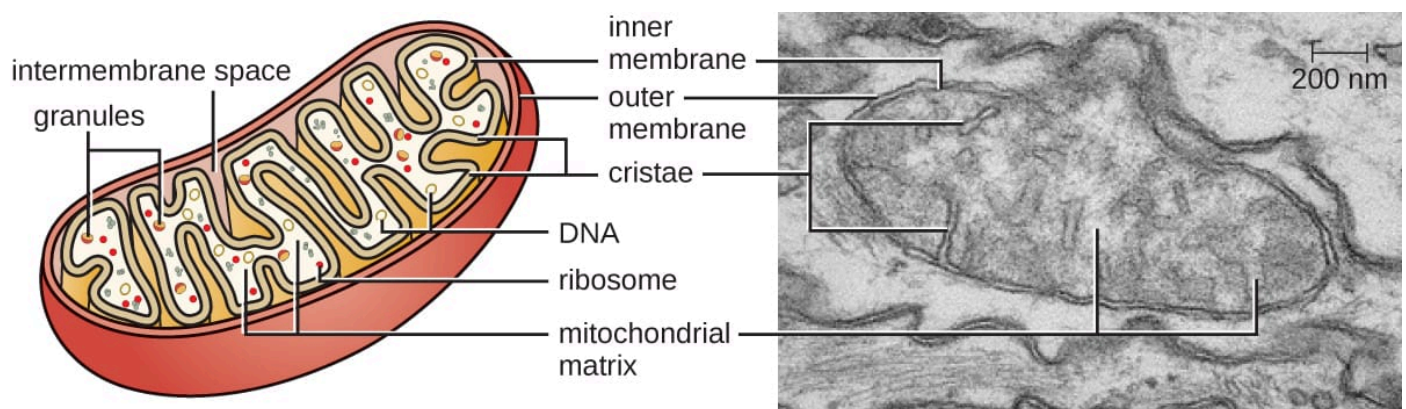


Figure 5.3.15: Each mitochondrion is surrounded by two membranes, the inner of which is extensively folded into cristae and is the site of the intermembrane space. The mitochondrial matrix contains the mitochondrial DNA, ribosomes, and metabolic enzymes. The transmission electron micrograph of a mitochondrion, on the right, shows both membranes, including cristae and the mitochondrial matrix. (credit “micrograph”: modification of work by Matthew Britton; scale-bar data from Matt Russell)

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Chloroplasts

Plant cells and algal cells contain **chloroplasts**, the organelles in which photosynthesis occurs (Figure 5.3.16). All chloroplasts have at least three membrane systems: the outer membrane, the inner membrane, and the thylakoid membrane system. Inside the outer and inner membranes is the chloroplast **stroma**, a gel-like fluid that makes up much of a chloroplast's volume, and in which the **thylakoid** system floats. The thylakoid system is a highly dynamic collection of folded membrane sacs. It is where the green photosynthetic pigment chlorophyll is found and the light reactions of photosynthesis occur. In most plant chloroplasts, the thylakoids are arranged in stacks called grana (singular: granum), whereas in some algal chloroplasts, the thylakoids are free floating.

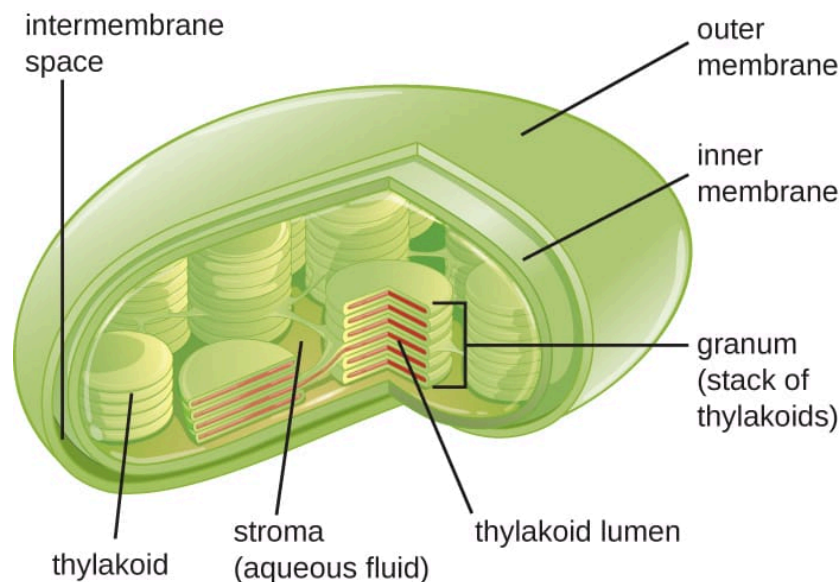


Figure 5.3.16: Photosynthesis takes place in chloroplasts, which have an outer membrane and an inner membrane. Stacks of thylakoids called grana form a third membrane layer.

Other organelles similar to mitochondria have arisen in other types of eukaryotes, but their roles differ. Hydrogenosomes are found in some anaerobic eukaryotes and serve as the location of anaerobic hydrogen production. Hydrogenosomes typically lack their own DNA and ribosomes. Kinetoplasts are a variation of the mitochondria found in some eukaryotic pathogens. In these organisms, each cell has a single, long, branched mitochondrion in which kinetoplast DNA, organized as multiple circular pieces of DNA, is found concentrated at one pole of the cell.

Mitochondria-Related Organelles in Protozoan Parasites

Many protozoans, including several protozoan parasites that cause infections in humans, can be identified by their unusual appearance. Distinguishing features may include complex cell morphologies, the presence of unique organelles, or the absence of common organelles. The protozoan parasites *Giardia lamblia* and *Trichomonas vaginalis* are two examples.

G. lamblia, a frequent cause of diarrhea in humans and many other animals, is an anaerobic parasite that possesses two nuclei and several flagella. Its Golgi apparatus and endoplasmic reticulum are greatly reduced, and it lacks mitochondria completely. However, it does have organelles known as mitosomes, double-membrane-bound organelles that appear to be severely reduced mitochondria. This has led scientists to believe that *G. lamblia*'s ancestors once possessed mitochondria that evolved to become mitosomes. *T. vaginalis*, which causes the sexually transmitted infection vaginitis, is another protozoan parasite that lacks conventional mitochondria. Instead, it possesses hydrogenosomes, mitochondrial-related, double-membrane-bound organelles that produce molecular hydrogen used in cellular metabolism. Scientists believe that hydrogenosomes, like mitosomes, also evolved from mitochondria.⁵

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- Centrosomes** are microtubule-organizing centers important in the formation of the mitotic spindle in mitosis.
- Mitochondria** are the site of cellular respiration. They have two membranes: an outer membrane and an inner membrane with cristae. The mitochondrial matrix, within the inner membrane, contains the mitochondrial DNA, 70S ribosomes, and metabolic enzymes.
- The plasma membrane of eukaryotic cells is structurally similar to that found in prokaryotic cells, and membrane components move according to the fluid mosaic model. However, eukaryotic membranes contain sterols, which alter membrane fluidity, as well as glycoproteins and glycolipids, which help the cell recognize other cells and infectious particles.
- In addition to active transport and passive transport, eukaryotic cell membranes can take material into the cell via **endocytosis**, or expel matter from the cell via **exocytosis**.
- Cells of fungi, algae, plants, and some protists have a **cell wall**, whereas cells of animals and some protozoans have a sticky **extracellular matrix** that provides structural support and mediates cellular signaling.
- Eukaryotic flagella are structurally distinct from prokaryotic flagella but serve a similar purpose (locomotion). **Cilia** are structurally similar to eukaryotic flagella, but shorter; they may be used for locomotion, feeding, or movement of extracellular particles.

Footnotes

- 1 A.E. Barnhill, M.T. Brewer, S.A. Carlson. "Adverse Effects of Antimicrobials via Predictable or Idiosyncratic Inhibition of Host Mitochondrial Components." *Antimicrobial Agents and Chemotherapy* 56 no. 8 (2012):4046–4051.
- 2 Fuchs E, Cleveland DW. "A Structural Scaffolding of Intermediate Filaments in Health and Disease." *Science* 279 no. 5350 (1998):514–519.
- 3 E. Fuchs, D.W. Cleveland. "A Structural Scaffolding of Intermediate Filaments in Health and Disease." *Science* 279 no. 5350 (1998):514–519.
- 4 E. Fuchs, D.W. Cleveland. "A Structural Scaffolding of Intermediate Filaments in Health and Disease." *Science* 279 no. 5350

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5. 5 N. Yarleth, J.H.P. Hackstein. “Hydrogenosomes: One Organelle, Multiple Origins.” *BioScience* 55 no. 8 (2005):657–658.
6. 6 M. Dudzick. “Protists.” OpenStax CNX. November 27, 2013. <http://cnx.org/contents/f7048bb6-e46...ef291cf7049c@1>

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5.4: Cell Boundary and External Structures of Eukaryotic Cells

Learning Objectives

- Explain the distinguishing characteristics of eukaryotic cells
- Describe internal and external structures of prokaryotic cells in terms of their physical structure, chemical structure, and function
- Identify and describe structures and organelles unique to eukaryotic cells
- Compare and contrast similar structures found in prokaryotic and eukaryotic cells

Plasma Membrane

The **plasma membrane** of eukaryotic cells is similar in structure to the prokaryotic plasma membrane in that it is composed mainly of phospholipids forming a bilayer with embedded peripheral and integral proteins (Figure 5.4.17). These membrane components move within the plane of the membrane according to the fluid mosaic model. However, unlike the prokaryotic membrane, eukaryotic membranes contain sterols, including cholesterol, that alter membrane fluidity. Additionally, many eukaryotic cells contain some specialized lipids, including sphingolipids, which are thought to play a role in maintaining membrane stability as well as being involved in signal transduction pathways and cell-to-cell communication.

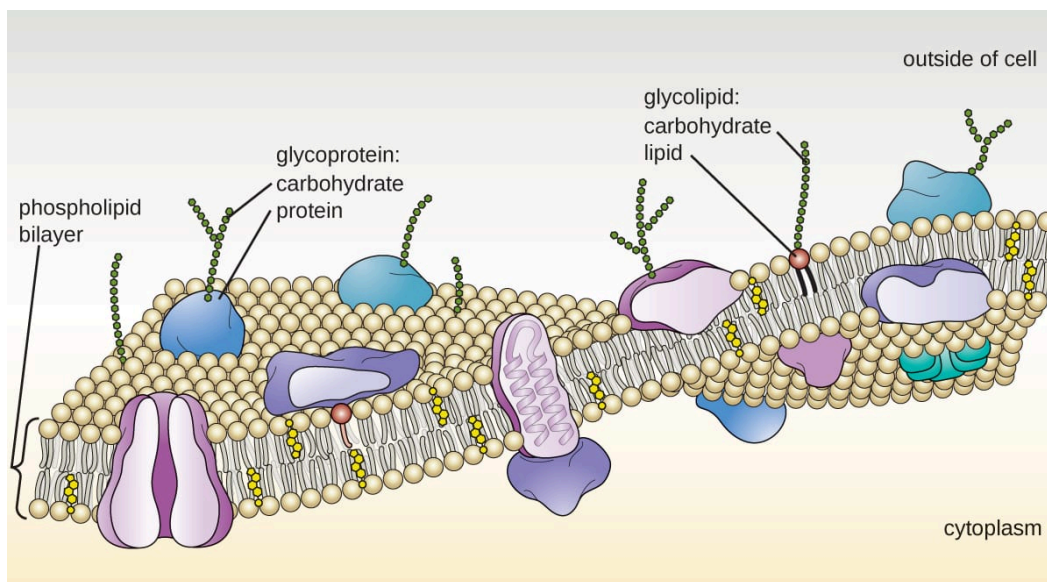


Figure 5.4.17: The eukaryotic plasma membrane is composed of a lipid bilayer with many embedded or associated proteins. It contains cholesterol for the maintenance of membrane, as well as glycoproteins and glycolipids that are important in the recognition other cells or pathogens.

Membrane Transport Mechanisms

The processes of simple diffusion, facilitated diffusion, and active transport are used in both eukaryotic and prokaryotic cells. However, eukaryotic cells also have the unique ability to perform various types of **endocytosis**, the uptake of matter through plasma membrane invagination and vacuole/vesicle formation (Figure 5.4.18). A type of endocytosis involving the engulfment of large particles through membrane invagination is called **phagocytosis**, which means “cell eating.” In phagocytosis, particles (or other cells) are enclosed in a pocket within the membrane, which then pinches off from the membrane to form a vacuole that completely surrounds the particle. Another type of endocytosis is called **pinocytosis**, which means “cell drinking.” In pinocytosis, small, dissolved materials and liquids are taken into the cell through small vesicles. Saprophytic fungi, for example, obtain their

nutrients from dead and decaying matter largely through pinocytosis.

Receptor-mediated endocytosis is a type of endocytosis that is initiated by specific molecules called ligands when they bind to cell surface receptors on the membrane. Receptor-mediated endocytosis is the mechanism that peptide and amine-derived hormones use to enter cells and is also used by various viruses and bacteria for entry into host cells.

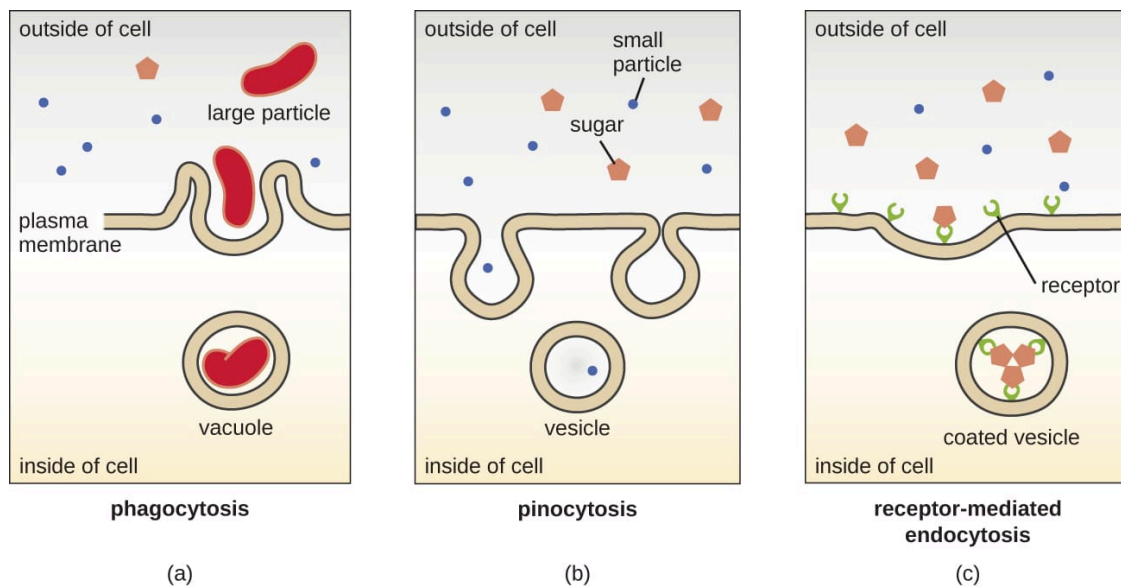


Figure 5.4.18: Three variations of endocytosis are shown. (a) In phagocytosis, the cell membrane surrounds the particle and pinches off to form an intracellular vacuole. (b) In pinocytosis, the cell membrane surrounds a small volume of fluid and pinches off, forming a vesicle. (c) In receptor-mediated endocytosis, the uptake of substances is targeted to a specific substance (a ligand) that binds at the receptor on the external cell membrane. (credit: modification of work by Mariana Ruiz Villarreal)

The process by which secretory vesicles release their contents to the cell's exterior is called **exocytosis**. Vesicles move toward the plasma membrane and then meld with the membrane, ejecting their contents out of the cell. Exocytosis is used by cells to remove waste products and may also be used to release chemical signals that can be taken up by other cells.

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Cell Wall

In addition to a plasma membrane, some eukaryotic cells have a cell wall. Cells of fungi, algae, plants, and even some protists have cell walls. Depending upon the type of eukaryotic cell, cell walls can be made of a wide range of materials, including cellulose (fungi and plants); biogenic silica, calcium carbonate, agar, and carrageenan (protists and algae); or chitin (fungi). In general, all cell walls provide structural stability for the cell and protection from environmental stresses such as desiccation, changes in osmotic pressure, and traumatic injury.⁶

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Extracellular Matrix

Cells of animals and some protozoans do not have cell walls to help maintain shape and provide structural stability. Instead, these types of eukaryotic cells produce an **extracellular matrix** for this purpose. They secrete a sticky mass of carbohydrates and proteins into the spaces between adjacent cells (Figure 5.4.19). Some protein components assemble into a basement membrane to which the remaining extracellular matrix components adhere. Proteoglycans typically form the bulky mass of the extracellular matrix while fibrous proteins, like collagen, provide strength. Both proteoglycans and collagen are attached to fibronectin proteins, which, in turn, are attached to integrin proteins. These integrin proteins interact with transmembrane proteins in the plasma membranes of eukaryotic cells that lack cell walls.

In animal cells, the extracellular matrix allows cells within tissues to withstand external stresses and transmits signals from the outside of the cell to the inside. The amount of extracellular matrix is quite extensive in various types of connective tissues, and variations in the extracellular matrix can give different types of tissues their distinct properties. In addition, a host cell's extracellular matrix is often the site where microbial pathogens attach themselves to establish infection. For example, *Streptococcus pyogenes*, the bacterium that causes strep throat and various other infections, binds to fibronectin in the extracellular matrix of the cells lining the oropharynx (upper region of the throat).

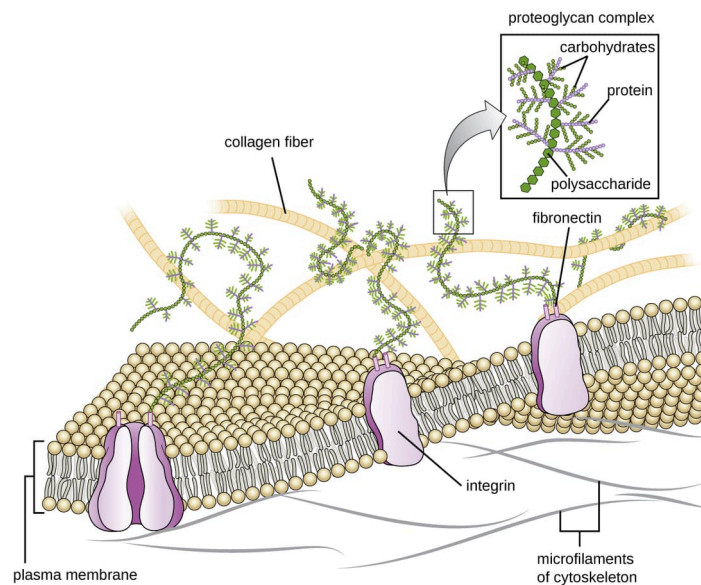


Figure 5.4.19: The extracellular matrix is composed of protein and carbohydrate components. It protects cells from physical stresses and transmits signals arriving at the outside edges of the tissue to cells deeper within the tissue.

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Flagella and Cilia

Some eukaryotic cells use **flagella** for locomotion; however, eukaryotic flagella are structurally distinct from those found in prokaryotic cells. Whereas the prokaryotic flagellum is a stiff, rotating structure, a eukaryotic flagellum is more like a flexible whip composed of nine parallel pairs of microtubules surrounding a central pair of microtubules. This arrangement is referred to as a 9+2 array (Figure 5.4.20). The parallel microtubules use **dynein** motor proteins to move relative to each other, causing the flagellum to bend.

Cilia (singular: **cilium**) are a similar external structure found in some eukaryotic cells. Unique to eukaryotes, cilia are shorter than flagella and often cover the entire surface of a cell; however, they are structurally similar to flagella (a 9+2 array of microtubules) and use the same mechanism for movement. A structure called a **basal body** is found at the base of each cilium and flagellum. The basal body, which attaches the cilium or flagellum to the cell, is composed of an array of triplet microtubules similar to that of a centriole but embedded in the plasma membrane. Because of their shorter length, cilia use a rapid, flexible, waving motion. In addition to motility, cilia may have other functions such as sweeping particles past or into cells. For example, ciliated protozoans use the sweeping of cilia to move food particles into their mouthparts, and ciliated cells in the mammalian respiratory tract beat in synchrony to sweep mucus and debris up and out of the lungs (Figure 5.4.20).

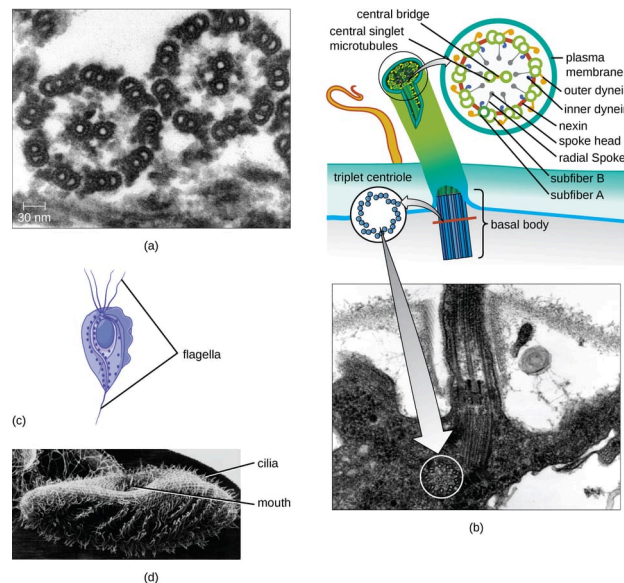


Figure 5.4.20: (a) Eukaryotic flagella and cilia are composed of a 9+2 array of microtubules, as seen in this transmission electron micrograph cross-section. (b) The sliding of these microtubules relative to each other causes a flagellum to bend. (c) An illustration of *Trichomonas vaginalis*, a flagellated protozoan parasite that causes vaginitis. (d) Many protozoans, like this *Paramecium*, have numerous cilia that aid in locomotion as well as in feeding. Note the mouth opening shown here. (credit d: modification of work by University of Vermont/National Institutes of Health)

Key Concepts and Summary

- The plasma membrane of eukaryotic cells is structurally similar to that found in prokaryotic cells, and membrane components move according to the fluid mosaic model. However, eukaryotic membranes contain sterols, which alter membrane fluidity,

as well as glycoproteins and glycolipids, which help the cell recognize other cells and infectious particles.

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Footnotes

1. A.E. Barnhill, M.T. Brewer, S.A. Carlson. "Adverse Effects of Antimicrobials via Predictable or Idiosyncratic Inhibition of Host Mitochondrial Components." *Antimicrobial Agents and Chemotherapy* 56 no. 8 (2012):4046–4051.
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5. N. Yarlett, J.H.P. Hackstein. "Hydrogenosomes: One Organelle, Multiple Origins." *BioScience* 55 no. 8 (2005):657–658.
6. M. Dudzick. "Protists." OpenStax CNX. November 27, 2013. <http://cnx.org/contents/f7048bb6-e46...ef291cf7049c@1>

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5.E: Eukaryotic Cell (Exercises)

5.1: 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
- 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

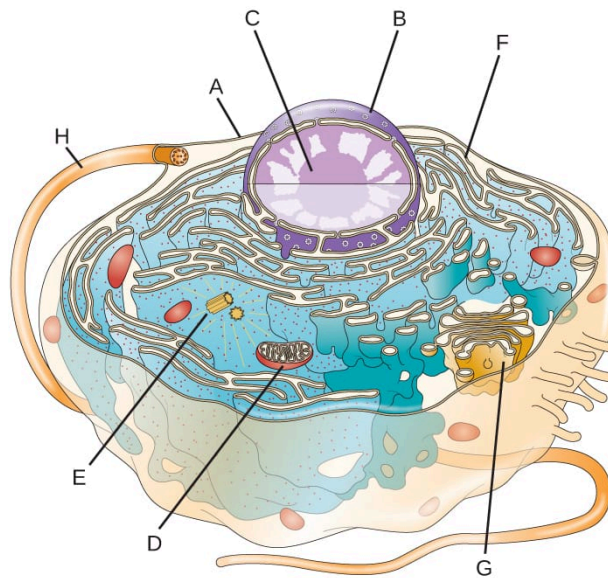
What existing evidence supports the theory that mitochondria are of prokaryotic origin?

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.



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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6: PROKARYOTIC PATHOGENS

Scientists have studied prokaryotes for centuries, but it wasn't until 1966 that scientist Thomas Brock (1926–) discovered that certain bacteria can live in boiling water. This led many to wonder whether prokaryotes may also live in other extreme environments, such as at the bottom of the ocean, at high altitudes, or inside volcanoes, or even on other planets.

Prokaryotes have an important role in changing, shaping, and sustaining the entire biosphere. They can produce proteins and other substances used by molecular biologists in basic research and in medicine and industry. For example, the bacterium *Shewanella* lives in the deep sea, where oxygen is scarce. It grows long appendages, which have special sensors used to seek the limited oxygen in its environment. It can also digest toxic waste and generate electricity. Other species of prokaryotes can produce more oxygen than the entire Amazon rainforest, while still others supply plants, animals, and humans with usable forms of nitrogen; and inhabit our body, protecting us from harmful microorganisms and producing some vitally important substances. This chapter will examine the diversity, structure, and function of prokaryotes.

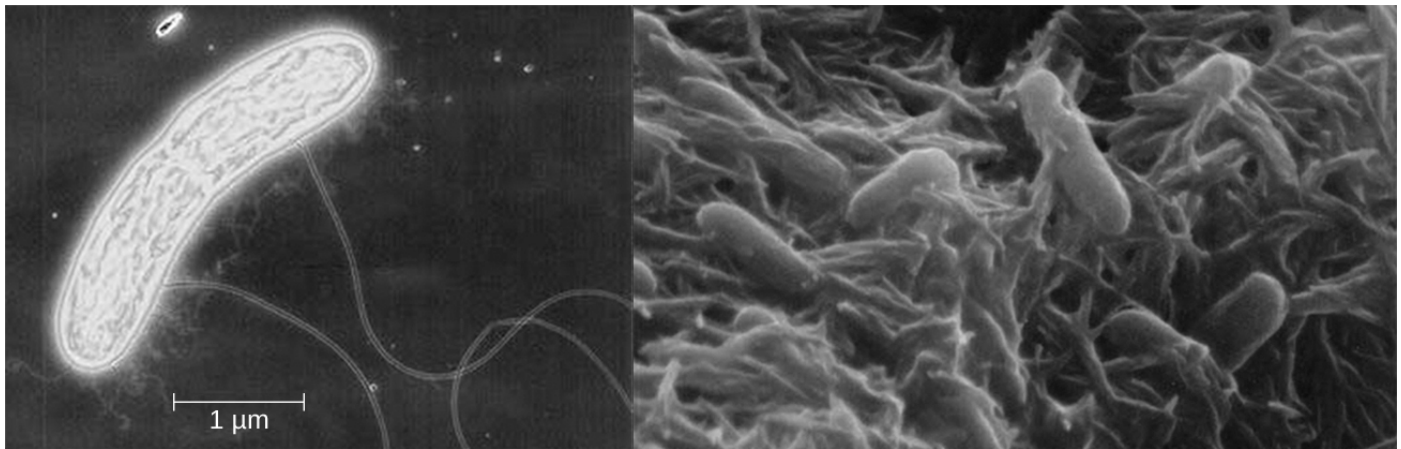


Figure 6.1: The bacterium *Shewanella* lives in the deep sea, where there is little oxygen diffused in the water. It is able to survive in this harsh environment by attaching to the sea floor and using long appendages, called “nanocables,” to sense oxygen. (credit a: modification of work by NASA; credit b: modification of work by Liza Gross)

6.1: Prokaryote Habitats and Functions

6.2: Symbiotic Relationships

6.3: Alpha and Beta Proteobacteria

6.4: Gamma, Delta and Epsilon Proteobacteria

6.5: Spirochetes and CFB

6.6: Gram-positive Bacteria

6.E: Prokaryotic Diversity (Exercises)

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6.1: Prokaryote Habitats and Functions

Learning Objectives

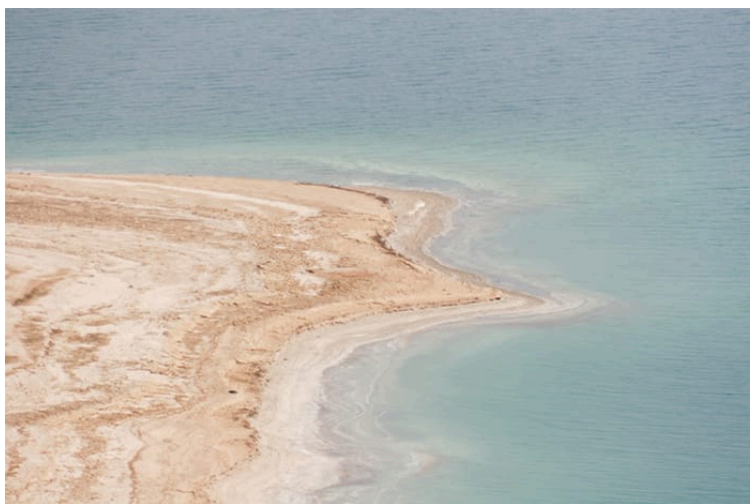
- Identify and describe unique examples of prokaryotes in various habitats on earth
- Identify and describe symbiotic relationships
- Compare normal/commensal/resident microbiota to transient microbiota
- Explain how prokaryotes are classified

Prokaryote Habitats and Functions

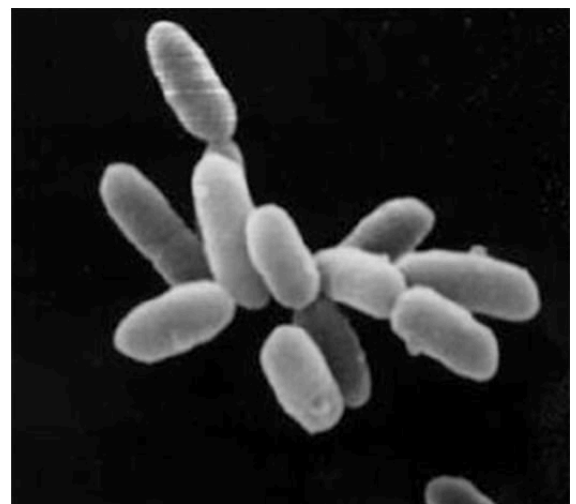
Prokaryotes are **ubiquitous**. They can be found everywhere on our planet, even in hot springs, in the Antarctic ice shield, and under extreme pressure two miles under water. One bacterium, *Paracoccus denitrificans*, has even been shown to survive when scientists removed it from its native environment (soil) and used a centrifuge to subject it to forces of gravity as strong as those found on the surface of Jupiter.

Prokaryotes also are abundant on and within the human body. According to a report by National Institutes of Health, prokaryotes, especially bacteria, outnumber human cells 10:1.¹ More recent studies suggest the ratio could be closer to 1:1, but even that ratio means that there are a great number of bacteria within the human body.² Bacteria thrive in the human mouth, nasal cavity, throat, ears, gastrointestinal tract, and vagina. Large colonies of bacteria can be found on healthy human skin, especially in moist areas (armpits, navel, and areas behind ears). However, even drier areas of the skin are not free from bacteria.

The existence of prokaryotes is very important for the stability and thriving of ecosystems. For example, they are a necessary part of soil formation and stabilization processes through the breakdown of organic matter and development of biofilms. One gram of soil contains up to 10 billion microorganisms (most of them prokaryotic) belonging to about 1,000 species. Many species of bacteria use substances released from plant roots, such as acids and carbohydrates, as nutrients. The bacteria metabolize these plant substances and release the products of bacterial metabolism back to the soil, forming humus and thus increasing the soil's fertility. In salty lakes such as the Dead Sea (Figure 6.1.1), salt-loving halobacteria decompose dead brine shrimp and nourish young brine shrimp and flies with the products of bacterial metabolism.



(a)



(b)

Figure 6.1.1: (a) Some prokaryotes, called halophiles, can thrive in extremely salty environments such as the Dead Sea, pictured here. (b) The archaeon *Halobacterium salinarum*, shown here in an electron micrograph, is a halophile that lives in the Dead Sea. (credit a: modification of work by Julien Menichini; credit b: modification of work by NASA)

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In addition to living in the ground and the water, prokaryotic microorganisms are abundant in the air, even high in the atmosphere. There may be up to 2,000 different kinds of bacteria in the air, similar to their diversity in the soil.

Prokaryotes can be found everywhere on earth because they are extremely resilient and adaptable. They are often metabolically flexible, which means that they might easily switch from one energy source to another, depending on the availability of the sources, or from one metabolic pathway to another. For example, certain prokaryotic cyanobacteria can switch from a conventional type of lipid metabolism, which includes production of fatty aldehydes, to a different type of lipid metabolism that generates biofuel, such as fatty acids and wax esters. Groundwater bacteria store complex high-energy carbohydrates when grown in pure groundwater, but they metabolize these molecules when the groundwater is enriched with phosphates. Some bacteria get their energy by reducing sulfates into sulfides, but can switch to a different metabolic pathway when necessary, producing acids and free hydrogen ions.

Prokaryotes perform functions vital to life on earth by capturing (or “fixing”) and recycling elements like carbon and nitrogen. Organisms such as animals require organic carbon to grow, but, unlike prokaryotes, they are unable to use inorganic carbon sources like carbon dioxide. Thus, animals rely on prokaryotes to convert carbon dioxide into organic carbon products that they can use. This process of converting carbon dioxide to organic carbon products is called **carbon fixation**.

Plants and animals also rely heavily on prokaryotes for **nitrogen fixation**, the conversion of atmospheric nitrogen into ammonia, a compound that some plants can use to form many different biomolecules necessary to their survival. Bacteria in the genus *Rhizobium*, for example, are nitrogen-fixing bacteria; they live in the roots of legume plants such as clover, alfalfa, and peas (Figure 6.1.2). Ammonia produced by *Rhizobium* helps these plants to survive by enabling them to make building blocks of nucleic acids. In turn, these plants may be eaten by animals—sustaining their growth and survival—or they may die, in which case the products of nitrogen fixation will enrich the soil and be used by other plants.

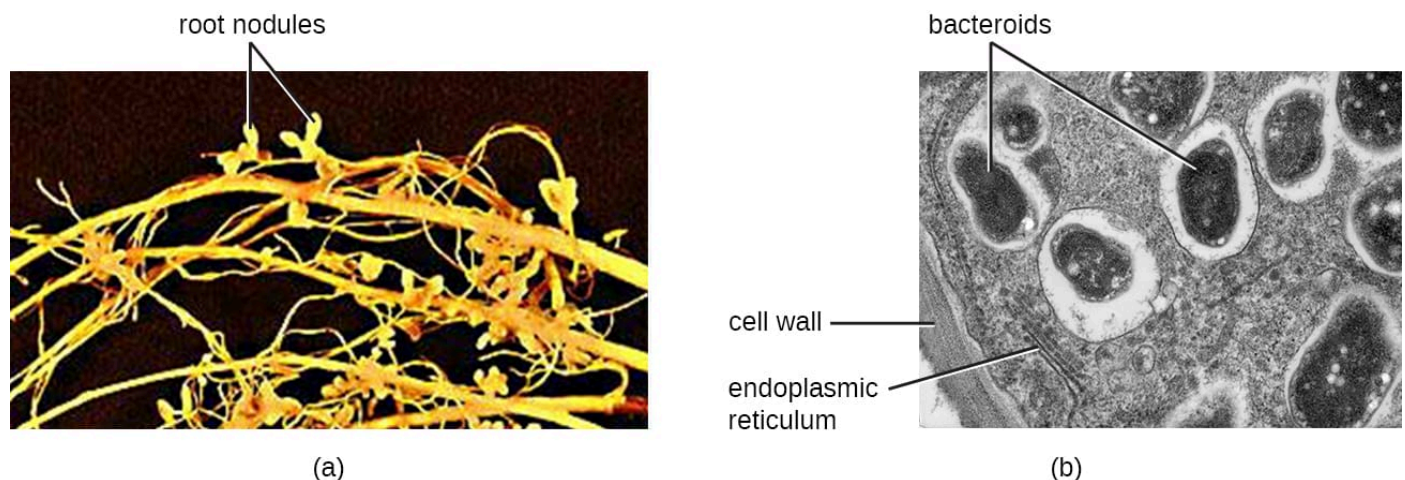


Figure 6.1.2: (a) Nitrogen-fixing bacteria such as *Rhizobium* live in the root nodules of legumes such as clover. (b) This micrograph of the root nodule shows bacteroids (bacterium-like cells or modified bacterial cells) within the plant cells. The bacteroids are visible as darker ovals within the larger plant cell. (credit a: modification of work by USDA)

Another positive function of prokaryotes is in cleaning up the environment. Recently, some researchers focused on the diversity and functions of prokaryotes in manmade environments. They found that some bacteria play a unique role in degrading toxic chemicals that pollute water and soil.³

Despite all of the positive and helpful roles prokaryotes play, some are **human pathogens** that may cause illness or infection when they enter the body. In addition, some bacteria can contaminate food, causing spoilage or foodborne illness, which makes them subjects of concern in food preparation and safety. Less than 1% of prokaryotes (all of them bacteria) are thought to be human pathogens, but collectively these species are responsible for a large number of the diseases that afflict humans.

Besides pathogens, which have a direct impact on human health, prokaryotes also affect humans in many indirect ways. For example, prokaryotes are now thought to be key players in the processes of **climate change**. In recent years, as temperatures in the earth's polar regions have risen, soil that was formerly frozen year-round (permafrost) has begun to thaw. Carbon trapped in the permafrost is gradually released and metabolized by prokaryotes. This produces massive amounts of carbon dioxide and methane, greenhouse gases that escape into the atmosphere and contribute to the greenhouse effect.

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Interactive Element

Case Study Preview: "Travel With Care"

After returning from a medical internship in Nigeria, Marsha assumes her fatigue and cough are just jet lag. But when her symptoms worsen - bloody sputum, night sweats, chest pain - her story becomes a cautionary tale about global travel, airborne pathogens, and a slow-growing bacterial threat: *Mycobacterium tuberculosis*.

In this case study, you'll follow Marsha's journey from symptoms to diagnosis and long-term treatment, using tools like acid-fast staining, radiography, and nucleic acid testing. Along the way, you'll uncover what makes *M. tuberculosis* so tough to kill - and why TB remains a global health threat.

Can you spot the red flags before the culture even grows? Understanding prokaryotic cell walls might just save a life.

[Chapter 4 Case Study - Travel With Care](#)

Key Concepts and Summary

- Prokaryotes are unicellular microorganisms whose cells have no nucleus.
- Prokaryotes can be found everywhere on our planet, even in the most extreme environments.
- Prokaryotes are very flexible metabolically, so they are able to adjust their feeding to the available natural resources.
- Prokaryotes live in **communities** that interact among themselves and with large organisms that they use as hosts (including humans).
- The totality of forms of prokaryotes (particularly bacteria) living on the human body is called the human microbiome, which varies between regions of the body and individuals, and changes over time.

- The totality of forms of prokaryotes (particularly bacteria) living in a certain region of the human body (e.g., mouth, throat, gut, eye, vagina) is called the **microbiota** of this region.
- Prokaryotes are classified into domains Archaea and Bacteria.
- In recent years, the traditional approaches to classification of prokaryotes have been supplemented by approaches based on molecular genetics.

Footnotes

1. 1 Medical Press. "Mouth Bacteria Can Change Their Diet, Supercomputers Reveal." August 12, 2014. medicalxpress.com/news/2014-08-mouth-bacteria-can-change-their-diet-supercomputers-reveal.html. Accessed February 24, 2015.
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6.2: Symbiotic Relationships

Learning Objectives

- Identify and describe unique examples of prokaryotes in various habitats on earth
- Identify and describe symbiotic relationships
- Compare normal/commensal/resident microbiota to transient microbiota
- Explain how prokaryotes are classified

Symbiotic Relationships

As we have learned, prokaryotic microorganisms can associate with plants and animals. Often, this association results in unique relationships between organisms. For example, bacteria living on the roots or leaves of a plant get nutrients from the plant and, in return, produce substances that protect the plant from pathogens. On the other hand, some bacteria are plant pathogens that use mechanisms of infection similar to bacterial pathogens of animals and humans.

Prokaryotes live in a **community**, or a group of interacting populations of organisms. A population is a group of individual organisms belonging to the same biological species and limited to a certain geographic area. Populations can have **cooperative interactions**, which benefit the populations, or **competitive interactions**, in which one population competes with another for resources. The study of these interactions between populations is called **microbial ecology**.

Any interaction between different species within a community is called **symbiosis**. Such interactions fall along a continuum between opposition and cooperation. Interactions in a symbiotic relationship may be beneficial or harmful, or have no effect on one or both of the species involved. Table 6.2.1 summarizes the main types of symbiotic interactions among prokaryotes.

Table 6.2.1: Types of Symbiotic Relationships

Type	Population A	Population B
Mutualism	Benefitted	Benefitted
Amensalism	Harmed	Unaffected
Commensalism	Benefitted	Unaffected
Neutralism	Unaffected	Unaffected
Parasitism	Benefitted	Harmed

Mutualism

When two species benefit from each other, the symbiosis is called **mutualism** (or syntrophy, or crossfeeding). For example, humans have a mutualistic relationship with the bacterium *Bacteroides thetaiotetraiotamicron*, which lives in the intestinal tract. *B. thetaiotetraiotamicron* digests complex polysaccharide plant materials that human digestive enzymes cannot break down, converting them into monosaccharides that can be absorbed by human cells. Humans also have a mutualistic relationship with certain strains of *Escherichia coli*, another bacterium found in the gut. *E. coli* relies on intestinal contents for nutrients, and humans derive certain vitamins from *E. coli*, particularly vitamin K, which is required for the formation of blood clotting factors. (This is only true for some strains of *E. coli*, however. Other strains are pathogenic and do not have a mutualistic relationship with humans.)

Amensalism

A type of symbiosis in which one population harms another but remains unaffected itself is called **amensalism**. In the case of bacteria, some amensalist species produce bactericidal substances that kill other species of bacteria. For example, the bacterium *Lucilia sericata* produces a protein that destroys *Staphylococcus aureus*, a bacterium commonly found on the surface of the

human skin. Too much handwashing can affect this relationship and lead to *S. aureus* diseases and transmission.

Commensalism

In another type of symbiosis, called **commensalism**, one organism benefits while the other is unaffected. This occurs when the bacterium *Staphylococcus epidermidis* uses the dead cells of the human skin as nutrients. Billions of these bacteria live on our skin, but in most cases (especially when our immune system is healthy), we do not react to them in any way.

Neutralism

If neither of the symbiotic organisms is affected in any way, we call this type of symbiosis **neutralism**. An example of neutralism is the coexistence of metabolically active (vegetating) bacteria and endospores (dormant, metabolically passive bacteria). For example, the bacterium *Bacillus anthracis* typically forms endospores in soil when conditions are unfavorable. If the soil is warmed and enriched with nutrients, some endospores germinate and remain in symbiosis with other endospores that have not germinated.

Parasitism

A type of symbiosis in which one organism benefits while harming the other is called **parasitism**. The relationship between humans and many pathogenic prokaryotes can be characterized as parasitic because these organisms invade the body, producing toxic substances or infectious diseases that cause harm. Diseases such as tetanus, diphtheria, pertussis, tuberculosis, and leprosy all arise from interactions between bacteria and humans.

Scientists have coined the term microbiome to refer to all prokaryotic and eukaryotic microorganisms that are associated with a certain organism. Within the human microbiome, there are resident microbiota and transient microbiota. The resident microbiota consists of microorganisms that constantly live in or on our bodies. The term transient microbiota refers to microorganisms that are only temporarily found in the human body, and these may include pathogenic microorganisms. Hygiene and diet can alter both the resident and transient microbiota.

The resident microbiota is amazingly diverse, not only in terms of the variety of species but also in terms of the preference of different microorganisms for different areas of the human body. For example, in the human mouth, there are thousands of commensal or mutualistic species of bacteria. Some of these bacteria prefer to inhabit the surface of the tongue, whereas others prefer the internal surface of the cheeks, and yet others prefer the front or back teeth or gums. The inner surface of the cheek has the least diverse microbiota because of its exposure to oxygen. By contrast, the crypts of the tongue and the spaces between teeth are two sites with limited oxygen exposure, so these sites have more diverse microbiota, including bacteria living in the absence of oxygen (e.g., *Bacteroides*, *Fusobacterium*). Differences in the oral microbiota between randomly chosen human individuals are also significant. Studies have shown, for example, that the prevalence of such bacteria as *Streptococcus*, *Haemophilus*, *Neisseria*, and others was dramatically different when compared between individuals.⁴

There are also significant differences between the microbiota of different sites of the same human body. The inner surface of the cheek has a predominance of *Streptococcus*, whereas in the throat, the palatine tonsil, and saliva, there are two to three times fewer *Streptococcus*, and several times more *Fusobacterium*. In the plaque removed from gums, the predominant bacteria belong to the genus *Fusobacterium*. However, in the intestine, both *Streptococcus* and *Fusobacterium* disappear, and the genus *Bacteroides* becomes predominant.

Not only can the microbiota vary from one body site to another, the microbiome can also change over time within the same individual. Humans acquire their first inoculations of normal flora during natural birth and shortly after birth. Before birth, there is a rapid increase in the population of *Lactobacillus* spp. in the vagina, and this population serves as the first colonization of microbiota during natural birth. After birth, additional microbes are acquired from health-care providers, parents, other relatives, and individuals who come in contact with the baby. This process establishes a microbiome that will continue to evolve over the course of the individual's life as new microbes colonize and are eliminated from the body. For example, it is estimated that within a 9-hour period, the microbiota of the small intestine can change so that half of the microbial inhabitants will be different.⁵ The importance of the initial *Lactobacillus* colonization during vaginal child birth is highlighted by studies demonstrating a higher incidence of diseases in individuals born by cesarean section, compared to those born vaginally. Studies have shown that babies born vaginally are predominantly colonized by vaginal lactobacillus, whereas babies born by cesarean section are more frequently colonized by microbes of the normal skin microbiota, including common hospital-acquired pathogens.

Throughout the body, resident microbiotas are important for human health because they occupy niches that might be otherwise taken by pathogenic microorganisms. For instance, *Lactobacillus* spp. are the dominant bacterial species of the normal vaginal microbiota for most women. *Lactobacillus* produce lactic acid, contributing to the acidity of the vagina and inhibiting the growth of pathogenic yeasts. However, when the population of the resident microbiota is decreased for some reason (e.g., because of taking antibiotics), the pH of the vagina increases, making it a more favorable environment for the growth of yeasts such as *Candida albicans*. Antibiotic therapy can also disrupt the microbiota of the intestinal tract and respiratory tract, increasing the risk for secondary infections and/or promoting the long-term carriage and shedding of pathogens.

Query 6.2.1

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Taxonomy and Systematics

Assigning prokaryotes to a certain species is challenging. They do not reproduce sexually, so it is not possible to classify them according to the presence or absence of interbreeding. Also, they do not have many morphological features. Traditionally, the classification of prokaryotes was based on their shape, staining patterns, and biochemical or physiological differences. More recently, as technology has improved, the nucleotide sequences in genes have become an important criterion of microbial classification.

In 1923, American microbiologist David Hendricks Bergey (1869–1937) published *A Manual in Determinative Bacteriology*. With this manual, he attempted to summarize the information about the kinds of bacteria known at that time, using Latin binomial classification. Bergey also included the morphological, physiological, and biochemical properties of these organisms. His manual has been updated multiple times to include newer bacteria and their properties. It is a great aid in bacterial taxonomy and methods of characterization of bacteria. A more recent sister publication, the five-volume *Bergey's Manual of Systematic Bacteriology*, expands on Bergey's original manual. It includes a large number of additional species, along with up-to-date descriptions of the taxonomy and biological properties of all named prokaryotic taxa. This publication incorporates the approved names of bacteria as determined by the List of Prokaryotic Names with Standing in Nomenclature (LPSN).

Classification by Staining Patterns

According to their staining patterns, which depend on the properties of their cell walls, bacteria have traditionally been classified into **gram-positive**, **gram-negative**, and “atypical,” meaning neither gram-positive nor gram-negative. As explained in [Staining Microscopic Specimens](#), gram-positive bacteria possess a thick peptidoglycan cell wall that retains the primary stain (crystal violet) during the decolorizing step; they remain purple after the gram-stain procedure because the crystal violet dominates the light red/pink color of the secondary counterstain, safranin. In contrast, gram-negative bacteria possess a thin peptidoglycan cell wall that does not prevent the crystal violet from washing away during the decolorizing step; therefore, they appear light red/pink after staining with the safranin. Bacteria that cannot be stained by the standard Gram stain procedure are called atypical bacteria. Included in the atypical category are species of *Mycoplasma* and *Chlamydia*, which lack a cell wall and therefore cannot retain the gram-stain reagents. *Rickettsia* are also considered atypical because they are too small to be evaluated by the Gram stain.

More recently, scientists have begun to further classify gram-negative and gram-positive bacteria. They have added a special group of deeply branching bacteria based on a combination of physiological, biochemical, and genetic features. They also now further classify gram-negative bacteria into Proteobacteria, *Cytophaga-Flavobacterium-Bacteroides* (CFB), and spirochetes.

The deeply branching bacteria are thought to be a very early evolutionary form of bacteria (see [Deeply Branching Bacteria](#)). They live in hot, acidic, ultraviolet-light-exposed, and anaerobic (deprived of oxygen) conditions. Proteobacteria is a phylum of very diverse groups of gram-negative bacteria; it includes some important human pathogens (e.g., *E. coli* and *Bordetella pertussis*). The CFB group of bacteria includes components of the normal human gut microbiota, like *Bacteroides*. The spirochetes are spiral-shaped bacteria and include the pathogen *Treponema pallidum*, which causes syphilis. We will characterize these groups

of bacteria in more detail later in the chapter.

Based on their prevalence of guanine and cytosine nucleotides, gram-positive bacteria are also classified into low G+C and high G+C gram-positive bacteria. The low G+C gram-positive bacteria have less than 50% of guanine and cytosine nucleotides in their DNA. They include human pathogens, such as those that cause anthrax (*Bacillus anthracis*), tetanus (*Clostridium tetani*), and listeriosis (*Listeria monocytogenes*). High G+C gram-positive bacteria, which have more than 50% guanine and cytosine nucleotides in their DNA, include the bacteria that cause diphtheria (*Corynebacterium diphtheriae*), tuberculosis (*Mycobacterium tuberculosis*), and other diseases.

The classifications of prokaryotes are constantly changing as new species are being discovered. We will describe them in more detail, along with the diseases they cause, in later sections and chapters.

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Human Microbiome Project

The Human Microbiome Project was launched by the National Institutes of Health (NIH) in 2008. One main goal of the project is to create a large repository of the gene sequences of important microbes found in humans, helping biologists and clinicians understand the dynamics of the human microbiome and the relationship between the human microbiota and diseases. A network of labs working together has been compiling the data from swabs of several areas of the skin, gut, and mouth from hundreds of individuals.

One of the challenges in understanding the human microbiome has been the difficulty of culturing many of the microbes that inhabit the human body. It has been estimated that we are only able to culture 1% of the bacteria in nature and that we are unable to grow the remaining 99%. To address this challenge, researchers have used metagenomic analysis, which studies genetic material harvested directly from microbial communities, as opposed to that of individual species grown in a culture. This allows researchers to study the genetic material of all microbes in the microbiome, rather than just those that can be cultured.⁶

One important achievement of the Human Microbiome Project is establishing the first reference database on microorganisms living in and on the human body. Many of the microbes in the microbiome are beneficial, but some are not. It was found, somewhat unexpectedly, that all of us have some serious microbial pathogens in our microbiota. For example, the conjunctiva of the human eye contains 24 genera of bacteria and numerous pathogenic species.⁷ A healthy human mouth contains a number of species of the genus *Streptococcus*, including pathogenic species *S. pyogenes* and *S. pneumoniae*.⁸ This raises the question of why certain prokaryotic organisms exist commensally in certain individuals but act as deadly pathogens in others. Also unexpected was the number of organisms that had never been cultured. For example, in one metagenomic study of the human gut microbiota, 174 new species of bacteria were identified.⁹

Another goal for the near future is to characterize the human microbiota in patients with different diseases and to find out whether there are any relationships between the contents of an individual's microbiota and risk for or susceptibility to specific diseases. Analyzing the microbiome in a person with a specific disease may reveal new ways to fight diseases.

Key Concepts and Summary

- Prokaryotes are unicellular microorganisms whose cells have no nucleus.
- Prokaryotes can be found everywhere on our planet, even in the most extreme environments.
- Prokaryotes are very flexible metabolically, so they are able to adjust their feeding to the available natural resources.

- Prokaryotes live in **communities** that interact among themselves and with large organisms that they use as hosts (including humans).
- The totality of forms of prokaryotes (particularly bacteria) living on the human body is called the human microbiome, which varies between regions of the body and individuals, and changes over time.
- The totality of forms of prokaryotes (particularly bacteria) living in a certain region of the human body (e.g., mouth, throat, gut, eye, vagina) is called the **microbiota** of this region.
- Prokaryotes are classified into domains Archaea and Bacteria.
- In recent years, the traditional approaches to classification of prokaryotes have been supplemented by approaches based on molecular genetics.

Footnotes

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2. 2 A. Abbott. "Scientists Bust Myth That Our Bodies Have More Bacteria Than Human Cells: Decades-Old Assumption about Microbiota Revisited." *Nature*. <http://www.nature.com/news/scientist...-cells-1.19136>. Accessed June 3, 2016.
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4. 4 E.M. Bik et al. "Bacterial Diversity in the Oral Cavity of 10 Healthy Individuals." *The ISME Journal* 4 no. 8 (2010):962–974.
5. 5 C.C. Boonjink et al. "High Temporal and Intra-Individual Variation Detected in the Human Ileal Microbiota." *Environmental Microbiology* 12 no. 12 (2010):3213–3227.
6. 6 National Institutes of Health. "Human Microbiome Project. Overview." commonfund.nih.gov/hmp/overview. Accessed June 7, 2016.
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8. 8 F.E. Dewhirst et al. "The Human Oral Microbiome." *Journal of Bacteriology* 192 no. 19 (2010):5002–5017.
9. 9 J.C. Lagier et al. "Microbial Culturomics: Paradigm Shift in the Human Gut Microbiome Study." *Clinical Microbiology and Infection* 18 no. 12 (2012):1185–1193.

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6.3: Alpha and Beta Proteobacteria

Learning Objectives

- Describe the unique features of each class within the phylum Proteobacteria: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria
- Give an example of a bacterium in each class of Proteobacteria

In 1987, the American microbiologist Carl Woese (1928–2012) suggested that a large and diverse group of bacteria that he called “purple bacteria and their relatives” should be defined as a separate phylum within the domain Bacteria based on the similarity of the nucleotide sequences in their genome.¹ This phylum of gram-negative bacteria subsequently received the name **Proteobacteria**. It includes many bacteria that are part of the normal human microbiota as well as many pathogens. The Proteobacteria are further divided into five classes: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria.

Alphaproteobacteria

The first class of Proteobacteria is the **Alphaproteobacteria**. The unifying characteristic of this class is that they are **oligotrophs**, organisms capable of living in low-nutrient environments such as deep oceanic sediments, glacial ice, or deep undersurface soil.

Among the Alphaproteobacteria are two taxa, chlamydias and rickettsias, that are **obligate intracellular pathogens**, meaning that part of their life cycle must occur inside other cells called host cells. When not growing inside a host cell, *Chlamydia* and *Rickettsia* are metabolically inactive outside of the host cell. They cannot synthesize their own adenosine triphosphate (ATP), and, therefore, rely on cells for their energy needs.

Rickettsia spp. include a number of serious human pathogens. For example, *R. rickettsii* causes Rocky Mountain spotted fever, a life-threatening form of meningoencephalitis (inflammation of the membranes that wrap the brain). *R. rickettsii* infects ticks and can be transmitted to humans via a bite from an infected tick (Figure 6.3.1).

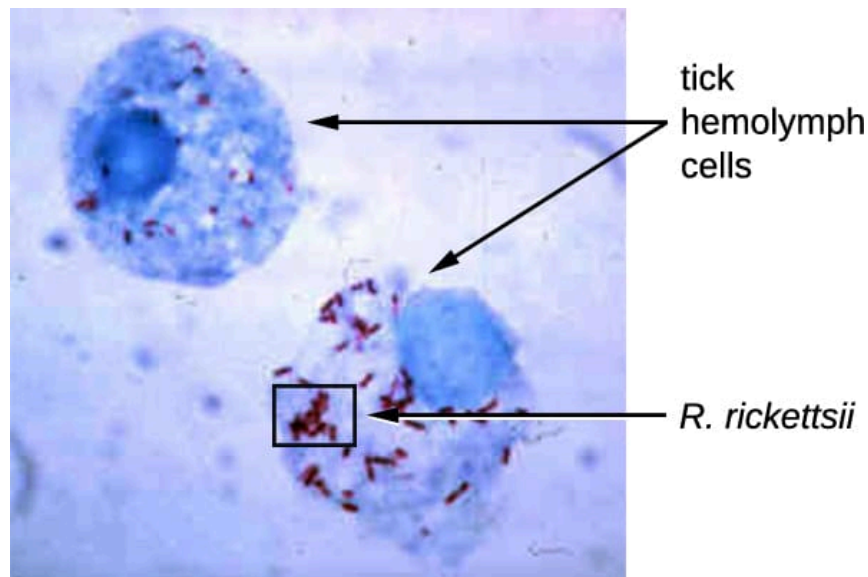


Figure 6.3.1: Rickettsias require special staining methods to see them under a microscope. Here, *R. rickettsii*, which causes Rocky Mountain spotted fever, is shown infecting the cells of a tick. (credit: modification of work by Centers for Disease Control and Prevention)

Another species of *Rickettsia*, *R. prowazekii*, is spread by lice. It causes epidemic typhus, a severe infectious disease common during warfare and mass migrations of people. *R. prowazekii* infects human endothelium cells, causing inflammation of the inner lining of blood vessels, high fever, abdominal pain, and sometimes delirium. A relative, *R. typhi*, causes a less severe disease

known as murine or endemic typhus, which is still observed in the southwestern United States during warm seasons.

Chlamydia is another taxon of the Alphaproteobacteria. Members of this genus are extremely resistant to the cellular defenses, giving them the ability to spread from host to host rapidly via elementary bodies. The metabolically and reproductively inactive elementary bodies are the endospore-like form of intracellular bacteria that enter an epithelial cell, where they become active. Figure 6.3.2 illustrates the life cycle of *Chlamydia*.

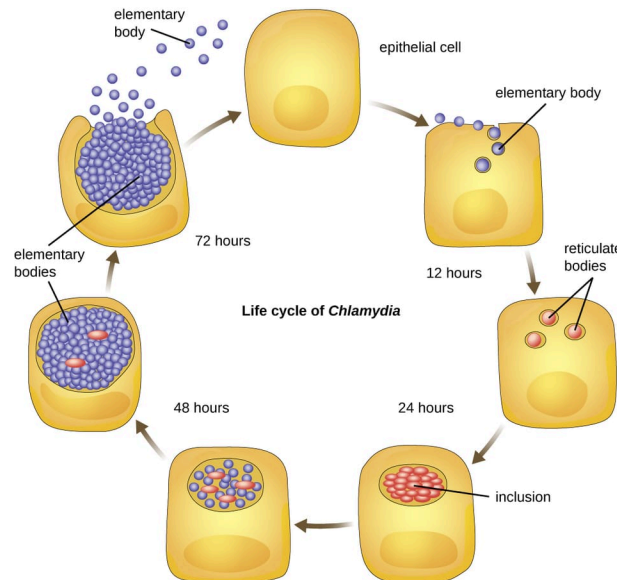


Figure 6.3.2: *Chlamydia* begins infection of a host when the metabolically inactive elementary bodies enter an epithelial cell. Once inside the host cell, the elementary bodies turn into active reticulate bodies. The reticulate bodies multiply and release more elementary bodies when the cell dies after the *Chlamydia* uses all of the host cell's ATP. (credit: modification of work by Centers for Disease Control and Prevention)

C. trachomatis is a human pathogen that causes trachoma, a disease of the eyes, often leading to blindness. *C. trachomatis* also causes the sexually transmitted disease lymphogranuloma venereum (LGV). This disease is often mildly symptomatic, manifesting as regional lymph node swelling, or it may be asymptomatic, but it is extremely contagious and is common on college campuses. Table 6.3.1 summarizes the characteristics of important genera of Alphaproteobacteria.

Table 6.3.1: Class Alphaproteobacteria

Genus	Microscopic Morphology	Unique Characteristics
<i>Agrobacterium</i>	Gram-negative bacillus	Plant pathogen; one species, <i>A. tumefaciens</i> , causes tumors in plants
<i>Bartonella</i>	Gram-negative, pleomorphic, flagellated coccobacillus	Facultative intracellular bacteria, transmitted by lice and fleas, cause trench fever and cat scratch disease in humans
<i>Brucella</i>	Gram-negative, small, flagellated coccobacillus	Facultative intracellular bacteria, transmitted by contaminated milk from infected cows, cause brucellosis in cattle and humans
<i>Caulobacter</i>	Gram-negative bacillus	Used in studies on cellular adaptation and differentiation because of its peculiar life cycle (during cell division, forms "swarm" cells and "stalked" cells)
<i>Chlamydia</i>	Gram-negative, coccoid or ovoid bacterium	Obligatory intracellular bacteria; some cause chlamydia, trachoma, and pneumonia
<i>Coxiella</i>	Small, gram-negative bacillus	Obligatory intracellular bacteria; cause Q fever; potential for use as biological weapon
<i>Ehrlichia</i>	Very small, gram-negative, coccoid or ovoid bacteria	Obligatory intracellular bacteria; can be transported from cell to cell; transmitted by ticks; cause ehrlichiosis (destruction of white blood cells and inflammation) in humans and dogs
<i>Hyphomicrobium</i>	Gram-negative bacilli; grows from a stalk	Similar to <i>Caulobacter</i>

Genus	Microscopic Morphology	Unique Characteristics
<i>Methylocystis</i>	Gram-negative, coccoid or short bacilli	Nitrogen-fixing aerobic bacteria
<i>Rhizobium</i>	Gram-negative, rectangular bacilli with rounded ends forming clusters	Nitrogen-fixing bacteria that live in soil and form symbiotic relationship with roots of legumes (e.g., clover, alfalfa, and beans)
<i>Rickettsia</i>	Gram-negative, highly pleomorphic bacteria (may be cocci, rods, or threads)	Obligate intracellular bacteria; transmitted by ticks; may cause Rocky Mountain spotted fever and typhus

Betaproteobacteria

Unlike Alphaproteobacteria, which survive on a minimal amount of nutrients, the class **Betaproteobacteria** are eutrophs (or copiotrophs), meaning that they require a copious amount of organic nutrients. Betaproteobacteria often grow between aerobic and anaerobic areas (e.g., in mammalian intestines). Some genera include species that are human pathogens, able to cause severe, sometimes life-threatening disease. The genus *Neisseria*, for example, includes the bacteria *N. gonorrhoeae*, the causative agent of the STI gonorrhea, and *N. meningitidis*, the causative agent of bacterial meningitis.

Neisseria are cocci that live on mucosal surfaces of the human body. They are fastidious, or difficult to culture, and they require high levels of moisture, nutrient supplements, and carbon dioxide. Also, *Neisseria* are microaerophilic, meaning that they require low levels of oxygen. For optimal growth and for the purposes of identification, *Neisseria* spp. are grown on chocolate agar (i.e., agar supplemented by partially hemolyzed red blood cells). Their characteristic pattern of growth in culture is diplococcal: pairs of cells resembling coffee beans (Figure 6.3.3).



Figure 6.3.3: *Neisseria meningitidis* growing in colonies on a chocolate agar plate. (credit: Centers for Disease Control and Prevention)

The pathogen responsible for pertussis (whooping cough) is also a member of Betaproteobacteria. The bacterium *Bordetella pertussis*, from the order Burkholderiales, produces several toxins that paralyze the movement of cilia in the human respiratory tract and directly damage cells of the respiratory tract, causing a severe cough. Table 6.3.2 summarizes the characteristics of important genera of Betaproteobacteria.

Table 6.3.2: Class Betaproteobacteria

Example Genus	Microscopic Morphology	Unique Characteristics
<i>Bordetella</i>	A small, gram-negative coccobacillus	Aerobic, very fastidious; <i>B. pertussis</i> causes pertussis (whooping cough)
<i>Burkholderia</i>	Gram-negative bacillus	Aerobic, aquatic, cause diseases in horses and humans (especially patients with cystic fibrosis); agents of nosocomial infections
<i>Leptothrix</i>	Gram-negative, sheathed, filamentous bacillus	Aquatic; oxidize iron and manganese; can live in wastewater treatment plants and clog pipes
<i>Neisseria</i>	Gram-negative, coffee bean-shaped coccus forming pairs	Require moisture and high concentration of carbon dioxide; oxidase positive, grow on chocolate agar; pathogenic species cause gonorrhea and meningitis
<i>Thiobacillus</i>	Gram-negative bacillus	Thermophilic, acidophilic, strictly aerobic bacteria; oxidize iron and sulfur

Query 6.3.1

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Key Concepts and Summary

- **Proteobacteria** is a phylum of gram-negative bacteria discovered by Carl Woese in the 1980s based on nucleotide sequence homology.
- Proteobacteria are further classified into the classes alpha-, beta-, gamma-, delta- and epsilonproteobacteria, each class having separate orders, families, genera, and species.
- **Alphaproteobacteria** are **oligotrophs**. The taxa chlamydias and rickettsias are **obligate intracellular pathogens**, feeding on cells of host organisms; they are metabolically inactive outside of the host cell. Some Alphaproteobacteria can convert atmospheric nitrogen to nitrites, making nitrogen usable by other forms of life.
- **Betaproteobacteria** are **eutrophs**. They include human pathogens of the genus *Neisseria* and the species *Bordetella pertussis*.

Footnotes

1. C.R. Woese. "Bacterial Evolution." *Microbiological Review* 51 no. 2 (1987):221–271.
2. H. Reichenbach. "Myxobacteria, Producers of Novel Bioactive Substances." *Journal of Industrial Microbiology & Biotechnology* 27 no. 3 (2001):149–156.
3. S. Suerbaum, P. Michetti. "*Helicobacter pylori* infection." *New England Journal of Medicine* 347 no. 15 (2002):1175–1186.

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6.4: Gamma, Delta and Epsilon Proteobacteria

Learning Objectives

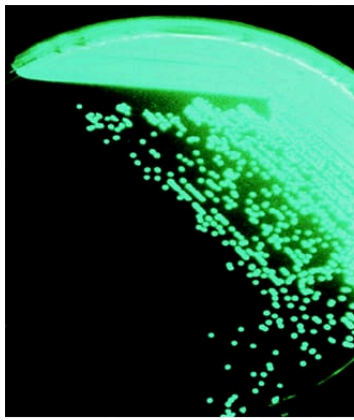
- Describe the unique features of each class within the phylum Proteobacteria: Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, and Epsilonproteobacteria
- Give an example of a bacterium in each class of Proteobacteria

Gammaproteobacteria

The most diverse class of gram-negative bacteria is **Gammaproteobacteria**, and it includes a number of human pathogens. For example, a large and diverse family, *Pseudomonaceae*, includes the genus *Pseudomonas*. Within this genus is the species *P. aeruginosa*, a pathogen responsible for diverse infections in various regions of the body. *P. aeruginosa* is a strictly aerobic, nonfermenting, highly motile bacterium. It often infects wounds and burns, can be the cause of chronic urinary tract infections, and can be an important cause of respiratory infections in patients with cystic fibrosis or patients on mechanical ventilators. Infections by *P. aeruginosa* are often difficult to treat because the bacterium is resistant to many antibiotics and has a remarkable ability to form biofilms. Other representatives of *Pseudomonas* include the fluorescent (glowing) bacterium *P. fluorescens* and the soil bacteria *P. putida*, which is known for its ability to degrade xenobiotics (substances not naturally produced or found in living organisms).

The *Pasteurellaceae* also includes several clinically relevant genera and species. This family includes several bacteria that are human and/or animal pathogens. For example, *Pasteurella haemolytica* causes severe pneumonia in sheep and goats. *P. multocida* is a species that can be transmitted from animals to humans through bites, causing infections of the skin and deeper tissues. The genus *Haemophilus* contains two human pathogens, *H. influenzae* and *H. ducreyi*. Despite its name, *H. influenzae* does not cause influenza (which is a viral disease). *H. influenzae* can cause both upper and lower respiratory tract infections, including sinusitis, bronchitis, ear infections, and pneumonia. Before the development of effective vaccination, strains of *H. influenzae* were a leading cause of more invasive diseases, like meningitis in children. *H. ducreyi* causes the STI known as chancroid.

The order Vibrionales includes the human pathogen *Vibrio cholerae*. This comma-shaped aquatic bacterium thrives in highly alkaline environments like shallow lagoons and sea ports. A toxin produced by *V. cholerae* causes hypersecretion of electrolytes and water in the large intestine, leading to profuse watery diarrhea and dehydration. *V. parahaemolyticus* is also a cause of gastrointestinal disease in humans, whereas *V. vulnificus* causes serious and potentially life-threatening cellulitis (infection of the skin and deeper tissues) and blood-borne infections. Another representative of Vibrionales, *Aliivibrio fischeri*, engages in a symbiotic relationship with squid. The squid provides nutrients for the bacteria to grow and the bacteria produce bioluminescence that protects the squid from predators (Figure 6.4.5).



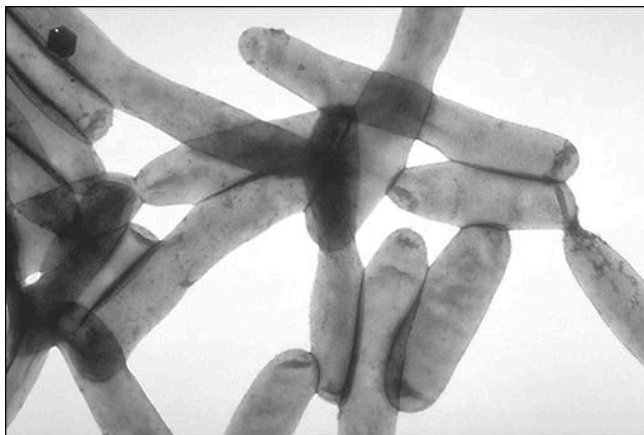
(a)



(b)

Figure 6.4.5: (a) *Aliivibrio fischeri* is a bioluminescent bacterium. (b) *A. fischeri* colonizes and lives in a mutualistic relationship with the Hawaiian bobtail squid (*Euprymna scolopes*). (credit a: modification of work by American Society for Microbiology; credit b: modification of work by Margaret McFall-Ngai)

The genus *Legionella* also belongs to the Gammaproteobacteria. *L. pneumophila*, the pathogen responsible for Legionnaires disease, is an aquatic bacterium that tends to inhabit pools of warm water, such as those found in the tanks of air conditioning units in large buildings (Figure 6.4.6). Because the bacteria can spread in aerosols, outbreaks of Legionnaires disease often affect residents of a building in which the water has become contaminated with *Legionella*. In fact, these bacteria derive their name from the first known outbreak of Legionnaires disease, which occurred in a hotel hosting an American Legion veterans' association convention in Philadelphia in 1976.



(a)



(b)

Figure 6.4.6: (a) *Legionella pneumophila*, the causative agent of Legionnaires disease, thrives in warm water. (b) Outbreaks of Legionnaires disease often originate in the air conditioning units of large buildings when water in or near the system becomes contaminated with *L. pneumophila*. (credit a: modification of work by Centers for Disease Control and Prevention)

Enterobacteriaceae is a large family of enteric (intestinal) bacteria belonging to the Gammaproteobacteria. They are facultative anaerobes and are able to ferment carbohydrates. Within this family, microbiologists recognize two distinct categories. The first category is called the coliforms, after its prototypical bacterium species, *Escherichia coli*. Coliforms are able to ferment lactose completely (i.e., with the production of acid and gas). The second category, noncoliforms, either cannot ferment lactose or can only ferment it incompletely (producing either acid or gas, but not both). The noncoliforms include some notable human pathogens, such as *Salmonella* spp., *Shigella* spp., and *Yersinia pestis*.

E. coli has been perhaps the most studied bacterium since it was first described in 1886 by Theodor Escherich (1857–1911). Many strains of *E. coli* are in mutualistic relationships with humans. However, some strains produce a potentially deadly toxin called Shiga toxin, which perforates cellular membranes in the large intestine, causing bloody diarrhea and peritonitis (inflammation of the inner linings of the abdominal cavity). Other *E. coli* strains may cause traveler's diarrhea, a less severe but very widespread disease.

The genus *Salmonella*, which belongs to the noncoliform group of *Enterobacteriaceae*, is interesting in that there is still no consensus about how many species it includes. Scientists have reclassified many of the groups they once thought to be species as serotypes (also called serovars), which are strains or variations of the same species of bacteria. Their classification is based on patterns of reactivity by animal antisera against molecules on the surface of the bacterial cells. A number of serotypes of *Salmonella* can cause salmonellosis, characterized by inflammation of the small and the large intestine, accompanied by fever, vomiting, and diarrhea. The species *S. enterobacterica* (serovar *typhi*) causes typhoid fever, with symptoms including fever, abdominal pain, and skin rashes (Figure 6.4.7). Table 6.4.3 summarizes the characteristics of important genera of Gammaproteobacteria.

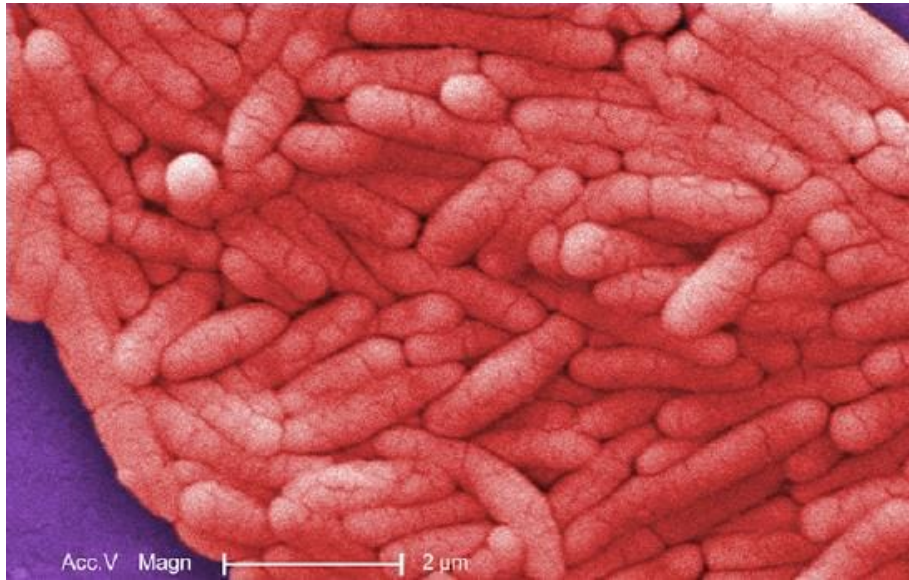


Figure 6.4.7: *Salmonella typhi* is the causative agent of typhoid fever. (credit: Centers for Disease Control and Prevention)

Table 6.4.3: Class Gammaproteobacteria

Example Genus	Microscopic Morphology	Unique Characteristics
<i>Beggiatoa</i>	Gram-negative bacteria; disc-shaped or cylindrical	Aquatic, live in water with high content of hydrogen disulfide; can cause problems for sewage treatment
<i>Enterobacter</i>	Gram-negative bacillus	Facultative anaerobe; cause urinary and respiratory tract infections in hospitalized patients; implicated in the pathogenesis of obesity
<i>Erwinia</i>	Gram-negative bacillus	Plant pathogen causing leaf spots and discoloration; may digest cellulose; prefer relatively low temperatures (25–30 °C)
<i>Escherichia</i>	Gram-negative bacillus	Facultative anaerobe; inhabit the gastrointestinal tract of warm-blooded animals; some strains are mutualists, producing vitamin K; others, like serotype <i>E. coli</i> O157:H7, are pathogens; <i>E. coli</i> has been a model organism for many studies in genetics and molecular biology
<i>Hemophilus</i>	Gram-negative bacillus	Pleomorphic, may appear as coccobacillus, aerobe, or facultative anaerobe; grow on blood agar; pathogenic species can cause respiratory infections, chancroid, and other diseases
<i>Klebsiella</i>	Gram-negative bacillus; appears rounder and thicker than other members of <i>Enterobacteriaceae</i>	Facultative anaerobe, encapsulated, nonmotile; pathogenic species may cause pneumonia, especially in people with alcoholism
<i>Legionella</i>	Gram-negative bacillus	Fastidious, grow on charcoal-buffered yeast extract; <i>L. pneumophila</i> causes Legionnaires disease
<i>Methylomonas</i>	Gram-negative bacillus	Use methane as source of carbon and energy

Example Genus	Microscopic Morphology	Unique Characteristics
<i>Proteus</i>	Gram-negative bacillus (pleomorphic)	Common inhabitants of the human gastrointestinal tract; motile; produce urease; opportunistic pathogens; may cause urinary tract infections and sepsis
<i>Pseudomonas</i>	Gram-negative bacillus	Aerobic; versatile; produce yellow and blue pigments, making them appear green in culture; opportunistic, antibiotic-resistant pathogens may cause wound infections, hospital-acquired infections, and secondary infections in patients with cystic fibrosis
<i>Serratia</i>	Gram-negative bacillus	Motile; may produce red pigment; opportunistic pathogens responsible for a large number of hospital-acquired infections
<i>Shigella</i>	Gram-negative bacillus	Nonmotile; dangerously pathogenic; produce Shiga toxin, which can destroy cells of the gastrointestinal tract; can cause dysentery
<i>Vibrio</i>	Gram-negative, comma- or curved rod-shaped bacteria	Inhabit seawater; flagellated, motile; may produce toxin that causes hypersecretion of water and electrolytes in the gastrointestinal tract; some species may cause serious wound infections
<i>Yersinia</i>	Gram-negative bacillus	Carried by rodents; human pathogens; <i>Y. pestis</i> causes bubonic plague and pneumonic plague; <i>Y. enterocolitica</i> can be a pathogen causing diarrhea in humans

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Deltaproteobacteria

The **Deltaproteobacteria** is a small class of gram-negative Proteobacteria that includes sulfate-reducing bacteria (SRBs), so named because they use sulfate as the final electron acceptor in the electron transport chain. Few SRBs are pathogenic. However, the SRB *Desulfovibrio orale* is associated with periodontal disease (disease of the gums).

Deltaproteobacteria also includes the genus *Bdellovibrio*, species of which are parasites of other gram-negative bacteria. *Bdellovibrio* invades the cells of the host bacterium, positioning itself in the periplasm, the space between the plasma membrane and the cell wall, feeding on the host's proteins and polysaccharides. The infection is lethal for the host cells.

Another type of Deltaproteobacteria, myxobacteria, lives in the soil, scavenging inorganic compounds. Motile and highly social, they interact with other bacteria within and outside their own group. They can form multicellular, macroscopic "fruiting bodies" (Figure 6.4.8), structures that are still being studied by biologists and bacterial ecologists.² These bacteria can also form metabolically inactive myxospores. Table 6.4.4 summarizes the characteristics of several important genera of Deltaproteobacteria.

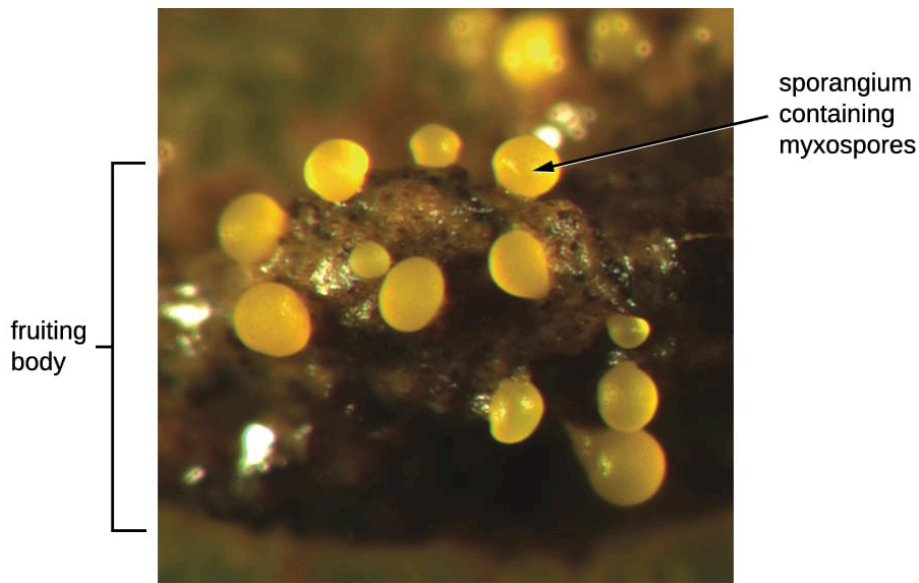


Figure 6.4.8: Myxobacteria form fruiting bodies. (credit: modification of work by Michiel Vos)

Table 6.4.4: Class Deltaproteobacteria

Genus	Microscopic Morphology	Unique characteristics
<i>Bdellovibrio</i>	Gram-negative, comma-shaped rod	Obligate aerobes; motile; parasitic (infecting other bacteria)
<i>Desulfovibrio</i> (formerly <i>Desulfuromonas</i>)	Gram-negative, comma-shaped rod	Reduce sulfur; can be used for removal of toxic and radioactive waste
<i>Myxobacterium</i>	Gram-negative, coccoid bacteria forming colonies (swarms)	Live in soil; can move by gliding; used as a model organism for studies of intercellular communication (signaling)

Epsilonproteobacteria

The smallest class of Proteobacteria is **Epsilonproteobacteria**, which are gram-negative microaerophilic bacteria (meaning they only require small amounts of oxygen in their environment). Two clinically relevant genera of Epsilonproteobacteria are *Campylobacter* and *Helicobacter*, both of which include human pathogens. *Campylobacter* can cause food poisoning that manifests as severe enteritis (inflammation in the small intestine). This condition, caused by the species *C. jejuni*, is rather common in developed countries, usually because of eating contaminated poultry products. Chickens often harbor *C. jejuni* in their gastrointestinal tract and feces, and their meat can become contaminated during processing.

Within the genus *Helicobacter*, the helical, flagellated bacterium *H. pylori* has been identified as a beneficial member of the stomach microbiota, but it is also the most common cause of chronic gastritis and ulcers of the stomach and duodenum (Figure 6.4.9). Studies have also shown that *H. pylori* is linked to stomach cancer.³ *H. pylori* is somewhat unusual in its ability to survive in the highly acidic environment of the stomach. It produces urease and other enzymes that modify its environment to make it less acidic. Table 6.4.5 summarizes the characteristics of the most clinically relevant genera of Epsilonproteobacteria.

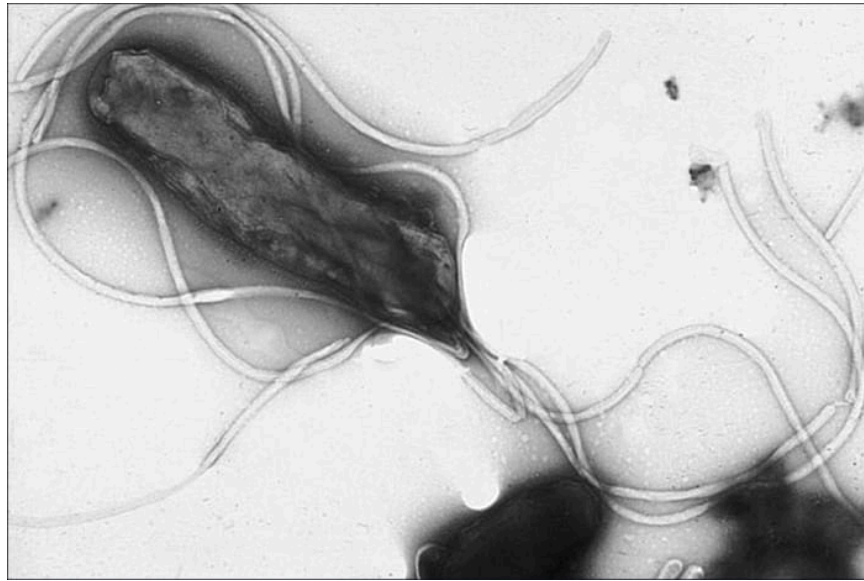


Figure 6.4.9: *Helicobacter pylori* can cause chronic gastritis, which can lead to ulcers and stomach cancer.

Table 6.4.5: Class Epsilonproteobacteria

Example Genus	Microscopic Morphology	Unique Characteristics
<i>Campylobacter</i>	Gram-negative, spiral-shaped rod	Aerobic (microaerophilic); often infects chickens; may infect humans via undercooked meat, causing severe enteritis
<i>Helicobacter</i>	Gram-negative, spiral-shaped rod	Aerobic (microaerophilic) bacterium; can damage the inner lining of the stomach, causing chronic gastritis, peptic ulcers, and stomach cancer

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Key Concepts and Summary

- **Gammaproteobacteria** are the largest and the most diverse group of Proteobacteria. Many are human pathogens that are aerobes or facultative anaerobes. Some Gammaproteobacteria are **enteric** bacteria that may be coliform or noncoliform. *Escherichia coli*, a member of Gammaproteobacteria, is perhaps the most studied bacterium.

- **Deltaproteobacteria** make up a small group able to reduce sulfate or elemental sulfur. Some are scavengers and form myxospores, with multicellular fruiting bodies.
- **Epsilonproteobacteria** make up the smallest group of Proteobacteria. The genera *Campylobacter* and *Helicobacter* are human pathogens.

Footnotes

1. C.R. Woese. "Bacterial Evolution." *Microbiological Review* 51 no. 2 (1987):221–271.
2. H. Reichenbach. "Myxobacteria, Producers of Novel Bioactive Substances." *Journal of Industrial Microbiology & Biotechnology* 27 no. 3 (2001):149–156.
3. S. Suerbaum, P. Michetti. "*Helicobacter pylori* infection." *New England Journal of Medicine* 347 no. 15 (2002):1175–1186.

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6.5: Spirochetes and CFB

Learning Objectives

- Describe the unique features of nonproteobacteria gram-negative bacteria
- Give an example of a nonproteobacteria bacterium in each category
- Describe the unique features of phototrophic bacteria
- Identify phototrophic bacteria

The majority of the gram-negative bacteria belong to the phylum Proteobacteria, discussed in the previous section. Those that do not are called the nonproteobacteria. In this section, we will describe three classes of gram-negative nonproteobacteria: the spirochetes, the CFB group, and the Planctomycetes. A diverse group of phototrophic bacteria that includes Proteobacteria and nonproteobacteria will be discussed at the end of this section.

Spirochetes

Spirochetes are characterized by their long (up to 250 μm), spiral-shaped bodies. Most spirochetes are also very thin, which makes it difficult to examine gram-stained preparations under a conventional brightfield microscope. Darkfield fluorescent microscopy is typically used instead. Spirochetes are also difficult or even impossible to culture. They are highly motile, using their axial filament to propel themselves. The axial filament is similar to a flagellum, but it wraps around the cell and runs inside the cell body of a spirochete in the periplasmic space between the outer membrane and the plasma membrane (Figure 6.5.1).

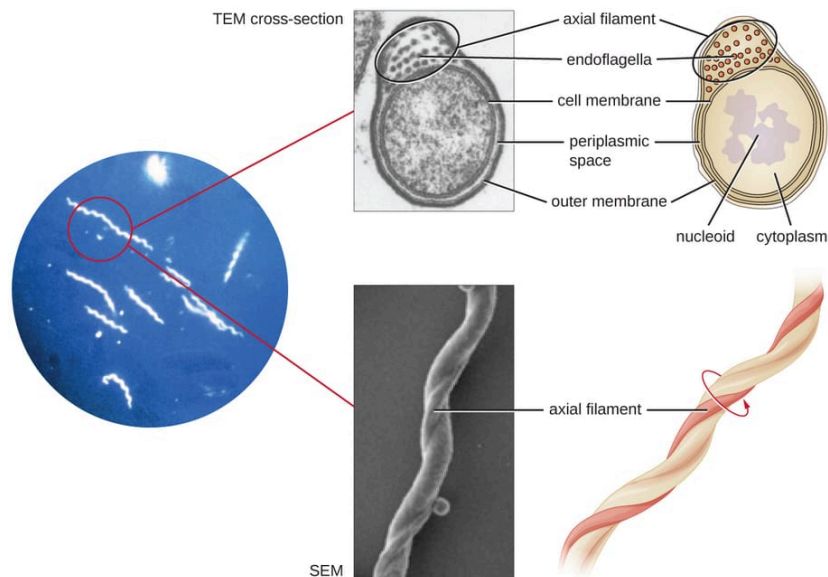


Figure 6.5.1: Spirochetes are typically observed using darkfield microscopy (left). However, electron microscopy (top center, bottom center) provides a more detailed view of their cellular morphology. The flagella found between the inner and outer membranes of spirochetes wrap around the bacterium, causing a twisting motion used for locomotion. (credit “spirochetes” micrograph: modification of work by Centers for Disease Control and Prevention; credit “SEM/TEM”: modification of work by Guyard C, Raffel SJ, Schruppf ME, Dahlstrom E, Sturdevant D, Ricklefs SM, Martens C, Hayes SF, Fischer ER, Hansen BT, Porcella SF, Schwan TG)

Several genera of spirochetes include human pathogens. For example, the genus *Treponema* includes a species *T. pallidum*, which is further classified into four subspecies: *T. pallidum pallidum*, *T. pallidum pertenue*, *T. pallidum carateum*, and *T. pallidum endemicum*. The subspecies *T. pallidum pallidum* causes the sexually transmitted infection known as syphilis, the third most prevalent sexually transmitted bacterial infection in the United States, after chlamydia and gonorrhea. The other subspecies of *T. pallidum* cause tropical infectious diseases of the skin, bones, and joints.

Another genus of spirochete, *Borrelia*, contains a number of pathogenic species. *B. burgdorferi* causes Lyme disease, which is

transmitted by several genera of ticks (notably *Ixodes* and *Amblyomma*) and often produces a “bull’s eye” rash, fever, fatigue, and, sometimes, debilitating arthritis. *B. recurrentis* causes a condition known as relapsing fever.

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Cytophaga, Fusobacterium, and Bacteroides

The gram-negative nonproteobacteria of the genera *Cytophaga*, *Fusobacterium*, and *Bacteroides* are classified together as a phylum and called the CFB group. Although they are phylogenetically diverse, bacteria of the **CFB group** share some similarities in the sequence of nucleotides in their DNA. They are rod-shaped bacteria adapted to anaerobic environments, such as the tissue of the gums, gut, and rumen of ruminating animals. CFB bacteria are avid fermenters, able to process cellulose in rumen, thus enabling ruminant animals to obtain carbon and energy from grazing.

Cytophaga are motile aquatic bacteria that glide. *Fusobacteria* inhabit the human mouth and may cause severe infectious diseases. The largest genus of the CFB group is *Bacteroides*, which includes dozens of species that are prevalent inhabitants of the human large intestine, making up about 30% of the entire gut microbiome (Figure 6.5.2). One gram of human feces contains up to 100 billion *Bacteroides* cells. Most *Bacteroides* are mutualistic. They benefit from nutrients they find in the gut, and humans benefit from their ability to prevent pathogens from colonizing the large intestine. Indeed, when populations of *Bacteroides* are reduced in the gut—as often occurs when a patient takes antibiotics—the gut becomes a more favorable environment for pathogenic bacteria and fungi, which can cause secondary infections.



Figure 6.5.2: Bacteroides comprise up to 30% of the normal microbiota in the human gut. (credit: NOAA)

Only a few species of *Bacteroides* are pathogenic. *B. melaninogenicus*, for example, can cause wound infections in patients with weakened immune systems.

Key Concepts and Summary

- Gram-negative nonproteobacteria include the taxa **spirochetes**; the *Cytophaga*, *Fusobacterium*, *Bacteroides* group;

Planctomycetes; and many representatives of **phototrophic bacteria**.

- Spirochetes are motile, spiral bacteria with a long, narrow body; they are difficult or impossible to culture.
- Several genera of spirochetes contain human pathogens that cause such diseases as syphilis and Lyme disease.
- *Cytophaga*, *Fusobacterium*, and *Bacteroides* are classified together as a phylum called the **CFB group**. They are rod-shaped anaerobic organoheterotrophs and avid fermenters. *Cytophaga* are aquatic bacteria with the gliding motility. *Fusobacteria* inhabit the human mouth and may cause severe infectious diseases. *Bacteroides* are present in vast numbers in the human gut, most of them being mutualistic but some are pathogenic.

Footnotes

1. R.C. Fuller et al. "Carbon Metabolism in *Chromatium*." *Journal of Biological Chemistry* 236 (1961):2140–2149.
2. T.T. Selao et al. "Comparative Proteomic Studies in *Rhodospirillum rubrum* Grown Under Different Nitrogen Conditions." *Journal of Proteome Research* 7 no. 8 (2008):3267–3275.
3. A. De los Rios et al. "Ultrastructural and Genetic Characteristics of Endolithic Cyanobacterial Biofilms Colonizing Antarctic Granite Rocks." *FEMS Microbiology Ecology* 59 no. 2 (2007):386–395.
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5. S. Zhang, D.A. Bryant. "The Tricarboxylic Acid Cycle in Cyanobacteria." *Science* 334 no. 6062 (2011):1551–1553.
6. A. Cain et al. "Cyanobacteria as a Biosorbent for Mercuric Ion." *Bioresource Technology* 99 no. 14 (2008):6578–6586.
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8. I. Stewart et al. Cyanobacterial Poisoning in Livestock, Wild Mammals and Birds – an Overview. *Advances in Experimental Medicine and Biology* 619 (2008):613–637.

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6.6: Gram-positive Bacteria

Learning Objectives

- Describe the unique features of each category of high G+C and low G+C gram-positive bacteria
- Identify similarities and differences between high G+C and low G+C bacterial groups
- Give an example of a bacterium of high G+C and low G+C group commonly associated with each category

Prokaryotes are identified as gram-positive if they have a multiple layer matrix of peptidoglycan forming the cell wall. Crystal violet, the primary stain of the Gram stain procedure, is readily retained and stabilized within this matrix, causing gram-positive prokaryotes to appear purple under a brightfield microscope after Gram staining. For many years, the retention of Gram stain was one of the main criteria used to classify prokaryotes, even though some prokaryotes did not readily stain with either the primary or secondary stains used in the Gram stain procedure.

Advances in nucleic acid biochemistry have revealed additional characteristics that can be used to classify gram-positive prokaryotes, namely the guanine to cytosine ratios (G+C) in DNA and the composition of 16S rRNA subunits. Microbiologists currently recognize two distinct groups of gram-positive, or weakly staining gram-positive, prokaryotes. The class Actinobacteria comprises the **high G+C gram-positive bacteria**, which have more than 50% guanine and cytosine nucleotides in their DNA. The class Bacilli comprises **low G+C gram-positive bacteria**, which have less than 50% of guanine and cytosine nucleotides in their DNA.

Actinobacteria: High G+C Gram-Positive Bacteria

The name Actinobacteria comes from the Greek words for *rays* and *small rod*, but Actinobacteria are very diverse. Their microscopic appearance can range from thin filamentous branching rods to coccobacilli. Some Actinobacteria are very large and complex, whereas others are among the smallest independently living organisms. Most Actinobacteria live in the soil, but some are aquatic. The vast majority are aerobic. One distinctive feature of this group is the presence of several different peptidoglycans in the cell wall.

The genus *Actinomyces* is a much studied representative of Actinobacteria. *Actinomyces* spp. play an important role in soil ecology, and some species are human pathogens. A number of *Actinomyces* spp. inhabit the human mouth and are opportunistic pathogens, causing infectious diseases like periodontitis (inflammation of the gums) and oral abscesses. The species *A. israelii* is an anaerobe notorious for causing endocarditis (inflammation of the inner lining of the heart) (Figure 6.6.1).

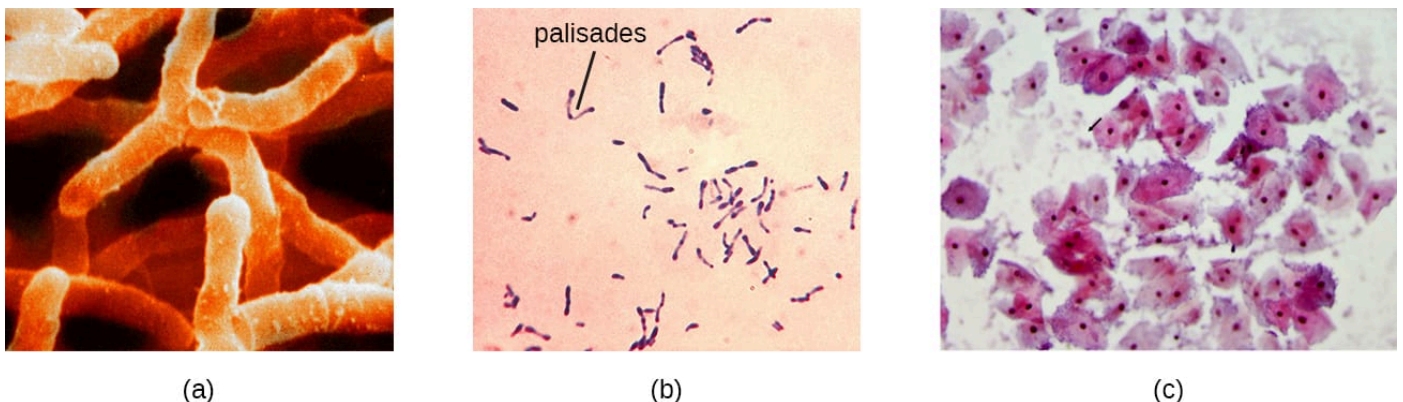


Figure 6.6.1: (a) *Actinomyces israelii* (false-color scanning electron micrograph [SEM]) has a branched structure. (b) *Corynebacterium diphtheriae* causes the deadly disease diphtheria. Note the distinctive palisades. (c) The gram-variable bacterium *Gardnerella vaginalis* causes bacterial vaginosis in women. This micrograph shows a Pap smear from a woman with vaginosis. (credit a: modification of work by "GrahamColm"/Wikimedia Commons; credit b: modification of work by Centers for Disease Control and Prevention; credit c: modification of work by Mwakigonja AR, Torres LM, Mwakyoma HA, Kaaya EE)

The genus *Mycobacterium* is represented by bacilli covered with a mycolic acid coat. This waxy coat protects the bacteria from

some antibiotics, prevents them from drying out, and blocks penetration by Gram stain reagents (see [Staining Microscopic Specimens](#)). Because of this, a special acid-fast staining procedure is used to visualize these bacteria. The genus *Mycobacterium* is an important cause of a diverse group of infectious diseases. *M. tuberculosis* is the causative agent of tuberculosis, a disease that primarily impacts the lungs but can infect other parts of the body as well. It has been estimated that one-third of the world's population has been infected with *M. tuberculosis* and millions of new infections occur each year. Treatment of *M. tuberculosis* is challenging and requires patients to take a combination of drugs for an extended time. Complicating treatment even further is the development and spread of multidrug-resistant strains of this pathogen.

Another pathogenic species, *M. leprae*, is the cause of Hansen's disease (leprosy), a chronic disease that impacts peripheral nerves and the integrity of the skin and mucosal surface of the respiratory tract. Loss of pain sensation and the presence of skin lesions increase susceptibility to secondary injuries and infections with other pathogens.

Bacteria in the genus *Corynebacterium* contain diaminopimelic acid in their cell walls, and microscopically often form *palisades*, or pairs of rod-shaped cells resembling the letter V. Cells may contain metachromatic granules, intracellular storage of inorganic phosphates that are useful for identification of *Corynebacterium*. The vast majority of *Corynebacterium* spp. are nonpathogenic; however, *C. diphtheria* is the causative agent of diphtheria, a disease that can be fatal, especially in children (Figure 6.6.1). *C. diphtheria* produces a toxin that forms a pseudomembrane in the patient's throat, causing swelling, difficulty breathing, and other symptoms that can become serious if untreated.

The genus *Bifidobacterium* consists of filamentous anaerobes, many of which are commonly found in the gastrointestinal tract, vagina, and mouth. In fact, *Bifidobacterium* spp. constitute a substantial part of the human gut microbiota and are frequently used as probiotics and in yogurt production.

The genus *Gardnerella*, contains only one species, *G. vaginalis*. This species is defined as "gram-variable" because its small coccobacilli do not show consistent results when Gram stained (Figure 6.6.1). Based on its genome, it is placed into the high G+C gram-positive group. *G. vaginalis* can cause bacterial vaginosis in women; symptoms are typically mild or even undetectable, but can lead to complications during pregnancy.

Table 6.6.1 summarizes the characteristics of some important genera of Actinobacteria.

Table 6.6.1: Actinobacteria: High G+C Gram-Positive

Example Genus	Microscopic Morphology	Unique Characteristics
<i>Actinomyces</i>	Gram-positive bacillus; in colonies, shows fungus-like threads (hyphae)	Facultative anaerobes; in soil, decompose organic matter; in the human mouth, may cause gum disease
<i>Arthrobacter</i>	Gram-positive bacillus (at the exponential stage of growth) or coccus (in stationary phase)	Obligate aerobes; divide by "snapping," forming V-like pairs of daughter cells; degrade phenol, can be used in bioremediation
<i>Bifidobacterium</i>	Gram-positive, filamentous actinobacterium	Anaerobes commonly found in human gut microbiota
<i>Corynebacterium</i>	Gram-positive bacillus	Aerobes or facultative anaerobes; form palisades; grow slowly; require enriched media in culture; <i>C. diphtheriae</i> causes diphtheria
<i>Frankia</i>	Gram-positive, fungus-like (filamentous) bacillus	Nitrogen-fixing bacteria; live in symbiosis with legumes
<i>Gardnerella</i>	Gram-variable coccobacillus	Colonize the human vagina, may alter the microbial ecology, thus leading to vaginosis
<i>Micrococcus</i>	Gram-positive coccus, form microscopic clusters	Ubiquitous in the environment and on the human skin; oxidase-positive (as opposed to morphologically similar <i>S. aureus</i>); some are opportunistic pathogens
<i>Mycobacterium</i>	Gram-positive, acid-fast bacillus	Slow growing, aerobic, resistant to drying and phagocytosis; covered with a waxy coat made of mycolic acid; <i>M. tuberculosis</i> causes tuberculosis; <i>M. leprae</i> causes leprosy

Example Genus	Microscopic Morphology	Unique Characteristics
<i>Nocardia</i>	Weakly gram-positive bacillus; forms acid-fast branches	May colonize the human gingiva; may cause severe pneumonia and inflammation of the skin
<i>Propionibacterium</i>	Gram-positive bacillus	Aerotolerant anaerobe; slow-growing; <i>P. acnes</i> reproduces in the human sebaceous glands and may cause or contribute to acne
<i>Rhodococcus</i>	Gram-positive bacillus	Strict aerobe; used in industry for biodegradation of pollutants; <i>R. fascians</i> is a plant pathogen, and <i>R. equi</i> causes pneumonia in foals
<i>Streptomyces</i>	Gram-positive, fungus-like (filamentous) bacillus	Very diverse genus (>500 species); aerobic, spore-forming bacteria; scavengers, decomposers found in soil (give the soil its “earthy” odor); used in pharmaceutical industry as antibiotic producers (more than two-thirds of clinically useful antibiotics)

Query 6.6.1

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Low G+C Gram-positive Bacteria

The low G+C gram-positive bacteria have less than 50% guanine and cytosine in their DNA, and this group of bacteria includes a number of genera of bacteria that are pathogenic.

Clostridia

One large and diverse class of low G+C gram-positive bacteria is Clostridia. The best studied genus of this class is *Clostridium*. These rod-shaped bacteria are generally obligate anaerobes that produce endospores and can be found in anaerobic habitats like soil and aquatic sediments rich in organic nutrients. The endospores may survive for many years.

Clostridium spp. produce more kinds of protein toxins than any other bacterial genus, and several species are human pathogens. *C. perfringens* is the third most common cause of food poisoning in the United States and is the causative agent of an even more serious disease called gas gangrene. Gas gangrene occurs when *C. perfringens* endospores enter a wound and germinate, becoming viable bacterial cells and producing a toxin that can cause the necrosis (death) of tissue. *C. tetani*, which causes tetanus, produces a neurotoxin that is able to enter neurons, travel to regions of the central nervous system where it blocks the inhibition of nerve impulses involved in muscle contractions, and cause a life-threatening spastic paralysis. *C. botulinum* produces botulinum neurotoxin, the most lethal biological toxin known. Botulinum toxin is responsible for rare but frequently fatal cases of botulism. The toxin blocks the release of acetylcholine in neuromuscular junctions, causing flaccid paralysis. In very small concentrations, botulinum toxin has been used to treat muscle pathologies in humans and in a cosmetic procedure to eliminate wrinkles. *C. difficile* is a common source of hospital-acquired infections (Figure 6.6.2) that can result in serious and even fatal cases of colitis (inflammation of the large intestine). Infections often occur in patients who are immunosuppressed or undergoing antibiotic therapy that alters the normal microbiota of the gastrointestinal tract.

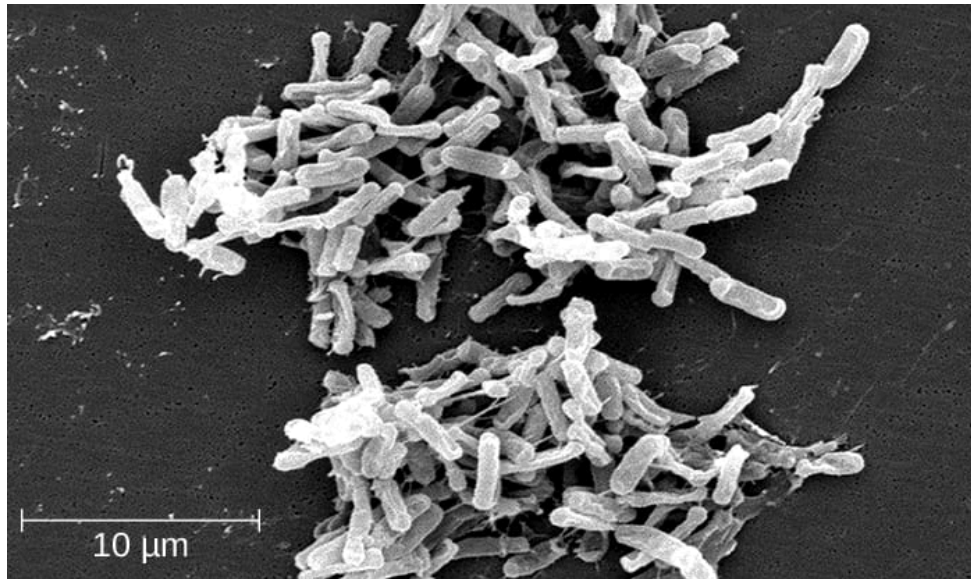


Figure 6.6.2: *Clostridium difficile*, a gram-positive, rod-shaped bacterium, causes severe colitis and diarrhea, often after the normal gut microbiota is eradicated by antibiotics. (credit: modification of work by Centers for Disease Control and Prevention)

Lactobacillales

The order Lactobacillales comprises low G+C gram-positive bacteria that include both bacilli and cocci in the genera *Lactobacillus*, *Leuconostoc*, *Enterococcus*, and *Streptococcus*. Bacteria of the latter three genera typically are spherical or ovoid and often form chains.

Streptococcus, the name of which comes from the Greek word for *twisted chain*, is responsible for many types of infectious diseases in humans. Species from this genus, often referred to as streptococci, are usually classified by serotypes called Lancefield groups, and by their ability to lyse red blood cells when grown on blood agar.

S. pyogenes belongs to the Lancefield group A, β -hemolytic *Streptococcus*. This species is considered a pyogenic pathogen because of the associated pus production observed with infections it causes (Figure 6.6.3). *S. pyogenes* is the most common cause of bacterial pharyngitis (strep throat); it is also an important cause of various skin infections that can be relatively mild (e.g., impetigo) or life threatening (e.g., necrotizing fasciitis, also known as flesh eating disease), life threatening.

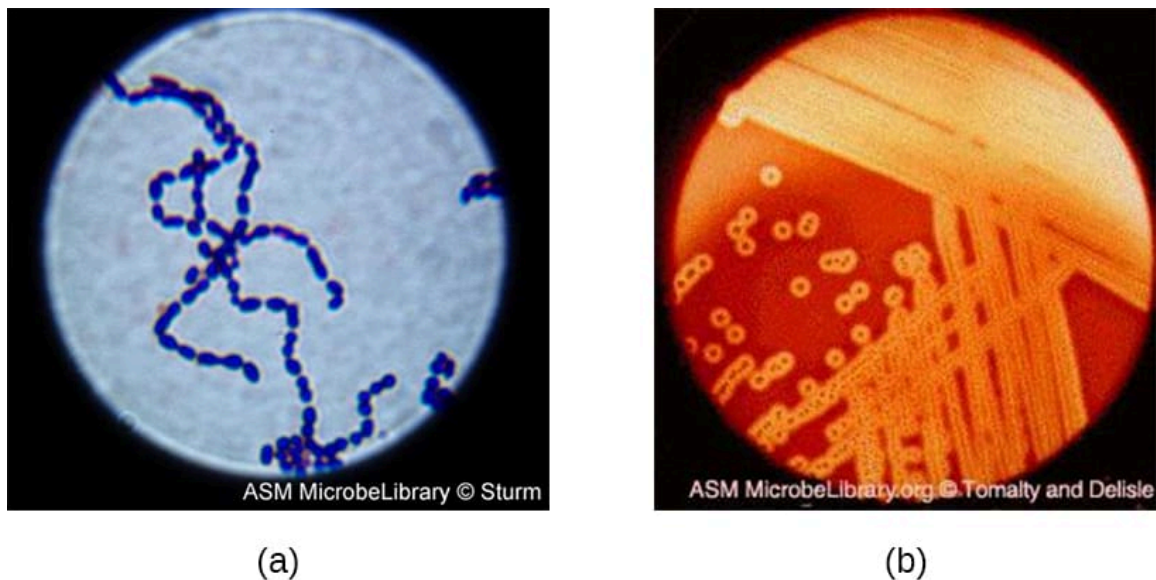


Figure 6.6.3: (a) A gram-stained specimen of *Streptococcus pyogenes* shows the chains of cocci characteristic of this organism's morphology. (b) *S. pyogenes* on blood agar shows characteristic lysis of red blood cells, indicated by the halo of clearing around colonies. (credit a, b: modification of work by American Society for Microbiology)

The nonpyogenic (i.e., not associated with pus production) streptococci are a group of streptococcal species that are not a taxon but are grouped together because they inhabit the human mouth. The nonpyogenic streptococci do not belong to any of the Lancefield groups. Most are commensals, but a few, such as *S. mutans*, are implicated in the development of dental caries.

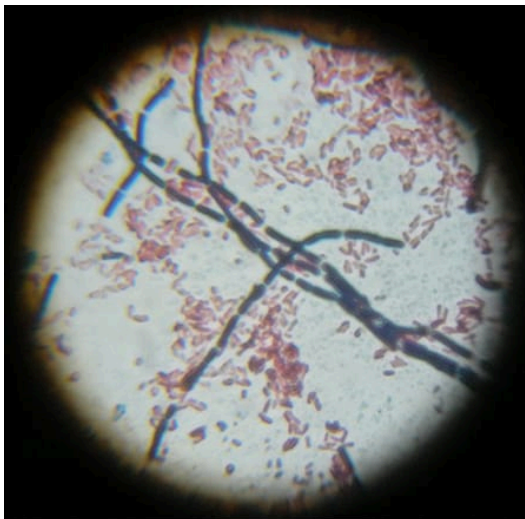
S. pneumoniae (commonly referred to as pneumococcus), is a *Streptococcus* species that also does not belong to any Lancefield group. *S. pneumoniae* cells appear microscopically as diplococci, pairs of cells, rather than the long chains typical of most streptococci. Scientists have known since the 19th century that *S. pneumoniae* causes pneumonia and other respiratory infections. However, this bacterium can also cause a wide range of other diseases, including meningitis, septicemia, osteomyelitis, and endocarditis, especially in newborns, the elderly, and patients with immunodeficiency.

Bacilli

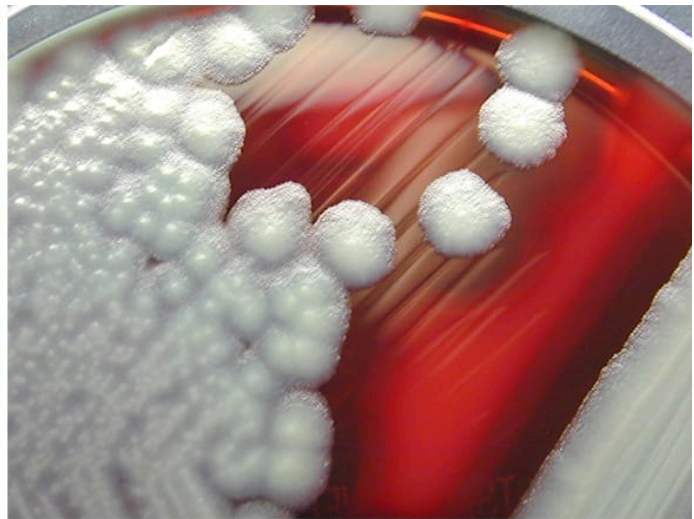
The name of the class Bacilli suggests that it is made up of bacteria that are bacillus in shape, but it is a morphologically diverse class that includes bacillus-shaped and coccus-shaped genera. Among the many genera in this class are two that are very important clinically: *Bacillus* and *Staphylococcus*.

Bacteria in the genus *Bacillus* are bacillus in shape and can produce endospores. They include aerobes or facultative anaerobes. A number of *Bacillus* spp. are used in various industries, including the production of antibiotics (e.g., barnase), enzymes (e.g., alpha-amylase, BamH1 restriction endonuclease), and detergents (e.g., subtilisin).

Two notable pathogens belong to the genus *Bacillus*. *B. anthracis* is the pathogen that causes anthrax, a severe disease that affects wild and domesticated animals and can spread from infected animals to humans. Anthrax manifests in humans as charcoal-black ulcers on the skin, severe enterocolitis, pneumonia, and brain damage due to swelling. If untreated, anthrax is lethal. *B. cereus*, a closely related species, is a pathogen that may cause food poisoning. It is a rod-shaped species that forms chains. Colonies appear milky white with irregular shapes when cultured on blood agar (Figure 6.6.4). One other important species is *B. thuringiensis*. This bacterium produces a number of substances used as insecticides because they are toxic for insects.



(a)



(b)

Figure 6.6.4: (a) In this gram-stained specimen, the violet rod-shaped cells forming chains are the gram-positive bacteria *Bacillus cereus*. The small, pink cells are the gram-negative bacteria *Escherichia coli*. (b) In this culture, white colonies of *B. cereus* have been grown on sheep blood agar. (credit a: modification of work by “Bibliomaniac 15”/Wikimedia Commons; credit b: modification of work by Centers for Disease Control and Prevention)

The genus *Staphylococcus* also belongs to the class Bacilli, even though its shape is coccus rather than a bacillus. The name *Staphylococcus* comes from a Greek word for *bunches of grapes*, which describes their microscopic appearance in culture (Figure 6.6.5). *Staphylococcus* spp. are facultative anaerobic, halophilic, and nonmotile. The two best-studied species of this genus are *S. epidermidis* and *S. aureus*.

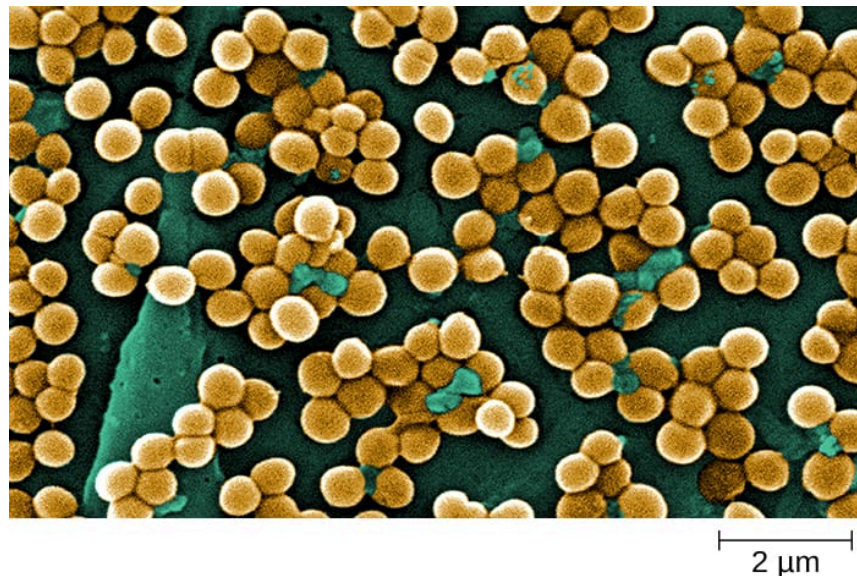


Figure 6.6.5: This SEM of *Staphylococcus aureus* illustrates the typical “grape-like” clustering of cells. (credit: modification of work by Centers for Disease Control and Prevention)

S. epidermidis, whose main habitat is the human skin, is thought to be nonpathogenic for humans with healthy immune systems, but in patients with immunodeficiency, it may cause infections in skin wounds and prostheses (e.g., artificial joints, heart valves). *S. epidermidis* is also an important cause of infections associated with intravenous catheters. This makes it a dangerous pathogen in hospital settings, where many patients may be immunocompromised.

Strains of *S. aureus* cause a wide variety of infections in humans, including skin infections that produce boils, carbuncles, cellulitis, or impetigo. Certain strains of *S. aureus* produce a substance called enterotoxin, which can cause severe enteritis, often called staph food poisoning. Some strains of *S. aureus* produce the toxin responsible for toxic shock syndrome, which can result in

cardiovascular collapse and death.

Many strains of *S. aureus* have developed resistance to antibiotics. Some antibiotic-resistant strains are designated as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). These strains are some of the most difficult to treat because they exhibit resistance to nearly all available antibiotics, not just methicillin and vancomycin. Because they are difficult to treat with antibiotics, infections can be lethal. MRSA and VRSA are also contagious, posing a serious threat in hospitals, nursing homes, dialysis facilities, and other places where there are large populations of elderly, bedridden, and/or immunocompromised patients.

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Biopiracy and Bioprospecting

In 1969, an employee of a Swiss pharmaceutical company was vacationing in Norway and decided to collect some soil samples. He took them back to his lab, and the Swiss company subsequently used the fungus *Tolypocladium inflatum* in those samples to develop cyclosporine A, a drug widely used in patients who undergo tissue or organ transplantation. The Swiss company earns more than \$1 billion a year for production of cyclosporine A, yet Norway receives nothing in return—no payment to the government or benefit for the Norwegian people. Despite the fact the cyclosporine A saves numerous lives, many consider the means by which the soil samples were obtained to be an act of “biopiracy,” essentially a form of theft. Do the ends justify the means in a case like this?

Nature is full of as-yet-undiscovered bacteria and other microorganisms that could one day be used to develop new life-saving drugs or treatments.¹ Pharmaceutical and biotechnology companies stand to reap huge profits from such discoveries, but ethical questions remain. To whom do biological resources belong? Should companies who invest (and risk) millions of dollars in research and development be required to share revenue or royalties for the right to access biological resources?

Compensation is not the only issue when it comes to bioprospecting. Some communities and cultures are philosophically opposed to bioprospecting, fearing unforeseen consequences of collecting genetic or biological material. Native Hawaiians, for example, are very protective of their unique biological resources.

For many years, it was unclear what rights government agencies, private corporations, and citizens had when it came to collecting samples of microorganisms from public land. Then, in 1993, the Convention on Biological Diversity granted each nation the rights to any genetic and biological material found on their own land. Scientists can no longer collect samples without a prior arrangement with the land owner for compensation. This convention now ensures that companies act ethically in obtaining the samples they use to create their products.

Key Concepts and Summary

- Gram-positive bacteria are a very large and diverse group of microorganisms. Understanding their taxonomy and knowing their unique features is important for diagnostics and treatment of infectious diseases.
- Gram-positive bacteria are classified into **high G+C gram-positive** and **low G+C gram-positive** bacteria, based on the prevalence of guanine and cytosine nucleotides in their genome
- Actinobacteria is the taxonomic name of the class of high G+C gram-positive bacteria. This class includes the genera *Actinomyces*, *Arthrobacter*, *Corynebacterium*, *Frankia*, *Gardnerella*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, *Rhodococcus*, and *Streptomyces*. Some representatives of these genera are used in industry; others are human or animal pathogens.
- Examples of high G+C gram-positive bacteria that are human pathogens include *Mycobacterium tuberculosis*, which causes tuberculosis; *M. leprae*, which causes leprosy (Hansen's disease); and *Corynebacterium diphtheriae*, which causes diphtheria.
- *Clostridia* spp. are low G+C gram-positive bacteria that are generally obligate anaerobes and can form endospores. Pathogens in this genus include *C. perfringens* (gas gangrene), *C. tetani* (tetanus), and *C. botulinum* (botulism).
- Lactobacillales include the genera *Enterococcus*, *Lactobacillus*, *Leuconostoc*, and *Streptococcus*. *Streptococcus* is responsible for many human diseases, including pharyngitis (strep throat), scarlet fever, rheumatic fever, glomerulonephritis, pneumonia, and other respiratory infections.
- Bacilli is a taxonomic class of low G+C gram-positive bacteria that include rod-shaped and coccus-shaped species, including the genera *Bacillus* and *Staphylococcus*. *B. anthracis* causes anthrax, *B. cereus* may cause opportunistic infections of the gastrointestinal tract, and *S. aureus* strains can cause a wide range of infections and diseases, many of which are highly resistant to antibiotics.
- *Mycoplasma* spp. are very small, **pleomorphic** low G+C gram-positive bacteria that lack cell walls. *M. pneumoniae* causes atypical pneumonia.

Footnotes

1. J. Andre. *Bioethics as Practice*. Chapel Hill, NC: University of North Carolina Press, 2002.

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6.E: Prokaryotic Diversity (Exercises)

6.1: Prokaryote Habitats, Relationships, and Microbiomes

Prokaryotes are unicellular microorganisms whose cells have no nucleus. Prokaryotes can be found everywhere on our planet, even in the most extreme environments. Prokaryotes are very flexible metabolically, so they are able to adjust their feeding to the available natural resources. Prokaryotes live in communities that interact among themselves and with large organisms that they use as hosts (including humans).

Multiple Choice

The term prokaryotes refers to which of the following?

- A. very small organisms
- B. unicellular organisms that have no nucleus
- C. multicellular organisms
- D. cells that resemble animal cells more than plant cells

Answer

B

The term microbiota refers to which of the following?

- A. all microorganisms of the same species
- B. all of the microorganisms involved in a symbiotic relationship
- C. all microorganisms in a certain region of the human body
- D. all microorganisms in a certain geographic region

Answer

C

Which of the following refers to the type of interaction between two prokaryotic populations in which one population benefits and the other is not affected?

- A. mutualism
- B. commensalism
- C. parasitism
- D. neutralism

Answer

B

True/False

Among prokaryotes, there are some that can live in every environment on earth.

Answer

True

Fill in the Blank

When prokaryotes live as interacting communities in which one population benefits to the harm of the other, the type of symbiosis is called _____.

Answer

parasitism

The domain _____ does not include prokaryotes.

Answer

Eukarya

Pathogenic bacteria that are part of the transient microbiota can sometimes be eliminated by _____ therapy.

Answer

antibiotic

Nitrogen-fixing bacteria provide other organisms with usable nitrogen in the form of _____.

Answer

ammonia

Short Answer

Compare commensalism and amensalism.

Give an example of the changes of human microbiota that result from medical intervention.

6.2: Proteobacteria

Proteobacteria is a phylum of gram-negative bacteria and are classified into the classes alpha-, beta-, gamma-, delta- and epsilonproteobacteria, each class having separate orders, families, genera, and species. Alphaproteobacteria are oligotrophs. The taxa chlamydias and rickettsias are obligate intracellular pathogens, feeding on cells of host organisms; they are metabolically inactive outside of the host cell. Some Alphaproteobacteria can convert atmospheric nitrogen to nitrites.

Multiple Choice

Which of the following describes Proteobacteria in domain Bacteria?

- A. phylum
- B. class
- C. species
- D. genus

Answer

A

All Alphaproteobacteria are which of the following?

- A. oligotrophs
- B. intracellular
- C. pathogenic

- D. all of the above
- E. none of the above

Answer

A

Class Betaproteobacteria includes all but which of the following genera?

- A. *Neisseria*.
- B. *Bordetella*.
- C. *Leptothrix*.
- D. *Campylobacter*.

Answer

D

Haemophilus influenzae is a common cause of which of the following?

- A. influenza
- B. dysentery
- C. upper respiratory tract infections
- D. hemophilia

Answer

C

Fill in the Blank

Rickettsias are _____ intracellular bacteria.

Answer

obligate

The species _____, which belongs to Epsilonproteobacteria, causes peptic ulcers of the stomach and duodenum.

Answer

Helicobacter pylori

The genus *Salmonella* belongs to the class _____ and includes pathogens that cause salmonellosis and typhoid fever.

Answer

Gammaproteobacteria

Short Answer

What is the metabolic difference between coliforms and noncoliforms? Which category contains several species of intestinal pathogens?

Why are *Mycoplasma* and *Chlamydia* classified as obligate intracellular pathogens?

Critical Thinking

The cell shown is found in the human stomach and is now known to cause peptic ulcers. What is the name of this bacterium?



(credit: American Society for Microbiology)

6.3: Nonproteobacteria Gram-negative Bacteria and Phototrophic Bacteria

Gram-negative nonproteobacteria include the taxa spirochetes; the Cytophaga, Fusobacterium, Bacteroides group; Planctomycetes; and many representatives of phototrophic bacteria. Spirochetes are motile, spiral bacteria with a long, narrow body; they are difficult or impossible to culture. Several genera of spirochetes contain human pathogens that cause such diseases as syphilis and Lyme disease. Cytophaga, Fusobacterium, and Bacteroides are classified together as a phylum called the CFB group.

Multiple Choice

Which of the following is the organelle that spirochetes use to propel themselves?

- A. plasma membrane
- B. axial filament
- C. pilum
- D. fimbria

Answer

B

Which of the following bacteria are the most prevalent in the human gut?

- A. cyanobacteria
- B. staphylococci
- C. *Borrelia*
- D. *Bacteroides*

Answer

D

Which of the following refers to photosynthesis performed by bacteria with the use of water as the donor of electrons?

- A. oxygenic
- B. anoxygenic
- C. heterotrophic
- D. phototrophic

Answer

A

Fill in the Blank

The bacterium that causes syphilis is called _____.

Answer

Treponema pallidum pallidum

Bacteria in the genus *Rhodospirillum* that use hydrogen for oxidation and fix nitrogen are _____ bacteria.

Answer

purple nonsulfur

Short Answer

Explain the term CFB group and name the genera that this group includes.

Name and briefly describe the bacterium that causes Lyme disease.

Characterize the phylum Cyanobacteria.

6.4: Gram-positive Bacteria

Gram-positive bacteria are a very large and diverse group of microorganisms. Understanding their taxonomy and knowing their unique features is important for diagnostics and treatment of infectious diseases. Gram-positive bacteria are classified into high G+C gram-positive and low G+C gram-positive bacteria, based on the prevalence of guanine and cytosine nucleotides in their genome.

Multiple Choice

Which of the following bacterial species is classified as high G+C gram-positive?

- A. *Corynebacterium diphtheriae*
- B. *Staphylococcus aureus*
- C. *Bacillus anthracis*
- D. *Streptococcus pneumonia*

Answer

A

Fill in the Blank

Streptococcus is the _____ of bacteria that is responsible for many human diseases.

Answer

genus

One species of *Streptococcus*, *S. pyogenes*, is classified as a _____ pathogen due to the characteristic production of pus in infections it causes.

Answer

pyogenic

Propionibacterium belongs to _____ G+C gram-positive bacteria. One of its species is used in the food industry and another causes acne.

Answer

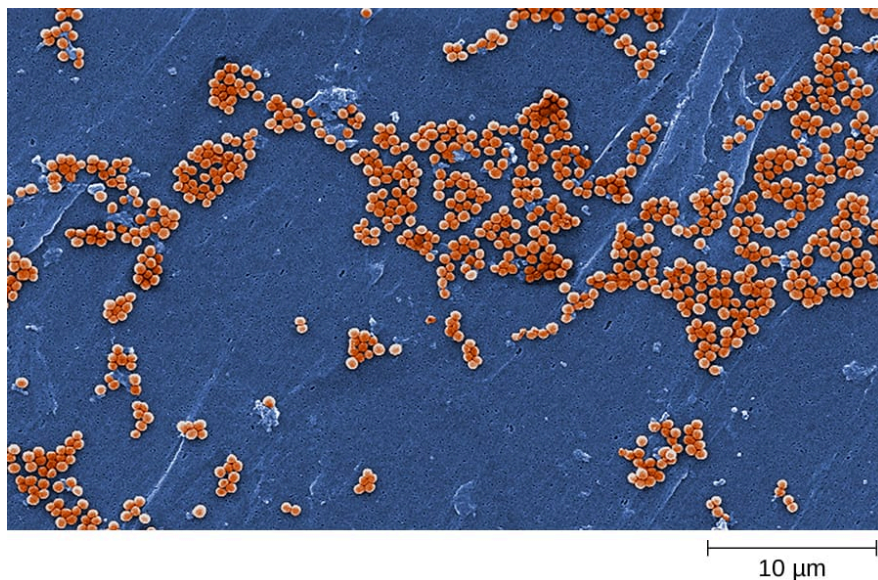
high

Short Answer

Name and describe two types of *S. aureus* that show multiple antibiotic resistance.

Critical Thinking

The microscopic growth pattern shown is characteristic of which genus of bacteria?



(credit: modification of work by Janice Haney Carr/Centers for Disease Control and Prevention)

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7: ACELLULAR PATHOGENS

Public health measures in the developed world have dramatically reduced mortality from viral epidemics. But when epidemics do occur, they can spread quickly with global air travel. In 2009, an outbreak of H1N1 influenza spread across various continents. In early 2014, cases of Ebola in Guinea led to a massive epidemic in western Africa. This included the case of an infected man who traveled to the United States, sparking fears the epidemic might spread beyond Africa.

Until the late 1930s and the advent of the electron microscope, no one had seen a virus. Yet treatments for preventing or curing viral infections were used and developed long before that. Historical records suggest that by the 17th century, and perhaps earlier, inoculation (also known as variolation) was being used to prevent the viral disease smallpox in various parts of the world. By the late 18th century, Englishman Edward Jenner was inoculating patients with cowpox to prevent smallpox, a technique he coined *vaccination*.¹

Today, the structure and genetics of viruses are well defined, yet new discoveries continue to reveal their complexities. In this chapter, we will learn about the structure, classification, and cultivation of viruses, and how they impact their hosts. In addition, we will learn about other infective particles such as viroids and prions.

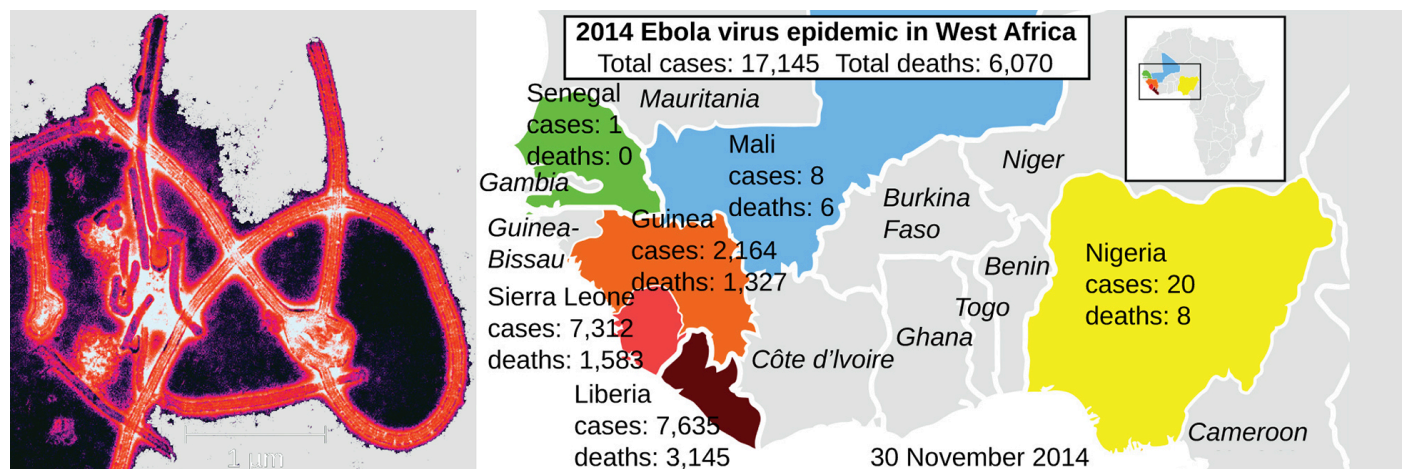


Figure 7.1: The year 2014 saw the first large-scale outbreak of Ebola virus (electron micrograph, left) in human populations in West Africa (right). Such epidemics are now widely reported and documented, but viral epidemics are sure to have plagued human populations since the origin of our species. (credit left: modification of work by Thomas W. Geisbert)

7.1: Viruses

7.2: Viral Structures

7.3: Bacteriophage Life Cycle

7.4: Animal Virus Life Cycle

7.5: Isolation, Culture, and Identification of Viruses

7.6: Viroids, Virusoids, and Prions

7.E: Acellular Pathogens (Exercises)

Footnotes

- 1 S. Riedel "Edward Jenner and the History of Smallpox and Vaccination." *Baylor University Medical Center Proceedings* 18, no. 1 (January 2005): 21–25.

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7.1: Viruses

Learning Objectives

- Describe the general characteristics of viruses as pathogens
- Identify the range of organisms that viruses can infect
- Explain the concept vectors
- Distinguish between mechanical and biological vectors

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 $\geq 0.2 \mu\text{m}$ 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.

Query 7.1.1

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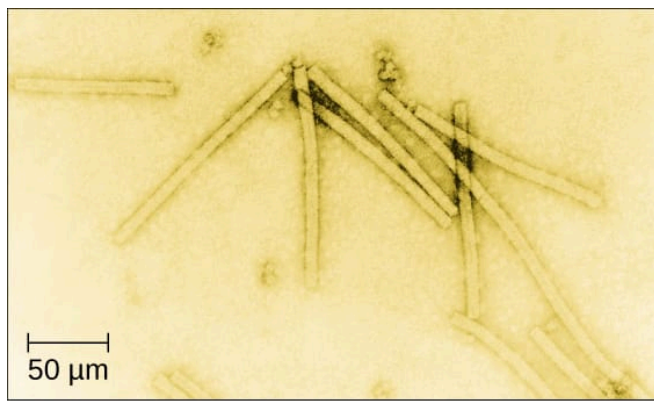
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Today, we can see viruses using electron microscopes (Figure 7.1.1) and we know much more about them. Viruses are distinct biological entities; however, their evolutionary origin is still a matter of speculation. In terms of taxonomy, they are not 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 7.1.1 summarizes the properties of viruses.

Table 7.1.1: 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



(a)



(b)

Figure 7.1.1: (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)

Video: Viruses

Explore the lytic and lysogenic viral replication cycles with the Amoeba Sisters! This video also discusses virus structures and why a host is critical for viral reproduction.

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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.

Query 7.1.1

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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

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Interactive Element

Case Study Preview: “Raging Rabies”

When David, a globe-trotting journalist, starts feeling weak, feverish, and agitated, he traces it back to what seemed like a harmless dog bite in rural China. Could it be rabies - or just paranoia? With time running out, his doctor races to diagnose a viral threat that’s nearly always fatal once symptoms set in.

In this case, you'll investigate how rabies is diagnosed using RT-PCR, antigen detection, and immunofluorescence - and explore why both passive and active immunization are crucial in treatment.

Can you outpace the virus before it reaches the brain? In this case, timing is everything.

[Chapter 6 Case Study - Raging Rabies](#)

Key Concepts and Summary

- Viruses are generally ultramicroscopic, typically from 20 nm to 900 nm in length. Some large viruses have been found.
- 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.

Footnotes

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.
2. US Department of Health and Human Services, Centers for Disease Control and Prevention. "Antibiotic Resistance Threats in the United States, 2013." www.cdc.gov/drugresistance/pd...s-2013-508.pdf (accessed September 22, 2015).
3. M. Clokie et al. "Phages in Nature." *Bacteriophage* 1, no. 1 (2011): 31-45.
4. A. Sulakvelidze et al. "Bacteriophage Therapy." *Antimicrobial Agents and Chemotherapy* 45, no. 3 (2001): 649-659.
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).
6. N. Philippe et al. "Pandoraviruses: Amoeba Viruses with Genomes up to 2.5 Mb Reaching that of Parasitic Eukaryotes." *Science* 341, no. 6143 (2013): 281-286.
7. J. Cohen. "What's Old Is New: 1918 Virus Matches 2009 H1N1 Strain." *Science* 327, no. 5973 (2010): 1563-1564.

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7.2: Viral Structures

Learning Objectives

- Describe the structural components of viruses and explain how they differ from prokaryotic and eukaryotic cells
- Compare and contrast DNA and RNA viruses in terms of replication strategies
- Describe how viruses are classified

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 7.2.2). The size of a virion can range from 20 nm for small viruses up to 900 nm for typical, large viruses (see Figure 7.2.3). 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

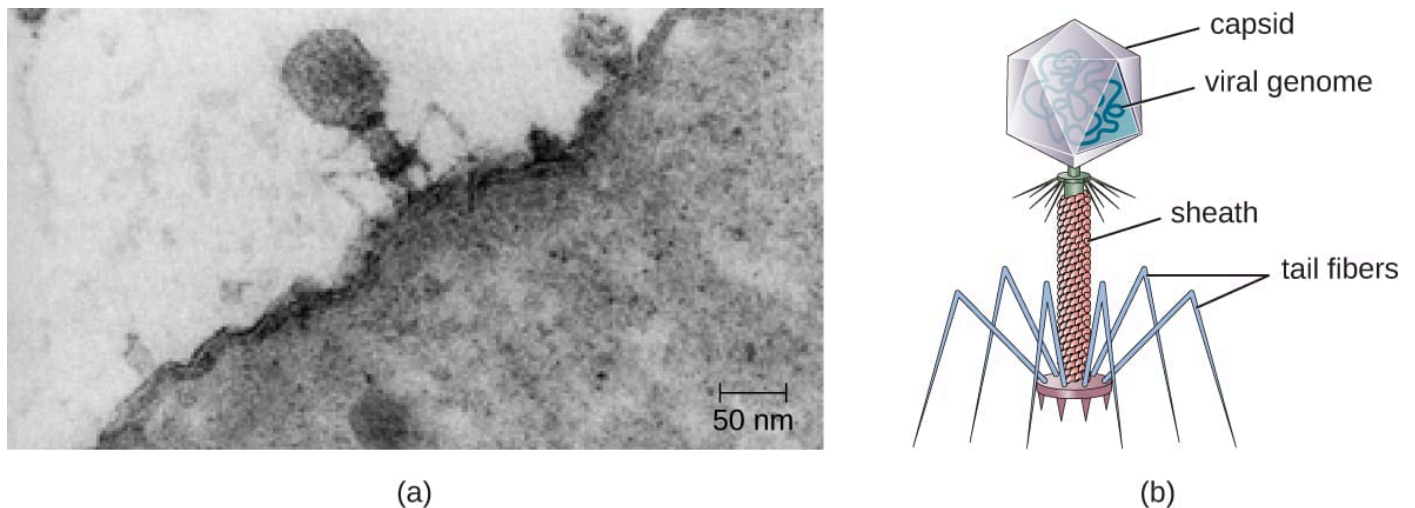


Figure 7.2.2: (a) In this transmission electron micrograph, a bacteriophage (a virus that infects bacteria) is dwarfed by the bacterial cell it infects. (b) An illustration of the bacteriophage in the micrograph. (credit a: modification of work by U.S. Department of Energy, Office of Science, LBL, PBD)

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.

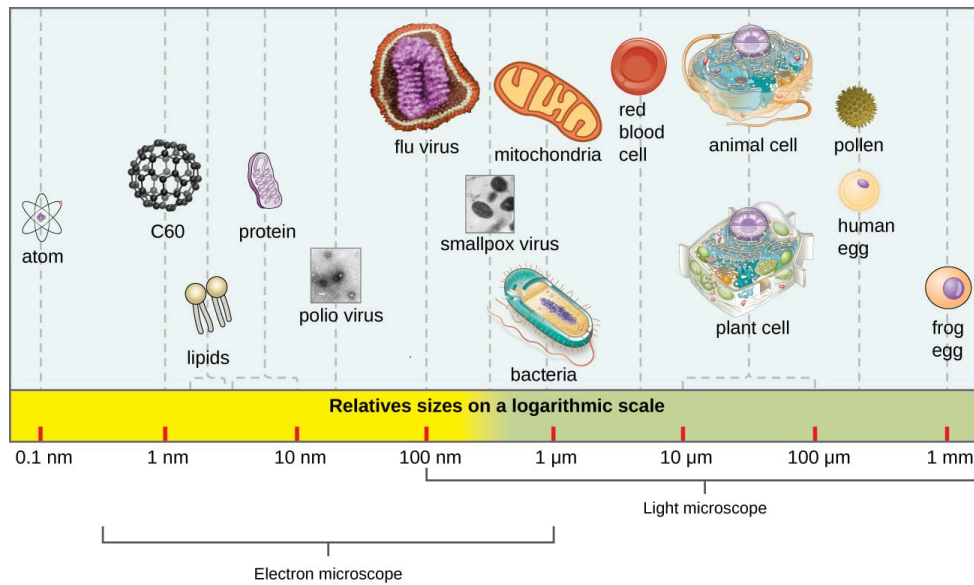


Figure 7.2.3: The size of a virus is small relative to the size of most bacterial and eukaryotic cells and their organelles.

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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 7.2.4). 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 7.2.4). 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.

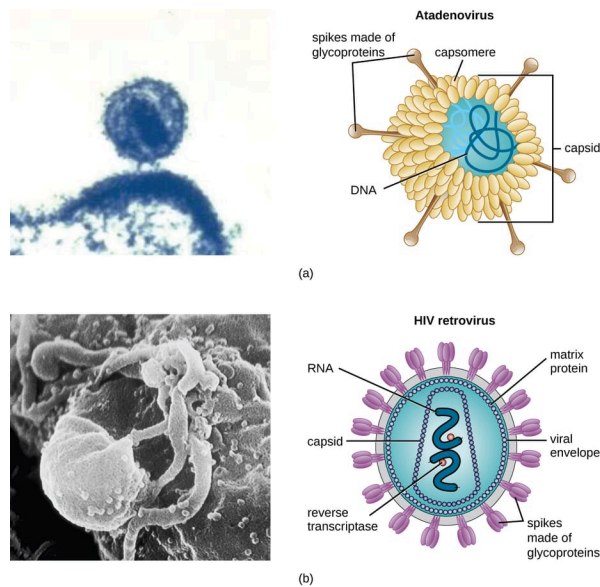


Figure 7.2.4: (a) The naked atadenovirus uses spikes made of glycoproteins from its capsid to bind to host cells. (b) The enveloped human immunodeficiency virus uses spikes made of glycoproteins embedded in its envelope to bind to host cells (credit a “micrograph”: modification of work by NIAID; credit 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 7.2.5). 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.

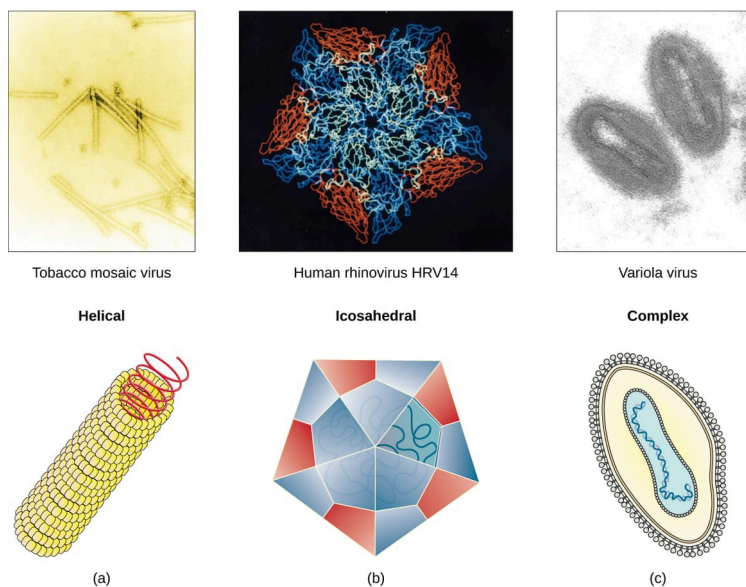


Figure 7.2.5: Viral capsids can be (a) helical, (b) polyhedral, or (c) have a complex shape. (credit a “micrograph”: modification of work by USDA ARS; credit b “micrograph”: modification of work by U.S. Department of Energy)

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Classification and Taxonomy of Viruses

Although viruses are not classified in the three domains of life, their numbers are great enough to require classification. Since 1971, the International Union of Microbiological Societies Virology Division has given the task of developing, refining, and maintaining a universal virus taxonomy to the International Committee on Taxonomy of Viruses (ICTV). Since viruses can mutate so quickly, it can be difficult to classify them into a genus and a species epithet using the binomial nomenclature system. Thus, the ICTV's viral nomenclature system classifies viruses into families and genera based on viral genetics, chemistry, morphology, and mechanism of multiplication. To date, the ICTV has classified known viruses in seven orders, 96 families, and 350 genera. Viral family names end in *-viridae* (e.g., *Parvoviridae*) and genus names end in *-virus* (e.g., *Parvovirus*). The names of viral orders, families, and genera are all italicized. When referring to a viral species, we often use a genus and species epithet such as *Pandoravirus dulcis* or *Pandoravirus salinus*.

The Baltimore classification system is an alternative to ICTV nomenclature. The Baltimore system classifies viruses according to their genomes (DNA or RNA, single versus double stranded, and mode of replication). This system thus creates seven groups of viruses that have common genetics and biology.

Link to Learning

Explore the latest virus [taxonomy](#) at the ICTV website.

Aside from formal systems of nomenclature, viruses are often informally grouped into categories based on chemistry, morphology, or other characteristics they share in common. Categories may include naked or enveloped structure, single-stranded (ss) or double-stranded (ds) DNA or ss or ds RNA genomes, segmented or nonsegmented genomes, and positive-strand (+) or negative-strand (–) RNA. For example, herpes viruses can be classified as a dsDNA enveloped virus; human immunodeficiency virus (HIV) is a +ssRNA enveloped virus, and tobacco mosaic virus is a +ssRNA virus. Other characteristics such as host specificity, tissue specificity, capsid shape, and special genes or enzymes may also be used to describe groups of similar viruses. Table 7.2.2 lists some of the most common viruses that are human pathogens by genome type.

Table 7.2.2: Common Pathogenic Viruses

Genome	Family	Example Virus	Clinical Features
dsDNA, enveloped	<i>Poxviridae</i>	<i>Orthopoxvirus</i>	Skin papules, pustules, lesions
	<i>Poxviridae</i>	<i>Parapoxvirus</i>	Skin lesions

Genome	Family	Example Virus	Clinical Features
	<i>Herpesviridae</i>	<i>Simplexvirus</i>	Cold sores, genital herpes, sexually transmitted disease
dsDNA, naked	<i>Adenoviridae</i>	<i>Atadenovirus</i>	Respiratory infection (common cold)
	<i>Papillomaviridae</i>	<i>Papillomavirus</i>	Genital warts, cervical, vulvar, or vaginal cancer
	<i>Reoviridae</i>	<i>Reovirus</i>	Gastroenteritis severe diarrhea (stomach flu)
ssDNA, naked	<i>Parvoviridae</i>	<i>Adeno-associated dependoparvovirus A</i>	Respiratory tract infection
	<i>Parvoviridae</i>	<i>Adeno-associated dependoparvovirus B</i>	Respiratory tract infection
dsRNA, naked	<i>Reoviridae</i>	<i>Rotavirus</i>	Gastroenteritis
+ssRNA, naked	<i>Picornaviridae</i>	<i>Enterovirus C</i>	Poliomyelitis
	<i>Picornaviridae</i>	<i>Rhinovirus</i>	Upper respiratory tract infection (common cold)
	<i>Picornaviridae</i>	<i>Hepatitis virus</i>	Hepatitis
+ssRNA, enveloped	<i>Togaviridae</i>	<i>Alphavirus</i>	Encephalitis, hemorrhagic fever
	<i>Togaviridae</i>	<i>Rubivirus</i>	Rubella
	<i>Retroviridae</i>	<i>Lentivirus</i>	Acquired immune deficiency syndrome (AIDS)
-ssRNA, enveloped	<i>Filoviridae</i>	<i>Zaire Ebolavirus</i>	Hemorrhagic fever
	<i>Orthomyxoviridae</i>	<i>Influenzavirus A, B, C</i>	Flu
	<i>Rhabdoviridae</i>	<i>Lyssavirus</i>	Rabies

Classification of Viral Diseases

While the ICTV has been tasked with the biological classification of viruses, it has also played an important role in the classification of diseases caused by viruses. To facilitate the tracking of virus-related human diseases, the ICTV has created classifications that link to the International Classification of Diseases (ICD), the standard taxonomy of disease that is maintained and updated by the World Health Organization (WHO). The ICD assigns an alphanumeric code of up to six characters to every type of viral infection, as well as all other types of diseases, medical conditions, and causes of death. This ICD code is used in conjunction with two other coding systems (the Current Procedural Terminology, and the Healthcare Common Procedure Coding System) to categorize patient conditions for treatment and insurance reimbursement.

For example, when a patient seeks treatment for a viral infection, ICD codes are routinely used by clinicians to order laboratory tests and prescribe treatments specific to the virus suspected of causing the illness. This ICD code is then used by medical laboratories to identify tests that must be performed to confirm the diagnosis. The ICD code is used by the health-care management system to verify that all treatments and laboratory work performed are appropriate for the given virus. Medical coders use ICD codes to assign the proper code for procedures performed, and medical billers, in turn, use this information to process claims for reimbursement by insurance companies. Vital-records keepers use ICD codes to record cause of death on death certificates, and epidemiologists used ICD codes to calculate morbidity and mortality statistics.

Key Concepts and Summary

- **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.
2. [2](#) US Department of Health and Human Services, Centers for Disease Control and Prevention. “Antibiotic Resistance Threats in the United States, 2013.” www.cdc.gov/drugresistance/pd...s-2013-508.pdf (accessed September 22, 2015).
3. [3](#) M. Clokie et al. “Phages in Nature.” *Bacteriophage* 1, no. 1 (2011): 31–45.
4. [4](#) A. Sulakvelidze et al. “Bacteriophage Therapy.” *Antimicrobial Agents and Chemotherapy* 45, no. 3 (2001): 649–659.
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).
6. [6](#) N. Philippe et al. “Pandoraviruses: Amoeba Viruses with Genomes up to 2.5 Mb Reaching that of Parasitic Eukaryotes.” *Science* 341, no. 6143 (2013): 281–286.
7. [7](#) J. Cohen. “What’s Old Is New: 1918 Virus Matches 2009 H1N1 Strain. *Science* 327, no. 5973 (2010): 1563–1564.

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7.3: Bacteriophage Life Cycle

Learning Objectives

- Describe the major steps of the bacteriophage lytic cycle: attachment, penetration, biosynthesis, maturation and release
- Explain the lysogenic cycle including the role of prophage integration and environmental triggers for induction
- Compare and contrast generalized and specialized transduction

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**.

Video: Lytic v. Lysogenic Cycles of Bacteriophages

McGraw Hill Video.

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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 7.3.1). **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.

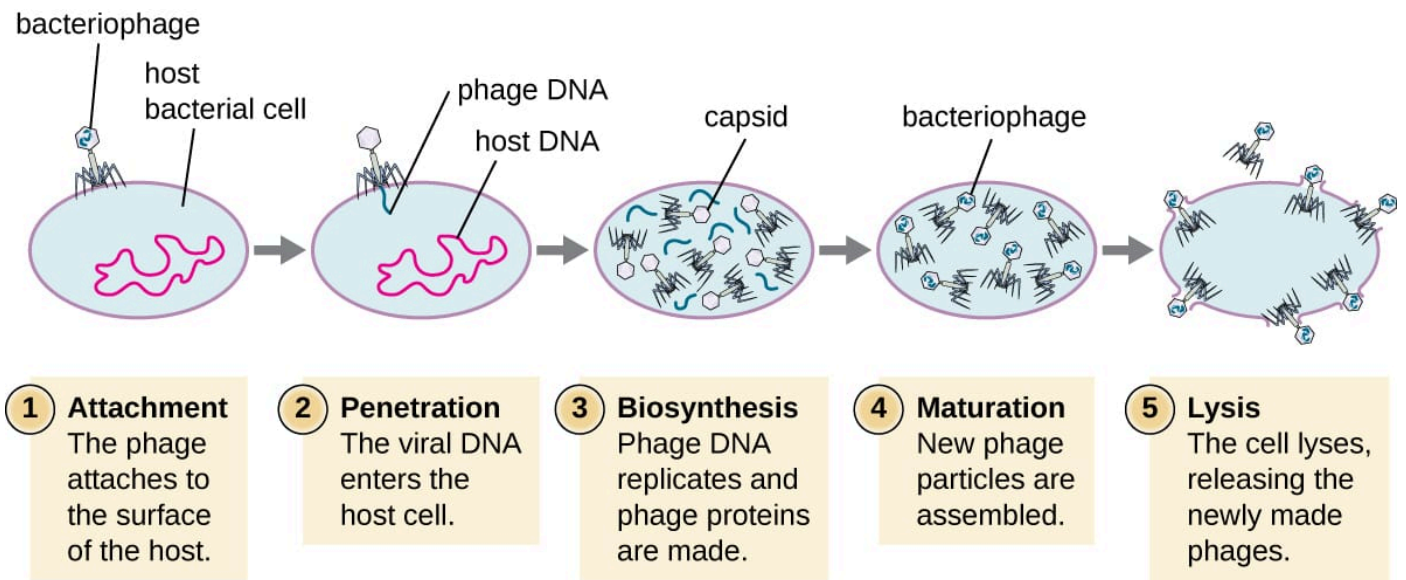


Figure 7.3.1: A virulent phage shows only the lytic cycle pictured here. In the lytic cycle, the phage replicates and lyses the host cell.

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 7.3.2).

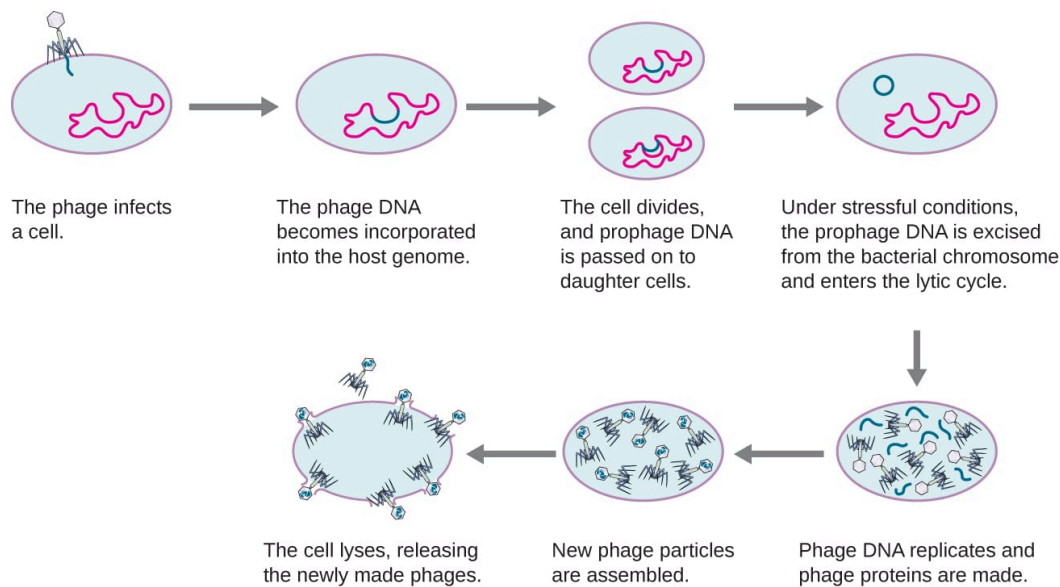


Figure 7.3.2: A temperate bacteriophage has both lytic and lysogenic cycles. In the lysogenic cycle, phage DNA is incorporated into the host genome, forming a prophage, which is passed on to subsequent generations of cells. Environmental stressors such as starvation or exposure to toxic chemicals may cause the prophage to be excised and enter the lytic cycle.

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Link to Learning

This [video](#) illustrates the stages of the lysogenic life cycle of a bacteriophage and the transition to a lytic phase.

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 7.3.3). 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.

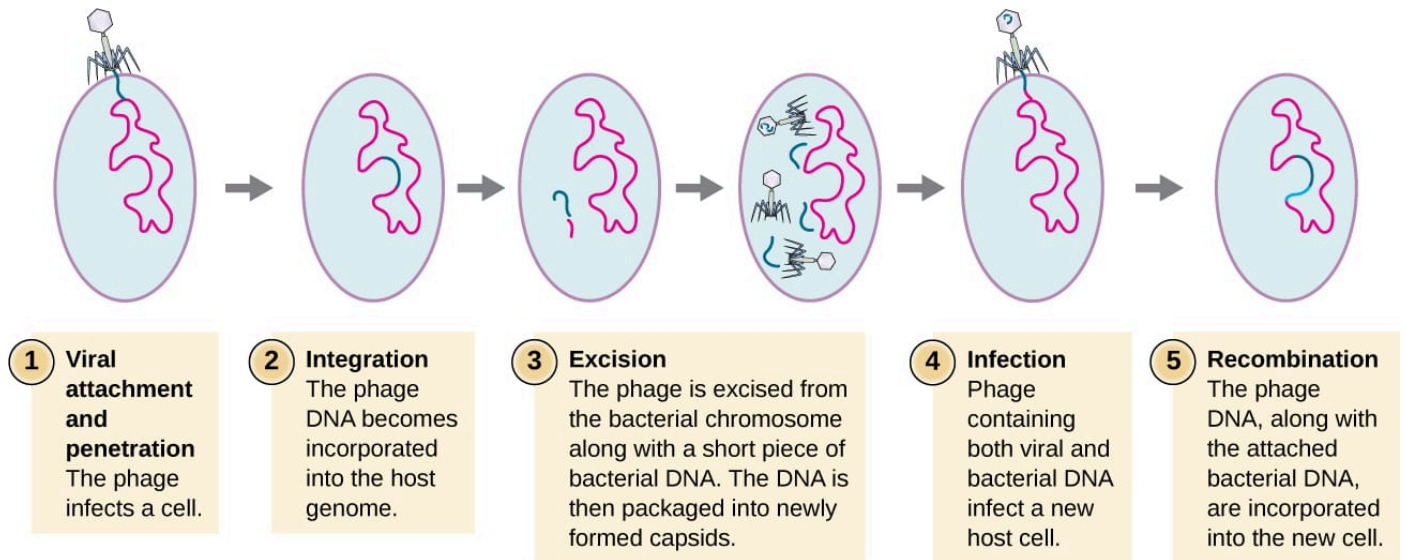


Figure 7.3.3: This flowchart illustrates the mechanism of specialized transduction. An integrated phage excises, bringing with it a piece of the DNA adjacent to its insertion point. On reinfection of a new bacterium, the phage DNA integrates along with the genetic material acquired from the previous host.

Key Concepts and 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.
- Bacteriophages transfer genetic information between hosts using either **generalized** or **specialized transduction**.

Footnotes

- 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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7.4: Animal Virus Life Cycle

Learning Objectives

- Describe the replication process of animal viruses
- Describe unique characteristics of retroviruses and latent viruses
- Discuss human viruses and their virus-host cell interactions

Life Cycle of Viruses with Animal Hosts

Video: The Viral Life Cycle

This CLEAR & SIMPLE tutorial describes the steps of the viral life cycle, including 1) Attachment 2) Entry 3) Replication 4) Biosynthesis 5) Assembly and 6) Release. Find more free tutorials, videos and readings for the science classroom at ricochetscience.com.

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Lytic animal viruses follow similar infection stages to bacteriophages: attachment, penetration, biosynthesis, maturation, and release (see Figure 7.4.4). 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.

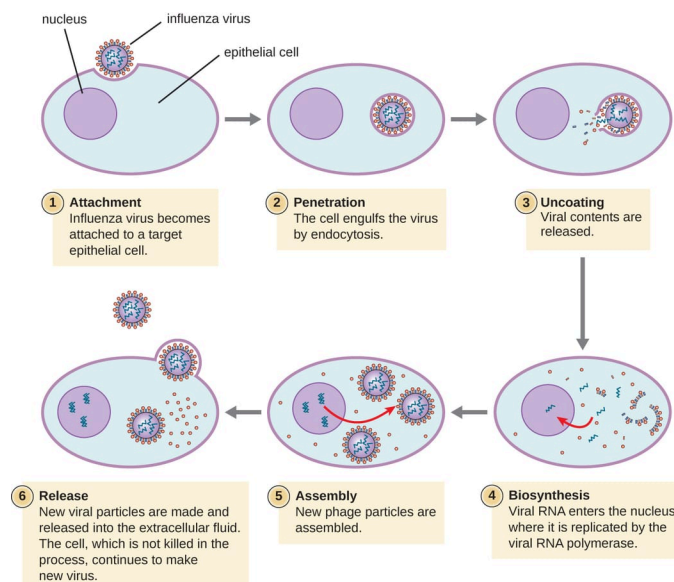
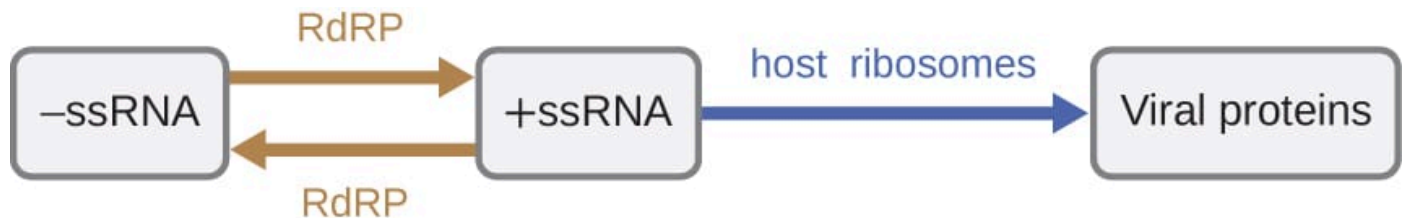


Figure 7.4.4: In influenza virus infection, viral glycoproteins attach the virus to a host epithelial cell. As a result, the virus is engulfed. Viral RNA and viral proteins are made and assembled into new virions that are released by budding.

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 7.4.5). 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.



RdRP = viral RNA-dependent RNA polymerase
 +ssRNA = positive (+) single strand
 –ssRNA = negative (–) single-strand RNA

Figure 7.4.5: 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 **retroviruses**, which are +ssRNA viruses (see Figure 7.4.6). 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.

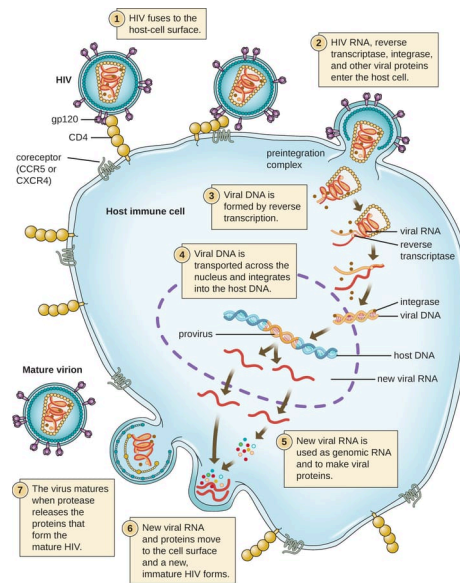


Figure 7.4.6: HIV, an enveloped, icosahedral retrovirus, attaches to a cell surface receptor of an immune cell and fuses with the cell membrane. Viral contents are released into the cell, where viral enzymes convert the single-stranded RNA genome into DNA and incorporate it into the host genome. (credit: modification of work by NIAID, NIH)

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Persistent Infections

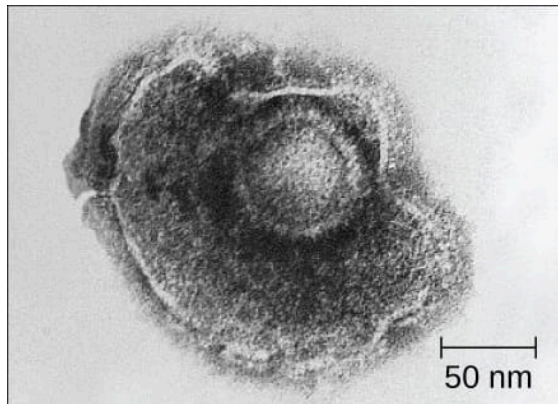
Persistent infection occurs when a virus is not completely cleared from the system of the host but stays in certain tissues or organs of the infected person. The virus may remain silent or undergo productive infection without seriously harming or killing the host. Mechanisms of persistent infection may 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 viruses** 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 7.4.7). 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.



(a)



(b)

Figure 7.4.7: (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.

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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 7.4.8).

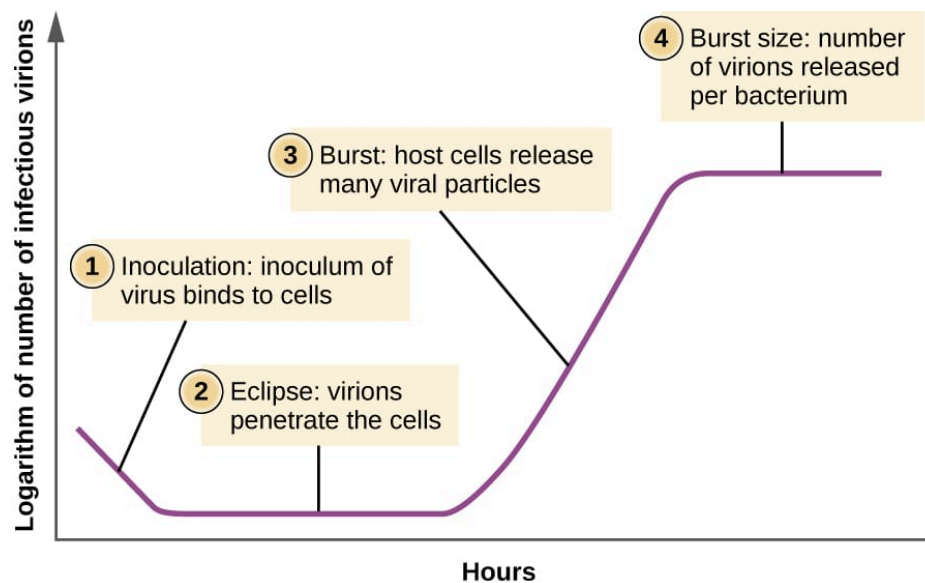


Figure 7.4.8: The one-step multiplication curve for a bacteriophage population follows three steps: 1) inoculation, during which the virions attach to host cells; 2) eclipse, during which entry of the viral genome occurs; and 3) burst, when sufficient numbers of new virions are produced and emerge from the host cell. The burst size is the maximum number of virions produced per bacterium.

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.



Figure 7.4.9: 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](https://www.cdc.gov/ebola/) website.

Key Concepts and Summary

- Animal viruses can undergo **latency**, similar to lysogeny for a bacteriophage.
- The growth curve of bacteriophage populations is a **one-step multiplication curve** and not a sigmoidal curve, as compared to the bacterial growth curve.

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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7.5: 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 7.5.1).

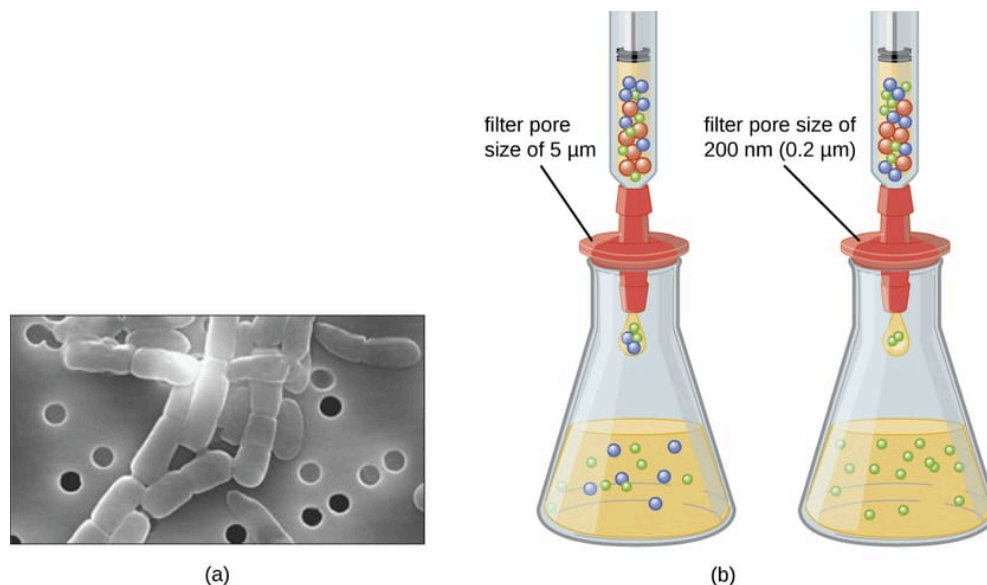


Figure 7.5.1: Membrane filters can be used to remove cells or viruses from a solution. (a) This scanning electron micrograph shows rod-shaped bacterial cells captured on the surface of a membrane filter. Note differences in the comparative size of the membrane pores and bacteria. Viruses will pass through 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)

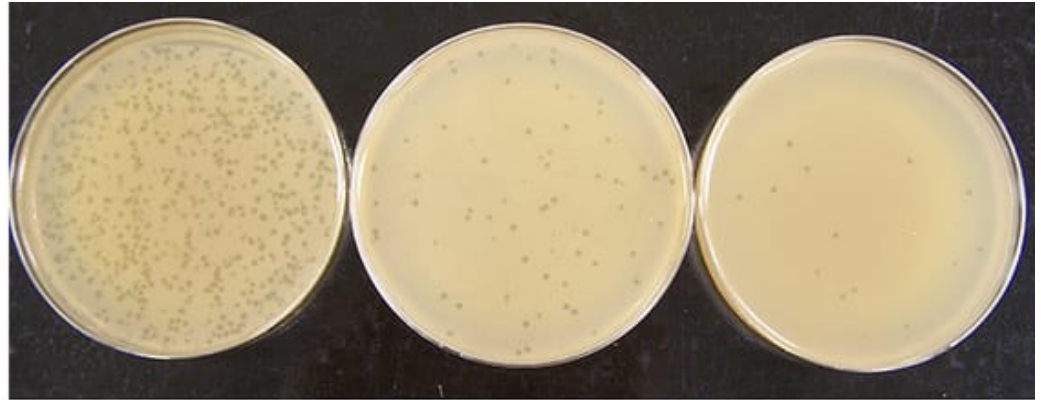
Cultivation of Viruses

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 7.5.2a). 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 7.5.1b). As the phage kills the bacteria, many plaques are observed

among the cloudy bacterial lawn.



(a)



(b)

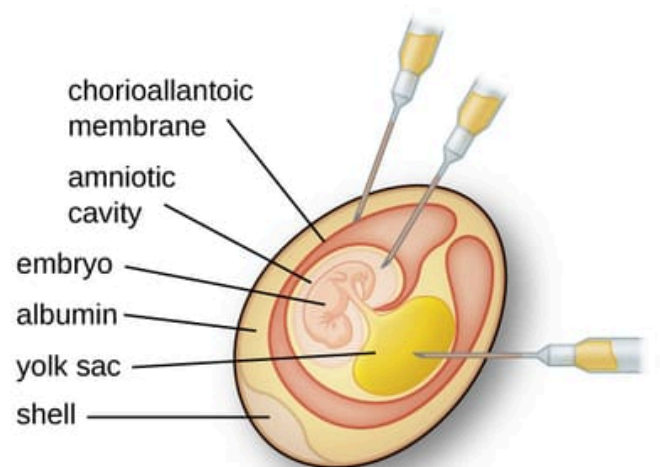
Figure 7.5.2: (a) Flasks like this may be used to culture human or animal cells for viral culturing. (b) These plates contain bacteriophage T4 grown on an *Escherichia coli* lawn. Clear plaques are visible where host bacterial cells have been lysed. Viral titers increase on the plates to the left. (credit 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 7.5.3). 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 7.5.3: (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 7.5.4).

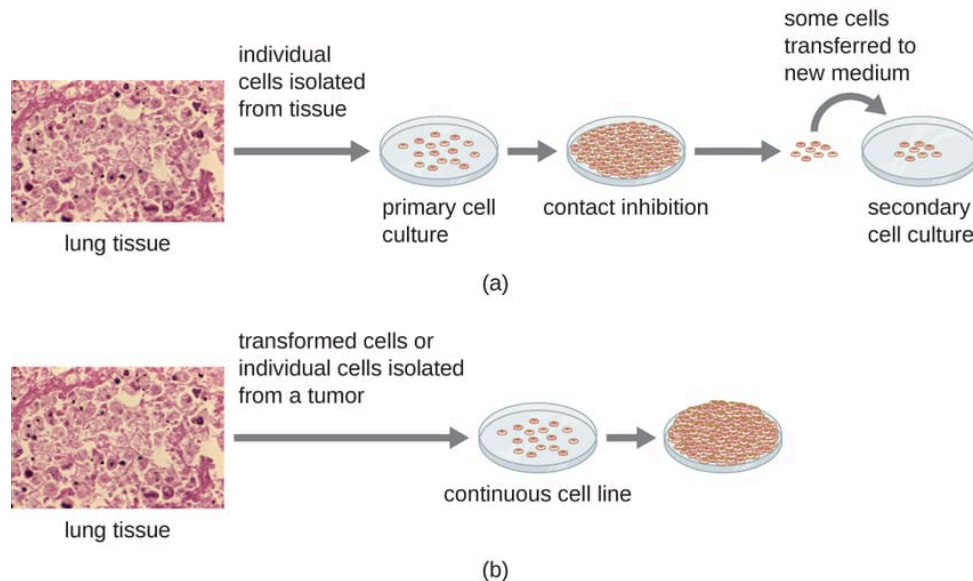


Figure 7.5.4: Cells for culture are prepared by separating them from their tissue matrix. (a) Primary cell cultures grow attached to the surface of the culture container. Contact inhibition slows the growth of the cells once they become too dense and begin touching each other. At this point, growth can 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.

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.

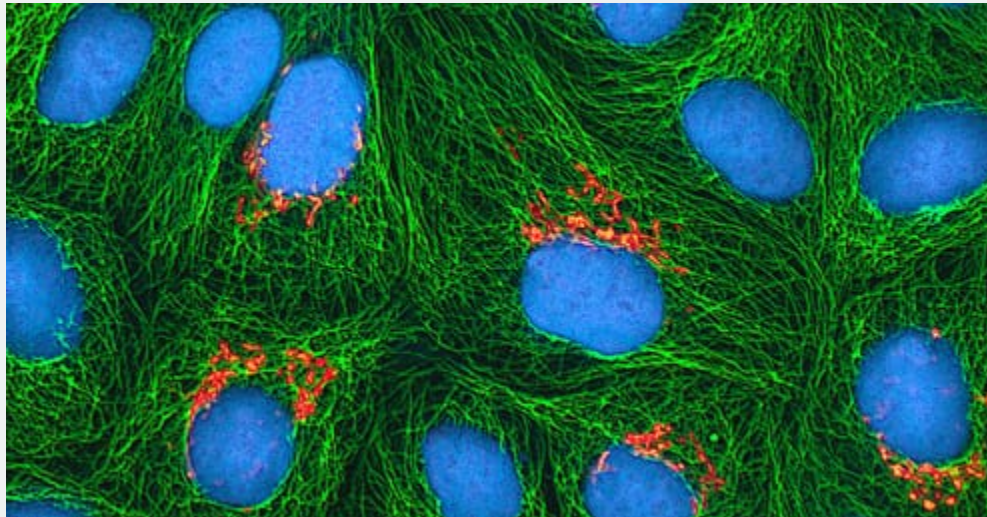


Figure 7.5.5: A multiphoton fluorescence image of HeLa cells in culture. Various fluorescent stains have been used to show the DNA (cyan), microtubules (green), and Golgi apparatus (orange). (credit: modification of work by National Institutes of Health)

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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 7.5.6).

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 7.5.6 lists CPEs for specific viruses.

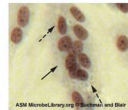
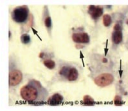
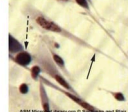
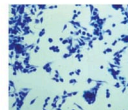
Cytopathic Effects of Specific Viruses		
Virus	Cytopathic Effect	Example
Paramyxovirus	Syncytium and faint basophilic cytoplasmic inclusion bodies	
Poxvirus	Pink eosinophilic cytoplasmic inclusion bodies (arrows) and cell swelling	
Herpesvirus	Cytoplasmic stranding (arrow) and nuclear inclusion bodies (dashed arrow)	
Adenovirus	Cell enlargement, rounding, and distinctive "grape-like" clusters	

Figure 7.5.6: (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.

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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 7.5.7). This assay is described in greater detail in [Agglutination Assays](#).

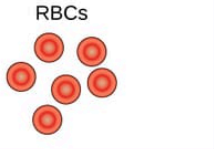
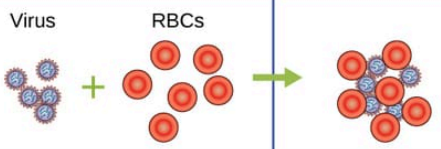
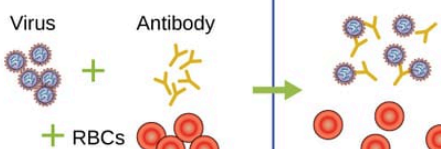
	Components	Interaction	Microtiter Results
A	RBCs		No reaction 
B	Virus + RBCs		Hemagglutination 
C	Virus + Antibody + RBCs		Hemagglutination inhibition 

Figure 7.5.7: 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)

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 Score

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 7.5.4

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 7.5.8). 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](#).

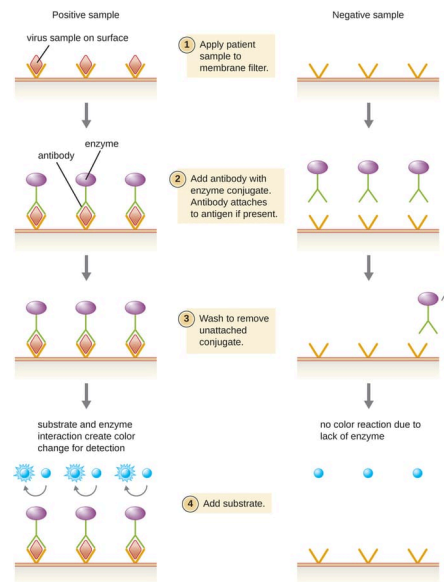


Figure 7.5.8: Similar to rapid, over-the-counter pregnancy tests, EIAs for viral antigens require a few drops of diluted patient serum or plasma applied to a membrane filter. The membrane filter has been previously modified and embedded with antibody to viral antigen and internal controls. Antibody conjugate 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 "Cavetri"/Wikimedia Commons)

Key Concepts and 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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7.6: Viroids, Virusoids, and Prions

Learning Objectives

- Define and differentiate viroids, virusoids and prions based on their structure, replication mechanisms and host range
- Explain how viroids and virusoids cause diseases in plants
- Summarize the unique protein-only nature of prions
- Analyze the public health implications of prion diseases such as CJD and mad cow disease

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 7.6.1). 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 7.6.1: 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.

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.

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.

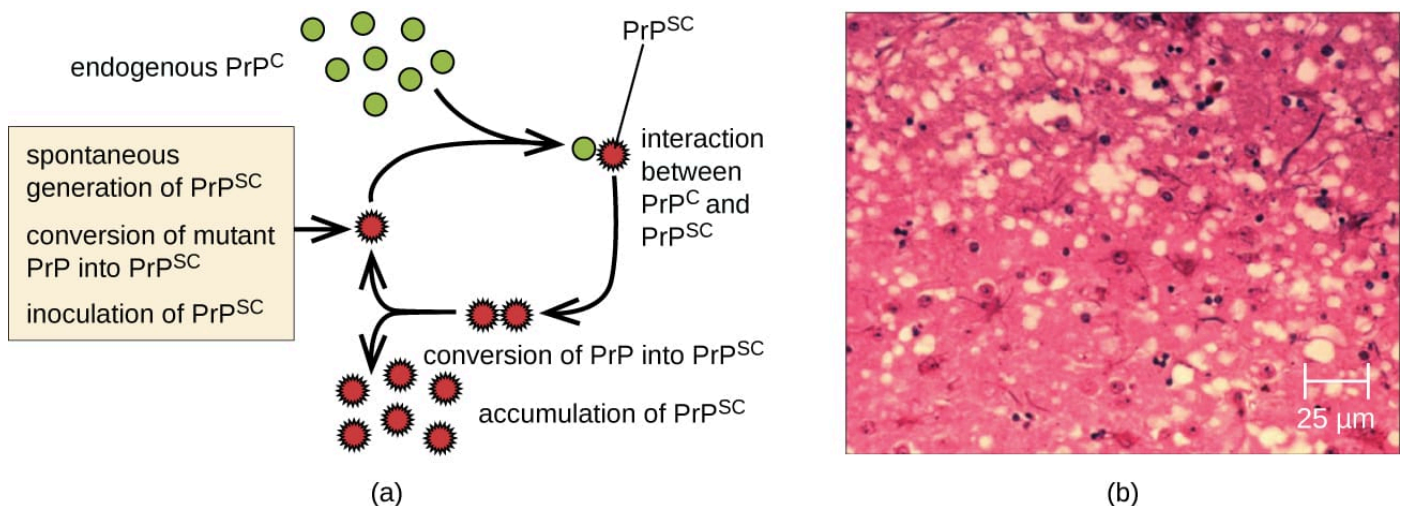


Figure 7.6.2: Endogenous normal prion protein (PrP^C) is converted into the disease-causing form (PrP^{Sc}) when it encounters this variant form of the protein. PrP^{Sc} may arise spontaneously in brain tissue, especially if a mutant form of the protein is present, or it may originate from misfolded 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 (PrP^C) found in the cell. This rogue prion protein (PrP^{Sc}), 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 7.6.2). 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 7.6.3). 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.

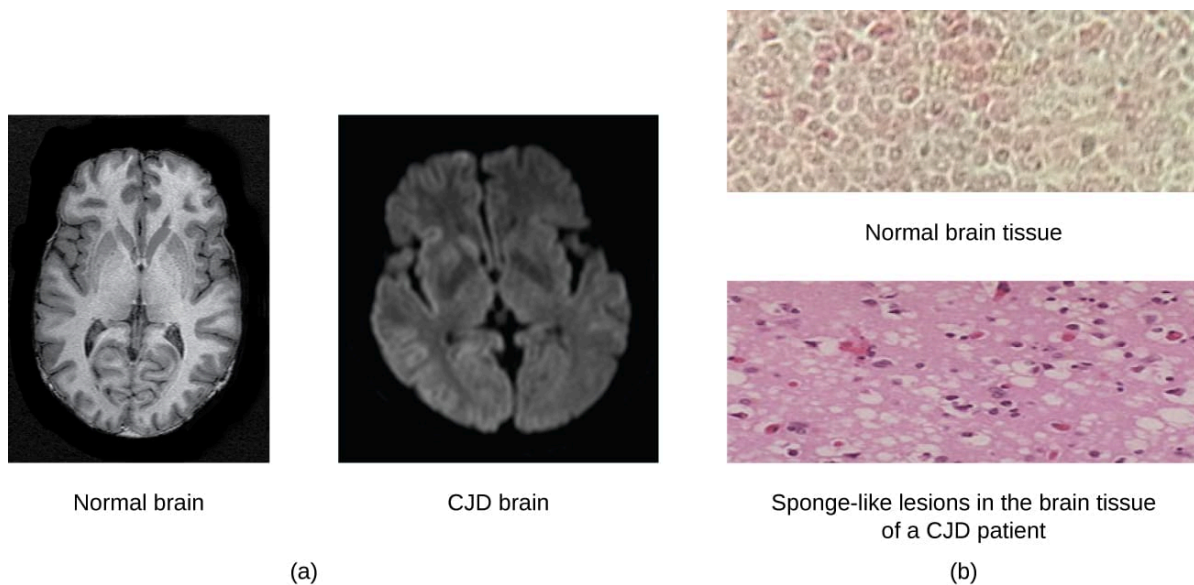


Figure 7.6.3: Creutzfeldt-Jakob disease (CJD) is a fatal disease that causes degeneration of neural tissue. (a) These brain scans compare a normal brain to one with CJD. (b) Compared to a normal brain, the brain tissue of a CJD patient is full of sponge-like lesions, which result from abnormal formations 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 7.6.3). 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 7.6.1 lists TSEs that affect humans and their modes of transmission.

Table 7.6.1: 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.

Query 7.6.1

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Key Concepts and 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. 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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7.E: Acellular Pathogens (Exercises)

7.1: Viruses

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.

Multiple Choice

The component(s) of a virus that is/are extended from the envelope for attachment is/are the:

- A. capsomeres
- B. spikes
- C. nucleic acid
- D. viral whiskers

Answer

B

Which of the following does a virus lack? Select all that apply.

- A. ribosomes
- B. metabolic processes
- C. nucleic acid
- D. glycoprotein

Answer

A and B

The envelope of a virus is derived from the host's

- A. nucleic acids
- B. membrane structures
- C. cytoplasm
- D. genome

Answer

B

In naming viruses, the family name ends with _____ and genus name ends with _____.

- A. *-virus; -viridae*
- B. *-viridae; -virus*
- C. *-virion; virus*
- D. *-virus; virion*

Answer

B

What is another name for a nonenveloped virus?

- A. enveloped virus
- B. provirus
- C. naked virus
- D. latent virus

Answer

C

True/False

True or False: Scientists have identified viruses that are able to infect fungal cells.

Answer

True

Fill in the Blank

A virus that infects a bacterium is called a/an _____.

Answer

bacteriophage

A/an _____ virus possesses characteristics of both a polyhedral and helical virus.

Answer

complex

A virus containing only nucleic acid and a capsid is called a/an _____ virus or _____ virus.

Answer

naked or nonenveloped

The _____ on the bacteriophage allow for binding to the bacterial cell.

Answer

tail fibers

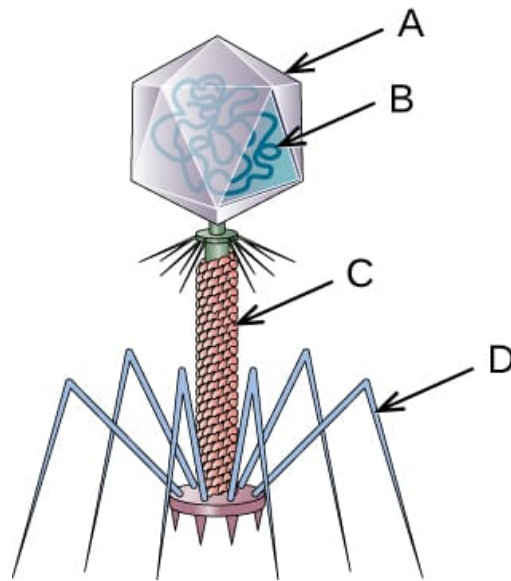
Short Answer

Discuss the geometric differences among helical, polyhedral, and complex viruses.

What was the meaning of the word “virus” in the 1880s and why was it used to describe the cause of tobacco mosaic disease?

Critical Thinking

Name each labeled part of the illustrated bacteriophage.



In terms of evolution, which do you think arises first? The virus or the host? Explain your answer.

Do you think it is possible to create a virus in the lab? Imagine that you are a mad scientist. Describe how you would go about creating a new virus.

7.2: The Viral Life Cycle

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.

Multiple Choice

Which of the following leads to the destruction of the host cells?

- A. lysogenic cycle
- B. lytic cycle
- C. prophage
- D. temperate phage

Answer

B

A virus obtains its envelope during which of the following phases?

- A. attachment
- B. penetration
- C. assembly
- D. release

Answer

D

Which of the following components is brought into a cell by HIV?

- A. a DNA-dependent DNA polymerase
- B. RNA polymerase
- C. ribosome
- D. reverse transcriptase

Answer

D

A positive-strand RNA virus:

- A. must first be converted to a mRNA before it can be translated.
- B. can be used directly to translate viral proteins.
- C. will be degraded by host enzymes.
- D. is not recognized by host ribosomes.

Answer

B

What is the name for the transfer of genetic information from one bacterium to another bacterium by a phage?

- A. transduction
- B. penetration
- C. excision
- D. translation

Answer

A

Fill in the Blank

An enzyme from HIV that can make a copy of DNA from RNA is called _____.

Answer

reverse transcriptase

For lytic viruses, _____ is a phase during a viral growth curve when the virus is not detected.

Answer

eclipse

Short Answer

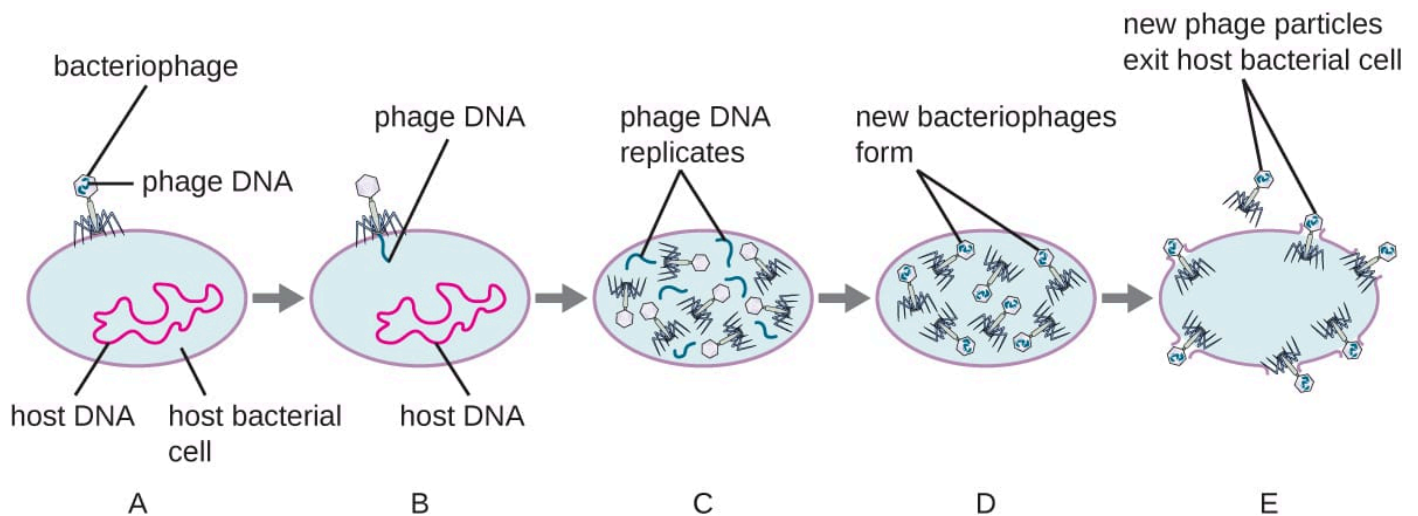
Briefly explain the difference between the mechanism of entry of a T-even bacteriophage and an animal virus.

Discuss the difference between generalized and specialized transduction.

Differentiate between lytic and lysogenic cycles.

Critical Thinking

Label the five stages of a bacteriophage infection in the figure:



Bacteriophages have lytic and lysogenic cycles. Discuss the advantages and disadvantages for the phage.

How does reverse transcriptase aid a retrovirus in establishing a chronic infection?

Discuss some methods by which plant viruses are transmitted from a diseased plant to a healthy one.

7.3: Isolation, Culture, and Identification of Viruses

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).

Multiple Choice

Which of the followings cannot be used to culture viruses?

- A. tissue culture
- B. liquid medium only
- C. embryo
- D. animal host

Answer

B

Which of the following tests can be used to detect the presence of a specific virus?

- A. EIA
- B. RT-PCR
- C. PCR
- D. all of the above

Answer

D

Which of the following is NOT a cytopathic effect?

- A. transformation

- B. cell fusion
- C. mononucleated cell
- D. inclusion bodies

Answer

C

Fill in the Blank

Viruses can be diagnosed and observed using a(n) _____ microscope.

Answer

Electron

Cell abnormalities resulting from a viral infection are called _____.

Answer

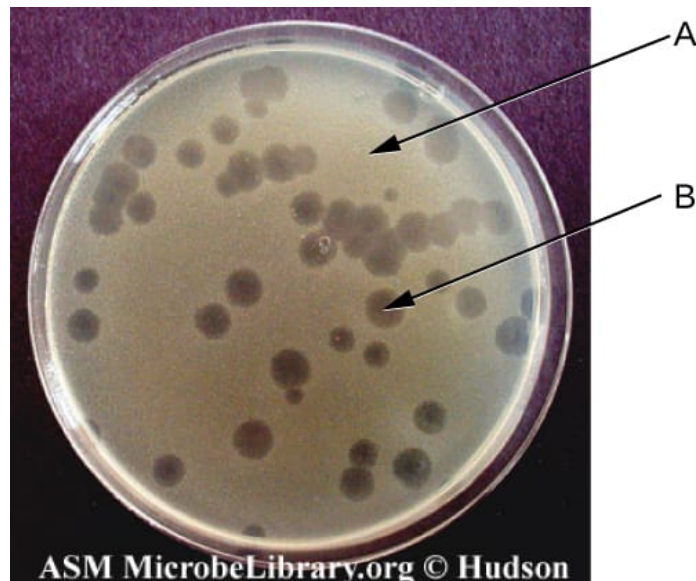
cytopathic effects

Short Answer

Briefly explain the various methods of culturing viruses.

Critical Thinking

Label the components indicated by arrows.



(credit: modification of work by American Society for Microbiology)

What are some characteristics of the viruses that are similar to a computer virus?

7.4: Viroids, Virusoids, and Prions

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.

Multiple Choice

Which of these infectious agents do not have nucleic acid?

- A. viroids
- B. viruses
- C. bacteria
- D. prions

Answer

D

Which of the following is true of prions?

- A. They can be inactivated by boiling at 100 °C.
- B. They contain a capsid.
- C. They are a rogue form of protein, PrP.
- D. They can be reliably inactivated by an autoclave.

Answer

C

Fill in the Blank

Both viroids and virusoids have a(n) _____ genome, but virusoids require a(n) _____ to reproduce.

Answer

RNA, helper virus

Short Answer

Describe the disease symptoms observed in animals infected with prions.

Critical Thinking

Does a prion replicate? Explain.

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8: MICROBIAL METABOLISM I

Throughout earth's history, microbial metabolism has been a driving force behind the development and maintenance of the planet's biosphere. Eukaryotic organisms such as plants and animals typically depend on organic molecules for energy, growth, and reproduction. Prokaryotes, on the other hand, can metabolize a wide range of organic as well as inorganic matter, from complex organic molecules like cellulose to inorganic molecules and ions such as atmospheric nitrogen (N_2), molecular hydrogen (H_2), sulfide (S^{2-}), manganese (II) ions (Mn^{2+}), ferrous iron (Fe^{2+}), and ferric iron (Fe^{3+}), to name a few. By metabolizing such substances, microbes chemically convert them to other forms. In some cases, microbial metabolism produces chemicals that can be harmful to other organisms; in others, it produces substances that are essential to the metabolism and survival of other life forms (Figure 8.1).



Figure 8.1: Prokaryotes have great metabolic diversity with important consequences to other forms of life. Acidic mine drainage (left) is a serious environmental problem resulting from the introduction of water and oxygen to sulfide-oxidizing bacteria during mining processes. These bacteria produce large amounts of sulfuric acid as a byproduct of their metabolism, resulting in a low-pH environment that can kill many aquatic plants and animals. On the other hand, some prokaryotes are essential to other life forms. Root nodules of many plants (right) house nitrogen-fixing bacteria that convert atmospheric nitrogen into ammonia, providing a usable nitrogen source for these plants. (credit left: modification of work by D. Hardesty, USGS Columbia Environment Research Center; credit right: modification of work by Celmo SR, Clairmont L, Madsen LH, and Guinel FC)

8.1: Energy, Matter, and Enzymes

8.2: Enzyme Structure and Function

8.3: Glycolysis

8.4: Transition Reaction and Krebs Cycle

8.E: Microbial Metabolism (Exercises)

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8.1: Energy, Matter, 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

The term used to describe all of the chemical reactions inside a cell is **metabolism** (Figure 8.1.1). 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.

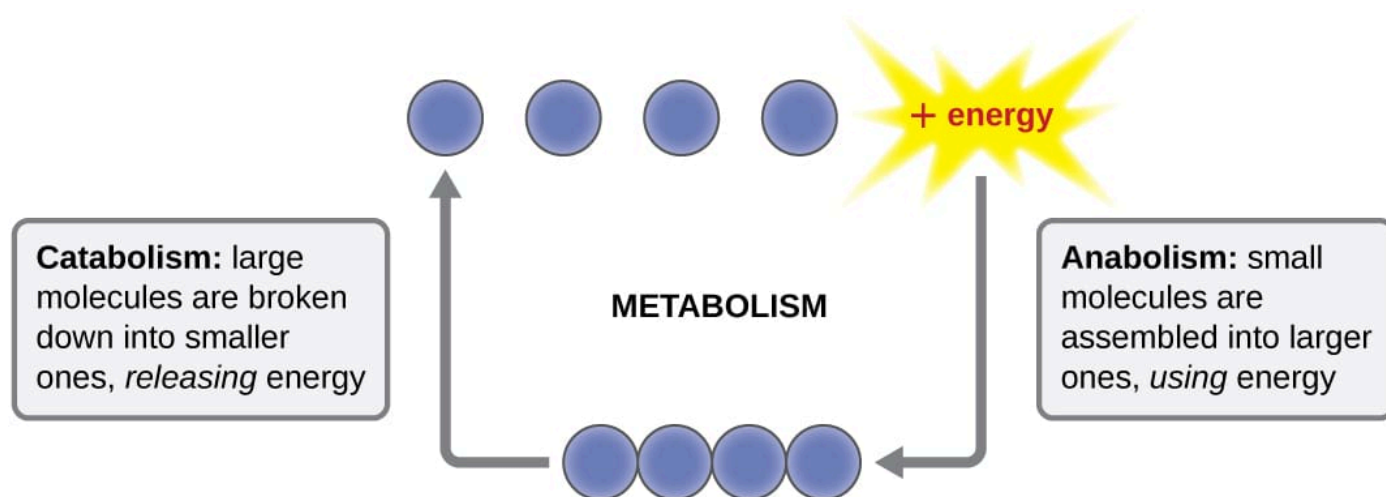
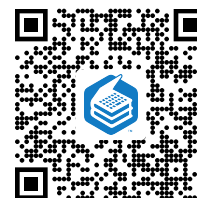


Figure 8.1.1: Metabolism includes catabolism and anabolism. Anabolic pathways require energy to synthesize larger molecules. Catabolic pathways generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance.

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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 (CO₂) 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 (H₂S) 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 8.1.1 summarizes this and the other classifications.

Table 8.1.1: 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

Video: Autotrophs and Heterotrophs

Curious about modes of nutrition? Join the Amoeba Sisters in learning about autotrophs and heterotrophs. Video explains these terms as well as how their carbon source differs. Photoautotrophs, photoheterotrophs, chemoautotrophs, and chemoheterotrophs, and their energy sources, are also discussed!

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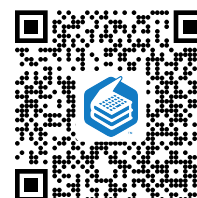
Oxidation and Reduction in Metabolism

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**.

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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 **$\text{NADP}^+/\text{NADPH}$** 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 8.1.2). 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 8.1.3).

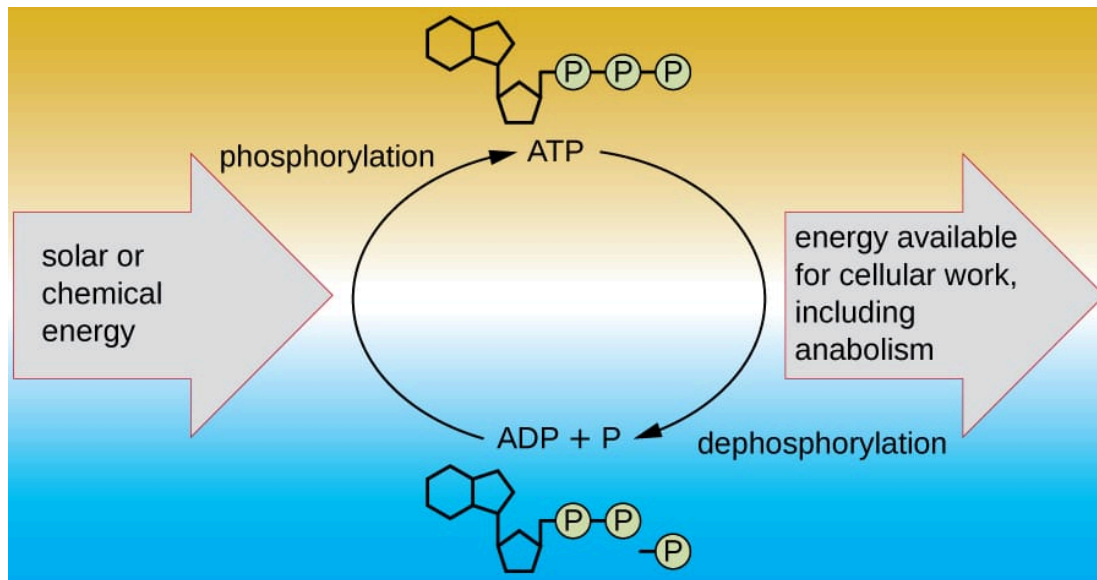


Figure 8.1.2: The energy released from dephosphorylation of ATP is used to drive cellular work, including anabolic pathways. ATP is regenerated through phosphorylation, harnessing the energy found in chemicals or from sunlight. (credit: modification of work by Robert Bear, David Rintoul)

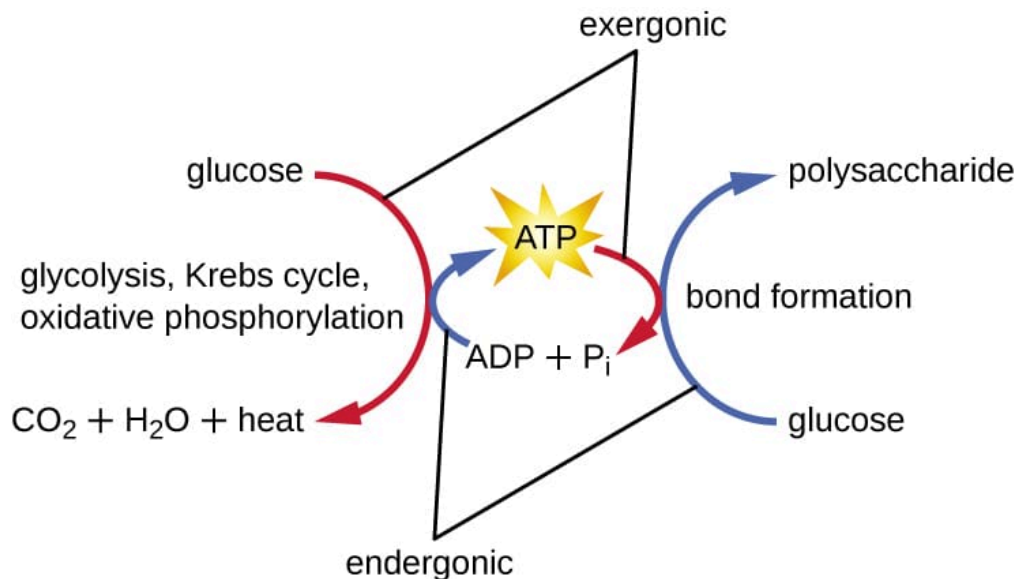


Figure 8.1.3: Exergonic reactions are coupled to endergonic ones, making the combination favorable. Here, the endergonic reaction of ATP phosphorylation is coupled to the exergonic reactions of catabolism. Similarly, the exergonic reaction of ATP dephosphorylation is coupled to the endergonic reaction of polypeptide formation, an example of anabolism.

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Case Study Preview: "Meningitis in the Sun"

When 15-month-old Hannah starts acting strangely during a summer trip to Gambia - fatigue, light sensitivity, and vomiting - her parents assume it's a mild virus. But when her symptoms worsen, a local physician suspects something more serious: meningitis. With her life on the line, doctors start treatment *before* test results come back.

In this case, you'll track how a tiny gram-negative diplococcus - *Neisseria meningitidis* - can rapidly invade the body and why quick diagnosis and treatment are critical. You'll also explore how biochemical tests are still essential tools in resource-limited settings and learn why travel vaccinations matter more than ever.

Sometimes the scariest bugs don't cause stomach aches - they go straight for the brain.

[Chapter 8 Case Study - Meningitis in the Sun](#)

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/FADH₂**, **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.

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8.2: Enzyme Structure and Function

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

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 8.2.4). 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 8.2.5). 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.

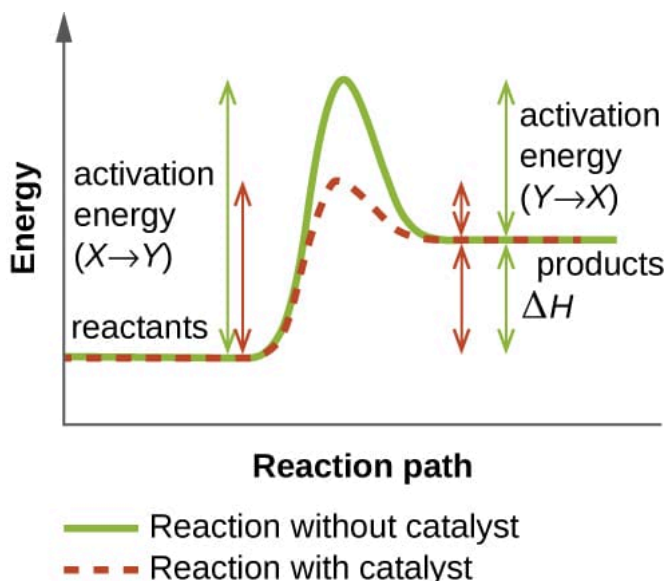


Figure 8.2.4: Enzymes lower the activation energy of a chemical reaction.

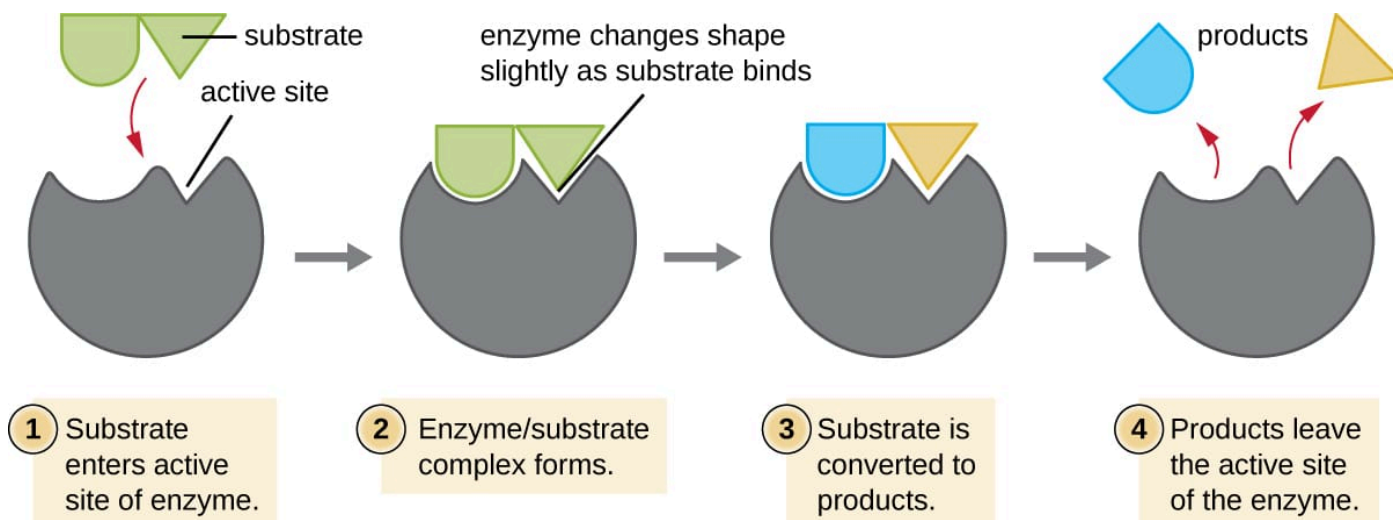


Figure 8.2.5: According to the induced-fit model, the active site of the enzyme undergoes conformational changes upon binding with the substrate.

Video: Enzymes

The Amoeba Sisters explain enzymes and how they interact with their substrates. Vocabulary covered includes active site, induced fit, coenzyme, and cofactor. Also the importance of ideal pH and temperatures for enzymes are discussed.

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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 8.2.6). 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.

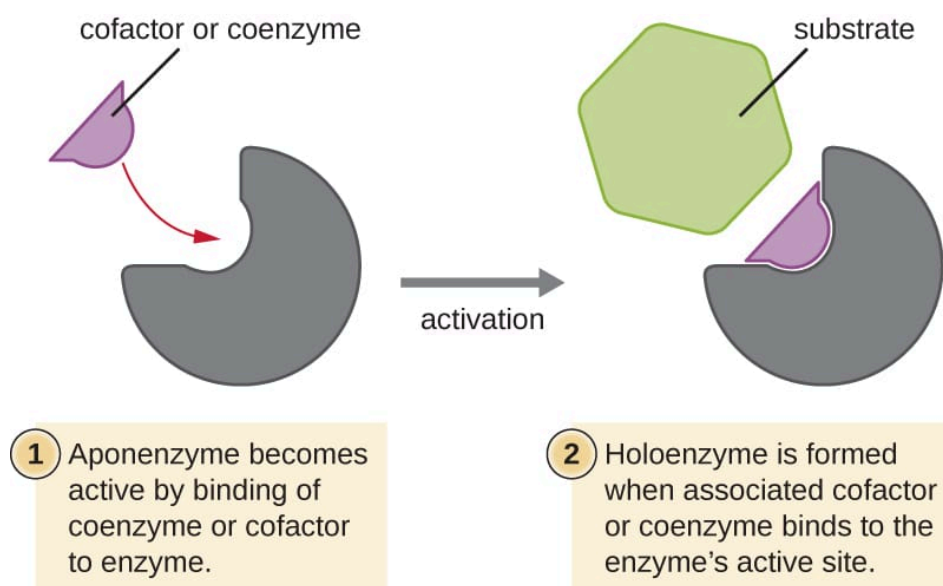


Figure 8.2.6: The binding of a coenzyme or cofactor to an apoenzyme is often required to form an active holoenzyme.

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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 8.2.7). 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 8.2.8). 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 8.2.8).

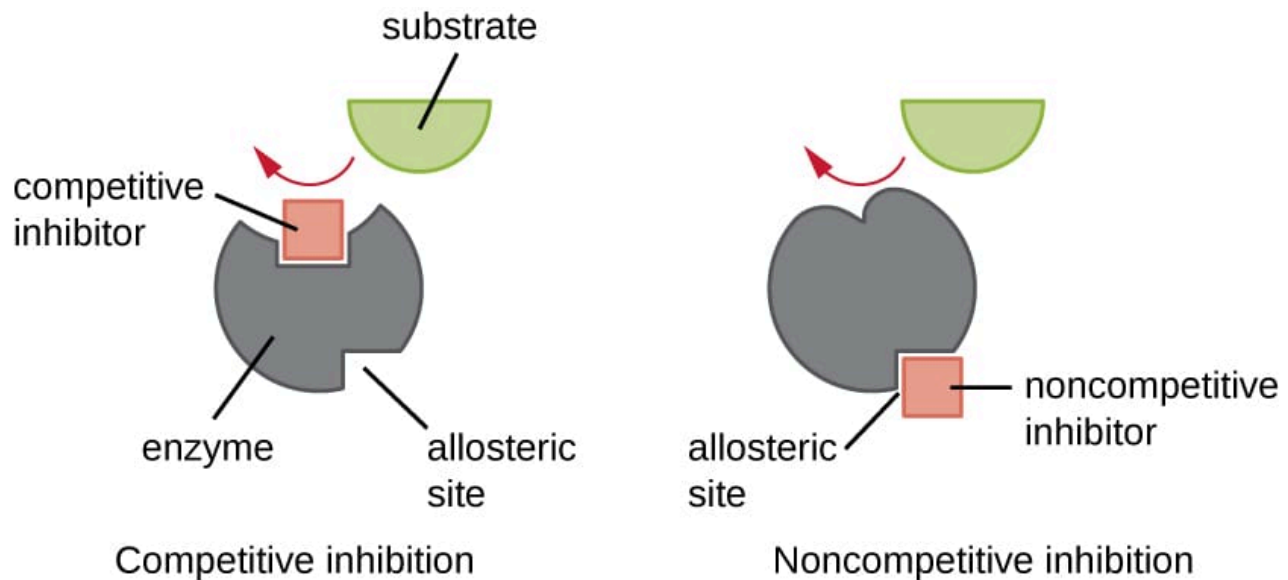


Figure 8.2.7: Enzyme activity can be regulated by either competitive inhibitors, which bind to the active site, or noncompetitive inhibitors, which bind to an allosteric site.

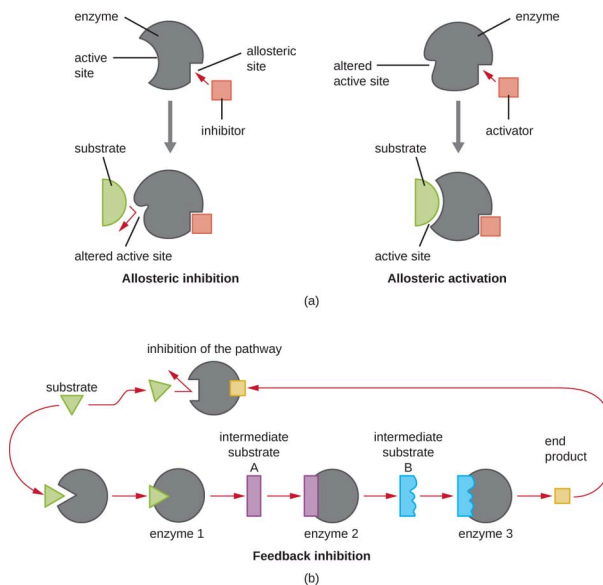


Figure 8.2.8: (a) Binding of an allosteric inhibitor reduces enzyme activity, but binding of an allosteric activator increases enzyme activity. (b) Feedback inhibition, where the end product of the pathway serves as a noncompetitive inhibitor to an enzyme early in the pathway, is an important mechanism of allosteric regulation in cells.

Video: Enzyme Examples, Cofactors/Coenzymes, inhibitors, and Feedback Inhibition

Already watched the Amoeba Sisters first video on enzymes and ready to explore a little more? In this video, the Amoeba Sisters cover a few examples of enzymes in the human body before emphasizing that enzymes are found in all the domains of life - and even in viruses! The importance of cofactors and coenzymes are discussed before moving into competitive and noncompetitive inhibitors. Feedback inhibition is also briefly described.

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Key Concepts and Summary

- **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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8.3: Glycolysis

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 chemoheterotrophs 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 8.3.1). 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 8.3.1), 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.

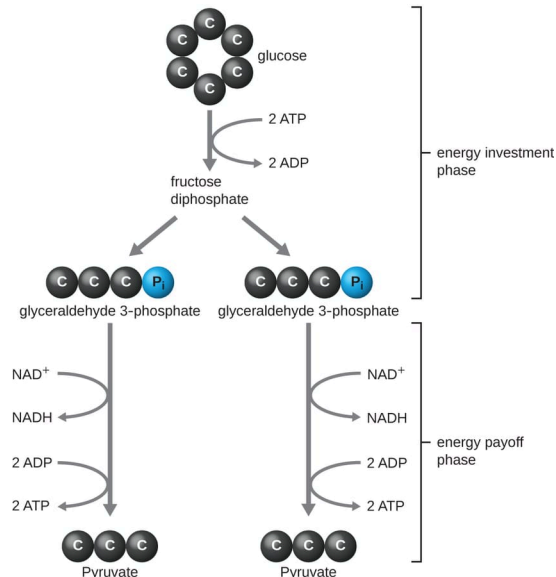


Figure 8.3.1: The energy investment phase of the Embden-Meyerhof-Parnas glycolysis pathway uses two ATP molecules to phosphorylate glucose, forming two glyceraldehyde 3-phosphate (G3P) molecules. The energy payoff phase harnesses the energy in the G3P molecules, producing four ATP molecules, two NADH molecules, and two pyruvates.

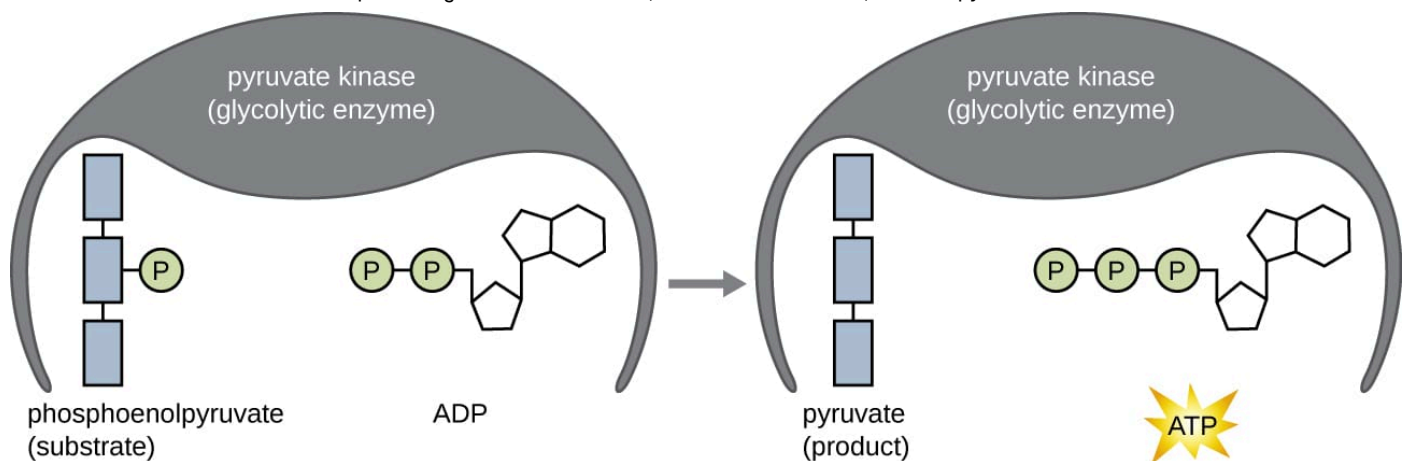


Figure 8.3.2: The ATP made during glycolysis is a result of substrate-level phosphorylation. One of the two enzymatic reactions in the energy payoff phase of Embden Meyerhof-Parnas glycolysis that produce ATP in this way is shown here.

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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](#).

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.

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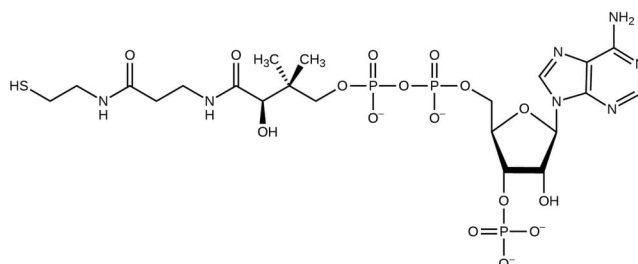
8.4: Transition Reaction and Krebs Cycle

Learning Objectives

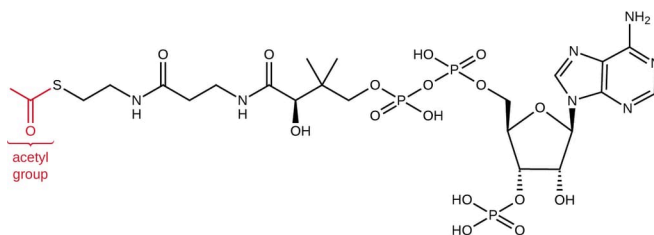
- Describe why glycolysis is not oxygen dependent
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- Define and describe the net yield of CO_2 , GTP/ATP, FADH_2 , and NADH from the Krebs cycle
- Explain how intermediate carbon molecules of the Krebs cycle can be used in a cell

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 8.4.3). 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 8.4.3: (a) Coenzyme A is shown here without an attached acetyl group. (b) Coenzyme A is shown here with an attached acetyl group.

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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 8.4.4). 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 CO₂ 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 FADH₂. (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 8.4.5).

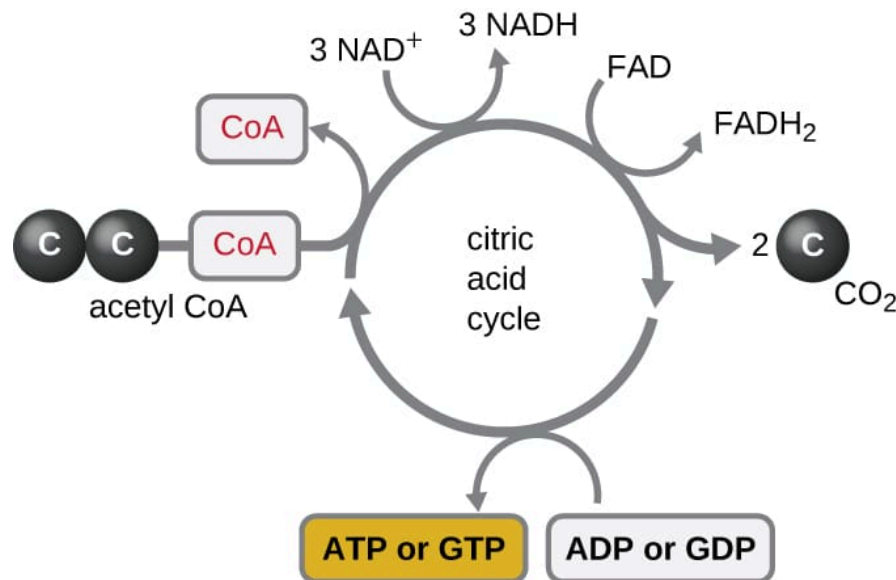


Figure 8.4.4: The Krebs cycle, also known as the citric acid cycle, is summarized here. Note incoming two-carbon acetyl results in the main outputs per turn of two CO₂, three NADH, one FADH₂, and one ATP (or GTP) molecules made by substrate-level phosphorylation. Two turns of the Krebs cycle are required to process all of the carbon from one glucose molecule.

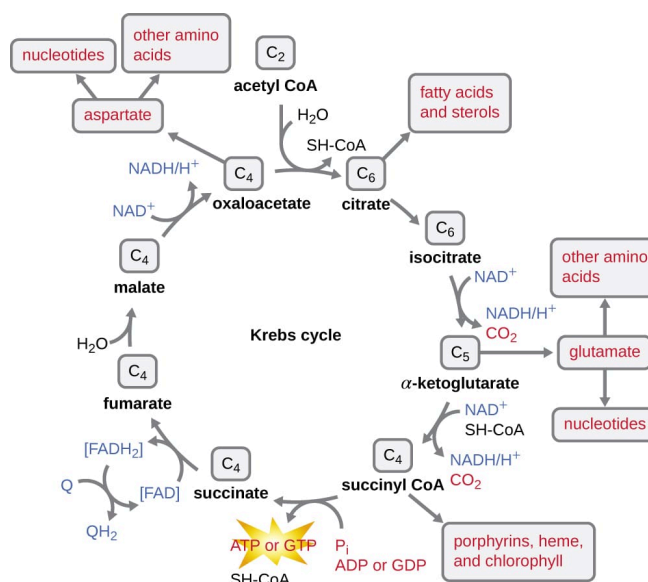
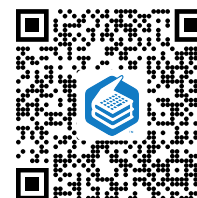


Figure 8.4.5: Many organisms use intermediates from the Krebs cycle, such as amino acids, fatty acids, and nucleotides, as building blocks for biosynthesis.

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Key Concepts and Summary

- 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 FADH₂, and one ATP by **substrate-level phosphorylation**, and releasing two CO₂ 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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8.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

Embsden-Meyerhof

Short Answer

What is substrate-level phosphorylation? When does it occur during the breakdown of glucose to CO₂?

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?

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9: MICROBIAL METABOLISM II

Life depends on the ability of cells to capture and convert energy from their surroundings, and two of the most vital processes that fulfill this role are cellular respiration and fermentation. These metabolic pathways enable organisms to transform energy stored in organic molecules - especially glucose - into ATP, the molecule that powers nearly all cellular work. Cellular respiration, an aerobic process, uses oxygen as the final electron acceptor in a complex series of redox reactions that includes glycolysis, the citric acid cycle, and the electron transport chain, resulting in the efficient production of large amounts of ATP. In contrast, fermentation allows ATP generation without oxygen, relying solely on glycolysis followed by pathways that regenerate NAD⁺ by converting pyruvate into simpler compounds such as lactic acid or ethanol. Although fermentation yields far less ATP than cellular respiration, it enables many microorganisms - and even human cells under certain conditions—to survive in anaerobic environments. Together, these pathways illustrate how cells have evolved to extract energy from organic molecules under a wide range of environmental conditions, ensuring their survival, growth, and reproduction.

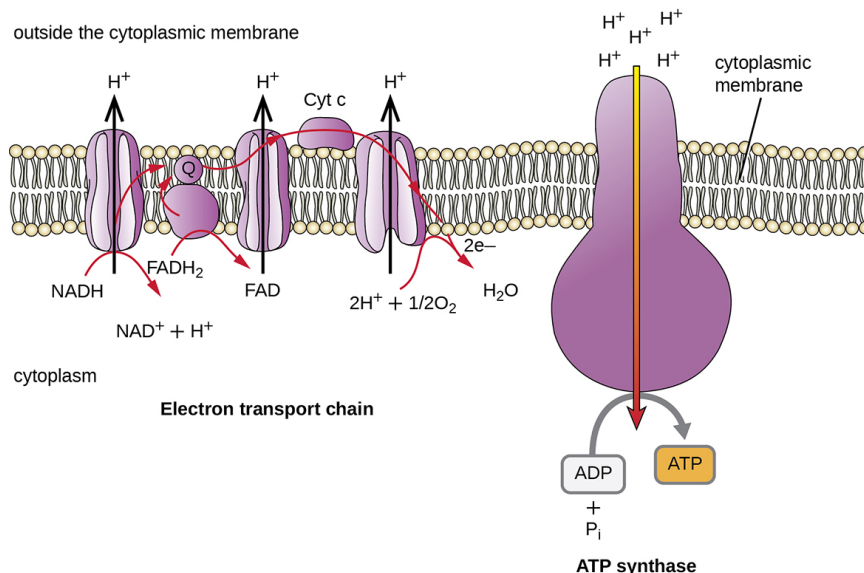


Figure 9.1: The bacterial electron transport chain is a

series of protein complexes, electron carriers, and ion pumps that is used to pump H⁺ out of the bacterial cytoplasm into the extracellular space. H⁺ flows back down the electrochemical gradient into the bacterial cytoplasm through ATP synthase, providing the energy for ATP production by oxidative phosphorylation. (credit: modification of work by Klaus Hoffmeier)

9.1: Cellular Respiration

9.2: Fermentation I

9.3: Fermentation II

9.4: Catabolism of Lipids and Proteins

9.E: Microbial Metabolism (Exercises)

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9.1: Cellular Respiration

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 FADH₂—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.

Video: Cellular Respiration

Explore the process of aerobic cellular respiration and why ATP production is so important in this updated cellular respiration video by The Amoeba Sisters! This video compares the cellular respiration equation to photosynthesis before exploring the basics of glycolysis, Krebs Cycle (Citric Acid Cycle), and the Electron Transport Chain (and chemiosmosis).

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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 FADH₂ 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 (O₂) that becomes reduced to water (H₂O) 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.

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 9.1.1). 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.

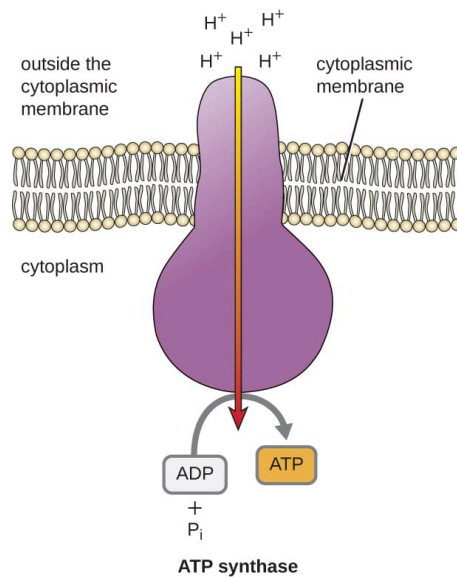


Figure 9.1.1: ATP synthase is a complex integral membrane protein through which H⁺ flows down an electrochemical gradient, providing the energy for ATP production by oxidative phosphorylation. (credit: modification of work by Klaus Hoffmeier)

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 FADH₂ 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 FADH₂ 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 9.1.2). 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 9.1.2 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 Phosphorylation	Net ATP Molecules Made by Oxidative Phosphorylation	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 9.1.2: Theoretical maximum yields of ATP from various processes during the complete aerobic respiration of one glucose molecule.

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Key Concepts and 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 FADH₂ 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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9.2: Fermentation I

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 9.2.1 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.

Video: Fermentation

What happens when you can't do aerobic cellular respiration because oxygen isn't available? Explore fermentation with The Amoeba Sisters! This video focuses on alcoholic fermentation and lactic acid fermentation, and it also mentions how this pathway is different from anaerobic cellular respiration.

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Table 9.2.1: 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	<i>Pseudomonas aeruginosa</i>	O_2	EMP glycolysis (SLP)	2
			Krebs cycle (SLP)	2
			Electron transport and chemiosmosis (OP):	34
			Total	38
Anaerobic respiration	<i>Paracoccus denitrificans</i>	NO_3^- , SO_4^{2-} , Fe^{+3} , CO_2 , other inorganics	EMP glycolysis (SLP)	2
			Krebs cycle (SLP)	2
			Electron transport and chemiosmosis (OP):	1–32
			Total	5–36
Fermentation	<i>Candida albicans</i>	Organics (usually pyruvate)	EMP glycolysis (SLP)	2
			Fermentation	0
			Total	2

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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.

Lactic Acid Fermentation

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:



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 CO₂ 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.

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Alcoholic Fermentation

Another familiar fermentation process is **alcohol fermentation**, which produces ethanol. The ethanol fermentation reaction is shown in Figure 9.2.1. In the first reaction, the enzyme pyruvate decarboxylase removes a carboxyl group from pyruvate, releasing CO₂ 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 CO₂ production. Outside of the food industry, ethanol fermentation of plant products is important in biofuel production.

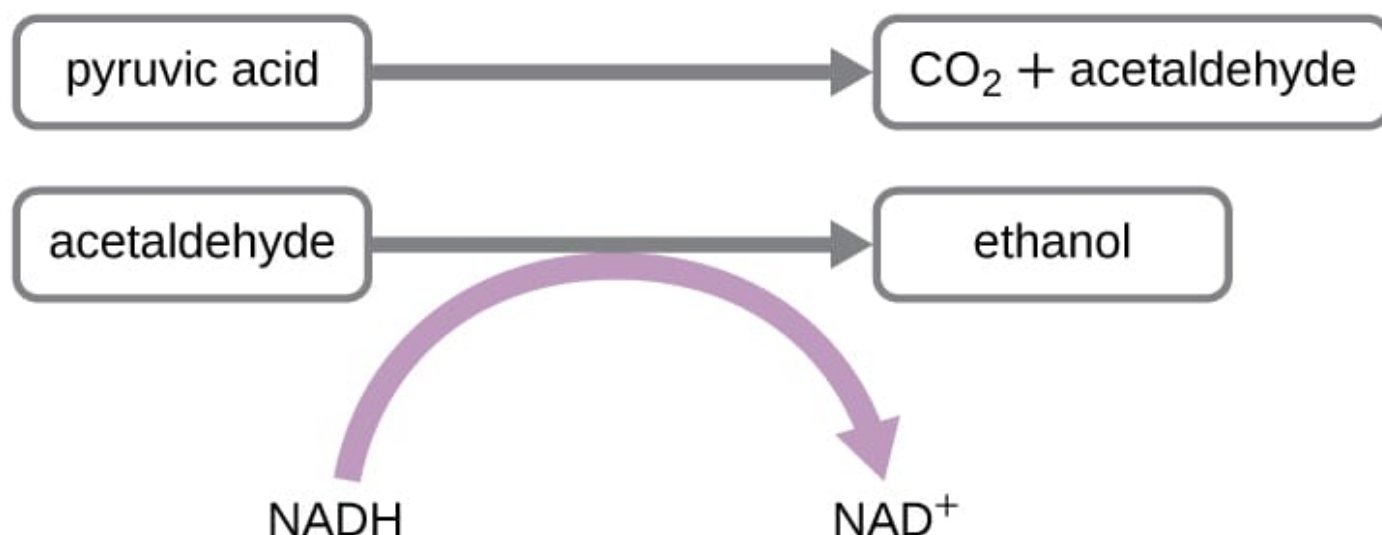


Figure 9.2.1: The chemical reactions of alcohol fermentation are shown here. Ethanol fermentation is important in the production of alcoholic beverages and bread.

Query 9.2.1

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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.

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9.3: Fermentation II

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

Other Fermentation Methods

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 9.3.2). 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 9.3.2: Common Fermentation Pathways

Pathway	End Products	Example Microbes	Commercial Products
Acetone-butanol-ethanol	Acetone, butanol, ethanol, CO_2	<i>Clostridium acetobutylicum</i>	Commercial solvents, gasoline alternative
Alcohol	Ethanol, CO_2	<i>Candida</i> , <i>Saccharomyces</i>	Beer, bread
Butanediol	Formic and lactic acid; ethanol; acetoin; 2,3 butanediol; CO_2 ; hydrogen gas	<i>Klebsiella</i> , <i>Enterobacter</i>	Chardonnay wine
Butyric acid	Butyric acid, CO_2 , hydrogen gas	<i>Clostridium butyricum</i>	Butter
Lactic acid	Lactic acid	<i>Streptococcus</i> , <i>Lactobacillus</i>	Sauerkraut, yogurt, cheese
Mixed acid	Acetic, formic, lactic, and succinic acids; ethanol, CO_2 , hydrogen gas	<i>Escherichia</i> , <i>Shigella</i>	Vinegar, cosmetics, pharmaceuticals
Propionic acid	Acetic acid, propionic acid, CO_2	<i>Propionibacterium</i> , <i>Bifidobacterium</i>	Swiss cheese

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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 9.3.2). Microbiologists can then compare the sample's profile to the database to identify the specific microbe.



Figure 9.3.2: 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 damsela* ssp. *piscicida*.

Key Concepts and Summary

- 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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9.4: 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.

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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.

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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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9.E: Microbial Metabolism (Exercises)

9.1: Cellular Respiration

Cellular respiration begins when electrons are transferred from NADH and FADH₂—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 CO₂.
- 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 H₂O 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?

9.2: 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?

9.3: 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?

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10: 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 10.1). 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 10.1: 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 in-dwelling 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)

10.1: Binary Fission and Generation Time

10.2: Growth Curve

10.3: Measurement of Bacterial Growth

10.4: Biofilm

10.5: Oxygen Requirements for Microbial Growth

10.6: The Effects of pH on Microbial Growth

10.7: Temperature and Microbial Growth

10.8: Other Environmental Conditions that Affect Growth

10.9: Media Used for Bacterial Growth

10.E: Microbial Growth (Exercises)

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10.1: Binary Fission and Generation Time

Learning Objectives

- Describe the process of binary fission and identify the key steps
- Define generation time and explain the significance in microbial growth
- Calculate the number of bacterial cells after a given number of generations using generation time

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 10.1.1. 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.

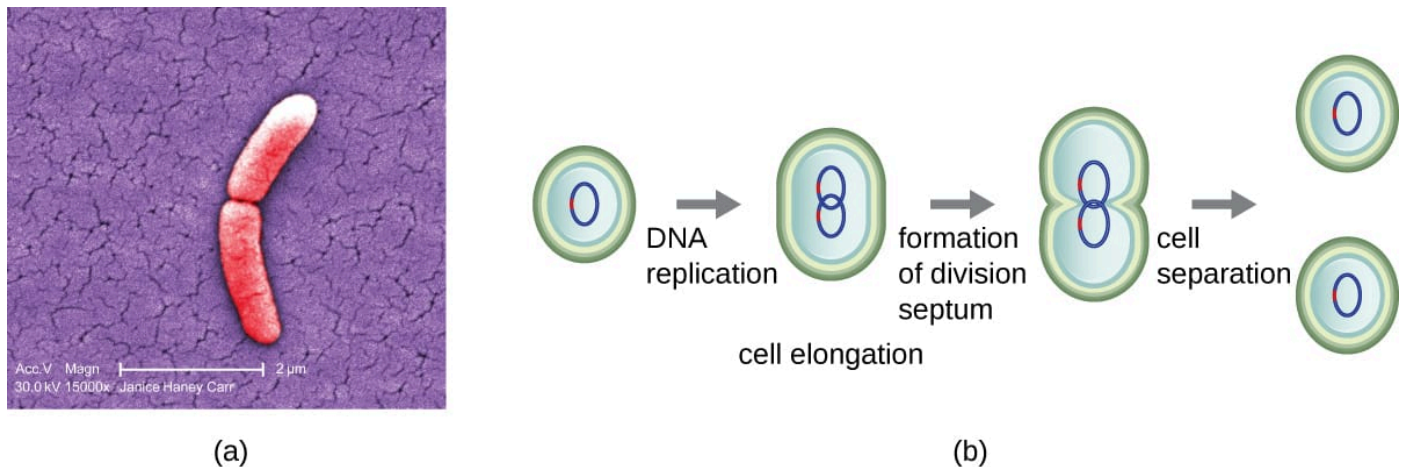


Figure 10.1.1: (a) The electron micrograph depicts two cells of *Salmonella typhimurium* after a binary fission event. (b) Binary fission in bacteria starts with the replication of DNA as the cell elongates. A division septum forms in the center of the cell. Two daughter cells of similar size form and 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 10.1.2). 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.

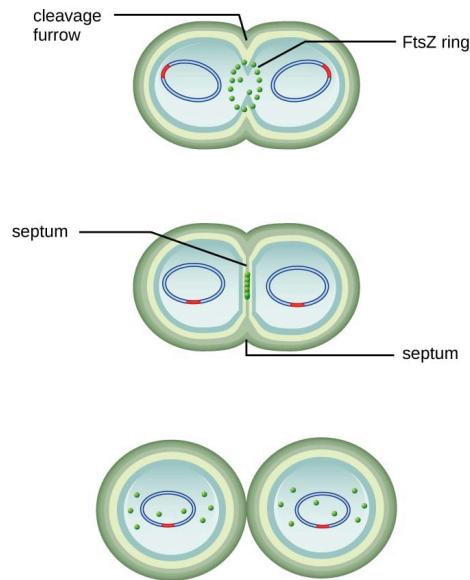


Figure 10.1.2: FtsZ proteins assemble to form a Z ring that is anchored to the plasma membrane. The Z ring pinches the cell envelope to separate the cytoplasm of the new cells.

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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 10.1.3 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:

$$N_n = N_0 2^n \quad (10.1.1)$$

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 10.1.3: 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.

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Case Study Preview: “The Raw Deal”

Jeni, a healthy 24-year-old in her second trimester, thought her flu-like symptoms were no big deal - until her doctor found a red flag: she drinks raw milk. With a possible *Listeria monocytogenes* infection on the table, Jeni's pregnancy puts both her and her fetus at serious risk.

In this case, you'll trace how *Listeria* is identified through blood agar, catalase testing, and molecular diagnostics, and why pregnant people are especially vulnerable. You'll also learn how *Listeria*'s ability to thrive in cold, salty environments makes it a stealthy threat in food safety - and a reason to rethink that unpasteurized cheese.

This time, it's not what you eat - it's what's hiding in it.

[Chapter 9 Case Study - The Raw Deal](#)

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.

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10.2: Growth Curve

Learning Objectives

- Identify and describe the four phases of a typical bacterial growth curve
- Explain the physiological changes that occur in bacterial populations during each phase
- Interpret a bacterial growth curve and correlate it with changes in cell number and metabolic activity

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 10.2.4).

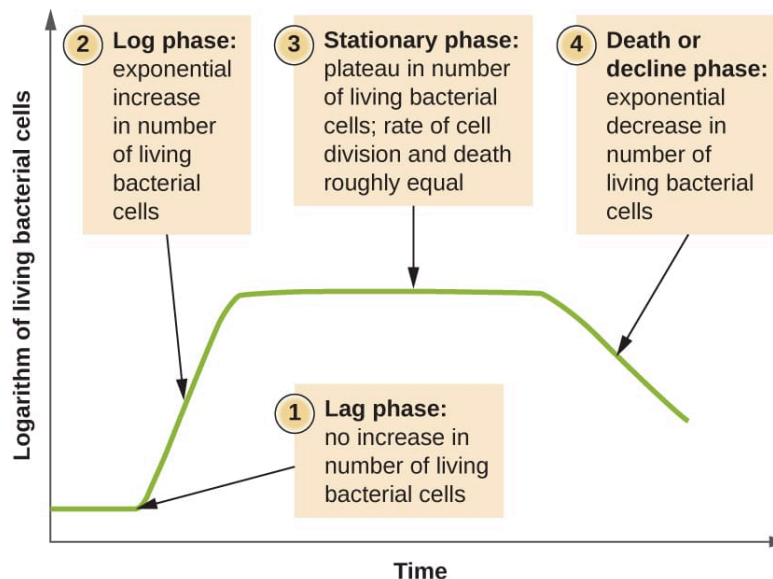


Figure 10.2.4: The growth curve of a bacterial culture is represented by the logarithm of the number of live cells plotted as a function of time. The graph can be divided into four phases according to the slope, each of which matches events in the cell. The four phases are lag, log, stationary, and death.

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 10.2.5, which gives the appearance of a linear relationship.

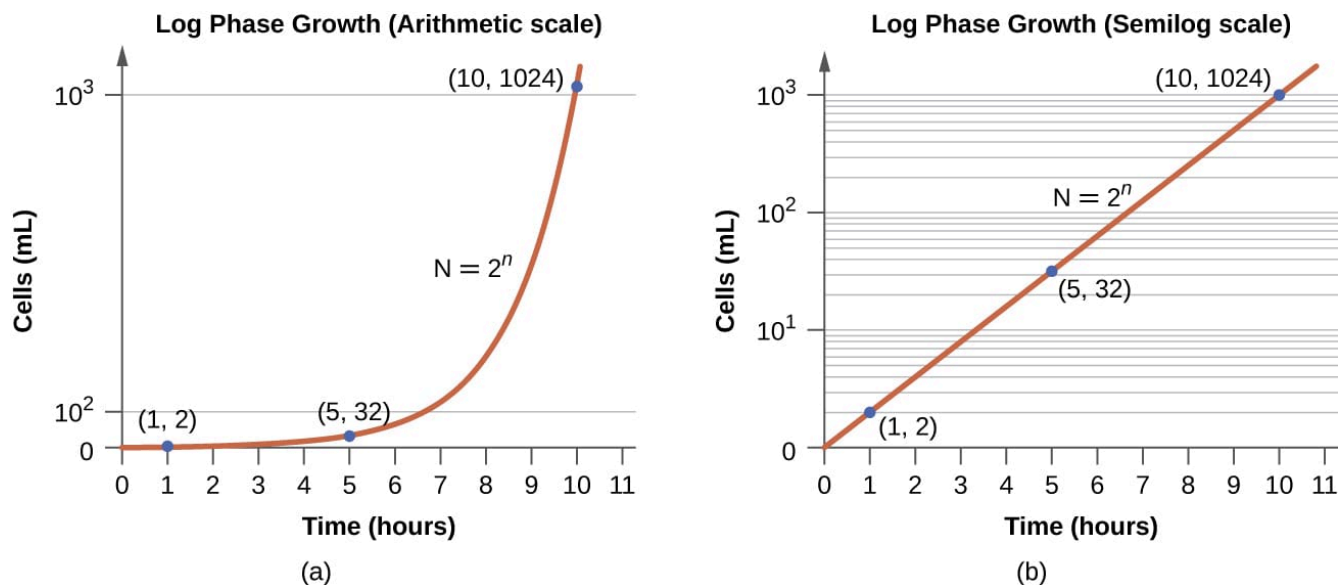


Figure 10.2.5: Both graphs illustrate population growth during the log phase for a bacterial sample with an initial population of one cell and a doubling time of 1 hour. (a) When plotted on an arithmetic scale, the growth rate resembles a curve. (b) When plotted on a semilogarithmic scale (meaning the 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 10.2.4). 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 10.2.4). 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.

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Sustaining Microbial Growth

The growth pattern shown in Figure 10.2.4 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 10.2.6) 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.

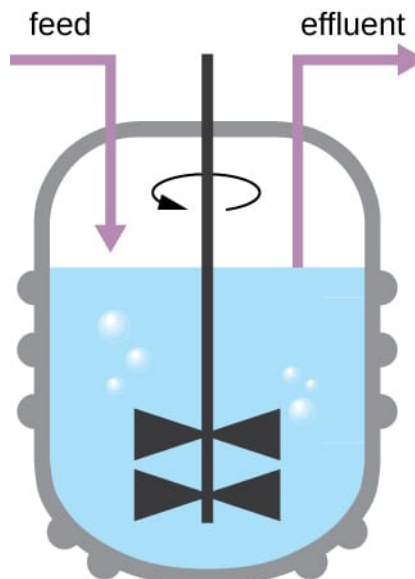


Figure 10.2.6: A chemostat is a culture vessel fitted with an opening to add nutrients (feed) and an outlet to remove contents (effluent), effectively diluting toxic wastes and dead cells. The addition and removal of fluids is adjusted to maintain the culture in the logarithmic phase of growth. If aerobic bacteria are grown, suitable oxygen levels are maintained.

Key Concepts and Summary

- Cells in a closed system follow a pattern of growth with four phases: **lag**, **logarithmic (exponential)**, **stationary**, and **death**.

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10.3: Measurement of Bacterial Growth

Learning Objectives

- Explain several laboratory methods used to determine viable and total cell counts in populations undergoing exponential growth
- Differentiate between direct and indirect methods used to measure bacterial growth
- Explain the principle of viable plate count methods including serial dilution and colony-forming units (CFUs)
- Compare the accuracy, sensitivity and applications of direct counts, plate counts and turbidity-based methods

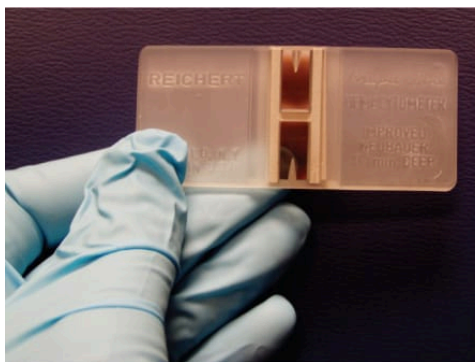
Measurement of Bacterial Growth

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.

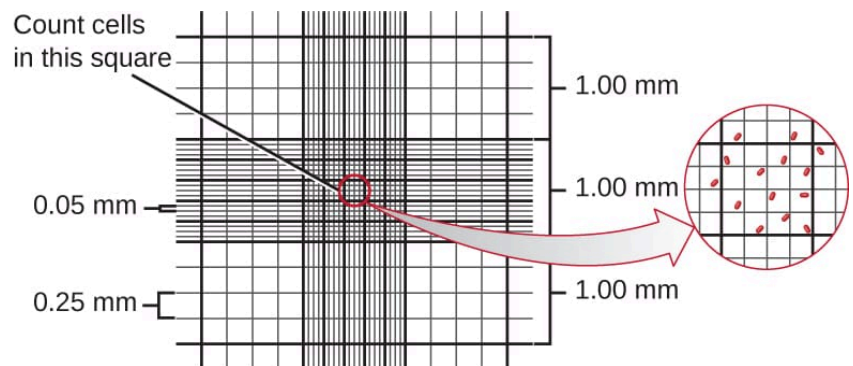
Direct Cell Count

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 10.3.7) 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.



(a)



(b)

Figure 10.3.7: (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 mm², then the volume is 0.008 mm³, 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 10.3.8).

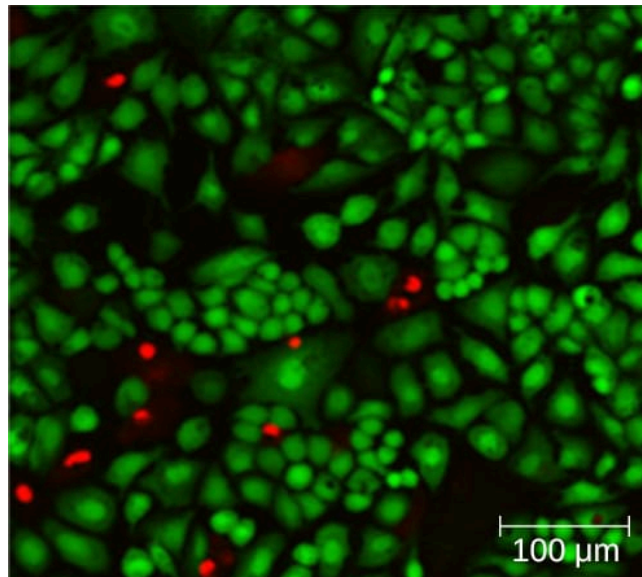


Figure 10.3.8: Fluorescence staining can be used to differentiate between viable and dead bacterial cells in a sample for purposes of counting. Viable cells are stained green, whereas dead cells are stained red. (credit: modification of work by Panzeri S, Cunha C, D'Alessandro T, Sandri M, Giavaresi 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 10.3.9); 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.

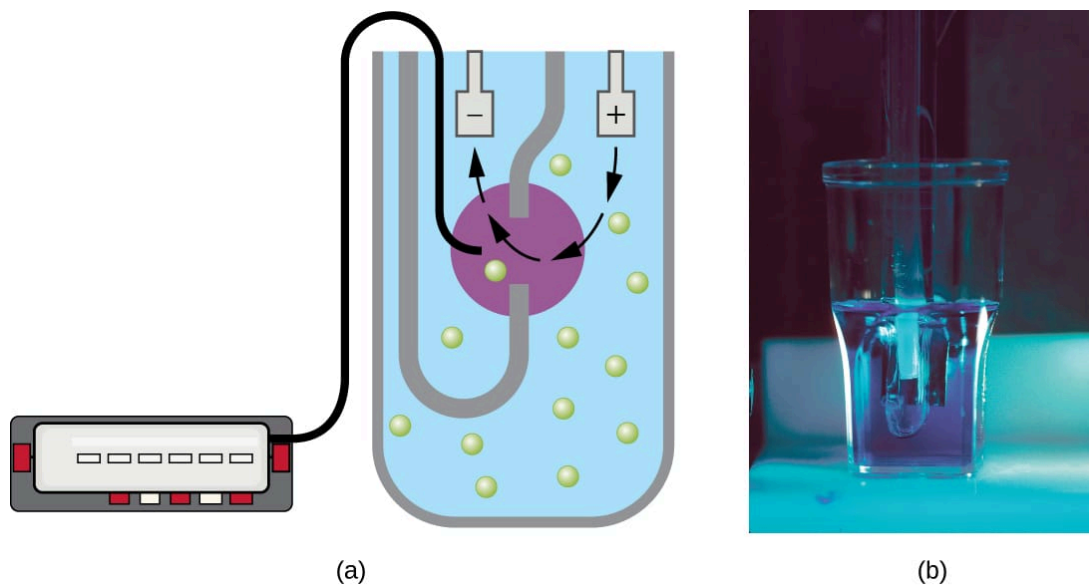


Figure 10.3.9: A Coulter counter is an electronic device that counts cells. It measures the change in resistance in an electrolyte solution that takes place when a cell passes through a small opening in the inside container wall. A detector automatically counts the number of cells passing through the opening. (credit b: modification of work by National Institutes of Health)

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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 10.3.10 illustrates the serial dilution method.

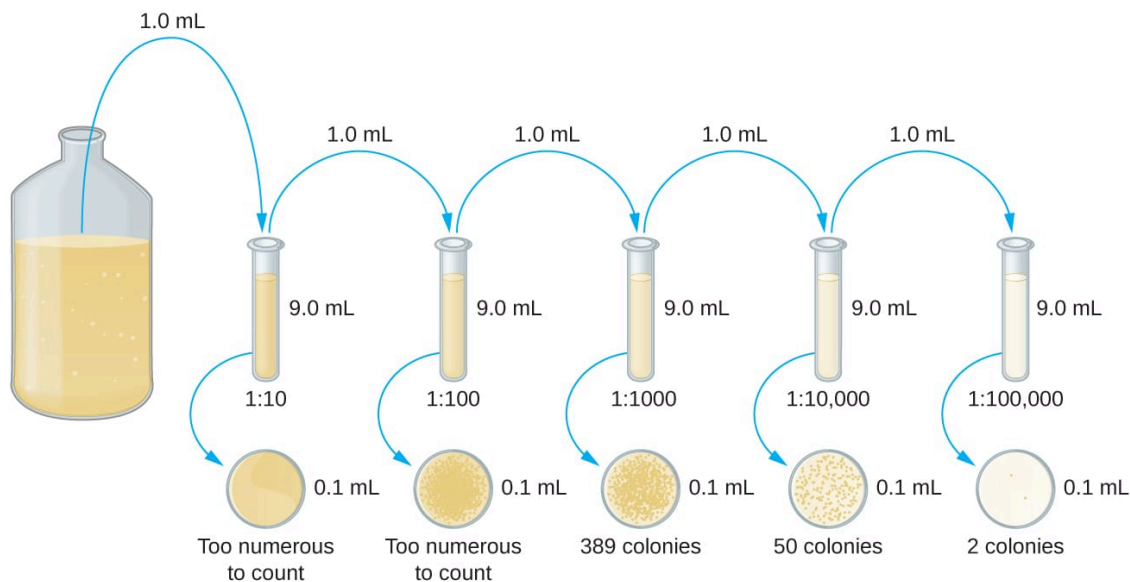


Figure 10.3.10: Serial dilution involves diluting a fixed volume of cells mixed with dilution solution using the previous dilution as an inoculum. The result is dilution of the original culture by an exponentially growing factor. (credit: modification of work by “Leberecht”/Wikimedia Commons)

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 10.3.11) or the **spread plate method** (Figure 10.3.12). 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.

Pour Plate Method

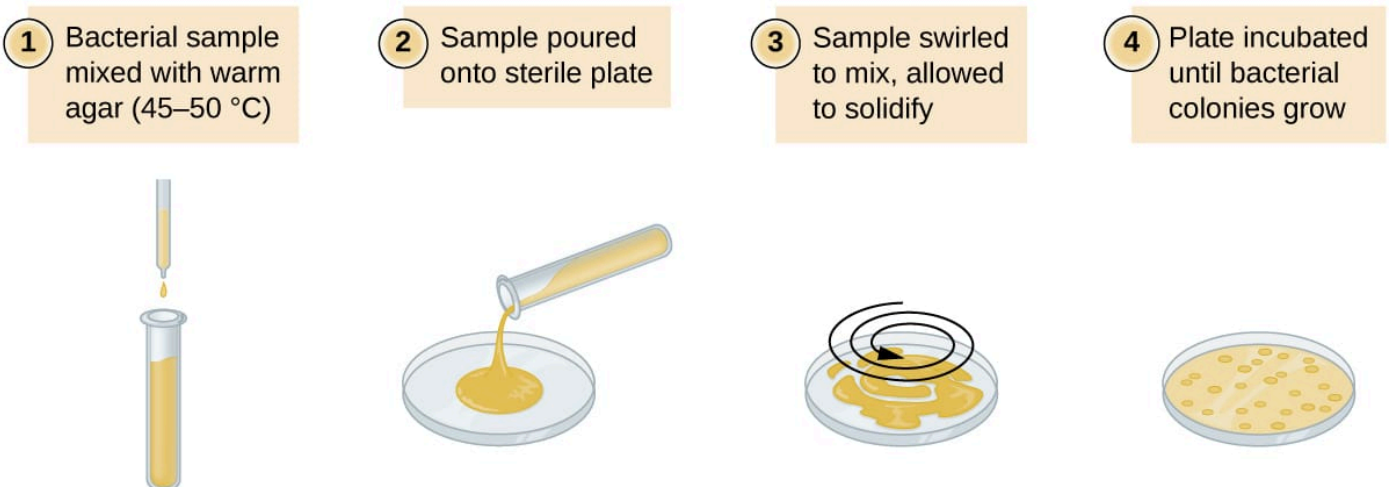


Figure 10.3.11: In the pour plate method of cell counting, the sample is mixed in liquid warm agar (45–50 °C) poured into a sterile Petri dish and further mixed by swirling. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number 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 $10 \times 10,000$. The number of CFU per mL is equal to $50 \times 100 \times 10,000 = 5,000,000$. 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.

Spread Plate Method

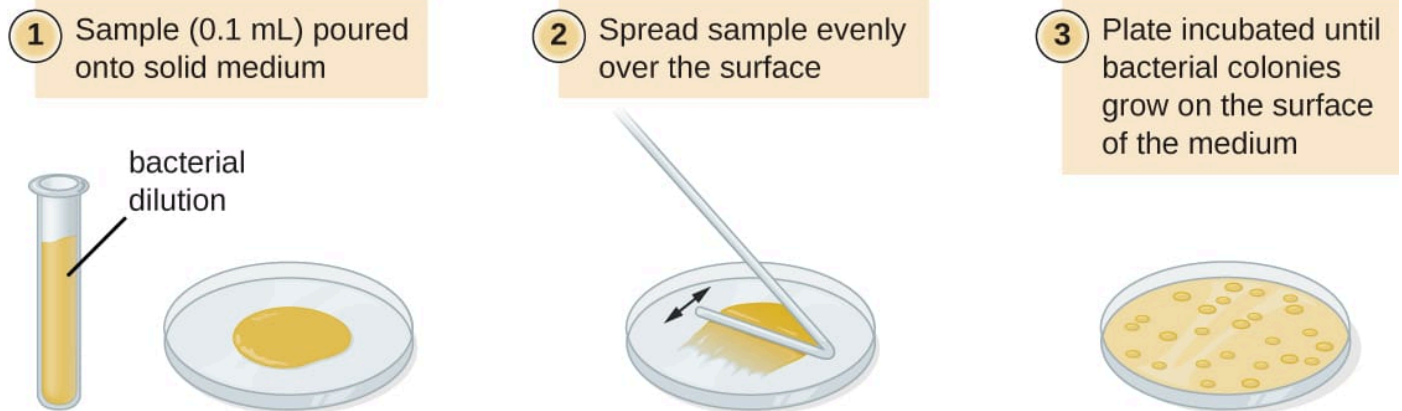


Figure 10.3.12: In the spread plate method of cell counting, the sample is poured onto solid agar and then spread using a sterile spreader. This process is repeated for each serial dilution prepared. The resulting colonies are counted and provide an estimate of the number of cells in the original volume 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.

In calculating serial dilution, use the equation:

$$V_1D_1 = V_2D_2$$

V_1 : original volume

D_1 : original dilution (undiluted samples are always expressed as 1)

V_2 : final volume

D_2 : final dilution

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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 10.3.13, 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.

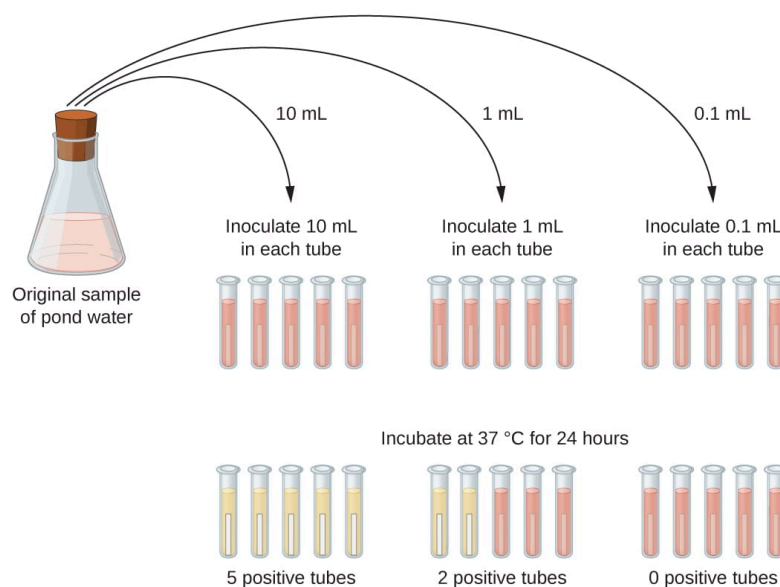


Figure 10.3.13: In the most probable number method, sets of five lactose broth tubes are inoculated with three different volumes of pond water: 10 mL, 1 mL, and 0.1mL. Bacterial growth is assessed through a change in the color of the broth from red to yellow as lactose is fermented.

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 10.3.14). 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.

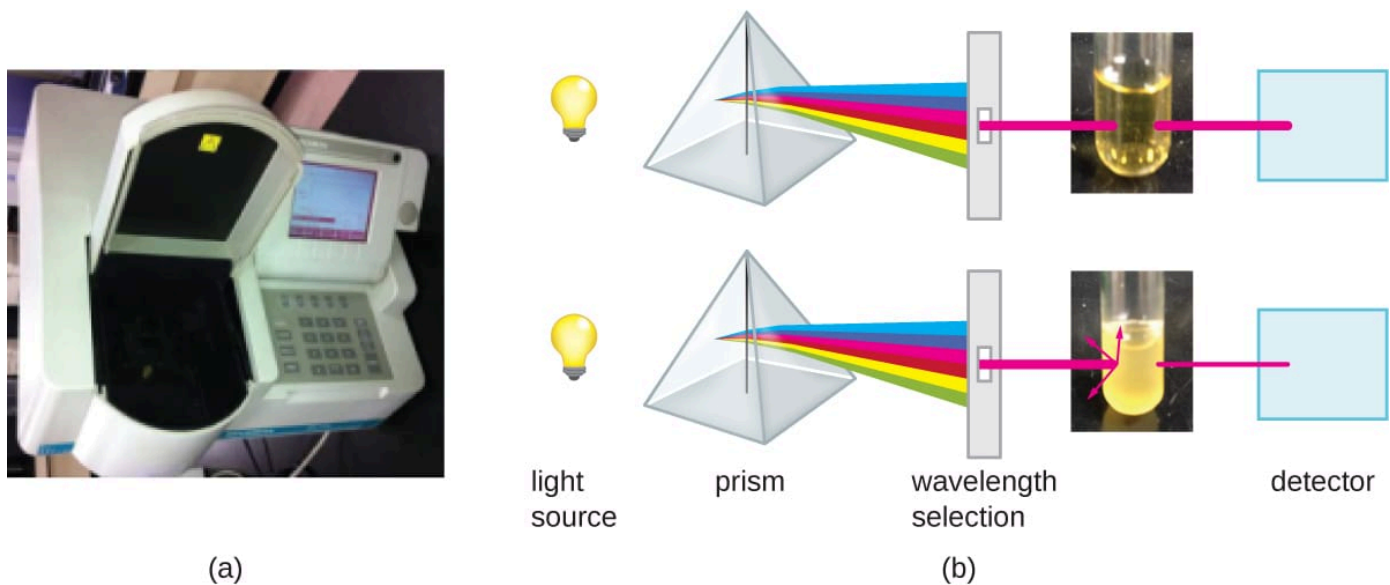


Figure 10.3.14: (a) A spectrophotometer is commonly used to measure the turbidity of a bacterial cell suspension as an indirect measure of cell density. (b) A spectrophotometer works by splitting white light from a source into a spectrum. The spectrophotometer allows choice of the wavelength of light 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.

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Key Concepts and Summary

- 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.

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10.4: Biofilm

Learning Objectives

- 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

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 10.4.16. 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.

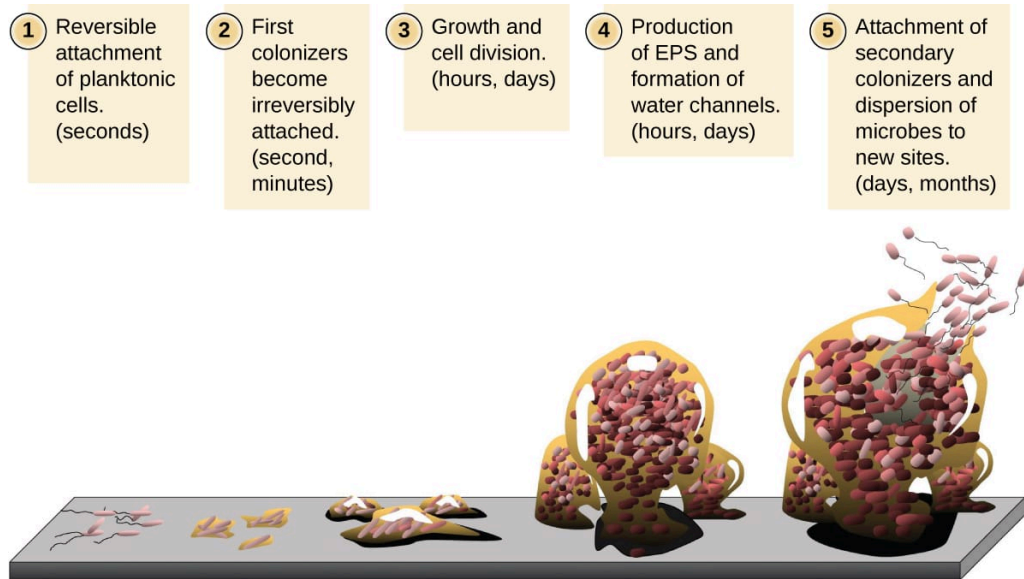


Figure 10.4.16: Stages in the formation and life cycle of a biofilm. (credit: modification of work by Public Library of Science and American Society for Microbiology)

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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 10.4.17). 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.

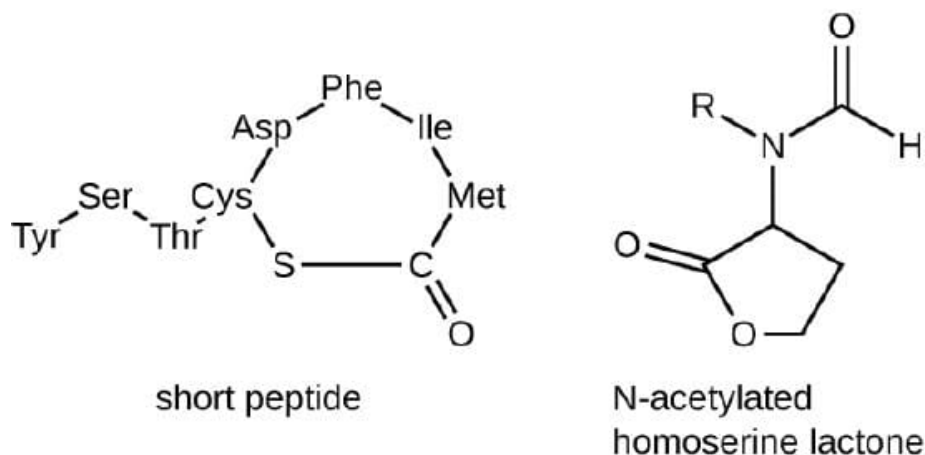


Figure 10.4.17: Short peptides in gram-positive bacteria and N-acetylated homoserine lactones in gram-negative bacteria act as autoinducers in quorum sensing and mediate the coordinated response of bacterial cells. The R side chain of the N-acetylated homoserine lactone is specific for the species of gram-negative bacteria. Some secreted homoserine lactones are recognized by more than one species.

Video: Biofilm Formation

Biofilm formation.

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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.

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Key Concepts and Summary

- **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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10.5: Oxygen Requirements for Microbial 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 ($O_2^{\bullet}$), superoxide ($O_2^{\bullet -}$), peroxides (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and hypochlorite ion (OCI^-), 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 10.5.1).



(a)



(b)

Figure 10.5.1: 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 10.5.2. 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 Oxygen Categories

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. gonorrhoeae*, the causative agent of sexually transmitted gonorrhea, are also obligate aerobes.

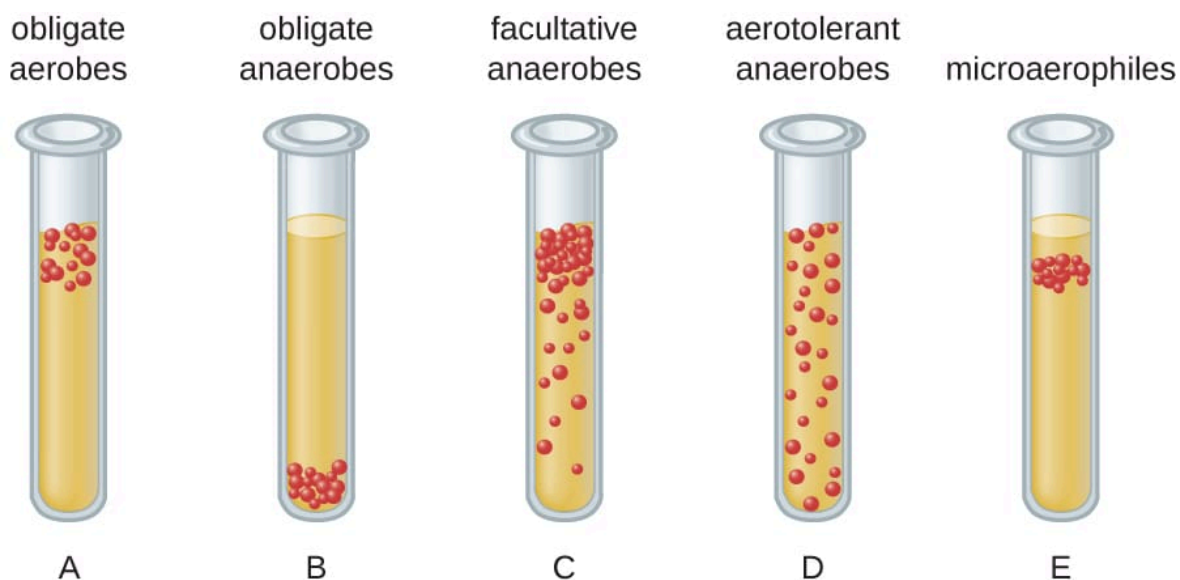


Figure 10.5.2: Diagram of bacterial cell distribution in thioglycolate tubes.

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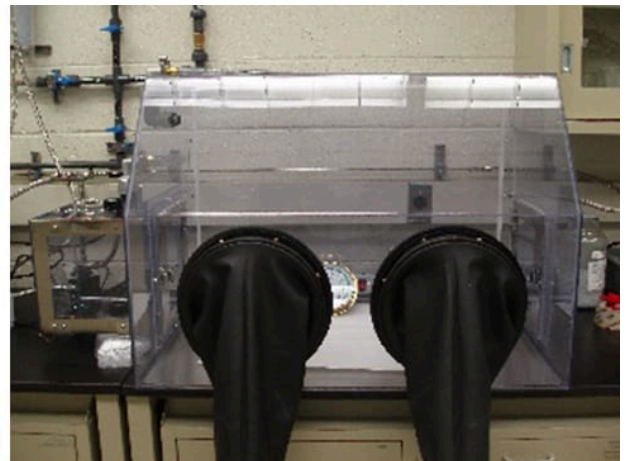
Many **obligate anaerobes** are found in the environment where anaerobic conditions exist, such as in deep sediments of soil, still waters, and at the bottom of the deep ocean where there is no photosynthetic life. Anaerobic conditions also exist naturally in the intestinal tract of animals. Obligate 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 10.5.3). Anaerobic jars include chemical packs that remove oxygen and release carbon dioxide (CO₂). 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 10.5.3).



(a)



(b)

Figure 10.5.3: (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.

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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 10.5.4). 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 10.5.4: 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 10.5.2

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**.



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:



The enzyme **catalase** converts hydrogen peroxide to water and oxygen as shown in Reaction 3.



Obligate anaerobes usually lack all three enzymes. Aerotolerant anaerobes do have SOD but no catalase. Reaction 3, shown occurring in Figure 10.5.5, 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.

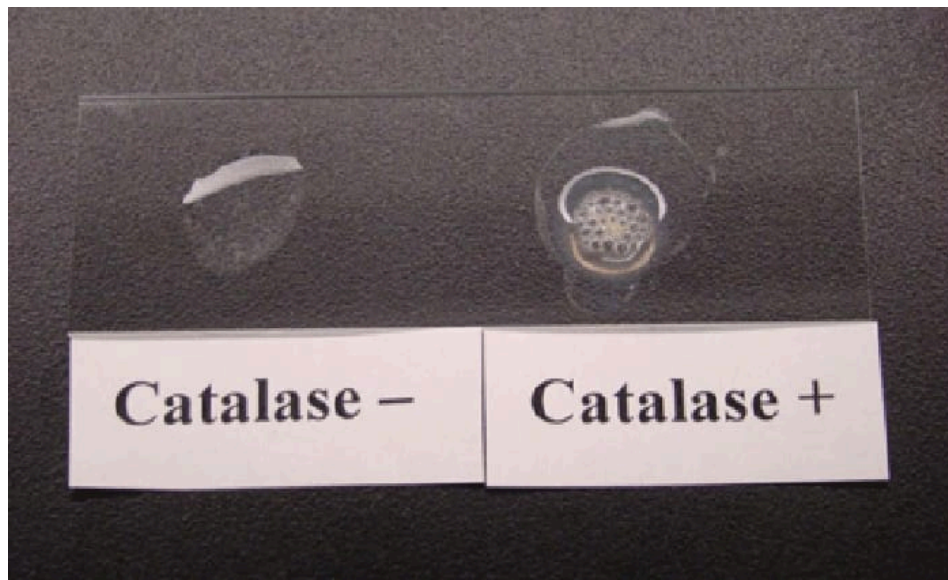


Figure 10.5.5: The catalase test detects the presence of the enzyme catalase by noting whether bubbles are released when hydrogen peroxide is added to a culture sample. Compare the positive result (right) with the negative result (left). (credit: Centers for Disease Control and Prevention)

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 .

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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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10.6: The Effects of pH on Microbial 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 10.6.1). 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 10.6.1: 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 10.6.2). 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.

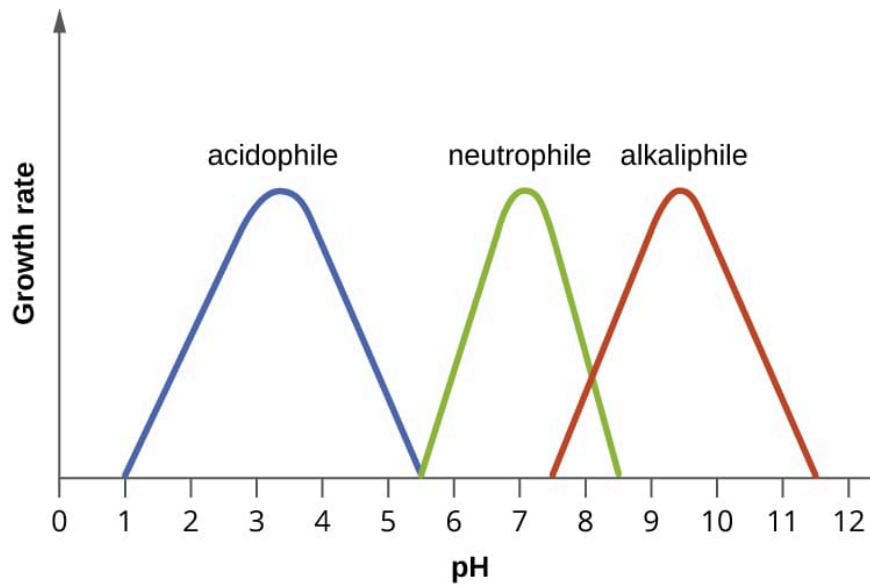


Figure 10.6.2: The curves show the approximate pH ranges for the growth of the different classes of pH-specific prokaryotes. Each curve has an optimal pH and extreme pH values at which growth is much reduced. Most bacteria are neutrophiles and grow best at near-neutral pH (center curve). Acidophiles 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 10.6.3). 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 10.6.3: 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)

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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.

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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10.7: Temperature and Microbial 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 10.7.1), 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.

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.

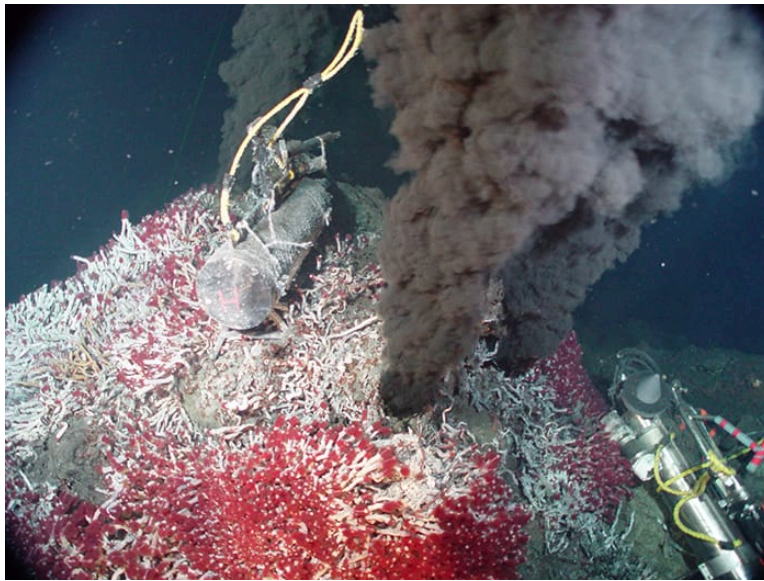


Figure 10.7.1: A black smoker at the bottom of the ocean belches hot, chemical-rich water, and heats the surrounding waters. Sea vents provide an extreme environment that is nonetheless teeming with macroscopic life (the red tubeworms) supported by an abundant microbial ecosystem. (credit: NOAA)

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 10.7.1). 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 10.7.2 shows the typical skewed curves of temperature-dependent growth for the categories of microorganisms we have discussed.

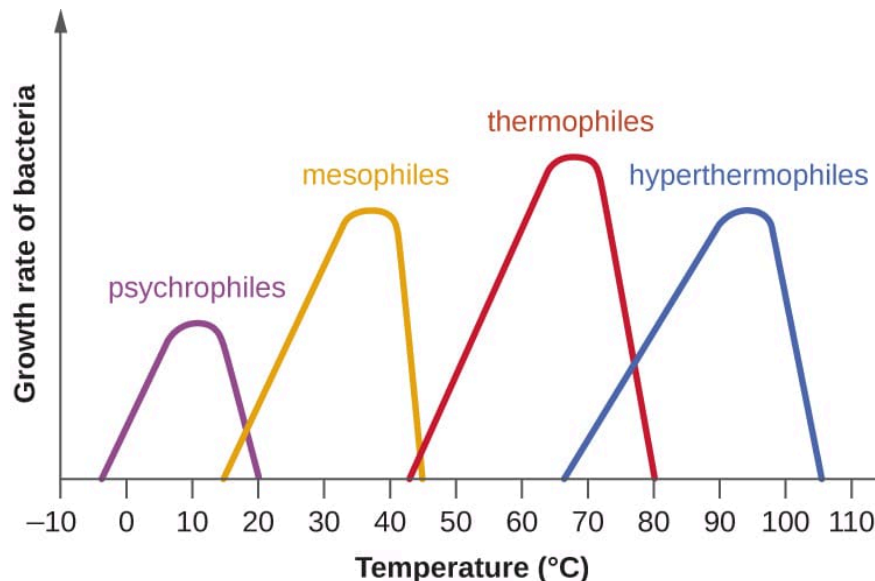


Figure 10.7.2: The graph shows growth rate of bacteria as a function of temperature. Notice that the curves are skewed toward the optimum temperature. The skewing of the growth curve is thought to reflect the rapid denaturation of proteins as the temperature rises past the optimum for growth of the microorganism.

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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.

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 10.7.3) 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 10.7.3: 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 **hyperthermophiles** 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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10.8: Other Environmental Conditions that Affect 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 10.8.1, 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 10.8.1: 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.

Query 10.8.1

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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.

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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10.9: 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 10.9.1, 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 10.9.1: 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)

Query 10.9.1

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Query 10.9.1

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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 10.9.2). 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 10.9.2

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?

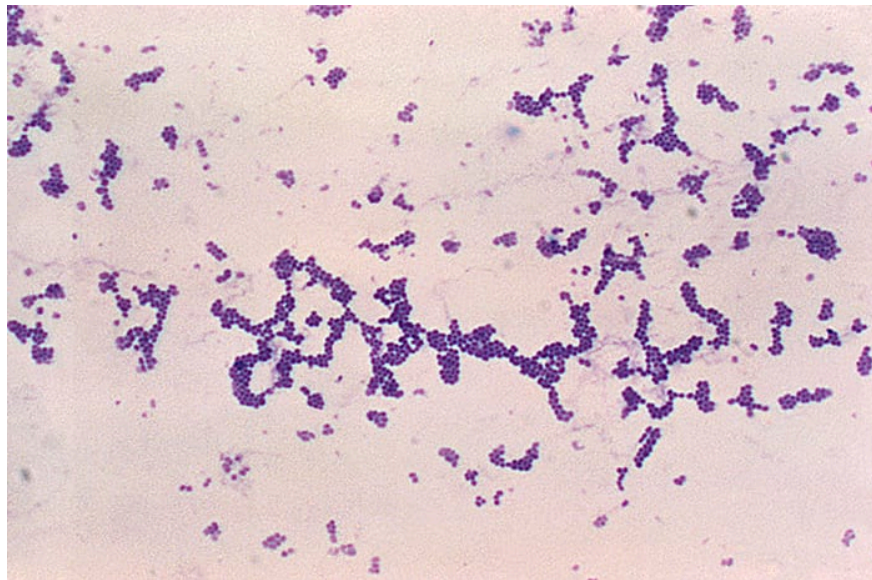


Figure 10.9.2: Gram-positive cocci in clusters. (credit: Centers for Disease Control and Prevention)

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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10.E: Microbial Growth (Exercises)

9.1: How Microbes Grow

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.

Multiple Choice

Which of the following methods would be used to measure the concentration of bacterial contamination in processed peanut butter?

- A. turbidity measurement
- B. total plate count
- C. dry weight measurement
- D. direct counting of bacteria on a calibrated slide under the microscope

Answer

B

In which phase would you expect to observe the most endospores in a *Bacillus* cell culture?

- A. death phase
- B. lag phase
- C. log phase
- D. log, lag, and death phases would all have roughly the same number of endospores.

Answer

A

During which phase would penicillin, an antibiotic that inhibits cell-wall synthesis, be most effective?

- A. death phase
- B. lag phase
- C. log phase
- D. stationary phase

Answer

C

Which of the following is the best definition of generation time in a bacterium?

- A. the length of time it takes to reach the log phase
- B. the length of time it takes for a population of cells to double
- C. the time it takes to reach stationary phase
- D. the length of time of the exponential phase

Answer

B

What is the function of the Z ring in binary fission?

- A. It controls the replication of DNA.
- B. It forms a contractile ring at the septum.
- C. It separates the newly synthesized DNA molecules.
- D. It mediates the addition of new peptidoglycan subunits.

Answer

B

If a culture starts with 50 cells, how many cells will be present after five generations with no cell death?

- A. 200
- B. 400
- C. 1600
- D. 3200

Answer

C

Filamentous cyanobacteria often divide by which of the following?

- A. budding
- B. mitosis
- C. fragmentation
- D. formation of endospores

Answer

C

Which is a reason for antimicrobial resistance being higher in a biofilm than in free-floating bacterial cells?

- A. The EPS allows faster diffusion of chemicals in the biofilm.
- B. Cells are more metabolically active at the base of a biofilm.
- C. Cells are metabolically inactive at the base of a biofilm.
- D. The structure of a biofilm favors the survival of antibiotic resistant cells.

Answer

C

Quorum sensing is used by bacterial cells to determine which of the following?

- A. the size of the population
- B. the availability of nutrients
- C. the speed of water flow
- D. the density of the population

Answer

D

Which of the following statements about autoinducers is incorrect?

- A. They bind directly to DNA to activate transcription.
- B. They can activate the cell that secreted them.
- C. N-acylated homoserine lactones are autoinducers in gram-negative cells.

D. Autoinducers may stimulate the production of virulence factors.

Answer

A

Fill in the Blank

Direct count of total cells can be performed using a _____ or a _____.

Answer

hemocytometer, Petroff-Hausser counting chamber

The _____ method allows direct count of total cells growing on solid medium.

Answer

plate count

A statistical estimate of the number of live cells in a liquid is usually done by _____.

Answer

most probable number

For this indirect method of estimating the growth of a culture, you measure _____ using a spectrophotometer.

Answer

turbidity

Active growth of a culture may be estimated indirectly by measuring the following products of cell metabolism: _____ or _____.

Answer

ATP, acid from fermentation

Matching

Match the definition with the name of the growth phase in the growth curve.

___Number of dying cells is higher than the number of cells dividing	A. Lag phase
___Number of new cells equal to number of dying cells	B. Log phase
___New enzymes to use available nutrients are induced	C. Stationary phase
___Binary fission is occurring at maximum rate	D. Death phase

Answer

D, C, A, B

Short Answer

Why is it important to measure the transmission of light through a control tube with only broth in it when making turbidity measures of bacterial cultures?

In terms of counting cells, what does a plating method accomplish that an electronic cell counting method does not?

Order the following stages of the development of a biofilm from the earliest to the last step.

1. secretion of EPS
2. reversible attachment
3. dispersal
4. formation of water channels
5. irreversible attachment

Infections among hospitalized patients are often related to the presence of a medical device in the patient. Which conditions favor the formation of biofilms on in-dwelling catheters and prostheses?

Critical Thinking

A patient in the hospital has an intravenous catheter inserted to allow for the delivery of medications, fluids, and electrolytes. Four days after the catheter is inserted, the patient develops a fever and an infection in the skin around the catheter. Blood cultures reveal that the patient has a blood-borne infection. Tests in the clinical laboratory identify the blood-borne pathogen as *Staphylococcus epidermidis*, and antibiotic susceptibility tests are performed to provide doctors with essential information for selecting the best drug for treatment of the infection. Antibacterial chemotherapy is initiated and delivered through the intravenous catheter that was originally inserted into the patient. Within 7 days, the skin infection is gone, blood cultures are negative for *S. epidermidis*, and the antibacterial chemotherapy is discontinued. However, 2 days after discontinuing the antibacterial chemotherapy, the patient develops another fever and skin infection and the blood cultures are positive for the same strain of *S. epidermidis* that had been isolated the previous week. This time, doctors remove the intravenous catheter and administer oral antibiotics, which successfully treat both the skin and blood-borne infection caused by *S. epidermidis*. Furthermore, the infection does not return after discontinuing the oral antibacterial chemotherapy. What are some possible reasons why intravenous chemotherapy failed to completely cure the patient despite laboratory tests showing the bacterial strain was susceptible to the prescribed antibiotic? Why might the second round of antibiotic therapy have been more successful? Justify your answers.

Why are autoinducers small molecules?

Refer to [Figure B.1](#) in [Appendix B](#). If the results from a pond water sample were recorded as 3, 2, 1, what would be the MPN of bacteria in 100 mL of pond water?

Refer to [Figure 9.1.14](#). Why does turbidity lose reliability at high cell concentrations when the culture reaches the stationary phase?

9.2: Oxygen Requirements for Microbial Growth

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 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.

Multiple Choice

An inoculated thioglycolate medium culture tube shows dense growth at the surface and turbidity throughout the rest of the tube. What is your conclusion?

- A. The organisms die in the presence of oxygen
- B. The organisms are facultative anaerobes.
- C. The organisms should be grown in an anaerobic chamber.
- D. The organisms are obligate aerobes.

Answer

B

An inoculated thioglycolate medium culture tube is clear throughout the tube except for dense growth at the bottom of the tube. What is your conclusion?

- A. The organisms are obligate anaerobes.
- B. The organisms are facultative anaerobes.
- C. The organisms are aerotolerant.
- D. The organisms are obligate aerobes.

Answer

A

Pseudomonas aeruginosa is a common pathogen that infects the airways of patients with cystic fibrosis. It does not grow in the absence of oxygen. The bacterium is probably which of the following?

- A. an aerotolerant anaerobe
- B. an obligate aerobe
- C. an obligate anaerobe
- D. a facultative anaerobe

Answer

B

Streptococcus mutans is a major cause of cavities. It resides in the gum pockets, does not have catalase activity, and can be grown outside of an anaerobic chamber. The bacterium is probably which of the following?

- A. a facultative anaerobe
- B. an obligate aerobe
- C. an obligate anaerobe
- D. an aerotolerant anaerobe

Answer

D

Why do the instructions for the growth of *Neisseria gonorrhoeae* recommend a CO₂-enriched atmosphere?

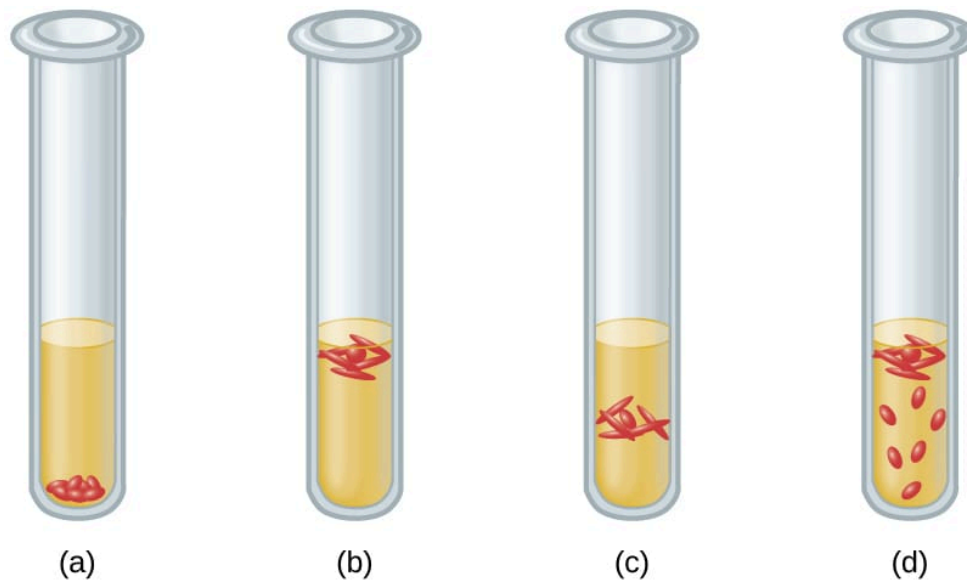
- A. It uses CO₂ as a final electron acceptor in respiration.
- B. It is an obligate anaerobe.
- C. It is a capnophile.
- D. It fixes CO₂ through photosynthesis.

Answer

C

Matching

Four tubes are illustrated with cultures grown in a medium that slows oxygen diffusion. Match the culture tube with the correct type of bacteria from the following list: facultative anaerobe, obligate anaerobe, microaerophile, aerotolerant anaerobe, obligate aerobe.



Answer

(a) obligate anaerobe, (b) obligate aerobe, (c) microaerophile, (d) facultative anaerobe

Short Answer

Why are some obligate anaerobes able to grow in tissues (e.g., gum pockets) that are not completely free of oxygen?

Why should *Haemophilus influenzae* be grown in a candle jar?

In terms of oxygen requirements, what type of organism would most likely be responsible for a foodborne illness associated with canned foods?

Critical Thinking

A microbiology instructor prepares cultures for a gram-staining practical laboratory by inoculating growth medium with a gram-positive coccus (nonmotile) and a gram-negative rod (motile). The goal is to demonstrate staining of a mixed culture. The flask is incubated at 35 °C for 24 hours without aeration. A sample is stained and reveals only gram-negative rods. Both cultures are known facultative anaerobes. Give a likely reason for success of the gram-negative rod. Assume that the cultures have comparable intrinsic growth rates.

9.3: The Effects of pH on Microbial Growth

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.

Multiple Choice

Bacteria that grow in mine drainage at pH 1-2 are probably which of the following?

- A. alkaliphiles
- B. acidophiles
- C. neutrophiles
- D. obligate anaerobes

Answer

B

Bacteria isolated from Lake Natron, where the water pH is close to 10, are which of the following?

- A. alkaliphiles
- B. facultative anaerobes
- C. neutrophiles
- D. obligate anaerobes

Answer

A

In which environment are you most likely to encounter an acidophile?

- A. human blood at pH 7.2
- B. a hot vent at pH 1.5
- C. human intestine at pH 8.5
- D. milk at pH 6.5

Answer

B

Fill in the Blank

A bacterium that thrives in a soda lake where the average pH is 10.5 can be classified as a(n) _____.

Answer

alkaliphile

Lactobacillus acidophilus grows best at pH 4.5. It is considered a(n) _____.

Answer

acidophile

Short Answer

Which macromolecule in the cell is most sensitive to changes in pH?

Which metabolic process in the bacterial cell is particularly challenging at high pH?

Critical Thinking

People who use proton pumps inhibitors or antacids are more prone to infections of the gastrointestinal tract. Can you explain the observation in light of what you have learned?

9.4: Temperature and Microbial Growth

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.

Multiple Choice

A soup container was forgotten in the refrigerator and shows contamination. The contaminants are probably which of the following?

- A. thermophiles
- B. acidophiles
- C. mesophiles
- D. psychrotrophs

Answer

D

Bacteria isolated from a hot tub at 39 °C are probably which of the following?

- A. thermophiles
- B. psychrotrophs
- C. mesophiles
- D. hyperthermophiles

Answer

C

In which environment are you most likely to encounter a hyperthermophile?

- A. hot tub
- B. warm ocean water in Florida
- C. hydrothermal vent at the bottom of the ocean
- D. human body

Answer

C

Which of the following environments would harbor psychrophiles?

- A. mountain lake with a water temperature of 12 °C
- B. contaminated plates left in a 35 °C incubator
- C. yogurt cultured at room temperature
- D. salt pond in the desert with a daytime temperature of 34 °C

Answer

A

Matching

Match the type of bacterium with its environment. Each choice may be used once, more than once, or not at all. Put the appropriate letter beside the environment.

___psychotroph	A. water heater set at 50 °C
___mesophile	B. hydrothermal vent
___thermophile	C. deep ocean waters

___hyperthermophile	D. human pathogen
___psychrophile	E. soil bacteria in temperate forest

Answer

C, D, E, B, A

Short Answer

How are hyperthermophile's proteins adapted to the high temperatures of their environment?

Why would NASA be funding microbiology research in Antarctica?

Critical Thinking

The bacterium that causes Hansen's disease (leprosy), *Mycobacterium leprae*, infects mostly the extremities of the body: hands, feet, and nose. Can you make an educated guess as to its optimum temperature of growth?

Refer to [Figure 9.4.2](#). Some hyperthermophiles can survive autoclaving temperatures. Are they a concern in health care?

9.5: Other Environmental Conditions that Affect Growth

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.

Multiple Choice

Which of the following is the reason jams and dried meats often do not require refrigeration to prevent spoilage?

- A. low pH
- B. toxic alkaline chemicals
- C. naturally occurring antibiotics
- D. low water activity

Answer

D

Bacteria living in salt marshes are most likely which of the following?

- A. acidophiles
- B. barophiles
- C. halotolerant
- D. thermophiles

Answer

C

Fill in the Blank

A bacterium that thrives in the Great Salt Lake but not in fresh water is probably a _____.

Answer

halophile

Bacteria isolated from the bottom of the ocean need high atmospheric pressures to survive. They are _____.

Answer

barophiles

Staphylococcus aureus can be grown on multipurpose growth medium or on mannitol salt agar that contains 7.5% NaCl. The bacterium is _____.

Answer

halotolerant

Short Answer

Fish sauce is a salty condiment produced using fermentation. What type of organism is likely responsible for the fermentation of the fish sauce?

9.6: Media Used for Bacterial Growth

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.

Multiple Choice

EMB agar is a medium used in the identification and isolation of pathogenic bacteria. It contains digested meat proteins as a source of organic nutrients. Two indicator dyes, eosin and methylene blue, inhibit the growth of gram-positive bacteria and distinguish between lactose fermenting and nonlactose fermenting organisms. Lactose fermenters form metallic green or deep purple colonies, whereas the nonlactose fermenters form completely colorless colonies. EMB agar is an example of which of the following?

- A. a selective medium only
- B. a differential medium only
- C. a selective medium and a chemically defined medium
- D. a selective medium, a differential medium, and a complex medium

Answer

D

Haemophilus influenzae must be grown on chocolate agar, which is blood agar treated with heat to release growth factors in the medium. *H. influenzae* is described as _____.

- A. an acidophile
- B. a thermophile
- C. an obligate anaerobe
- D. fastidious

Answer

D

Fill in the Blank

Blood agar contains many unspecified nutrients, supports the growth of a large number of bacteria, and allows differentiation of bacteria according to hemolysis (breakdown of blood). The medium is _____ and _____.

Answer

complex, differential

Rogosa agar contains yeast extract. The pH is adjusted to 5.2 and discourages the growth of many microorganisms; however, all the colonies look similar. The medium is _____ and _____.

Answer

complex, selective

Short Answer

What is the major difference between an enrichment culture and a selective culture?

Critical Thinking

Haemophilus, influenzae grows best at 35–37 °C with ~5% CO₂ (or in a candle-jar) and requires hemin (X factor) and nicotinamide-adenine-dinucleotide (NAD, also known as V factor) for growth.¹ Using the vocabulary learned in this chapter, describe *H. influenzae*.

Footnotes

1. ¹ Centers for Disease Control and Prevention, World Health Organization. “*CDC Laboratory Methods for the Diagnosis of Meningitis Caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenza*. WHO Manual, 2nd edition.” 2011. <http://www.cdc.gov/meningitis/lab-manual-manual.pdf>

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11: DNA AND RNA

Children inherit some characteristics from each parent. Siblings typically look similar to each other, but not exactly the same—except in the case of identical twins. How can we explain these phenomena? The answers lie in heredity (the transmission of traits from one generation to the next) and genetics (the science of heredity). Because humans reproduce sexually, 50% of a child's genes come from the mother's egg cell and the remaining 50% from the father's sperm cell. Sperm and egg are formed through the process of meiosis, where DNA recombination occurs. Thus, there is no predictable pattern as to which 50% comes from which parent. Thus, siblings have only some genes, and their associated characteristics, in common. Identical twins are the exception, because they are genetically identical.

Genetic differences among related microbes also dictate many observed biochemical and virulence differences. For example, some strains of the bacterium *Escherichia coli* are harmless members of the normal microbiota in the human gastrointestinal tract. Other strains of the same species have genes that give them the ability to cause disease. In bacteria, such genes are not inherited via sexual reproduction, as in humans. Often, they are transferred via plasmids, small circular pieces of double-stranded DNA that can be exchanged between prokaryotes.

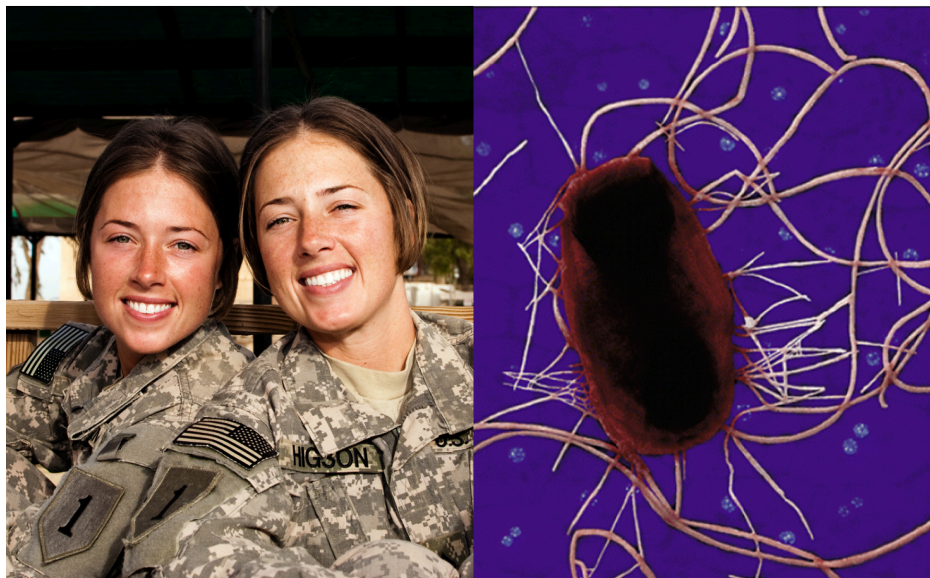


Figure 11.1: Siblings within a family share some genes with each other and with each parent. Identical twins, however, are genetically identical. Bacteria like *Escherichia coli* may acquire genes encoding virulence factors, converting them into pathogenic strains, like this uropathogenic *E. coli*. (Credit left: Army twins, Myrtle Beach, S.C. / U.S. Army; Public Domain; credit right: modification of work by American Society for Microbiology)

11.1: Using Microbiology to Discover the Secrets of Life

11.2: DNA As the Molecule Responsible for Heredity

11.3: Structure and Function of DNA

11.4: Structure and Function of RNA

11.5: The Structure and Function of Cellular Genomes

11.E: Biochemistry of the Genome (Exercises)

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11.1: Using Microbiology to Discover the Secrets of Life

Learning Objectives

- Describe the discovery of nucleic acid and nucleotides
- Explain the historical experiments that led to the characterization of DNA
- Describe how microbiology and microorganisms have been used to discover the biochemistry of genes

Through the early 20th century, DNA was not yet recognized as the genetic material responsible for heredity, the passage of traits from one generation to the next. In fact, much of the research was dismissed until the mid-20th century. The scientific community believed, incorrectly, that the process of inheritance involved a blending of parental traits that produced an intermediate physical appearance in offspring; this hypothetical process appeared to be correct because of what we know now as continuous variation, which results from the action of many genes to determine a particular characteristic, like human height. Offspring appear to be a “blend” of their parents’ traits when we look at characteristics that exhibit continuous variation. The blending theory of inheritance asserted that the original parental traits were lost or absorbed by the blending in the offspring, but we now know that this is not the case.

Two separate lines of research, begun in the mid to late 1800s, ultimately led to the discovery and characterization of DNA and the foundations of genetics, the science of heredity. These lines of research began to converge in the 1920s, and research using microbial systems ultimately resulted in significant contributions to elucidating the molecular basis of genetics.

Discovery and Characterization of DNA

Modern understanding of DNA has evolved from the discovery of nucleic acid to the development of the double-helix model. In the 1860s, Friedrich Miescher (1844–1895), a physician by profession, was the first person to isolate phosphorus-rich chemicals from leukocytes (white blood cells) from the pus on used bandages from a local surgical clinic. He named these chemicals (which would eventually be known as RNA and DNA) “nuclein” because they were isolated from the nuclei of the cells. His student Richard Altmann (1852–1900) subsequently termed it “nucleic acid” 20 years later when he discovered the acidic nature of nuclein. In the last two decades of the 19th century, German biochemist Albrecht Kossel (1853–1927) isolated and characterized the five different nucleotide bases composing nucleic acid. These are adenine, guanine, cytosine, thymine (in DNA), and uracil (in RNA). Kossel received the Nobel Prize in Physiology or Medicine in 1910 for his work on nucleic acids and for his considerable work on proteins, including the discovery of histidine.

Foundations of Genetics

Despite the discovery of DNA in the late 1800s, scientists did not make the association with heredity for many more decades. To make this connection, scientists, including a number of microbiologists, performed many experiments on plants, animals, and bacteria.

Mendel’s Pea Plants

While Miescher was isolating and discovering DNA in the 1860s, Austrian monk and botanist **Johann Gregor Mendel** (1822–1884) was experimenting with garden peas, demonstrating and documenting basic patterns of inheritance, now known as **Mendel’s laws**.

In 1856, Mendel began his decade-long research into inheritance patterns. He used the diploid **garden pea**, *Pisum sativum*, as his primary model system because it naturally self-fertilizes and is highly inbred, producing “true-breeding” pea plant lines—plants that always produce offspring that look like the parent. By experimenting with true-breeding pea plants, Mendel avoided the appearance of unexpected traits in offspring that might occur if he used plants that were not true-breeding. Mendel performed **hybridizations**, which involve mating two true-breeding individuals (P generation) that have different traits, and examined the characteristics of their offspring (first filial generation, F₁) as well as the offspring of self-fertilization of the F₁ generation (second filial generation, F₂) (Figure 11.1.1).

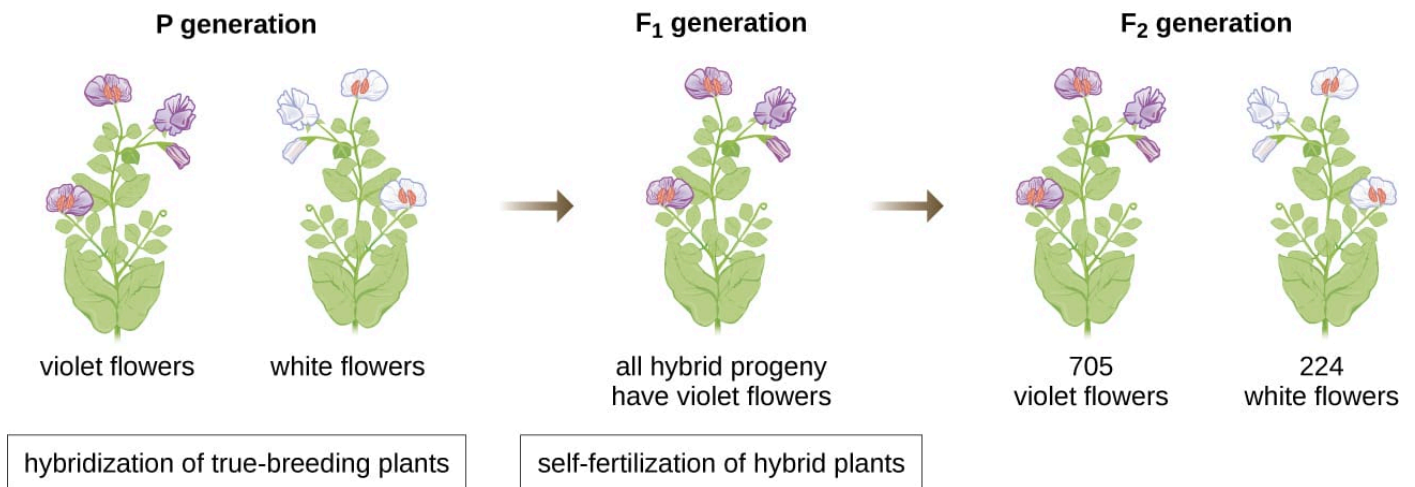


Figure 11.1.1: In one of his experiments on inheritance patterns, Mendel crossed plants that were true-breeding for violet flower color with plants true-breeding for white flower color (the P generation). The resulting hybrids in the F₁ generation all had violet flowers. In the F₂ generation, approximately three-quarters of the plants had violet flowers, and one-quarter had white flowers.

In 1865, Mendel presented the results of his experiments with nearly 30,000 pea plants to the local natural history society. He demonstrated that traits are transmitted faithfully from parents to offspring independently of other traits. In 1866, he published his work, "Experiments in Plant Hybridization,"¹ in the *Proceedings of the Natural History Society of Brunn*. Mendel's work went virtually unnoticed by the scientific community, which believed, incorrectly, in the theory of blending of traits in continuous variation.

He was not recognized for his extraordinary scientific contributions during his lifetime. In fact, it was not until 1900 that his work was rediscovered, reproduced, and revitalized by scientists on the brink of discovering the chromosomal basis of heredity.

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The Chromosomal Theory of Inheritance

Mendel carried out his experiments long before chromosomes were visualized under a microscope. However, with the improvement of microscopic techniques during the late 1800s, cell biologists could stain and visualize subcellular structures with dyes and observe their actions during meiosis. They were able to observe chromosomes replicating, condensing from an amorphous nuclear mass into distinct X-shaped bodies and migrating to separate cellular poles. The speculation that chromosomes might be the key to understanding heredity led several scientists to examine Mendel's publications and re-evaluate his model in terms of the behavior of chromosomes during mitosis and meiosis.

In 1902, **Theodor Boveri** (1862–1915) observed that in sea urchins, nuclear components (chromosomes) determined proper embryonic development. That same year, **Walter Sutton** (1877–1916) observed the separation of chromosomes into daughter cells during meiosis. Together, these observations led to the development of the Chromosomal Theory of Inheritance, which identified chromosomes as the genetic material responsible for Mendelian inheritance.

Despite compelling correlations between the behavior of chromosomes during meiosis and Mendel's observations, the Chromosomal Theory of Inheritance was proposed long before there was any direct evidence that traits were carried on chromosomes. **Thomas Hunt Morgan** (1866–1945) and his colleagues spent several years carrying out crosses with the fruit fly, *Drosophila melanogaster*. They performed meticulous microscopic observations of fly chromosomes and correlated these

observations with resulting fly characteristics. Their work provided the first experimental evidence to support the Chromosomal Theory of Inheritance in the early 1900s. In 1915, Morgan and his “Fly Room” colleagues published *The Mechanism of Mendelian Heredity*, which identified chromosomes as the cellular structures responsible for heredity. For his many significant contributions to genetics, Morgan received the Nobel Prize in Physiology or Medicine in 1933.

In the late 1920s, **Barbara McClintock** (1902–1992) developed chromosomal staining techniques to visualize and differentiate between the different chromosomes of maize (corn). In the 1940s and 1950s, she identified a breakage event on chromosome 9, which she named the dissociation locus (*Ds*). *Ds* could change position within the chromosome. She also identified an activator locus (*Ac*). *Ds* chromosome breakage could be activated by an *Ac* element (transposase enzyme). At first, McClintock’s finding of these jumping genes, which we now call transposons, was not accepted by the scientific community. It wasn’t until the 1960s and later that transposons were discovered in bacteriophages, bacteria, and *Drosophila*. Today, we know that transposons are mobile segments of DNA that can move within the genome of an organism. They can regulate gene expression, protein expression, and virulence (ability to cause disease).

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Microbes and Viruses in Genetic Research

Microbiologists have also played a crucial part in our understanding of genetics. Experimental organisms such as Mendel’s garden peas, Morgan’s fruit flies, and McClintock’s corn had already been used successfully to pave the way for an understanding of genetics. However, microbes and viruses were (and still are) excellent model systems for the study of genetics because, unlike peas, fruit flies, and corn, they are propagated more easily in the laboratory, growing to high population densities in a small amount of space and in a short time. In addition, because of their structural simplicity, microbes and viruses are more readily manipulated genetically.

Fortunately, despite significant differences in size, structure, reproduction strategies, and other biological characteristics, there is biochemical unity among all organisms; they have in common the same underlying molecules responsible for heredity and the use of genetic material to give cells their varying characteristics. In the words of French scientist Jacques Monod, “What is true for *E.*

coli is also true for the elephant,” meaning that the biochemistry of life has been maintained throughout evolution and is shared in all forms of life, from simple unicellular organisms to large, complex organisms. This biochemical continuity makes microbes excellent models to use for genetic studies.

In a clever set of experiments in the 1930s and 1940s, German scientist **Joachim Hämmerling** (1901–1980), using the single-celled alga *Acetabularia* as a microbial model, established that the genetic information in a eukaryotic cell is housed within the nucleus. *Acetabularia* spp. are unusually large algal cells that grow asymmetrically, forming a “foot” containing the nucleus, which is used for substrate attachment; a stalk; and an umbrella-like cap—structures that can all be easily seen with the naked eye. In an early set of experiments, Hämmerling removed either the cap or the foot of the cells and observed whether new caps or feet were regenerated (Figure 11.1.2). He found that when the foot of these cells was removed, new feet did not grow; however, when caps were removed from the cells, new caps were regenerated. This suggested that the hereditary information was located in the nucleus-containing foot of each cell.

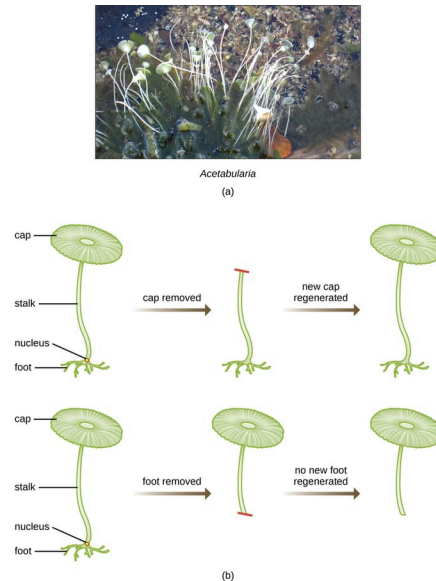


Figure 11.1.2: (a) The cells of the single-celled alga *Acetabularia* measure 2–6 cm and have a cell morphology that can be observed with the naked eye. Each cell has a cap, a stalk, and a foot, which contains the nucleus. (b) Hämmerling found that if he removed the cap, a new cap would regenerate; but if he removed the foot, a new foot would not regenerate. He concluded that the genetic information needed for regeneration was found in the nucleus. (credit a: modification of work by James St. John)

In another set of experiments, Hämmerling used two species of *Acetabularia* that have different cap morphologies, *A. crenulata* and *A. mediterranea* (Figure 11.1.3). He cut the caps from both types of cells and then grafted the stalk from an *A. crenulata* onto an *A. mediterranea* foot, and vice versa. Over time, he observed that the grafted cell with the *A. crenulata* foot and *A. mediterranea* stalk developed a cap with the *A. crenulata* morphology. Conversely, the grafted cell with the *A. mediterranea* foot and *A. crenulata* stalk developed a cap with the *A. mediterranea* morphology. He microscopically confirmed the presence of nuclei in the feet of these cells and attributed the development of these cap morphologies to the nucleus of each grafted cell. Thus, he showed experimentally that the nucleus was the location of genetic material that dictated a cell's properties.

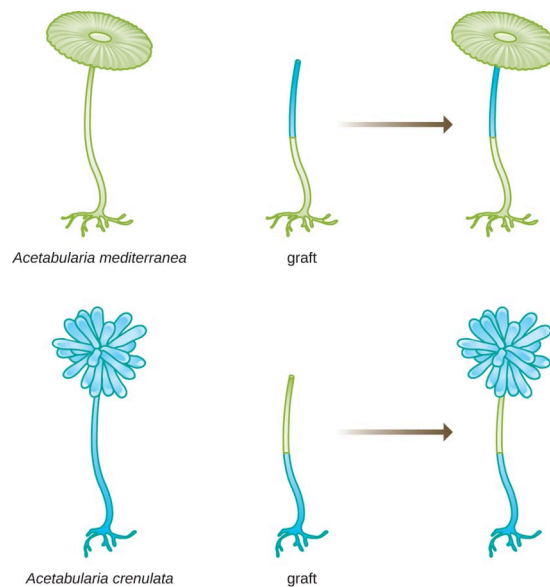


Figure 11.1.3: In a second set of experiments, Hämmerling used two morphologically different species and grafted stalks from each species to the feet of the other. He found that the properties of the regenerated caps were dictated by the species of the nucleus-containing foot.

Another microbial model, the red bread mold *Neurospora crassa*, was used by **George Beadle** and **Edward Tatum** to demonstrate the relationship between genes and the proteins they encode. Beadle had worked with fruit flies in Morgan's laboratory but found them too complex to perform certain types of experiments. *N. crassa*, on the other hand, is a simpler organism and has the ability to grow on a minimal medium because it contains enzymatic pathways that allow it to use the medium to produce its own vitamins and amino acids.

Beadle and Tatum irradiated the mold with X-rays to induce changes to a sequence of nucleic acids, called **mutations**. They mated the irradiated mold spores and attempted to grow them on both a complete medium and a minimal medium. They looked for mutants that grew on a complete medium, supplemented with vitamins and amino acids, but did not grow on the minimal medium lacking these supplements. Such molds theoretically contained mutations in the genes that encoded biosynthetic pathways. Upon finding such mutants, they systematically tested each to determine which vitamin or amino acid it was unable to produce (Figure 11.1.4) and published this work in 1941.

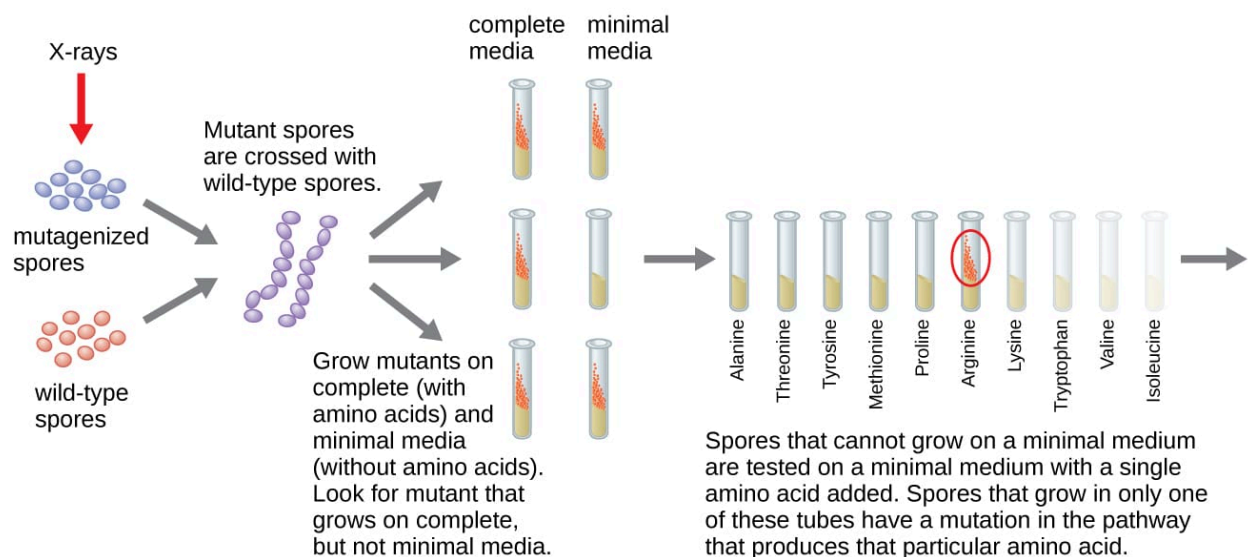


Figure 11.1.4: Beadle and Tatum's experiment involved the mating of irradiated and nonirradiated mold spores. These spores were grown on both complete medium and a minimal medium to determine which amino acid or vitamin the mutant was unable to produce on its own.

Subsequent work by Beadle, Tatum, and colleagues showed that they could isolate different classes of mutants that required a particular supplement, like the amino acid arginine (Figure 11.1.5). With some knowledge of the arginine biosynthesis pathway,

they identified three classes of arginine mutants by supplementing the minimal medium with intermediates (citrulline or ornithine) in the pathway. The three mutants differed in their abilities to grow in each of the media, which led the group of scientists to propose, in 1945, that each type of mutant had a defect in a different gene in the arginine biosynthesis pathway. This led to the so-called **one gene–one enzyme hypothesis**, which suggested that each gene encodes one enzyme.

Subsequent knowledge about the processes of transcription and translation led scientists to revise this to the “one gene–one polypeptide” hypothesis. Although there are some genes that do not encode polypeptides (but rather encode for transfer RNAs [tRNAs] or ribosomal RNAs [rRNAs], which we will discuss later), the one gene–one enzyme hypothesis is true in many cases, especially in microbes. Beadle and Tatum’s discovery of the link between genes and corresponding characteristics earned them the 1958 Nobel Prize in Physiology and Medicine and has since become the basis for modern molecular genetics.

Beadle and Tatum Experiments				
Bread Mold	Minimal Medium (MM)	MM + Ornithine	MM + Citrulline	MM + Arginine
Wild type	grew	grew	grew	grew
Mutant 1	did not grow	grew	grew	grew
Mutant 2	did not grow	did not grow	grew	grew
Mutant 3	did not grow	did not grow	did not grow	grew

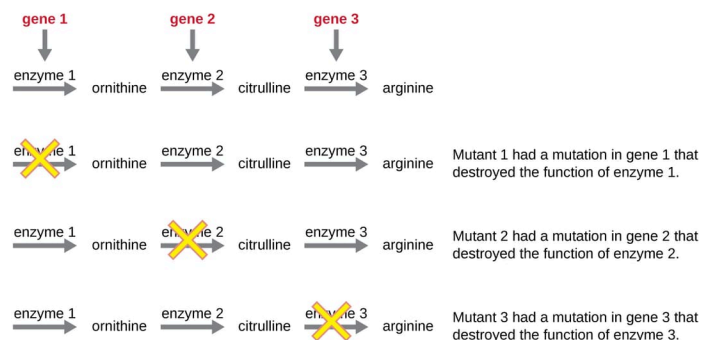


Figure 11.1.5: Three classes of arginine mutants were identified, each differing in their ability to grow in the presence of intermediates in the arginine biosynthesis pathway. From this, Beadle and Tatum concluded that each mutant was defective in a different gene encoding a different enzyme in the arginine biosynthesis pathway, leading to them to their one gene–one enzyme hypothesis.

Link to Learning

To learn more about the experiments of Beadle and Tatum, visit this [website](#) from the DNA Learning Center.

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Case Study Preview: “Tacos, Toilets, and Toxins”

Alex thought his spring break trip to Mexico would be unforgettable - and it was, just not in the way he hoped. Two days after returning home, he was stuck on the toilet with cramping and relentless watery diarrhea. The culprit? ETEC, or enterotoxigenic *E. coli*, better known as the bacterial prankster behind “traveler’s diarrhea.”

In this case, you’ll follow Alex’s misadventure through diagnosis via DNA-based detection of virulence factors, explore how *E. coli* evolves by swapping genes with other pathogens, and compare ETEC with its much scarier cousin EHEC. You’ll also learn which foods to avoid abroad and why bismuth and bottled water might just save your vacation.

Spoiler alert: it wasn’t the tacos, it was the toxins.

[Chapter 10 Case Study - Tacos, Toilets, and Toxins](#)

Key Concepts and Summary

- **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. Miescher’s student Richard Altmann discovered its acidic nature, renaming it **nucleic acid**. Albrecht Kossell characterized the **nucleotide bases** found within nucleic acids.
- Although Walter Sutton and Theodor Boveri proposed the **Chromosomal Theory of Inheritance** in 1902, it was not scientifically demonstrated until the 1915 publication of the work of Thomas Hunt Morgan and his colleagues.
- Using *Acetabularia*, a large algal cell, as his model system, Joachim Hämmerling demonstrated in the 1930s and 1940s that the nucleus was the location of hereditary information in these cells.
- In the 1940s, George Beadle and Edward Tatum used the mold *Neurospora crassa* to show that each protein’s production was under the control of a single gene, demonstrating the **“one gene–one enzyme” hypothesis**.

Footnotes

1. J.G. Mendel. “Versuche über Pflanzenhybriden.” *Verhandlungen des naturforschenden Vereines in Brünn, Bd. Abhandlungen* 4 (1865):3–7. (For English translation, see <http://www.mendelweb.org/Mendel.plain.html>)
2. G.W. Beadle, E.L. Tatum. “Genetic Control of Biochemical Reactions in *Neurospora*.” *Proceedings of the National Academy of Sciences* 27 no. 11 (1941):499–506.
3. F. Griffith. “The Significance of Pneumococcal Types.” *Journal of Hygiene* 27 no. 2 (1928):8–159.
4. A.D. Hershey, M. Chase. “Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage.” *Journal of General Physiology* 36 no. 1 (1952):39–56.

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11.2: DNA As the Molecule Responsible for Heredity

Learning Objectives

- Explain how scientists established the link between DNA and heredity
- Describe key experiments (Griffith, Avery-MacLeod-McCarty, Hershey-Chase) that demonstrated DNA's role in heredity

DNA as the Molecule Responsible for Heredity

By the beginning of the 20th century, a great deal of work had already been done on characterizing DNA and establishing the foundations of genetics, including attributing heredity to chromosomes found within the nucleus. Despite all of this research, it was not until well into the 20th century that these lines of research converged and scientists began to consider that DNA could be the genetic material that offspring inherited from their parents. DNA, containing only four different nucleotides, was thought to be structurally too simple to encode such complex genetic information. Instead, protein was thought to have the complexity required to serve as cellular genetic information because it is composed of 20 different amino acids that could be combined in a huge variety of combinations. Microbiologists played a pivotal role in the research that determined that DNA is the molecule responsible for heredity.

Griffith's Transformation Experiments

British bacteriologist **Frederick Griffith** (1879–1941) was perhaps the first person to show that hereditary information could be transferred from one cell to another “horizontally” (between members of the same generation), rather than “vertically” (from parent to offspring). In 1928, he reported the first demonstration of bacterial transformation, a process in which external DNA is taken up by a cell, thereby changing its characteristics.³ He was working with two strains of *Streptococcus pneumoniae*, a bacterium that causes pneumonia: a rough (R) strain and a smooth (S) strain. The R strain is nonpathogenic and lacks a capsule on its outer surface; as a result, colonies from the R strain appear rough when grown on plates. The S strain is pathogenic and has a capsule outside its cell wall, allowing it to escape phagocytosis by the host immune system. The capsules cause colonies from the S strain to appear smooth when grown on plates.

In a series of experiments, Griffith analyzed the effects of live R, live S, and heat-killed S strains of *S. pneumoniae* on live mice (Figure 11.2.6). When mice were injected with the live S strain, the mice died. When he injected the mice with the live R strain or the heat-killed S strain, the mice survived. But when he injected the mice with a mixture of live R strain and heat-killed S strain, the mice died. Upon isolating the live bacteria from the dead mouse, he only recovered the S strain of bacteria. When he then injected this isolated S strain into fresh mice, the mice died. Griffith concluded that something had passed from the heat-killed S strain into the live R strain and “transformed” it into the pathogenic S strain; he called this the “transforming principle.” These experiments are now famously known as Griffith's transformation experiments.

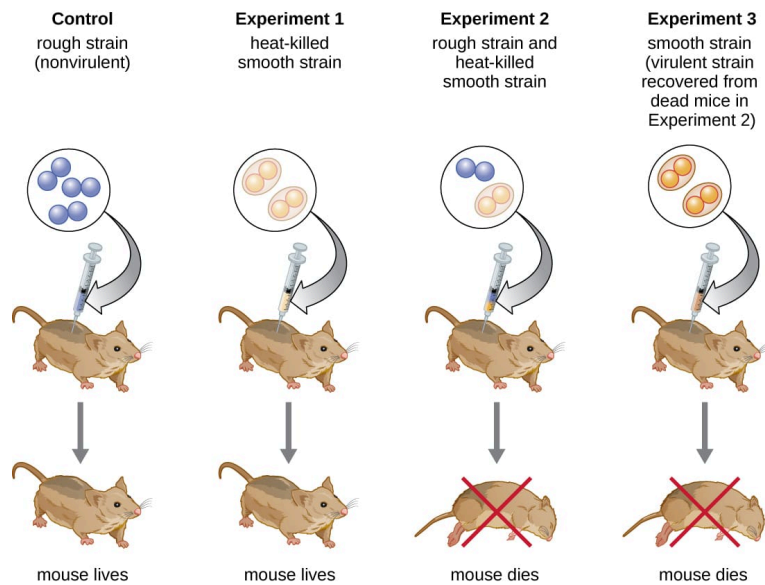


Figure 11.2.6: In his famous series of experiments, Griffith used two strains of *S. pneumoniae*. The S strain is pathogenic and causes death. Mice injected with the nonpathogenic R strain or the heat-killed S strain survive. However, a combination of the heat-killed S strain and the live R strain causes the mice to die. The S strain recovered from the dead mouse showed that something had passed from the heat-killed S strain to the R strain, transforming the R strain into an S strain in the process.

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In 1944, **Oswald Avery**, **Colin MacLeod**, and **Maclyn McCarty** were interested in exploring Griffith's transforming principle further. They isolated the S strain from infected dead mice, heat-killed it, and inactivated various components of the S extract, conducting a systematic elimination study (Figure 11.2.7). They used enzymes that specifically degraded proteins, RNA, and DNA and mixed the S extract with each of these individual enzymes. Then, they tested each extract/enzyme combination's resulting ability to transform the R strain, as observed by the diffuse growth of the S strain in culture media and confirmed visually by growth on plates. They found that when DNA was degraded, the resulting mixture was no longer able to transform the R strain bacteria, whereas no other enzymatic treatment was able to prevent transformation. This led them to conclude that DNA was the transforming principle. Despite their results, many scientists did not accept their conclusion, instead believing that there were protein contaminants within their extracts.

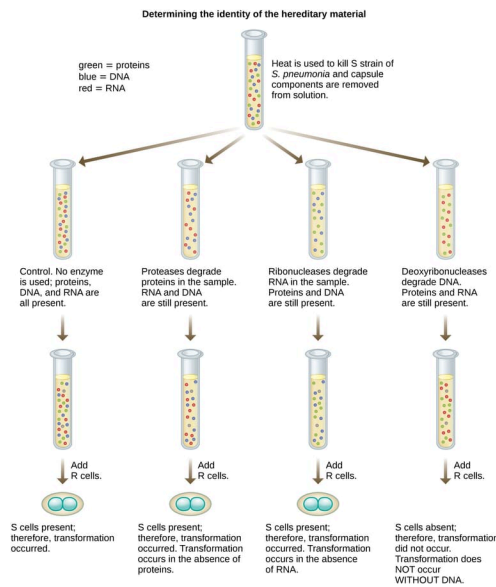


Figure 11.2.7: Oswald Avery, Colin MacLeod, and Maclyn McCarty followed up on Griffith's experiment and experimentally determined that the transforming principle was DNA.

Hershey and Chase's Proof of DNA as Genetic Material

Alfred Hershey and **Martha Chase** performed their own experiments in 1952 and were able to provide confirmatory evidence that DNA, not protein, was the genetic material (Figure 11.2.8).⁴ Hershey and Chase were studying a bacteriophage, a virus that infects bacteria. Viruses typically have a simple structure: a protein coat, called the capsid, and a nucleic acid core that contains the genetic material, either DNA or RNA (see [Viruses](#)). The particular bacteriophage they were studying was the T2 bacteriophage, which infects *E. coli* cells. As we now know today, T2 attaches to the surface of the bacterial cell and then it injects its nucleic acids inside the cell. The phage DNA makes multiple copies of itself using the host machinery, and eventually the host cell bursts, releasing a large number of bacteriophages.

Hershey and Chase labeled the protein coat in one batch of phage using radioactive sulfur, ³⁵S, because sulfur is found in the amino acids methionine and cysteine but not in nucleic acids. They labeled the DNA in another batch using radioactive phosphorus, ³²P, because phosphorus is found in DNA and RNA but not typically in protein.

Each batch of phage was allowed to infect the cells separately. After infection, Hershey and Chase put each phage bacterial suspension in a blender, which detached the phage coats from the host cell, and spun down the resulting suspension in a centrifuge. The heavier bacterial cells settled down and formed a pellet, whereas the lighter phage particles stayed in the supernatant. In the tube with the protein labeled, the radioactivity remained only in the supernatant. In the tube with the DNA labeled, the radioactivity was detected only in the bacterial cells. Hershey and Chase concluded that it was the phage DNA that was injected into the cell that carried the information to produce more phage particles, thus proving that DNA, not proteins, was the source of the genetic material. As a result of their work, the scientific community more broadly accepted DNA as the molecule responsible for heredity.

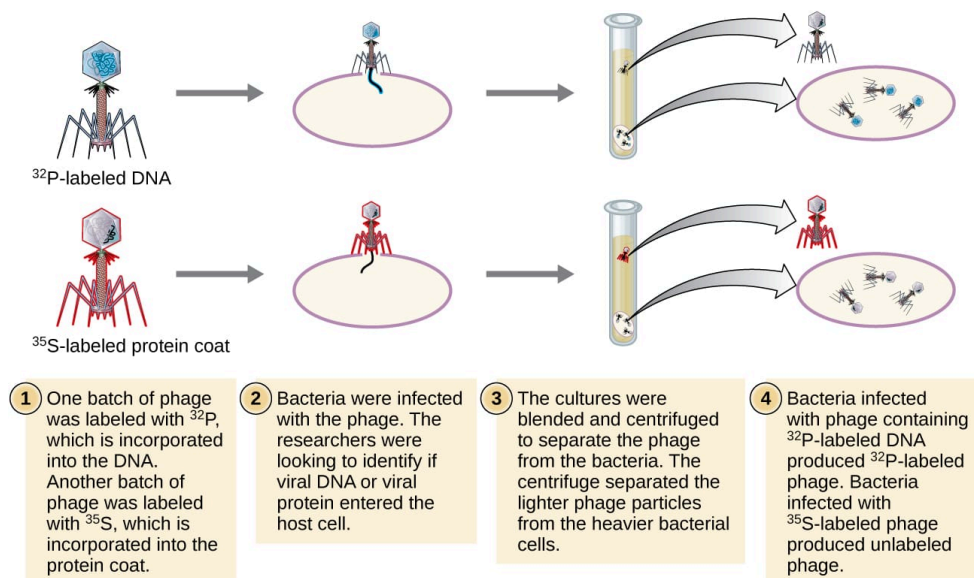


Figure 11.2.8: Martha Chase and Alfred Hershey conducted an experiment separately labeling the DNA and proteins of the T2 bacteriophage to determine which component was the genetic material responsible for the production of new phage particles.

By the time Hershey and Chase published their experiment in the early 1950s, microbiologists and other scientists had been researching heredity for over 80 years. Building on one another's research during that time culminated in the general agreement that DNA was the genetic material responsible for heredity (Figure 11.2.9). This knowledge set the stage for the age of molecular biology to come and the significant advancements in biotechnology and systems biology that we are experiencing today.

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Link to Learning

To learn more about the experiments involved in the history of genetics and the discovery of DNA as the genetic material of cells, visit this [website](#) from the DNA Learning Center.

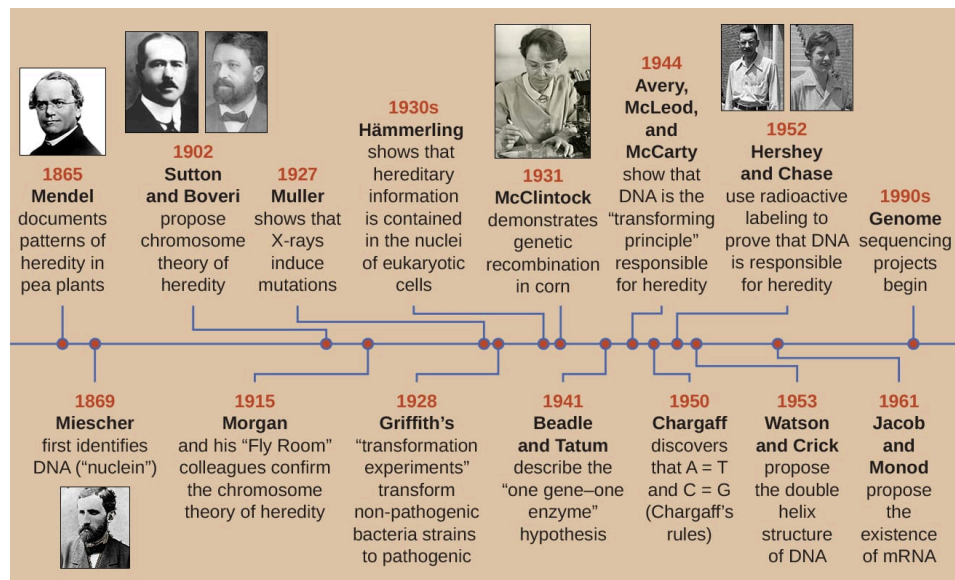


Figure 11.2.9: A timeline of key events leading up to the identification of DNA as the molecule responsible for heredity.

Key Concepts and Summary

- In 1928, Frederick Griffith showed that dead encapsulated bacteria could pass genetic information to live nonencapsulated bacteria and transform them into harmful strains. In 1944, Oswald Avery, Colin McLeod, and Maclyn McCarty identified the compound as DNA.
- The nature of DNA as the molecule that stores genetic information was unequivocally demonstrated in the experiment of Alfred Hershey and Martha Chase published in 1952. Labeled DNA from bacterial viruses entered and infected bacterial cells, giving rise to more viral particles. The labeled protein coats did not participate in the transmission of genetic information.

Footnotes

- 1 J.G. Mendel. "Versuche über Pflanzenhybriden." *Verhandlungen des naturforschenden Vereines in Brünn, Bd. Abhandlungen* 4 (1865):3–7. (For English translation, see <http://www.mendelweb.org/Mendel.plain.html>)
- 2 G.W. Beadle, E.L. Tatum. "Genetic Control of Biochemical Reactions in *Neurospora*." *Proceedings of the National Academy of Sciences* 27 no. 11 (1941):499–506.
- 3 F. Griffith. "The Significance of Pneumococcal Types." *Journal of Hygiene* 27 no. 2 (1928):8–159.
- 4 A.D. Hershey, M. Chase. "Independent Functions of Viral Protein and Nucleic Acid in Growth of Bacteriophage." *Journal of General Physiology* 36 no. 1 (1952):39–56.

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11.3: 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 11.3.1). 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.

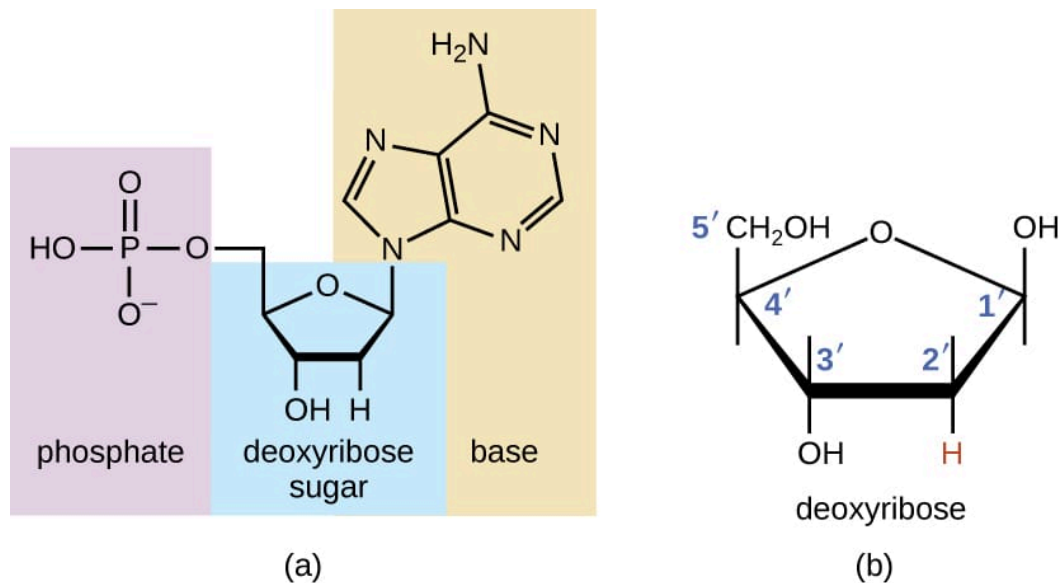


Figure 11.3.1: (a) Each deoxyribonucleotide is made up of a sugar called deoxyribose, a phosphate group, and a nitrogenous base—in this case, adenine. (b) The five carbons within deoxyribose are designated as 1', 2', 3', 4', and 5'.

The deoxyribonucleotide is named according to the nitrogenous bases (Figure 11.3.2). 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.

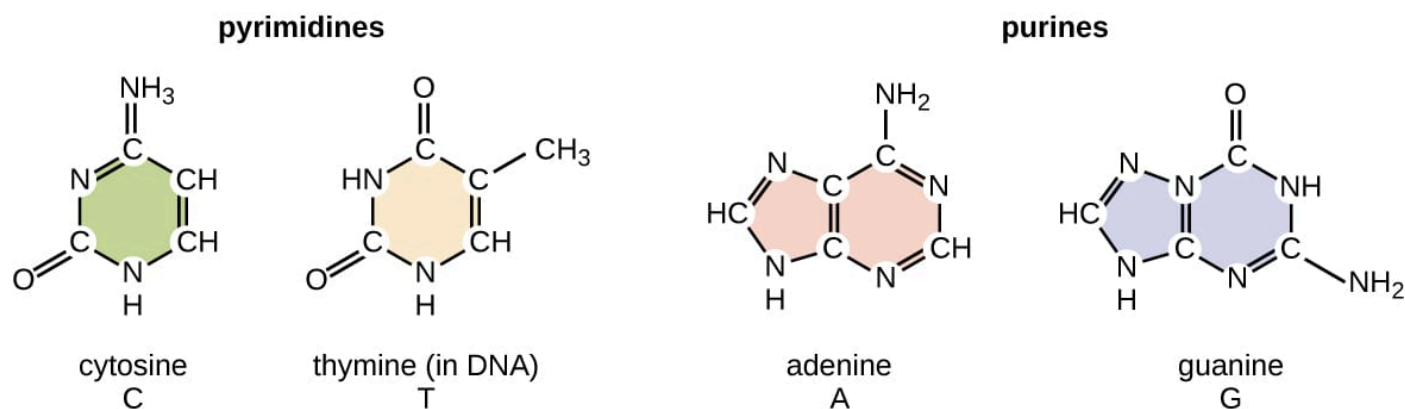


Figure 11.3.2: Nitrogenous bases within DNA are categorized into the two-ringed purines adenine and guanine and the single-ringed pyrimidines cytosine and thymine. Thymine is unique to DNA.

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 11.3.3). 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.

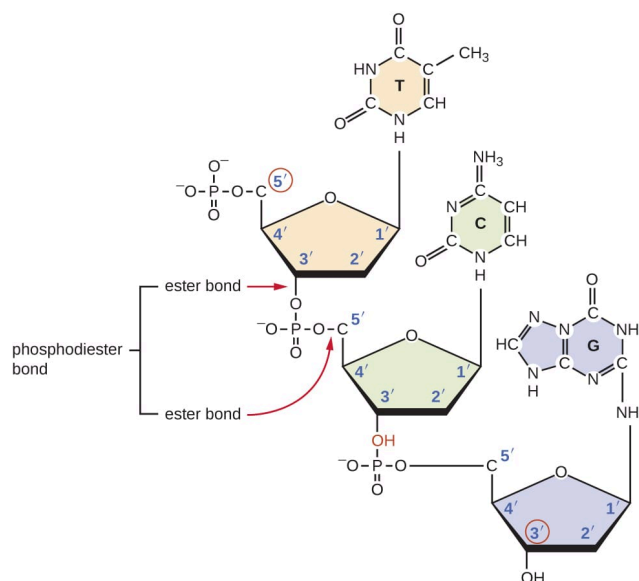


Figure 11.3.3: Phosphodiester bonds form between the phosphate group attached to the 5' carbon of one nucleotide and the hydroxyl group of the 3' carbon in the next nucleotide, bringing about polymerization of nucleotides in to nucleic acid strands. Note the 5' and 3' ends of this nucleic acid strand.

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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 11.3.4). 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.

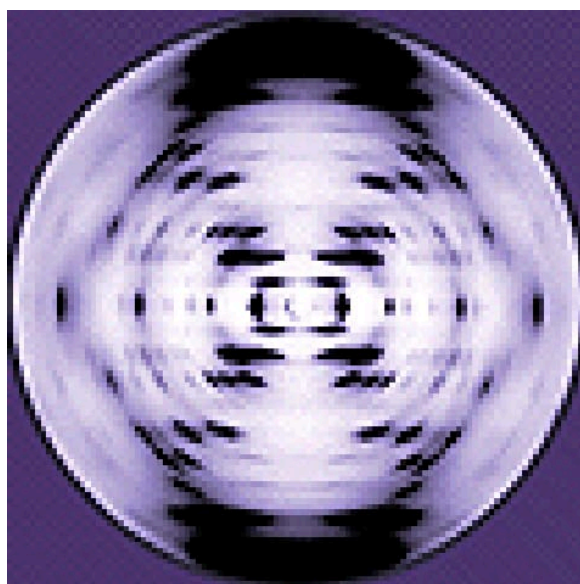


Figure 11.3.4: The X-ray diffraction pattern of DNA shows its helical nature. (credit: National Institutes of Health)

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 diffraction images of DNA fibers to piece together the purine-pyrimidine pairing of the double helical DNA molecule (Figure 11.3.5). 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 11.3.5: 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.

Video: The DNA Double Helix Discovery - HHMI Biointeractive Video

The discovery of the structure of the DNA double helix was one of the most important of the 20th century. In this educational video, explore Watson and Crick's quest to understand DNA's structure, and Rosalind Franklin's key insights via x-ray crystallography.

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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 11.3.6). 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 11.3.6). 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.

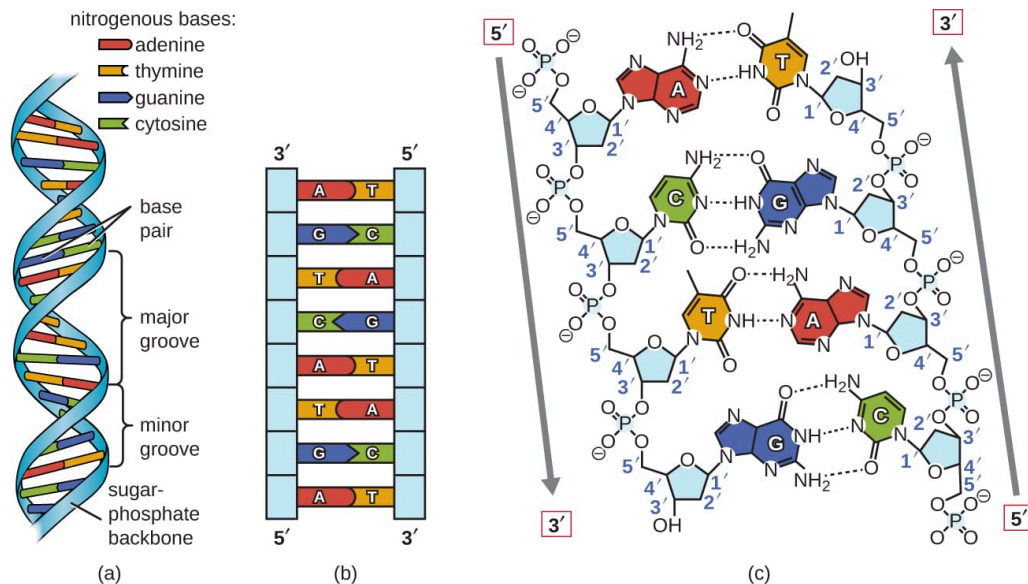


Figure 11.3.6: Watson and Crick proposed the double helix model for DNA. (a) The sugar-phosphate backbones are on the outside of the double helix and purines and pyrimidines form the “rungs” of the DNA helix ladder. (b) The two DNA strands are antiparallel to each other. (c) The direction of each strand 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 11.3.7). 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.

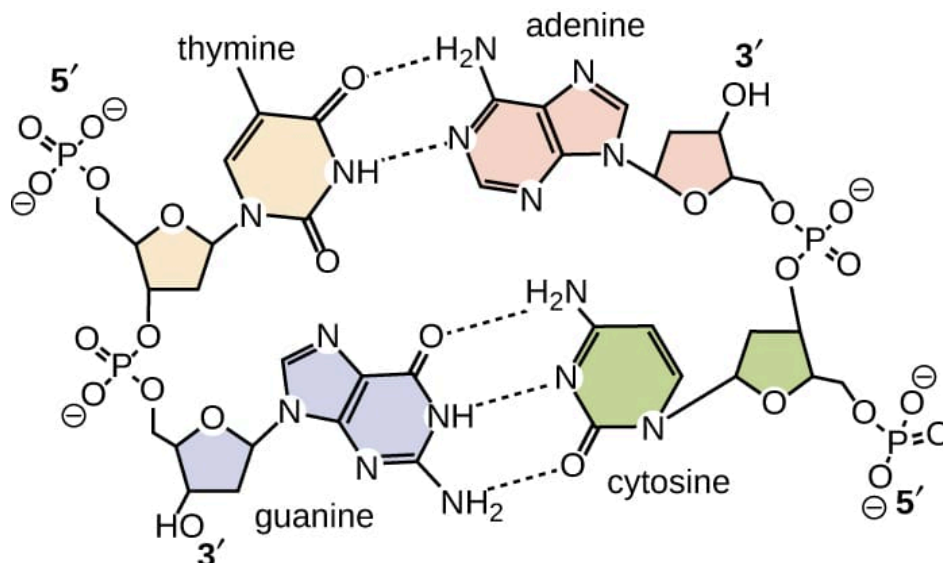


Figure 11.3.7: Hydrogen bonds form between complementary nitrogenous bases on the interior of DNA.

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 11.3.8). 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.

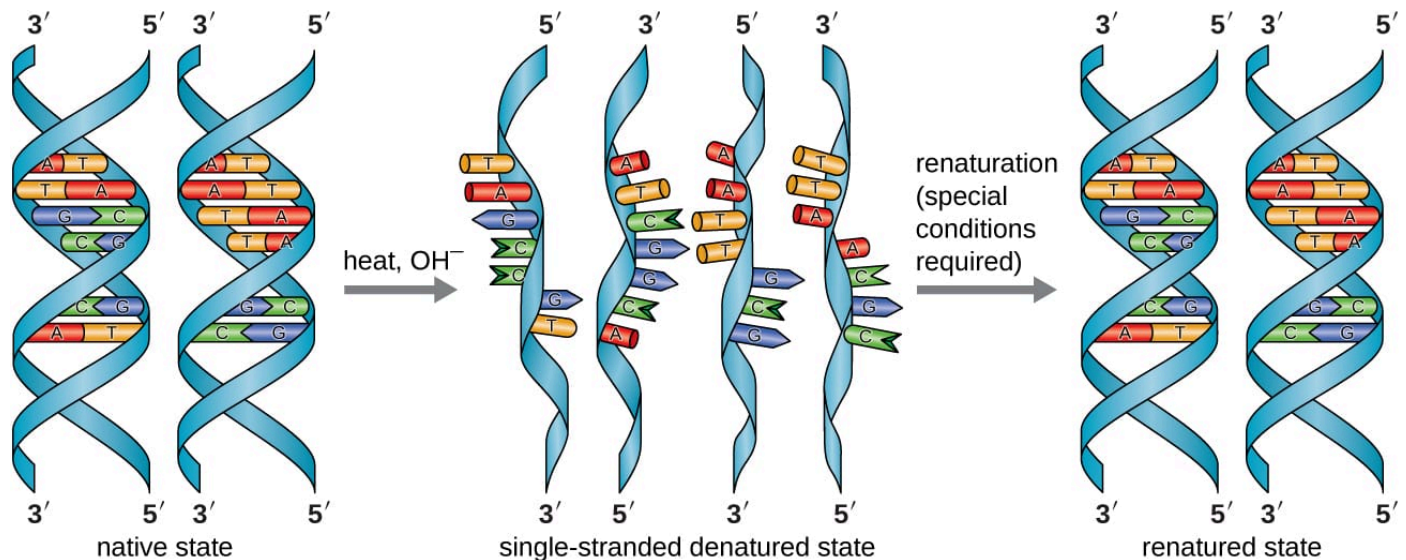


Figure 11.3.8: In the laboratory, the double helix can be denatured to single-stranded DNA through exposure to heat or chemicals, and then renatured through cooling or removal of chemical denaturants to allow the DNA strands to reanneal. (credit: modification of work by Hernández-Lemus E, Nicasio-Collazo LA, Castañeda-Priego R)

Link to Learning

View an [animation](#) on DNA structure from the DNA Learning Center to learn more.

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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.

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 11.3.9).

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 science⁹ were met with widespread condemnation.

Perhaps girls should be supported more from a young age in the areas of science and math (Figure 11.3.9). 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.



(a)



(b)

Figure 11.3.9: (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)

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 N. Kresge et al. "Chargaff's Rules: The Work of Erwin Chargaff." *Journal of Biological Chemistry* 280 (2005):e21.
- 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 J.D. Watson, F.H.C. Crick. "A Structure for Deoxyribose Nucleic Acid." *Nature* 171 no. 4356 (1953):737-738.
- 4 M.H.F. Wilkins et al. "Molecular Structure of Deoxypentose Nucleic Acids." *Nature* 171 no. 4356 (1953):738-740.
- 5 R. Franklin, R.G. Gosling. "Molecular Configuration in Sodium Thymonucleate." *Nature* 171 no. 4356 (1953):740-741.
- 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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11.4: 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.

Video: DNA vs RNA

Why is RNA just as cool as DNA? Join the Amoeba Sisters as they compare and contrast RNA with DNA and learn why DNA should be sharing the limelight!

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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.

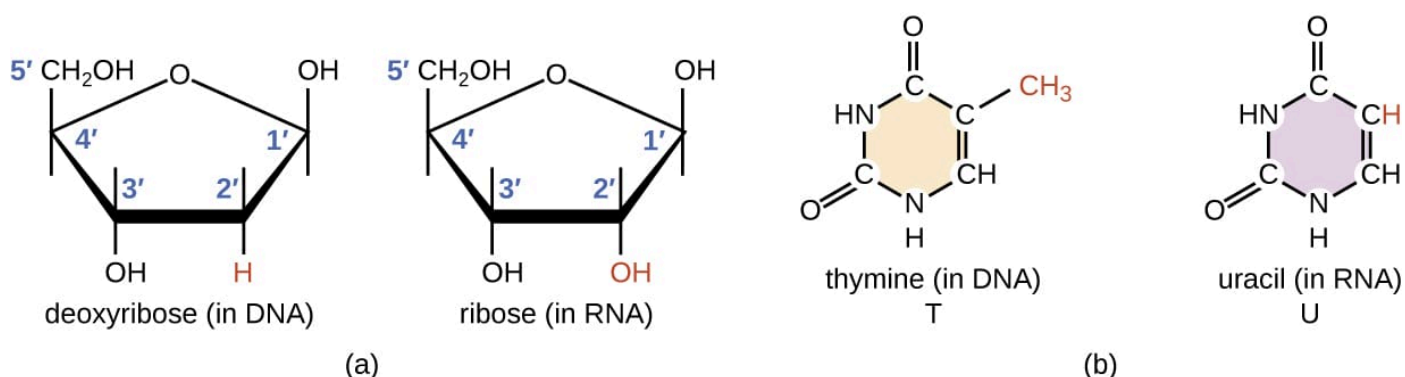


Figure 11.4.1: (a) Ribonucleotides contain the pentose sugar ribose instead of the deoxyribose found in deoxyribonucleotides. (b) RNA contains the pyrimidine uracil in place of thymine found in DNA.

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 11.4.1 and Figure 11.4.2).

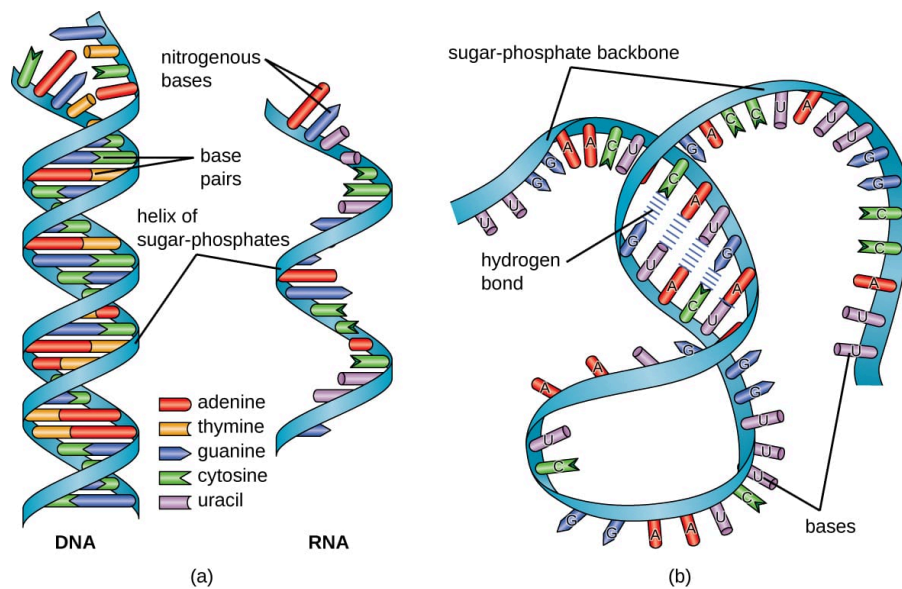


Figure 11.4.2: (a) DNA is typically double stranded, whereas RNA is typically single stranded. (b) Although it is single stranded, RNA can fold upon itself, with the folds stabilized by short areas of complementary base pairing within the molecule, forming a three-dimensional structure.

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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 11.4.3) 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.

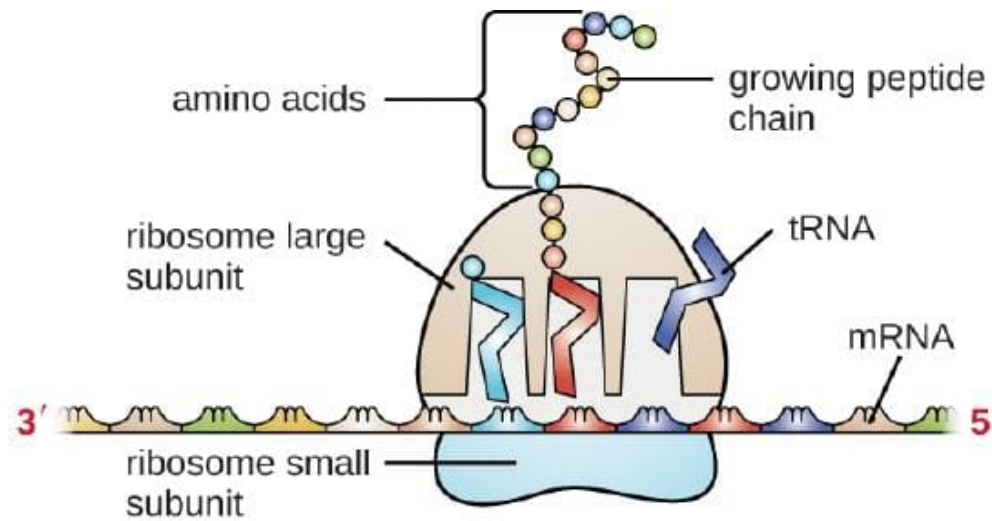


Figure 11.4.3: A generalized illustration of how mRNA and tRNA are used in protein synthesis within a cell.

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 11.4.4). Any mutations in the tRNA or rRNA can result in global problems for the cell because both are necessary for proper protein synthesis (Table 11.4.1).

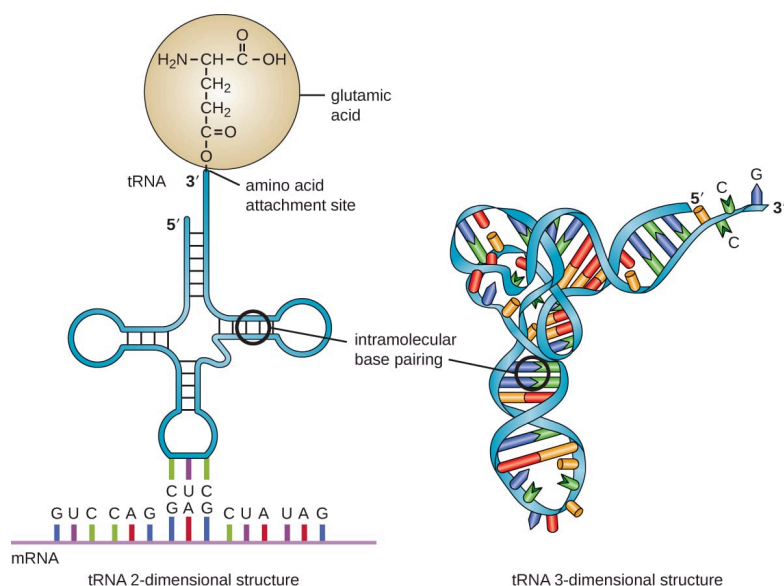


Figure 11.4.4: A tRNA molecule is a single-stranded molecule that exhibits significant intracellular base pairing, giving it its characteristic three-dimensional shape.

Table 11.4.1: Structure and Function of RNA

	mRNA	rRNA	tRNA
Structure	Short, unstable, single-stranded RNA corresponding 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

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](#).

Query 11.4.1

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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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11.5: 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 11.5.1).

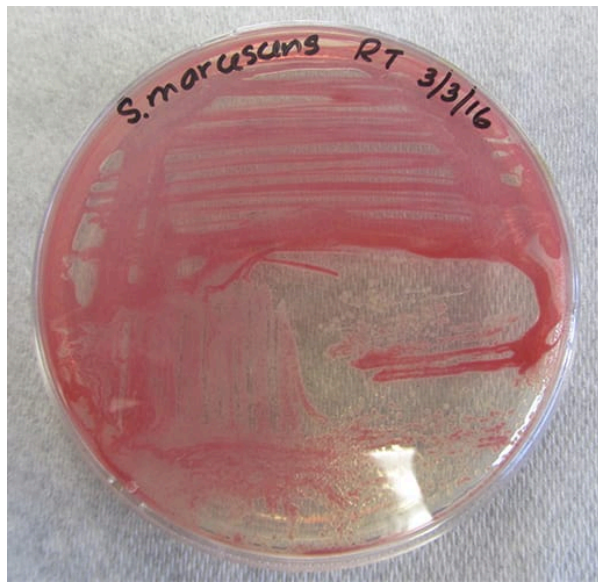
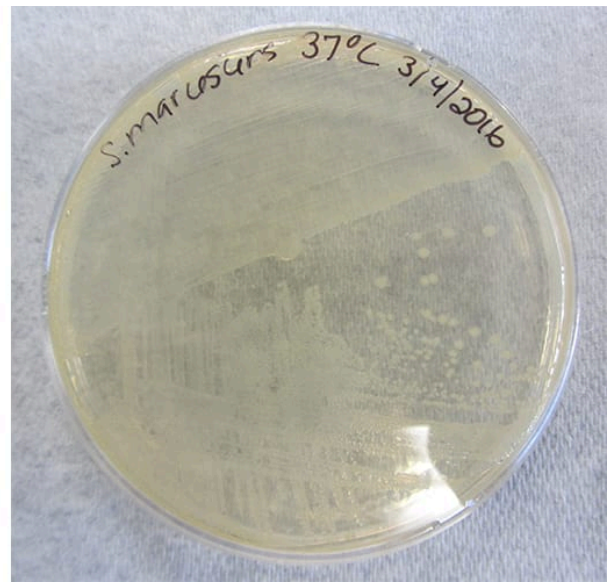


Figure 11.5.1: Both plates contain strains of *Serratia marcescens* that have the gene for red pigment. However, this gene is expressed at 28 °C (left) but not at 37 °C (right). (credit: modification of work by Ann Auman)

Query 11.5.1

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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 pairs¹ 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

Query 11.5.1

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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 11.5.2).

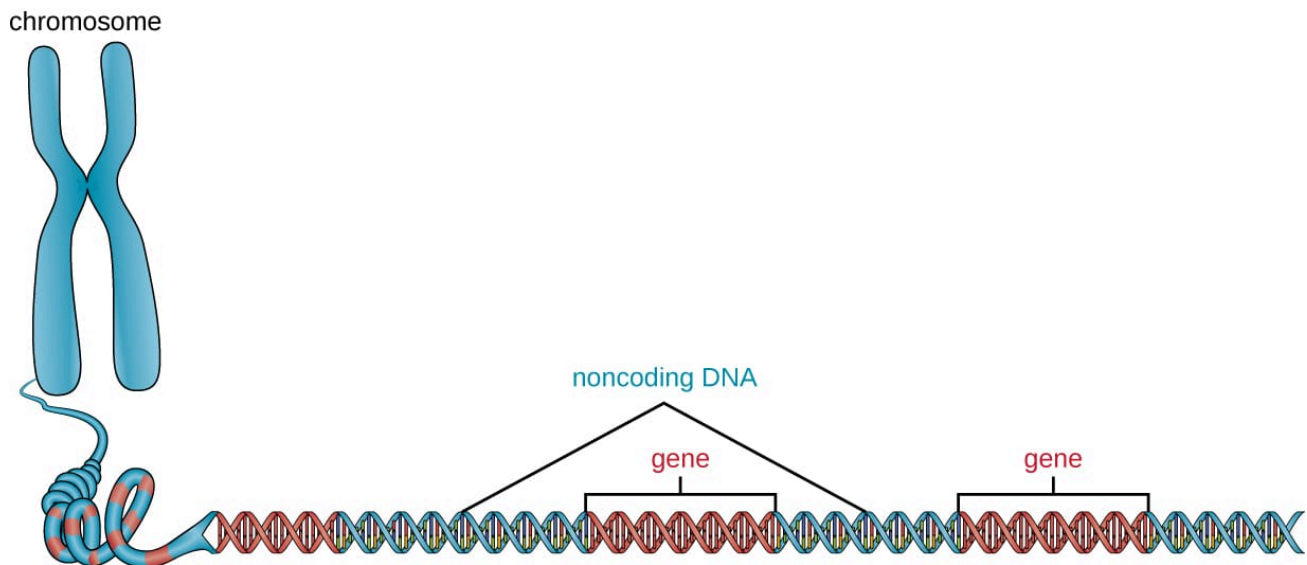


Figure 11.5.2: Chromosomes typically have a significant amount of noncoding DNA, often found in intergenic regions.

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.

Query 11.5.1

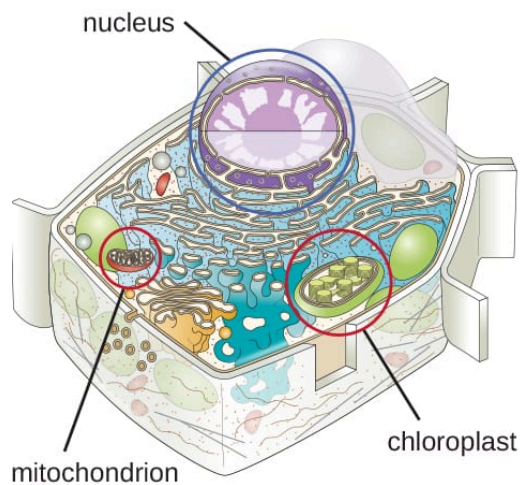
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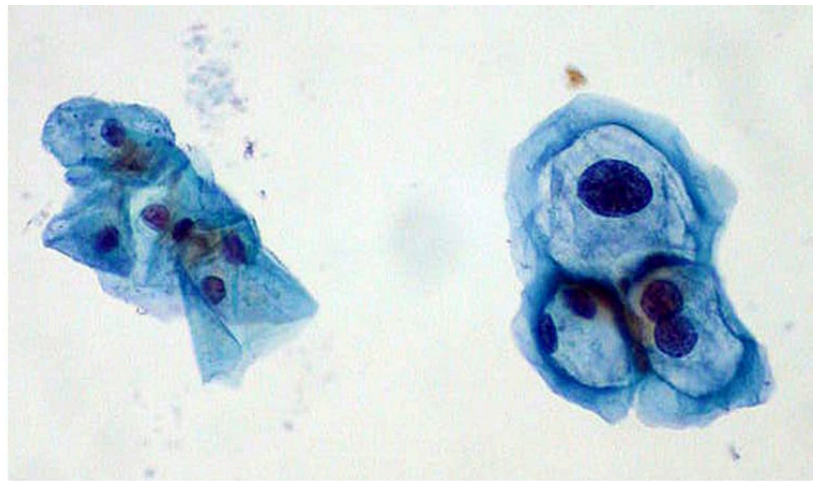


Extrachromosomal DNA

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 11.5.3). 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.



(a)



(b)

Figure 11.5.3: 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](#).

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 11.5.4). 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.

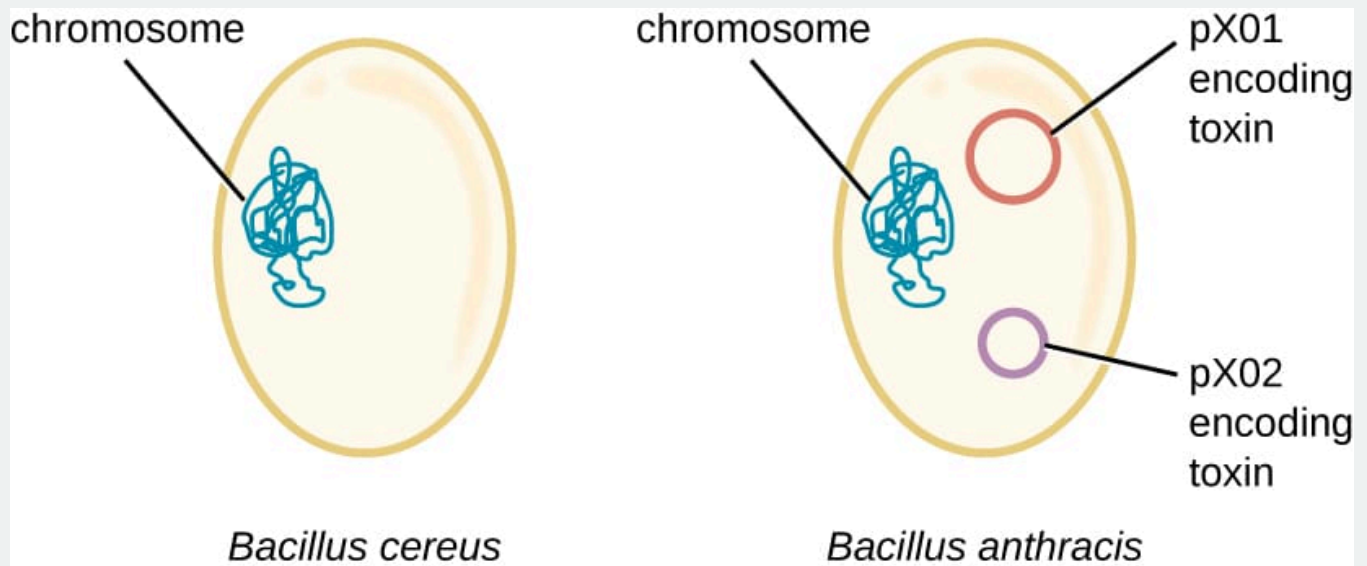


Figure 11.5.4: Genome sequencing of *Bacillus anthracis* and its close relative *B. cereus* reveals that the pathogenicity of *B. anthracis* is due to the maintenance of two plasmids, pX01 and pX02, which encode virulence factors.

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](#).

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 11.5.5). 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.

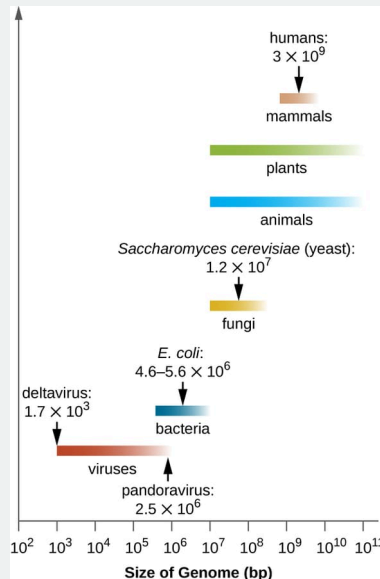


Figure 11.5.5: There is great variability as well as overlap among the genome sizes of various groups of organisms and viruses.

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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11.E: Biochemistry of the Genome (Exercises)

11.1: 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

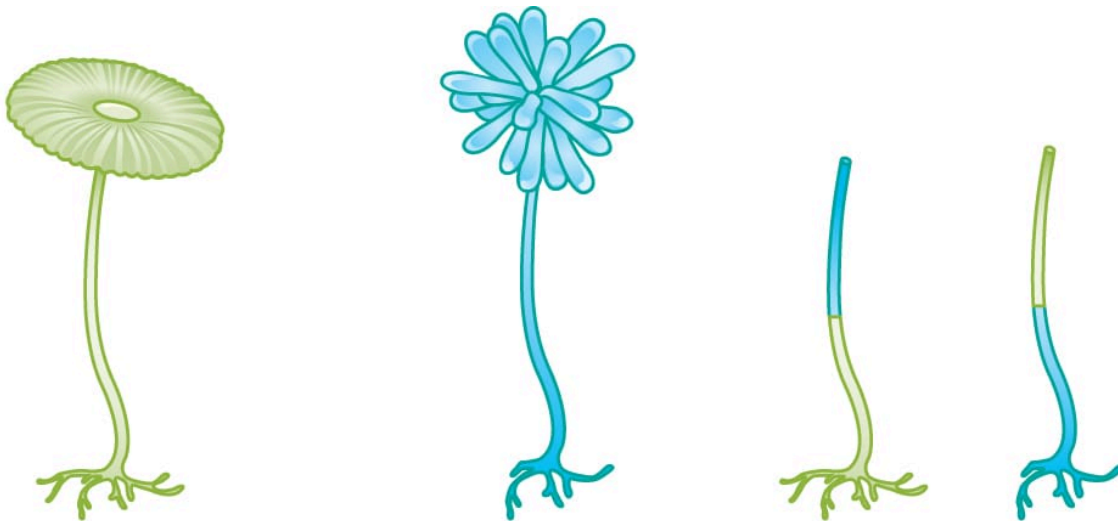
Why do bacteria and viruses make good model systems for various genetic studies?

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?



Acetabularia mediterranea

Acetabularia crenulata

graft A

graft B

Why are Hershey and Chase credited with identifying DNA as the carrier of heredity even though DNA had been discovered many years before?

11.2: 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?

11.3: 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?

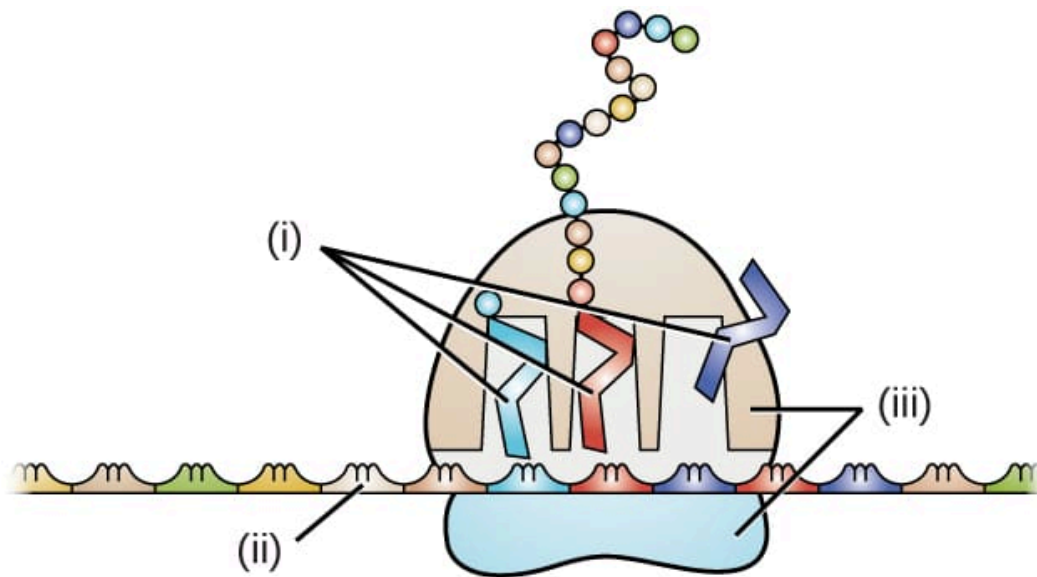
How do complementary base pairs contribute to intramolecular base pairing within an RNA molecule?

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?

11.4: 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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12: CENTRAL DOGMA

In 1954, French scientist and future Nobel laureate Jacques Monod (1910–1976) famously said, “What is true in *E. coli* is true in the elephant,” suggesting that the biochemistry of life was maintained throughout evolution and is shared in all forms of known life. Since Monod’s famous statement, we have learned a great deal about the mechanisms of gene regulation, expression, and replication in living cells. All cells use DNA for information storage, share the same genetic code, and use similar mechanisms to replicate and express it. Although many aspects of genetics are universally shared, variations do exist among contemporary genetic systems. We now know that within the shared overall theme of the genetic mechanism, there are significant differences among the three domains of life: Eukarya, Archaea, and Bacteria. Additionally, viruses, cellular parasites but not themselves living cells, show dramatic variation in their genetic material and the replication and gene expression processes. Some of these differences have allowed us to engineer clinical tools such as antibiotics and antiviral drugs that specifically inhibit the reproduction of pathogens yet are harmless to their hosts.

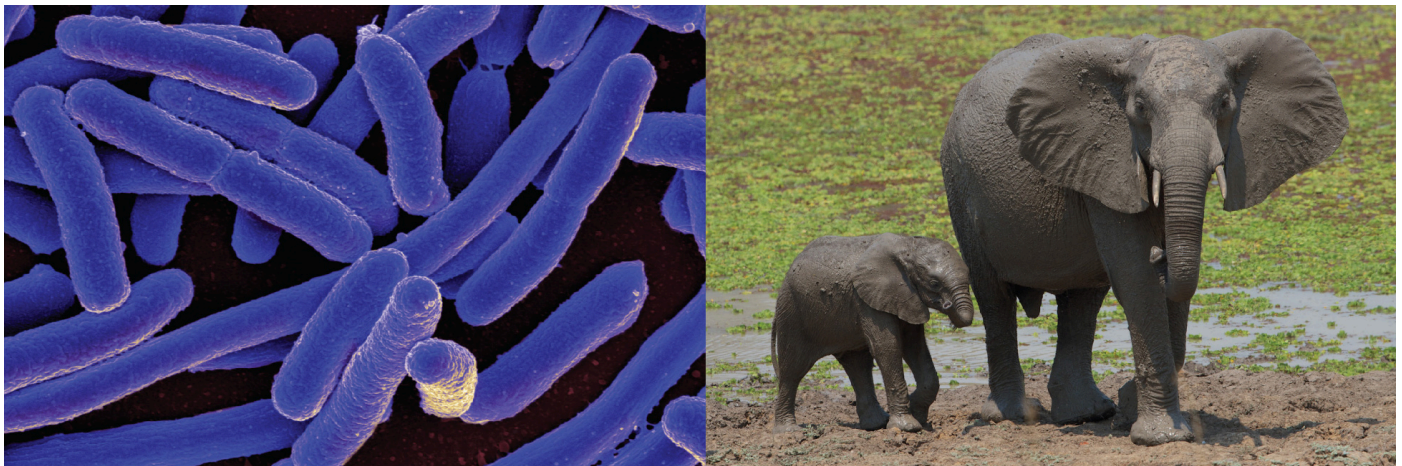


Figure 12.1: *Escherichia coli* (left) may not appear to have much in common with an elephant (right), but the genetic blueprints for these vastly different organisms are both encoded in DNA. (credit left: modification of work by NIAID; credit right: modification of work by Tom Lubbock)

12.1: What Are Genes?

12.2: DNA Replication in Bacteria

12.3: DNA Replication in Eukaryotes

12.4: RNA Transcription

12.5: Protein Synthesis (Translation)

12.6: The Mechanism of Protein Synthesis

12.E: Mechanisms of Microbial Genetics (Exercises)

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12.1: 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

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 12.1.1). 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 12.1.1: 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 12.1.2).

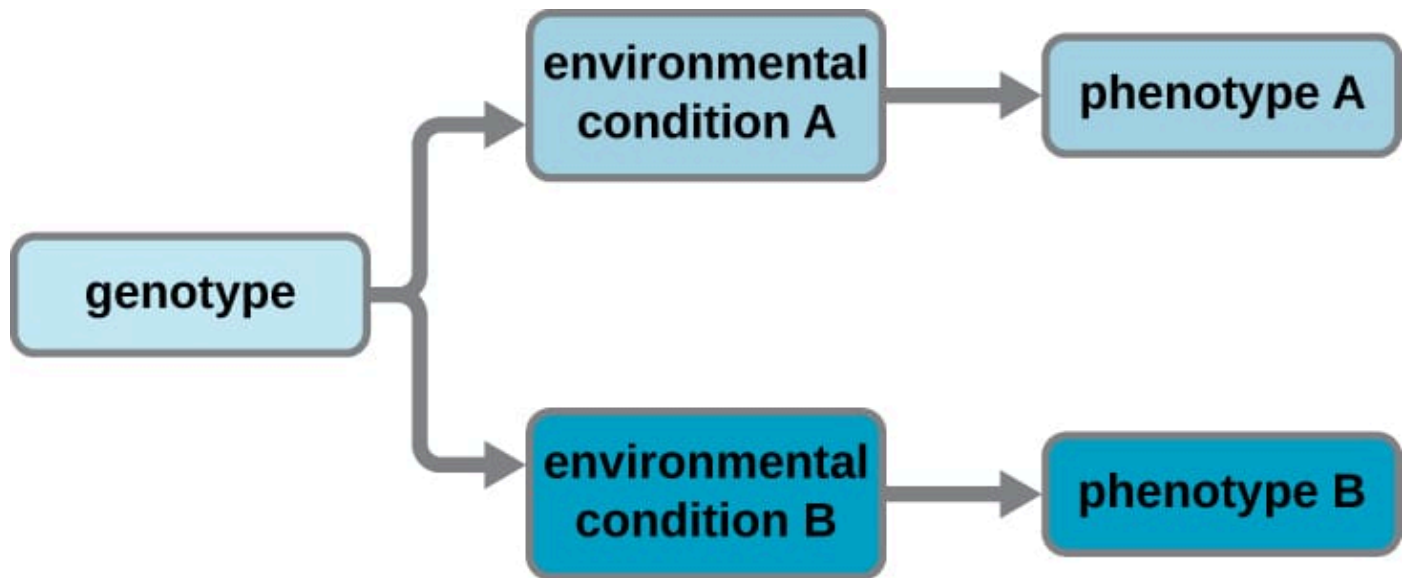


Figure 12.1.2: Phenotype is determined by the specific genes within a genotype that are expressed under specific conditions. Although multiple cells may have the same genotype, they may exhibit a wide range of phenotypes resulting from differences in patterns of gene expression in response to different environmental conditions.

DNA, Chromosomes, Genes, and Traits

Explore DNA structure/function, chromosomes, genes, and traits and how this relates to heredity! Video can replace old DNA structure & function video and in addition covers foundational concepts of heredity.

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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.

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Interactive Element

Case Study Preview: “The Flesh-Eating Flub”

Mark thought a scratch from gardening was no big deal - until his leg turned gray and started blistering with a smell you don't forget. Behind the rapidly spreading infection: *Staphylococcus aureus*, a notorious culprit in necrotizing fasciitis. Despite antibiotics, Mark's condition spiraled, requiring emergency surgery, a ventilator, and eventually skin grafts.

In this case, you'll explore how bacterial toxins and antibiotic resistance complicate treatment, why early wound care matters (seriously - just wash the cut!), and how the CDC tracks virulent strains through genomic analysis.

Moral of the story? Never underestimate a bramble and a bad bacterium.

[Chapter 11 Case Study - The Flesh-Eating Flub](#)

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. 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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12.2: DNA Replication in Bacteria

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

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 12.2.1.

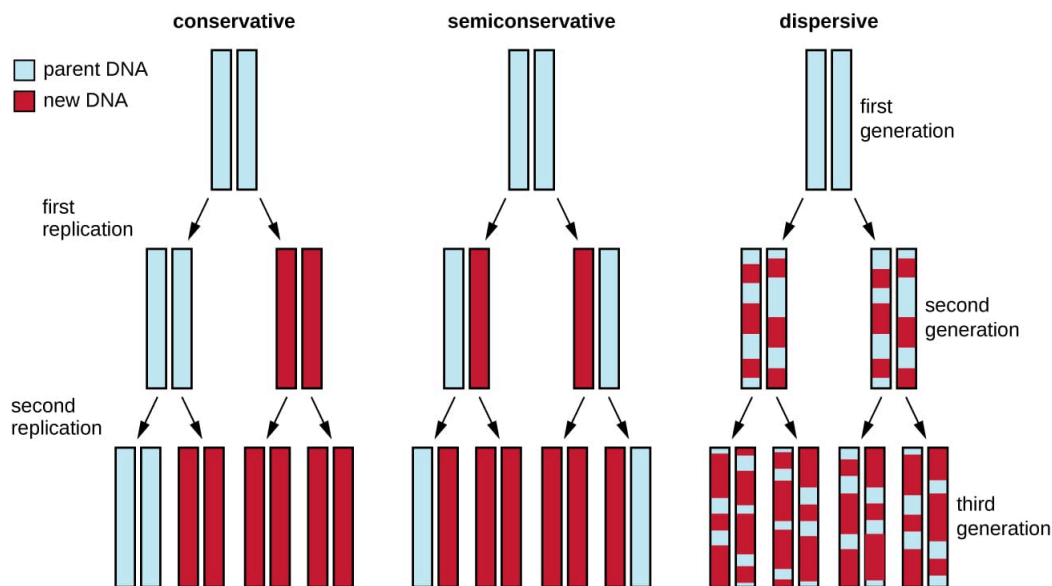


Figure 12.2.1: There were three models suggested for DNA replication. In the conservative model, parental DNA strands (blue) remained associated in one DNA molecule while new daughter strands (red) remained associated in newly formed DNA molecules. In the semiconservative model, parental strands separated 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 12.2.2). 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.

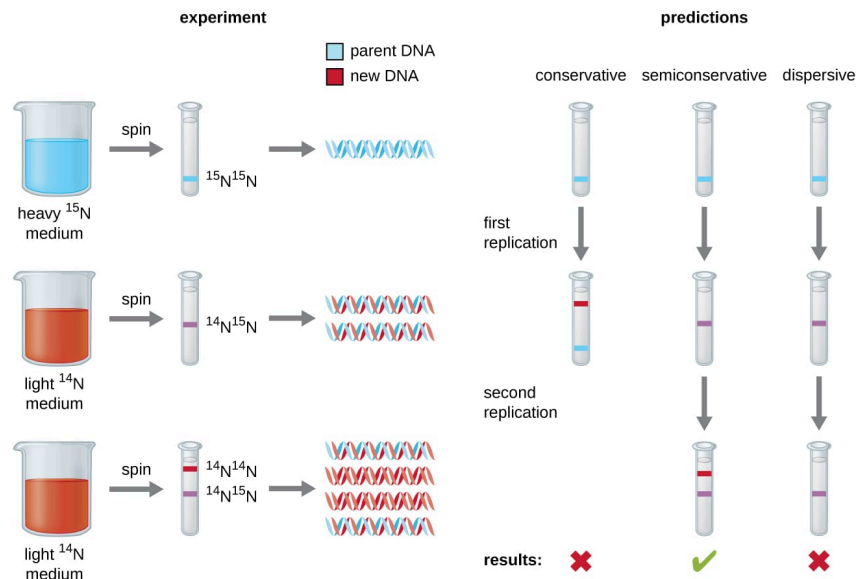


Figure 12.2.2: Meselson and Stahl experimented with *E. coli* grown first in heavy nitrogen (^{15}N) then in ^{14}N . DNA grown in ^{15}N (blue band) was heavier than DNA grown in ^{14}N (red band), and sedimented to a lower level on ultracentrifugation. After one round of replication, the DNA sedimented 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.

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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 12.2.1). 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 12.2.3). 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.

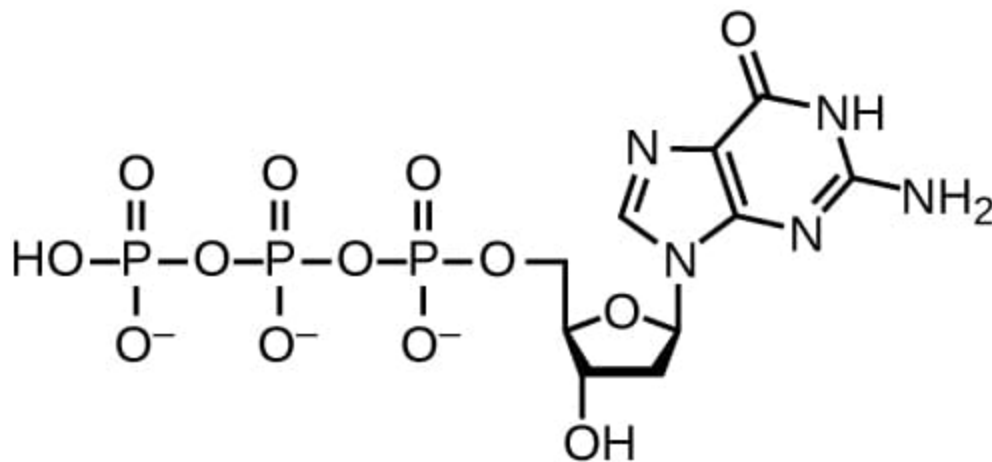


Figure 12.2.3: This structure shows the guanosine triphosphate deoxyribonucleotide that is incorporated into a growing DNA strand by cleaving the two end phosphate groups from the molecule and transferring the energy to the sugar phosphate bond. The other three nucleotides form analogous structures.

Video: DNA Replication

Explore the steps of DNA replication, the enzymes involved, and the difference between the leading and lagging strand!

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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 12.2.1).

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 12.2.4). 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.

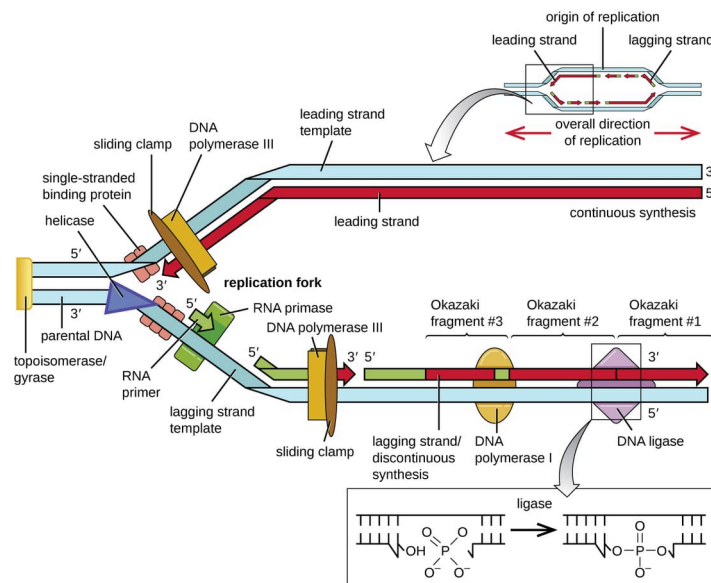


Figure 12.2.4: At the origin of replication, topoisomerase II relaxes the supercoiled chromosome. Two replication forks are formed by the opening of the double-stranded DNA at the origin, and helicase separates the DNA strands, which are coated by single-stranded binding proteins to keep the strands 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.

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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 12.2.1: 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

Enzyme or Factor	Function
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

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.

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12.3: DNA Replication in Eukaryotes

Learning Objectives

- Identify the differences between DNA replication in bacteria and eukaryotes
- Describe the role of telomerase in replicating the ends of linear chromosomes
- Explain the process of rolling circle replication

DNA Replication in Eukaryotes

Eukaryotic genomes are much more complex and larger than prokaryotic genomes and are typically composed of multiple linear chromosomes (Table 12.3.2). 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 12.3.5); 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.

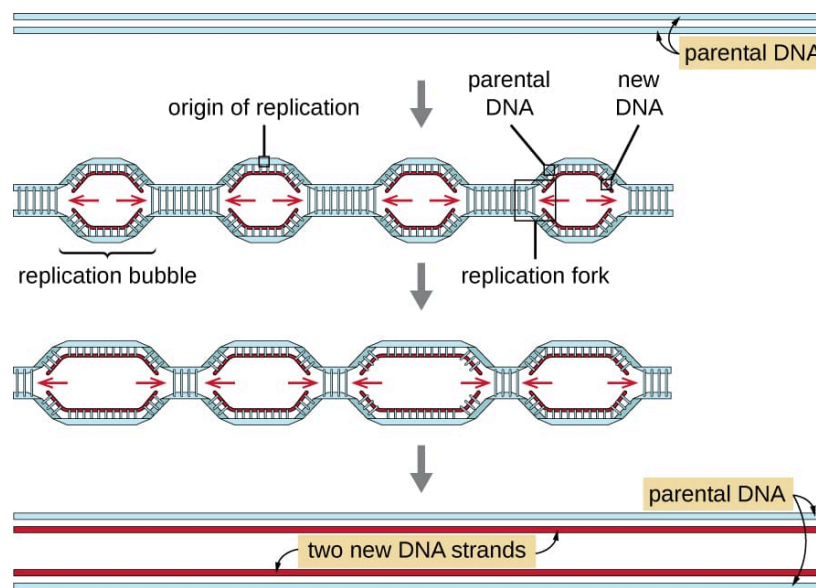


Figure 12.3.5: Eukaryotic chromosomes are typically linear, and each contains multiple origins of replication.

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 12.3.6) 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.

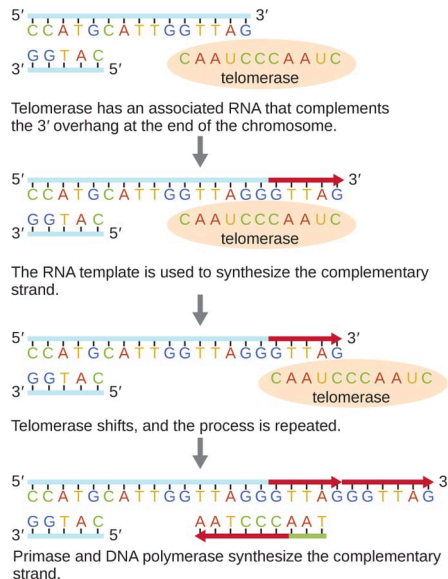


Figure 12.3.6: In eukaryotes, the ends of the linear chromosomes are maintained by the action of the telomerase enzyme.

Table 12.3.2: 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

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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 12.3.7). 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.

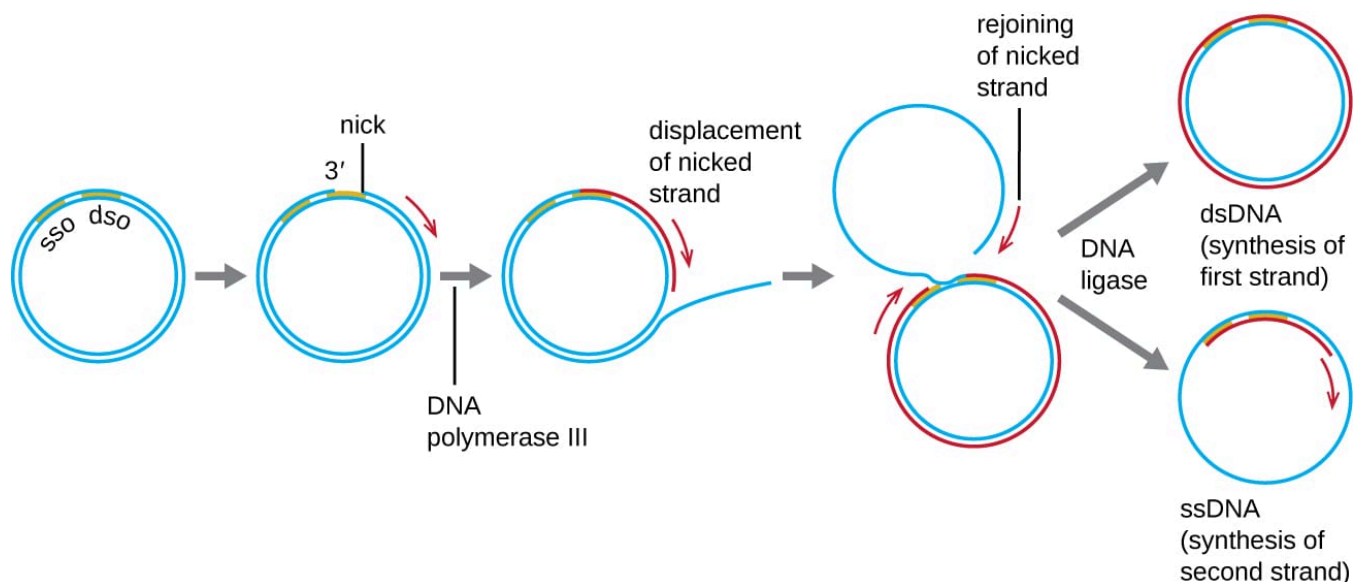


Figure 12.3.7: The process of rolling circle replication results in the synthesis of a single new copy of the circular DNA molecule, as shown here.

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Key Concepts and Summary

- 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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12.4: RNA Transcription

Learning Objectives

- Explain how RNA is synthesized using DNA as a template
- Distinguish between transcription in prokaryotes and eukaryotes
- Interpret the DNA template strand to predict the sequence of the corresponding mRNA

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 12.4.1).

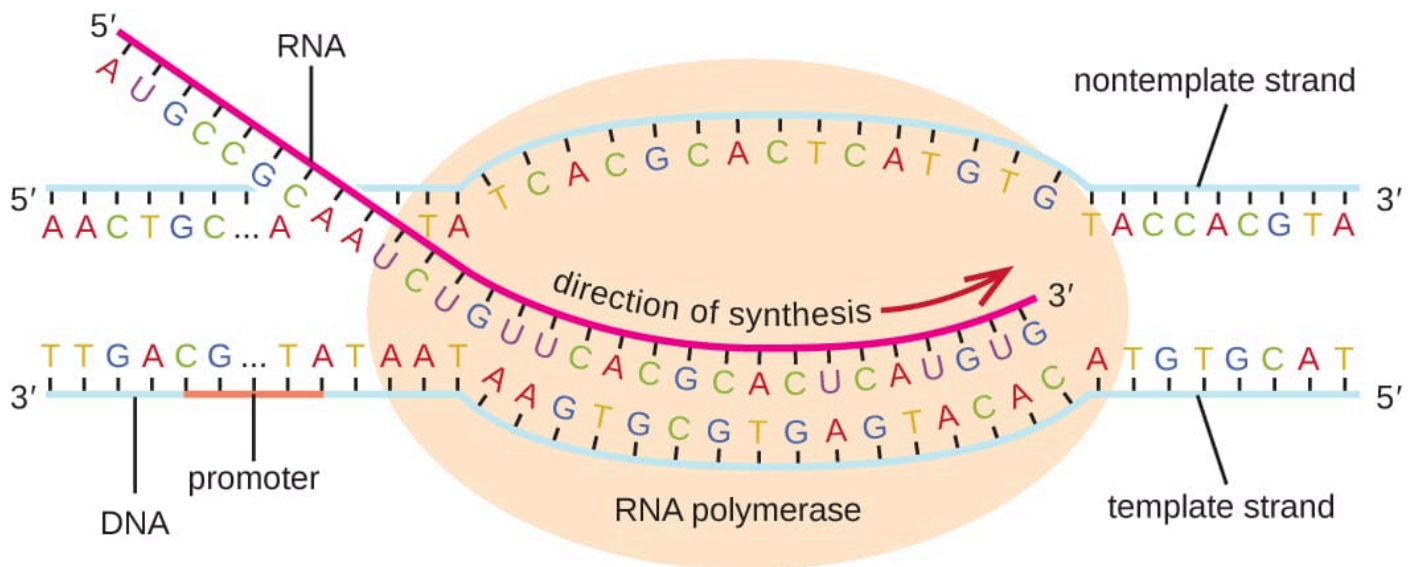


Figure 12.4.1: During elongation, the bacterial RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to 3' direction, and unwinds and rewinds the DNA as it is read.

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.

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Transcription in Eukaryotes

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few significant differences (see Table 12.4.1). 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 expressed) and intervening sequences called **introns** (*int*-ron denotes their *intervening* 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 12.4.1: 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.

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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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12.5: Protein Synthesis (Translation)

Learning Objectives

- Describe the genetic code and explain why it is considered almost universal
- Interpret a codon chart to determine the amino acid sequence encoded by an mRNA strand
- Describe the components of the translation machinery

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.

Video: Protein Synthesis

Explore the steps of transcription and translation in protein synthesis! This video explains several reasons why proteins are so important before explaining the roles of mRNA, rRNA, and tRNA in the steps of protein synthesis! Expand details for contents and resources.

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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 12.5.1). 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 codons** or **nonsense codons**. 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.

		second letter					
		U	C	A	G		
first letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA stop UAG stop	UGU } Cys UGC } UGA stop UGG Trp	U C A G	third letter
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G	
	A	AUU } AUC } Ile AUA } AUG Met	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA } Arg AGG }	U C A G	
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G	

Figure 12.5.1: 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)

Video: How to Read a Codon Chart

Join the Amoeba Sisters as they show how to work through a rectangular and circular codon chart. Also, find our video companion under the topic "codon charts" on our handout page <https://www.amoebasisters.com/handouts>

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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 12.5.2). 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.

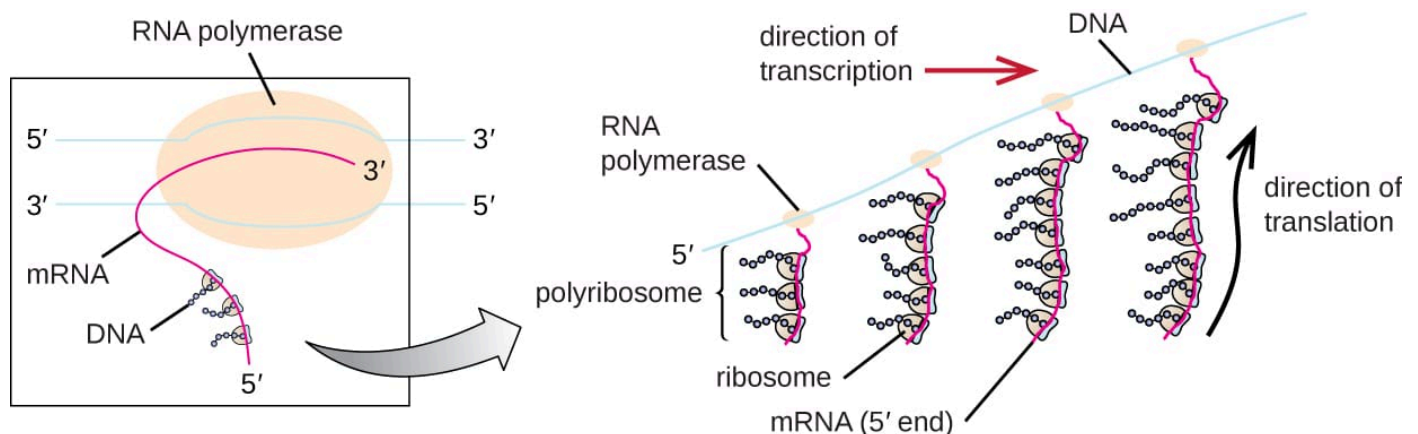


Figure 12.5.2: In prokaryotes, multiple RNA polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

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 12.5.3). 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 anticodon at 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.

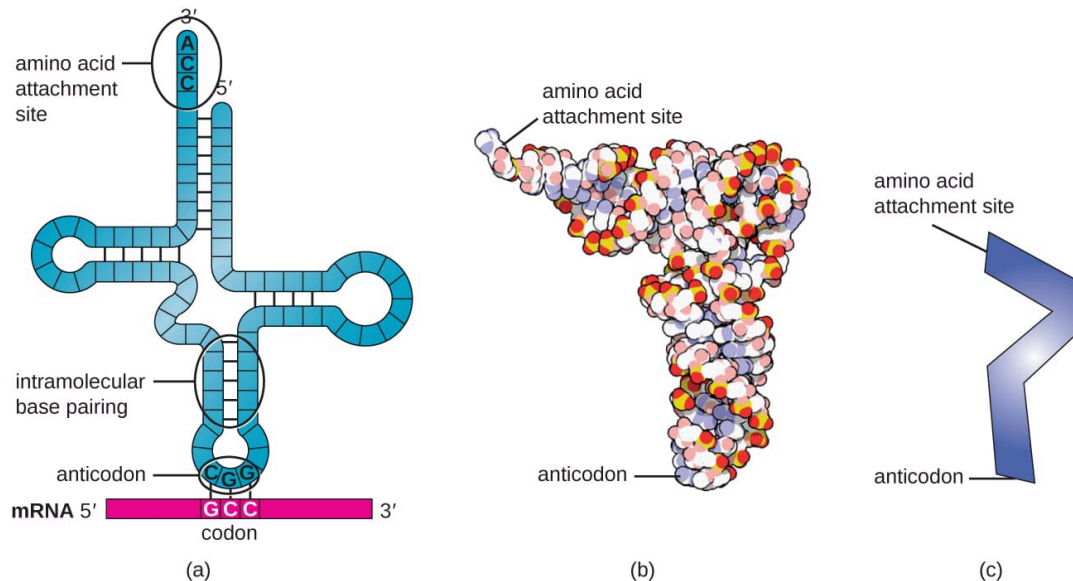


Figure 12.5.3: (a) After folding caused by intramolecular base pairing, a tRNA molecule has one end that contains the anticodon, which interacts with the mRNA codon, and the CCA amino acid binding end. (b) A space-filling model is helpful for visualizing the three-dimensional shape of tRNA. (c) Simplified models are useful when drawing complex processes such as protein synthesis.

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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.

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12.6: The Mechanism of Protein Synthesis

Learning Objectives

- Outline the key steps of protein synthesis including initiation, elongation and termination
- Explain the roles of the ribosomal A, P and E sites during the elongation phase of translation
- Compare translation in eukaryotes and prokaryotes
- Interpret diagrams or models that represent the molecular events of translation

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.

Video: Translation

NDSU Virtual Cell Animations project animation "Translation".

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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 12.6.4). 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-tRNA_i
- 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-tRNA_i, mRNA, and the 40S subunit.

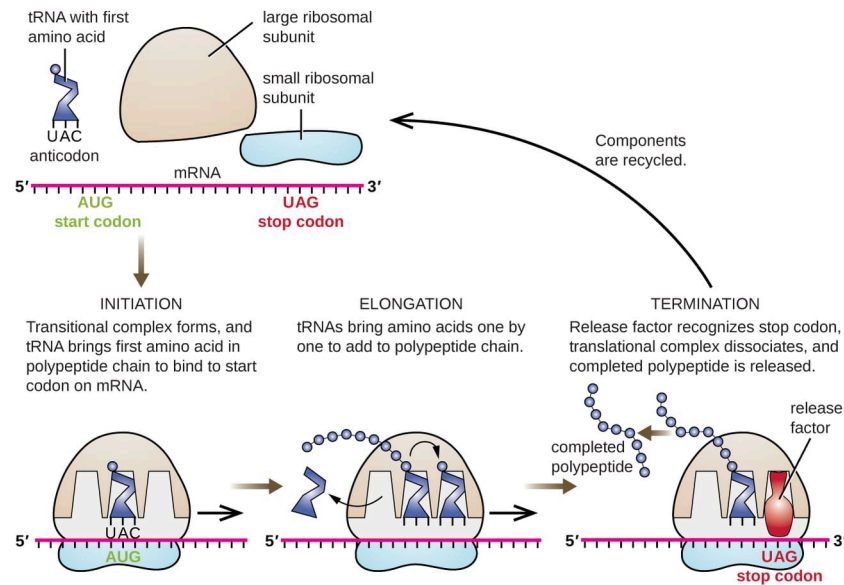


Figure 12.6.4: Translation in bacteria begins with the formation of the initiation complex, which includes the small ribosomal subunit, the mRNA, the initiator tRNA carrying N-formyl-methionine, and initiation factors. Then the 50S subunit binds, forming an intact ribosome.

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 initiation complex.

In summary, there are several key features that distinguish prokaryotic gene expression from that seen in eukaryotes. These are illustrated in Figure 12.6.5 and listed in Figure 12.6.6.

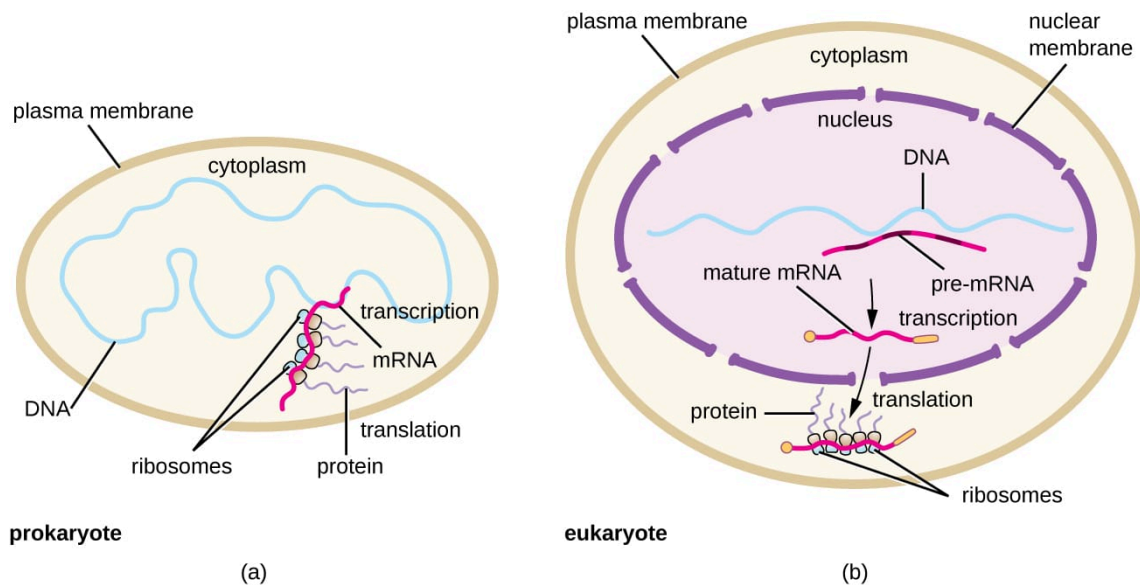


Figure 12.6.5: (a) In prokaryotes, the processes of transcription and translation occur simultaneously in the cytoplasm, allowing for a rapid cellular response to an environmental cue. (b) In eukaryotes, transcription is localized to the nucleus and translation is localized to the cytoplasm, separating these processes and necessitating RNA processing for stability.

Comparison of Translation in Bacteria Versus Eukaryotes		
Property	Bacteria	Eukaryotes
Ribosomes	70S <ul style="list-style-type: none"> 30S (small subunit) with 16S rRNA subunit 50S (large subunit) with 5S and 23S rRNA subunits 	80S <ul style="list-style-type: none"> 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 12.6.6: Comparison of translation in bacteria versus eukaryotes

Query 12.6.1

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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.

Key Concepts and Summary

- **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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12.E: Mechanisms of Microbial Genetics (Exercises)

12.1: 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?

12.2: 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'-TCCGAAGCTG-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?

12.3: 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.

12.4: 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?

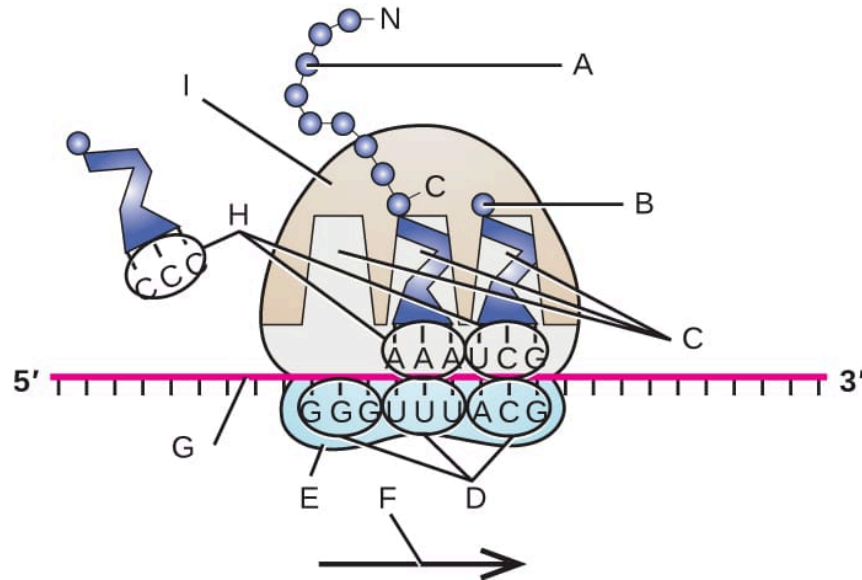
What is meant by the genetic code being nearly universal?

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?

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13: HORIZONTAL GENE TRANSFER AND OPERONS

In the intricate world of genetics, the flow and regulation of genetic information are crucial for the survival and adaptation of all living organisms. While DNA replication and gene expression are highly conserved processes, the introduction of variation through mutations and the exchange of genetic material via horizontal gene transfer have been key drivers of evolution and diversity. Mutations, whether spontaneous or induced, can alter the genetic code, sometimes resulting in advantageous traits that allow organisms to thrive in changing environments.

Beyond mutations, horizontal gene transfer enables the exchange of genetic material between organisms, even across species boundaries, profoundly influencing microbial evolution and the spread of traits like antibiotic resistance. Additionally, cells use precise mechanisms, such as operons in prokaryotes, to regulate gene expression in response to environmental cues, ensuring efficient use of resources and rapid adaptation to changing conditions.

In this chapter, we will delve into the processes of mutation, horizontal gene transfer, and the regulation of gene expression, exploring how these genetic mechanisms underpin both the stability and the adaptability of life.

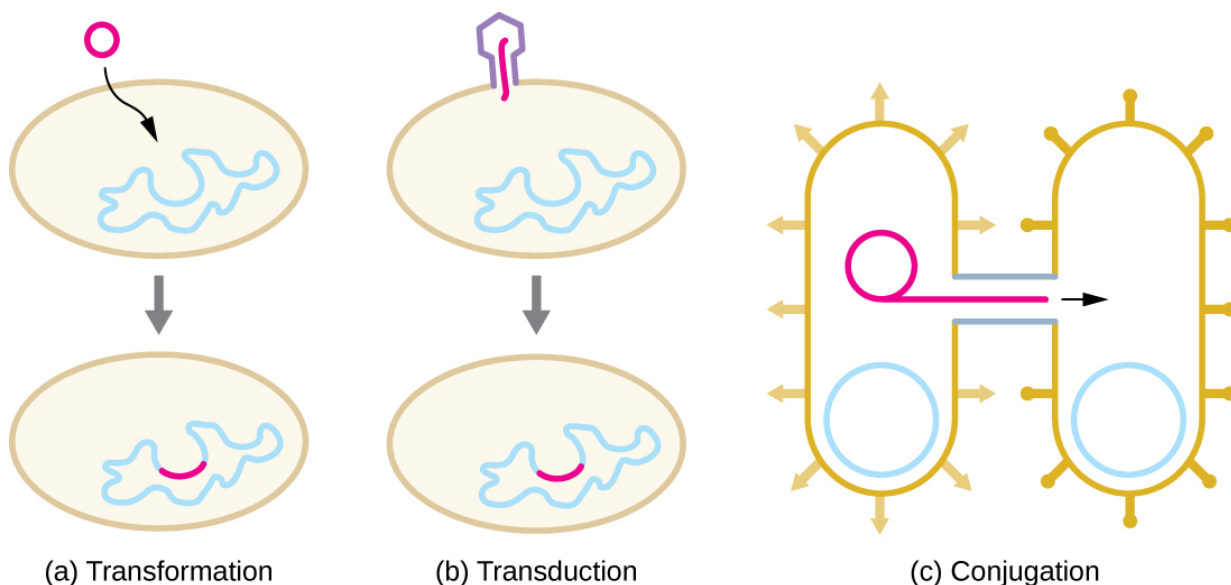


Figure 13.1:

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.

13.1: Mutations

13.2: DNA Repair

13.3: Horizontal Gene Transfer- Transformation and Transduction

13.4: Horizontal Gene Transfer- Conjugation and Transposition

13.5: Gene Regulation - Repressible Operon

13.6: Gene Regulation - Inducible Operon

13.E: Mechanisms of Microbial Genetics (Exercises)

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13.1: Mutations

Learning Objectives

- Compare point mutations and frameshift mutations
- Describe the differences between missense, nonsense, and silent mutations
- Explain how different mutagens act
- 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.

Mutations

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>

Join the Amoeba Sisters as they explain gene and chromosome mutations, and explore the significance of these changes.

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**.

Effects of Mutations on Protein Structure and Function

Point mutations may have a wide range of effects on protein function (Figure 13.1.1). 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 13.1.1). 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.

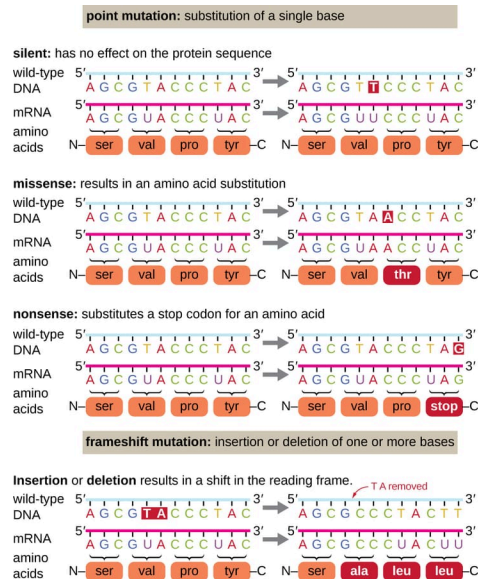


Figure 13.1.1: Mutations can lead to changes in the protein sequence encoded by the DNA.

Query 13.1.1

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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 worldwide² (Figure 13.1.2). Unfortunately, this is also a part of the world where prevention strategies and drugs to treat the infection are the most lacking.

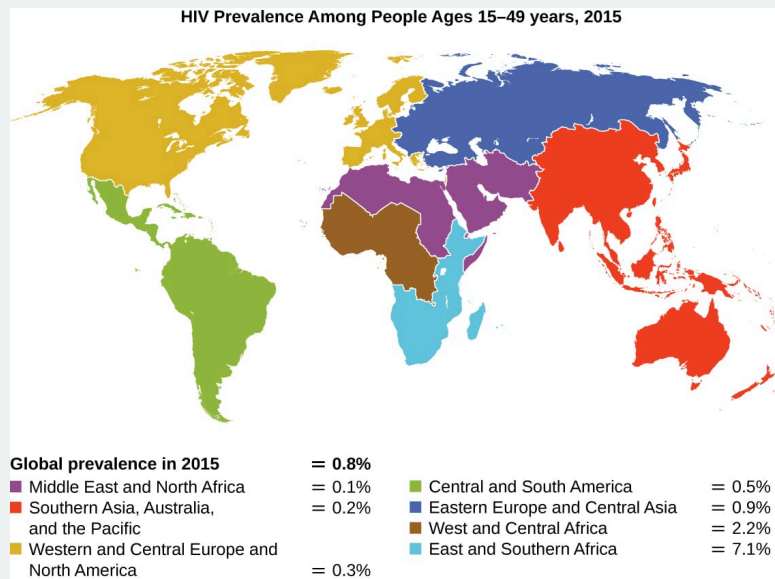


Figure 13.1.2: HIV is highly prevalent in sub-Saharan Africa, but its prevalence is quite low in some other parts of the world.

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 13.1.1). 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 13.1.3). 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 13.1.4). 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.

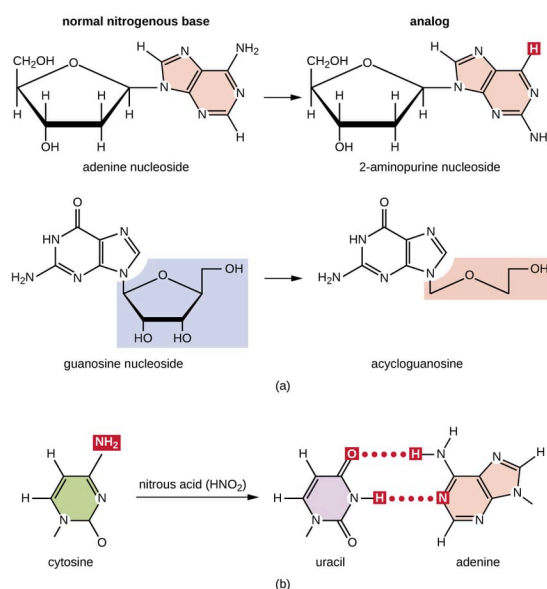


Figure 13.1.3: (a) 2-aminopurine nucleoside (2AP) structurally is a nucleoside analog to adenine nucleoside, whereas 5-bromouracil (5BU) is a nucleoside analog to thymine nucleoside. 2AP base pairs with C, converting an AT base pair to a GC base pair after several rounds of replication. 5BU pairs with 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.

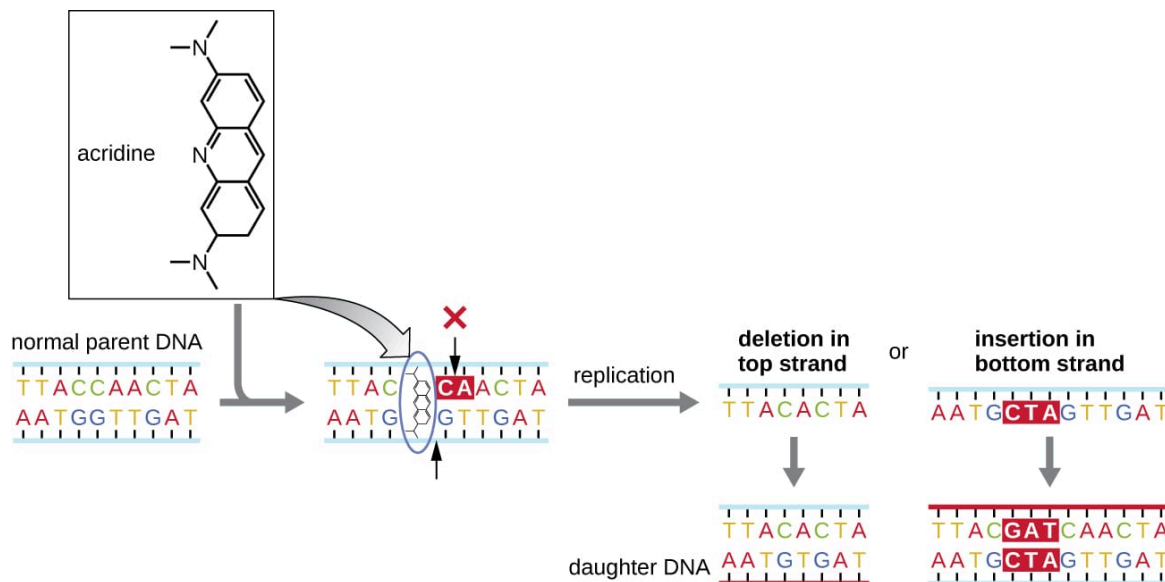


Figure 13.1.4: Intercalating agents, such as acridine, introduce atypical spacing between base pairs, resulting in DNA polymerase introducing either a deletion or an insertion, leading to a potential frameshift mutation.

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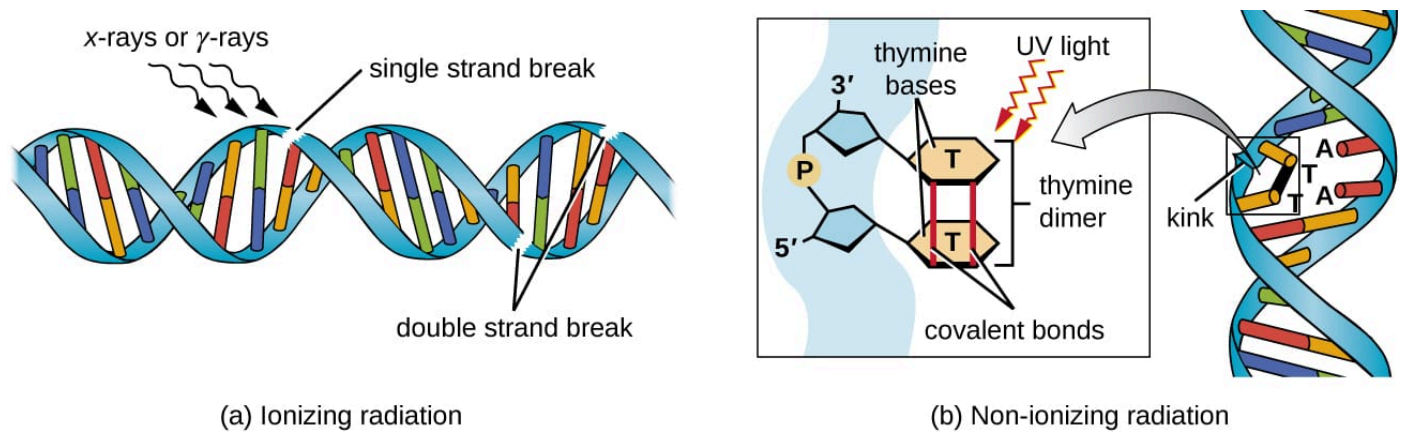
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Radiation

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 13.1.5). 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 13.1.5: (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.

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Table 13.1.1: 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			

Mutagenic Agents	Mode of Action	Effect on DNA	Resulting Type of Mutation
Ultraviolet	Forms pyrimidine (usually thymine) dimers	Causes DNA replication errors	Frameshift or point

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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.

Footnotes

- 1 World Health Organization. "Global Health Observatory (GHO) Data, HIV/AIDS." <http://www.who.int/gho/hiv/en/>. Accessed August 5, 2016.
- 2 World Health Organization. "Global Health Observatory (GHO) Data, HIV/AIDS." <http://www.who.int/gho/hiv/en/>. Accessed August 5, 2016.
- 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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13.2: DNA Repair

Learning Objectives

- Define DNA repair and why it is essential for cell survival
- Compare and contrast dark repair with light repair
- Explain how cells recognize and fix thymine-dimers caused by UV radiation

What happens when you DNA is damaged?

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The DNA in just one of your cells gets damaged tens of thousands of times per day. Because DNA provides the blueprint for the proteins your cells need to function, this damage can cause serious issues—including cancer. Fortunately, your cells have ways of fixing most of these problems, most of the time. Monica Menesini details the processes of DNA damage and repair.

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 13.2.6). 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.

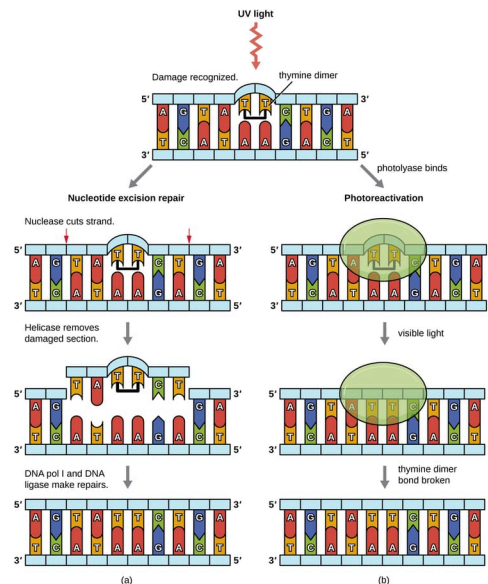


Figure 13.2.6: Bacteria have two mechanisms for repairing thymine dimers. (a) In nucleotide excision repair, an enzyme complex recognizes the distortion in the DNA complex around the thymine dimer and cuts and removes the damaged DNA strand. The correct nucleotides are replaced by DNA pol I and the nucleotide 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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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 13.2.7), 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.

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 13.2.8). 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.

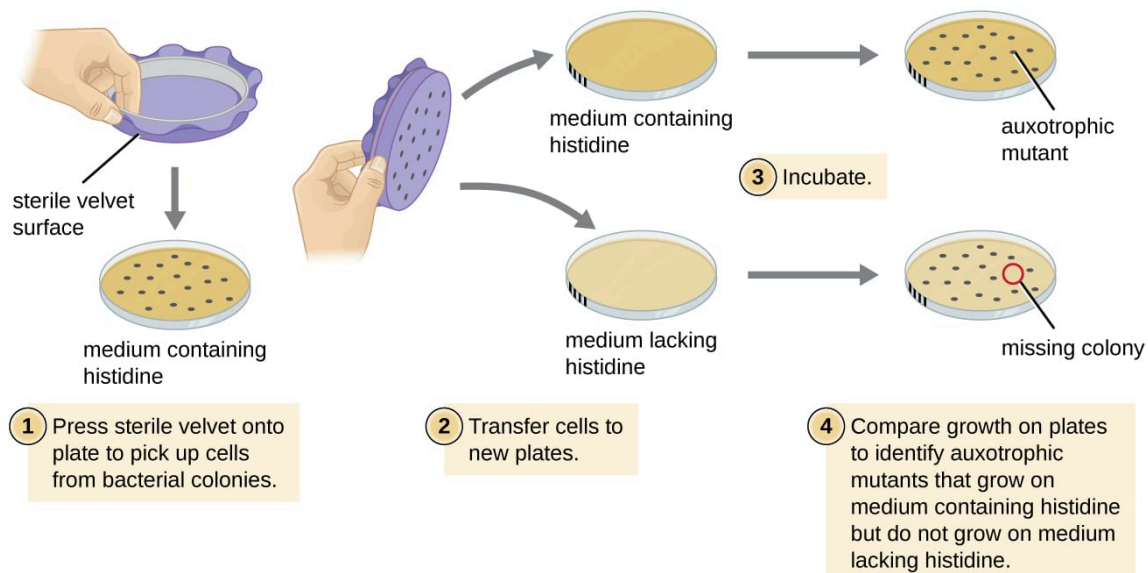


Figure 13.2.7: Identification of auxotrophic mutants, like histidine auxotrophs, is done using replica plating. After mutagenesis, colonies that grow on nutritionally complete medium but not on medium lacking histidine are identified as histidine auxotrophs.

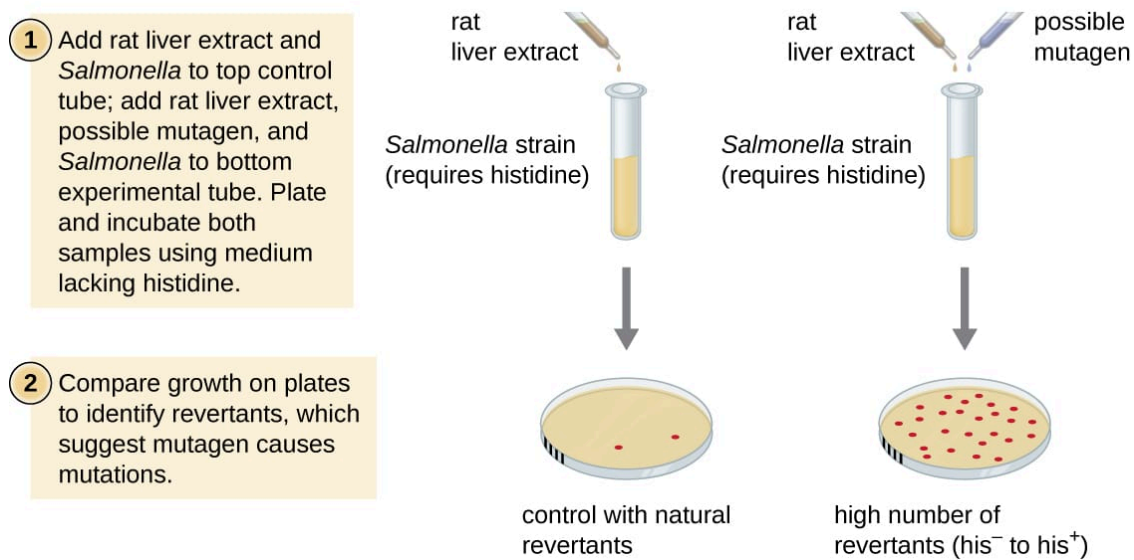


Figure 13.2.8: The Ames test is used to identify mutagenic, potentially carcinogenic chemicals. A *Salmonella* histidine auxotroph is used as the test strain, exposed to a potential mutagen/carcinogen. The number of reversion mutants capable of growing in the absence of supplied histidine is counted and compared with the number of natural reversion mutants that arise in the absence of the potential mutagen.

Key Concepts and Summary

- 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.

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13.3: Horizontal Gene Transfer- Transformation and Transduction

Learning Objectives

- Compare the processes of transformation and transduction
- Explain how asexual gene transfer results in prokaryotic genetic diversity
- Evaluate how scientists use horizontal gene transfer mechanisms in genetic engineering and recombinant DNA technology

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 13.3.1:

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

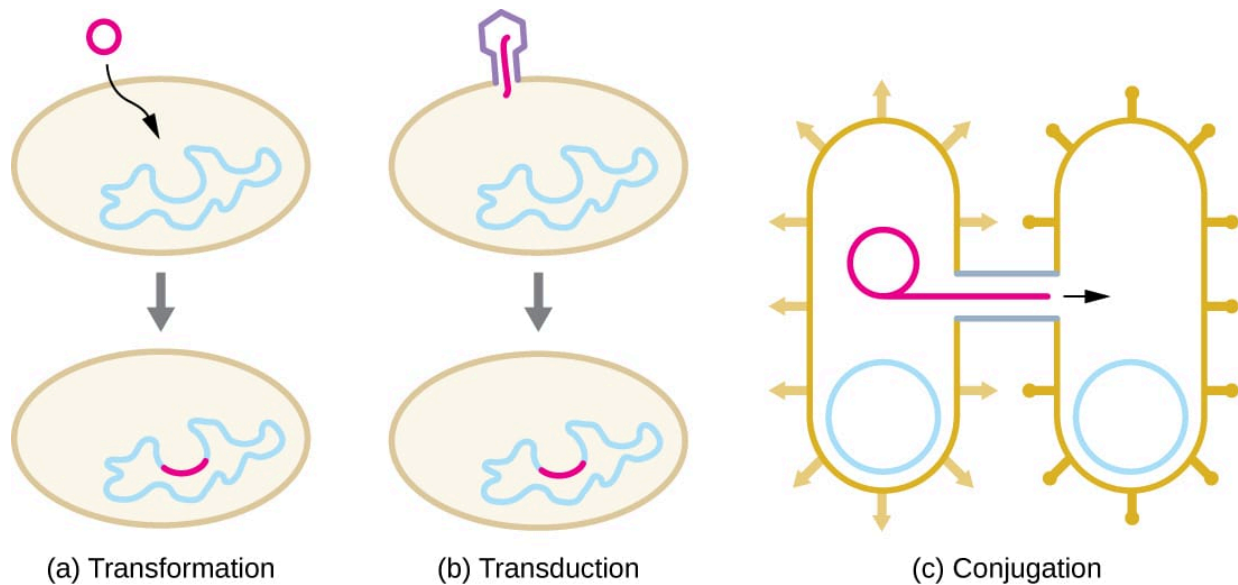


Figure 13.3.1: There are three prokaryote-specific mechanisms leading to horizontal gene transfer in prokaryotes. a) In transformation, the cell takes up DNA directly from the environment. The DNA may remain separate as a plasmid or be incorporated into the host genome. b) In transduction, a bacteriophage 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.

What is Horizontal Gene Transfer?

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A Horizontal Gene Transfer (sometimes called lateral gene transfer or sideways gene transfer) is the passing of one or more genes through routes other than parent-to-offspring. In this animation you will learn what that looks like and how it effects evolution.

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](#).

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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.

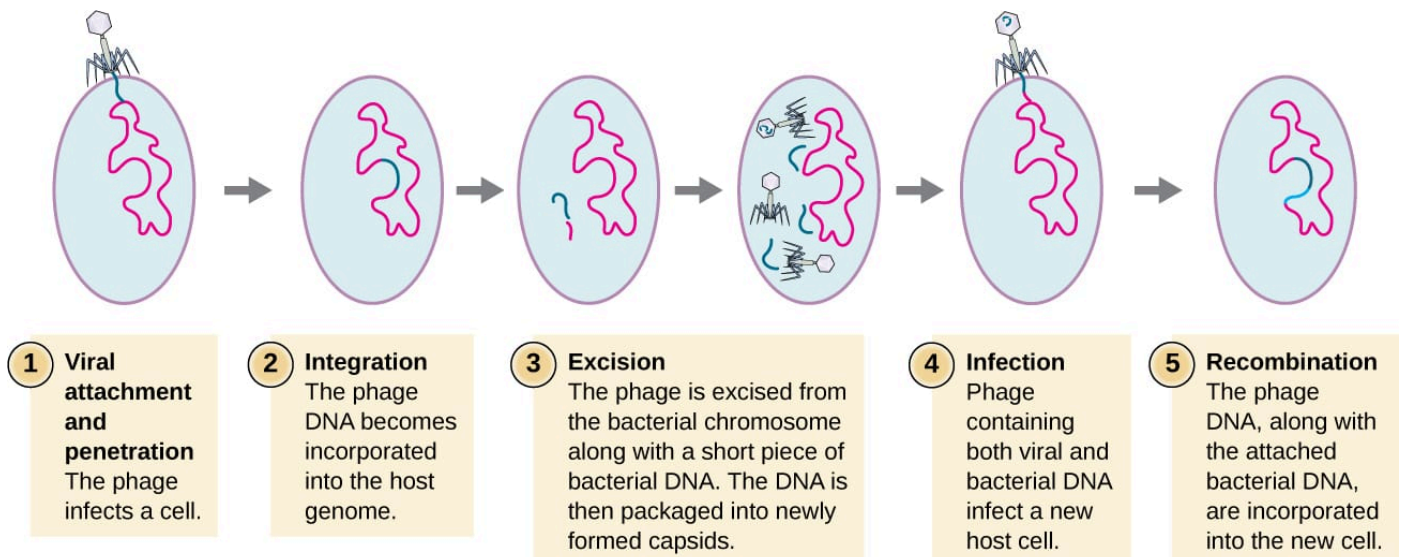


Figure 13.3.1: This flowchart illustrates the mechanism of specialized transduction. An integrated phage excises, bringing with it a piece of the DNA adjacent to its insertion point. On reinfection of a new bacterium, the phage DNA integrates along with the genetic material acquired from the previous host.

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 13.3.2). 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.

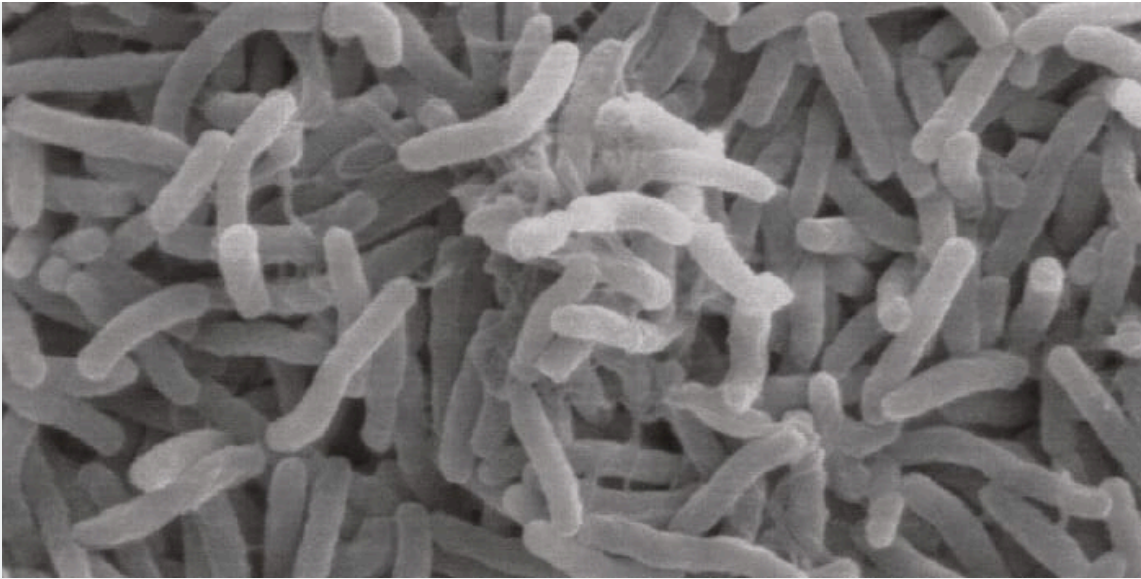


Figure 13.3.2: A scanning electron micrograph of *Vibrio cholerae* shows its characteristic curved rod shape.

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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.

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13.4: Horizontal Gene Transfer- Conjugation and Transposition

Learning Objectives

- Describe the process of bacterial conjugation
- Explain how conjugation contributes to the spread of antibiotic resistance in bacterial population
- Define transposable elements and evaluate the impact of transposition on genome evolution

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 13.4.3). 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.

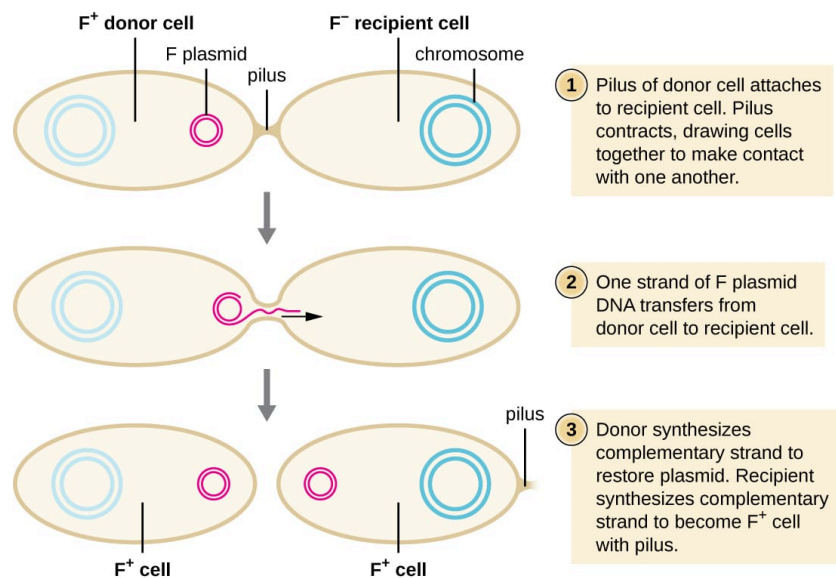


Figure 13.4.3: Typical conjugation of the F plasmid from an F⁺ cell to an F⁻ cell is brought about by the conjugation pilus bringing the two cells into contact. A single strand of the F plasmid is transferred to the F⁻ cell, which is then made double stranded.

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 13.4.4). “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 13.4.5). 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.

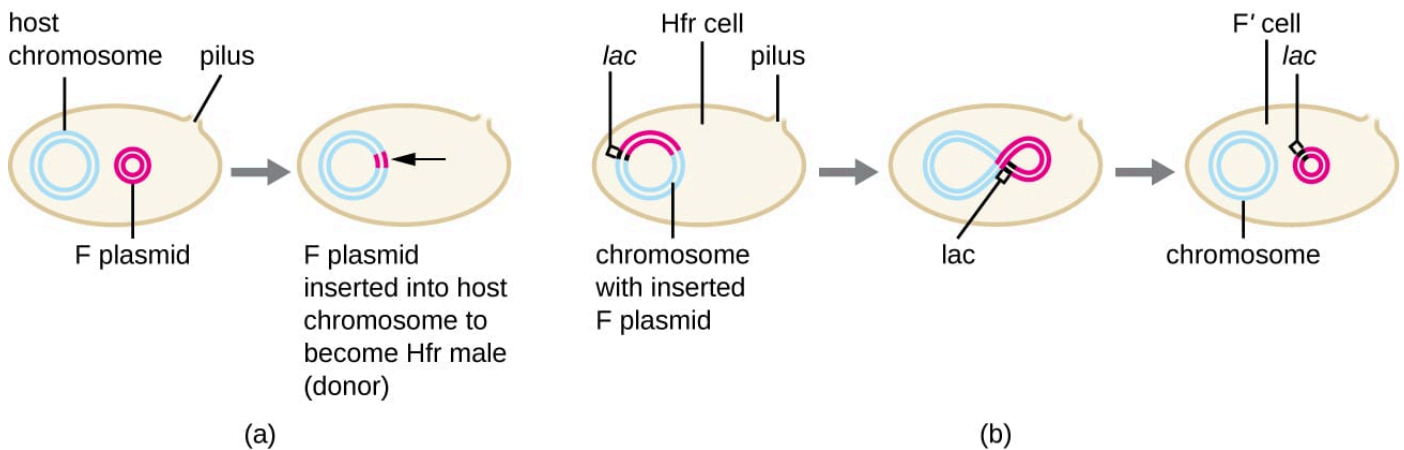


Figure 13.4.4: (a) The F plasmid can occasionally integrate into the bacterial chromosome, producing an Hfr cell. (b) Imprecise excision of the F plasmid from the chromosome of an Hfr cell may lead to the production of an F' plasmid that carries chromosomal DNA adjacent to the integration site. This F' plasmid can be transferred to an F⁻ cell by conjugation.

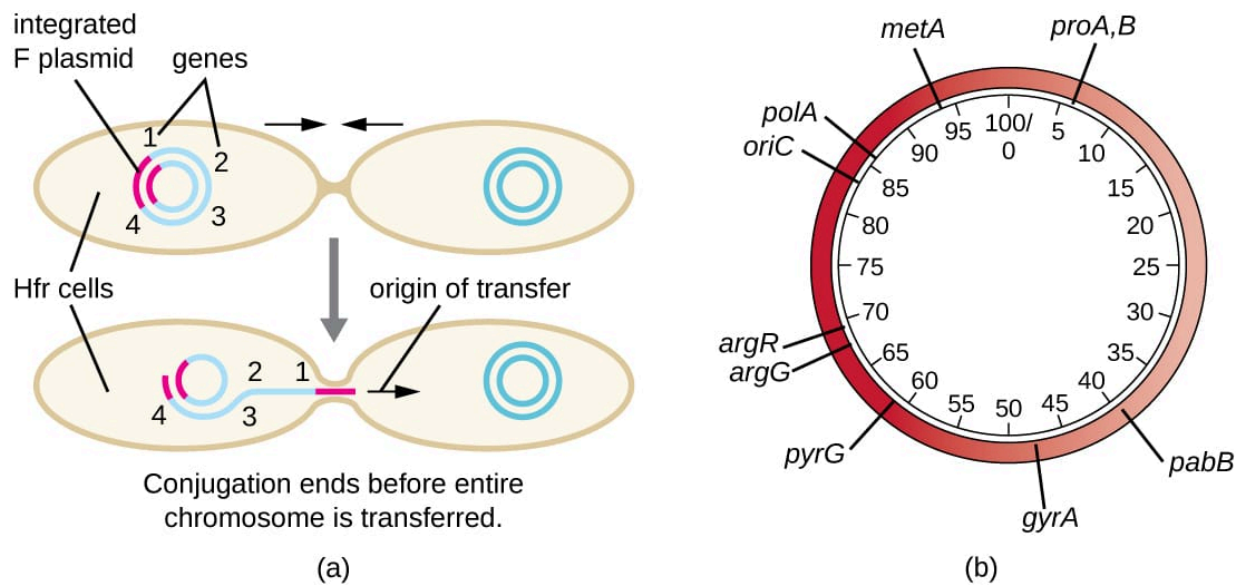


Figure 13.4.5: (a) An Hfr cell may attempt to transfer the entire bacterial chromosome to an F^- cell, treating the chromosome like an extremely large F plasmid. However, contact between cells during conjugation is temporary. Chromosomal genes closest to the integration site (gene 1) that are first displaced 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 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](#).

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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 13.4.6). 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.

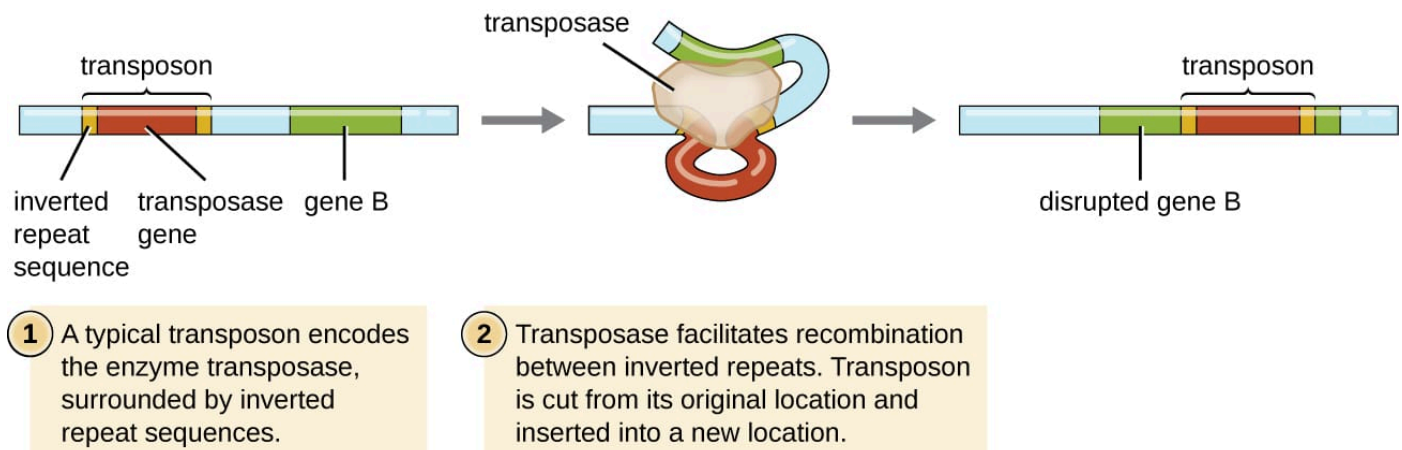


Figure 13.4.6: Transposons are segments of DNA that have the ability to move from one location to another because they code for the enzyme transposase. In this example, a nonreplicative transposon has disrupted gene B. The consequence of that the transcription of gene B may now have been interrupted.

Table 13.4.1 summarizes the processes discussed in this section.

Table 13.4.1: 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

Term	Definition
Transposition	Process whereby DNA independently excises from one location in a DNA molecule and integrates elsewhere

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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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13.5: Gene Regulation - Repressible Operon

Learning Objectives

- Distinguish structural genes and regulatory genes and their different roles in cells
- Define operon and identify its components
- Explain how repressible operon works using Trp operon as an example

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 13.5.1). 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.

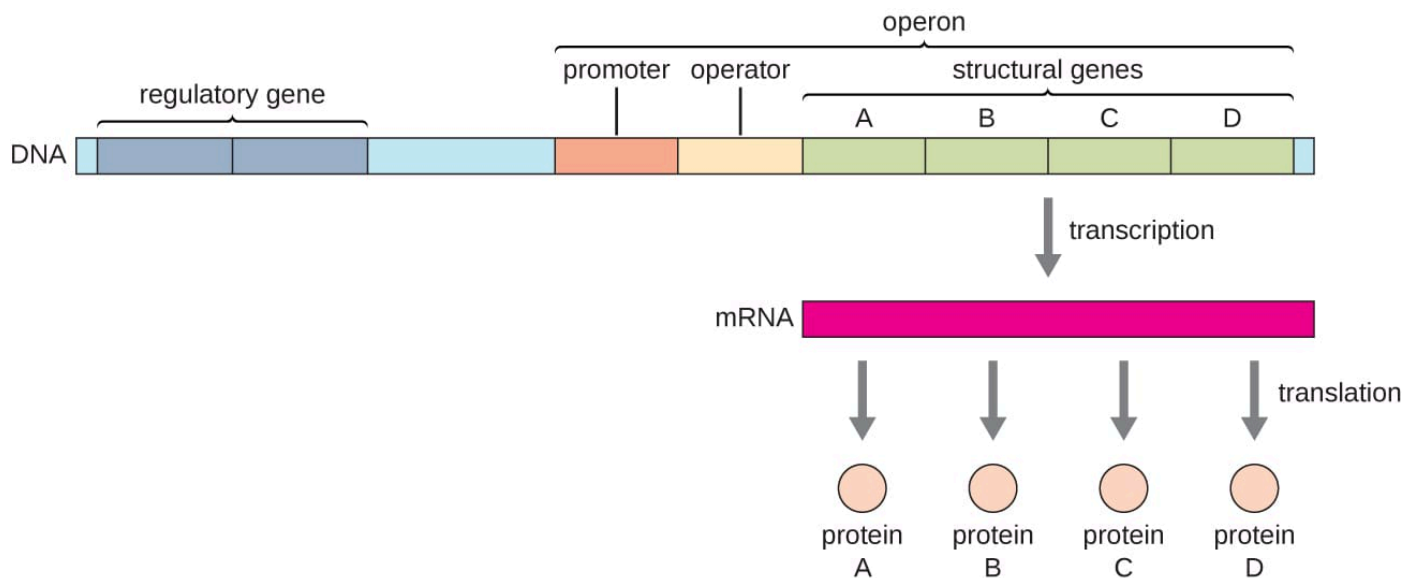


Figure 13.5.1: In prokaryotes, structural genes of related function are often organized together on the genome and transcribed together under the control of a single promoter. The operon's regulatory region includes both the promoter and the operator. If a repressor binds to the operator, then the structural genes will not be transcribed. Alternatively, activators may bind to the regulatory region, enhancing transcription.

Gene Regulation and the Operon

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Explore gene expression with the Amoeba Sisters, including the fascinating Lac Operon found in bacteria! Learn how genes can be turned "on" and "off" and why this is essential for cellular function.

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Regulation by Repression

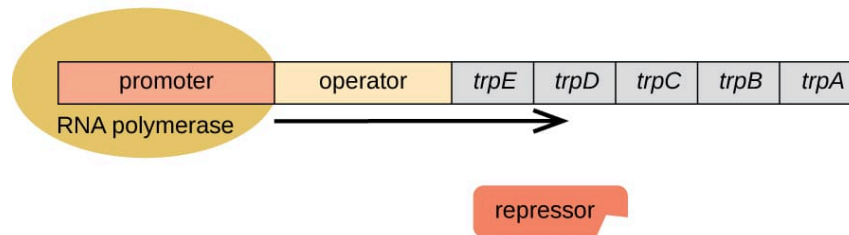
Prokaryotic operons are commonly controlled by the binding of repressors to operator regions, thereby preventing the transcription of the structural genes. Such operons are classified as either **repressible operons** or inducible operons. Repressible operons, like the tryptophan (*trp*) operon, typically 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 13.5.2). 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.

In the absence of tryptophan, the *trp* repressor dissociates from the operator, and RNA synthesis proceeds.



When tryptophan is present, the *trp* repressor binds the operator, and RNA synthesis is blocked.

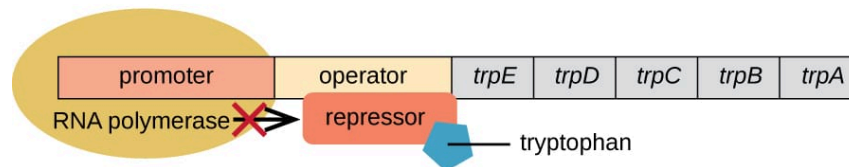


Figure 13.5.2: The five structural genes needed to synthesize tryptophan in *E. coli* are located next to each other in the *trp* operon. When tryptophan is absent, the repressor protein does not bind to the operator, and the genes are transcribed. When tryptophan is plentiful, tryptophan binds the repressor 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.

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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.

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13.6: Gene Regulation - Inducible Operon

Learning Objectives

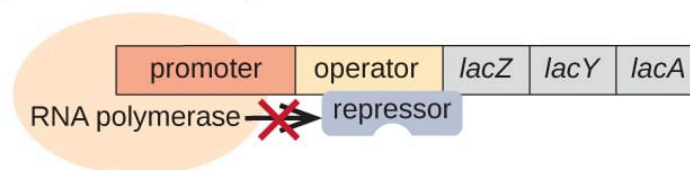
- Describe the structure and components of the *lac* operon
- Differentiate between positive and negative regulation of the *lac* operon
- Identify the conditions under which the *lac* operon is activated or repressed

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 13.6.3). 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.

In the absence of lactose, the *lac* repressor binds the operator, and transcription is blocked.



In the presence of lactose, the *lac* repressor is released from the operator, and transcription proceeds at a slow rate.

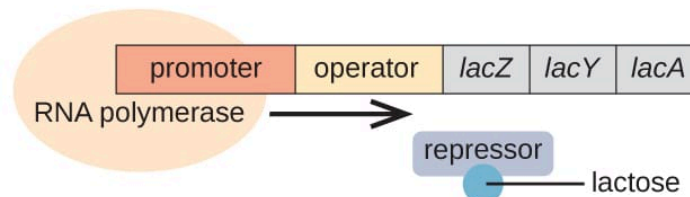


Figure 13.6.3: The three structural genes that are needed to degrade lactose in *E. coli* are located next to each other in the *lac* operon. When lactose is absent, the repressor protein binds to the operator, physically blocking the RNA polymerase from transcribing the *lac* structural genes. When lactose is available, a lactose molecule binds the repressor protein, preventing the repressor from binding to the operator sequence, and the genes are transcribed.

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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 13.6.4. 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 13.6.5).

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 13.6.6). 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 13.6.1 for a summary of the regulation of the *lac* operon.

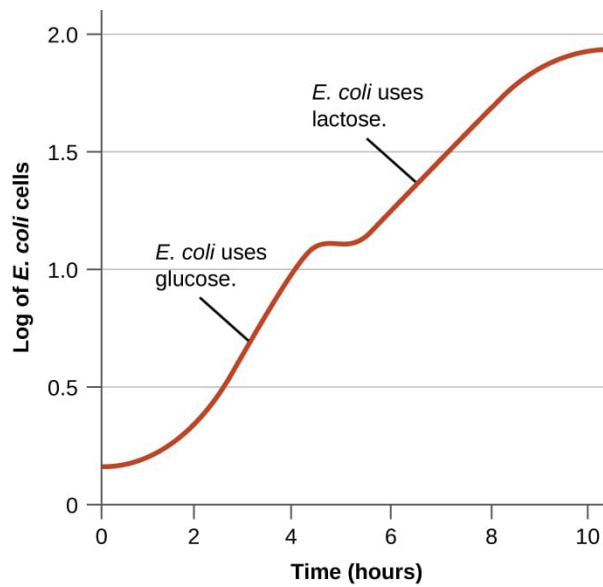


Figure 13.6.4: When grown in the presence of two substrates, *E. coli* uses the preferred substrate (in this case glucose) until it is depleted. Then, enzymes needed for the metabolism of the second substrate are expressed and growth resumes, although at a slower rate.

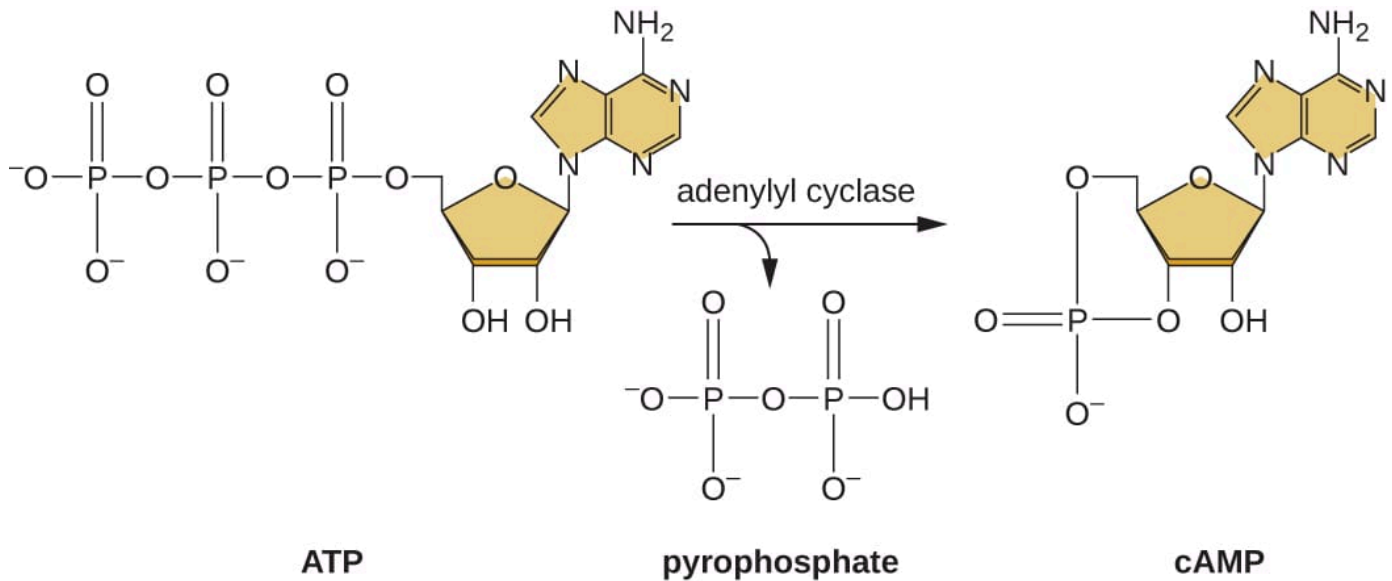
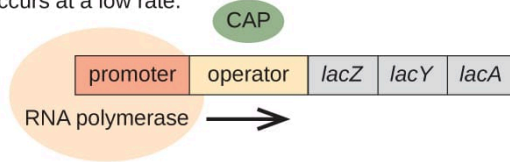
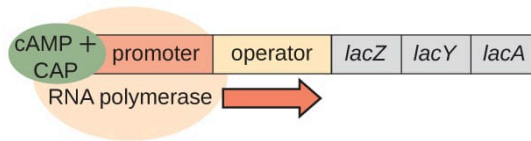


Figure 13.6.5: When ATP levels decrease due to depletion of glucose, some remaining ATP is converted to cAMP by adenylyl cyclase. Thus, increased cAMP levels signal glucose depletion.

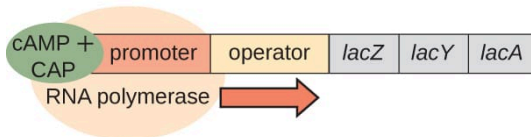
In the absence of cAMP, CAP does not bind the promoter. Transcription occurs at a low rate.



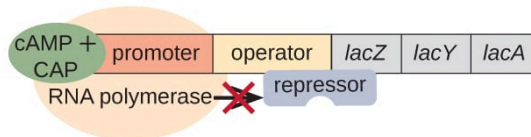
cAMP-CAP complex stimulates RNA polymerase activity and increases RNA synthesis.



In the presence of cAMP, CAP binds the promoter and increases RNA polymerase activity.



However, even in the presence of cAMP-CAP complex, RNA synthesis is blocked when repressor is bound to the operator.



(a)

(b)

Figure 13.6.6: (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 13.6.1: 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.

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Key Concepts and Summary

- 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**.

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13.E: Mechanisms of Microbial Genetics (Exercises)

13.1: 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?

13.2: 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.

13.3: 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

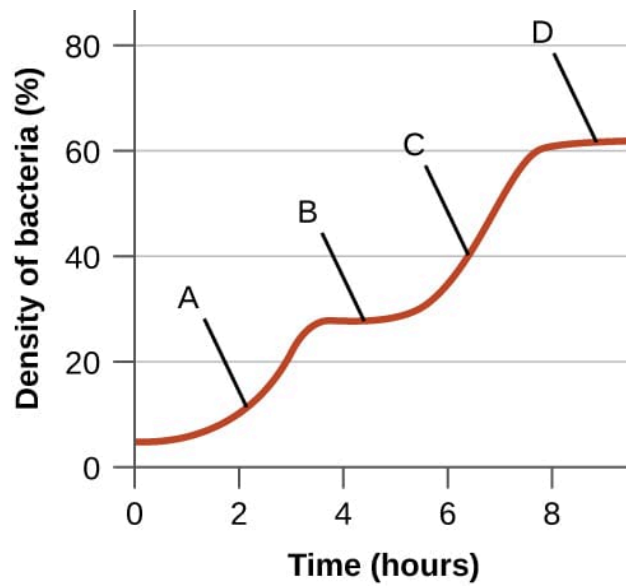
catabolite repression

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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14: ANTIBIOTICS

In nature, some microbes produce substances that inhibit or kill other microbes that might otherwise compete for the same resources. Humans have successfully exploited these abilities, using microbes to mass-produce substances that can be used as antimicrobial drugs. Since their discovery, antimicrobial drugs have saved countless lives, and they remain an essential tool for treating and controlling infectious disease. But their widespread and often unnecessary use has had an unintended side effect: the rise of multidrug-resistant microbial strains. In this chapter, we will discuss how antimicrobial drugs work, why microbes develop resistance, and what health professionals can do to encourage responsible use of antimicrobials.



Figure 14.1: First mass produced in the 1940s, penicillin was instrumental in saving millions of lives during World War II and was considered a wonder drug.¹ Today, overprescription of antibiotics (especially for childhood illnesses) has contributed to the evolution of drug-resistant pathogens. (credit left: modification of work by Chemical Heritage Foundation; Credit right: DFID / Flickr; CC-BY)

14.1: Discovering Antimicrobial Drugs

14.2: Fundamentals of Antimicrobial Therapy

14.3: Inhibitors of Cell Wall Biosynthesis

14.4: Inhibitors of Protein Synthesis

14.5: Other Antibiotics

14.E: Antimicrobial Drugs (Exercises)

Footnotes

1. ¹ "Treatment of War Wounds: A Historical Review." *Clinical Orthopaedics and Related Research* 467 no. 8 (2009):2168–2191.

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14.1: Discovering Antimicrobial Drugs

Learning Objectives

- Compare and contrast natural, semisynthetic, and synthetic antimicrobial drugs
- Describe the chemotherapeutic approaches of ancient societies
- Describe the historically important individuals and events that led to the development of antimicrobial drugs

Most people associate the term chemotherapy with treatments for cancer. However, chemotherapy is actually a broader term that refers to any use of chemicals or drugs to treat disease. Chemotherapy may involve drugs that target cancerous cells or tissues, or it may involve **antimicrobial drugs** that target infectious microorganisms. Antimicrobial drugs typically work by destroying or interfering with microbial structures and enzymes, either killing microbial cells or inhibiting of their growth. But before we examine how these drugs work, we will briefly explore the history of humans' use of antimicrobials for the purpose of chemotherapy.

Use of Antimicrobials in Ancient Societies

Although the discovery of antimicrobials and their subsequent widespread use is commonly associated with modern medicine, there is evidence that humans have been exposed to antimicrobial compounds for millennia. Chemical analyses of the skeletal remains of people from Nubia¹ (now found in present-day Sudan) dating from between 350 and 550 AD have shown residue of the antimicrobial agent tetracycline in high enough quantities to suggest the purposeful fermentation of tetracycline-producing *Streptomyces* during the beer-making process. The resulting beer, which was thick and gruel-like, was used to treat a variety of ailments in both adults and children, including gum disease and wounds. The antimicrobial properties of certain plants may also have been recognized by various cultures around the world, including Indian and Chinese herbalists (Figure 14.1.1) who have long used plants for a wide variety of medical purposes. Healers of many cultures understood the antimicrobial properties of fungi and their use of moldy bread or other mold-containing products to treat wounds has been well documented for centuries.² Today, while about 80% of the world's population still relies on plant-derived medicines,³ scientists are now discovering the active compounds conferring the medicinal benefits contained in many of these traditionally used plants.



Figure 14.1.1: For millennia, Chinese herbalists have used many different species of plants for the treatment of a wide variety of human ailments.

The First Antimicrobial Drugs

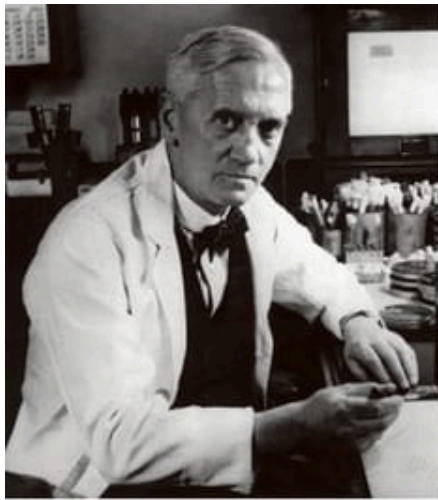
Societies relied on traditional medicine for thousands of years; however, the first half of the 20th century brought an era of strategic drug discovery. In the early 1900s, the German physician and scientist Paul Ehrlich (1854–1915) set out to discover or synthesize chemical compounds capable of killing infectious microbes without harming the patient. In 1909, after screening more than 600 arsenic-containing compounds, Ehrlich's assistant Sahachiro Hata (1873–1938) found one such “magic bullet.” Compound 606 targeted the bacterium *Treponema pallidum*, the causative agent of syphilis. Compound 606 was found to successfully cure syphilis in rabbits and soon after was marketed under the name Salvarsan as a remedy for the disease in humans (Figure 14.1.2). Ehrlich's innovative approach of systematically screening a wide variety of compounds remains a common strategy for the discovery of new antimicrobial agents even today.



Figure 14.1.2: Paul Ehrlich was influential in the discovery of Compound 606, an antimicrobial agent that proved to be an effective treatment for syphilis.

A few decades later, German scientists Josef Klarer, Fritz Mietzsch, and Gerhard Domagk discovered the antibacterial activity of a synthetic dye, prontosil, that could treat streptococcal and staphylococcal infections in mice. Domagk's own daughter was one of the first human recipients of the drug, which completely cured her of a severe streptococcal infection that had resulted from a poke with an embroidery needle. Gerhard Domagk (1895–1964) was awarded the Nobel Prize in Medicine in 1939 for his work with prontosil and sulfanilamide, the active breakdown product of prontosil in the body. Sulfanilamide, the first synthetic antimicrobial created, served as the foundation for the chemical development of a family of sulfa drugs. A **synthetic antimicrobial** is a drug that is developed from a chemical not found in nature. The success of the sulfa drugs led to the discovery and production of additional important classes of synthetic antimicrobials, including the quinolones and oxazolidinones.

A few years before the discovery of prontosil, scientist Alexander Fleming (1881–1955) made his own accidental discovery that turned out to be monumental. In 1928, Fleming returned from holiday and examined some old plates of staphylococci in his research laboratory at St. Mary's Hospital in London. He observed that contaminating mold growth (subsequently identified as a strain of *Penicillium notatum*) inhibited staphylococcal growth on one plate. Fleming, therefore, is credited with the discovery of **penicillin**, the first **natural antibiotic**, (Figure 14.1.3). Further experimentation showed that penicillin from the mold was antibacterial against streptococci, meningococci, and *Corynebacterium diphtheriae*, the causative agent of diphtheria.



(a)



(b)

Figure 14.1.3: (a) Alexander Fleming was the first to discover a naturally produced antimicrobial, penicillin, in 1928. (b) Howard Florey and Ernst Chain discovered how to scale up penicillin production. Then they figured out how to purify it and showed its efficacy as an antimicrobial in animal and human trials in the early 1940s.

Fleming and his colleagues were credited with discovering and identifying penicillin, but its isolation and mass production were accomplished by a team of researchers at Oxford University under the direction of Howard Florey (1898–1968) and Ernst Chain (1906–1979) (Figure 14.1.3). In 1940, the research team purified penicillin and reported its success as an antimicrobial agent against streptococcal infections in mice. Their subsequent work with human subjects also showed penicillin to be very effective. Because of their important work, Fleming, Florey, and Chain were awarded the Nobel Prize in Physiology and Medicine in 1945.

In the early 1940s, scientist Dorothy Hodgkin (1910–1994), who studied crystallography at Oxford University, used X-rays to analyze the structure of a variety of natural products. In 1946, she determined the structure of penicillin, for which she was awarded the Nobel Prize in Chemistry in 1964. Once the structure was understood, scientists could modify it to produce a variety of **semisynthetic penicillins**. A semisynthetic antimicrobial is a chemically modified derivative of a natural antibiotic. The chemical modifications are generally designed to increase the range of bacteria targeted, increase stability, decrease toxicity, or confer other properties beneficial for treating infections.

Penicillin is only one example of a natural antibiotic. Also in the 1940s, Selman Waksman (1888–1973) (Figure 14.1.4), a prominent soil microbiologist at Rutgers University, led a research team that discovered several antimicrobials, including actinomycin, streptomycin, and neomycin. The discoveries of these antimicrobials stemmed from Waksman's study of fungi and the Actinobacteria, including soil bacteria in the genus *Streptomyces*, known for their natural production of a wide variety of antimicrobials. His work earned him the Nobel Prize in Physiology and Medicine in 1952. The actinomycetes are the source of more than half of all natural antibiotics⁴ and continue to serve as an excellent reservoir for the discovery of novel antimicrobial agents. Some researchers argue that we have not yet come close to tapping the full antimicrobial potential of this group.⁵



Figure 14.1.4: Selman Waksman was the first to show the vast antimicrobial production capabilities of a group of soil bacteria, the actinomycetes.

Query 14.1.1

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Interactive Element

Case Study Preview: “Unfortunate UTI: A Resistant Return”

After a trip to Cambodia and hospitalization in Vietnam, Marisa returns home with more than just memories - she's in pain, feverish, and rushed to the ER. What initially seems like a standard urinary tract infection turns out to be a dangerous case of *carbapenem-resistant Klebsiella pneumoniae* (CRKP), carrying the infamous NDM-1 enzyme. Standard treatments fail, and Marisa's infection requires isolation, targeted antibiotics, and weeks of monitoring.

In this case, you'll explore how catheterization, international hospitalization, and antibiotic pressure create a perfect storm for drug-resistant superbugs. You'll also learn how diagnostic tests, infection control protocols, and antibiotic stewardship are critical in containing these global threats.

Sometimes the real souvenir is hiding in your microbiome.

[Chapter 14 Case Study - Unfortunate UTI: A Resistant Return](#)

Key Concepts and Summary

- **Antimicrobial drugs** produced by purposeful fermentation and/or contained in plants have been used as traditional medicines in many cultures for millennia.
- The purposeful and systematic search for a chemical “magic bullet” that specifically target infectious microbes was initiated by Paul Ehrlich in the early 20th century.
- The discovery of the **natural antibiotic**, penicillin, by Alexander Fleming in 1928 started the modern age of antimicrobial

discovery and research.

- Sulfanilamide, the first **synthetic antimicrobial**, was discovered by Gerhard Domagk and colleagues and is a breakdown product of the synthetic dye, prontosil.

Footnotes

1. M.L. Nelson et al. "Brief Communication: Mass Spectroscopic Characterization of Tetracycline in the Skeletal Remains of an Ancient Population from Sudanese Nubia 350–550 CE." *American Journal of Physical Anthropology* 143 no. 1 (2010):151–154.
2. M. Wainwright. "Moulds in Ancient and More Recent Medicine." *Mycologist* 3 no. 1 (1989):21–23.
3. S. Verma, S.P. Singh. "Current and Future Status of Herbal Medicines." *Veterinary World* 1 no. 11 (2008):347–350.
4. J. Berdy. "Bioactive Microbial Metabolites." *The Journal of Antibiotics* 58 no. 1 (2005):1–26.
5. M. Baltz. "Antimicrobials from Actinomycetes: Back to the Future." *Microbe* 2 no. 3 (2007):125–131.

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14.2: Fundamentals of Antimicrobial Therapy

Learning Objectives

- Contrast bacteriostatic versus bactericidal antibacterial activities
- Contrast broad-spectrum drugs versus narrow-spectrum drugs
- Explain the significance of superinfections
- Discuss the significance of dosage and the route of administration of a drug
- Identify factors and variables that can influence the side effects of a drug
- Describe the significance of positive and negative interactions between drugs

Several factors are important in choosing the most appropriate antimicrobial drug therapy, including bacteriostatic versus bactericidal mechanisms, spectrum of activity, dosage and route of administration, the potential for side effects, and the potential interactions between drugs. The following discussion will focus primarily on antibacterial drugs, but the concepts translate to other antimicrobial classes.

Bacteriostatic Versus Bactericidal

Antibacterial drugs can be either bacteriostatic or bactericidal in their interactions with target bacteria. **Bacteriostatic** drugs cause a reversible inhibition of growth, with bacterial growth restarting after elimination of the drug. By contrast, **bactericidal** drugs kill their target bacteria. The decision of whether to use a bacteriostatic or bactericidal drugs depends on the type of infection and the immune status of the patient. In a patient with strong immune defenses, bacteriostatic and bactericidal drugs can be effective in achieving clinical cure. However, when a patient is immunocompromised, a bactericidal drug is essential for the successful treatment of infections. Regardless of the immune status of the patient, life-threatening infections such as acute endocarditis require the use of a bactericidal drug.

Spectrum of Activity

The spectrum of activity of an antibacterial drug relates to diversity of targeted bacteria. A **narrow-spectrum antimicrobial** targets only specific subsets of bacterial pathogens. For example, some narrow-spectrum drugs only target gram-positive bacteria, whereas others target only gram-negative bacteria. If the pathogen causing an infection has been identified, it is best to use a narrow-spectrum antimicrobial and minimize collateral damage to the normal microbiota. A **broad-spectrum antimicrobial** targets a wide variety of bacterial pathogens, including both gram-positive and gram-negative species, and is frequently used as empiric therapy to cover a wide range of potential pathogens while waiting on the laboratory identification of the infecting pathogen. Broad-spectrum antimicrobials are also used for polymicrobial infections (mixed infection with multiple bacterial species), or as prophylactic prevention of infections with surgery/invasive procedures. Finally, broad-spectrum antimicrobials may be selected to treat an infection when a narrow-spectrum drug fails because of development of drug resistance by the target pathogen.

The risk associated with using broad-spectrum antimicrobials is that they will also target a broad spectrum of the normal microbiota, increasing the risk of a **superinfection**, a secondary infection in a patient having a preexisting infection. A superinfection develops when the antibacterial intended for the preexisting infection kills the protective microbiota, allowing another pathogen resistant to the antibacterial to proliferate and cause a secondary infection (Figure 14.2.1). Common examples of superinfections that develop as a result of antimicrobial usage include yeast infections (candidiasis) and pseudomembranous colitis caused by *Clostridium difficile*, which can be fatal.

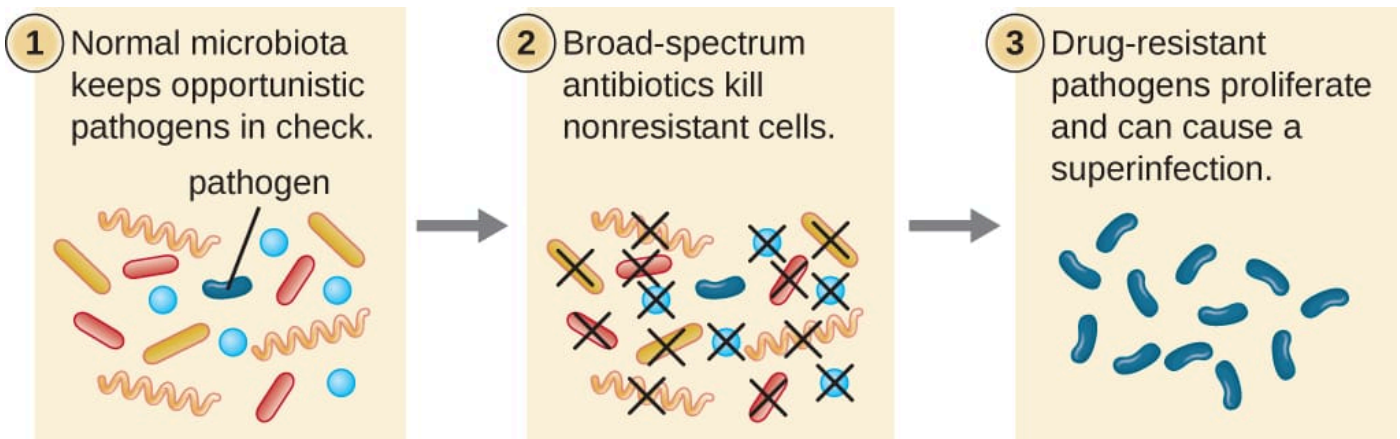


Figure 14.2.1: Broad-spectrum antimicrobial use may lead to the development of a superinfection. (credit: modification of work by Centers for Disease Control and Prevention)

Query 14.2.1

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Dosage and Route of Administration

The amount of medication given during a certain time interval is the **dosage**, and it must be determined carefully to ensure that optimum therapeutic drug levels are achieved at the site of infection without causing significant toxicity (side effects) to the patient. Each drug class is associated with a variety of potential side effects, and some of these are described for specific drugs later in this chapter. Despite best efforts to optimize dosing, allergic reactions and other potentially serious side effects do occur. Therefore, the goal is to select the optimum dosage that will minimize the risk of side effects while still achieving clinical cure, and there are important factors to consider when selecting the best dose and dosage interval. For example, in children, dose is based upon the patient's mass. However, the same is not true for adults and children 12 years of age and older, for which there is typically a single standard dose regardless of the patient's mass. With the great variability in adult body mass, some experts have argued that mass should be considered for all patients when determining appropriate dosage.¹ An additional consideration is how drugs are metabolized and eliminated from the body. In general, patients with a history of liver or kidney dysfunction may experience reduced drug metabolism or clearance from the body, resulting in increased drug levels that may lead to toxicity and make them more prone to side effects.

There are also some factors specific to the drugs themselves that influence appropriate dose and time interval between doses. For example, the half-life, or rate at which 50% of a drug is eliminated from the plasma, can vary significantly between drugs. Some drugs have a short half-life of only 1 hour and must be given multiple times a day, whereas other drugs have half-lives exceeding 12 hours and can be given as a single dose every 24 hours. Although a longer half-life can be considered an advantage for an antibacterial when it comes to convenient dosing intervals, the longer half-life can also be a concern for a drug that has serious side effects because drug levels may remain toxic for a longer time. Last, some drugs are dose dependent, meaning they are more effective when administered in large doses to provide high levels for a short time at the site of infection. Others are time dependent, meaning they are more effective when lower optimum levels are maintained over a longer period of time.

The **route of administration**, the method used to introduce a drug into the body, is also an important consideration for drug therapy. Drugs that can be administered orally are generally preferred because patients can more conveniently take these drugs at home. However, some drugs are not absorbed easily from the gastrointestinal (GI) tract into the bloodstream. These drugs are often useful for treating diseases of the intestinal tract, such as tapeworms treated with niclosamide, or for decontaminating the bowel, as with colistin. Some drugs that are not absorbed easily, such as bacitracin, polymyxin, and several antifungals, are available as topical preparations for treatment of superficial skin infections. Sometimes, patients may not initially be able to take

oral medications because of their illness (e.g., vomiting, intubation for respirator). When this occurs, and when a chosen drug is not absorbed in the GI tract, administration of the drug by a parenteral route (intravenous or intramuscular injection) is preferred and typically is performed in health-care settings. For most drugs, the plasma levels achieved by intravenous administration is substantially higher than levels achieved by oral or intramuscular administration, and this can also be an important consideration when choosing the route of administration for treating an infection (Figure 14.2.2).

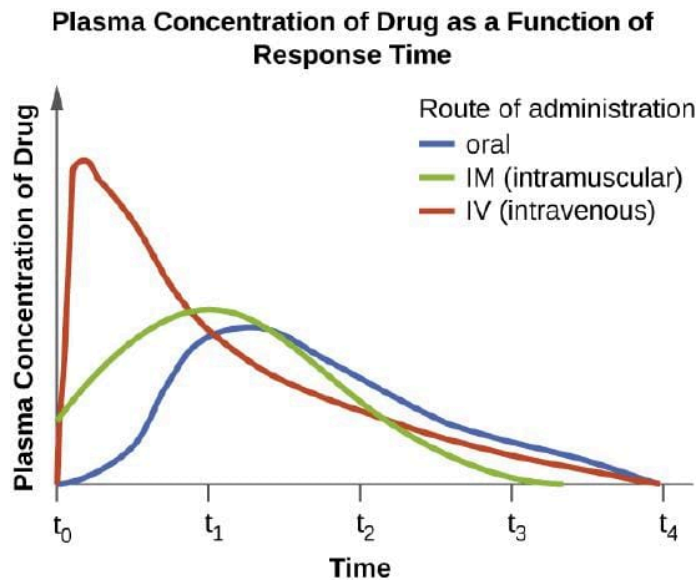


Figure 14.2.2: On this graph, t_0 represents the time at which a drug dose is administered. The curves illustrate how plasma concentration of the drug changes over specific intervals of time (t_1 through t_4). As the graph shows, when a drug is administered intravenously, the concentration peaks very quickly and then gradually decreases. When drugs are administered orally or intramuscularly, it takes longer for the concentration to reach its peak.

Query 14.2.1

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Drug Interactions

For the optimum treatment of some infections, two antibacterial drugs may be administered together to provide a **synergistic interaction** that is better than the efficacy of either drug alone. A classic example of synergistic combinations is trimethoprim and sulfamethoxazole (Bactrim). Individually, these two drugs provide only bacteriostatic inhibition of bacterial growth, but combined, the drugs are bactericidal.

Whereas synergistic drug interactions provide a benefit to the patient, **antagonistic interactions** produce harmful effects. Antagonism can occur between two antimicrobials or between antimicrobials and nonantimicrobials being used to treat other conditions. The effects vary depending on the drugs involved, but antagonistic interactions may cause loss of drug activity, decreased therapeutic levels due to increased metabolism and elimination, or increased potential for toxicity due to decreased metabolism and elimination. As an example, some antibacterials are absorbed most effectively from the acidic environment of the stomach. If a patient takes antacids, however, this increases the pH of the stomach and negatively impacts the absorption of these antimicrobials, decreasing their effectiveness in treating an infection. Studies have also shown an association between use of some antimicrobials and failure of oral contraceptives.²

Resistance Police

In the United States and many other countries, most antimicrobial drugs are self-administered by patients at home. Unfortunately, many patients stop taking antimicrobials once their symptoms dissipate and they feel better. If a 10-day course of treatment is prescribed, many patients only take the drug for 5 or 6 days, unaware of the negative consequences of not completing the full course of treatment. A shorter course of treatment not only fails to kill the target organisms to expected levels, it also selects for drug-resistant variants within the target population and within the patient's microbiota.

Patients' nonadherence especially amplifies drug resistance when the recommended course of treatment is long. Treatment for tuberculosis (TB) is a case in point, with the recommended treatment lasting from 6 months to a year. The CDC estimates that about one-third of the world's population is infected with TB, most living in underdeveloped or underserved regions where antimicrobial drugs are available over the counter. In such countries, there may be even lower rates of adherence than in developed areas. Nonadherence leads to antibiotic resistance and more difficulty in controlling pathogens. As a direct result, the emergence of multidrug-resistant and extensively drug-resistant strains of TB is becoming a huge problem.

Overprescription of antimicrobials also contributes to antibiotic resistance. Patients often demand antibiotics for diseases that do not require them, like viral colds and ear infections. Pharmaceutical companies aggressively market drugs to physicians and clinics, making it easy for them to give free samples to patients, and some pharmacies even offer certain antibiotics free to low-income patients with a prescription.

In recent years, various initiatives have aimed to educate parents and clinicians about the judicious use of antibiotics. However, a recent study showed that, between 2000 and 2013, the parental expectation for antimicrobial prescriptions for children actually increased (Figure 14.2.3).

One possible solution is a regimen called directly observed therapy (DOT), which involves the supervised administration of medications to patients. Patients are either required to visit a health-care facility to receive their medications, or health-care providers must administer medication in patients' homes or another designated location. DOT has been implemented in many cases for the treatment of TB and has been shown to be effective; indeed, DOT is an integral part of WHO's global strategy for eradicating TB.^{3,4} But is this a practical strategy for all antibiotics? Would patients taking penicillin, for example, be more or less likely to adhere to the full course of treatment if they had to travel to a health-care facility for each dose? And who would pay for the increased cost associated with DOT? When it comes to overprescription, should someone be policing physicians or drug companies to enforce best practices? What group should assume this responsibility, and what penalties would be effective in discouraging overprescription?

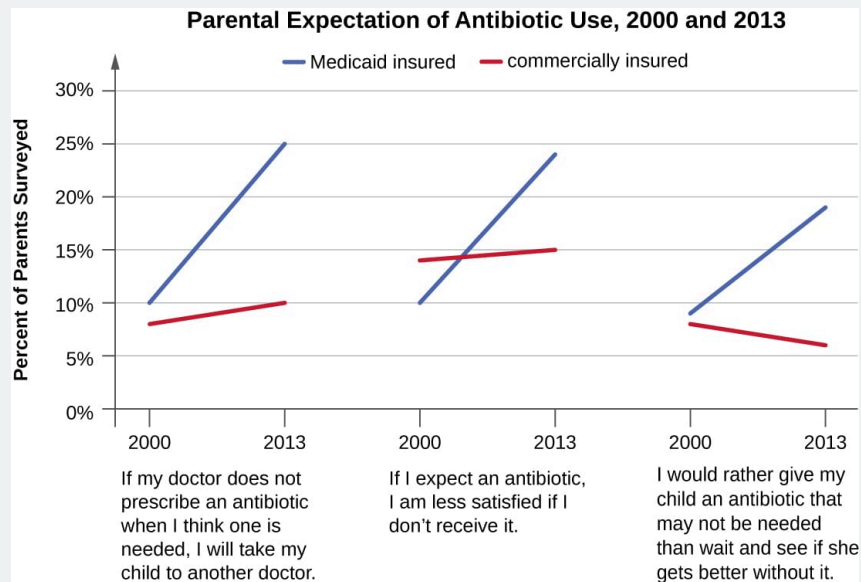


Figure 14.2.3: This graph indicates trends in parental expectations related to prescription of antibiotics based on a recent study.⁵ Among parents of Medicaid-insured children, there was a clear upward trend in parental expectations for prescription antibiotics. Expectations were relatively stable (and lesser) among parents whose children were commercially insured, suggesting that these parents were somewhat better informed than those with Medicaid-insured children.

Key Concepts and Summary

- Antimicrobial drugs can be **bacteriostatic** or **bactericidal**, and these characteristics are important considerations when selecting the most appropriate drug.
- The use of **narrow-spectrum** antimicrobial drugs is preferred in many cases to avoid **superinfection** and the development of antimicrobial resistance.
- **Broad-spectrum** antimicrobial use is warranted for serious systemic infections when there is no time to determine the causative agent, when narrow-spectrum antimicrobials fail, or for the treatment or prevention of infections with multiple types of microbes.
- The **dosage** and **route of administration** are important considerations when selecting an antimicrobial to treat and infection. Other considerations include the patient's age, mass, ability to take oral medications, liver and kidney function, and possible interactions with other drugs the patient may be taking.

Footnotes

1. M.E. Falagas, D.E. Karageorgopoulos. "Adjustment of Dosing of Antimicrobial Agents for Bodyweight in Adults." *The Lancet* 375 no. 9710 (2010):248–251.
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4. World Health Organization. "Tuberculosis (TB): The Five Elements of DOTS." <http://www.who.int/tb/dots/whatisdots/en/>. Accessed June 2, 2016.
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14.3: Inhibitors of Cell Wall Biosynthesis

Learning Objectives

- Describe the mechanisms of action associated with drugs that inhibit cell wall biosynthesis.
- Explain the mode of action of β -lactams.
- Describe why β -lactamase (penicillinase) offers drug resistance for bacteria.
- Explain the mode of action of non- β -lactam cell wall inhibitors.

An important quality for an antimicrobial drug is **selective toxicity**, meaning that it selectively kills or inhibits the growth of microbial targets while causing minimal or no harm to the host. Most antimicrobial drugs currently in clinical use are antibacterial because the prokaryotic cell provides a greater variety of unique targets for selective toxicity, in comparison to fungi, parasites, and viruses. Each class of antibacterial drugs has a unique mode of action (the way in which a drug affects microbes at the cellular level), and these are summarized in Figure 14.3.1 and Table 14.3.1.

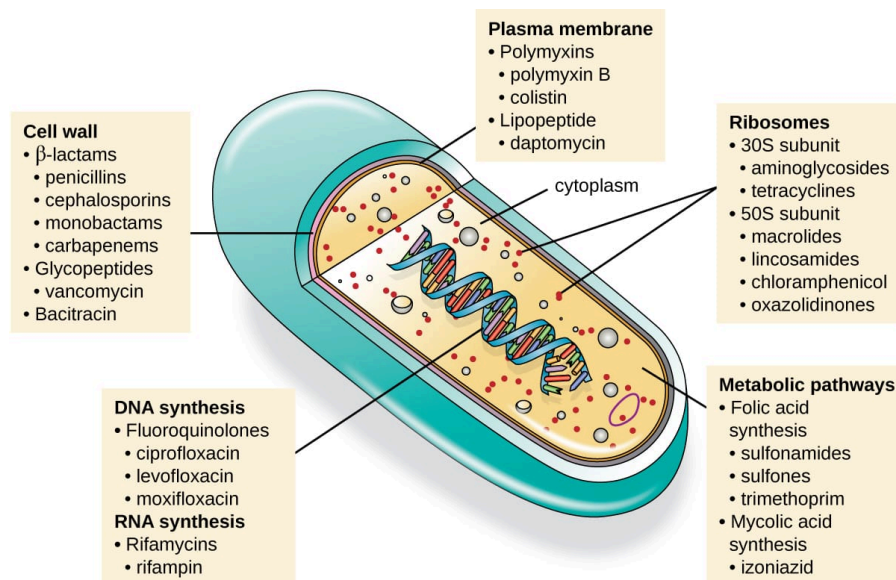


Figure 14.3.1: There are several classes of antibacterial compounds that are typically classified based on their bacterial target.

Table 14.3.1: Common Antibacterial Drugs by Mode of Action

Mode of Action	Target	Drug Class
Inhibit cell wall biosynthesis	Penicillin-binding proteins	β -lactams: penicillins, cephalosporins, monobactams, carbapenems
	Peptidoglycan subunits	Glycopeptides
	Peptidoglycan subunit transport	Bacitracin
Inhibit biosynthesis of proteins	30S ribosomal subunit	Aminoglycosides, tetracyclines
	50S ribosomal subunit	Macrolides, lincosamides, chloramphenicol, oxazolidinones
Disrupt membranes	Lipopolysaccharide, inner and outer membranes	Polymyxin B, colistin, daptomycin
Inhibit nucleic acid synthesis	RNA	Rifamycin
	DNA	Fluoroquinolones

Mode of Action	Target	Drug Class
Antimetabolites	Folic acid synthesis enzyme	Sulfonamides, trimethoprim
	Mycolic acid synthesis enzyme	Isonicotinic acid hydrazide
Mycobacterial adenosine triphosphate (ATP) synthase inhibitor	Mycobacterial ATP synthase	Diarylquinoline

Inhibitors of Cell Wall Biosynthesis

Several different classes of antibacterials block steps in the biosynthesis of peptidoglycan, making cells more susceptible to osmotic lysis (Table 14.3.2). Therefore, antibacterials that target cell wall biosynthesis are bactericidal in their action. Because human cells do not make peptidoglycan, this mode of action is an excellent example of selective toxicity.

Penicillin, the first antibiotic discovered, is one of several antibacterials within a class called **β -lactams**. This group of compounds includes the penicillins, cephalosporins, monobactams, and carbapenems, and is characterized by the presence of a β -lactam ring found within the central structure of the drug molecule (Figure 14.3.2). The β -lactam antibacterials block the crosslinking of peptide chains during the biosynthesis of new peptidoglycan in the bacterial cell wall. They are able to block this process because the β -lactam structure is similar to the structure of the peptidoglycan subunit component that is recognized by the crosslinking transpeptidase enzyme, also known as a penicillin-binding protein (PBP). Although the β -lactam ring must remain unchanged for these drugs to retain their antibacterial activity, strategic chemical changes to the R groups have allowed for development of a wide variety of semisynthetic β -lactam drugs with increased potency, expanded spectrum of activity, and longer half-lives for better dosing, among other characteristics.

Penicillin G and penicillin V are natural antibiotics from fungi and are primarily active against gram-positive bacterial pathogens, and a few gram-negative bacterial pathogens such as *Pasteurella multocida*. Figure 14.3.2 summarizes the semisynthetic development of some of the penicillins. Adding an amino group ($-\text{NH}_2$) to penicillin G created the aminopenicillins (i.e., ampicillin and amoxicillin) that have increased spectrum of activity against more gram-negative pathogens. Furthermore, the addition of a hydroxyl group ($-\text{OH}$) to amoxicillin increased acid stability, which allows for improved oral absorption. Methicillin is a semisynthetic penicillin that was developed to address the spread of enzymes (penicillinases) that were inactivating the other penicillins. Changing the R group of penicillin G to the more bulky dimethoxyphenyl group provided protection of the β -lactam ring from enzymatic destruction by penicillinases, giving us the first penicillinase-resistant penicillin.

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Similar to the penicillins, **cephalosporins** contain a β -lactam ring (Figure 14.3.2) and block the transpeptidase activity of penicillin-binding proteins. However, the β -lactam ring of cephalosporins is fused to a six-member ring, rather than the five-member ring found in penicillins. This chemical difference provides cephalosporins with an increased resistance to enzymatic inactivation by **β -lactamases**. The drug cephalosporin C was originally isolated from the fungus *Cephalosporium acremonium* in the 1950s and has a similar spectrum of activity to that of penicillin against gram-positive bacteria but is active against more gram-negative bacteria than penicillin. Another important structural difference is that cephalosporin C possesses two R groups, compared with just one R group for penicillin, and this provides for greater diversity in chemical alterations and development of semisynthetic cephalosporins. The family of semisynthetic cephalosporins is much larger than the penicillins, and these drugs have been classified into generations based primarily on their spectrum of activity, increasing in spectrum from the narrow-spectrum, first-generation cephalosporins to the broad-spectrum, fourth-generation cephalosporins. A new fifth-generation cephalosporin has been developed that is active against **methicillin-resistant *Staphylococcus aureus* (MRSA)**.

The carbapenems and monobactams also have a β -lactam ring as part of their core structure, and they inhibit the transpeptidase activity of penicillin-binding proteins. The only monobactam used clinically is aztreonam. It is a narrow-spectrum antibacterial

with activity only against gram-negative bacteria. In contrast, the carbapenem family includes a variety of semisynthetic drugs (imipenem, meropenem, and doripenem) that provide very broad-spectrum activity against gram-positive and gram-negative bacterial pathogens.

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The drug **vancomycin**, a member of a class of compounds called the **glycopeptides**, was discovered in the 1950s as a natural antibiotic from the actinomycete *Amycolatopsis orientalis*. Similar to the β -lactams, vancomycin inhibits cell wall biosynthesis and is bactericidal. However, in contrast to the β -lactams, the structure of vancomycin is not similar to that of cell-wall peptidoglycan subunits and does not directly inactivate penicillin-binding proteins. Rather, vancomycin is a very large, complex molecule that binds to the end of the peptide chain of cell wall precursors, creating a structural blockage that prevents the cell wall subunits from being incorporated into the growing N-acetylglucosamine and N-acetylmuramic acid (NAM-NAG) backbone of the peptidoglycan structure (transglycosylation). Vancomycin also structurally blocks transpeptidation. Vancomycin is bactericidal against gram-positive bacterial pathogens, but it is not active against gram-negative bacteria because of its inability to penetrate the protective outer membrane.

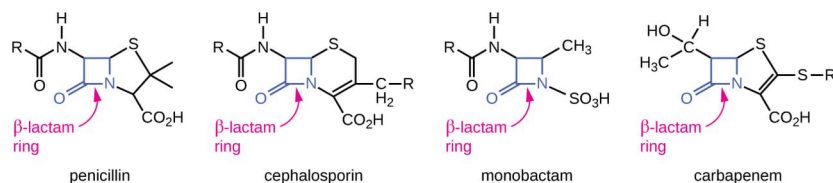
The drug **bacitracin** consists of a group of structurally similar peptide antibiotics originally isolated from *Bacillus subtilis*. Bacitracin blocks the activity of a specific cell-membrane molecule that is responsible for the movement of peptidoglycan precursors from the cytoplasm to the exterior of the cell, ultimately preventing their incorporation into the cell wall. Bacitracin is effective against a wide range of bacteria, including gram-positive organisms found on the skin, such as *Staphylococcus* and *Streptococcus*. Although it may be administered orally or intramuscularly in some circumstances, bacitracin has been shown to be nephrotoxic (damaging to the kidneys). Therefore, it is more commonly combined with neomycin and polymyxin in topical ointments such as Neosporin.

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R group					
Drug name	penicillin G	penicillin V	ampicillin	amoxicillin	methicillin
Spectrum of activity	G+ and a few G-	similar to penicillin G	G+ and more G- than penicillin	similar to ampicillin	G+ only, including β-lactamase producers
Route of administration	parenteral	oral	parenteral and oral	oral (better than ampicillin)	parenteral

Figure 14.3.2: Penicillins, cephalosporins, monobactams, and carbapenems all contain a β-lactam ring, the site of attack by inactivating β-lactamase enzymes. Although they all share the same nucleus, various penicillins differ from each other in the structure of their R groups. Chemical changes to the R groups provided increased spectrum of activity, acid stability, and resistance to β-lactamase degradation.

Table 14.3.2: Drugs that Inhibit Bacterial Cell Wall Synthesis

Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
Interact directly with PBPs and inhibit transpeptidase activity	Penicillins	Penicillin G, penicillin V	Natural	Narrow-spectrum against gram-positive and a few gram-negative bacteria
		Ampicillin, amoxicillin	Semisynthetic	Narrow-spectrum against gram-positive bacteria but with increased gram-negative spectrum
		Methicillin	Semisynthetic	Narrow-spectrum against gram-positive bacteria only, including strains producing penicillinase
	Cephalosporins	Cephalosporin C	Natural	Narrow-spectrum similar to penicillin but with increased gram-negative spectrum
		First-generation cephalosporins	Semisynthetic	Narrow-spectrum similar to cephalosporin C
		Second-generation cephalosporins	Semisynthetic	Narrow-spectrum but with increased gram-negative spectrum compared with first generation
		Third- and fourth-generation cephalosporins	Semisynthetic	Broad-spectrum against gram-positive and gram-negative bacteria, including some β-lactamase producers
		Fifth-generation cephalosporins	Semisynthetic	Broad-spectrum against gram-positive and gram-negative bacteria, including MRSA
	Monobactams	Aztreonam	Semisynthetic	Narrow-spectrum against gram-negative bacteria, including some β-lactamase producers

Mechanism of Action	Drug Class	Specific Drugs	Natural or Semisynthetic	Spectrum of Activity
	Carbapenems	Imipenem, meropenem, doripenem	Semisynthetic	Broadest spectrum of the β -lactams against gram-positive and gram-negative bacteria, including many β -lactamase producers
Large molecules that bind to the peptide chain of peptidoglycan subunits, blocking transglycosylation and transpeptidation	Glycopeptides	Vancomycin	Natural	Narrow spectrum against gram-positive bacteria only, including multidrug-resistant strains
Block transport of peptidoglycan subunits across cytoplasmic membrane	Bacitracin	Bacitracin	Natural	Broad-spectrum against gram-positive and gram-negative bacteria

Key Concepts and Summary

- Antibacterial compounds exhibit **selective toxicity**, largely due to differences between prokaryotic and eukaryotic cell structure.
- Cell wall synthesis inhibitors, including the **β -lactams**, the **glycopeptides**, and **bacitracin**, interfere with peptidoglycan synthesis, making bacterial cells more prone to osmotic lysis.

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14.4: Inhibitors of Protein Synthesis

Learning Objectives

- Distinguish the protein synthesis inhibitors that target the large vs. the small ribosomal subunits.
- Compare and contrast aminoglycosides and tetracyclines in how they inhibit protein synthesis by binding to the 30S subunit.
- Explain the mechanisms of action of 50S large subunit inhibitors.

Inhibitors of Protein Biosynthesis

The cytoplasmic ribosomes found in animal cells (80S) are structurally distinct from those found in bacterial cells (70S), making protein biosynthesis a good selective target for antibacterial drugs. Several types of protein biosynthesis inhibitors are discussed in this section and are summarized in Figure 14.4.3.

Protein Synthesis Inhibitors That Bind the 30S Subunit

Aminoglycosides are large, highly polar antibacterial drugs that bind to the 30S subunit of bacterial ribosomes, impairing the proofreading ability of the ribosomal complex. This impairment causes mismatches between codons and anticodons, resulting in the production of proteins with incorrect amino acids and shortened proteins that insert into the cytoplasmic membrane. Disruption of the cytoplasmic membrane by the faulty proteins kills the bacterial cells. The aminoglycosides, which include drugs such as streptomycin, gentamicin, neomycin, and kanamycin, are potent broad-spectrum antibacterials. However, aminoglycosides have been shown to be nephrotoxic (damaging to kidney), neurotoxic (damaging to the nervous system), and ototoxic (damaging to the ear).

Another class of antibacterial compounds that bind to the 30S subunit is the **tetracyclines**. In contrast to aminoglycosides, these drugs are bacteriostatic and inhibit protein synthesis by blocking the association of tRNAs with the ribosome during translation. Naturally occurring tetracyclines produced by various strains of *Streptomyces* were first discovered in the 1940s, and several semisynthetic tetracyclines, including doxycycline and tigecycline have also been produced. Although the tetracyclines are broad spectrum in their coverage of bacterial pathogens, side effects that can limit their use include phototoxicity, permanent discoloration of developing teeth, and liver toxicity with high doses or in patients with kidney impairment.

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Protein Synthesis Inhibitors That Bind the 50S Subunit

There are several classes of antibacterial drugs that work through binding to the 50S subunit of bacterial ribosomes. The **macrolide** antibacterial drugs have a large, complex ring structure and are part of a larger class of naturally produced secondary metabolites called polyketides, complex compounds produced in a stepwise fashion through the repeated addition of two-carbon units by a mechanism similar to that used for fatty acid synthesis. Macrolides are broad-spectrum, bacteriostatic drugs that block elongation of proteins by inhibiting peptide bond formation between specific combinations of amino acids. The first macrolide was **erythromycin**. It was isolated in 1952 from *Streptomyces erythreus* and prevents translocation. Semisynthetic macrolides include azithromycin and telithromycin. Compared with erythromycin, **azithromycin** has a broader spectrum of activity, fewer side effects, and a significantly longer half-life (1.5 hours for erythromycin versus 68 hours for azithromycin) that allows for once-daily

dosing and a short 3-day course of therapy (i.e., Zpac formulation) for most infections. Telithromycin is the first semisynthetic within the class known as ketolides. Although telithromycin shows increased potency and activity against macrolide-resistant pathogens, the US Food and Drug Administration (FDA) has limited its use to treatment of community-acquired pneumonia and requires the strongest “black box warning” label for the drug because of serious hepatotoxicity.

The **lincosamides** include the naturally produced **lincomycin** and semisynthetic **clindamycin**. Although structurally distinct from macrolides, lincosamides are similar in their mode of action to the macrolides through binding to the 50S ribosomal subunit and preventing peptide bond formation. Lincosamides are particularly active against streptococcal and staphylococcal infections.

The drug **chloramphenicol** represents yet another structurally distinct class of antibacterials that also bind to the 50S ribosome, inhibiting peptide bond formation. Chloramphenicol, produced by *Streptomyces venezuelae*, was discovered in 1947; in 1949, it became the first broad-spectrum antibiotic that was approved by the FDA. Although it is a natural antibiotic, it is also easily synthesized and was the first antibacterial drug synthetically mass produced. As a result of its mass production, broad-spectrum coverage, and ability to penetrate into tissues efficiently, chloramphenicol was historically used to treat a wide range of infections, from meningitis to typhoid fever to conjunctivitis. Unfortunately, serious side effects, such as lethal gray baby syndrome, and suppression of bone marrow production, have limited its clinical role. Chloramphenicol also causes anemia in two different ways. One mechanism involves the targeting of mitochondrial ribosomes within hematopoietic stem cells, causing a reversible, dose-dependent suppression of blood cell production. Once chloramphenicol dosing is discontinued, blood cell production returns to normal. This mechanism highlights the similarity between 70S ribosomes of bacteria and the 70S ribosomes within our mitochondria. The second mechanism of anemia is idiosyncratic (i.e., the mechanism is not understood), and involves an irreversible lethal loss of blood cell production known as aplastic anemia. This mechanism of aplastic anemia is not dose dependent and can develop after therapy has stopped. Because of toxicity concerns, chloramphenicol usage in humans is now rare in the United States and is limited to severe infections unable to be treated by less toxic antibiotics. Because its side effects are much less severe in animals, it is used in veterinary medicine.

The **oxazolidinones**, including linezolid, are a new broad-spectrum class of synthetic protein synthesis inhibitors that bind to the 50S ribosomal subunit of both gram-positive and gram-negative bacteria. However, their mechanism of action seems somewhat different from that of the other 50S subunit-binding protein synthesis inhibitors already discussed. Instead, they seem to interfere with formation of the initiation complex (association of the 50S subunit, 30S subunit, and other factors) for translation, and they prevent translocation of the growing protein from the ribosomal A site to the P site. Table 14.4.3 summarizes the protein synthesis inhibitors.

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Major classes of protein synthesis-inhibiting antibacterials

Chloramphenicol, macrolides, and lincosamides

- Bind to the 50S ribosomal subunit
- Prevent peptide bond formation
- Stop protein synthesis

Aminoglycosides

- Bind to the 30S ribosomal subunit
- Impair proofreading, resulting in production of faulty proteins

Tetracyclines

- Bind to the 30S ribosomal subunit
- Block the binding of tRNAs, thereby inhibiting protein synthesis

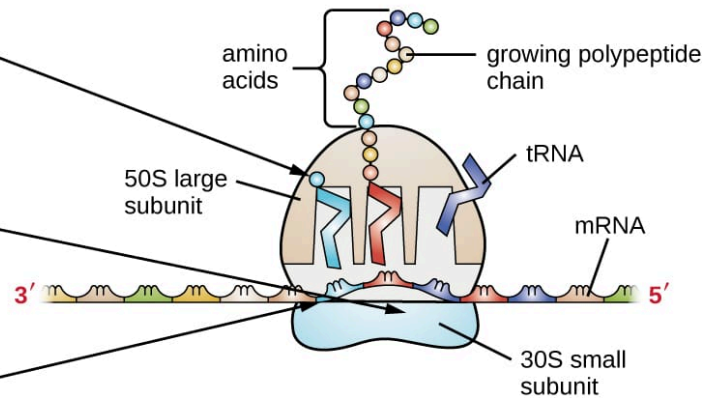


Figure 14.4.3: The major classes of protein synthesis inhibitors target the 30S or 50S subunits of cytoplasmic ribosomes.

Table 14.4.3: Drugs That Inhibit Bacterial Protein Synthesis

Molecular Target	Mechanism of Action	Drug Class	Specific Drugs	Bacteriostatic or Bactericidal	Spectrum of Activity
30S subunit	Causes mismatches between codons and anticodons, leading to faulty proteins that insert into and disrupt cytoplasmic membrane	Aminoglycosides	Streptomycin, gentamicin, neomycin, kanamycin	Bactericidal	Broad spectrum
	Blocks association of tRNAs with ribosome	Tetracyclines	Tetracycline, doxycycline, tigecycline	Bacteriostatic	Broad spectrum
50S subunit	Blocks peptide bond formation between amino acids	Macrolides	Erythromycin, azithromycin, telithromycin	Bacteriostatic	Broad spectrum
		Lincosamides	Lincomycin, clindamycin	Bacteriostatic	Narrow spectrum
	Interferes with the formation of the initiation complex between 50S and 30S subunits and other factors.	Not applicable	Chloramphenicol	Bacteriostatic	Broad spectrum
		Oxazolidinones	Linezolid	Bacteriostatic	Broad spectrum

Key Concepts and Summary

- There are a variety of broad-spectrum, bacterial protein synthesis inhibitors that selectively target the prokaryotic 70S ribosome, including those that bind to the 30S subunit (**aminoglycosides** and **tetracyclines**) and others that bind to the 50S subunit (**macrolides**, **lincosamides**, **chloramphenicol**, and **oxazolidinones**).

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14.5: Other Antibiotics

Learning Objectives

- Explain how polymyxins inhibit membrane function.
- Contrast rifampin and fluoroquinolones in their actions to block nucleic acid synthesis.
- Summarize metabolite inhibitors and why they have high selective toxicity.

Inhibitors of Membrane Function

A small group of antibacterials target the bacterial membrane as their mode of action (Table 14.5.4). The **polymyxins** are natural polypeptide antibiotics that were first discovered in 1947 as products of *Bacillus polymyxa*; only **polymyxin B** and **polymyxin E (colistin)** have been used clinically. They are lipophilic with detergent-like properties and interact with the lipopolysaccharide component of the outer membrane of gram-negative bacteria, ultimately disrupting both their outer and inner membranes and killing the bacterial cells. Unfortunately, the membrane-targeting mechanism is not a selective toxicity, and these drugs also target and damage the membrane of cells in the kidney and nervous system when administered systemically. Because of these serious side effects and their poor absorption from the digestive tract, polymyxin B is used in over-the-counter topical antibiotic ointments (e.g., Neosporin), and oral colistin was historically used only for bowel decontamination to prevent infections originating from bowel microbes in immunocompromised patients or for those undergoing certain abdominal surgeries. However, the emergence and spread of multidrug-resistant pathogens has led to increased use of intravenous colistin in hospitals, often as a drug of last resort to treat serious infections. The antibacterial **daptomycin** is a cyclic lipopeptide produced by *Streptomyces roseosporus* that seems to work like the polymyxins, inserting in the bacterial cell membrane and disrupting it. However, in contrast to polymyxin B and colistin, which target only gram-negative bacteria, daptomycin specifically targets gram-positive bacteria. It is typically administered intravenously and seems to be well tolerated, showing reversible toxicity in skeletal muscles.

Table 14.5.4: Drugs That Inhibit Bacterial Membrane Function

Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity	Clinical Use
Interacts with lipopolysaccharide in the outer membrane of gram-negative bacteria, killing the cell through the eventual disruption of the outer membrane and cytoplasmic membrane	Polymyxins	Polymyxin B	Narrow spectrum against gram-negative bacteria, including multidrug-resistant strains	Topical preparations to prevent infections in wounds
		Polymyxin E (colistin)	Narrow spectrum against gram-negative bacteria, including multidrug-resistant strains	Oral dosing to decontaminate bowels to prevent infections in immunocompromised patients or patients undergoing invasive surgery/procedures.
				Intravenous dosing to treat serious systemic infections caused by multidrug-resistant pathogens
Inserts into the cytoplasmic membrane of gram-positive bacteria, disrupting the membrane and killing the cell	Lipopeptide	Daptomycin	Narrow spectrum against gram-positive bacteria, including multidrug-resistant strains	Complicated skin and skin-structure infections and bacteremia caused by gram-positive pathogens, including MRSA

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Inhibitors of Nucleic Acid Synthesis

Some antibacterial drugs work by inhibiting nucleic acid synthesis (Table 14.5.5). For example, **metronidazole** is a semisynthetic member of the nitroimidazole family that is also an antiprotozoan. It interferes with DNA replication in target cells. The drug **rifampin** is a semisynthetic member of the rifamycin family and functions by blocking RNA polymerase activity in bacteria. The RNA polymerase enzymes in bacteria are structurally different from those in eukaryotes, providing for selective toxicity against bacterial cells. It is used for the treatment of a variety of infections, but its primary use, often in a cocktail with other antibacterial drugs, is against mycobacteria that cause tuberculosis. Despite the selectivity of its mechanism, rifampin can induce liver enzymes to increase metabolism of other drugs being administered (antagonism), leading to hepatotoxicity (liver toxicity) and negatively influencing the bioavailability and therapeutic effect of the companion drugs.

One member of the **quinolone family**, a group of synthetic antimicrobials, is **nalidixic acid**. It was discovered in 1962 as a byproduct during the synthesis of chloroquine, an antimalarial drug. Nalidixic acid selectively inhibits the activity of bacterial DNA gyrase, blocking DNA replication. Chemical modifications to the original quinolone backbone have resulted in the production of fluoroquinolones, like ciprofloxacin and levofloxacin, which also inhibit the activity of DNA gyrase. Ciprofloxacin and levofloxacin are effective against a broad spectrum of gram-positive or gram-negative bacteria, and are among the most commonly prescribed antibiotics used to treat a wide range of infections, including urinary tract infections, respiratory infections, abdominal infections, and skin infections. However, despite their selective toxicity against DNA gyrase, side effects associated with different fluoroquinolones include phototoxicity, neurotoxicity, cardiotoxicity, glucose metabolism dysfunction, and increased risk for tendon rupture.

Table 14.5.5: Drugs That Inhibit Bacterial Nucleic Acid Synthesis

Mechanisms of Action	Drug Class	Specific Drugs	Spectrum of activity	Clinical Use
Inhibits bacterial RNA polymerase activity and blocks transcription, killing the cell	Rifamycin	Rifampin	Narrow spectrum with activity against gram-positive and limited numbers of gram-negative bacteria. Also active against <i>Mycobacterium tuberculosis</i> .	Combination therapy for treatment of tuberculosis
Inhibits the activity of DNA gyrase and blocks DNA replication, killing the cell	Fluoroquinolones	Ciprofloxacin, ofloxacin, moxifloxacin	Broad spectrum against gram-positive and gram-negative bacteria	Wide variety of skin and systemic infections

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Inhibitors of Metabolic Pathways

Some synthetic drugs control bacterial infections by functioning as **antimetabolites**, competitive inhibitors for bacterial metabolic enzymes (Table 14.5.6). The **sulfonamides (sulfa drugs)** are the oldest synthetic antibacterial agents and are structural analogues of *para*-aminobenzoic acid (PABA), an early intermediate in folic acid synthesis (Figure 14.5.4). By inhibiting the enzyme involved in the production of dihydrofolic acid, sulfonamides block bacterial biosynthesis of folic acid and, subsequently, pyrimidines and purines required for nucleic acid synthesis. This mechanism of action provides bacteriostatic inhibition of growth against a wide spectrum of gram-positive and gram-negative pathogens. Because humans obtain folic acid from food instead of synthesizing it intracellularly, sulfonamides are selectively toxic for bacteria. However, allergic reactions to sulfa drugs are common. The sulfones are structurally similar to sulfonamides but are not commonly used today except for the treatment of Hansen's disease (leprosy).

Trimethoprim is a synthetic antimicrobial compound that serves as an antimetabolite within the same folic acid synthesis pathway as sulfonamides. However, trimethoprim is a structural analogue of dihydrofolic acid and inhibits a later step in the metabolic pathway (Figure 14.5.4). Trimethoprim is used in combination with the sulfa drug sulfamethoxazole to treat urinary tract infections, ear infections, and bronchitis. As discussed, the combination of trimethoprim and sulfamethoxazole is an example of **antibacterial synergy**. When used alone, each antimetabolite only decreases production of folic acid to a level where bacteriostatic inhibition of growth occurs. However, when used in combination, inhibition of both steps in the metabolic pathway decreases folic acid synthesis to a level that is lethal to the bacterial cell. Because of the importance of folic acid during fetal development, sulfa drugs and trimethoprim use should be carefully considered during early pregnancy.

The drug isoniazid is an antimetabolite with specific toxicity for mycobacteria and has long been used in combination with rifampin or streptomycin in the treatment of tuberculosis. It is administered as a prodrug, requiring activation through the action of an intracellular bacterial peroxidase enzyme, forming isoniazid-nicotinamide adenine dinucleotide (NAD) and isoniazid-nicotinamide adenine dinucleotide phosphate (NADP), ultimately preventing the synthesis of mycolic acid, which is essential for mycobacterial cell walls. Possible side effects of isoniazid use include hepatotoxicity, neurotoxicity, and hematologic toxicity (anemia).

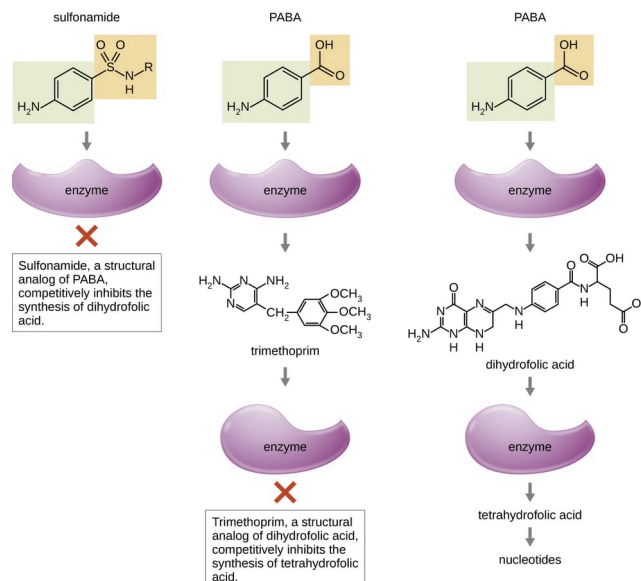


Figure 14.5.4: Sulfonamides and trimethoprim are examples of antimetabolites that interfere in the bacterial synthesis of folic acid by blocking purine and pyrimidine biosynthesis, thus inhibiting bacterial growth.

Table 14.5.6: Antimetabolite Drugs

Metabolic Pathway Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
Folic acid synthesis	Inhibits the enzyme involved in production of dihydrofolic acid	Sulfonamides	Sulfamethoxazole	Broad spectrum against gram-positive and gram-negative bacteria
		Sulfones	Dapsone	

Metabolic Pathway Target	Mechanism of Action	Drug Class	Specific Drugs	Spectrum of Activity
	Inhibits the enzyme involved in the production of tetrahydrofolic acid	Not applicable	Trimethoprim	Broad spectrum against gram-positive and gram-negative bacteria
Mycolic acid synthesis	Interferes with the synthesis of mycolic acid	Not applicable	Isoniazid	Narrow spectrum against <i>Mycobacterium</i> spp., including <i>M. tuberculosis</i>

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Inhibitor of ATP Synthase

Bedaquiline, representing the synthetic antibacterial class of compounds called the diarylquinolones, uses a novel mode of action that specifically inhibits mycobacterial growth. Although the specific mechanism has yet to be elucidated, this compound appears to interfere with the function of ATP synthases, perhaps by interfering with the use of the hydrogen ion gradient for ATP synthesis by oxidative phosphorylation, leading to reduced ATP production. Due to its side effects, including hepatotoxicity and potentially lethal heart arrhythmia, its use is reserved for serious, otherwise untreatable cases of tuberculosis.

Key Concepts and Summary

- **Polymyxins** are lipophilic polypeptide antibiotics that target the lipopolysaccharide component of gram-negative bacteria and ultimately disrupt the integrity of the outer and inner membranes of these bacteria.
- The nucleic acid synthesis inhibitors rifamycins and **fluoroquinolones** target bacterial RNA transcription and DNA replication, respectively.
- Some antibacterial drugs are **antimetabolites**, acting as competitive inhibitors for bacterial metabolic enzymes.

Sulfonamides and **trimethoprim** are antimetabolites that interfere with bacterial folic acid synthesis. **Isoniazid** is an antimetabolite that interferes with mycolic acid synthesis in mycobacteria.

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14.E: Antimicrobial Drugs (Exercises)

14.1: Discovering Antimicrobial Drugs

Antimicrobial drugs produced by purposeful fermentation and/or contained in plants have been used as traditional medicines in many cultures for millennia. The purposeful and systematic search for a chemical “magic bullet” that specifically target infectious microbes was initiated by Paul Ehrlich in the early 20th century. The discovery of the natural antibiotic, penicillin, by Alexander Fleming in 1928 started the modern age of antimicrobial discovery and research.

Multiple Choice

A scientist discovers that a soil bacterium he has been studying produces an antimicrobial that kills gram-negative bacteria. She isolates and purifies the antimicrobial compound, then chemically converts a chemical side chain to a hydroxyl group. When she tests the antimicrobial properties of this new version, she finds that this antimicrobial drug can now also kill gram-positive bacteria. The new antimicrobial drug with broad-spectrum activity is considered to be which of the following?

- A. resistant
- B. semisynthetic
- C. synthetic
- D. natural

Answer

B

Which of the following antimicrobial drugs is synthetic?

- A. sulfanilamide
- B. penicillin
- C. actinomycin
- D. neomycin

Answer

A

Fill in the Blank

The group of soil bacteria known for their ability to produce a wide variety of antimicrobials is called the _____.

Answer

actinomycetes

Short Answer

Where do antimicrobials come from naturally? Why?

Why was Salvarsan considered to be a “magic bullet” for the treatment of syphilis?

Critical Thinking

In nature, why do antimicrobial-producing microbes commonly also have antimicrobial resistance genes?

14.2: Antibacterial Drugs

Antimicrobial drugs can be bacteriostatic or bactericidal, and these characteristics are important considerations when selecting the most appropriate drug. The use of narrow-spectrum antimicrobial drugs is preferred in many cases to avoid superinfection and the development of antimicrobial resistance. Broad-spectrum antimicrobial use is warranted for serious systemic infections when there is no time to determine the causative agent or when narrow-spectrum antimicrobials fail.

Multiple Choice

Which of the following combinations would most likely contribute to the development of a superinfection?

- A. long-term use of narrow-spectrum antimicrobials
- B. long-term use of broad-spectrum antimicrobials
- C. short-term use of narrow-spectrum antimicrobials
- D. short-term use of broad-spectrum antimicrobials

Answer

B

Which of the following routes of administration would be appropriate and convenient for home administration of an antimicrobial to treat a systemic infection?

- A. oral
- B. intravenous
- C. topical
- D. parenteral

Answer

A

Which clinical situation would be appropriate for treatment with a narrow-spectrum antimicrobial drug?

- A. treatment of a polymicrobial mixed infection in the intestine
- B. prophylaxis against infection after a surgical procedure
- C. treatment of strep throat caused by culture identified *Streptococcus pyogenes*
- D. empiric therapy of pneumonia while waiting for culture results

Answer

C

Fill in the Blank

The bacterium known for causing pseudomembranous colitis, a potentially deadly superinfection, is _____.

Answer

Clostridium difficile

True/False

Narrow-spectrum antimicrobials are commonly used for prophylaxis following surgery.

Answer

False

Short Answer

When prescribing antibiotics, what aspects of the patient's health history should the clinician ask about and why?

When is using a broad-spectrum antimicrobial drug warranted?

Critical Thinking

Why are yeast infections a common type of superinfection that results from long-term use of broad-spectrum antimicrobials?

Too often patients will stop taking antimicrobial drugs before the prescription is finished. What are factors that cause a patient to stop too soon, and what negative impacts could this have?

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15: ANTIBIOTIC RESISTANCE AND OTHER ANTIMICROBIALS

In nature, microbes have evolved mechanisms to resist antimicrobial substances produced by their competitors, ensuring their survival in competitive environments. These natural abilities have been amplified by human activity, as the widespread use—and misuse—of antibiotics and other antimicrobials has driven the rapid emergence and spread of antimicrobial resistance (AMR). Multidrug-resistant organisms, such as MRSA and drug-resistant tuberculosis, now pose significant challenges to global health, making once-treatable infections increasingly difficult to manage.

In this chapter, we will explore the mechanisms by which microbes develop resistance, the factors contributing to the AMR crisis, and the implications for public health. Additionally, we will examine alternatives to antibiotics, including antifungal, antiviral, and antiparasitic drugs, highlighting their mechanisms of action and the challenges of resistance in these domains. Finally, we will discuss strategies for combating antimicrobial resistance, from stewardship programs to the development of novel therapies.

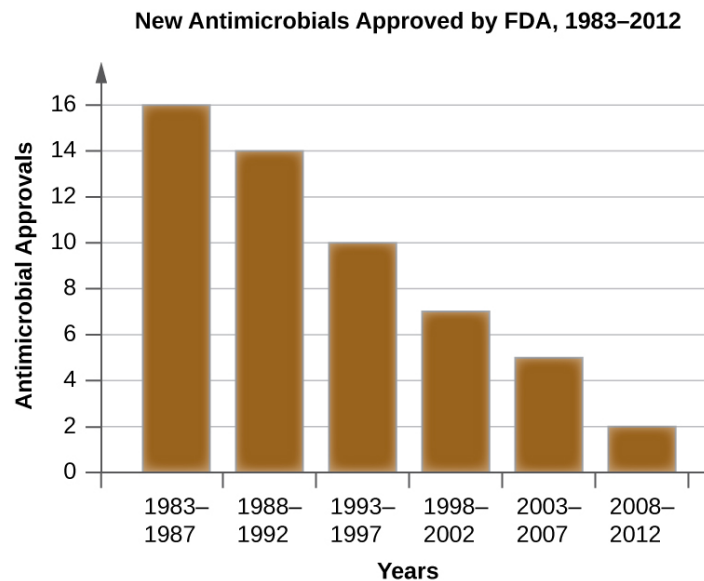


Figure 15.1: In recent decades, approvals of new antimicrobials by the FDA have steadily fallen. In the five-year period from 1983–1987, 16 new antimicrobial drugs were approved, compared to just two from 2008–2012.

15.1: Antifungal and Antiviral Drugs

15.2: Drug Resistance

15.3: Multidrug Resistant Microbes

15.4: Testing the Effectiveness of Antimicrobials

15.5: Current Strategies for Antimicrobial Discovery

15.E: Antimicrobial Drugs (Exercises)

Footnotes

- 1 “Treatment of War Wounds: A Historical Review.” *Clinical Orthopaedics and Related Research* 467 no. 8 (2009):2168–2191.

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15.1: Antifungal and Antiviral Drugs

Learning Objectives

- Explain how disruption of ergosterol biosynthesis is an effective mode of action for antifungals
- Describe how certain nucleoside analogs act as effective antiviral drugs
- Explain antiretroviral therapy (ART) for treating HIV infections

Because fungi, protozoa, and helminths are eukaryotic, their cells are very similar to human cells, making it more difficult to develop drugs with selective toxicity. Additionally, viruses replicate within human host cells, making it difficult to develop drugs that are selectively toxic to viruses or virus-infected cells. Despite these challenges, there are antimicrobial drugs that target fungi, protozoa, helminths, and viruses, and some even target more than one type of microbe. Table 15.1.1, Table 15.1.2, Table 15.1.3, and Table 15.1.4 provide examples for antimicrobial drugs in these various classes.

Antifungal Drugs

The most common mode of action for antifungal drugs is the disruption of the cell membrane. Antifungals take advantage of small differences between fungi and humans in the biochemical pathways that synthesize sterols. The sterols are important in maintaining proper membrane fluidity and, hence, proper function of the cell membrane. For most fungi, the predominant membrane sterol is **ergosterol**. Because human cell membranes use **cholesterol**, instead of ergosterol, antifungal drugs that target ergosterol synthesis are selectively toxic (Figure 15.1.1).

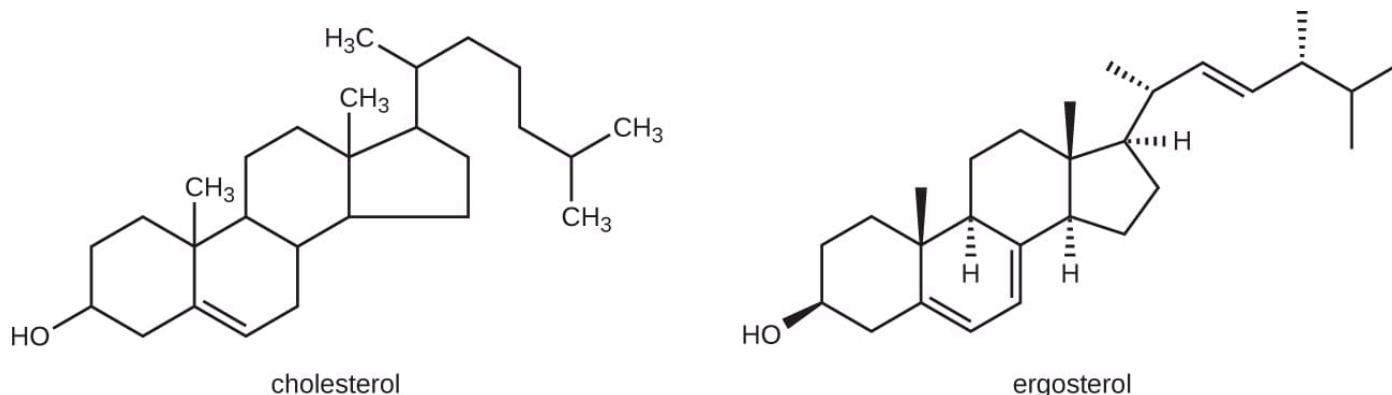


Figure 15.1.1: The predominant sterol found in human cells is cholesterol, whereas the predominant sterol found in fungi is ergosterol, making ergosterol a good target for antifungal drug development.

The **imidazoles** are synthetic fungicides that disrupt ergosterol biosynthesis; they are commonly used in medical applications and also in agriculture to keep seeds and harvested crops from molding. Examples include miconazole, ketoconazole, and clotrimazole, which are used to treat fungal skin infections such as ringworm, specifically tinea pedis (athlete's foot), tinea cruris (jock itch), and tinea corporis. These infections are commonly caused by dermatophytes of the genera *Trichophyton*, *Epidermophyton*, and *Microsporum*. Miconazole is also used predominantly for the treatment of vaginal yeast infections caused by the fungus *Candida*, and ketoconazole is used for the treatment of tinea versicolor and dandruff, which both can be caused by the fungus *Malassezia*.

The **triazole** drugs, including **fluconazole**, also inhibit ergosterol biosynthesis. However, they can be administered orally or intravenously for the treatment of several types of systemic yeast infections, including oral thrush and cryptococcal meningitis, both of which are prevalent in patients with AIDS. The triazoles also exhibit more selective toxicity, compared with the imidazoles, and are associated with fewer side effects.

The **allylamines**, a structurally different class of synthetic antifungal drugs, inhibit an earlier step in ergosterol biosynthesis. The most commonly used allylamine is **terbinafine** (marketed under the brand name Lamisil), which is used topically for the treatment of dermatophytic skin infections like athlete's foot, ringworm, and jock itch. Oral treatment with terbinafine is also used for the

treatment of fingernail and toenail fungus, but it can be associated with the rare side effect of hepatotoxicity.

The **polyenes** are a class of antifungal agents naturally produced by certain actinomycete soil bacteria and are structurally related to macrolides. These large, lipophilic molecules bind to ergosterol in fungal cytoplasmic membranes, thus creating pores. Common examples include nystatin and **amphotericin B**. Nystatin is typically used as a topical treatment for yeast infections of the skin, mouth, and vagina, but may also be used for intestinal fungal infections. The drug amphotericin B is used for systemic fungal infections like aspergillosis, cryptococcal meningitis, histoplasmosis, blastomycosis, and candidiasis. Amphotericin B was the only antifungal drug available for several decades, but its use is associated with some serious side effects, including nephrotoxicity (kidney toxicity).

Amphotericin B is often used in combination with flucytosine, a fluorinated pyrimidine analog that is converted by a fungal-specific enzyme into a toxic product that interferes with both DNA replication and protein synthesis in fungi. Flucytosine is also associated with hepatotoxicity (liver toxicity) and bone marrow depression.

Beyond targeting ergosterol in fungal cell membranes, there are a few antifungal drugs that target other fungal structures (Figure 15.1.2). The echinocandins, including caspofungin, are a group of naturally produced antifungal compounds that block the synthesis of $\beta(1\rightarrow3)$ glucan found in fungal cell walls but not found in human cells. This drug class has the nickname “penicillin for fungi.” Caspofungin is used for the treatment of aspergillosis as well as systemic yeast infections.

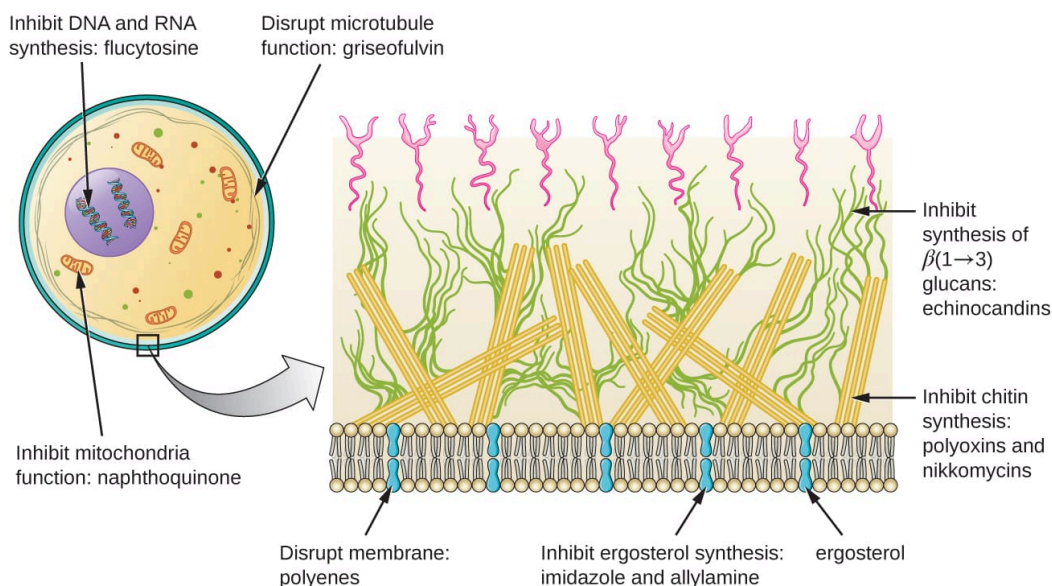


Figure 15.1.2: Antifungal drugs target several different cell structures. (credit right: modification of work by “Maya and Rike”/Wikimedia Commons)

Although chitin is only a minor constituent of fungal cell walls, it is also absent in human cells, making it a selective target. The polyoxins and nikkomycins are naturally produced antifungals that target chitin synthesis. Polyoxins are used to control fungi for agricultural purposes, and nikkomycin Z is currently under development for use in humans to treat yeast infections and Valley fever (coccidioidomycosis), a fungal disease prevalent in the southwestern US.¹

The naturally produced antifungal griseofulvin is thought to specifically disrupt fungal cell division by interfering with microtubules involved in spindle formation during mitosis. It was one of the first antifungals, but its use is associated with hepatotoxicity. It is typically administered orally to treat various types of dermatophytic skin infections when other topical antifungal treatments are ineffective.

There are a few drugs that act as antimetabolites against fungal processes. For example, atovaquone, a representative of the naphthoquinone drug class, is a semisynthetic antimetabolite for fungal and protozoal versions of a mitochondrial cytochrome important in electron transport. Structurally, it is an analog of coenzyme Q, with which it competes for electron binding. It is particularly useful for the treatment of *Pneumocystis* pneumonia caused by *Pneumocystis jirovecii*. The antibacterial sulfamethoxazole-trimethoprim combination also acts as an antimetabolite against *P. jirovecii*.

Table 15.1.1 shows the various therapeutic classes of antifungal drugs, categorized by mode of action, with examples of each.

Table 15.1.1: Common Antifungal Drugs

Mechanism of Action	Drug Class	Specific Drugs	Clinical Uses
Inhibit ergosterol synthesis	Imidazoles	Miconazole, ketoconazole, clotrimazole	Fungal skin infections and vaginal yeast infections
	Triazoles	Fluconazole	Systemic yeast infections, oral thrush, and cryptococcal meningitis
	Allylamines	Terbinafine	Dermatophytic skin infections (athlete's foot, ring worm, jock itch), and infections of fingernails and toenails
Bind ergosterol in the cell membrane and create pores that disrupt the membrane	Polyenes	Nystatin	Used topically for yeast infections of skin, mouth, and vagina; also used for fungal infections of the intestine
		Amphotericin B	Variety systemic fungal infections
Inhibit cell wall synthesis	Echinocandins	Caspofungin	Aspergillosis and systemic yeast infections
	Not applicable	Nikkomycin Z	Coccidioidomycosis (Valley fever) and yeast infections
Inhibit microtubules and cell division	Not applicable	Griseofulvin	Dermatophytic skin infections

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Treating a Fungal Infection of the Lungs

Jack, a 48-year-old engineer, is HIV positive but generally healthy thanks to antiretroviral therapy (ART). However, after a particularly intense week at work, he developed a fever and a dry cough. He assumed that he just had a cold or mild flu due to overexertion and didn't think much of it. However, after about a week, he began to experience fatigue, weight loss, and shortness of breath. He decided to visit his physician, who found that Jack had a low level of blood oxygenation. The physician ordered blood testing, a chest X-ray, and the collection of an induced sputum sample for analysis. His X-ray showed a fine cloudiness and several pneumatoceles (thin-walled pockets of air), which indicated *Pneumocystis pneumonia* (PCP), a type of pneumonia caused by the fungus *Pneumocystis jirovecii*. Jack's physician admitted him to the hospital and prescribed Bactrim, a combination of sulfamethoxazole and trimethoprim, to be administered intravenously.

P. jirovecii is a yeast-like fungus with a life cycle similar to that of protozoans. As such, it was classified as a protozoan until the 1980s. It lives only in the lung tissue of infected persons and is transmitted from person to person, with many people exposed as children. Typically, *P. jirovecii* only causes pneumonia in immunocompromised individuals. Healthy people may carry the fungus in their lungs with no symptoms of disease. PCP is particularly problematic among HIV patients with compromised immune systems.

PCP is usually treated with oral or intravenous Bactrim, but atovaquone or pentamidine (another antiparasitic drug) are alternatives. If not treated, PCP can progress, leading to a collapsed lung and nearly 100% mortality. Even with antimicrobial drug therapy, PCP still is responsible for 10% of HIV-related deaths.

The cytological examination, using direct immunofluorescence assay (DFA), of a smear from Jack's sputum sample confirmed the presence of *P. jirovecii* (Figure 15.1.3). Additionally, the results of Jack's blood tests revealed that his white blood cell count had dipped, making him more susceptible to the fungus. His physician reviewed his ART regimen and made adjustments. After a few days of hospitalization, Jack was released to continue his antimicrobial therapy at home. With the adjustments to his ART therapy, Jack's CD4 counts began to increase and he was able to go back to work.

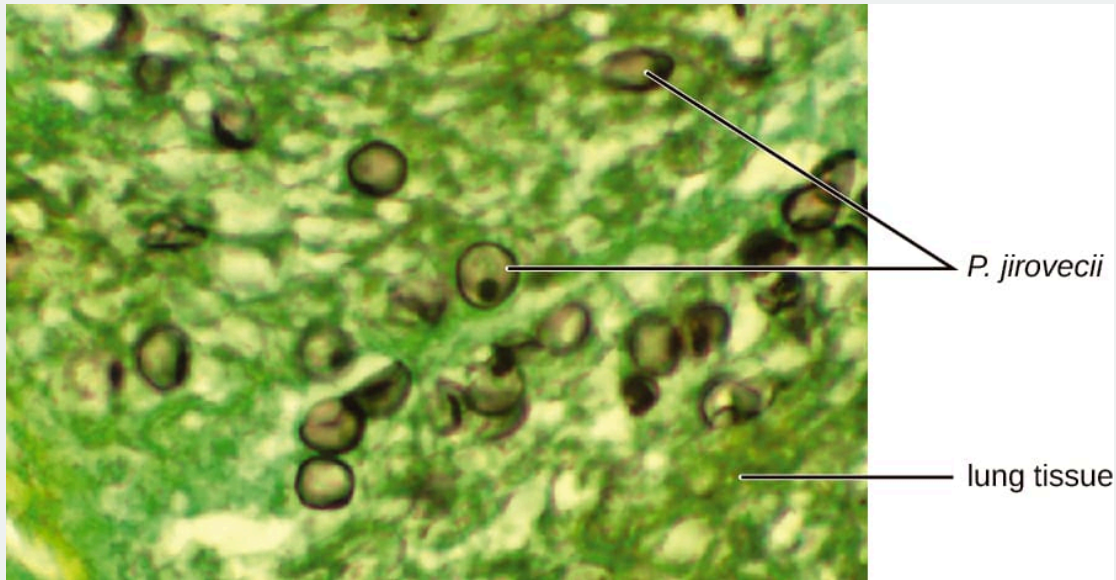


Figure 15.1.3: Microscopic examination of an induced sputum sample or bronchoalveolar lavage sample typically reveals the organism, as shown here. (credit: modification of work by the Centers for Disease Control and Prevention)

Antiviral Drugs

Unlike the complex structure of fungi, protozoa, and helminths, viral structure is simple, consisting of nucleic acid, a protein coat, viral enzymes, and, sometimes, a lipid envelope. Furthermore, viruses are obligate intracellular pathogens that use the host's cellular machinery to replicate. These characteristics make it difficult to develop drugs with selective toxicity against viruses.

Many antiviral drugs are **nucleoside analogs** and function by inhibiting nucleic acid biosynthesis. For example, **acyclovir** (marketed as Zovirax) is a synthetic analog of the nucleoside guanosine (Figure 15.1.4). It is activated by the herpes simplex viral enzyme thymidine kinase and, when added to a growing DNA strand during replication, causes chain termination. Its specificity for virus-infected cells comes from both the need for a viral enzyme to activate it and the increased affinity of the activated form for viral DNA polymerase compared to host cell DNA polymerase. Acyclovir and its derivatives are frequently used for the treatment of herpes virus infections, including genital herpes, chickenpox, shingles, Epstein-Barr virus infections, and cytomegalovirus infections. Acyclovir can be administered either topically or systemically, depending on the infection. One possible side effect of its use includes nephrotoxicity. The drug adenine-arabinside, marketed as vidarabine, is a synthetic analog to deoxyadenosine that has a mechanism of action similar to that of acyclovir. It is also effective for the treatment of various human herpes viruses. However, because of possible side effects involving low white blood cell counts and neurotoxicity, treatment with acyclovir is now preferred.

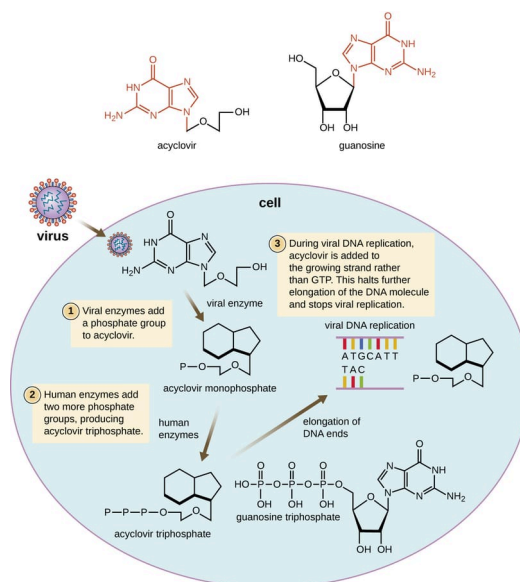


Figure 15.1.4: Acyclovir is a structural analog of guanosine. It is specifically activated by the viral enzyme thymidine kinase and then preferentially binds to viral DNA polymerase, leading to chain termination during DNA replication.

Ribavirin, another synthetic guanosine analog, works by a mechanism of action that is not entirely clear. It appears to interfere with both DNA and RNA synthesis, perhaps by reducing intracellular pools of guanosine triphosphate (GTP). Ribavirin also appears to inhibit the RNA polymerase of hepatitis C virus. It is primarily used for the treatment of the RNA viruses like hepatitis C (in combination therapy with interferon) and respiratory syncytial virus. Possible side effects of ribavirin use include anemia and developmental effects on unborn children in pregnant patients. In recent years, another nucleotide analog, sofosbuvir (Solvaldi), has also been developed for the treatment of hepatitis C. Sofosbuvir is a uridine analog that interferes with viral polymerase activity. It is commonly coadministered with ribavirin, with and without interferon.

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Inhibition of nucleic acid synthesis is not the only target of synthetic antivirals. Although the mode of action of **amantadine** and its relative **rimantadine** are not entirely clear, these drugs appear to bind to a transmembrane protein that is involved in the escape of the influenza virus from endosomes. Blocking escape of the virus also prevents viral RNA release into host cells and subsequent viral replication. Increasing resistance has limited the use of amantadine and rimantadine in the treatment of influenza A. Use of amantadine can result in neurological side effects, but the side effects of rimantadine seem less severe. Interestingly, because of their effects on brain chemicals such as dopamine and NMDA (N-methyl D-aspartate), amantadine and rimantadine are also used for the treatment of Parkinson's disease.

Neuraminidase inhibitors, including oseltamivir (Tamiflu), zanamivir (Relenza), and peramivir (Rapivab), specifically target **influenza viruses** by blocking the activity of influenza virus neuraminidase, preventing the release of the virus from infected cells. These three antivirals can decrease flu symptoms and shorten the duration of illness, but they differ in their modes of administration: oseltamivir is administered orally, zanamivir is inhaled, and peramivir is administered intravenously. Resistance to these neuraminidase inhibitors still seems to be minimal.

Pleconaril is a synthetic antiviral under development that showed promise for the treatment of picornaviruses. Use of pleconaril for the treatment of the common cold caused by rhinoviruses was not approved by the FDA in 2002 because of lack of proven effectiveness, lack of stability, and association with irregular menstruation. Its further development for this purpose was halted in 2007. However, pleconaril is still being investigated for use in the treatment of life-threatening complications of enteroviruses, such as meningitis and sepsis. It is also being investigated for use in the global eradication of a specific enterovirus, polio.⁶ Pleconaril seems to work by binding to the viral capsid and preventing the uncoating of viral particles inside host cells during viral infection.

Viruses with complex life cycles, such as HIV, can be more difficult to treat. First, HIV targets CD4-positive white blood cells, which are necessary for a normal immune response to infection. Second, HIV is a retrovirus, meaning that it converts its RNA genome into a DNA copy that integrates into the host cell's genome, thus hiding within host cell DNA. Third, the HIV reverse transcriptase lacks proofreading activity and introduces mutations that allow for rapid development of antiviral drug resistance. To help prevent the emergence of resistance, a combination of specific synthetic antiviral drugs is typically used in ART for HIV (Figure 15.1.5).

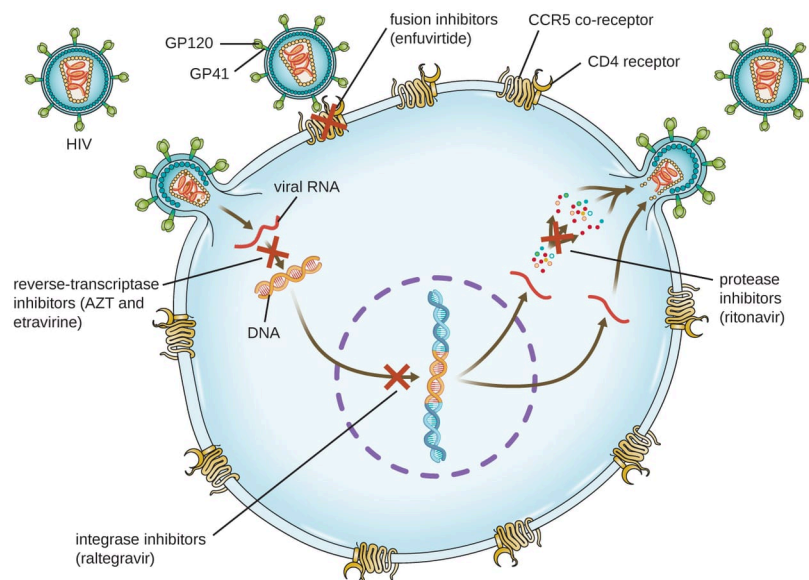


Figure 15.1.5: Antiretroviral therapy (ART) is typically used for the treatment of HIV. The targets of drug classes currently in use are shown here. (credit: modification of work by Thomas Spletstoesser)

The **reverse transcriptase inhibitors** block the early step of converting viral RNA genome into DNA, and can include competitive nucleoside analog inhibitors (e.g., azidothymidine/zidovudine, or AZT) and non-nucleoside noncompetitive inhibitors (e.g., efavirine) that bind reverse transcriptase and cause an inactivating conformational change. Drugs called **protease inhibitors** (e.g., ritonavir) block the processing of viral proteins and prevent viral maturation. Protease inhibitors are also being developed for the treatment of other viral types.⁷ For example, simeprevir (Olysio) has been approved for the treatment of hepatitis C and is administered with ribavirin and interferon in combination therapy. The **integrase inhibitors** (e.g., raltegravir), block the activity of the HIV integrase responsible for the recombination of a DNA copy of the viral genome into the host cell chromosome. Additional drug classes for HIV treatment include the CCR5 antagonists and the **fusion inhibitors** (e.g., enfuvirtide), which prevent the binding of HIV to the host cell coreceptor (chemokine receptor type 5 [CCR5]) and the merging of the viral envelope with the host cell membrane, respectively. Table 15.1.4 shows the various therapeutic classes of antiviral drugs, categorized by mode of action, with examples of each.

Query 15.1.1

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Table 15.1.4: Common Antiviral Drugs

Mechanism of Action	Drug	Clinical Uses
Nucleoside analog inhibition of nucleic acid synthesis	Acyclovir	Herpes virus infections
	Azidothymidine/zidovudine (AZT)	HIV infections
	Ribavirin	Hepatitis C virus and respiratory syncytial virus infections
	Vidarabine	Herpes virus infections
	Sofosbuvir	Hepatitis C virus infections
Non-nucleoside noncompetitive inhibition	Etravirine	HIV infections
Inhibit escape of virus from endosomes	Amantadine, rimantadine	Infections with influenza virus
Inhibit neuraminidase	Oseltamivir, zanamivir, peramivir	Infections with influenza virus
Inhibit viral uncoating	Pleconaril	Serious enterovirus infections
Inhibition of protease	Ritonavir	HIV infections
	Simeprevir	Hepatitis C virus infections
Inhibition of integrase	Raltegravir	HIV infections
Inhibition of membrane fusion	Enfuvirtide	HIV infections

Link to Learning

To learn more about the various classes of antiretroviral drugs used in the ART of HIV infection, explore each of the drugs in the HIV drug classes provided by US Department of Health and Human Services at [this](#) website.

Key Concepts and Summary

- Because fungi, protozoans, and helminths are eukaryotic organisms like human cells, it is more challenging to develop antimicrobial drugs that specifically target them. Similarly, it is hard to target viruses because human viruses replicate inside of human cells.
- Antifungal drugs** interfere with ergosterol synthesis, bind to ergosterol to disrupt fungal cell membrane integrity, or target cell wall-specific components or other cellular proteins.
- Antiviral drugs** inhibit viral entry, inhibit viral uncoating, inhibit nucleic acid biosynthesis, prevent viral escape from endosomes in host cells, and prevent viral release from infected cells.
- Because it can easily mutate to become drug resistant, HIV is typically treated with a combination of several **antiretroviral drugs**, which may include **reverse transcriptase inhibitors**, **protease inhibitors**, **integrase inhibitors**, and drugs that interfere with viral binding and fusion to initiate infection.

Footnotes

1. 1 Centers for Disease Control and Prevention. "Valley Fever: Awareness Is Key." www.cdc.gov/features/valleyfever/. Accessed June 1, 2016.
2. 2 B. Chu et al. "A Benzimidazole Derivative Exhibiting Antitumor Activity Blocks EGFR and HER2 Activity and Upregulates DR5 in Breast Cancer Cells." *Cell Death and Disease* 6 (2015):e1686
3. 3 J.-X. Pan et al. "Niclosamide, An Old Anthelmintic Agent, Demonstrates Antitumor Activity by Blocking Multiple Signaling Pathways of Cancer Stem Cells." *Chinese Journal of Cancer* 31 no. 4 (2012):178–184.
4. 4 F. Imperi et al. "New Life for an Old Drug: The Anthelmintic Drug Niclosamide Inhibits *Pseudomonas aeruginosa* Quorum Sensing." *Antimicrobial Agents and Chemotherapy* 57 no. 2 (2013):996–1005.
5. 5 A. Jurgeit et al. "Niclosamide Is a Proton Carrier and Targets Acidic Endosomes with Broad Antiviral Effects." *PLoS Pathogens* 8 no. 10 (2012):e1002976.
6. 6 M.J. Abzug. "The Enteroviruses: Problems in Need of Treatments." *Journal of Infection* 68 no. S1 (2014):108–14.
7. 7 B.L. Pearlman. "Protease Inhibitors for the Treatment of Chronic Hepatitis C Genotype-1 Infection: The New Standard of Care." *Lancet Infectious Diseases* 12 no. 9 (2012):717–728.

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15.2: Drug Resistance

Learning Objectives

- Explain the concept of drug resistance
- Describe how microorganisms develop or acquire drug resistance
- Describe the different mechanisms of antimicrobial drug resistance

Antimicrobial resistance is not a new phenomenon. In nature, microbes are constantly evolving in order to overcome the antimicrobial compounds produced by other microorganisms. Human development of antimicrobial drugs and their widespread clinical use has simply provided another selective pressure that promotes further evolution. Several important factors can accelerate the evolution of **drug resistance**. These include the overuse and misuse of antimicrobials, inappropriate use of antimicrobials, subtherapeutic dosing, and patient noncompliance with the recommended course of treatment.

Exposure of a pathogen to an antimicrobial compound can select for chromosomal mutations conferring resistance, which can be transferred vertically to subsequent microbial generations and eventually become predominant in a microbial population that is repeatedly exposed to the antimicrobial. Alternatively, many genes responsible for drug resistance are found on plasmids or in transposons that can be transferred easily between microbes through horizontal gene transfer (see [How Asexual Prokaryotes Achieve Genetic Diversity](#)). Transposons also have the ability to move resistance genes between plasmids and chromosomes to further promote the spread of resistance.

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Mechanisms for Drug Resistance

There are several common mechanisms for drug resistance, which are summarized in Figure 15.2.1. These mechanisms include enzymatic modification of the drug, modification of the antimicrobial target, and prevention of drug penetration or accumulation.

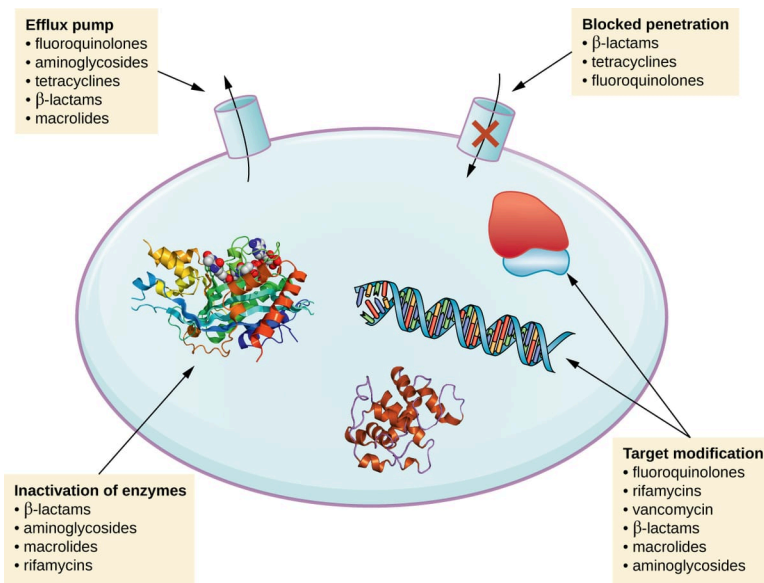


Figure 15.2.1: There are multiple strategies that microbes use to develop resistance to antimicrobial drugs. (Not shown: target overproduction, target mimicry, and enzymatic bypass). (credit: modification of work by Gerard D Wright)

Drug Modification or Inactivation

Resistance genes may code for enzymes that chemically modify an antimicrobial, thereby inactivating it, or destroy an antimicrobial through hydrolysis. Resistance to many types of antimicrobials occurs through this mechanism. For example, aminoglycoside resistance can occur through enzymatic transfer of chemical groups to the drug molecule, impairing the binding of the drug to its bacterial target. For β -lactams, bacterial resistance can involve the enzymatic hydrolysis of the β -lactam bond within the β -lactam ring of the drug molecule. Once the β -lactam bond is broken, the drug loses its antibacterial activity. This mechanism of resistance is mediated by **β -lactamases**, which are the most common mechanism of β -lactam resistance. Inactivation of rifampin commonly occurs through glycosylation, phosphorylation, or adenosine diphosphate (ADP) ribosylation, and resistance to macrolides and lincosamides can also occur due to enzymatic inactivation of the drug or modification.

Prevention of Cellular Uptake or Efflux

Microbes may develop resistance mechanisms that involve inhibiting the accumulation of an antimicrobial drug, which then prevents the drug from reaching its cellular target. This strategy is common among gram-negative pathogens and can involve changes in outer membrane lipid composition, porin channel selectivity, and/or porin channel concentrations. For example, a common mechanism of carbapenem resistance among *Pseudomonas aeruginosa* is to decrease the amount of its OprD porin, which is the primary portal of entry for carbapenems through the outer membrane of this pathogen. Additionally, many gram-positive and gram-negative pathogenic bacteria produce **efflux pumps** that actively transport an antimicrobial drug out of the cell and prevent the accumulation of drug to a level that would be antibacterial. For example, resistance to β -lactams, tetracyclines, and fluoroquinolones commonly occurs through active efflux out of the cell, and it is rather common for a single efflux pump to have the ability to translocate multiple types of antimicrobials.

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Target Modification

Because antimicrobial drugs have very specific targets, structural changes to those targets can prevent drug binding, rendering the drug ineffective. Through spontaneous mutations in the genes encoding antibacterial drug targets, bacteria have an evolutionary advantage that allows them to develop resistance to drugs. This mechanism of resistance development is quite common. Genetic changes impacting the active site of penicillin-binding proteins (PBPs) can inhibit the binding of β -lactam drugs and provide resistance to multiple drugs within this class. This mechanism is very common among strains of *Streptococcus pneumoniae*, which alter their own PBPs through genetic mechanisms. In contrast, strains of *Staphylococcus aureus* develop resistance to methicillin (MRSA) through the acquisition of a new low-affinity PBP, rather than structurally alter their existing PBPs. Not only does this new low-affinity PBP provide resistance to methicillin but it provides resistance to virtually all β -lactam drugs, with the exception of the newer fifth-generation cephalosporins designed specifically to kill MRSA. Other examples of this resistance strategy include alterations in

- ribosome subunits, providing resistance to macrolides, tetracyclines, and aminoglycosides;
- lipopolysaccharide (LPS) structure, providing resistance to polymyxins;
- RNA polymerase, providing resistance to rifampin;
- DNA gyrase, providing resistance to fluoroquinolones;
- metabolic enzymes, providing resistance to sulfa drugs, sulfones, and trimethoprim; and
- peptidoglycan subunit peptide chains, providing resistance to glycopeptides.

Target Overproduction or Enzymatic Bypass

When an antimicrobial drug functions as an antimetabolite, targeting a specific enzyme to inhibit its activity, there are additional ways that microbial resistance may occur. First, the microbe may overproduce the target enzyme such that there is a sufficient amount of antimicrobial-free enzyme to carry out the proper enzymatic reaction. Second, the bacterial cell may develop a bypass that circumvents the need for the functional target enzyme. Both of these strategies have been found as mechanisms of sulfonamide resistance. Vancomycin resistance among *S. aureus* has been shown to involve the decreased cross-linkage of peptide chains in the bacterial cell wall, which provides an increase in targets for vancomycin to bind to in the outer cell wall. Increased binding of vancomycin in the outer cell wall provides a blockage that prevents free drug molecules from penetrating to where they can block new cell wall synthesis.

Target Mimicry

A recently discovered mechanism of resistance called target mimicry involves the production of proteins that bind and sequester drugs, preventing the drugs from binding to their target. For example, *Mycobacterium tuberculosis* produces a protein with regular pentapeptide repeats that appears to mimic the structure of DNA. This protein binds fluoroquinolones, sequestering them and keeping them from binding to DNA, providing *M. tuberculosis* resistance to fluoroquinolones. Proteins that mimic the A-site of the bacterial ribosome have been found to contribute to aminoglycoside resistance as well.¹

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Key Concepts and Summary

- **Antimicrobial resistance** is on the rise and is the result of selection of drug-resistant strains in clinical environments, the overuse and misuse of antibacterials, the use of subtherapeutic doses of antibacterial drugs, and poor patient compliance with antibacterial drug therapies.

- Drug resistance genes are often carried on plasmids or in transposons that can undergo vertical transfer easily and between microbes through horizontal gene transfer.
- Common modes of antimicrobial drug resistance include drug modification or inactivation, prevention of cellular uptake or efflux, target modification, target overproduction or enzymatic bypass, and target mimicry.

Footnotes

1. D.H. Fong, A.M. Berghuis. "Substrate Promiscuity of an Aminoglycoside Antibiotic Resistance Enzyme Via Target Mimicry." *EMBO Journal* 21 no. 10 (2002):2323–2331.
2. 2 Centers for Disease Control and Prevention. "Antibiotic/Antimicrobial Resistance." <http://www.cdc.gov/drugresistance/index.html>. Accessed June 2, 2016.

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15.3: Multidrug Resistant Microbes

Learning Objectives

- Explain multidrug-resistant microbes (MDRs), also known as superbugs
- Describe MRSA and their resistance mechanisms
- Distinguish between MDR- and XDR-TB
- Explain how clavulanic acid (Augmentin) is used in combination with β -lactam drugs

Multidrug-Resistant Microbes and Cross Resistance

From a clinical perspective, our greatest concerns are **multidrug-resistant microbes (MDRs)** and cross resistance. MDRs are colloquially known as “superbugs” and carry one or more resistance mechanism(s), making them resistant to multiple antimicrobials. In **cross-resistance**, a single resistance mechanism confers resistance to multiple antimicrobial drugs. For example, having an efflux pump that can export multiple antimicrobial drugs is a common way for microbes to be resistant to multiple drugs by using a single resistance mechanism. In recent years, several clinically important superbugs have emerged, and the CDC reports that superbugs are responsible for more than 2 million infections in the US annually, resulting in at least 23,000 fatalities.² Several of the superbugs discussed in the following sections have been dubbed the ESKAPE pathogens. This acronym refers to the names of the pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) but it is also fitting in that these pathogens are able to “escape” many conventional forms of antimicrobial therapy. As such, infections by ESKAPE pathogens can be difficult to treat and they cause a large number of nosocomial infections.

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Methicillin, a semisynthetic penicillin, was designed to resist inactivation by β -lactamases. Unfortunately, soon after the introduction of methicillin to clinical practice, methicillin-resistant strains of *S. aureus* appeared and started to spread. The mechanism of resistance, acquisition of a new low-affinity PBP, provided *S. aureus* with resistance to all available β -lactams. Strains of **methicillin-resistant *S. aureus* (MRSA)** are widespread opportunistic pathogens and a particular concern for skin and other wound infections, but may also cause pneumonia and septicemia. Although originally a problem in health-care settings (hospital-acquired MRSA [HA-MRSA]), MRSA infections are now also acquired through contact with contaminated members of the general public, called community-associated MRSA (CA-MRSA). Approximately one-third of the population carries *S. aureus* as a member of their normal nasal microbiota without illness, and about 6% of these strains are methicillin resistant.³⁴

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Clavulanic Acid: Penicillin's Little Helper

With the introduction of penicillin in the early 1940s, and its subsequent mass production, society began to think of antibiotics as miracle cures for a wide range of infectious diseases. Unfortunately, as early as 1945, penicillin resistance was first documented and started to spread. Greater than 90% of current *S. aureus* clinical isolates are resistant to penicillin.⁵

Although developing new antimicrobial drugs is one solution to this problem, scientists have explored new approaches, including the development of compounds that inactivate resistance mechanisms. The development of clavulanic acid represents an early example of this strategy. Clavulanic acid is a molecule produced by the bacterium *Streptococcus clavuligerus*. It contains a β -lactam ring, making it structurally similar to penicillin and other β -lactams, but shows no clinical effectiveness when administered on its own. Instead, clavulanic acid binds irreversibly within the active site of β -lactamases and prevents them from inactivating a coadministered penicillin.

Clavulanic acid was first developed in the 1970s and was mass marketed in combination with amoxicillin beginning in the 1980s under the brand name Augmentin. As is typically the case, resistance to the amoxicillin-clavulanic acid combination soon appeared. Resistance most commonly results from bacteria increasing production of their β -lactamase and overwhelming the inhibitory effects of clavulanic acid, mutating their β -lactamase so it is no longer inhibited by clavulanic acid, or from acquiring a new β -lactamase that is not inhibited by clavulanic acid. Despite increasing resistance concerns, clavulanic acid and related β -lactamase inhibitors (sulbactam and tazobactam) represent an important new strategy: the development of compounds that directly inhibit antimicrobial resistance-conferring enzymes.

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Vancomycin-Resistant Enterococci and *Staphylococcus aureus*

Vancomycin is only effective against gram-positive organisms, and it is used to treat wound infections, septic infections, endocarditis, and meningitis that are caused by pathogens resistant to other antibiotics. It is considered one of the last lines of defense against such resistant infections, including MRSA. With the rise of antibiotic resistance in the 1970s and 1980s, vancomycin use increased, and it is not surprising that we saw the emergence and spread of **vancomycin-resistant enterococci (VRE)**, **vancomycin-resistant *S. aureus* (VRSA)**, and **vancomycin-intermediate *S. aureus* (VISA)**. The mechanism of vancomycin resistance among enterococci is target modification involving a structural change to the peptide component of the peptidoglycan subunits, preventing vancomycin from binding. These strains are typically spread among patients in clinical settings by contact with health-care workers and contaminated surfaces and medical equipment.

VISA and VRSA strains differ from each other in the mechanism of resistance and the degree of resistance each mechanism confers. VISA strains exhibit intermediate resistance, with a minimum inhibitory concentration (MIC) of 4–8 $\mu\text{g/mL}$, and the mechanism involves an increase in vancomycin targets. VISA strains decrease the crosslinking of peptide chains in the cell wall, providing an increase in vancomycin targets that trap vancomycin in the outer cell wall. In contrast, VRSA strains acquire vancomycin resistance through horizontal transfer of resistance genes from VRE, an opportunity provided in individuals coinfecting with both VRE and MRSA. VRSA exhibit a higher level of resistance, with MICs of 16 $\mu\text{g/mL}$ or higher.⁶ In the case of all three types of vancomycin-resistant bacteria, rapid clinical identification is necessary so proper procedures to limit spread can be implemented. The oxazolidinones like linezolid are useful for the treatment of these vancomycin-resistant, opportunistic pathogens, as well as MRSA.

Extended-Spectrum β -Lactamase-Producing Gram-Negative Pathogens

Gram-negative pathogens that produce **extended-spectrum β -lactamases (ESBLs)** show resistance well beyond just penicillins. The spectrum of β -lactams inactivated by ESBLs provides for resistance to all penicillins, cephalosporins, monobactams, and the β -lactamase-inhibitor combinations, but not the carbapenems. An even greater concern is that the genes encoding for ESBLs are usually found on mobile plasmids that also contain genes for resistance to other drug classes (e.g., fluoroquinolones, aminoglycosides, tetracyclines), and may be readily spread to other bacteria by horizontal gene transfer. These multidrug-resistant bacteria are members of the intestinal microbiota of some individuals, but they are also important causes of opportunistic infections in hospitalized patients, from whom they can be spread to other people.

Carbapenem-Resistant Gram-Negative Bacteria

The occurrence of **carbapenem-resistant Enterobacteriaceae (CRE)** and carbapenem resistance among other gram-negative bacteria (e.g., *P. aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*) is a growing health-care concern. These pathogens develop resistance to carbapenems through a variety of mechanisms, including production of carbapenemases (broad-spectrum β -lactamases that inactivate all β -lactams, including carbapenems), active efflux of carbapenems out of the cell, and/or prevention of carbapenem entry through porin channels. Similar to concerns with ESBLs, carbapenem-resistant, gram-negative pathogens are usually resistant to multiple classes of antibacterials, and some have even developed pan-resistance (resistance to all available antibacterials). Infections with carbapenem-resistant, gram-negative pathogens commonly occur in health-care settings through interaction with contaminated individuals or medical devices, or as a result of surgery.

Multidrug-Resistant Mycobacterium tuberculosis

The emergence of **multidrug-resistant Mycobacterium tuberculosis (MDR-TB)** and **extensively drug-resistant Mycobacterium tuberculosis (XDR-TB)** is also of significant global concern. MDR-TB strains are resistant to both rifampin and isoniazid, the drug combination typically prescribed for treatment of tuberculosis. XDR-TB strains are additionally resistant to any fluoroquinolone and at least one of three other drugs (amikacin, kanamycin, or capreomycin) used as a second line of treatment, leaving these patients very few treatment options. Both types of pathogens are particularly problematic in immunocompromised persons, including those suffering from HIV infection. The development of resistance in these strains often results from the incorrect use of antimicrobials for tuberculosis treatment, selecting for resistance.

Factory Farming and Drug Resistance

Although animal husbandry has long been a major part of agriculture in America, the rise of concentrated animal feeding operations (CAFOs) since the 1950s has brought about some new environmental issues, including the contamination of water and air with biological waste, and ethical issues regarding animal rights also are associated with growing animals in this way. Additionally, the increase in CAFOs involves the extensive use of antimicrobial drugs in raising livestock. Antimicrobials are used to prevent the development of infectious disease in the close quarters of CAFOs; however, the majority of antimicrobials used in factory farming are for the promotion of growth—in other words, to grow larger animals.

The mechanism underlying this enhanced growth remains unclear. These antibiotics may not necessarily be the same as those used clinically for humans, but they are structurally related to drugs used for humans. As a result, use of antimicrobial drugs in animals can select for antimicrobial resistance, with these resistant bacteria becoming cross-resistant to drugs typically used in humans. For example, tylosin use in animals appears to select for bacteria also cross-resistant to other macrolides, including erythromycin, commonly used in humans.

Concentrations of the drug-resistant bacterial strains generated by CAFOs become increased in water and soil surrounding these farms. If not directly pathogenic in humans, these resistant bacteria may serve as a reservoir of mobile genetic elements that can then pass resistance genes to human pathogens. Fortunately, the cooking process typically inactivates any antimicrobials remaining in meat, so humans typically are not directly ingesting these drugs. Nevertheless, many people are calling for more judicious use of these drugs, perhaps charging farmers user fees to reduce indiscriminate use. In fact, in 2012, the FDA published guidelines for farmers who voluntarily phase out the use of antimicrobial drugs except under veterinary supervision and when necessary to ensure animal health. Although following the guidelines is voluntary at this time, the FDA does recommend what it calls “judicious” use of antimicrobial drugs in food-producing animals in an effort to decrease antimicrobial resistance.

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Key Concepts and Summary

- Problematic microbial strains showing extensive antimicrobial resistance are emerging; many of these strains can reside as members of the normal microbiota in individuals but also can cause opportunistic infection. The transmission of many of these highly resistant microbial strains often occurs in clinical settings, but can also be community-acquired.

Footnotes

1. D.H. Fong, A.M. Berghuis. "Substrate Promiscuity of an Aminoglycoside Antibiotic Resistance Enzyme Via Target Mimicry." *EMBO Journal* 21 no. 10 (2002):2323–2331.
2. Centers for Disease Control and Prevention. "Antibiotic/Antimicrobial Resistance." <http://www.cdc.gov/drugresistance/index.html>. Accessed June 2, 2016.
3. A.S. Kalokhe et al. "Multidrug-Resistant Tuberculosis Drug Susceptibility and Molecular Diagnostic Testing: A Review of the Literature." *American Journal of the Medical Sciences* 345 no. 2 (2013):143–148.
4. Centers for Disease Control and Prevention. "Methicillin-Resistant *Staphylococcus aureus* (MRSA): General Information About MRSA in the Community." <http://www.cdc.gov/mrsa/community/index.html>. Accessed June 2, 2016
5. F.D. Lowy. "Antimicrobial Resistance: The Example of *Staphylococcus aureus*." *Journal of Clinical Investigation* 111 no. 9 (2003):1265–1273.
6. Centers for Disease Control and Prevention. "Healthcare-Associated Infections (HAI): General Information about VISA/VRSA." www.cdc.gov/HAI/organisms/vis...visa_vrsa.html. Accessed June 2, 2016.

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15.4: Testing the Effectiveness of Antimicrobials

Learning Objectives

- Describe how the Kirby-Bauer disk diffusion test determines the susceptibility of a microbe to an antibacterial drug.
- Explain the significance of the minimal inhibitory concentration and the minimal bactericidal concentration relative to the effectiveness of an antimicrobial drug.

Testing the effectiveness of antimicrobial drugs against specific organisms is important in identifying their spectrum of activity and the therapeutic dosage. This type of test, generally described as antimicrobial susceptibility testing (AST), is commonly performed in a clinical laboratory. In this section, we will discuss common methods of testing the effectiveness of antimicrobials.

The Kirby-Bauer Disk Diffusion Test

The **Kirby-Bauer disk diffusion test** has long been used as a starting point for determining the susceptibility of specific microbes to various antimicrobial drugs. The Kirby-Bauer assay starts with a Mueller-Hinton agar plate on which a confluent lawn is inoculated with a patient's isolated bacterial pathogen. Filter paper disks impregnated with known amounts of antibacterial drugs to be tested are then placed on the agar plate. As the bacterial inoculum grows, antibiotic diffuses from the circular disk into the agar and interacts with the growing bacteria. Antibacterial activity is observed as a clear circular **zone of inhibition** around the drug-impregnated disk, similar to the disk-diffusion assay. The diameter of the zone of inhibition, measured in millimeters and compared to a standardized chart, determines the susceptibility or resistance of the bacterial pathogen to the drug.

There are multiple factors that determine the size of a zone of inhibition in this assay, including drug solubility, rate of drug diffusion through agar, the thickness of the agar medium, and the drug concentration impregnated into the disk. Due to a lack of standardization of these factors, interpretation of the Kirby-Bauer disk diffusion assay provides only limited information on susceptibility and resistance to the drugs tested. The assay cannot distinguish between bacteriostatic and bactericidal activities, and differences in zone sizes cannot be used to compare drug potencies or efficacies. Comparison of zone sizes to a standardized chart will only provide information on the antibacterials to which a bacterial pathogen is susceptible or resistant.

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Antibiograms: Taking Some of the Guesswork Out of Prescriptions

Unfortunately, infectious diseases don't take a time-out for lab work. As a result, physicians rarely have the luxury of conducting susceptibility testing before they write a prescription. Instead, they rely primarily on the empirical evidence (i.e., the signs and symptoms of disease) and their professional experience to make an educated guess as to the diagnosis, causative agent(s), and drug most likely to be effective. This approach allows treatment to begin sooner so the patient does not have to wait for lab test results. In many cases, the prescription is effective; however, in an age of increased antimicrobial resistance, it is becoming increasingly more difficult to select the most appropriate empiric therapy. Selecting an inappropriate empiric therapy not only puts the patient at risk but may promote greater resistance to the drug prescribed.

Recently, studies have shown that antibiograms are useful tools in the decision-making process of selecting appropriate empiric therapy. An antibiogram is a compilation of local antibiotic susceptibility data broken down by bacterial pathogen. In a November 2014 study published in the journal *Infection Control and Hospital Epidemiology*, researchers determined that 85% of the prescriptions ordered in skilled nursing facilities were decided upon empirically, but only 35% of those prescriptions were deemed appropriate when compared with the eventual pathogen identification and susceptibility profile obtained from the clinical laboratory. However, in one nursing facility where use of antibiograms was implemented to direct selection of empiric therapy, appropriateness of empiric therapy increased from 32% before antibiogram implementation to 45% after implementation of antibiograms.¹ Although these data are preliminary, they do suggest that health-care facilities can reduce the number of inappropriate prescriptions by using antibiograms to select empiric therapy, thus benefiting patients and minimizing opportunities for antimicrobial resistance to develop.

Dilution Tests

As discussed, the limitations of the Kirby-Bauer disk diffusion test do not allow for a direct comparison of antibacterial potencies to guide selection of the best therapeutic choice. However, antibacterial dilution tests can be used to determine a particular drug's **minimal inhibitory concentration (MIC)**, the lowest concentration of drug that inhibits visible bacterial growth, and **minimal bactericidal concentration (MBC)**, the lowest drug concentration that kills $\geq 99.9\%$ of the starting inoculum. Determining these concentrations helps identify the correct drug for a particular pathogen. For the macrobroth dilution assay, a dilution series of the drug in broth is made in test tubes and the same number of cells of a test bacterial strain is added to each tube (Figure 15.4.1). The MIC is determined by examining the tubes to find the lowest drug concentration that inhibits visible growth; this is observed as turbidity (cloudiness) in the broth. Tubes with no visible growth are then inoculated onto agar media without antibiotic to determine the MBC. Generally, serum levels of an antibacterial should be at least three to five times above the MIC for treatment of an infection.

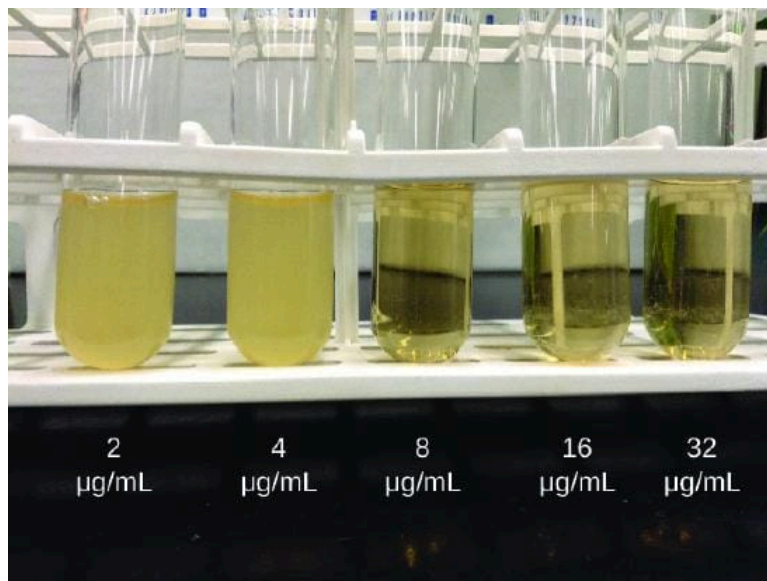


Figure 15.4.1: In a dilution test, the lowest dilution that inhibits turbidity (cloudiness) is the MIC. In this example, the MIC is 8 $\mu\text{g/mL}$. Broth from samples without turbidity can be inoculated onto plates lacking the antimicrobial drug. The lowest dilution that kills $\geq 99.9\%$ of the starting inoculum is observed on the plates is the MBC. (credit: modification of work by Suzanne Wakim)

The MIC assay can also be performed using 96-well microdilution trays, which allow for the use of small volumes and automated dispensing devices, as well as the testing of multiple antimicrobials and/or microorganisms in one tray (Figure 15.4.2). MICs are interpreted as the lowest concentration that inhibits visible growth, the same as for the macrobroth dilution in test tubes. Growth may also be interpreted visually or by using a spectrophotometer or similar device to detect turbidity or a color change if an appropriate biochemical substrate that changes color in the presence of bacterial growth is also included in each well.

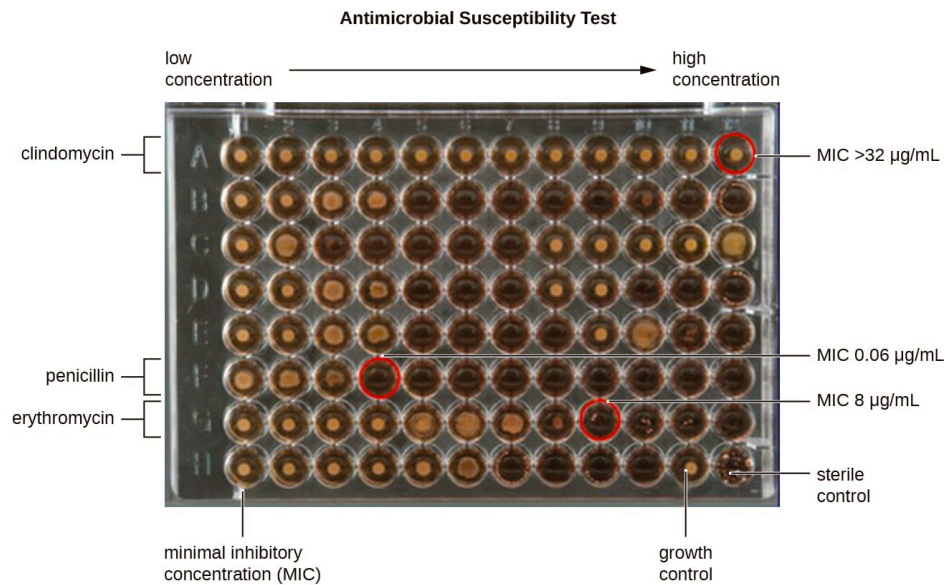


Figure 15.4.2: A microdilution tray can also be used to determine MICs of multiple antimicrobial drugs in a single assay. In this example, the drug concentrations increase from left to right and the rows with clindamycin, penicillin, and erythromycin have been indicated to the left of the plate. For penicillin and erythromycin, the lowest concentrations that inhibited visible growth are indicated by red circles and were 0.06 µg/mL for penicillin and 8 µg/mL for erythromycin. For clindamycin, visible bacterial growth was observed at every concentration up to 32 µg/mL and the MIC is interpreted as >32 µg/mL. (credit: modification of work by Centers for Disease Control and Prevention)

The **Etest** is an alternative method used to determine MIC, and is a combination of the Kirby-Bauer disk diffusion test and dilution methods. Similar to the Kirby-Bauer assay, a confluent lawn of a bacterial isolate is inoculated onto the surface of an agar plate. Rather than using circular disks impregnated with one concentration of drug, however, commercially available plastic strips that contain a gradient of an antibacterial are placed on the surface of the inoculated agar plate (Figure 15.4.3). As the bacterial inoculum grows, antibiotic diffuses from the plastic strips into the agar and interacts with the bacterial cells. Because the rate of drug diffusion is directly related to concentration, an elliptical zone of inhibition is observed with the Etest drug gradient, rather than a circular zone of inhibition observed with the Kirby-Bauer assay. To interpret the results, the intersection of the elliptical zone with the gradient on the drug-containing strip indicates the MIC. Because multiple strips containing different antimicrobials can be placed on the same plate, the MIC of multiple antimicrobials can be determined concurrently and directly compared. However, unlike the macrobroth and microbroth dilution methods, the MBC cannot be determined with the Etest.

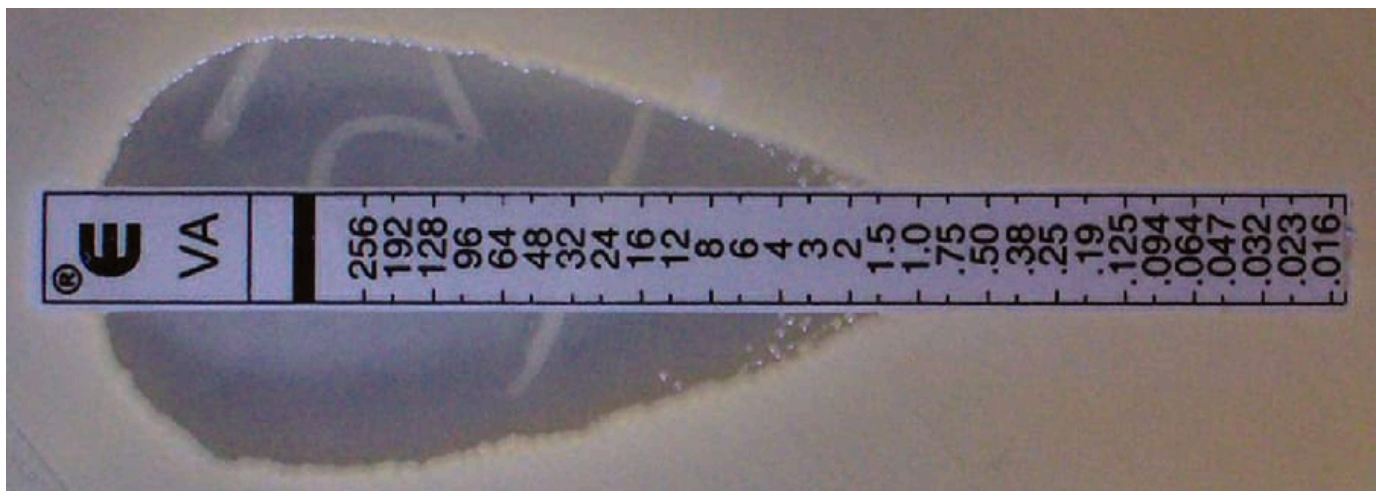


Figure 15.4.3: The Etest can be used to determine the MIC of an antibiotic. In this Etest, vancomycin is shown to have a MIC of 1.5 µg/mL against *Staphylococcus aureus*.

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Key Concepts and Summary

- The **Kirby-Bauer disk diffusion** test helps determine the susceptibility of a microorganism to various antimicrobial drugs. However, the **zones of inhibition** measured must be correlated to known standards to determine susceptibility and resistance, and do not provide information on bactericidal versus bacteriostatic activity, or allow for direct comparison of drug potencies.
- Antibiograms are useful for monitoring local trends in antimicrobial resistance/susceptibility and for directing appropriate selection of empiric antibacterial therapy.
- There are several laboratory methods available for determining the **minimum inhibitory concentration (MIC)** of an antimicrobial drug against a specific microbe. The **minimal bactericidal concentration (MBC)** can also be determined, typically as a follow-up experiment to MIC determination using the tube dilution method.

Footnotes

1. J.P. Furuno et al. "Using Antibiograms to Improve Antibiotic Prescribing in Skilled Nursing Facilities." *Infection Control and Hospital Epidemiology* 35 no. Suppl S3 (2014):S56–61.

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15.5: Current Strategies for Antimicrobial Discovery

Learning Objectives

- Describe the methods and strategies used for discovery of new antimicrobial agents.

With the continued evolution and spread of antimicrobial resistance, and now the identification of pan-resistant bacterial pathogens, the search for new antimicrobials is essential for preventing the postantibiotic era. Although development of more effective semisynthetic derivatives is one strategy, resistance to them develops rapidly because bacterial pathogens are already resistant to earlier-generation drugs in the family and can easily mutate and develop resistance to the new semisynthetic drugs. Today, scientists continue to hunt for new antimicrobial compounds and explore new avenues of antimicrobial discovery and synthesis. They check large numbers of soils and microbial products for antimicrobial activity by using high-throughput screening methods, which use automation to test large numbers of samples simultaneously. The recent development of the iChip¹ allows researchers to investigate the antimicrobial-producing capabilities of soil microbes that are difficult to grow by standard cultivation techniques in the laboratory. Rather than grow the microbes in the laboratory, they are grown *in situ*—right in the soil. Use of the iChip has resulted in the discovery of teixobactin, a novel antimicrobial from Mount Ararat, Turkey. Teixobactin targets two distinct steps in gram-positive cell wall synthesis and for which antimicrobial resistance appears not yet to have evolved.

Although soils have been widely examined, other environmental niches have not been tested as fully. Since 70% of the earth is covered with water, marine environments could be mined more fully for the presence of antimicrobial-producing microbes. In addition, researchers are using combinatorial chemistry, a method for making a very large number of related compounds from simple precursors, and testing them for antimicrobial activity. An additional strategy that needs to be explored further is the development of compounds that inhibit resistance mechanisms and restore the activity of older drugs, such as the strategy described earlier for β -lactamase inhibitors like clavulanic acid. Finally, developing inhibitors of virulence factor production and function could be a very important avenue. Although this strategy would not be directly antibacterial, drugs that slow the progression of an infection could provide an advantage for the immune system and could be used successfully in combination with antimicrobial drugs.

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The (Free?) Market for New Antimicrobials

There used to be plenty of antimicrobial drugs on the market to treat infectious diseases. However, the spread of antimicrobial resistance has created a need for new antibiotics to replace those that are no longer as effective as they once were. Unfortunately, pharmaceutical companies are not particularly motivated to fill this need. As of 2009, all but five pharmaceutical companies had moved away from antimicrobial drug development.² As a result, the number of FDA approvals of new antimicrobials has fallen drastically in recent decades (Figure 15.5.1).

Given that demand usually encourages supply, one might expect pharmaceutical companies to be rushing to get back in the business of developing new antibiotics. But developing new drugs is a lengthy process and requires large investments in research and development. Pharmaceutical companies can typically get a higher return on their investment by developing products for chronic, nonmicrobial diseases like diabetes; such drugs must be taken for life, and therefore generate more

long-term revenue than an antibiotic that does its job in a week or two. But what will happen when drugs like vancomycin, a superantimicrobial reserved for use as a last resort, begin to lose their effectiveness against ever more drug-resistant superbugs? Will drug companies wait until all antibiotics have become useless before beginning to look for new ones?

Recently, it has been suggested that large pharmaceutical companies should be given financial incentives to pursue such research. In September 2014, the White House released an executive order entitled “Combating Antibiotic Resistant Bacteria,” calling upon various government agencies and the private sector to work together to “accelerate basic and applied research and development for new antimicrobials, other therapeutics, and vaccines.”³ As a result, as of March 2015, President Obama’s proposed fiscal year 2016 budget doubled the amount of federal funding to \$1.2 billion for “combating and preventing antibiotic resistance,” which includes money for antimicrobial research and development.⁴ Similar suggestions have also been made on a global scale. In December 2014, a report chaired by former Goldman Sachs economist Jim O’Neill was published in *The Review on Antimicrobial Resistance*.⁵

These developments reflect the growing belief that for-profit pharmaceutical companies must be subsidized to encourage development of new antimicrobials. But some ask whether pharmaceutical development should be motivated by profit at all. Given that millions of lives may hang in the balance, some might argue that drug companies have an ethical obligation to devote their research and development efforts to high-utility drugs, as opposed to highly profitable ones. Yet this obligation conflicts with the fundamental goals of a for-profit company. Are government subsidies enough to ensure that drug companies make the public interest a priority, or should government agencies assume responsibility for developing critical drugs that may have little or no return on investment?

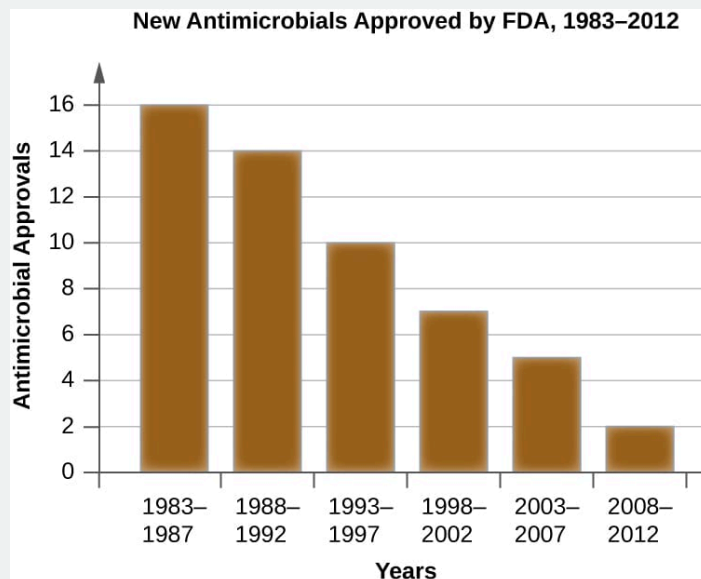


Figure 15.5.1: In recent decades, approvals of new antimicrobials by the FDA have steadily fallen. In the five-year period from 1983–1987, 16 new antimicrobial drugs were approved, compared to just two from 2008–2012.

Link to Learning

To further examine the scope of the problem, view [this](#) video.

To [learn more](#) about the history of antimicrobial drug discovery, visit Michigan State University’s Antimicrobial Resistance Learning Site.

Query 15.5.1

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Key Concepts and Summary

- Current research into the development of antimicrobial drugs involves the use of high-throughput screening and combinatorial chemistry technologies.
- New technologies are being developed to discover novel antibiotics from soil microorganisms that cannot be cultured by standard laboratory methods.
- Additional strategies include searching for antibiotics from sources other than soil, identifying new antibacterial targets, using combinatorial chemistry to develop novel drugs, developing drugs that inhibit resistance mechanisms, and developing drugs that target virulence factors and hold infections in check.

Footnotes

1. L. Losee et al. "A New Antibiotic Kills Pathogens Without Detectable Resistance." *Nature* 517 no. 7535 (2015):455–459.
2. H.W. Boucher et al. "Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America." *Clinical Infectious Diseases* 48 no. 1 (2009):1–12.
3. The White House. *National Action Plan for Combating Antibiotic-Resistant Bacteria*. Washington, DC: The White House, 2015.
4. White House Office of the Press Secretary. "Fact Sheet: Obama Administration Releases National Action Plan to Combat Antibiotic-Resistant Bacteria." March 27, 2015. <https://www.whitehouse.gov/the-press...lan-combat-ant>
5. Review on Antimicrobial Resistance. <http://amr-review.org>. Accessed June 1, 2016.

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15.E: Antimicrobial Drugs (Exercises)

15.1: Drugs Targeting Other Microorganisms

Antibacterial compounds exhibit selective toxicity, largely due to differences between prokaryotic and eukaryotic cell structure. Cell wall synthesis inhibitors, including the β -lactams, the glycopeptides, and bacitracin, interfere with peptidoglycan synthesis, making bacterial cells more prone to osmotic lysis. There are a variety of broad-spectrum, bacterial protein synthesis inhibitors that selectively target the prokaryotic 70S ribosome, including those that bind to the 30S and 50S subunits.

Multiple Choice

Which of the following terms refers to the ability of an antimicrobial drug to harm the target microbe without harming the host?

- A. mode of action
- B. therapeutic level
- C. spectrum of activity
- D. selective toxicity

Answer

D

Which of the following is not a type of β -lactam antimicrobial?

- A. penicillins
- B. glycopeptides
- C. cephalosporins
- D. monobactams

Answer

B

Which of the following does not bind to the 50S ribosomal subunit?

- A. tetracyclines
- B. lincosamides
- C. macrolides
- D. chloramphenicol

Answer

A

Which of the following antimicrobials inhibits the activity of DNA gyrase?

- A. polymyxin B
- B. clindamycin
- C. nalidixic acid
- D. rifampin

Answer

C

Fill in the Blank

Selective toxicity antimicrobials are easier to develop against bacteria because they are _____ cells, whereas human cells are eukaryotic.

Answer

prokaryotic

True/False

β -lactamases can degrade vancomycin.

Answer

false

Short Answer

If human cells and bacterial cells perform transcription, how are the rifamycins specific for bacterial infections?

What bacterial structural target would make an antibacterial drug selective for gram-negative bacteria? Provide one example of an antimicrobial compound that targets this structure.

Critical Thinking

In considering the cell structure of prokaryotes compared with that of eukaryotes, propose one possible reason for side effects in humans due to treatment of bacterial infections with protein synthesis inhibitors.

15.2: Clinical Considerations

Because fungi, protozoans, and helminths are eukaryotic organisms like human cells, it is more challenging to develop antimicrobial drugs that specifically target them. Similarly, it is hard to target viruses because human viruses replicate inside of human cells.

Multiple Choice

Which of the following is not an appropriate target for antifungal drugs?

- A. ergosterol
- B. chitin
- C. cholesterol
- D. $\beta(1 \rightarrow 3)$ glucan

Answer

C

Which of the following drug classes specifically inhibits neuronal transmission in helminths?

- A. quinolines
- B. avermectins
- C. amantadines
- D. imidazoles

Answer

B

Which of the following is a nucleoside analog commonly used as a reverse transcriptase inhibitor in the treatment of HIV?

- A. acyclovir
- B. ribavirin
- C. adenine-arabinoside
- D. azidothymidine

Answer

D

Which of the following is an antimalarial drug that is thought to increase ROS levels in target cells?

- A. artemisinin
- B. amphotericin b
- C. praziquantel
- D. pleconaril

Answer

A

Fill in the Blank

Antiviral drugs, like Tamiflu and Relenza, that are effective against the influenza virus by preventing viral escape from host cells are called _____.

Answer

neuraminidase inhibitors

True/False

Echinocandins, known as “penicillin for fungi,” target $\beta(1 \rightarrow 3)$ glucan in fungal cell walls.

Answer

true

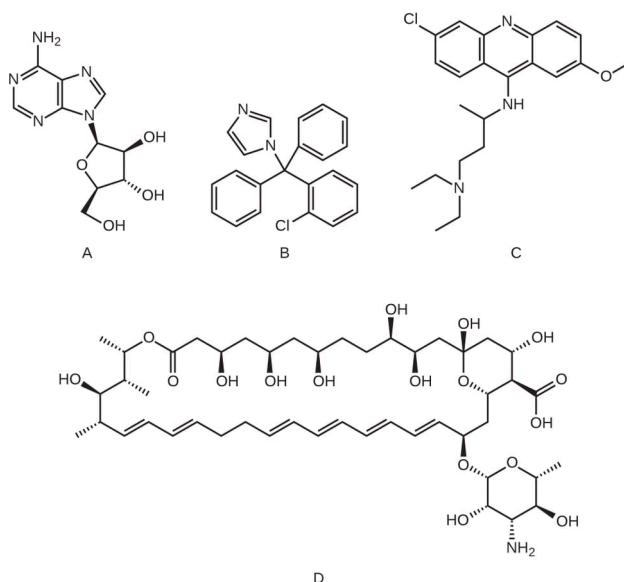
Short Answer

How does the biology of HIV necessitate the need to treat HIV infections with multiple drugs?

Niclosamide is insoluble and thus is not readily absorbed from the stomach into the bloodstream. How does the insolubility of niclosamide aid its effectiveness as a treatment for tapeworm infection?

Critical Thinking

Which of the following molecules is an example of a nucleoside analog?



Why can't drugs used to treat influenza, like amantadines and neuraminidase inhibitors, be used to treat a wider variety of viral infections?

15.3: Testing the Effectiveness of Antimicrobials

Antimicrobial resistance is on the rise and is the result of selection of drug-resistant strains in clinical environments, the overuse and misuse of antibacterials, the use of subtherapeutic doses of antibacterial drugs, and poor patient compliance with antibacterial drug therapies. Drug resistance genes are often carried on plasmids or in transposons that can undergo vertical transfer easily and between microbes through horizontal gene transfer.

Multiple Choice

Which of the following resistance mechanisms describes the function of β -lactamase?

- A. efflux pump
- B. target mimicry
- C. drug inactivation
- D. target overproduction

Answer

C

Which of the following resistance mechanisms is commonly effective against a wide range of antimicrobials in multiple classes?

- A. efflux pump
- B. target mimicry
- C. target modification
- D. target overproduction

Answer

A

Which of the following resistance mechanisms is the most nonspecific to a particular class of antimicrobials?

- A. drug modification
- B. target mimicry

- C. target modification
- D. efflux pump

Answer

D

Which of the following types of drug-resistant bacteria do not typically persist in individuals as a member of their intestinal microbiota?

- A. MRSA
- B. VRE
- C. CRE
- D. ESBL-producing bacteria

Answer

A

Fill in the Blank

Staphylococcus aureus, including MRSA strains, may commonly be carried as a normal member of the _____ microbiota in some people.

Answer

nasal

Short Answer

Why does the length of time of antimicrobial treatment for tuberculosis contribute to the rise of resistant strains?

What is the difference between multidrug resistance and cross-resistance?

15.4: The Emergence of Drug Resistance

The Kirby-Bauer disk diffusion test helps determine the susceptibility of a microorganism to various antimicrobial drugs. However, the zones of inhibition measured must be correlated to known standards to determine susceptibility and resistance, and do not provide information on bactericidal versus bacteriostatic activity, or allow for direct comparison of drug potencies. Antibigrams are useful for monitoring local trends in antimicrobial resistance/susceptibility.

Multiple Choice

In the Kirby-Bauer disk diffusion test, the _____ of the zone of inhibition is measured and used for interpretation.

- A. diameter
- B. microbial population
- C. circumference
- D. depth

Answer

A

Which of the following techniques cannot be used to determine the minimum inhibitory concentration of an antimicrobial drug against a particular microbe?

- A. Etest
- B. microbroth dilution test
- C. Kirby-Bauer disk diffusion test
- D. macrobroth dilution test

Answer

C

The utility of an antibiogram is that it shows antimicrobial susceptibility trends

- A. over a large geographic area.
- B. for an individual patient.
- C. in research laboratory strains.
- D. in a localized population.

Answer

D

Fill in the Blank

The method that can determine the MICs of multiple antimicrobial drugs against a microbial strain using a single agar plate is called the _____.

Answer

Etest

True/False

If drug A produces a larger zone of inhibition than drug B on the Kirby-Bauer disk diffusion test, drug A should always be prescribed.

Answer

false

Short Answer

How is the information from a Kirby-Bauer disk diffusion test used for the recommendation of the clinical use of an antimicrobial drug?

What is the difference between MIC and MBC?

Critical Thinking

Can an Etest be used to find the MBC of a drug? Explain.

15.5: Current Strategies for Antimicrobial Discovery

With the continued evolution and spread of antimicrobial resistance, and now the identification of pan-resistant bacterial pathogens, the search for new antimicrobials is essential for preventing the postantibiotic era.

Multiple Choice

Which of the following has yielded compounds with the most antimicrobial activity?

- A. water
- B. air
- C. volcanoes
- D. soil

Answer

D

True/False

The rate of discovery of antimicrobial drugs has decreased significantly in recent decades.

Answer

true

Critical Thinking

Who should be responsible for discovering and developing new antibiotics? Support your answer with reasoning.

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16: MICROBIAL MECHANISMS OF PATHOGENICITY

Jane woke up one spring morning feeling not quite herself. Her throat felt a bit dry and she was sniffling. She wondered why she felt so lousy. Was it because of a change in the weather? The pollen count? Was she coming down with something? Did she catch a bug from her coworker who sneezed on her in the elevator yesterday?

The signs and symptoms we associate with illness can have many different causes. Sometimes they are the direct result of a pathogenic infection, but in other cases they result from a response by our immune system to a pathogen or another perceived threat. For example, in response to certain pathogens, the immune system may release pyrogens, chemicals that cause the body temperature to rise, resulting in a fever. This response creates a less-than-favorable environment for the pathogen, but it also makes us feel sick.

Medical professionals rely heavily on analysis of signs and symptoms to determine the cause of an ailment and prescribe treatment. In some cases, signs and symptoms alone are enough to correctly identify the causative agent of a disease, but since few diseases produce truly unique symptoms, it is often necessary to confirm the identity of the infectious agent by other direct and indirect diagnostic methods.



Figure 16.1: Although medical professionals rely heavily on signs and symptoms to diagnose disease and prescribe treatment, many diseases can produce similar signs and symptoms. (credit left: modification of work by U.S. Navy)

16.1: Characteristics of Infectious Diseases

16.2: Periods of Disease

16.3: Koch Postulate and Molecular Koch Postulate

16.4: Pathogenicity and Virulence

16.E: Microbial Mechanisms of Pathogenicity (Exercises)

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16.1: Characteristics of Infectious Diseases

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

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.

YouTube Video

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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 16.1.1 lists some of the prefixes and suffixes commonly used in naming syndromes.

Table 16.1.1: 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.

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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 of 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 16.1.1). Other types of noninfectious diseases are listed in Table 16.1.2.

Table 16.1.2: 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)

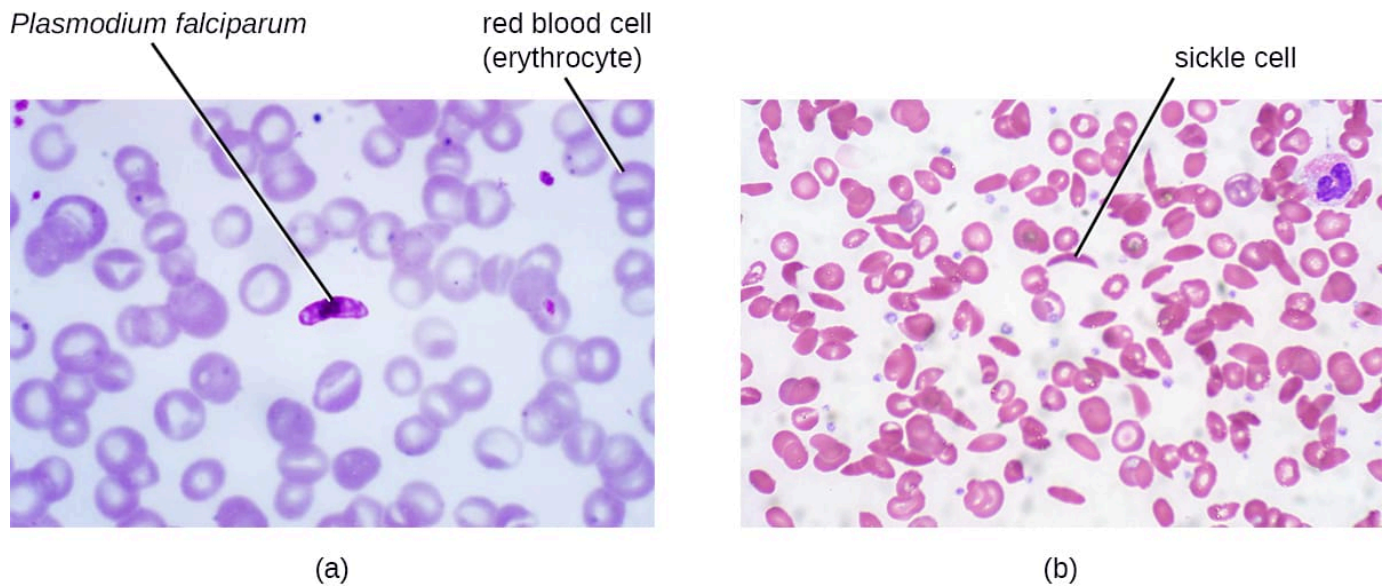


Figure 16.1.1: Blood smears showing two diseases of the blood. (a) Malaria is an infectious, zoonotic disease caused by the protozoan pathogen *Plasmodium falciparum* (shown here) and several other species of the genus *Plasmodium*. It is transmitted by mosquitoes to humans. (b) Sickle cell disease is 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)

Query 16.1.1

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Case Study Preview: “Hot Dog Headache: A *Listeria* Close Call”

When 10-year-old Michael develops fever, nausea, and aches after a party, his parents think it's just the flu. But as his condition worsens - featuring spiking fevers, severe headaches, and eventually neurological symptoms - the truth turns out to be far more dangerous. A contaminated undercooked hot dog, coupled with an unsuspecting immune system, sets off a rare but serious foodborne illness: *Listeria monocytogenes* infection.

This case highlights how a seemingly minor food safety lapse can lead to systemic infection and meningitis. Students will explore the pathogenesis of listeriosis, bacterial virulence factors like listeriolysin O and ActA, the stages of disease progression, and the importance of timely diagnostics and treatment with dual antibiotics.

Sometimes the scariest things aren't what you catch from people—it's what you eat.

[Chapter 15 Case Study - Hot Dog Headache: A *Listeria* Close Call](#)

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

Footnotes

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. J.G. Kusters et al. Pathogenesis of *Helicobacter pylori* Infection. *Clinical Microbiology Reviews* 19 no. 3 (2006):449–490.
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16.2: Periods of Disease

Learning Objectives

- Identify and describe the stages of an acute infectious disease in terms of number of pathogens present and severity of signs and symptoms
- Name some of the factors that can affect the length of the incubation period of a particular disease
- Explain the difference between acute, latent and chronic diseases

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 16.2.2). 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.

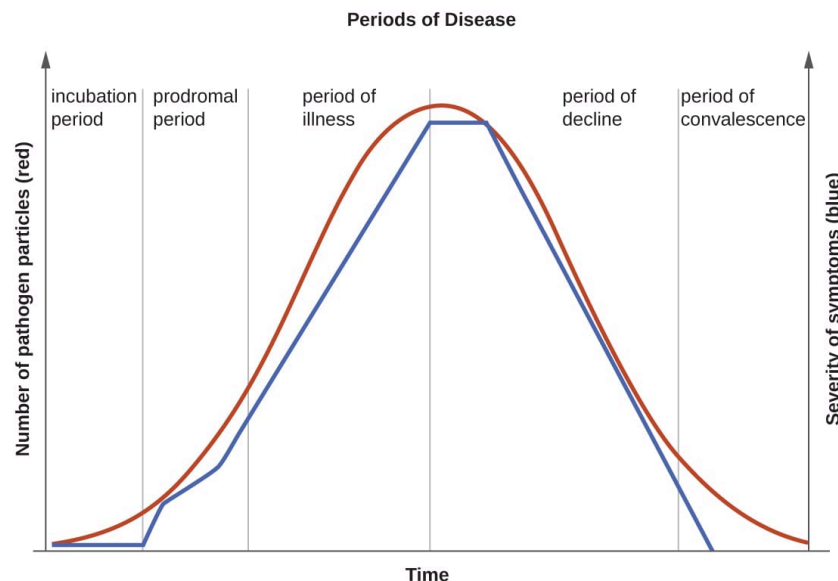


Figure 16.2.2: The progression of an infectious disease can be divided into five periods, which are related to the number of pathogen particles (red) and the severity of signs and symptoms (blue).

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 be 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).

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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.

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Key Concepts and Summary

- 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. 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. J.G. Kusters et al. Pathogenesis of *Helicobacter pylori* Infection. *Clinical Microbiology Reviews* 19 no. 3 (2006):449–490.
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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16.3: Koch Postulate and Molecular Koch Postulate

Learning Objectives

- Explain the significance and limitations of Koch's postulates
- Compare and contrast Koch's postulates and Molecular Koch's postulates
- Analyze case studies where Koch's postulates and Molecular Koch's postulates have been applied

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 16.3.1).

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.

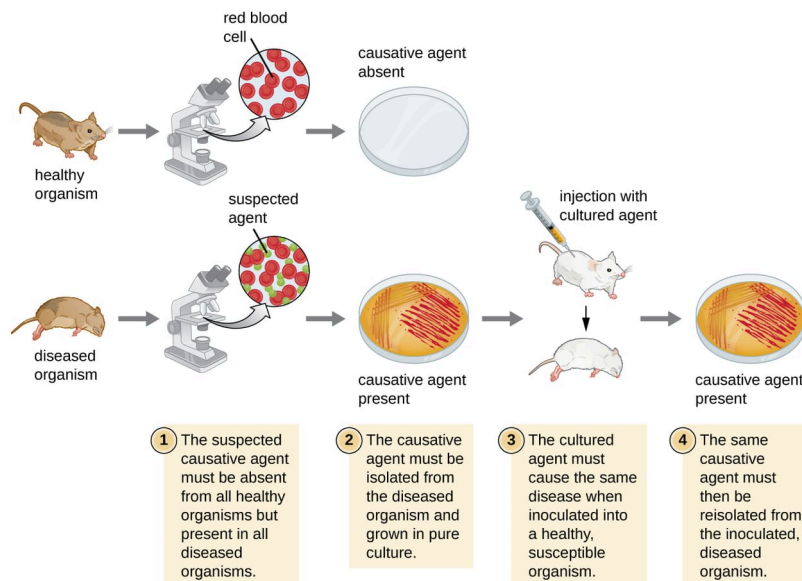


Figure 16.3.1: The steps for confirming that a pathogen is the cause of a particular disease using Koch's postulates.

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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.

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Molecular Koch's Postulates

In 1988, Stanley Falkow (1934–) proposed a revised form of Koch's postulates known as molecular Koch's postulates. These are listed in the left column of Table 16.3.1. The premise for molecular Koch's postulates is not in the ability to isolate a particular pathogen but rather to identify a gene that may cause the organism to be pathogenic.

Falkow's modifications to Koch's original postulates explain not only infections caused by intracellular pathogens but also the existence of pathogenic strains of organisms that are usually nonpathogenic. For example, the predominant form of the bacterium *Escherichia coli* is a member of the normal microbiota of the human intestine and is generally considered harmless. However, there are pathogenic strains of *E. coli* such as enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (O157:H7) (EHEC). We

now know ETEC and EHEC exist because of the acquisition of new genes by the once-harmless *E. coli*, which, in the form of these pathogenic strains, is now capable of producing toxins and causing illness. The pathogenic forms resulted from minor genetic changes. The right-side column of Table 16.3.1 illustrates how molecular Koch's postulates can be applied to identify EHEC as a pathogenic bacterium.

Table 16.3.1: Molecular Koch's Postulates Applied to EHEC

Molecular Koch's Postulates	Application to EHEC
(1) The phenotype (sign or symptom of disease) should be associated only with pathogenic strains of a species.	EHEC causes intestinal inflammation and diarrhea, whereas nonpathogenic strains of <i>E. coli</i> do not.
(2) Inactivation of the suspected gene(s) associated with pathogenicity should result in a measurable loss of pathogenicity.	One of the genes in EHEC encodes for Shiga toxin, a bacterial toxin (poison) that inhibits protein synthesis. Inactivating this gene reduces the bacteria's ability to cause disease.
(3) Reversion of the inactive gene should restore the disease phenotype.	By adding the gene that encodes the toxin back into the genome (e.g., with a phage or plasmid), EHEC's ability to cause disease is restored.

As with Koch's original postulates, the molecular Koch's postulates have limitations. For example, genetic manipulation of some pathogens is not possible using current methods of molecular genetics. In a similar vein, some diseases do not have suitable animal models, which limits the utility of both the original and molecular postulates.

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Key Concepts and Summary

- Koch's postulates** are used to determine whether a particular microorganism is a pathogen. **Molecular Koch's postulates** are used to determine what genes contribute to a pathogen's ability to cause disease.

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16.4: Pathogenicity and Virulence

Learning Objectives

- Explain the concept of pathogenicity (virulence) in terms of infectious and lethal dose
- Define and differentiate between pathogenicity and virulence
- Distinguish between primary and opportunistic pathogens and identify specific examples of each

Pathogenicity and Virulence

The ability of a microbial agent to cause disease is called **pathogenicity**, and the degree to which an organism is pathogenic is called **virulence**. Virulence is a continuum. On one end of the spectrum are organisms that are avirulent (not harmful) and on the other are organisms that are highly virulent. Highly virulent pathogens will almost always lead to a disease state when introduced to the body, and some may even cause multi-organ and body system failure in healthy individuals. Less virulent pathogens may cause an initial infection, but may not always cause severe illness. Pathogens with low virulence would more likely result in mild signs and symptoms of disease, such as low-grade fever, headache, or muscle aches. Some individuals might even be asymptomatic.

Definition: Term

Pathogenicity refers to the ability of a microorganism to cause disease. It is a qualitative trait—either a microbe is pathogenic (capable of causing disease) or non-pathogenic (not capable of causing disease).

Virulence refers to the **degree or extent of pathogenicity**, meaning how severe the disease caused by the pathogen is. It is a quantitative measure and can vary between different strains of the same species.

An example of a highly virulent microorganism is *Bacillus anthracis*, the pathogen responsible for anthrax. *B. anthracis* can produce different forms of disease, depending on the route of transmission (e.g., cutaneous injection, inhalation, ingestion). The most serious form of anthrax is inhalation anthrax. After *B. anthracis* spores are inhaled, they germinate. An active infection develops and the bacteria release potent toxins that cause edema (fluid buildup in tissues), hypoxia (a condition preventing oxygen from reaching tissues), and necrosis (cell death and inflammation). Signs and symptoms of inhalation anthrax include high fever, difficulty breathing, vomiting and coughing up blood, and severe chest pains suggestive of a heart attack. With inhalation anthrax, the toxins and bacteria enter the bloodstream, which can lead to multi-organ failure and death of the patient. If a gene (or genes) involved in pathogenesis is inactivated, the bacteria become less virulent or nonpathogenic.

Virulence of a pathogen can be quantified using controlled experiments with laboratory animals. Two important indicators of virulence are the **median infectious dose (ID₅₀)** and the **median lethal dose (LD₅₀)**, both of which are typically determined experimentally using animal models. The ID₅₀ is the number of pathogen cells or virions required to cause active infection in 50% of inoculated animals. The LD₅₀ is the number of pathogenic cells, virions, or amount of toxin required to kill 50% of infected animals. To calculate these values, each group of animals is inoculated with one of a range of known numbers of pathogen cells or virions. In graphs like the one shown in Figure 16.4.2, the percentage of animals that have been infected (for ID₅₀) or killed (for LD₅₀) is plotted against the concentration of pathogen inoculated. Figure 16.4.2 represents data graphed from a hypothetical experiment measuring the LD₅₀ of a pathogen. Interpretation of the data from this graph indicates that the LD₅₀ of the pathogen for the test animals is 10⁴ pathogen cells or virions (depending upon the pathogen studied).

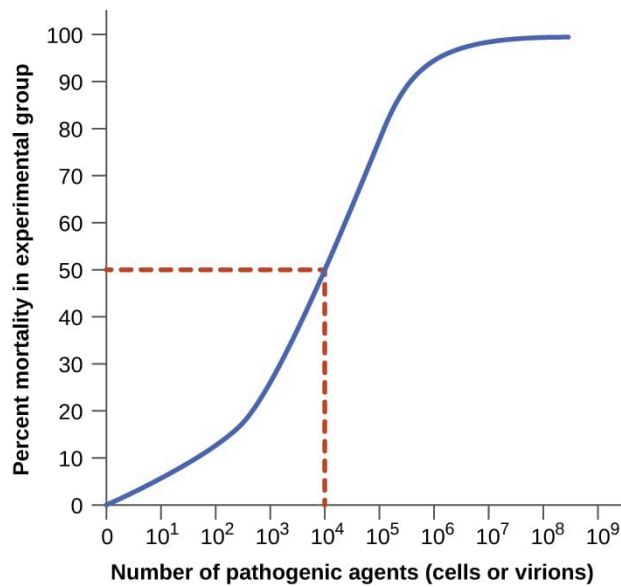


Figure 16.4.2: A graph like this is used to determine LD_{50} by plotting pathogen concentration against the percent of infected test animals that have died. In this example, the $LD_{50} = 10^4$ pathogenic particles.

Table 16.4.2 lists selected foodborne pathogens and their ID_{50} values in humans (as determined from epidemiologic data and studies on human volunteers). Keep in mind that these are *median* values. The actual infective dose for an individual can vary widely, depending on factors such as route of entry; the age, health, and immune status of the host; and environmental and pathogen-specific factors such as susceptibility to the acidic pH of the stomach. It is also important to note that a pathogen's infective dose does not necessarily correlate with disease severity. For example, just a single cell of *Salmonella enterica* serotype Typhimurium can result in an active infection. The resultant disease, *Salmonella* gastroenteritis or salmonellosis, can cause nausea, vomiting, and diarrhea, but has a mortality rate of less than 1% in healthy adults. In contrast, *S. enterica* serotype Typhi has a much higher ID_{50} , typically requiring as many as 1,000 cells to produce infection. However, this serotype causes typhoid fever, a much more systemic and severe disease that has a mortality rate as high as 10% in untreated individuals.

Table 16.4.2: ID_{50} for Selected Foodborne Diseases¹

Pathogen	ID_{50}
Viruses	
Hepatitis A virus	10–100
Norovirus	1–10
Rotavirus	10–100
Bacteria	
<i>Escherichia coli</i> , enterohemorrhagic (EHEC, serotype O157)	10–100
<i>E. coli</i> , enteroinvasive (EIEC)	200–5,000
<i>E. coli</i> , enteropathogenic (EPEC)	10,000,000–10,000,000,000
<i>E. coli</i> , enterotoxigenic (ETEC)	10,000,000–10,000,000,000
<i>Salmonella enterica</i> serovar Typhi	<1,000
<i>S. enterica</i> serovar Typhimurium	≥ 1
<i>Shigella dysenteriae</i>	10–200
<i>Vibrio cholerae</i> (serotypes O139, O1)	1,000,000
<i>V. parahemolyticus</i>	100,000,000
Protozoa	

Pathogen	ID ₅₀
<i>Giardia lamblia</i>	1
<i>Cryptosporidium parvum</i>	10–100

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Query 16.4.1

Primary Pathogens versus Opportunistic Pathogens

Pathogens can be classified as either primary pathogens or opportunistic pathogens. A **primary pathogen** can cause disease in a host regardless of the host's resident microbiota or immune system. An **opportunistic pathogen**, by contrast, can only cause disease in situations that compromise the host's defenses, such as the body's protective barriers, immune system, or normal microbiota. Individuals susceptible to opportunistic infections include the very young, the elderly, women who are pregnant, patients undergoing chemotherapy, people with immunodeficiencies (such as acquired immunodeficiency syndrome [AIDS]),

patients who are recovering from surgery, and those who have had a breach of protective barriers (such as a severe wound or burn).

An example of a primary pathogen is enterohemorrhagic *E. coli* (EHEC), which produces a virulence factor known as Shiga toxin. This toxin inhibits protein synthesis, leading to severe and bloody diarrhea, inflammation, and renal failure, even in patients with healthy immune systems. *Staphylococcus epidermidis*, on the other hand, is an opportunistic pathogen that is among the most frequent causes of nosocomial disease.² *S. epidermidis* is a member of the normal microbiota of the skin, where it is generally avirulent. However, in hospitals, it can also grow in biofilms that form on catheters, implants, or other devices that are inserted into the body during surgical procedures. Once inside the body, *S. epidermidis* can cause serious infections such as endocarditis, and it produces virulence factors that promote the persistence of such infections.

Other members of the normal microbiota can also cause opportunistic infections under certain conditions. This often occurs when microbes that reside harmlessly in one body location end up in a different body system, where they cause disease. For example, *E. coli* normally found in the large intestine can cause a urinary tract infection if it enters the bladder. This is the leading cause of urinary tract infections among women.

Members of the normal microbiota may also cause disease when a shift in the environment of the body leads to overgrowth of a particular microorganism. For example, the yeast *Candida* is part of the normal microbiota of the skin, mouth, intestine, and vagina, but its population is kept in check by other organisms of the microbiota. If an individual is taking antibacterial medications, however, bacteria that would normally inhibit the growth of *Candida* can be killed off, leading to a sudden growth in the population of *Candida*, which is not affected by antibacterial medications because it is a fungus. An overgrowth of *Candida* can manifest as oral thrush (growth on mouth, throat, and tongue), a vaginal yeast infection, or cutaneous candidiasis. Other scenarios can also provide opportunities for *Candida* infections. Untreated diabetes can result in a high concentration of glucose in the saliva, which provides an optimal environment for the growth of *Candida*, resulting in thrush. Immunodeficiencies such as those seen in patients with HIV, AIDS, and cancer also lead to higher incidence of thrush. Vaginal yeast infections can result from decreases in estrogen levels during the menstruation or menopause. The amount of glycogen available to lactobacilli in the vagina is controlled by levels of estrogen; when estrogen levels are low, lactobacilli produce less lactic acid. The resultant increase in vaginal pH allows overgrowth of *Candida* in the vagina.

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Key Concepts and Summary

- **Virulence**, the degree to which a pathogen can cause disease, can be quantified by calculating either the **ID₅₀** or **LD₅₀** of a pathogen on a given population.
- **Primary pathogens** are capable of causing pathological changes associated with disease in a healthy individual, whereas **opportunistic pathogens** can only cause disease when the individual is compromised by a break in protective barriers or immunosuppression.
- Infections and disease can be caused by pathogens in the environment or microbes in an individual's **resident microbiota**.

Footnotes

- 1 Food and Drug Administration. "Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins." 2nd ed. Silver Spring, MD: US Food and Drug Administration; 2012.
- 2 M. Otto. "Staphylococcus epidermidis--The 'Accidental' Pathogen." *Nature Reviews Microbiology* 7 no. 8 (2009):555–567.
- 3 The O in TORCH stands for "other."
- 4 D. Davies. "Understanding Biofilm Resistance to Antibacterial Agents." *Nature Reviews Drug Discovery* 2 (2003):114–122.

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16.E: Microbial Mechanisms of Pathogenicity (Exercises)

16.1: Characteristics of Infectious Diseases

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).

Multiple Choice

Which of the following would be a sign of an infection?

- A. muscle aches
- B. headache
- C. fever
- D. nausea

Answer

C

Which of the following is an example of a noncommunicable infectious disease?

- A. infection with a respiratory virus
- B. food poisoning due to a preformed bacterial toxin in food
- C. skin infection acquired from a dog bite
- D. infection acquired from the stick of a contaminated needle

Answer

B

During an oral surgery, the surgeon nicked the patient's gum with a sharp instrument. This allowed *Streptococcus*, a bacterium normally present in the mouth, to gain access to the blood. As a result, the patient developed bacterial endocarditis (an infection of the heart). Which type of disease is this?

- A. iatrogenic
- B. nosocomial
- C. vectors
- D. zoonotic

Answer

A

Which period is the stage of disease during which the patient begins to present general signs and symptoms?

- A. convalescence
- B. incubation
- C. illness
- D. prodromal

Answer

D

A communicable disease that can be easily transmitted from person to person is which type of disease?

- A. contagious
- B. iatrogenic
- C. acute
- D. nosocomial

Answer

A

Fill in the Blank

A difference between an acute disease and chronic disease is that chronic diseases have an extended period of _____.

Answer

illness

A person steps on a rusty nail and develops tetanus. In this case, the person has acquired a(n) _____ disease.

Answer

noncommunicable

Short Answer

Brian goes to the hospital after not feeling well for a week. He has a fever of 38 °C (100.4 °F) and complains of nausea and a constant migraine. Distinguish between the signs and symptoms of disease in Brian's case.

Critical Thinking

Two periods of acute disease are the periods of illness and period of decline. (a) In what way are both of these periods similar? (b) In terms of quantity of pathogen, in what way are these periods different? (c) What initiates the period of decline?

In July 2015, a report¹ was released indicating the gram-negative bacterium *Pseudomonas aeruginosa* was found on hospital sinks 10 years after the initial outbreak in a neonatal intensive care unit. *P. aeruginosa* usually causes localized ear and eye infections but can cause pneumonia or septicemia in vulnerable individuals like newborn babies. Explain how the current discovery of the presence of this reported *P. aeruginosa* could lead to a recurrence of nosocomial disease.

16.2: How Pathogens Cause Disease

Koch's postulates are used to determine whether a particular microorganism is a pathogen. Molecular Koch's postulates are used to determine what genes contribute to a pathogen's ability to cause disease. Virulence, the degree to which a pathogen can cause disease, can be quantified by calculating either the ID₅₀ or LD₅₀ of a pathogen on a given population. Primary pathogens are capable of causing pathological changes associated with disease in a healthy individual.

Multiple Choice

Which of the following is a pathogen that could not be identified by the original Koch's postulates?

- A. *Staphylococcus aureus*

- B. *Pseudomonas aeruginosa*
- C. Human immunodeficiency virus
- D. *Salmonella enterica* serovar Typhimurium

Answer

C

Pathogen A has an ID_{50} of 50 particles, pathogen B has an ID_{50} of 1,000 particles, and pathogen C has an ID_{50} of 1×10^6 particles. Which pathogen is most virulent?

- A. pathogen A
- B. pathogen B
- C. pathogen C

Answer

A

Fill in the Blank

A(n) _____ pathogen causes disease only when conditions are favorable for the microorganism because of transfer to an inappropriate body site or weakened immunity in an individual.

Answer

opportunistic

The concentration of pathogen needed to kill 50% of an infected group of test animals is the _____.

Answer

LD_{50}

A(n) _____ infection is a small region of infection from which a pathogen may move to another part of the body to establish a second infection.

Answer

focal

Cilia, fimbriae, and pili are all examples of structures used by microbes for _____.

Answer

adhesion

Critical Thinking

Diseases that involve biofilm-producing bacteria are of serious concern. They are not as easily treated compared with those involving free-floating (or planktonic) bacteria. Explain three reasons why biofilm formers are more pathogenic.

A microbiologist has identified a new gram-negative pathogen that causes liver disease in rats. She suspects that the bacterium's fimbriae are a virulence factor. Describe how molecular Koch's postulates could be used to test this hypothesis.

Acupuncture is a form of alternative medicine that is used for pain relief. Explain how acupuncture could facilitate exposure to pathogens.



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17: PATHOGENICITY AND VIRULENCE FACTORS

Pathogenicity refers to a microorganism's ability to cause disease, while virulence describes the severity of disease it causes. Bacterial pathogens use adhesion factors (fimbriae, adhesins, biofilms) to attach to host cells, and they evade immune defenses using capsules, antigenic variation, and intracellular survival. Many bacteria produce toxins, such as exotoxins (e.g., cholera toxin, botulinum toxin) that directly harm cells, and endotoxins (lipopolysaccharides) that trigger inflammatory responses. Enzymes like hyaluronidase and coagulase help bacteria invade tissues. True pathogens (e.g., *Mycobacterium tuberculosis*) cause disease in healthy individuals, while opportunistic pathogens (e.g., *Pseudomonas aeruginosa*) require a weakened immune system to establish infection.

Viruses, unlike bacteria, rely on host cells for replication and use viral attachment proteins (e.g., HIV gp120, SARS-CoV-2 spike protein) to enter cells. To evade immunity, they undergo antigenic variation (influenza virus), establish latency (herpesvirus), or inhibit immune signaling (Epstein-Barr virus). Some viruses cause cytopathic effects (CPEs) such as apoptosis, syncytia formation, or immune-mediated damage. Unlike bacteria, viruses do not produce toxins, but they manipulate the host's immune system to enhance their survival. The severity of viral disease depends on both viral virulence factors and host defenses, such as genetic resistance (e.g., CCR5 mutation protects against HIV) and microbiome interactions.

Both bacterial and viral infections depend on a complex interplay between microbial virulence factors and host susceptibility. Bacterial pathogens can be treated with antibiotics (if susceptible), while viral infections often require antivirals or vaccines for prevention. Host immune status, genetic predisposition, and microbiome balance play critical roles in determining infection outcomes. Understanding the mechanisms of pathogenicity and virulence helps in developing therapies, vaccines, and public health strategies to combat infectious diseases.

nonencapsulated bacteria

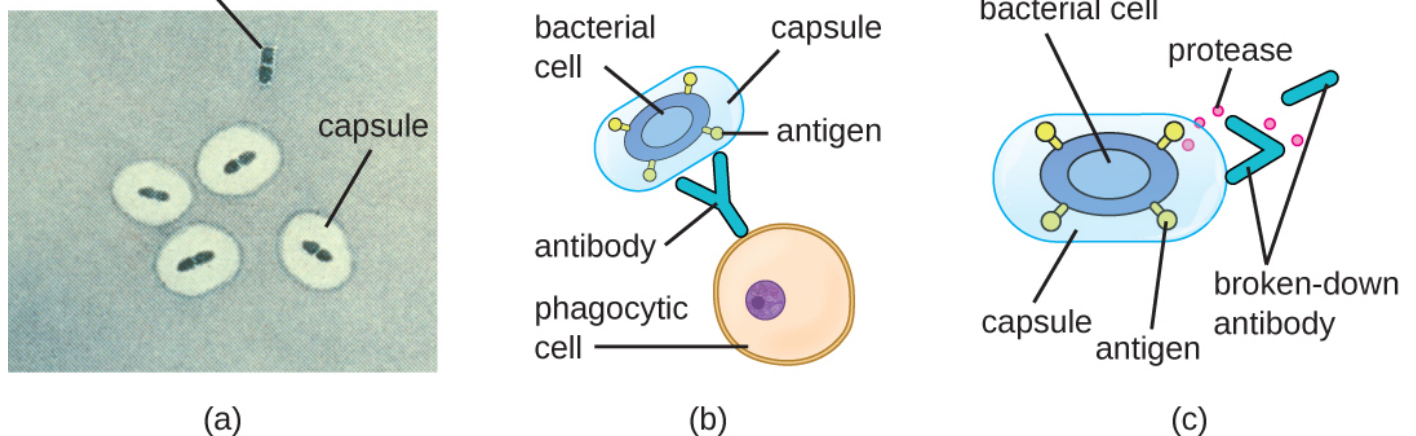


Figure 17.1: (a) A micrograph of capsules around bacterial cells. (b) Antibodies normally function by binding to antigens, molecules on the surface of pathogenic bacteria. Phagocytes then bind to the antibody, initiating phagocytosis. (c) Some bacteria also produce proteases, virulence factors that break down host antibodies to evade phagocytosis. (credit a: modification of work by Centers for Disease Control and Prevention)

17.1: Stages of Pathogenesis

17.2: Bacterial Virulence Factors- Adhesins and Exoenzymes

17.3: Bacterial Virulence Factors- Toxins

17.4: Immune Evasion and Viral Virulence Factors

17.E: Pathogenicity and Virulence Factors (Exercises)

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17.1: Stages of Pathogenesis

Learning Objectives

- Identify and explain the key stages of pathogenesis, including exposure, adhesion, invasion, infection, and transmission
- Compare and contrast the mechanisms used by different pathogens to progress through each stage of pathogenesis
- Explain how host factors influence the progression of pathogenesis

Stages of Pathogenesis

To cause disease, a pathogen must successfully achieve four steps or stages of pathogenesis: exposure (contact), adhesion (colonization), invasion, and infection. The pathogen must be able to gain entry to the host, travel to the location where it can establish an infection, evade or overcome the host's immune response, and cause damage (i.e., disease) to the host. In many cases, the cycle is completed when the pathogen exits the host and is transmitted to a new host.

Exposure

An encounter with a potential pathogen is known as **exposure** or **contact**. The food we eat and the objects we handle are all ways that we can come into contact with potential pathogens. Yet, not all contacts result in infection and disease. For a pathogen to cause disease, it needs to be able to gain access into host tissue. An anatomic site through which pathogens can pass into host tissue is called a **portal of entry**. These are locations where the host cells are in direct contact with the external environment. Major portals of entry are identified in Figure 17.1.3 and include the skin, mucous membranes, and parenteral routes.

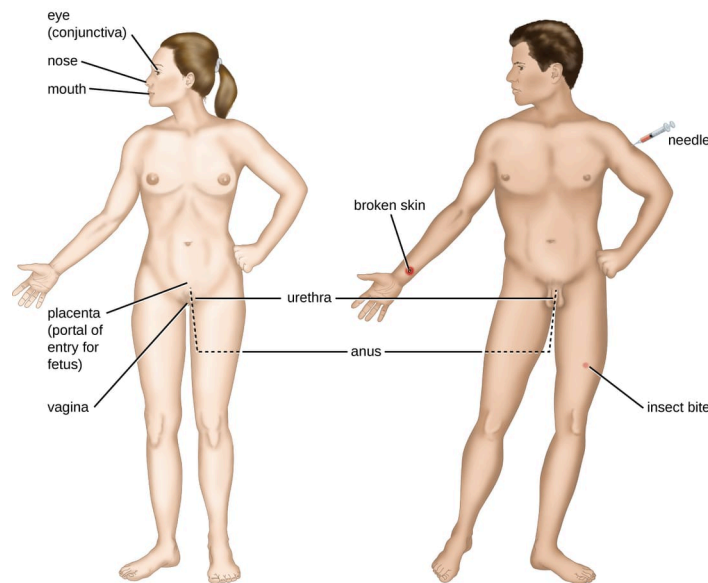


Figure 17.1.3: Shown are different portals of entry where pathogens can gain access into the body. With the exception of the placenta, many of these locations are directly exposed to the external environment.

Mucosal surfaces are the most important portals of entry for microbes; these include the mucous membranes of the respiratory tract, the gastrointestinal tract, and the genitourinary tract. Although most mucosal surfaces are in the interior of the body, some are contiguous with the external skin at various body openings, including the eyes, nose, mouth, urethra, and anus.

Most pathogens are suited to a particular portal of entry. A pathogen's portal specificity is determined by the organism's environmental adaptations and by the enzymes and toxins they secrete. The respiratory and gastrointestinal tracts are particularly vulnerable portals of entry because particles that include microorganisms are constantly inhaled or ingested, respectively.

Pathogens can also enter through a breach in the protective barriers of the skin and mucous membranes. Pathogens that enter the body in this way are said to enter by the **parenteral route**. For example, the skin is a good natural barrier to pathogens, but breaks in the skin (e.g., wounds, insect bites, animal bites, needle pricks) can provide a parenteral portal of entry for microorganisms.

In pregnant women, the placenta normally prevents microorganisms from passing from the mother to the fetus. However, a few pathogens are capable of crossing the blood-placental barrier. The gram-positive bacterium *Listeria monocytogenes*, which causes the foodborne disease listeriosis, is one example that poses a serious risk to the fetus and can sometimes lead to spontaneous abortion. Other pathogens that can pass the placental barrier to infect the fetus are known collectively by the acronym TORCH (Table 17.1.3).

Transmission of infectious diseases from mother to baby is also a concern at the time of birth when the baby passes through the birth canal. Babies whose mothers have active chlamydia or gonorrhea infections may be exposed to the causative pathogens in the vagina, which can result in eye infections that lead to blindness. To prevent this, it is standard practice to administer antibiotic drops to infants' eyes shortly after birth.

Table 17.1.3: Pathogens Capable of Crossing the Placental Barrier (TORCH Infections)

	Disease	Pathogen
T	Toxoplasmosis	<i>Toxoplasma gondii</i> (protozoan)
O³	Syphilis	<i>Treponema pallidum</i> (bacterium)
	Chickenpox	Varicella-zoster virus (human herpesvirus 3)
	Hepatitis B	Hepatitis B virus (hepadnavirus)
	HIV	Retrovirus
	Fifth disease (erythema infectiosum)	Parvovirus B19
R	Rubella (German measles)	Togavirus
C	Cytomegalovirus	Human herpesvirus 5
H	Herpes	Herpes simplex viruses (HSV) 1 and 2

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Adhesion

Following the initial exposure, the pathogen adheres at the portal of entry. The term **adhesion** refers to the capability of pathogenic microbes to attach to the cells of the body using adhesion factors, and different pathogens use various mechanisms to adhere to the cells of host tissues.

Molecules (either proteins or carbohydrates) called **adhesins** are found on the surface of certain pathogens and bind to specific receptors (glycoproteins) on host cells. Adhesins are present on the fimbriae and flagella of bacteria, the cilia of protozoa, and the capsids or membranes of viruses. Protozoans can also use hooks and barbs for adhesion; spike proteins on viruses also enhance viral adhesion. The production of glycocalyxes (slime layers and capsules) (Figure 17.1.4), with their high sugar and protein content, can also allow certain bacterial pathogens to attach to cells.

Biofilm growth can also act as an adhesion factor. A biofilm is a community of bacteria that produce a glycocalyx, known as extrapolymeric substance (EPS), that allows the biofilm to attach to a surface. Persistent *Pseudomonas aeruginosa* infections are common in patients suffering from cystic fibrosis, burn wounds, and middle-ear infections (otitis media) because *P. aeruginosa* produces a biofilm. The EPS allows the bacteria to adhere to the host cells and makes it harder for the host to physically remove the pathogen. The EPS not only allows for attachment but provides protection against the immune system and antibiotic treatments, preventing antibiotics from reaching the bacterial cells within the biofilm. In addition, not all bacteria in a biofilm are rapidly growing; some are in stationary phase. Since antibiotics are most effective against rapidly growing bacteria, portions of bacteria in a biofilm are protected against antibiotics.⁴

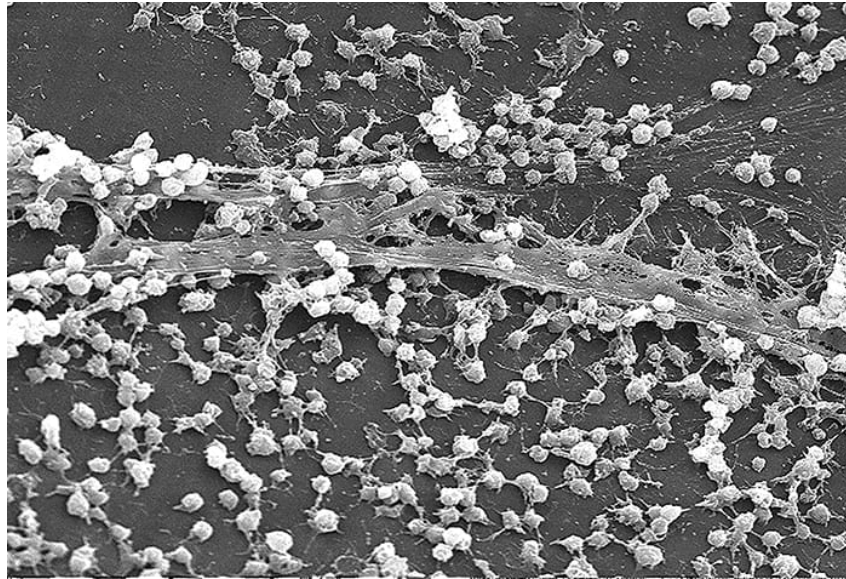


Figure 17.1.4: Glycocalyx produced by bacteria in a biofilm allows the cells to adhere to host tissues and to medical devices such as the catheter surface shown here. (credit: modification of work by Centers for Disease Control and Prevention)

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Invasion

Once adhesion is successful, invasion can proceed. **Invasion** involves the dissemination of a pathogen throughout local tissues or the body. Pathogens may produce exoenzymes or toxins, which serve as virulence factors that allow them to colonize and damage host tissues as they spread deeper into the body. Pathogens may also produce virulence factors that protect them against immune system defenses. A pathogen's specific virulence factors determine the degree of tissue damage that occurs. Figure 17.1.5 shows the invasion of *H. pylori* into the tissues of the stomach, causing damage as it progresses.

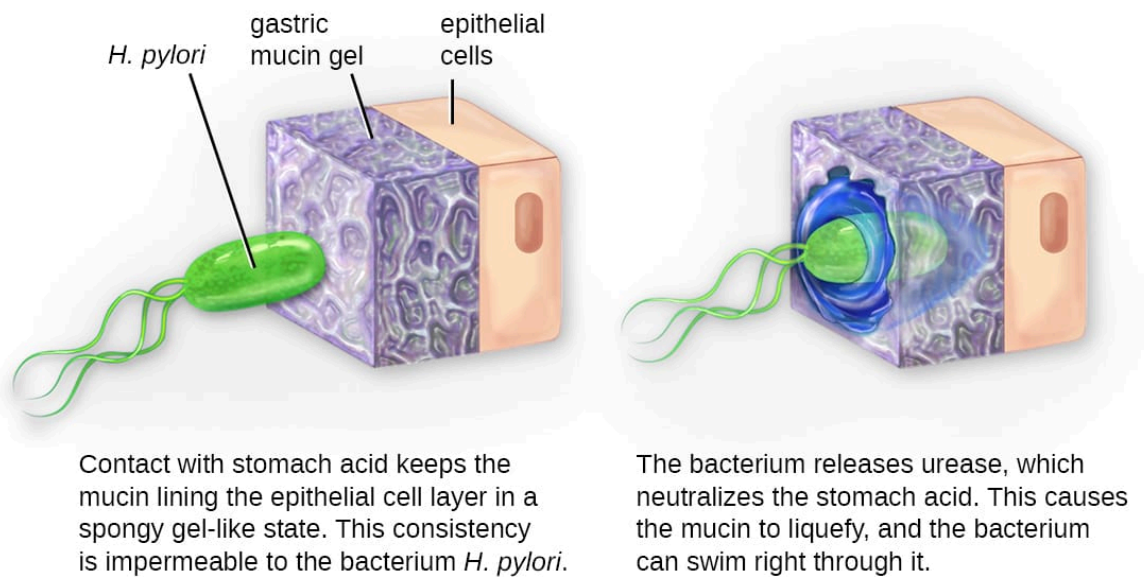


Figure 17.1.5: *H. pylori* is able to invade the lining of the stomach by producing virulence factors that enable it pass through the mucin layer covering epithelial cells. (credit: modification of work by Zina Deretsky, National Science Foundation)

Intracellular pathogens achieve invasion by entering the host's cells and reproducing. Some are obligate intracellular pathogens (meaning they can only reproduce inside of host cells) and others are facultative intracellular pathogens (meaning they can reproduce either inside or outside of host cells). By entering the host cells, intracellular pathogens are able to evade some mechanisms of the immune system while also exploiting the nutrients in the host cell.

Entry to a cell can occur by endocytosis. For most kinds of host cells, pathogens use one of two different mechanisms for endocytosis and entry. One mechanism relies on effector proteins secreted by the pathogen; these effector proteins trigger entry into the host cell. This is the method that *Salmonella* and *Shigella* use when invading intestinal epithelial cells. When these pathogens come in contact with epithelial cells in the intestine, they secrete effector molecules that cause protrusions of membrane ruffles that bring the bacterial cell in. This process is called membrane ruffling. The second mechanism relies on surface proteins expressed on the pathogen that bind to receptors on the host cell, resulting in entry. For example, *Yersinia pseudotuberculosis* produces a surface protein known as invasin that binds to beta-1 integrins expressed on the surface of host cells.

Some host cells, such as white blood cells and other phagocytes of the immune system, actively endocytose pathogens in a process called phagocytosis. Although phagocytosis allows the pathogen to gain entry to the host cell, in most cases, the host cell kills and degrades the pathogen by using digestive enzymes. Normally, when a pathogen is ingested by a phagocyte, it is enclosed within a phagosome in the cytoplasm; the phagosome fuses with a lysosome to form a phagolysosome, where digestive enzymes kill the pathogen (see [Pathogen Recognition and Phagocytosis](#)). However, some intracellular pathogens have the ability to survive and multiply within phagocytes. Examples include *Listeria monocytogenes* and *Shigella*; these bacteria produce proteins that lyse the phagosome before it fuses with the lysosome, allowing the bacteria to escape into the phagocyte's cytoplasm where they can multiply. Bacteria such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, and *Salmonella* species use a slightly different mechanism to evade being digested by the phagocyte. These bacteria prevent the fusion of the phagosome with the lysosome, thus remaining alive and dividing within the phagosome.

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Infection

Following invasion, successful multiplication of the pathogen leads to infection. Infections can be described as local, focal, or systemic, depending on the extent of the infection. A **local infection** is confined to a small area of the body, typically near the portal of entry. For example, a hair follicle infected by *Staphylococcus aureus* infection may result in a boil around the site of infection, but the bacterium is largely contained to this small location. Other examples of local infections that involve more extensive tissue involvement include urinary tract infections confined to the bladder or pneumonia confined to the lungs.

In a **focal infection**, a localized pathogen, or the toxins it produces, can spread to a secondary location. For example, a dental hygienist nicking the gum with a sharp tool can lead to a local infection in the gum by *Streptococcus* bacteria of the normal oral microbiota. These *Streptococcus* spp. may then gain access to the bloodstream and make their way to other locations in the body, resulting in a secondary infection.

When an infection becomes disseminated throughout the body, we call it a **systemic infection**. For example, infection by the varicella-zoster virus typically gains entry through a mucous membrane of the upper respiratory system. It then spreads throughout the body, resulting in the classic red skin lesions associated with chickenpox. Since these lesions are not sites of initial infection, they are signs of a systemic infection.

Sometimes a **primary infection**, the initial infection caused by one pathogen, can lead to a **secondary infection** by another pathogen. For example, the immune system of a patient with a primary infection by HIV becomes compromised, making the patient more susceptible to secondary diseases like oral thrush and others caused by opportunistic pathogens. Similarly, a primary infection by Influenzavirus damages and decreases the defense mechanisms of the lungs, making patients more susceptible to a secondary pneumonia by a bacterial pathogen like *Haemophilus influenzae* or *Streptococcus pneumoniae*. Some secondary infections can even develop as a result of treatment for a primary infection. Antibiotic therapy targeting the primary pathogen can cause collateral damage to the normal microbiota, creating an opening for opportunistic pathogens (see Case in Point: A Secondary Yeast Infection below).

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Transmission of Disease

For a pathogen to persist, it must put itself in a position to be transmitted to a new host, leaving the infected host through a **portal of exit** (Figure 17.1.6). As with portals of entry, many pathogens are adapted to use a particular portal of exit. Similar to portals of entry, the most common portals of exit include the skin and the respiratory, urogenital, and gastrointestinal tracts. Coughing and sneezing can expel pathogens from the respiratory tract. A single sneeze can send thousands of virus particles into the air. Secretions and excretions can transport pathogens out of other portals of exit. Feces, urine, semen, vaginal secretions, tears, sweat, and shed skin cells can all serve as vehicles for a pathogen to leave the body. Pathogens that rely on insect vectors for transmission exit the body in the blood extracted by a biting insect. Similarly, some pathogens exit the body in blood extracted by needles.

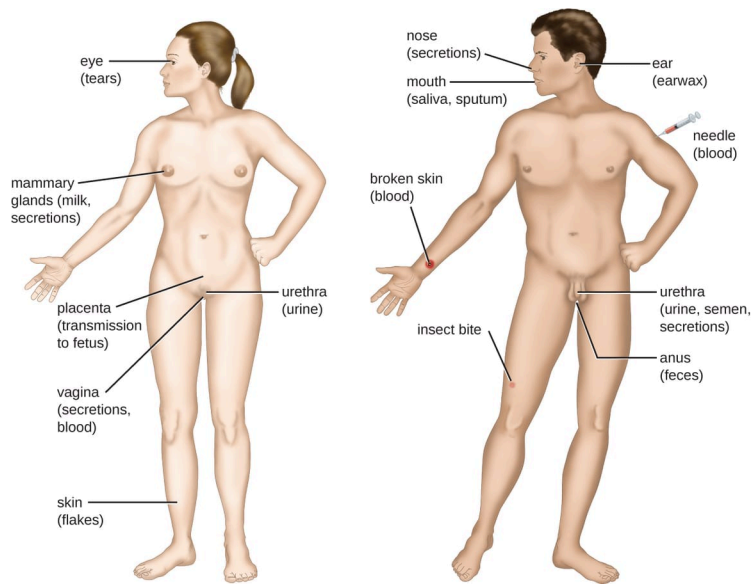


Figure 17.1.6: Pathogens leave the body of an infected host through various portals of exit to infect new hosts.

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Key Concepts and Summary

- Infections and disease can be caused by pathogens in the environment or microbes in an individual's **resident microbiota**.
- Infections can be classified as **local**, **focal**, or **systemic** depending on the extent to which the pathogen spreads in the body.
- A **secondary infection** can sometimes occur after the host's defenses or normal microbiota are compromised by a **primary infection** or antibiotic treatment.
- Pathogens enter the body through **portals of entry** and leave through **portals of exit**. The stages of pathogenesis include **exposure**, **adhesion**, **invasion**, **infection**, and **transmission**.

Footnotes

- 1 Food and Drug Administration. "Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins." 2nd ed. Silver Spring, MD: US Food and Drug Administration; 2012.
- 2 M. Otto. "Staphylococcus epidermidis--The 'Accidental' Pathogen." *Nature Reviews Microbiology* 7 no. 8 (2009):555–567.
- 3 The O in TORCH stands for "other."

4. [4](#) D. Davies. "Understanding Biofilm Resistance to Antibacterial Agents." *Nature Reviews Drug Discovery* 2 (2003):114–122.

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17.2: Bacterial Virulence Factors- Adhesins and Exoenzymes

Learning Objectives

- Explain how virulence factors contribute to signs and symptoms of infectious disease
- Describe the importance of adhesion and how it contributes to colonization
- Differentiate between various bacterial exoenzymes
- Evaluate how exoenzymes enhance pathogenicity

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 17.2.1 lists common adhesins found in some of the pathogens we have discussed or will be seeing later in this chapter.

Table 17.2.1: Some Bacterial Adhesins and Their Host Attachment Sites

Pathogen	Disease	Adhesin	Attachment Site
<i>Streptococcus pyogenes</i>	Strep throat	Protein F	Respiratory epithelial cells
<i>Streptococcus mutans</i>	Dental caries	Adhesin P1	Teeth
<i>Neisseria gonorrhoeae</i>	Gonorrhea	Type IV pili	Urethral epithelial cells
Enterotoxigenic <i>E. coli</i> (ETEC)	Traveler's diarrhea	Type 1 fimbriae	Intestinal epithelial cells
<i>Vibrio cholerae</i>	Cholera	N-methylphenylalanine pili	Intestinal epithelial cells

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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 toxemia describes 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 17.2.1). 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 17.2.1: 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.

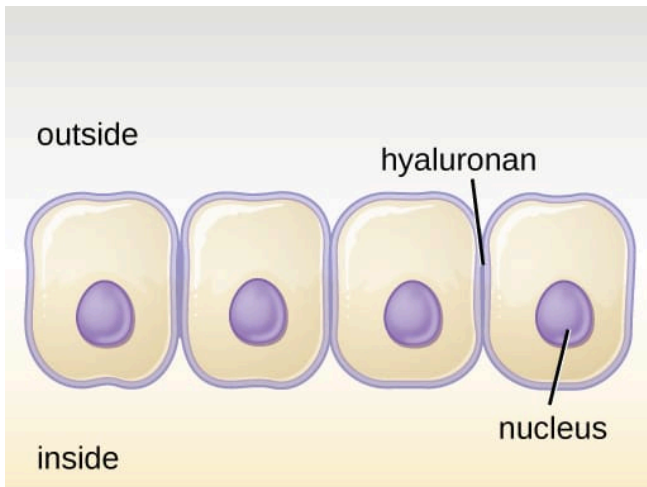
Exoenzymes

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 17.2.2. 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 17.2.2). This allows the pathogen to pass through the tissue layers at the portal of entry and disseminate elsewhere in the body (Figure 17.2.2).

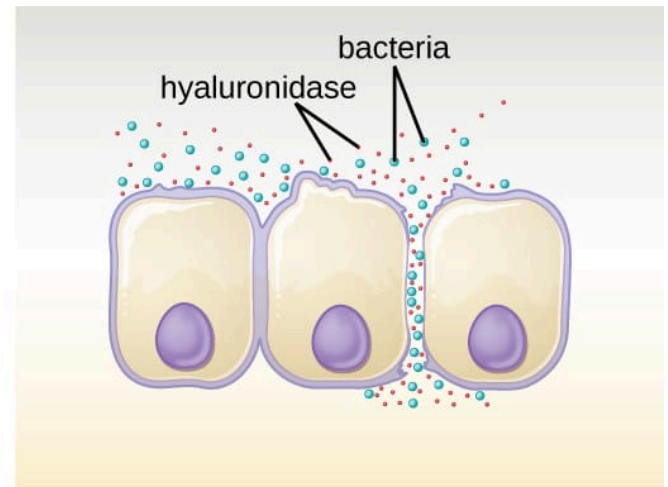
Table 17.2.2: Some Classes of Exoenzymes and Their Targets

Class	Example	Function
Glycohydrolases	Hyaluronidase S in <i>Staphylococcus aureus</i>	Degrades hyaluronic acid that cements cells together to promote spreading through tissues
Nucleases	DNAse produced by <i>S. aureus</i>	Degrades DNA released by dying cells (bacteria and host cells) that can trap the bacteria, thus promoting spread
Phospholipases	Phospholipase C of <i>Bacillus anthracis</i>	Degrades phospholipid bilayer of host cells, causing cellular lysis, and degrade membrane of phagosomes to enable escape into the cytoplasm

Class	Example	Function
Proteases	Collagenase in <i>Clostridium perfringens</i>	Degrades collagen in connective tissue to promote spread



(a)



(b)

Figure 17.2.2: (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.

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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 17.2.3).

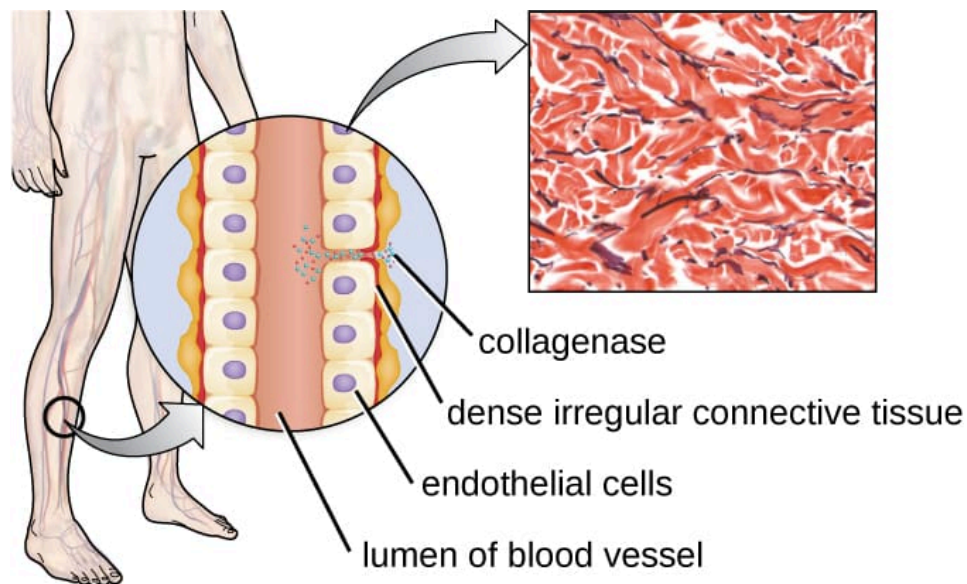


Figure 17.2.3: The illustration depicts a blood vessel with a single layer of endothelial cells surrounding the lumen and dense connective tissue (shown in red) surrounding the endothelial cell layer. Collagenase produced by *C. perfringens* degrades the collagen between the endothelial cells, allowing 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)

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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.

Footnotes

1. V. Meka. "Panton-Valentine Leukocidin." <http://www.antimicrobe.org/h04c.file...L-S-aureus.asp>

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17.3: Bacterial Virulence Factors- Toxins

Learning Objectives

- Differentiate between endotoxins and exotoxins
- Describe and differentiate between various types of exotoxins
- Explain how superantigens attack the body

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 17.3.4). 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 17.3.4: 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* amoebocyte lysate (LAL) test. In this procedure, the blood cells (amoebocytes) of the horseshoe crab (*Limulus polyphemus*) is mixed with a patient's serum. The amoebocytes 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 17.3.3. 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 LD₅₀ of 0.24 mg/kg. By contrast, very small concentrations of exotoxins can be lethal. For example, botulinum toxin, which causes botulism, has an LD₅₀ of 0.000001 mg/kg (240,000 times more lethal than endotoxin).

Table 17.3.3: 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 17.3.4 provides examples of well-characterized toxins within each of these three categories.

Table 17.3.4: Some Common Exotoxins and Associated Bacterial Pathogens

Category	Example	Pathogen	Mechanism and Disease
Intracellular-targeting toxins	Cholera toxin	<i>Vibrio cholerae</i>	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	<i>Clostridium tetani</i>	Inhibits the release of inhibitory neurotransmitters in the central nervous system, causing spastic paralysis
	Botulinum toxin	<i>Clostridium botulinum</i>	Inhibits release of the neurotransmitter acetylcholine from neurons, resulting in flaccid paralysis
	Diphtheria toxin	<i>Corynebacterium diphtheriae</i>	Inhibition of protein synthesis, causing cellular death
Membrane-disrupting toxins	Streptolysin	<i>Streptococcus pyogenes</i>	Proteins that assemble into pores in cell membranes, disrupting their function and killing the cell
	Pneumolysin	<i>Streptococcus pneumoniae</i>	
	Alpha-toxin	<i>Staphylococcus aureus</i>	
	Alpha-toxin	<i>Clostridium perfringens</i>	Phospholipases that degrade cell membrane phospholipids, disrupting membrane function and killing the cell
	Phospholipase C	<i>Pseudomonas aeruginosa</i>	
	Beta-toxin	<i>Staphylococcus aureus</i>	
Superantigens	Toxic shock syndrome toxin	<i>Staphylococcus aureus</i>	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	<i>Streptococcus pyogenes</i>	
	Streptococcal pyrogenic toxins	<i>Streptococcus pyogenes</i>	

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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 17.3.5). 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.

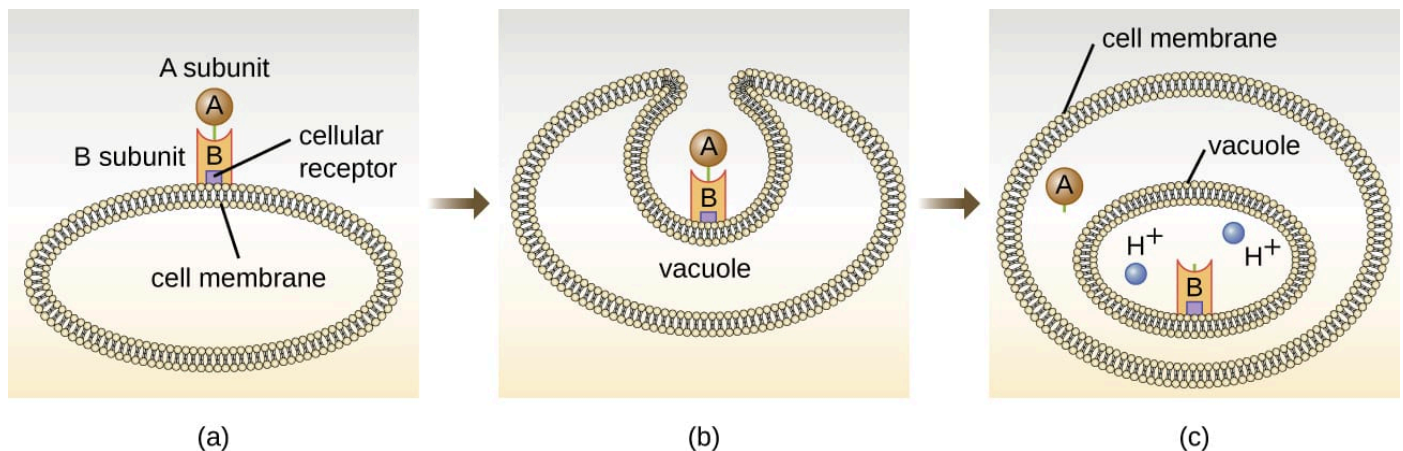


Figure 17.3.5: (a) In A-B toxins, the B component binds to the host cell through its interaction with specific cell surface receptors. (b) The toxin is brought in through endocytosis. (c) Once inside the vacuole, the A component (active component) separates from the B component and the A component gains 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 17.3.6).

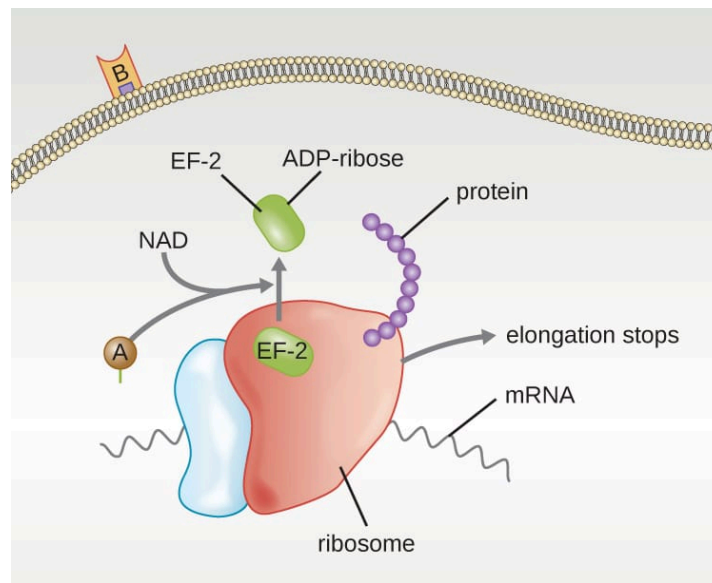


Figure 17.3.6: The mechanism of the diphtheria toxin inhibiting protein synthesis. The A subunit inactivates elongation factor 2 by transferring an ADP-ribose. This stops protein elongation, inhibiting protein synthesis and killing the cell.

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 adenylyl 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.

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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 17.3.7 shows the actions of both botulinum and tetanus toxins.

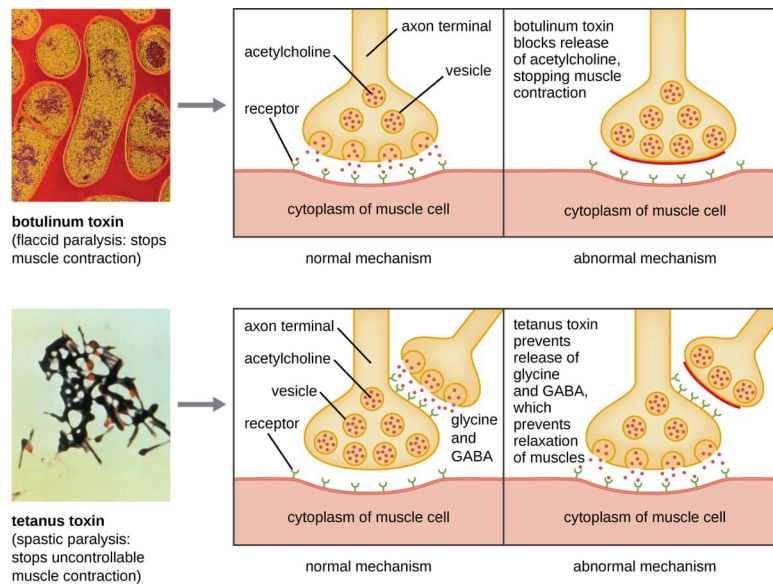


Figure 17.3.7: Mechanisms of botulinum and tetanus toxins. (credit micrographs: modification of work by Centers for Disease Control and Prevention)

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.

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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.

Footnotes

- 1 V. Meka. "Panton-Valentine Leukocidin." <http://www.antimicrobe.org/h04c.file...L-S-aureus.asp>

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17.4: Immune Evasion and Viral Virulence Factors

Learning Objectives

- Describe the mechanisms that bacteria and viruses use for immune evasion
- Evaluate the mechanisms viruses use for adhesion and antigenic variation
- Analyze the roles of antigenic drift and antigenic shift in viral evolution

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 17.4.8). 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 17.4.8).

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.

nonencapsulated bacteria

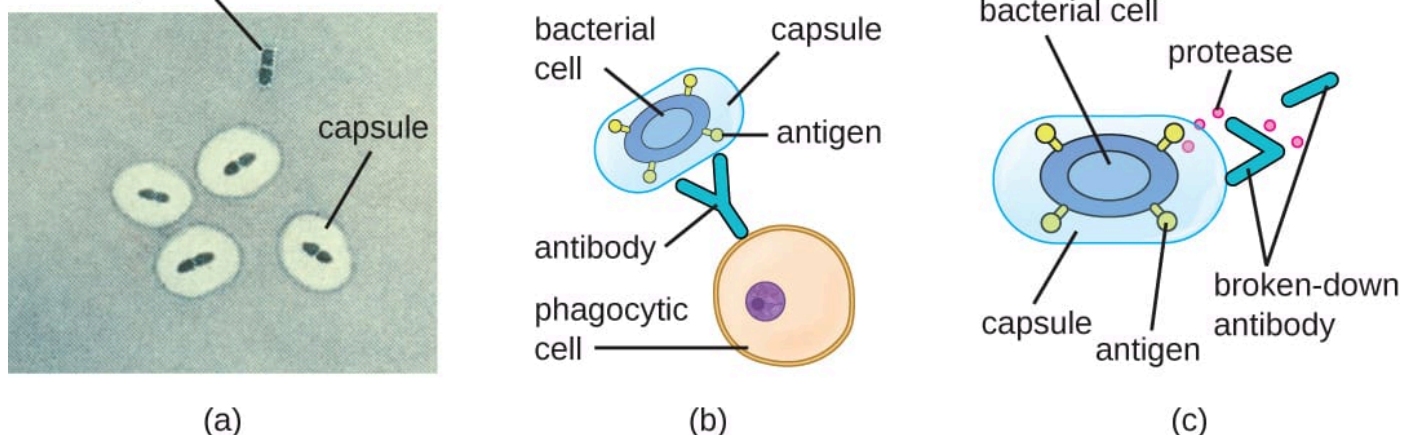


Figure 17.4.8: (a) A micrograph of capsules around bacterial cells. (b) Antibodies normally function by binding to antigens, molecules on the surface of pathogenic bacteria. Phagocytes then bind to the antibody, initiating phagocytosis. (c) Some bacteria also produce proteases, virulence factors that 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.

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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 Influenza virus 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 **gp120**, 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 17.4.5 lists the adhesins for some common viral pathogens and the specific sites to which these adhesins allow viruses to attach.

Table 17.4.5: 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

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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 17.4.9). 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 Influenza virus. 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 Influenza virus 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.

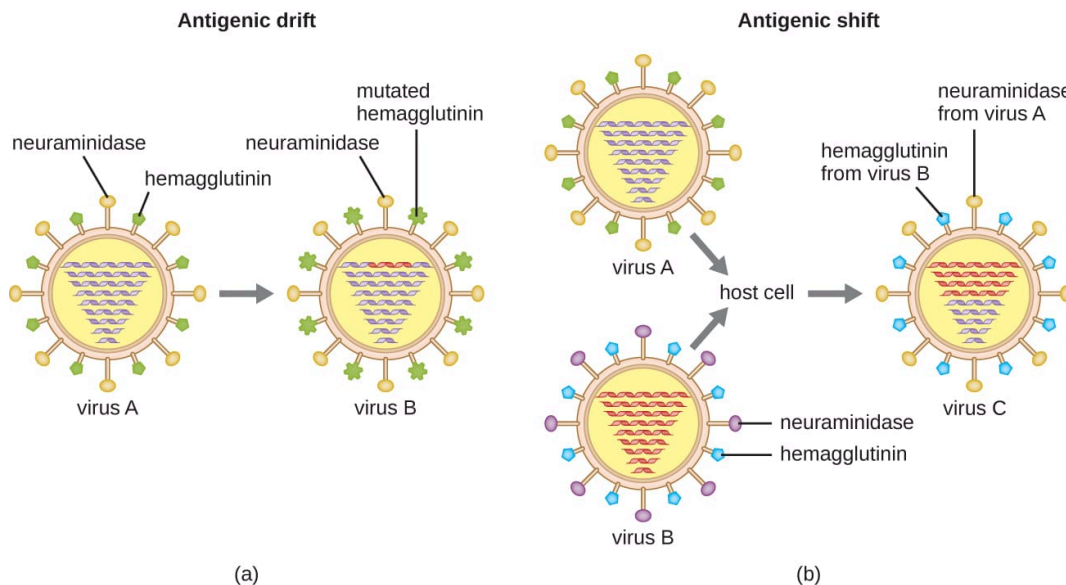


Figure 17.4.9: Antigenic drift and antigenic shift in influenza viruses. (a) In antigenic drift, mutations in the genes for the surface proteins neuraminidase and/or hemagglutinin result in small antigenic changes over time. (b) In antigenic shift, simultaneous infection of a cell with two different 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.

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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. V. Meka. "Panton-Valentine Leukocidin." <http://www.antimicrobe.org/h04c.file...L-S-aureus.asp>

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17.E: Pathogenicity and Virulence Factors (Exercises)

17.1: Virulence Factors

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.

Multiple Choice

Which of the following would be a virulence factor of a pathogen?

- A. a surface protein allowing the pathogen to bind to host cells
- B. a secondary host the pathogen can infect
- C. a surface protein the host immune system recognizes
- D. the ability to form a provirus

Answer

A

You have recently identified a new toxin. It is produced by a gram-negative bacterium. It is composed mostly of protein, has high toxicity, and is not heat stable. You also discover that it targets liver cells. Based on these characteristics, how would you classify this toxin?

- A. superantigen
- B. endotoxin
- C. exotoxin
- D. leukocidin

Answer

C

Which of the following applies to hyaluronidase?

- A. It acts as a spreading factor.
- B. It promotes blood clotting.
- C. It is an example of an adhesin.
- D. It is produced by immune cells to target pathogens.

Answer

A

Phospholipases are enzymes that do which of the following?

- A. degrade antibodies
- B. promote pathogen spread through connective tissue.
- C. degrade nucleic acid to promote spread of pathogen
- D. degrade cell membranes to allow pathogens to escape phagosomes

Answer

D

Fill in the Blank

The glycoprotein adhesion gp120 on HIV must interact with _____ on some immune cells as the first step in the process of infecting the cell.

Answer

CD4

Adhesins are usually located on _____ of the pathogen and are composed mainly of _____ and _____.

Answer

surface; proteins; sugars

The Shiga and diphtheria toxins target _____ in host cells.

Answer

protein synthesis

Antigenic _____ is the result of reassortment of genes responsible for the production of influenza virus spike proteins between different virus particles while in the same host, whereas antigenic _____ is the result of point mutations in the spike proteins.

Answer

shift; drift

Critical Thinking

Two types of toxins are hemolysins and leukocidins. (a) How are these toxins similar? (b) How do they differ?

Imagine that a mutation in the gene encoding the cholera toxin was made. This mutation affects the A-subunit, preventing it from interacting with any host protein. (a) Would the toxin be able to enter into the intestinal epithelial cell? (b) Would the toxin be able to cause diarrhea?

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18: DISEASE AND EPIDEMIOLOGY

In the United States and other developed nations, public health is a key function of government. A healthy citizenry is more productive, content, and prosperous; high rates of death and disease, on the other hand, can severely hamper economic productivity and foster social and political instability. The burden of disease makes it difficult for citizens to work consistently, maintain employment, and accumulate wealth to better their lives and support a growing economy.

In this chapter, we will explore the intersections between microbiology and epidemiology, the science that underlies public health. Epidemiology studies how disease originates and spreads throughout a population, with the goal of preventing outbreaks and containing them when they do occur. Over the past two centuries, discoveries in epidemiology have led to public health policies that have transformed life in developed nations, leading to the eradication (or near eradication) of many diseases that were once causes of great human suffering and premature death. However, the work of epidemiologists is far from finished. Numerous diseases continue to plague humanity, and new diseases are always emerging. Moreover, in the developing world, lack of infrastructure continues to pose many challenges to efforts to contain disease.



Figure 18.1: Signs like this may seem self-explanatory today, but a few short centuries ago, people lacked a basic understanding of how diseases spread. Microbiology has greatly contributed to the field of epidemiology, which focuses on containing the spread of disease. (credit: modification of work by Tony Webster)

18.1: The Language of Epidemiologists

18.2: Pioneers of Epidemiology

18.3: Types of Epidemiological Studies

18.4: How Diseases Spread

18.5: Global Public Health

18.E: Disease and Epidemiology (Exercises)

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18.1: The Language of Epidemiologists

Learning Objectives

- Explain the difference between prevalence and incidence of disease
- Describe how morbidity and mortality rates are expressed
- 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

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 18.1.1. 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 rate can 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).

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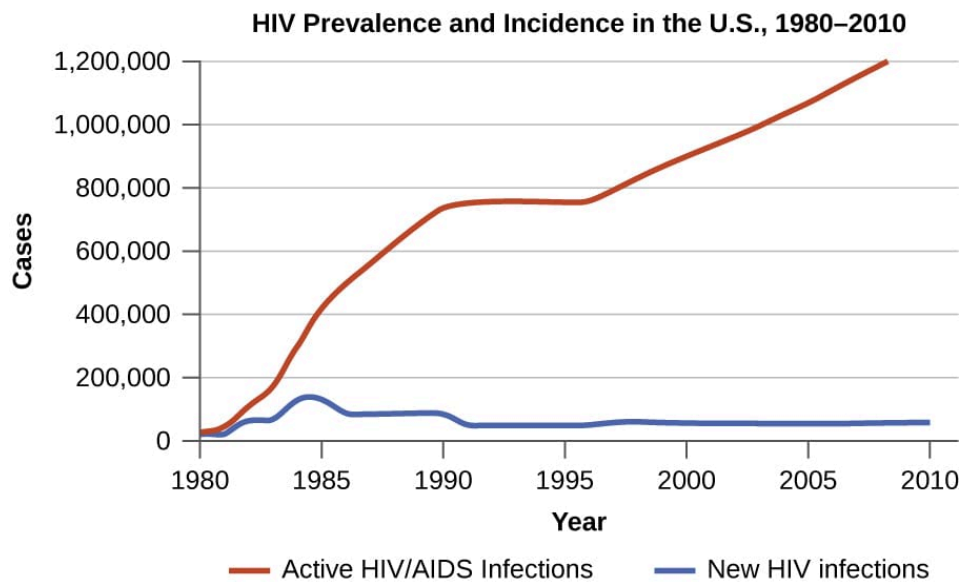


Figure 18.1.1: This graph compares the incidence of HIV (the number of new cases reported each year) with the prevalence (the total number of cases each year). Prevalence and incidence can also be expressed as a rate or proportion for a given population.

Incidence rate and prevalence rate are often calculated as the number of people per 100,000 population, or as a percentage. For example, in 2003, it was reported that there were 44,232 new cases of AIDS in the US. The estimated mid-year population of the US in 2003 was 290 million (290,809,777). The incidence rate is calculated as: $(44,232 / 290,809,777) \times 100,000 = 15.21$ new cases of AIDS per 100,000 population. In another example, a survey of 1,150 women who gave birth in Maine in 2000, a total of 468 reported taking a multivitamin at least 4 times a week during the month before becoming pregnant. The prevalence rate is calculated as $(468 / 1,150) \times 100 = 40.7\%$.

Here is an opportunity to practice the calculations as depicted in the HIV figure above.

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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 unusually large number of seasonal influenza cases in particular regions, and such

situations would qualify as epidemics (Figure 18.1.2 and Figure 18.1.3).

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.

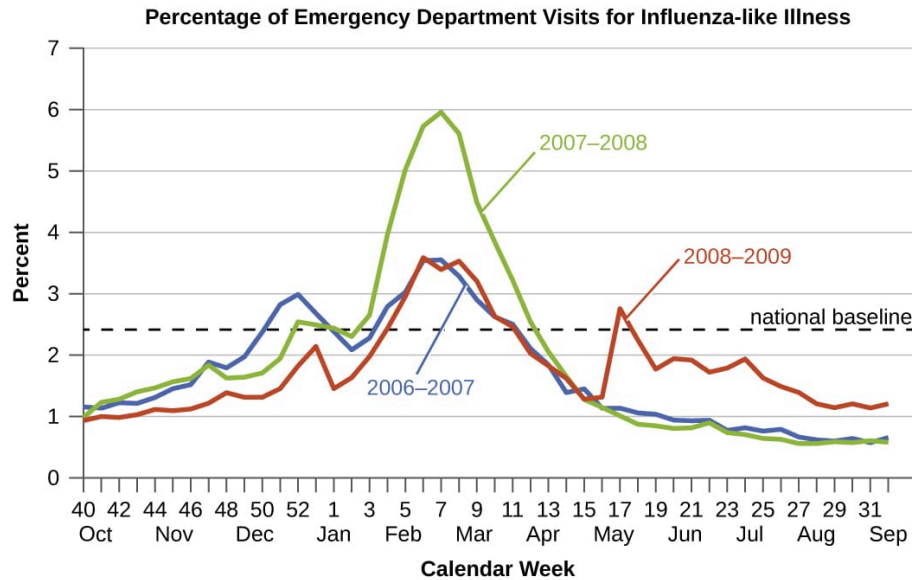


Figure 18.1.2: The 2007–2008 influenza season in the United States saw much higher than normal numbers of visits to emergency departments for influenza-like symptoms as compared to the previous and the following years. (credit: modification of work by Centers for Disease Control and Prevention)

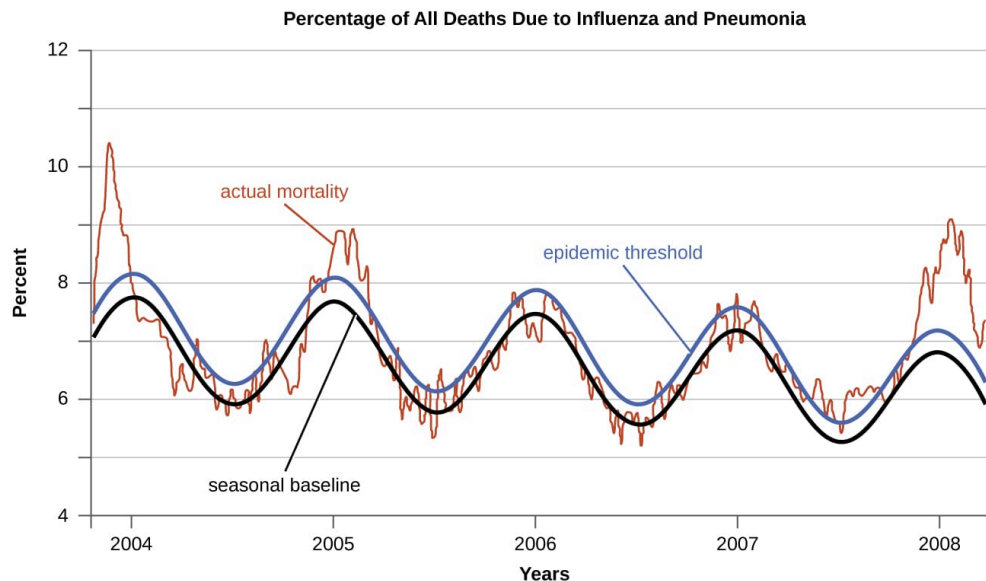


Figure 18.1.3: The seasonal epidemic threshold (blue curve) is set by the CDC-based data from the previous five years. When actual mortality rates exceed this threshold, a disease is considered to be epidemic. As this graph shows, pneumonia- and influenza-related mortality saw pronounced epidemics during the winters of 2003–2004, 2005, and 2008. (credit: modification of work by Centers for Disease Control and Prevention)

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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](#).

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 18.1.1 is an example of the kind of data contained in the *MMWR*.

Table 18.1.1: 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
<i>Chlamydia trachomatis</i> 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.

Interactive Element

Case Study Preview: “Turkey, Duck, and Disaster: The Stuffing That Stuffed Everyone Up”

When hospitals in western Florida report a sudden surge of vomiting and diarrhea cases right after Thanksgiving, public health officials race to find the source. With symptoms pointing to acute gastroenteritis and lab tests revealing *Salmonella*, investigators follow the clues back to a surprising culprit: the holiday favorite turducken - a chicken inside a duck inside a turkey. It turns out the bird within a bird within a bird harbored more than just seasonal cheer.

In this case study, you'll step into the role of an outbreak investigator, exploring routes of transmission, the importance of food safety practices, diagnostic testing, and the power of public health responses like recalls and inspections. From contamination at a regional processing plant to a full-scale product recall, this real-world scenario shows how fast thinking and detailed epidemiology can stop an outbreak in its tracks.

It wasn't the turkey or the duck - or even the chicken - it was the stuffing between the cracks.

[Chapter 16 Case Study - Turkey, Duck, and Disaster: The Stuffing That Stuffed Everyone Up](#)

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. 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. Centers for Disease Control and Prevention. “Diagnoses of HIV Infection in the United States and Dependent Areas, 2014.” *HIV Surveillance Report* 26 (2015).
3. Centers for Disease Control and Prevention. “Tetanus Surveillance—United States, 2001–2008.” *Morbidity and Mortality Weekly Report* 60, no. 12 (2011): 365.
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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18.2: Pioneers of Epidemiology

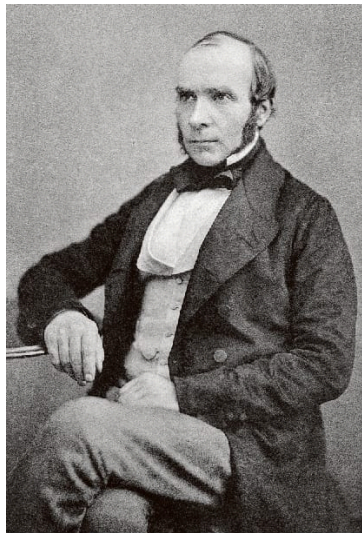
Learning Objectives

- Describe the contributions of John Snow to the field of epidemiology
- Explain how Florence Nightingale used statistical analysis and sanitation reforms to reduce mortality rate in military hospitals
- Analyze the impact of Joseph Lister's introduction to aseptic techniques in surgery

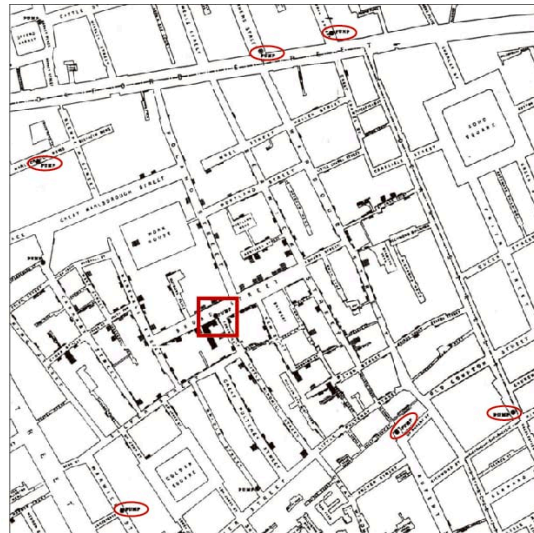
Epidemiology has its roots in the work of physicians who looked for patterns in disease occurrence as a way to understand how to prevent it. The idea that disease could be transmitted was an important precursor to making sense of some of the patterns. In 1546, Girolamo Fracastoro first proposed the germ 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 18.2.1) 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 18.2.1: (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.

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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 18.2.2). In addition, because of person-to-person transmission, propagated spread cannot be easily stopped at a single source like point source spread.

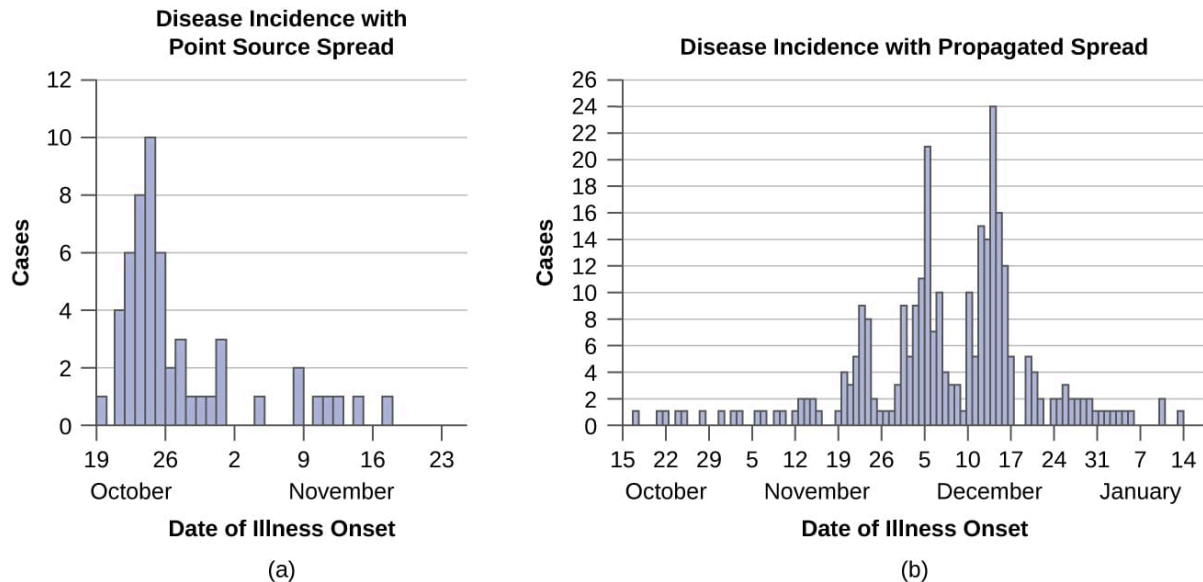


Figure 18.2.2: (a) Outbreaks that can be attributed to point source spread often have a short duration. (b) Outbreaks attributed to propagated spread can have a more extended duration. (credit a, b: modification of work by Centers for Disease Control and Prevention)

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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 18.2.3). 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 18.2.4). 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.

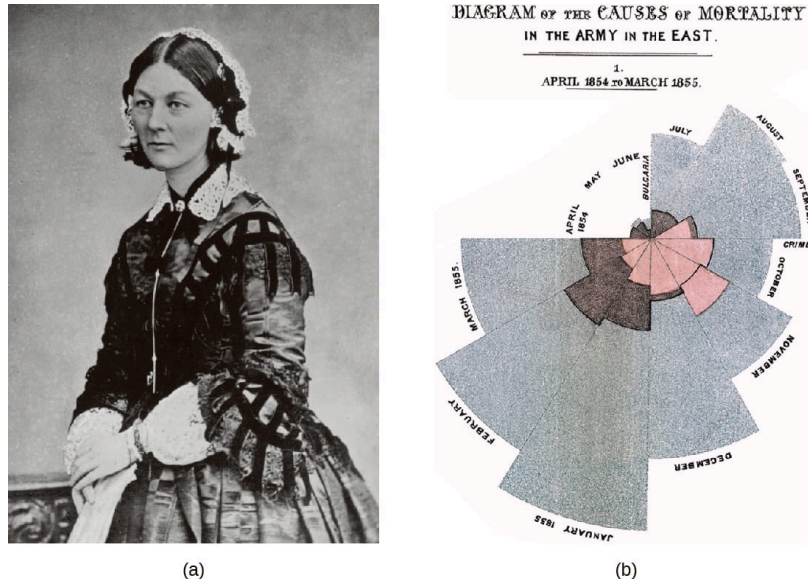


Figure 18.2.3: (a) Florence Nightingale reported on the data she collected as a nurse in the Crimean War. (b) Nightingale's diagram shows the number of fatalities in soldiers by month of the conflict from various causes. The total number dead in a particular month is equal to the area of the wedge for that month. The colored sections of the wedge represent different causes of death: wounds (pink), preventable infectious diseases (gray), and all other causes (brown).

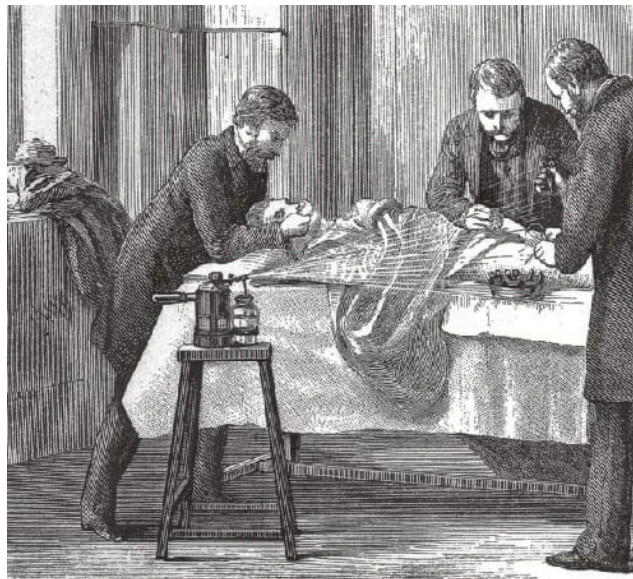


Figure 18.2.4: Joseph Lister initiated the use of a carbolic acid (phenol) during surgeries. This illustration of a surgery shows a pressurized canister of carbolic acid being sprayed over the surgical site.

Link to Learning

This [CDC resource](#) further breaks down the pattern expected from a point-source outbreak.

Learn more about [Nightingale's wedge chart](#) here.

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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.

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-Square, and at Deptford." *Medical Times and Gazette* 9 (1854): 321–322.
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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18.3: Types of Epidemiological Studies

Learning Objectives

- Define the major types of epidemiological studies including observational (descriptive, analytical) and experimental studies
- Distinguish between retrospective and prospective cohort studies and how they are used to study disease outcomes
- Differentiate between cohort, case-control, and cross-sectional studies
- Explain the advantages and limitations of each type of epidemiological study

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.

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Key Concepts and Summary

- **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.

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18.4: How Diseases Spread

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."

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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 18.4.1) 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 18.4.2). 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 18.4.1: 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)

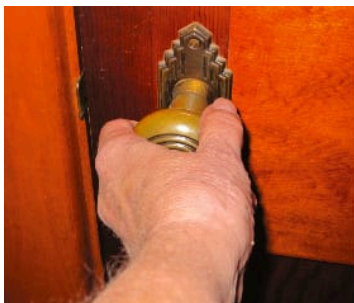


Figure 18.4.2: Fomites are nonliving objects that facilitate the indirect transmission of pathogens. Contaminated doorknobs, towels, and syringes are all common examples of fomites. (credit left: modification of work by Kate Ter Haar; credit middle: modification of work by Vernon Swanepoel; credit right: modification of work by "Zaldylmg"/Flickr)

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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 18.4.3).

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 18.4.3: 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)

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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 18.4.4).

Biological transmission occurs when the pathogen reproduces within a **biological vector** that transmits the pathogen from one host to another (Figure 18.4.4). Arthropods are the main vectors responsible for biological transmission (Figure 18.4.5). 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.

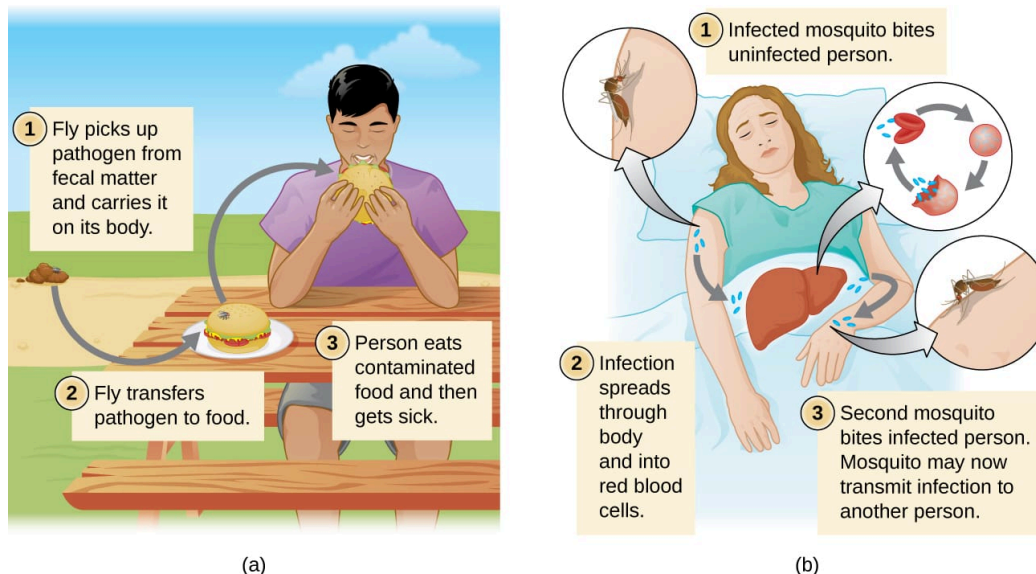


Figure 18.4.4: (a) A mechanical vector carries a pathogen on its body from one host to another, not as an infection. (b) A biological vector carries a pathogen from one host to another after becoming infected itself.

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.	Yellow fever virus	Yellow fever
	Haemagogus spp.		
	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 18.4.5: (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)

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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 18.4.6). 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, 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. 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 18.4.6). 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.

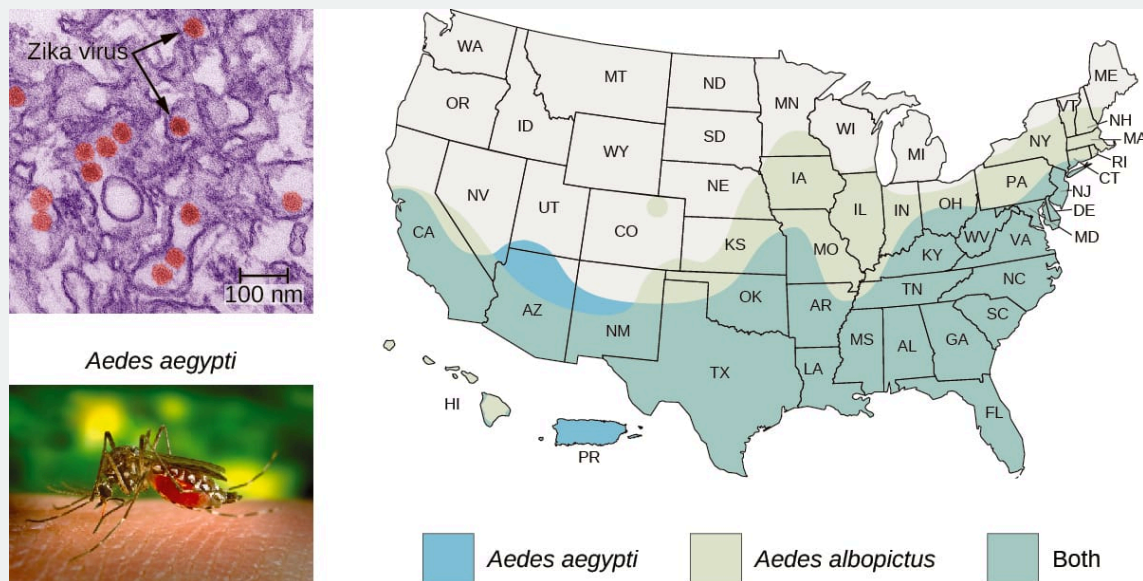


Figure 18.4.6: The Zika virus is an enveloped virus transmitted by mosquitoes, especially *Aedes aegypti*. The range of this mosquito includes much of the United States, from the Southwest and Southeast to as far north as the Mid-Atlantic. The range of *A. albopictus*, another vector, extends even farther 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 18.4.7). 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 18.4.7: (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](#).

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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 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 Filio Marineli, Gregory Tsoucalas, Marianna Karamanou, and George Androustos. "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 World Health Organization. Fact sheet No. 391—*Drinking Water*. June 2005. www.who.int/mediacentre/factsheets/fs391/en.
- 4 Blandine Massonnet-Bruneel, Nicole Corre-Catelin, Renaud Lacroix, Rosemary S. Lees, Kim Phuc Hoang, Derric Nimmo, Luke Alphey, and Paul Reiter. "Fitness of Transgenic Mosquito *Aedes aegypti* Males Carrying a Dominant Lethal Genetic System." *PLOS ONE* 8, no. 5 (2013): e62711.
- 5 Richard Levine. "Cases of Dengue Drop 91 Percent Due to Genetically Modified Mosquitoes." *Entomology Today*. entomologytoday.org/2016/07/...ied-mosquitoes.
- 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 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 Constância F.J. Ayres. "Identification of Zika Virus Vectors and Implications for Control." *The Lancet Infectious Diseases* 16, no. 3 (2016): 278–279.
- 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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18.5: 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 2015–16¹, 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.

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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 18.5.1). 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 18.5.1). 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.

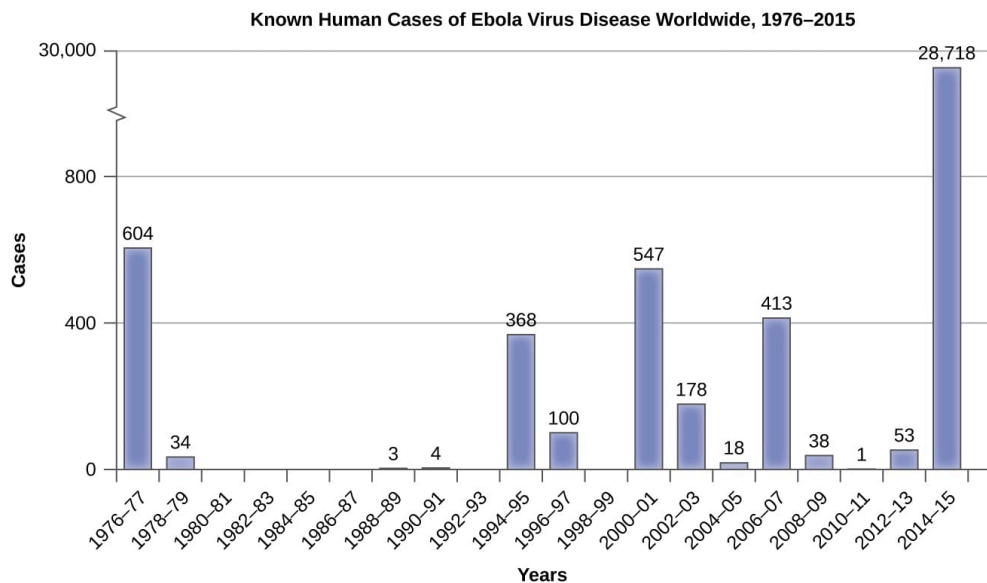


Figure 18.5.1: Even before the Ebola epidemic of 2014–15, Ebola was considered an emerging disease because of several smaller outbreaks between the mid-1990s and 2000s.

Table 18.5.1: 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

Disease	Pathogen	Year Discovered	Affected Regions	Transmission
Lyme disease	<i>Borrelia burgdorferi</i> 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

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 18.5.2).

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 18.5.2: 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.

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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 World Health Organization. "Programme Budget 2014–2015." www.who.int/about/finances-accountability/budget/en.

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18.E: Disease and Epidemiology (Exercises)

18.1: The Language of Epidemiologists

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).

Matching

Match each term with its description.

___sporadic disease	A. the number of disease cases per 100,000 individuals
___endemic disease	B. a disease in higher than expected numbers around the world
___pandemic disease	C. the number of deaths from a disease for every 10,000 individuals
___morbidity rate	D. a disease found occasionally in a region with cases occurring mainly in isolation from each other
___mortality rate	E. a disease found regularly in a region

Answer

D, E, B, A, C

Fill in the Blank

The _____ collects data and conducts epidemiologic studies in the United States.

Answer

Centers for Disease Control and Prevention, or CDC

Short Answer

During an epidemic, why might the prevalence of a disease at a particular time not be equal to the sum of the incidences of the disease?

In what publication would you find data on emerging/reemerging diseases in the United States?

Critical Thinking

Why might an epidemiological population in a state not be the same size as the number of people in a state? Use an example.

18.2: Tracking Infectious Diseases

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.

Matching

Match each type of epidemiology study with its description.

___experimental	A. examination of past case histories and medical test results conducted on patients in an outbreak
___analytical	B. examination of current case histories, interviews with patients and their contacts, interpretation of medical test results; frequently conducted while outbreak is still in progress
___prospective	C. use of a set of test subjects (human or animal) and control subjects that are treated the same as the test subjects except for the specific treatment being studied
___descriptive	D. observing groups of individuals to look for associations with disease
___retrospective	E. a comparison of a cohort of individuals through the course of the study

Answer

C, D, E, B, A

Match each pioneer of epidemiology with his or her contribution.

___Florence Nightingale	A. determined the source of a cholera outbreak in London
___Robert Koch	B. showed that surgical wound infection rates could be dramatically reduced by using carbolic acid to disinfect surgical tools, bandages, and surgical sites
___Joseph Lister	C. compiled data on causes of mortality in soldiers, leading to innovations in military medical care
___John Snow	D. developed a methodology for conclusively determining the etiology of disease

Answer

C, D, B, A

Fill in the Blank

_____occurs when an infected individual passes the infection on to other individuals, who pass it on to still others, increasing the penetration of the infection into the susceptible population.

Answer

Propagated spread

A batch of food contaminated with botulism exotoxin, consumed at a family reunion by most of the members of a family, would be an example of a _____ outbreak.

Answer

point source

Short Answer

What activity did John Snow conduct, other than mapping, that contemporary epidemiologists also use when trying to understand how to control a disease?

18.3: How Diseases Spread

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.

Multiple Choice

Which is the most common type of biological vector of human disease?

- A. viruses
- B. bacteria
- C. mammals
- D. arthropods

Answer

D

A mosquito bites a person who subsequently develops a fever and abdominal rash. What type of transmission would this be?

- A. mechanical vector transmission
- B. biological vector transmission
- C. direct contact transmission
- D. vehicle transmission

Answer

B

Cattle are allowed to pasture in a field that contains the farmhouse well, and the farmer's family becomes ill with a gastrointestinal pathogen after drinking the water. What type of transmission of infectious agents would this be?

- A. biological vector transmission
- B. direct contact transmission
- C. indirect contact transmission
- D. vehicle transmission

Answer

D

A blanket from a child with chickenpox is likely to be contaminated with the virus that causes chickenpox (Varicella-zoster virus). What is the blanket called?

- A. fomite
- B. host
- C. pathogen
- D. vector

Answer

A

Fill in the Blank

A patient in the hospital with a urinary catheter develops a bladder infection. This is an example of a(n) _____ infection.

Answer

nosocomial or healthcare-associated

A _____ is an animal that can transfer infectious pathogens from one host to another.

Answer

vector

Short Answer

Differentiate between droplet vehicle transmission and airborne transmission.

Critical Thinking

Many people find that they become ill with a cold after traveling by airplane. The air circulation systems of commercial aircraft use HEPA filters that should remove any infectious agents that pass through them. What are the possible reasons for increased incidence of colds after flights?

18.4: Global Public Health

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.

Multiple Choice

Which of the following would NOT be considered an emerging disease?

- A. Ebola hemorrhagic fever
- B. West Nile virus fever/encephalitis
- C. Zika virus disease
- D. Tuberculosis

Answer

D

Which of the following would NOT be considered a reemerging disease?

- A. Drug-resistant tuberculosis
- B. Drug-resistant gonorrhea
- C. Malaria
- D. West Nile virus fever/encephalitis

Answer

D

Which of the following factors can lead to reemergence of a disease?

- A. A mutation that allows it to infect humans
- B. A period of decline in vaccination rates
- C. A change in disease reporting procedures
- D. Better education on the signs and symptoms of the disease

Answer

B

Why are emerging diseases with very few cases the focus of intense scrutiny?

- A. They tend to be more deadly
- B. They are increasing and therefore not controlled
- C. They naturally have higher transmission rates
- D. They occur more in developed countries

Answer

B

Fill in the Blank

The _____ collects data and conducts epidemiologic studies at the global level.

Answer

WHO (World Health Organization)

Critical Thinking

An Atlantic crossing by boat from England to New England took 60–80 days in the 18th century. In the late 19th century the voyage took less than a week. How do you think these time differences for travel might have impacted the spread of infectious diseases from Europe to the Americas, or vice versa?

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19: MICROBIOMES

The human microbiome and the health of animals and the environment are increasingly recognized as essential components of public health. Microorganisms are not only agents of disease but also vital partners in maintaining the health of individuals and ecosystems. The growing burden of chronic and emerging infectious diseases, antibiotic resistance, and the effects of climate change have shown that human health cannot be viewed in isolation.

In this chapter, we will explore the connections between the microbiome and One Health, an interdisciplinary approach that links the health of people, animals, and the environment. We will examine how microbial communities support digestion, immunity, and development - and how disruptions to these communities can contribute to disease. We will also investigate how zoonotic diseases emerge, why antibiotic resistance spreads between sectors, and what global cooperation is needed to tackle these challenges. Advances in microbiome research and the One Health framework are reshaping how we understand, monitor, and promote health in a rapidly changing world.

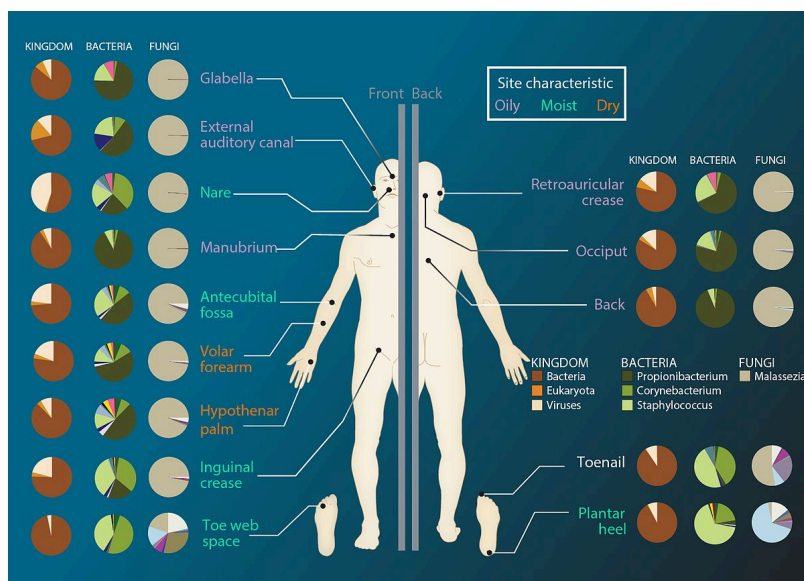


Figure 19.1: Skin metagenomics and defines relative abundance of viral, bacterial and fungal components of the microbial community. Credit: Darryl Leja, NHGRI.

19.1: An Introduction to Microbiomes

19.2: The Human Microbiome

19.3: Analyzing Microbiomes

19.4: Human Health and Disease

19.5: The Gut Microbiome

19.6: C diff Infection (CDI) and Fecal Microbiota Transplant (FMT)

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19.1: An Introduction to Microbiomes

Learning Objectives

- Contrast the terms microbiome and microbiota
- Describe the major types of microbes that make up the human microbiome
- Discuss how multi-omics approaches (e.g., genomics, metagenomics, transcriptomics, proteomics, and metabolomics) are used to study the microbiome

An Introduction to Microbiomes

Microorganisms represent the fundamentals of life and interact with almost every facet of it. Yet, for the majority of their existence they have been largely ignored, or rather unseen by humans. The unraveling of the complex interactions between all life forms is an endless duty and seems to generate more questions than answers. However, the cracks in knowledge produced by peering into the unknown offers insight into our life and the world around us. These minute creatures have shaped Earth's evolution since their dawn almost three-and-a-half billion years ago, and continuously affect our environment and health. The importance of the planet's collection of microbes is realized more and more each day, and our unceasing investigation of them will surely unlock secrets of life we could never imagine.

The study of microbiomes is fairly novel in the context of understanding and applying their communal existence to ourselves and surroundings. Though scientists have recognized symbiotic relationships and traditionally focused on individual microbes and their interactions with human health, environmental impact, industrial applications, etc., their respective communities and influence as a whole in these areas have only just begun to be elucidated. That is in no small part due to the daunting task of cataloging the immense and complex interplay between the multitude of different microorganisms in a given environment, though rapid advances in technology have begun to ease analysis.

What is a microbiome?

A **microbiome** can be best described as a collective polymicrobial community, or '**microbiota**', and its associated activity with genetic and physio-chemical constituents in a defined spatiotemporal habitat (Figure 1). These members of the microbiota include bacteria, archaea, algae, protozoa, fungi, and viruses (though the latter is somewhat debated since viruses and their derivatives aren't technically living). Within this symbiotic context with a particular eukaryotic host, the entire entity is termed a 'holobiont' and the aggregate of genetic material termed the 'hologenome'. Interactions between these partners may have long occurred, shaping the evolution of each, whereas others may be novel or transient, sometimes resulting in prompt change and infectious diseases. The change in the normal microbiota, or **dysbiosis**, can result in a variety of different diseases. As so, their study has been especially important in the fields of life sciences, human health, and medicine.

Diagram showing the definition of a microbiome, which includes all microorganisms and their interactions in a defined area and time.

Figure 1. A schematic highlighting the composition of the term microbiome containing both the microbiota (community of microorganisms) and their "theatre of activity" (structural elements, metabolites/signal molecules, and the surrounding environmental conditions). (Berg et al., 2020 adapted by Dylan Parks).

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Quick Quiz

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Our fascination with microorganisms began before we even fully understood them or were even able to see them. Their implications concerning human health were primarily explored during the 'golden age of microbiology' with the work of Louis Pasteur and Robert Koch. Their experiments and discoveries shed light on not only the ubiquitous nature of microbes, but their importance in our everyday lives. Other significant milestones and historical microbiological development can be viewed in Figure 2. Human health and infectious diseases were central to the field of microbiology, though food microbiology, industrial applications, and microbial ecology became increasingly explored. Over the last couple centuries, a microbial catalog of knowledge has slowly grown, but much of these findings were limited to those organisms that could be cultured and measured.

The advent of sequencing and **'multi-omics' technologies** has since allowed researchers to document microorganisms that were previously missed or ignored with traditional techniques, and with further advances, larger microbial communities and symbioses can be better understood. Multi-omics refers to the analysis of genes, proteins, metabolism, etc. on a system level. For example, **genomics** refers to the sequencing of the whole genome; **proteomics** refers to the analysis of all the proteins in cells; and **metabolomics** is the study of all metabolites in cells.

The 'microbiome' was first defined in the late 1980s when a group of microbial ecologists were studying the rhizosphere, which provided context to better describe these polymicrobial communities (Whipps et al., 1988). Many other similar definitions have been published since then with varying specifics on genetic expression, symbioses, and ecological interactions (Lederberg & McCray, 2001, Marchesi & Ravel, 2015, Berg et al., 2020). The 'holobiont' concept stems from Adolf Meyer-Abich's 'theory of holobiosis' proposed in 1943 and was independently conceived and popularized in the early 1990s by Lynn Margulis, though it only described the host and a single symbiont (Margulis, 1991, Baedke et al., 2020). Since then has been expanded to include the entire microbiota in multiple symbiotic contexts (Simon et al., 2019). In recent decades there has been a steady increase in microbiome publications as the subject has grown in popularity. Along with that, there has been more analytical breakdown as certain microbiomes are being described with emphasis on specific members, such as the 'bacteriome', 'archaeome', 'mycobiome', 'protistome', and 'virome', and these terms are best used to refer to the distinct contribution of those particular microbes within the entire microbiome context. In general, though, most microbiomes are delineated by their specific host or type of environment, with the human microbiome being the most popular example.

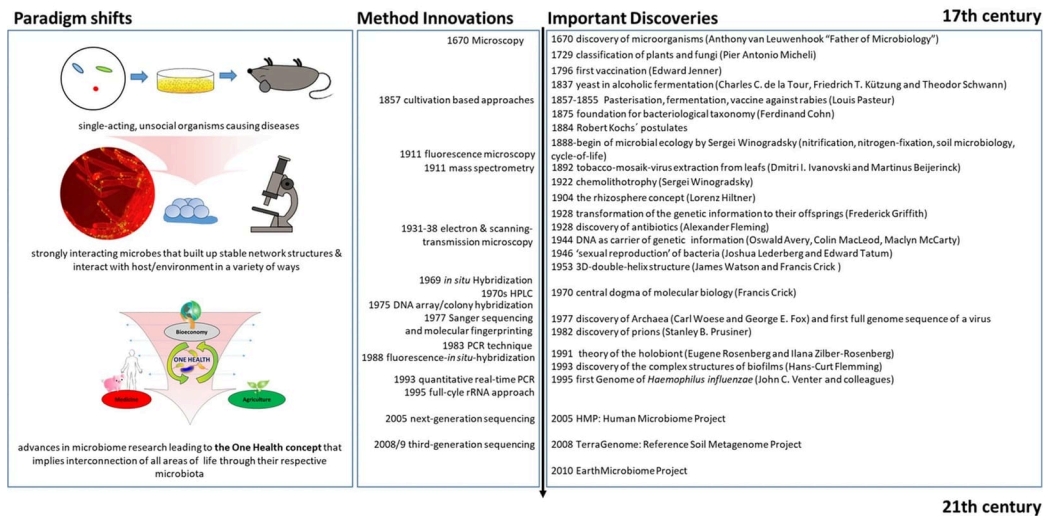


Figure 2. The history of microbiome research from seventieth century until our days, highlighting the shift of the paradigm from microbes as unsocial organisms causing diseases to the holistic view of microorganisms being the center of the One Health Concept: positively interconnecting all areas of our lives. (Berg et al., 2020).

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Key Concepts and Summary

- **Microbiota** refers to the community of microorganisms (bacteria, archaea, fungi, viruses, etc.) living in a specific environment, such as the human gut or soil.
- **Microbiome** includes both the microbiota and their collective genetic material, metabolic activity, and environmental context—also known as their “theater of activity.”
- The concept of the **holobiont** represents a host organism together with its associated microbiota; their combined genetic information is referred to as the hologenome.
- Microbiomes are diverse, dynamic ecosystems that influence and are influenced by their hosts, often shaping health, disease, and evolution.

- The term **dysbiosis** refers to an imbalance in the normal microbiota, which may contribute to disease.

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19.2: The Human Microbiome

Learning Objectives

- Describe the goals and major accomplishments of the Human Microbiome Project (HMP) and iHMP
- Explain how microbial communities vary across different body sites
- Analyze how local conditions (e.g., moist, dry, or oily) influence microbiome composition

The Human Microbiome

It was evident that the human microbiome and its involvement in a micro and macro scale needed to be characterized. The **Human Microbiome Project (HMP)** set out in 2007 with this as one of its primary goals (Turnbaugh et al., 2007). The program also set out with initiatives to develop a set of microbial genome sequences, explain the relationship between disease and microbiome changes and evaluate the data with multi-omics approaches, develop new tools and technology for computational analysis, establish a data analysis and coordinating center and research repositories, as well as address ethical, social, and legal implications of HMP research (Human Microbiome Project). The second phase of the HMP launched in 2014, called the **Integrative Human Microbiome Project (iHMP)**, having the main mission to completely characterize the human microbiota with a key focus on human health and disease using three projects: pregnancy and preterm birth, onset of inflammatory bowel disease (IBD), and onset of type 2 diabetes (NIH Human Microbiome Project, The Integrative HMP (iHMP) Research Network Consortium, 2019). Aside from these, the human microbiome and disruption of the microbiota has been linked to several other important conditions and diseases including multiple sclerosis, diabetes (types 1 and 2), allergies, asthma, autism, and cancer (Backhed et al., 2012, Hsiao et al., 2013, Petersen and Round, 2014, Trompette et al., 2014, Garrett, 2015, Lloyd-Price et al., 2016).

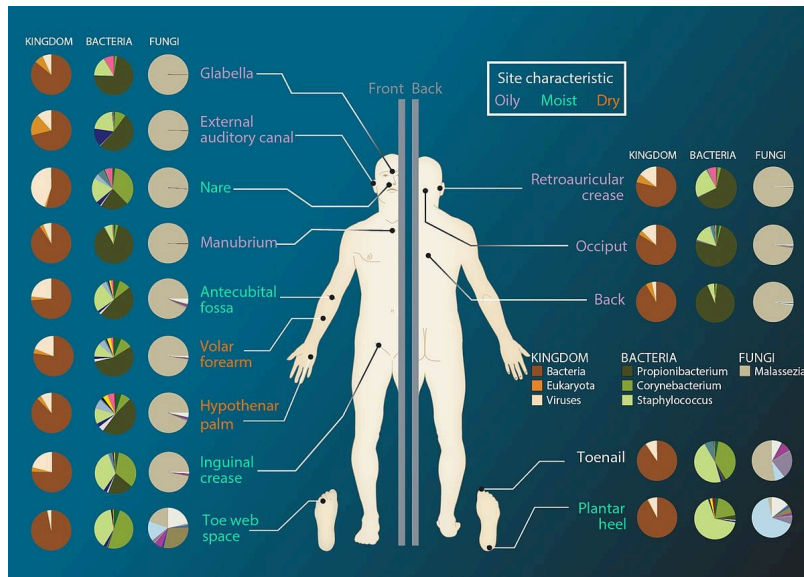


Figure 19.2.1: This diagram shows the microbial composition across different sites on the human body, highlighting variation by location and skin type (oily, moist, or dry). Each site's pie charts represent the relative abundance of bacteria, fungi, and other microbial kingdoms. Dominant bacterial genera include *Propionibacterium*, *Corynebacterium*, and *Staphylococcus*, while *Malassezia* is the primary fungal genus. The figure illustrates how microbial communities are shaped by local environmental conditions. "Microbiome Sites" by National Human Genome Research Institute is licensed under CC BY 2.0.

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It makes sense that the human microbiome can have such an impact on human health and behavior if you consider that we are essentially a collection of organisms forming a living entity. In a way, our symbionts may even actually define more of who we are than just our own unique biological makeup. For instance, the ratio of microbial cells associated with a human body could equal, if not exceed (traditional estimates were tenfold), the number of human cells ([Sender et al., 2016](#)). Even more interesting is viewing our genetic makeup; the human genome contains about 20,000 genes, but its hologenome contains > 33 million genes brought by its microbiota ([Huttenhower et al., 2012](#), [Lloyd-Price et al., 2016](#), [Simon et al., 2019](#)). Furthermore, the composition and rate of change of each person's microbiota is distinctive from one individual to another since it is influenced by variables like age, lifestyle, diet, antibiotics, occupation, environment, etc. ([Gilbert et al., 2018](#)). The genetic wealth and member diversity contributed from the microbiota has roles in adaptation, survival, development, growth, and reproduction of the holobiont and can affect fitness in the short term as well as have long lasting effects concerning the evolution of both partners ([Rosenberg, and Zilber-Rosenberg, 2011](#)).

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Co-evolution of the host-microbiota symbiosis can be considered even more unique when viewing the microbial consortium at different locations or organs in the host, as their makeup is governed by and reflects specific physiological processes in those areas. For example, the bacteria found in the human gut microbiota are primarily from the phyla Bacteroides and Firmicutes, whereas Actinobacteria and Proteobacteria command the skin microbiome, though there is some overlap and it is important to note that there are differences depending on exact location (e.g. dry vs. moist areas of the skin) ([Grice and Segre, 2011](#), [Jandhyala et al. 2015](#)). Though there are differences between various microbiota within a holobiont, they can still influence each other to some degree. In the case of the gut and skin microbiotas in humans, deemed the 'gut-skin axis', there are indications that both the health of the gastrointestinal (GI) tract and skin, as well as their response to stressors, are correlated ([Levcovich et al., 2013](#), [O'Neill et al., 2016](#), [Salem et al. 2018](#)). Even more interesting is the effects certain microbiota can have on germ-free organs like the brain. Studies on the '**gut-brain axis**' show that the microbiota in the GI tract, and in some cases disruption of it, are associated with many mental illnesses and neurodegenerative disorders including depression, anxiety, autism, schizophrenia, Parkinson's disease, and Alzheimer's disease ([Clapp et al. 2017](#), [Foster et al. 2017](#), [Cryan et al. 2019](#)). A variety of different 'axes' which demonstrate interplay between microbiota, organs, and locations have been identified in the human body and much of what is known about their connections is novel and early in its research.

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Key Concepts and Summary

- The **Human Microbiome Project (HMP)** was launched in 2007 to characterize the human microbiome, explore its connection to health and disease, and develop tools for microbial genomics and data analysis.
- The **Integrative Human Microbiome Project (iHMP)**, launched in 2014, focused on microbiome changes related to pregnancy/preterm birth, inflammatory bowel disease (IBD), and type 2 diabetes.
- Disruptions to the human microbiome have been associated with a wide range of diseases and conditions, including allergies, asthma, cancer, and neurological disorders.
- The microbiome is site-specific, varying by body location (e.g., gut, skin), and is influenced by environmental conditions such as moisture and pH.
- Humans are **holobionts** - a complex ecosystem of host and microbial cells—where the microbiota contributes the majority of genetic content (the hologenome).
- The microbiome is shaped by lifestyle, diet, age, antibiotic use, and environment, resulting in highly individualized microbial communities.
- Key symbiotic pathways such as the gut-skin axis and **gut-brain axis** illustrate the deep interconnection between microbiota and host systems, including immune, nervous, and integumentary systems.

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19.3: Analyzing Microbiomes

Learning Objectives

- Differentiate between culture-dependent and culture-independent methods for analyzing microbiomes
- Compare and contrast metagenomics, metatranscriptomics, metaproteomics, and metabolomics in terms of purpose, methodology and limitations
- Discuss the role of culturomics in recovering rare or slow-growing microorganisms that may be missed by sequencing approaches

Analyzing Microbiomes

Choosing the correct approach when characterizing a microbiome is important when considering what questions are trying to be answered. Who are the players? What is the function? What genes are being expressed? How do they interact?

Even though when assessing a microbiome all members of the microbiota should be considered, some insight can be gained by viewing individual groups, such as the prokaryotic component, which can be further compartmentalized into the bacteriome and archaeome. In some cases, it is difficult to completely characterize all of the members due to inherent biological differences between these communities, and in other situations, certain groups may be absent or irrelevant to the study. In so, analyzing these individual pieces could help reveal their distinct importance and contribution to the overall big picture.

The type of analysis also depends on the research question. If the sole interest is to identify all the bacteria in a sample, then a metagenomic approach may work best. Whereas, if an interest lies in what the microorganisms are doing, then a metatranscriptomics or metaproteomics approach would be more appropriate. If there is an interest in one or a few particular members of the microbiome, culture-based techniques (culturomics) could be implemented, which can often catch rare microbes that are missed using sequencing techniques because of data filtering protocols (Lagier et al., 2012, Allaband et al., 2019). However, it is usually difficult to characterize an entire microbiome based on just a few microbes and achieving multiple isolations is extremely difficult as a 'one-size-fits-all' type media does not exist.

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Sample Collection

Depending on the type of research questions and the particular microbiome of interest, there are different ways to collect microbial communities that will be subjected to analysis. That is, the approach to collecting microbes from the human gut is much different than getting a soil sample. Really, there is no perfect method for any one microbiome, though some may be more difficult to obtain than others, and will depend on a variety of factors such as feasibility, cost, patient acceptance, and downstream analytical methods (Allaband et al., 2019). It can also be very difficult to capture all microbes within a microbiome sample, as inevitably some will be missed or cannot be collected by a particular technique. For example, when analyzing the gut microbiome, stool collection is preferred due to its ease and frequent accessibility, but this method often misses microbes in the small intestine and others which are mucosally adherent as opposed to excreted (Eckburg et al., 2005, de Carcer et al., 2010, Allaband et al., 2019). Additionally, collection and storage materials, storage time, and transport options should be considered as these can compromise sample integrity and create problems for various analytical approaches (Costello et al., 2009, Caporaso et al., 2011, McDonald et al., 2018).

Approaches to Characterization

What is the Difference between Culture-Dependent and Independent Methods of Microbial Community Characterization?

-Excerpt taken from [Findley and Grice, 2014](#) available under the [Creative Commons CC0 public domain dedication](#):

Up until the 1980s, microbiologists routinely relied on culture-dependent methods for microbial isolation, identification, and characterization. Colony morphology, stains (i.e., Gram stain), biochemical characteristics (i.e., coagulase test), motility tests, antibiotic resistance profiles, and other characteristics guided bacterial and/or fungal identification and taxonomy. However, this approach has several limitations, including an inability to mimic *in vivo* conditions and selection against slow-growing and/or fastidious organisms. With recent advances in sequencing technologies and development of bioinformatics tools and reference databases, researchers are now better equipped to capture microbial diversity without the biases of culture-based approaches.

Culture-independent methods of microbial identification rely on a targeted amplicon strategy, which employs highly conserved microbe-specific molecular markers and does not rely on growing isolates in pure culture. The 16S ribosomal RNA (rRNA) gene is used for bacterial identification, while fungi and other microeukaryotes are identified using either the 18S rRNA gene or the Internal Transcribed Spacer (ITS) region. A complementary approach to amplicon-based surveys is whole genome shotgun metagenomics. With this approach, one can identify the microbiota present and gain insight into the functional potential of the microbiota in an untargeted manner.

Within the culture-independent targeted approach, PCR amplification techniques, like quantitative PCR (qPCR) and reverse transcriptase PCR (RT-PCR) can be implemented to detect and quantify specific organisms, genes, or expression values within the microbiome sample. Untargeted approaches typically give whole community characterization and include metagenomics, metatranscriptomics, metaproteomics, and metabolomics which have a variety of applications depending on the type of study.

Culturomics

Cultivating all the microorganisms within a certain microbiome can be quite challenging and time-consuming, but nonetheless can provide important information that would otherwise be missed with other approaches. Creating media that mimics the original environment can help to capture organisms that exist at lower concentrations than that of the detectable threshold of molecular tools ([Lagier et al., 2012](#)). The application of **matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)** has enhanced culture-dependent studies by allowing fast and reliable identification of bacterial taxa in a cost-effective manner ([Seng et al., 2009](#), [Lagier et al., 2012](#)). Even though there have been advances in **culturomics**, the approach still has certain biases based on the types of microbes that can be easily grown (e.g. aerobic microbes are much easier to grow than anaerobic ones in most cases), as well as the selection for those that grow more rapidly and outcompete the others in a sample ([Allaband et al., 2019](#)). Thusly, it is imperative to close the gap between microbial richness observed in nature versus what is cultivable in the lab in order to better understand *in vivo* microbial functioning within a microbiome ([Sarhan et al., 2019](#)).

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Metagenomics and DNA sequencing

Next-generation sequencing (NGS), also known as **high-throughput sequencing (HTS)**, allows the generation of thousands of sequencing reads in a cost-effective manner (Nkrumah-Elie, et al., 2018). These reads can then be bioinformatically processed and annotated to identify microbial taxa, genes, potential functions, etc. depending on the sample type and experimental conditions (Rivera-Pinto et al., 2018). However, differences in wet lab work, sequencing platforms, processing pipelines, and statistical approaches can affect data output, which make reproducibility come into question (Gloor et al., 2016). Usually, information about microbiome compositions are provided as relative abundances instead of the true abundances due to sequencing platform imitations. This data is considered an arbitrary sum and is referred to as compositional data (Jiang et al., 2019). As sequence generation and pipelines improve and become standardized, the development and accessibility for analytical tools can help to streamline and produce consistent and more accurate results while aggregating data for microbiome characterization and meta-analysis without advanced expertise (Markowitz et al., 2007, Dhariwal et al., 2017, Gonzalez et al., 2018, Chong et al., 2020, Mitchell et al., 2020, Bharti and Grimm, 2021, Chen et al., 2021).

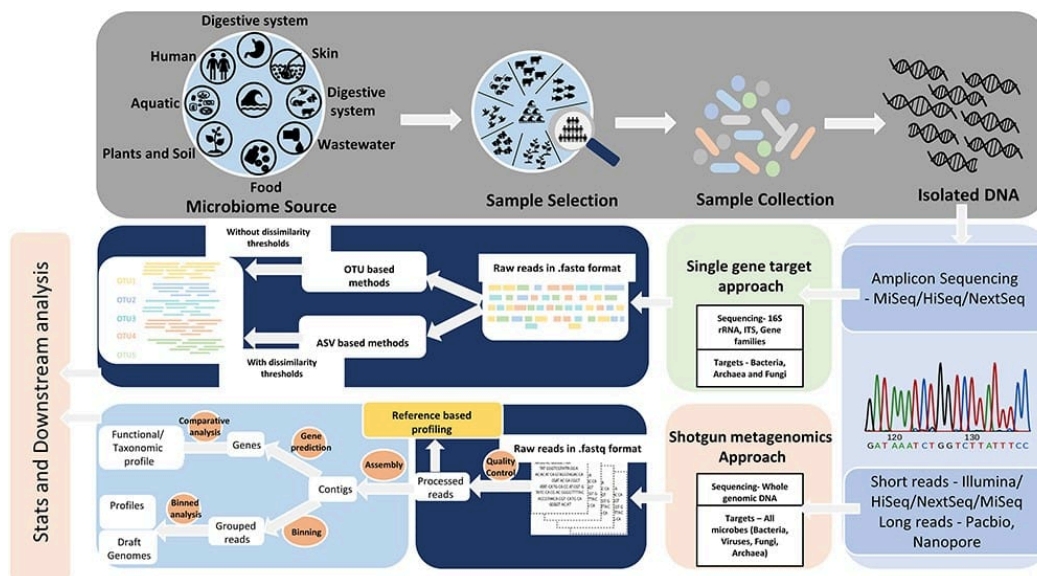


Figure 1. An illustration of targeted amplicon and metagenomic sequencing approaches. A schematic overview demonstrating diverse sample types along with commonly utilized sequencing platforms, as well as systematic and stepwise data processing steps. (Bharti and Grimm, 2021).

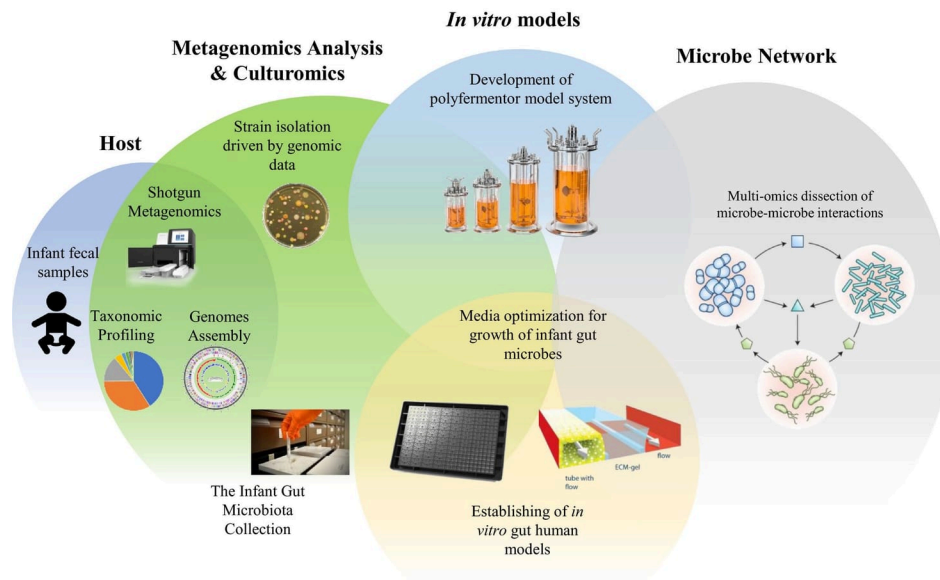


Figure 2. Schematic representations of the in vitro models used for underpinning the microbe-microbe and host-microbe cross talks.

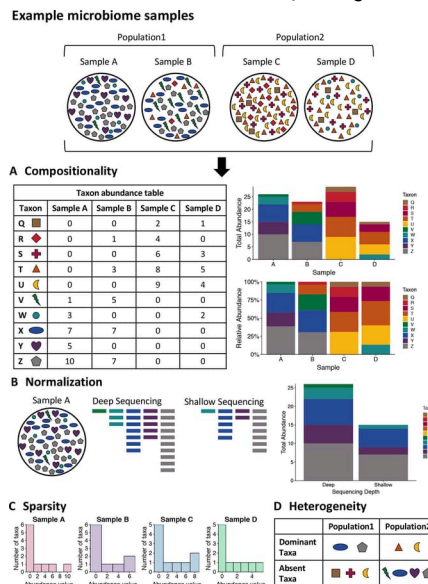


Figure 3. Visualizing the unique challenges of microbiome data. A mock set of bacterial samples from two populations where each colored shape is a bacterial taxon. (A) Compositionality. The taxon abundance table depicts the count of each observed taxon in each sample. When sequencing microbiome samples, the resulting counts of taxa are not representative of the actual taxa counts in the sample due to constraints of sequencing. Due to this, relative abundances are generally used in analysis of microbiome data. The bar plots illustrate the difference in community representation between raw counts (top) and relative abundances (bottom). (B) Normalization. Due to the constraints of sequencing, the overall sequencing depth of a sample can impact the results. For example, shallow sequencing may miss rare taxa such as the green taxon V in the example sample A that is present in low abundance in the community. (C) Sparsity. Microbiome data are often very sparse, where most observations are zero. This is illustrated by the histogram of taxa counts for each sample where most counts are zero and there are few taxa with high counts. This can also be seen in the table for part A, where many entries are zero. (D) Heterogeneity. The table summarizes the taxonomic heterogeneity in the mock dataset between the two populations. Each sample has a unique taxonomic composition, but there are also population specific signatures. The samples in each population are dominated by a few taxa, and these dominant taxa are different for the two populations. Additionally, there are taxa that are highly abundant in one sample and absent from the rest, such as the purple taxon Y in sample A.

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Metatranscriptomics and RNA sequencing

While metagenomic studies have been instrumental in identifying various microbes and genes in a given sample, it does not differentiate between viable cells or when those genes are being expressed (Gosalbes, et al., 2011, Bashiardes et al., 2016).

Metatranscriptomic strategies of microbiome analysis allow researchers to capture gene activity which can delineate the functional dynamics of a particular community and its interactions with its host or environment. These RNA-based approaches can complement metagenomics, as the extent of expression of those annotated genes can be measured (Franzosa et al., 2014). This is considerably important concerning certain disease states because the presence of a pathogen does not always produce deleterious conditions, but rather it is the functional activity of certain organisms.

RNA-seq experiments usually begin with isolation of total RNA in a sample, then selection is based upon whether it is prokaryotic or eukaryotic in origin, and mRNA must be isolated from other RNA species (i.e. rRNA and tRNA) (Bashiardes et al., 2016). cDNA is then synthesized from the mRNA, adapters are ligated to create a library, amplification and sequencing follows, and the generated reads are mapped to a reference genome where expression can be measured (Giannoukos et al., 2012, Bashiardes et al., 2016). These experiments can be difficult and sensitive, as care must be taken with sample collection, and avoiding contamination and degradation from ribonucleases is important to maintain integrity of the samples as well. Furthermore, metatranscriptomics may not always give the whole picture of community expression due to the high complexity of organisms, the delicateness of RNA, the wide range of transcript expression, and various limitations with current technology (Shakya et al., 2019).

Metaproteomics

Metaproteomics gives a snapshot of the entire protein content of a microbiome sample under various conditions, which can further provide insight into gene activity and metabolic functions. This strategy identifies peptides by matching tandem mass spectrometry (MS/MS) data to protein sequence databases, which can be further assigned to functional groups via annotation databases (Sajulga et al., 2020). This approach can be convoluted though, as many peptides match to similar proteins produced by different organisms, and proteins can be multi-functional making it difficult for assignment to any one group. Additionally, bioinformatic analysis of metaproteomes isn't exactly standardized which makes reproducibility and comparison of studies problematic (Schiebenhoefer et al., 2019, Sajulga et al., 2020).

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Metabolomics

Metabolomics analyzes the non-protein metabolites that are produced and/or regulated by a given microbiome, which adds another angle of characterization to community-host-environmental interaction and functional dynamics. By understanding how various microbes process metabolites like natural products, nutrients, and medications, we can better determine how they influence their ecosystem through molecular interactions. Researchers typically obtain these molecules through gas or

liquid chromatography and subsequently analyze them by mass spectrometry (Allaband et al., 2019, Bauermeister et al., 2021). Metabolomics strategies can either be targeted or untargeted, where the former is more sensitive and compares metabolites to a pre-determined bank with reference standards, however, it cannot detect novel molecules. Since most metabolites produced by microbiomes are unknown, an untargeted approach using tandem mass spectrometry is useful in detecting many small molecules, though annotation of modified metabolites is challenging (Watrous et al., 2012, Wang et al., 2016, Allaband et al., 2019). As with other microbiome analytical approaches, various data analysis tools and pipelines of the metabolome need to be standardized to a degree to improve overall research context. Moreover, major advancements in the known inventory of microorganism-derived metabolites and their functions are required to enhance metabolomics studies (Lee-Sarwar et al., 2020, Bauermeister et al., 2021).

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Conclusion and Future Directions

While various approaches to characterizing a microbiome each have their advantages and drawbacks, network analyses integrating all the -omics (i.e. metagenomics, metatranscriptomics, metaproteomics, and metabolomics) could be best to gain a holistic view of compositional and functional dynamics (Bashiardes et al., 2016, Jiang et al., 2019). Novel and improved approaches to collect and analyze various microbiomes will help to minimize loss of information and provide a better picture of how these communities interact with and influence their host or environment. Also, the establishment of more comprehensive publicly available databases, and protocols for streamlining computational procedures will allow more consistent and reproducible research for upon which knowledge can be accumulated.

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Key Concepts and Summary

- Microbiome analysis must align with the research question, whether identifying community members (metagenomics), studying gene expression (metatranscriptomics), examining protein function (metaproteomics), or analyzing biochemical outputs (metabolomics).
- Sample collection methods depend on the microbiome of interest (e.g., gut vs. soil) and are influenced by feasibility, patient compliance, and methodological constraints.
- **Culture-dependent approaches (culturomics)** can isolate rare microbes and provide phenotypic data but have growth and selection biases.
- Culture-independent methods, such as 16S rRNA and ITS sequencing, allow broad identification of microbial taxa without the need for culturing, reducing bias against unculturable organisms.
- **Metagenomics (DNA sequencing)** gives insight into the microbial composition and genetic potential but is limited by compositional data issues and platform variability.
- **Metatranscriptomics (RNA sequencing)** captures active gene expression and community dynamics but is sensitive to RNA degradation and complex workflows.
- **Metaproteomics** identifies expressed proteins and functions but suffers from issues with peptide ambiguity and lack of standardization in data analysis.
- **Metabolomics** investigates small molecules produced or modified by microbes, shedding light on metabolic activity and microbe-host-environment interactions.
- **Multi-omics** integration is essential for a complete picture of microbiome structure, function, and dynamics, but requires better standardization of methods and databases.

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19.4: Human Health and Disease

Learning Objectives

- Discuss the factors that contribute to microbiome disruption (dysbiosis) and its relationship to human disease
- Identify how host genetics and environmental factors influence microbiome composition and stability

Human Health and Disease

The human microbiome plays a vital role in maintaining homeostasis of various organ systems and protecting against infectious agents. It can be subcategorized into local or regional microbiomes throughout the body, such as the gut, oral, skin, lung, and vaginal microbiomes. These manicured microecosystems are highly organized and complex, with each person having their own distinct makeup and distribution of various microorganisms. Though, individual microbiomes are unique, there is still capacity to understand key role players in the community and their potential for adaptation to improve human health in general.

Image showing taxonomy, function, ecology, and dynamics of healthy human microbiomes

Figure 1. Possible definitions of a healthy microbiome: composition, function, dynamics, and ecology. **a** Early definitions of a “healthy” microbiome generally focused on sets of taxa that might be expected to be found prevalently in healthy people. While purely taxonomic cores of any type have remained elusive, even in relatively narrowly defined populations, each body-site habitat possesses strong phylogenetic enrichments. Typical genera (or families in the gut) in healthy populations at different sites are shown here. **b** Metagenomic measurements have allowed the functional potential of the microbiome at different sites to be assessed. These studies have yielded more consistently shared functional cores of body-wide and niche-specific pathways that are maintained in health. LPS lipopolysaccharide, PAMP pathogen-associated molecular pattern. **c** Ecological assembly patterns provide another possible definition of a healthy microbiome, because each host may draw from a “typical” meta-population of potential microbes through a mix of partially stochastic processes. These processes may include the order in which microbes colonize their respective human habitat (affected by geography and early exposures, for example), the prolonged availability of each microbe in the host’s local environment, and host selection. **d** The healthy microbiome can also be characterized in terms of its dynamics, depicted here in a simplified model as a conceptual energy landscape. The infant microbiome (yellow point) starts out in an unstable state and gradually descends towards one of potentially several healthy adult attractor states. Perturbations (dashed red arrows) can either be resisted (green point) or can move the microbiome out of the healthy state, after which a resilient microbiome will return to a healthy state (not necessarily the original healthy state) or fall into an unhealthy state (red). (Lloyd-Price et al., 2016).

Disruption, or **dysbiosis**, of the microbiome can cause serious diseases and allow for opportunistic infections to occur. This disturbance can be caused by a variety of factors including changes in diet, exercise, geographical location, age, habits, as well as medical intervention like antimicrobial chemotherapy. This change in microbial composition subsequently leads to the development and exacerbation of a number of diseases impacting essentially every aspect of human physiology including the digestive, respiratory, integumentary, central nervous, and reproductive systems. Alternatively, dysbiosis may be the result of a particular disease, and in other cases disease-dysbiosis may be bidirectional (Silverman et al., 2019). The human microbiome can be influenced by many of the above mentioned factors, but also others like life partners, pet ownership, and occupation, which do not necessarily correlate with or contribute to dysbiosis (Kiecolt-Glaser et al., 2019, Kates et al., 2020).

A major driving feature of many of these elements is the host’s genetics, which can also directly impact their microbiome (Tabrett and Horton, 2020). Part of the gut microbiome and even individual types of bacteria have been shown to be heritable (Kurilshikov et al., 2017), and it is likely that other areas of the human microbiome, like the skin microbiome, could be passed from parent to offspring as well (Si et al., 2015). The host genotype is intimately linked to its microbiome and has been shown to affect dysbiosis-induced diseases such as inflammatory bowel disease and atopic dermatitis (Knights et al., 2013, Dabrowska and Witkiewicz, 2016, Woo and Sibley, 2020). Host genetics and their microbiome can even indirectly affect one’s susceptibility to other diseases. For example, the attractiveness of mosquitos to particular individuals is an heritable trait which is influenced by the many factors, including the skin microbiome; therefore altering the potential of contracting a mosquito-borne pathogen, such as a *Plasmodium* parasite which causes malaria (Martinez et al., 2020). It seems there is a combination between environmental factors, host genetics, behavior, and others that continually build and adapt the human microbiome, however, is worthy to note that the former may play a larger role in shaping particular microbiomes, such as the gut microbiome (Rothschild et al., 2018).

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Key Concepts and Summary

- The human microbiome is essential for maintaining homeostasis, regulating immune function, and protecting against infection across various organ systems (e.g., gut, skin, oral, lung, vaginal).
- Each individual hosts a unique yet functionally consistent microbiome, with site-specific taxa and functional pathways contributing to health.
- **Dysbiosis**, or disruption of the microbiome, can contribute to or result from a wide range of diseases, with possible bidirectional interactions between host health and microbiome composition.
- Microbiome composition is influenced by a wide range of environmental factors such as diet, exercise, geography, antibiotics, and even pets or partners.
- **Host genetics** play a significant role in shaping the microbiome, influencing the risk of diseases like IBD, atopic dermatitis, and mosquito-borne infections through microbial mediation.
- A "healthy" microbiome can be defined by various characteristics: typical microbial composition, shared functional capabilities, ecological assembly processes, and resilience to disturbance.
- The **developmental trajectory** of the microbiome (e.g., from infancy to adulthood) and its response to perturbation (resilience or susceptibility to dysbiosis) are key to understanding long-term health outcomes.

Media Attributions

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19.5: The Gut Microbiome

Learning Objectives

- Identify key factors that influence the gut microbiome, including diet, genetics, environment and behavior
- Discuss the role of the gut microbiome in maintaining host health and homeostasis
- Evaluate the relationship between gut microbiome dysbiosis and diseases such as IBD, diabetes and obesity

The Gut Microbiome

The microbiome associated with the human gastrointestinal tract, termed the **gut microbiome**, may arguably be the most important component of the collective human microbiome. It has been shown to affect numerous other regions of the body and serves a role in many types of diseases throughout it. This is because many of the microbial products are absorbed in the alimentary canal and distributed throughout the body via the cardiovascular system.

Sample procurement to characterize the gut microbiome usually comes in the form of **fecal matter**, which readily available and less invasive. However, there are novel attempts to characterize gut microbiome constituents by sampling the mucosal-luminal interface (Yan et al., 2020). DNA and other features of interest can then be extracted from the microbes to provide information regarding gut microbiome composition and function.

Composition

The gut microbiome is quite versatile and its composition can vary widely among individuals with different ethnicities, across geographic locations, and with age (Yatsunen et al., 2012, Gaulke and Sharpton, 2018). The host's genetics do play a role in the composition of the gut microbiome, as certain members are heritable, while other's abundances are the causal result of congenital diseases (Xu et al., 2020). Though diet, which is closely related to the aforementioned variables, may be the single strongest influencing factor when it comes to structuring this digestive community and alterations are reflected in both short-term and long-term dietary interventions (David et al., 2014, Xu and Knight, 2015). Indeed, the type of diet, such as a high-fat diet, will have a direct impact on the gut microbiome. More specifically, the unique macromolecules within the gut can modify microbial abundance and predicted functions. For example, a certain oil in the diet could result in higher microbial richness, however, this diversity may not alone be a predictor of better health outcomes (Abulizi et al., 2019). Dietary vitamin content and even receptors required for processing them are in part modulated by the microbiome. For example, vitamin D deficiency and downregulation of its affiliated receptor is associated with pathogenesis of various diseases and their restoration promotes healthy host-microbe homeostasis (Jin et al., 2015). It is also interesting to note that diet usually changes throughout the year as certain food items become available or absent depending on the season. An increased gut prevalence of Bacteroidetes, which can digest complex plant carbohydrates, could be explained by a diet consisting heavily of produce during a harvest season (Davenport et al., 2014). The composition and diversity of the gut microbiome can even be linked to personality traits. Those individuals with larger social networks have a more diverse gut microbiome, and those affected by stress and anxiety show an altered composition with reduced diversity (Johnson, 2020). The connection between the gut microbiome and mental states and behaviors will be discussed more in the section "Mental Health".

Video Quiz

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Dysbiosis and Disease

The gastrointestinal mucosal immune system modulates and responds to the gut microbiome, where the resident members aid in its development and transient pathogens cause dysfunction, leading to various diseases (Shi et al., 2017). Inflammatory diseases such as systemic lupus erythematosus, rheumatoid arthritis, IBD, and ankylosing spondylitis are implicated in the impaired interaction between the intestinal microbiota and mucosal immune system (Arbuckle et al., 2003, Mikuls et al., 2012, Costello et al., 2015). Microbial dysbiosis within these cases are marked by changes in composition and diversity of specific groups of organisms (Shi et al., 2017). So, the gut microbiome can not only serve as an indicator for such diseases, but may also eventually become a target for treatment, with maintaining proper homeostasis a major goal.

Gut microbiome dysbiosis, whether it causes a particular disease or manifests as a result of it, is quickly becoming the focus of many gastrointestinal illnesses. Specific members of the gut microbiome (e.g. bacteriome, mycobiome, virome) can even vary and be affected differently depending on the disease and its severity. For example, while much of the focus is usually on bacteria in the gut, it is important to not discount the contributions of other microorganisms like bacteriophages and fungi. The gut phageome can vary in diversity, complexion, and in so has been shown to contribute to diseases like IBD, malnutrition, and AIDS (Norman et al., 2015, Reyes et al., 2015, Monaco et al., 2016, Shkoporov and Hill, 2019). Similarly, the gut mycobiome has shown to have roles in IBD, colorectal cancer, and even neurological diseases (Forbes et al., 2019, Gu et al., 2019, Qin et al., 2021). Though, it is likely the complex interactions between all members of the gut microbiome with the host undoubtedly play a role in various degrees for the progression of gastrointestinal and other diseases.

Those with **Inflammatory Bowel Disease (IBD)** experience substantial fluctuations in the gut microbiome, which is implicated due to signs and symptoms of the disease state, diet, as well as increased medication during flare ups (Walters et al., 2014, Halfvarson et al., 2017). IBD is a blanket term for two disorders, **ulcerative colitis** and **Crohn's disease**, which are characterized by chronic inflammation of the gastrointestinal tract and commonly results in frequent diarrhea, abdominal pain, bloody stool, weight loss, and fatigue. It is likely that the gut microbiome plays both a role in the development of these conditions and is affected by the induced changes. The gut virome component responds to disease-induced environmental change of IBD patients by shifting from virulent to temperate bacteriophage core, which subsequently affects the bacteriome and dysbiosis condition (Clooney et al., 2019). A familial study of patients with Crohn's disease showed an increase in the number of pathogenic bacteria, and a decrease in beneficial bacteria. In particular, three potentially pathogenic biofilm-forming species from both the bacteriome (*Serratia marcescens* and *Escherichia coli*) and mycobiome (*Candida tropicalis*) interact with each other and impact the host immune system by increasing levels of proinflammatory cytokines and mucolytic enzymes which cause oxidative and tissue damage (Hoarau et al., 2016).

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Diabetes mellitus is another disease in which its progression is partially in response to gut microbiome dysbiosis. While there are other factors that play into diabetes such as culture, genetics, age, lifestyle, etc., this can be interlinked with an individual's microbiome. Studies over both type 1 and type 2 diabetes have shown that a change in the gastrointestinal microbial ecology is associated with diabetic subjects as compared with their healthy counterparts (Giongo et al., 2010, Larsen et al., 2010, Musso

et al., 2011, Sohail et al., 2017). Type 1 diabetes stems from destruction of pancreatic beta cells, which results in decreased insulin production and elevated blood glucose levels. The gut microbiome may contribute to the disease by dysbiosis-associated immune regulation causing destruction of the beta cells and/or gut leakiness, endotoxemia, and chronic low-grade inflammation associated with certain enteric microbes (Cani et al., 2007, Lee et al., 2011, McDermott and Huffnagle, 2014, Sohail et al., 2017). Type 2 diabetes is characterized by the body's improper regulation and secretion of insulin and is associated by hyperglycemia. Physiological changes in the GI tract could be induced by dysbiosis in the gut microbiome leading to gut permeability and insulin resistance (Everard and Cani, 2013). In general, the gut microbiota composition is less in terms of diversity and richness for those with type 2 diabetes, though an increase in abundance of certain groups like *Bifidobacterium* could improve conditions associated with pathogenesis (Cani et al., 2007, Sohail et al., 2017). It seems that an alteration of the microbial gut profile has considerable effects on host metabolism, gastrointestinal physiology, gut fermentation capabilities, and immunity which can have many other downstream implications (Boulange et al., 2016).

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Obesity is commonly associated with type 2 diabetes as well as other comorbidities that are linked to gut microbiome dysbiosis. As mentioned earlier, diet strongly affects the composition and function of the gut microbial community and subsequently impacts the host's metabolic capabilities. In fact, a high-fat/calorie or improper diet that results in dysbiosis is evident earlier than the signs of the host's metabolic abnormalities, and so gut microbiome dysbiosis may be the principle ingredient responsible for the progression of obesity and type 2 diabetes (Nagpal et al., 2018). A high-sugar diet seems to promote an abundance of *Mollicutes*, a class within Firmicutes, which in turn suppresses Bacteroidetes (Turnbaugh et al., 2008), and this higher ratio of Firmicutes/Bacteroidetes has been proposed as a biomarker and hallmark indicator for obesity (de Bandt et al., 2011, Zou et al., 2020). However, it is important to consider other factors such as physical activity and medication that could cause a variation in diversity of the gut microbiome, as this ratio does not always definitively denote obesity (Magne et al., 2020). Though, there are similarities between many of the microbiome-linked contributing factors of pathogenesis progression for diet-induced diseases. For example, a high-fat diet induces increased gut permeability that allows exogenously produced bacterial compounds (e.g. lipopolysaccharides) to diffuse through the intestinal barrier, which then can interact with immune cells and lead to inflammation (Cani et al., 2007, Nagpal and Yadav, 2017). However, diet isn't the sole factor that can promote gut leakiness, and it seems that obesity in general causes an altered gastrointestinal state (Nagpal et al., 2018).

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Key Concepts and Summary

- The gut microbiome is a diverse and dynamic community of microorganisms that plays a vital role in digestion, immune function, metabolism, and overall health. Its composition varies based on factors like diet, genetics, geography, and age, with diet being a major influence.
- Disruption of this microbial balance, known as **dysbiosis**, is linked to a wide range of diseases, including inflammatory bowel disease (IBD), diabetes, and obesity. These conditions are often marked by changes in microbial diversity, increased gut permeability, and inflammation.
- In addition to bacteria, the **gut virome** and **mycobiome** also affect health, and their roles are increasingly recognized in disease processes. Understanding the gut microbiome offers opportunities for new treatments and personalized health strategies.

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19.6: C diff Infection (CDI) and Fecal Microbiota Transplant (FMT)

Learning Objectives

- Explain how antibiotics and other medications disrupt the gut microbiome and contribute to dysbiosis
- Describe how *Clostridioides difficile* infections (CDIs) are linked to antibiotic use
- Explain the principles and uses of probiotics and fecal microbiota transplant (FMT) in restoring gut microbiome health

Chemotherapeutic Intervention and C. diff

Medication and antimicrobial drugs can also have drastic effects on the gut microbiome that invoke risk of secondary infections, allergies, and other diseases like obesity ([Becattini et al., 2016](#)). Though many of these prescribed treatments are necessary to combat infectious diseases, the aftermath may have more serious consequences. Not only does antimicrobial therapy disrupt the resident microbiome, but misuse, suboptimal dosing, and patient noncompliance can create conditions conducive to fostering antimicrobial resistance through selective pressure.

Clostridioides difficile (commonly called **C. diff**) infections are directly associated with antibiotic-induced dysbiosis in the gastrointestinal tract. *C. difficile* is part of the normal microbiota, however, as an opportunistic pathogen it can invade or colonize empty niches brought about by dysbiosis and cause potentially fatal episodes of pseudomembranous colitis, which is associated with abdominal cramping, pain, sepsis, and bouts of diarrhea ([Kho and Lal, 2018](#)). Infection and transmission of this organism has been well known for its prevalence in hospital settings, primarily affecting the elderly and immunocompromised, however, community-associated infections have recently increased in what was once considered low-risk populations ([Rouphael et al., 2008](#), [Baker et al., 2010](#), [Hensgens et al., 2012](#), [Benson et al., 2015](#), [Johanesen et al., 2015](#)). It is also alarming that this organism has resistance mechanisms to many commonly prescribed antimicrobials, including β -lactams, aminoglycosides, lincomycin, tetracyclines, and erythromycin ([George et al., 1978](#)), and more recently ‘hypervirulent’ strains have developed resistance to fluoroquinolones ([He et al., 2013](#), [Johanesen et al., 2015](#)). *C. difficile* infections have an enrichment of fungi that associate with the bacteriome and perhaps antifungal therapy could help improve treatment success if administered in conjunction with specific antibacterial drugs ([Stewart et al., 2019](#)). Though, these infections can have lingering impacts on the gut microbiome, as further antimicrobial therapy that is usually required can perpetuate the situation. The inflammation as a result of the disease induces the production of antimicrobial peptides by epithelial cells and neutrophils which inhibit the growth of the natural resident commensal microbes ([Leber et al., 2015](#)).

While many events of gut dysbiosis are directly linked to the chemotherapeutic effects on microbes since they are prescribed to target microbes responsible for the infection, some medications which are meant to address other diseases, like antidepressants for mental health, have undesired effects on the microbiome ([Maier and Typas, 2017](#)). In the cases of multi-drug combinations (e.g. non-steroidal anti-inflammatory drugs (NSAIDs), antidepressants, laxatives, proton-pump inhibitors (PPIs), etc.), it is not the number of drugs that affect gut microbiome diversity, but rather the types of drugs ([Rogers and Aronoff, 2016](#)). Though these scenarios become complicated as it is difficult to ascertain whether the alterations observed on the microbiome are from the drug’s mechanism of action, a downstream side effect, or originate from the condition that is being treated, and it is likely a complex combination of all factors for each disease and medication ([Rogers and Aronoff, 2016](#), [Maier and Typas, 2017](#), [Jackson et al., 2018](#)).

Fecal Microbiota Transplant

Although pharmaceutical drugs are of dire importance to treat various diseases, whether they are infectious in nature or not, other avenues must be pursued for those that may benefit from restoration of the gut microbiome. Probiotics and **fecal microbiota transplant (FMT)** can serve as viable options for the prevention and treatment of gut microbiome dysbiosis. Probiotics are considered foodstuffs with microorganisms, usually bacteria (many being lactic acid bacteria) and yeast, and their byproducts that have a beneficial effect on human health when introduced into the body. Many probiotics are commercially available to consumers in the forms of products like yogurt, kefir, buttermilk, sauerkraut, pickles, premade vitamin supplements and many others (more information about probiotics and fermented foods can be found in the section “Food and Fermentation: Your Microbiome is What

you Eat”). Specifically, probiotics can be used for the treatment and prevention of many of the aforementioned gut microbiome dysbiosis-associated diseases, especially those induced by antibiotics (Kim et al., 2019). The beneficial microbes outcompete pathogens for resources or prevent them from establishing a niche in which to grow (Ouwehand et al., 1999).

In more extreme cases of gut microbiome dysfunction and disease, like those from *C. difficile* infection (CDI) in which antibiotics are ineffective and can potentially exacerbate the problem, other measures must be taken. Fecal microbiota transplant therapy takes a stool sample containing the gut microbiome from a healthy donor and relocates it into the infected patient’s colon. The introduced microbiota then helps move the gut microbiome towards homeostasis by restoring the structure of beneficial microbes and metabolites (Fujimoto et al., 2021). This procedure is usually reserved for those patients with recurrent CDI and has shown to be highly successful and is considered safer and more effective than prolonged antibiotic usage (Mattila et al., 2012, Cammarota et al., 2015), though is also being investigated for first-line treatment of CDI (Camacho-Ortiz et al., 2017). FMT has gained traction for its success and is being further considered as a therapeutic option in other treatment protocols, such as those for cancer patients undergoing cancer immunotherapy to help improve response or manage toxicity (McQuade et al., 2020), and individuals undergoing allogeneic hematopoietic stem cell transplant for hematological disorders that experience graft-versus-host disease complications from it (Zhang et al., 2021). However, precautions must be taken for FMT, as the donor’s sample could potentially harbor other pathogenic microbes, like multi-drug resistant *Escherichia coli*, that can result in pathogenesis, further complications, and even death for the recipient (DeFilipp et al., 2019, Martinez-Gili et al., 2020). More comprehensive research and FMT trials must be performed in order to optimize this procedure to better match donors with recipients and to further understand the exact mechanisms of microbiome rehabilitation.

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Conclusion

Gut microbiome intervention may be the key to future treatments of diseases associated with dysbiosis like IBD, diabetes, obesity, colorectal cancer, etc., and offers a viable alternative to many traditional pharmaceutical interventions. Though, the gut microbiome is plastic and continually changes with its host's environment and lifestyle, so stabilization is constant work. Additionally, creating an ideal 'cocktail' of microbes that will maintain homeostasis when implemented can be challenge. While there are general members of the gut microbiome that exist at a constant level and show some correlation to normal health, there may not be a true 'standard' gut microbiome due to the vast differences between people across the world. So, this type of therapy may require a more unique and individualized approach that depends on the disease and characteristics of both the host and their microbiome.

Key Concepts and Summary

- Medications—especially antibiotics—can disrupt the gut microbiome, increasing the risk of infections like *Clostridioides difficile* (C. diff), which thrives in the absence of competing microbes and can cause severe, recurrent disease. Beyond antibiotics, other drugs (e.g., antidepressants, NSAIDs) can also influence microbial balance, often in complex, condition-dependent ways.
- To counteract dysbiosis, treatments like probiotics and **fecal microbiota transplant (FMT)** are used. FMT is particularly effective for recurrent C. diff infections and is being explored in other contexts like cancer therapy. However, it carries risks and must be carefully managed.
- Because everyone's microbiome is unique, personalized approaches may be necessary. Microbiome-based therapies offer promising alternatives to traditional treatments for conditions linked to dysbiosis, such as IBD, diabetes, and obesity—but more research is needed to optimize and safely implement them.

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20: INNATE IMMUNITY 1

Despite relatively constant exposure to pathogenic microbes in the environment, humans do not generally suffer from constant infection or disease. Under most circumstances, the body is able to defend itself from the threat of infection thanks to a complex immune system designed to repel, kill, and expel disease-causing invaders. Immunity as a whole can be described as two interrelated parts: nonspecific innate immunity, which is the subject of this chapter, and specific adaptive host defenses, which are discussed in the next chapter.

The nonspecific innate immune response provides a first line of defense that can often prevent infections from gaining a solid foothold in the body. These defenses are described as *nonspecific* because they do not target any specific pathogen; rather, they defend against a wide range of potential pathogens. They are called *innate* because they are built-in mechanisms of the human organism. Unlike the specific adaptive defenses, they are not acquired over time and they have no “memory” (they do not improve after repeated exposures to specific pathogens).

Broadly speaking, nonspecific innate defenses provide an immediate (or very rapid) response against potential pathogens. However, these responses are neither perfect nor impenetrable. They can be circumvented by pathogens on occasion, and sometimes they can even cause damage to the body, contributing to the signs and symptoms of infection.

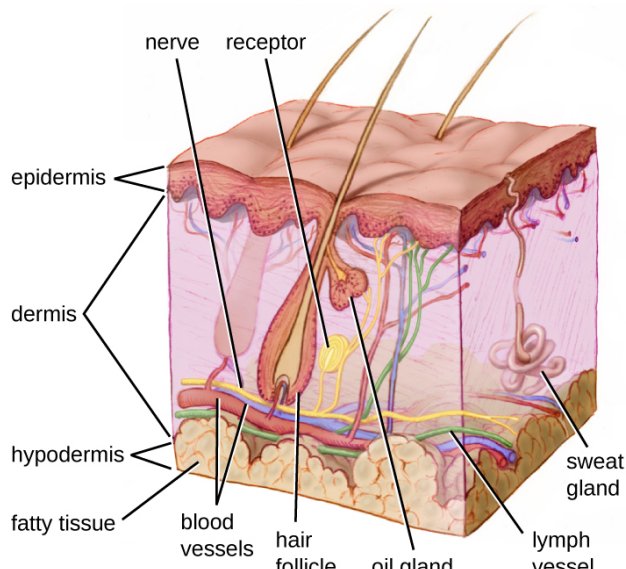


Figure 20.1: Human skin has three layers, the epidermis, the dermis, and the

hypodermis, which provide a thick barrier between microbes outside the body and deeper tissues. Dead skin cells on the surface of the epidermis are continually shed, taking with them microbes on the skin's surface. (credit: modification of work by National Institutes of Health)

[20.1: Physical Defenses Overview](#)

[20.2: Physical Barriers - Skin and Mucous Membranes](#)

[20.3: Chemical Defenses - Overview](#)

[20.4: Chemical Defenses - Antimicrobial Peptides \(AMP\) and Plasma Protein Mediators](#)

[20.5: Chemical Defenses - Complement System](#)

[20.6: Chemical Defenses - Cytokines](#)

[20.E: Innate Immune Defenses I \(Exercises\)](#)

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20.1: Physical Defenses Overview

Learning Objectives

- Describe the various physical barriers and mechanical defenses that protect the human body against infection and disease
- Describe the role of microbiota as a first-line defense against infection and disease

Nonspecific innate immunity can be characterized as a multifaceted system of defenses that targets invading pathogens in a nonspecific manner. In this chapter, we have divided the numerous defenses that make up this system into three categories: **physical defenses**, **chemical defenses**, and **cellular defenses**. However, it is important to keep in mind that these defenses do not function independently, and the categories often overlap. Table 20.1.1 provides an overview of the nonspecific defenses discussed in this chapter.

Table 20.1.1: Overview of Nonspecific Innate Immune Defenses

Overview of Nonspecific Innate Immune Defenses	
Physical defenses	Physical barriers
	Mechanical defenses
	Microbiome
Chemical defenses	Chemicals and enzymes in body fluids
	Antimicrobial peptides
	Plasma protein mediators
	Cytokines
	Inflammation-eliciting mediators
Cellular defenses	Granulocytes
	Agranulocytes

Physical defenses provide the body's most basic form of nonspecific defense. They include physical barriers to microbes, such as the skin and mucous membranes, as well as mechanical defenses that physically remove microbes and debris from areas of the body where they might cause harm or infection. In addition, the microbiome provides a measure of physical protection against disease, as microbes of the normal microbiota compete with pathogens for nutrients and cellular binding sites necessary to cause infection.

Query 20.1.1

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Interactive Element

Case Study Preview: “When Breathing Isn’t Easy: A Hidden Genetic Threat”

Angela arrives at the ER gasping for air, her throat swelling and her voice nearly gone. She has no known allergies, no asthma history - just a haunting family memory: her father died suddenly of similar symptoms at age 27. With no clear allergic trigger and no microbial infection, doctors dig deeper. What they uncover is a rare but dangerous condition: type I hereditary angioedema, caused by a deficiency in C1 esterase inhibitors that leads to uncontrolled inflammation.

In this case, you'll follow Angela's journey from misdiagnosis to lifesaving insight, learning how complement proteins, genetic inheritance, and the body's inflammatory responses can sometimes become their own worst enemies.

It wasn't an allergy. It was her immune system missing a critical off switch.

[Chapter 17 Case Study - When Breathing Isn't Easy: A Hidden Genetic Threat](#)

Key Concepts and Summary

- **Nonspecific innate immunity** provides a first line of defense against infection by nonspecifically blocking entry of microbes and targeting them for destruction or removal from the body.
- The physical defenses of innate immunity include physical barriers, mechanical actions that remove microbes and debris, and the microbiome, which competes with and inhibits the growth of pathogens.
- The skin, mucous membranes, and endothelia throughout the body serve as physical barriers that prevent microbes from reaching potential sites of infection. Tight cell junctions in these tissues prevent microbes from passing through.
- Microbes trapped in dead skin cells or **mucus** are removed from the body by mechanical actions such as shedding of skin cells, mucociliary sweeping, coughing, **peristalsis**, and flushing of bodily fluids (e.g., urination, tears)
- The resident microbiota provide a physical defense by occupying available cellular binding sites and competing with pathogens for available nutrients.

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20.2: Physical Barriers - Skin and Mucous Membranes

Learning Objectives

- Summarize cellular junctions in physical barriers
- Describe how skin acts as a barrier to protect us from infections from the outside world
- Evaluate the roles of mucous membranes in protecting against infections from inside of the body
- Identify the importance of endothelia in CNS immune defense

Physical Barriers

Physical barriers play an important role in preventing microbes from reaching tissues that are susceptible to infection. At the cellular level, barriers consist of cells that are tightly joined to prevent invaders from crossing through to deeper tissue. For example, the endothelial cells that line blood vessels have very tight cell-to-cell junctions, blocking microbes from gaining access to the bloodstream.

Cell junctions are generally composed of cell membrane proteins that may connect with the extracellular matrix or with complementary proteins from neighboring cells. Tissues in various parts of the body have different types of cell junctions. These include **tight junctions**, **desmosomes**, and **gap junctions**, as illustrated in Figure 20.2.1. Invading microorganisms may attempt to break down these substances chemically, using enzymes such as proteases that can cause structural damage to create a point of entry for pathogens.

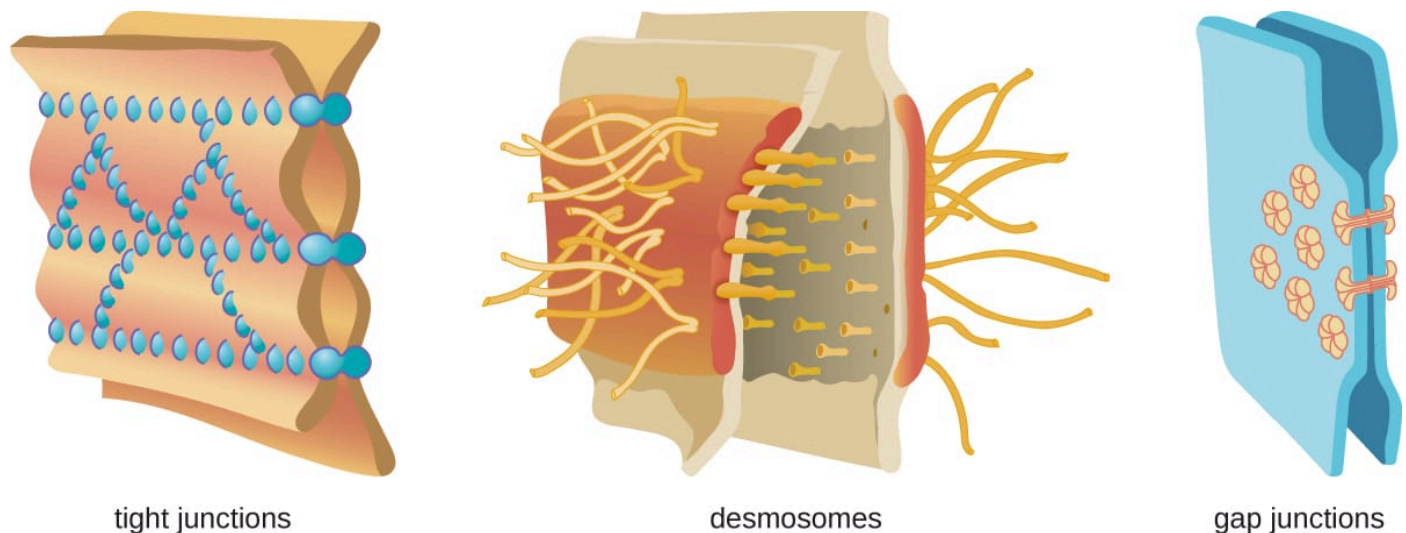


Figure 20.2.1: There are multiple types of cell junctions in human tissue, three of which are shown here. Tight junctions rivet two adjacent cells together, preventing or limiting material exchange through the spaces between them. Desmosomes have intermediate fibers that act like shoelaces, tying two cells together, allowing small materials to pass through the resulting spaces. Gap junctions are channels between two cells that permit their communication via signals. (credit: modification of work by Mariana Ruiz Villareal)

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The Skin Barrier

One of the body's most important physical barriers is the **skin barrier**, which is composed of three layers of closely packed cells. The thin upper layer is called the **epidermis**. A second, thicker layer, called the **dermis**, contains hair follicles, sweat glands, nerves, and blood vessels. A layer of fatty tissue called the **hypodermis** lies beneath the dermis and contains blood and lymph vessels (Figure 20.2.2).

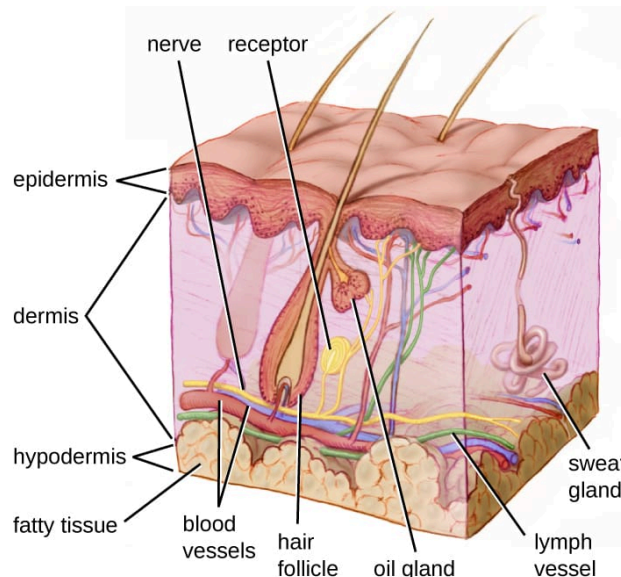


Figure 20.2.2: Human skin has three layers, the epidermis, the dermis, and the hypodermis, which provide a thick barrier between microbes outside the body and deeper tissues. Dead skin cells on the surface of the epidermis are continually shed, taking with them microbes on the skin's surface. (credit: modification of work by National Institutes of Health)

The topmost layer of skin, the epidermis, consists of cells that are packed with **keratin**. These dead cells remain as a tightly connected, dense layer of protein-filled cell husks on the surface of the skin. The keratin makes the skin's surface mechanically tough and resistant to degradation by bacterial enzymes. **Fatty acids** on the skin's surface create a dry, salty, and acidic environment that inhibits the growth of some microbes and is highly resistant to breakdown by bacterial enzymes. In addition, the **dead cells** of the epidermis are frequently shed, along with any microbes that may be clinging to them. Shed skin cells are continually replaced with new cells from below, providing a new barrier that will soon be shed in the same way.

Infections can occur when the skin barrier is compromised or broken. A wound can serve as a point of entry for opportunistic pathogens, which can infect the skin tissue surrounding the wound and possibly spread to deeper tissues.

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Mucous Membranes

The **mucous membranes** lining the nose, mouth, lungs, and urinary and digestive tracts provide another nonspecific barrier against potential pathogens. Mucous membranes consist of a layer of epithelial cells bound by tight junctions. The epithelial cells secrete a moist, sticky substance called **mucus**, which covers and protects the more fragile cell layers beneath it and traps debris and particulate matter, including microbes. Mucus secretions also contain **antimicrobial peptides**.

In many regions of the body, mechanical actions serve to flush mucus (along with trapped or dead microbes) out of the body or away from potential sites of infection. For example, in the respiratory system, inhalation can bring microbes, dust, mold spores, and other small airborne debris into the body. This debris becomes trapped in the mucus lining the respiratory tract, a layer known as the mucociliary blanket. The epithelial cells lining the upper parts of the respiratory tract are called ciliated epithelial cells because they have hair-like appendages known as cilia. Movement of the cilia propels debris-laden mucus out and away from the lungs. The expelled mucus is then swallowed and destroyed in the stomach, or coughed up, or sneezed out (Figure 20.2.4). This system of removal is often called the **mucociliary escalator**.

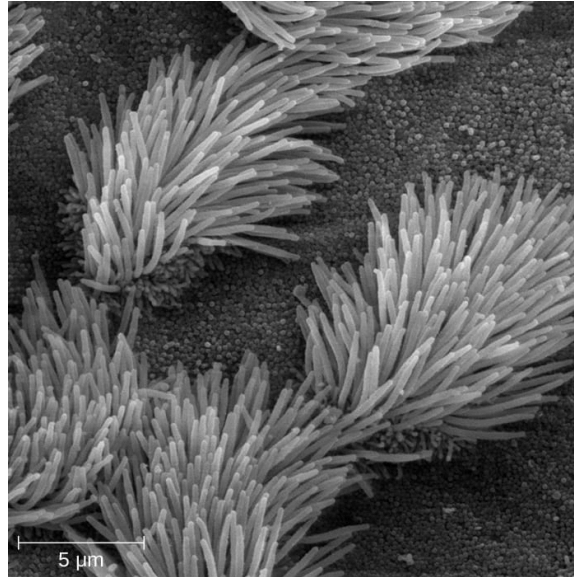


Figure 20.2.4: This scanning electron micrograph shows ciliated and nonciliated epithelial cells from the human trachea. The mucociliary escalator pushes mucus away from the lungs, along with any debris or microorganisms that may be trapped in the sticky mucus, and the mucus moves up to the esophagus where it can be removed by swallowing.

The mucociliary escalator is such an effective barrier to microbes that the lungs, the lowermost (and most sensitive) portion of the respiratory tract, were long considered to be a sterile environment in healthy individuals. Only recently has research suggested that healthy lungs may have a small normal microbiota. Disruption of the mucociliary escalator by the damaging effects of smoking or diseases such as cystic fibrosis can lead to increased colonization of bacteria in the lower respiratory tract and frequent infections, which highlights the importance of this physical barrier to host defenses.

Like the respiratory tract, the digestive tract is a portal of entry through which microbes enter the body, and the mucous membranes lining the digestive tract provide a nonspecific physical barrier against ingested microbes. The intestinal tract is lined with epithelial cells, interspersed with mucus-secreting **goblet cells** (Figure 20.2.5). This mucus mixes with material received from the stomach, trapping foodborne microbes and debris. The mechanical action of **peristalsis**, a series of muscular contractions in the digestive tract, moves the sloughed mucus and other material through the intestines, rectum, and anus, excreting the material in feces.

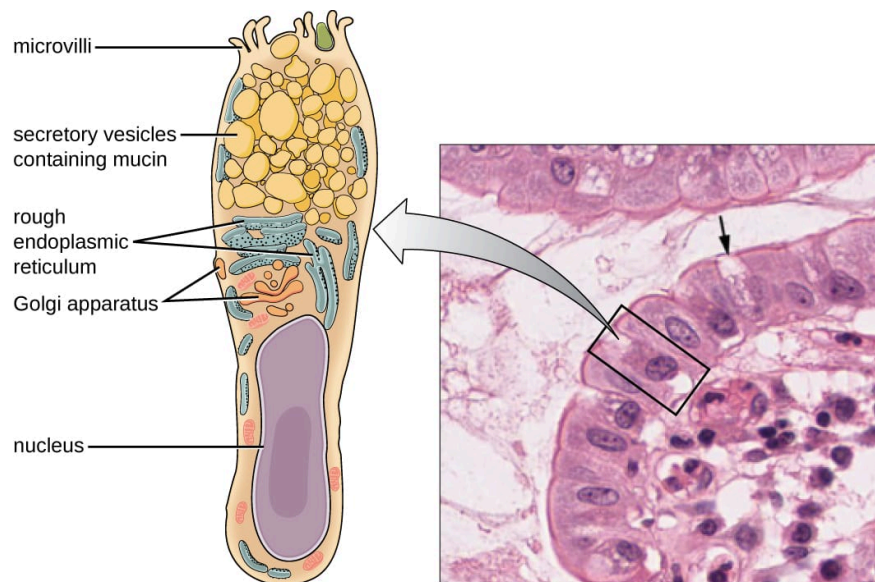


Figure 20.2.5: Goblet cells produce and secrete mucus. The arrows in this micrograph point to the mucus-secreting goblet cells (magnification 1600 $\times$) in the intestinal epithelium. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

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Endothelia

The **epithelial cells** lining the urogenital tract, blood vessels, lymphatic vessels, and certain other tissues are known as endothelia. These tightly packed cells provide a particularly effective frontline barrier against invaders. The endothelia of the **blood-brain barrier**, for example, protect the central nervous system (CNS), which consists of the brain and the spinal cord. The CNS is one of the most sensitive and important areas of the body, as microbial infection of the CNS can quickly lead to serious and often fatal inflammation. The cell junctions in the blood vessels traveling through the CNS are some of the tightest and toughest in the body, preventing any transient microbes in the bloodstream from entering the CNS. This keeps the cerebrospinal fluid that surrounds and bathes the brain and spinal cord sterile under normal conditions.

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Key Concepts and Summary

- The physical defenses of innate immunity include physical barriers, mechanical actions that remove microbes and debris, and the microbiome, which competes with and inhibits the growth of pathogens.
- The skin, mucous membranes, and endothelia throughout the body serve as physical barriers that prevent microbes from reaching potential sites of infection. Tight cell junctions in these tissues prevent microbes from passing through.
- Microbes trapped in dead skin cells or **mucus** are removed from the body by mechanical actions such as shedding of skin cells, mucociliary sweeping, coughing, **peristalsis**, and flushing of bodily fluids (e.g., urination, tears)

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20.3: Chemical Defenses - Overview

Learning Objectives

- Describe how enzymes in body fluids provide protection against infection or disease
- Recognize the chemical defense mechanisms in several organ systems

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 20.3.1). 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.

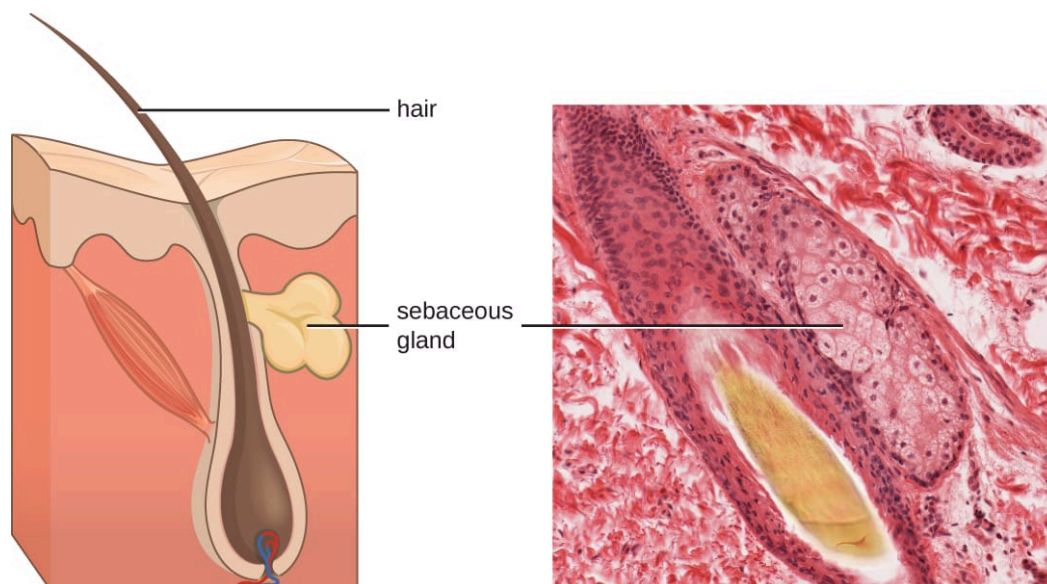


Figure 20.3.1: Sebaceous glands secrete sebum, a chemical mediator that lubricates and protect the skin from invading microbes. Sebum is also a food source for resident microbes that produce oleic acid, an exogenously produced mediator. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

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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.

Chemical Defense Mechanisms in Organ Systems

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.

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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.

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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.

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20.4: Chemical Defenses - Antimicrobial Peptides (AMP) and Plasma Protein Mediators

Learning Objectives

- Describe the functions of antimicrobial peptides (AMPs)
- Distinguish between defensins and bacteriocins

Antimicrobial Peptides (AMPs)

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.

Functions of AMPs

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.

Defensins versus Bacteriocins

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 20.4.1.

Table 20.4.1: 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

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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.

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 20.4.1).

Table 20.4.2: 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

Key Concepts and Summary

- **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.

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20.5: Chemical Defenses - Complement System

Learning Objectives

- Identify the three mechanisms that trigger complement activation
- Describe the outcome of complement activation

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.

Complement Activation

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 20.5.2).

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 20.5.2).

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 20.5.2).

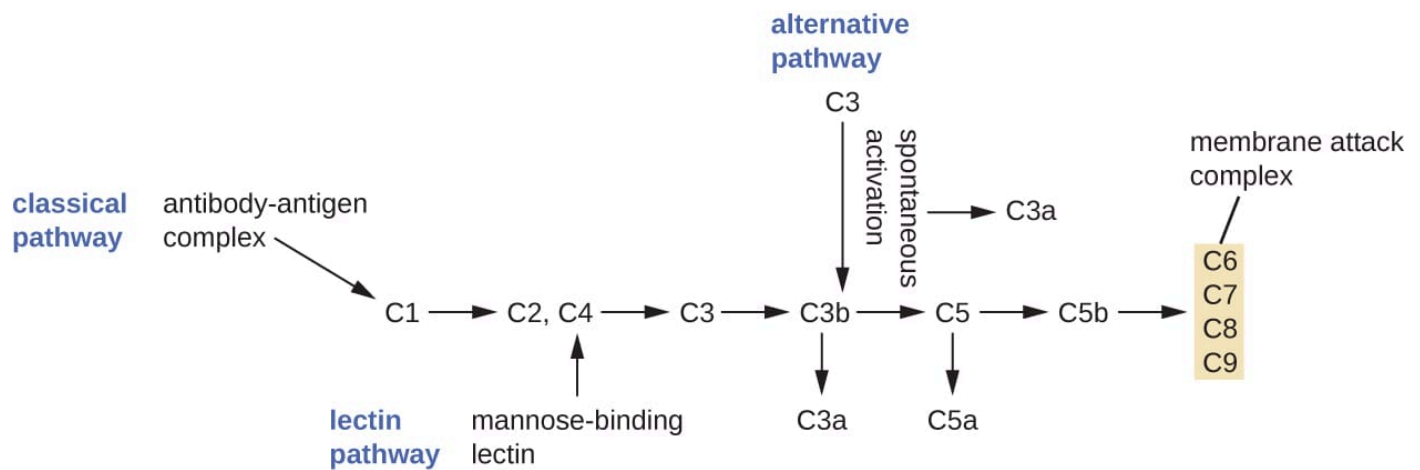


Figure 20.5.2: The three complement activation pathways have different triggers, as shown here, but all three result in the activation of the complement protein C3, which produces C3a and C3b. The latter binds to the surface of the target cell and then works with other complement proteins to cleave C5 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.

Outcome of Complement Activation

Although each complement activation pathway is initiated in a different way, they all provide the same protective outcomes: **opsonization, inflammation, chemotaxis, and cytotoxicity**. 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 20.5.2). 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.

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Key Concepts and Summary

- 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 cytotoxicity through the formation of a **membrane attack complex (MAC)**.

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20.6: Chemical Defenses - Cytokines

Learning Objectives

- Distinguish the three classes of cytokines
- Describe the actions of inflammation-eliciting mediators

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 20.6.3). 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.

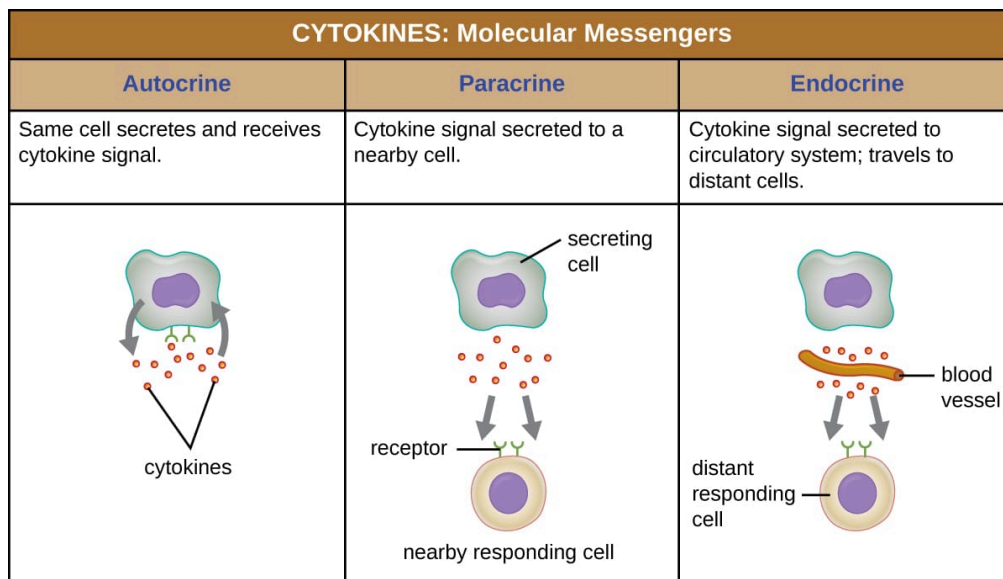


Figure 20.6.3: Autocrine, paracrine, and endocrine actions describe which cells are targeted by cytokines and how far the cytokines must travel to bind to their intended target cells' receptors.

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 20.6.4).

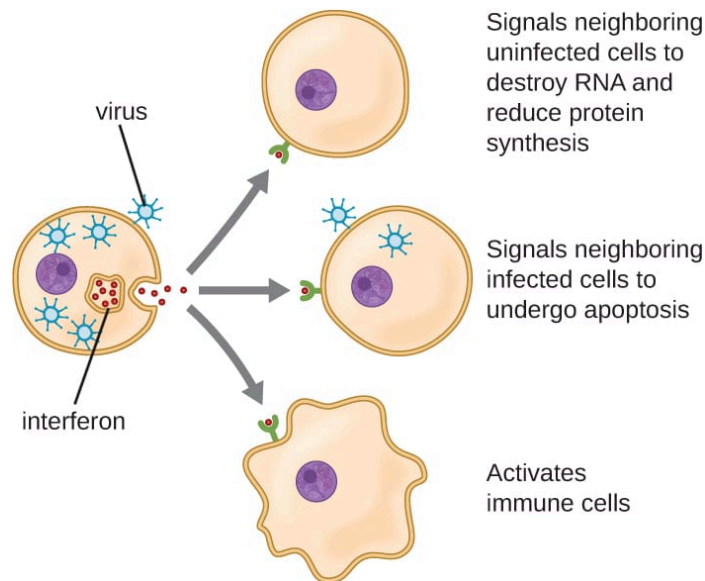


Figure 20.6.4: Interferons are cytokines released by a cell infected with a virus. Interferon- α and interferon- β signal uninfected neighboring cells to inhibit mRNA synthesis, destroy RNA, and reduce protein synthesis (top arrow). Interferon- α and interferon- β also promote apoptosis in cells infected 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.

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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.

Table 20.6.3 provides a summary of the chemical defenses discussed in this section.

Table 20.6.3: 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

- **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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20.E: Innate Immune Defenses I (Exercises)

20.1: Physical Defenses

Nonspecific innate immunity provides a first line of defense against infection by nonspecifically blocking entry of microbes and targeting them for destruction or removal from the body. The physical defenses of innate immunity include physical barriers, mechanical actions that remove microbes and debris, and the microbiome, which competes with and inhibits the growth of pathogens. The skin, mucous membranes, and endothelia throughout the body serve as physical barriers.

Multiple Choice

Which of the following best describes the innate nonspecific immune system?

- A. a targeted and highly specific response to a single pathogen or molecule
- B. a generalized and nonspecific set of defenses against a class or group of pathogens
- C. a set of barrier mechanisms that adapts to specific pathogens after repeated exposure
- D. the production of antibody molecules against pathogens

Answer

B

Which of the following constantly sheds dead cells along with any microbes that may be attached to those cells?

- A. epidermis
- B. dermis
- C. hypodermis
- D. mucous membrane

Answer

A

Which of the following uses a particularly dense suite of tight junctions to prevent microbes from entering the underlying tissue?

- A. the mucociliary escalator
- B. the epidermis
- C. the blood-brain barrier
- D. the urethra

Answer

C

Fill in the Blank

The muscular contraction of the intestines that results in movement of material through the digestive tract is called _____.

Answer

peristalsis

_____ are the hair-like appendages of cells lining parts of the respiratory tract that sweep debris away from the lungs.

Answer

cilia

Secretions that bathe and moisten the interior of the intestines are produced by _____ cells.

Answer

goblet

Short Answer

Differentiate a physical barrier from a mechanical removal mechanism and give an example of each.

Identify some ways that pathogens can breach the physical barriers of the innate immune system.

20.2: Chemical Defenses

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.

Multiple Choice

Which of the following serve as chemical signals between cells and stimulate a wide range of nonspecific defenses?

- A. cytokines
- B. antimicrobial peptides
- C. complement proteins
- D. antibodies

Answer

A

Bacteriocins and defensins are types of which of the following?

- A. leukotrienes
- B. cytokines
- C. inflammation-eliciting mediators
- D. antimicrobial peptides

Answer

D

Which of the following chemical mediators is secreted onto the surface of the skin?

- A. cerumen
- B. sebum
- C. gastric acid
- D. prostaglandin

Answer

B

Identify the complement activation pathway that is triggered by the binding of an acute-phase protein to a pathogen.

- A. classical
- B. alternate
- C. lectin
- D. cathelicidin

Answer

C

Histamine, leukotrienes, prostaglandins, and bradykinin are examples of which of the following?

- A. chemical mediators primarily found in the digestive system
- B. chemical mediators that promote inflammation
- C. antimicrobial peptides found on the skin
- D. complement proteins that form MACs

Answer

B

Fill in the Blank

_____ are antimicrobial peptides produced by members of the normal microbiota.

Answer

bacteriocins

_____ is the fluid portion of a blood sample that has been drawn in the presence of an anticoagulant compound.

Answer

plasma

The process by which cells are drawn or attracted to an area by a microbe invader is known as _____.

Answer

chemotaxis

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21: INNATE IMMUNITY II

Cellular defenses are part of the innate immune system and include various white blood cells that protect the body against infection. Key players are phagocytes, such as neutrophils and macrophages, which engulf and destroy pathogens through phagocytosis. During this process, the cell engulfs a pathogen into a vesicle called a phagosome, which then fuses with a lysosome containing digestive enzymes to break down the invader. Other cells, like natural killer (NK) cells, destroy infected or abnormal cells by inducing apoptosis.

Inflammation is a localized response to injury or infection characterized by redness, heat, swelling, and pain. It serves to contain the infection, recruit immune cells, and promote tissue repair. Fever is a systemic response often triggered by pyrogens, which reset the body's thermostat in the hypothalamus to a higher temperature. Elevated body temperature can slow pathogen growth and enhance immune function, aiding in a faster resolution of infections.

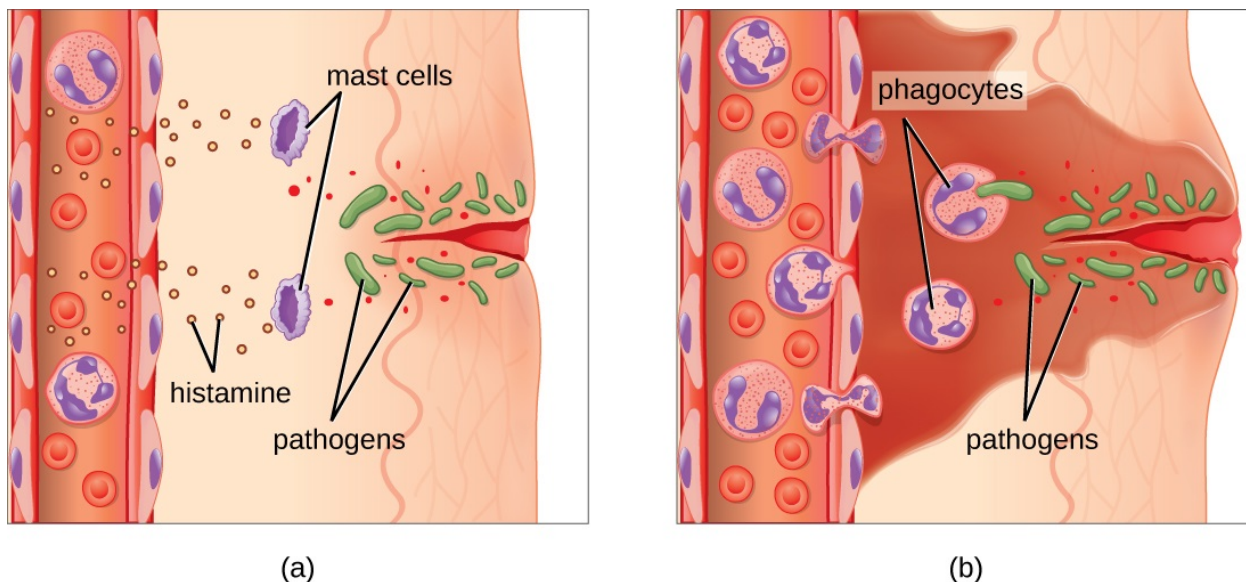


Figure 21.1:

(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.

[21.1: Cellular Defenses - Overview](#)

[21.2: Cellular Defenses - Granulocytes](#)

[21.3: Cellular Defenses - Agranulocytes](#)

[21.4: Phagocytosis](#)

[21.5: Inflammation](#)

[21.6: Fever](#)

[21.7: Pathogen Recognition](#)

[21.E: Innate Immune Defenses II \(Exercises\)](#)

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21.1: Cellular Defenses - Overview

Learning Objectives

- Identify and describe the components of blood
- Explain the process by which the formed elements of blood are formed (hematopoiesis)
- Describe the characteristics of formed elements found in peripheral blood, as well as their respective functions within the innate immune system

In the previous section, we discussed some of the chemical mediators found in plasma, the fluid portion of blood. The nonfluid portion of blood consists of various types of **formed elements**, so called because they are all formed from the same stem cells found in bone marrow. **The three major categories of formed elements are: red blood cells (RBCs), also called erythrocytes; platelets, also called thrombocytes; and white blood cells (WBCs), also called leukocytes.**

Red blood cells are primarily responsible for carrying oxygen to tissues. Platelets are cellular fragments that participate in blood clot formation and tissue repair. Several different types of WBCs participate in various nonspecific mechanisms of innate and adaptive immunity. In this section, we will focus primarily on the innate mechanisms of various types of WBCs.

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Hematopoiesis

All of the formed elements of blood are derived from **hematopoietic stem cells (HSCs)** in the bone marrow. As the HSCs make copies of themselves in the bone marrow, individual cells receive different cues from the body that control how they develop and mature. As a result, the HSCs differentiate into different types of blood cells that, once mature, circulate in peripheral blood. This process of differentiation, called hematopoiesis, is shown in more detail in Figure 21.1.1.

In terms of sheer numbers, the vast majority of HSCs become erythrocytes. Much smaller numbers become leukocytes and platelets. Leukocytes can be further subdivided into granulocytes, which are characterized by numerous granules visible in the cytoplasm, and agranulocytes, which lack granules. Figure 21.1.2 provides an overview of the various types of formed elements, including their relative numbers, primary function, and lifespans.

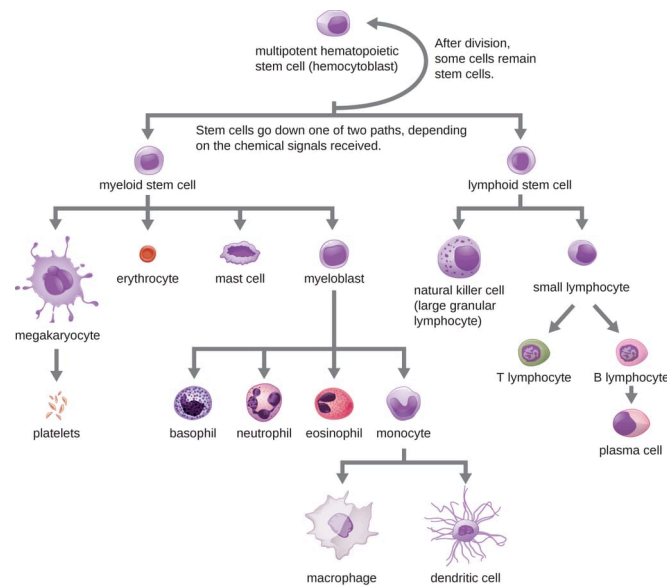


Figure 21.1.1: All the formed elements of the blood arise by differentiation of hematopoietic stem cells in the bone marrow.

Formed Element	Major Subtypes	Numbers Present per Microliter (μL) and Mean (Range)	Appearance in a Standard Blood Smear	Summary of Functions	Comments
Erythrocytes (red blood cells)		5.2 million (4.4-6.0 million)	Flattened biconcave disk; no nucleus; pale red	Transport oxygen and some carbon dioxide between tissue and lungs	Lifespan of approximately 120 days
Leukocytes (white blood cells)		7000 (5000-10,000)	Circular; dark-staining nucleus	All function in body defenses	Exit capillaries and move into tissues; lifespan of usually a few hours or days
	Granulocytes, including neutrophils, eosinophils, and basophils	Total leukocytes (%) 4360 (1050-9950)	Abundant granules in cytoplasm; nucleus normally indented	Non-specific (innate) resistance to disease	Classified according to membrane-bound granules in cytoplasm
	Neutrophils	50-70 4150 (1050-7300)	Nucleus lobes increase with age; pale lilac granules	Phagocytic; particularly effective against bacteria; release cytotoxic chemicals from granules	Most common leukocyte; lifespan of minutes to days
	Eosinophils	1-3 165 (0-700)	Nucleus generally two-lobed; bright red-orange granules	Phagocytic cells; particularly effective with antigen-antibody complexes; release antihistamines; combat parasitic infections	Lifespan of minutes to days
	Basophils	<1 44 (0-150)	Nucleus generally two-lobed but difficult to see due to presence of heavy, dense, dark purple granules	Pro-inflammatory	Least common leukocyte; lifespan unknown
	Agranulocytes, including lymphocytes and monocytes	2640 (1700-4950)	Lack abundant granules in cytoplasm; have a simple-shaped nucleus that may be indented	Body defenses	Group consists of two major cell types from different lineages
	Lymphocytes	20-40 2185 (1500-4000)	Small cells with a single, often large, nucleus occupying much of the cell's volume; stains purple; seen in large (natural killer cells) and small (B and T cells) variants	Primarily specific (adaptive) immunity: T cells directly attack other cells (cellular immunity); B cells release antibodies (humoral immunity); natural killer cells are similar to T cells but non-specific	Initial cells originate in bone marrow, but secondary production occurs in lymphatic tissue; several distinct subtypes; memory cells form after exposure to a pathogen and rapidly increase responses to subsequent exposure; lifespan of many years
	Monocytes	1-6 450 (200-950)	Largest leukocyte; has an indented or horseshoe-shaped nucleus	Very effective phagocytic cells engulfing pathogens or worn-out cells; also serve as antigen-presenting cells (APCs) or other components of the immune system	Produced in red bone marrow; referred to as macrophages and dendritic cells after leaving the circulation
Platelets		350,000 (150,000-500,000)	Cellular fragments surrounded by a plasma membrane and containing granules; stains purple	Hemostatic; release growth factors for repair and healing of tissue	Formed from megakaryocytes that remain in the red bone marrow and shed platelets into circulation

Figure 21.1.2: Formed elements of blood include erythrocytes (red blood cells), leukocytes (white blood cells), and platelets.

Key Concepts and Summary

- The **formed elements** of the blood include red blood cells (**erythrocytes**), white blood cells (**leukocytes**), and **platelets (thrombocytes)**. Of these, leukocytes are primarily involved in the immune response.
- All formed elements originate in the bone marrow as stem cells (HSCs) that differentiate through **hematopoiesis**.

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21.2: Cellular Defenses - Granulocytes

Learning Objectives

- Distinguish the morphology and function of different granulocytes
- Name three antimicrobial mechanisms of neutrophils (PMNs)

Granulocytes

The various types of granulocytes can be distinguished from one another in a blood smear by the appearance of their nuclei and the contents of their granules, which confer different traits, functions, and staining properties. The **neutrophils**, also called **polymorphonuclear neutrophils (PMNs)**, have a nucleus with three to five lobes and small, numerous, lilac-colored granules. Each lobe of the nucleus is connected by a thin strand of material to the other lobes. The **eosinophils** have fewer lobes in the nucleus (typically 2–3) and larger granules that stain reddish-orange. The **basophils** have a two-lobed nucleus and large granules that stain dark blue or purple (Figure 21.2.3).

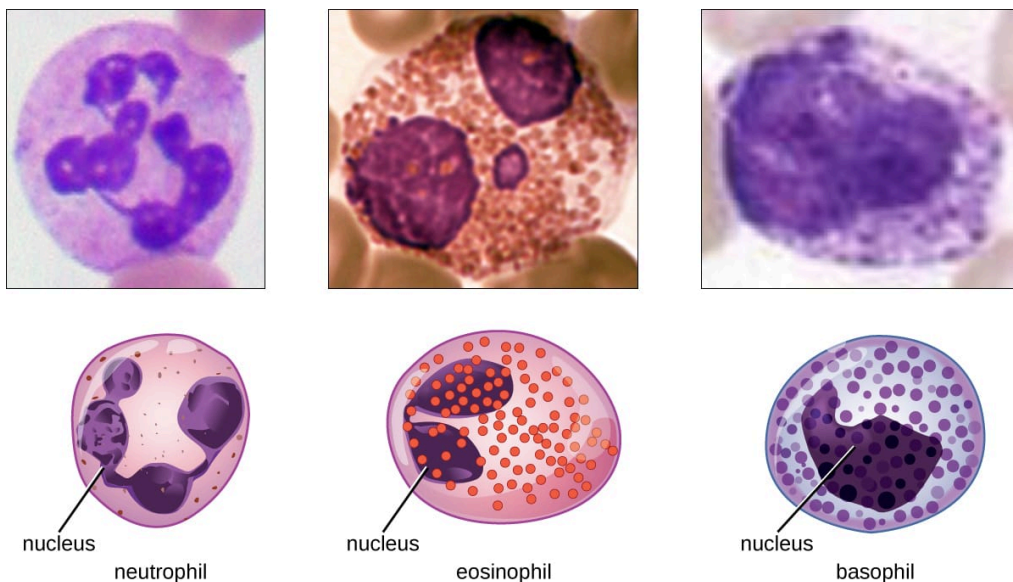


Figure 21.2.3: Granulocytes can be distinguished by the number of lobes in their nuclei and the staining properties of their granules. (credit "neutrophil" micrograph: modification of work by Ed Uthman)

Neutrophils (PMNs)

Neutrophils (PMNs) are frequently involved in the elimination and destruction of extracellular bacteria. They are capable of migrating through the walls of blood vessels to areas of bacterial infection and tissue damage, where they seek out and kill infectious bacteria. **PMN granules contain a variety of defensins and hydrolytic enzymes** that help them destroy bacteria through phagocytosis (described in more detail in [Pathogen Recognition and Phagocytosis](#)). In addition, when many neutrophils are brought into an infected area, they can be stimulated to release toxic molecules into the surrounding tissue to better clear infectious agents. This is called degranulation.

Another mechanism used by neutrophils is **neutrophil extracellular traps (NETs)**, which are extruded meshes of chromatin that are closely associated with antimicrobial granule proteins and components. Chromatin is DNA with associated proteins (usually histone proteins, around which DNA wraps for organization and packing within a cell). By creating and releasing a mesh or lattice-like structure of chromatin that is coupled with antimicrobial proteins, the neutrophils can mount a highly concentrated and efficient attack against nearby pathogens. Proteins frequently associated with NETs include lactoferrin, gelatinase, cathepsin G, and myeloperoxidase. Each has a different means of promoting antimicrobial activity, helping neutrophils eliminate pathogens.

The toxic proteins in NETs may kill some of the body's own cells along with invading pathogens. However, this collateral damage can be repaired after the danger of the infection has been eliminated.

As neutrophils fight an infection, a visible accumulation of leukocytes, cellular debris, and bacteria at the site of infection can be observed. This buildup is what we call **pus** (also known as purulent or suppurative discharge or drainage). The presence of pus is a sign that the immune defenses have been activated against an infection; historically, some physicians believed that inducing pus formation could actually promote the healing of wounds. The practice of promoting "laudable pus" (by, for instance, wrapping a wound in greasy wool soaked in wine) dates back to the ancient physician Galen in the 2nd century AD, and was practiced in variant forms until the 17th century (though it was not universally accepted). Today, this method is no longer practiced because we now know that it is not effective. Although a small amount of pus formation can indicate a strong immune response, artificially inducing pus formation does not promote recovery.

Eosinophils

Eosinophils are granulocytes that protect against protozoa and helminths; they also play a role in allergic reactions. The granules of eosinophils, which readily absorb the acidic reddish dye eosin, contain histamine, degradative enzymes, and a compound known as major basic protein (MBP) (Figure 21.2.3). MBP binds to the surface carbohydrates of parasites, and this binding is associated with disruption of the cell membrane and membrane permeability.

Basophils

Basophils have cytoplasmic granules of varied size and are named for their granules' ability to absorb the basic dye methylene blue (Figure 21.2.3). Their stimulation and degranulation can result from multiple triggering events. Activated complement fragments C3a and C5a, produced in the activation cascades of complement proteins, act as anaphylatoxins by inducing degranulation of basophils and inflammatory responses. This cell type is important in allergic reactions and other responses that involve inflammation. One of the most abundant components of basophil granules is histamine, which is released along with other chemical factors when the basophil is stimulated. These chemicals can be chemotactic and can help to open the gaps between cells in the blood vessels. Other mechanisms for basophil triggering require the assistance of antibodies, as discussed in [B Lymphocytes and Humoral Immunity](#).

Mast Cells

Hematopoiesis also gives rise to mast cells, which appear to be derived from the same common myeloid progenitor cell as neutrophils, eosinophils, and basophils. Functionally, mast cells are very similar to basophils, containing many of the same components in their granules (e.g., histamine) and playing a similar role in allergic responses and other inflammatory reactions. However, unlike basophils, mast cells leave the circulating blood and are most frequently found residing in tissues. They are often associated with blood vessels and nerves or found close to surfaces that interface with the external environment, such as the skin and mucous membranes in various regions of the body (Figure 21.2.4).

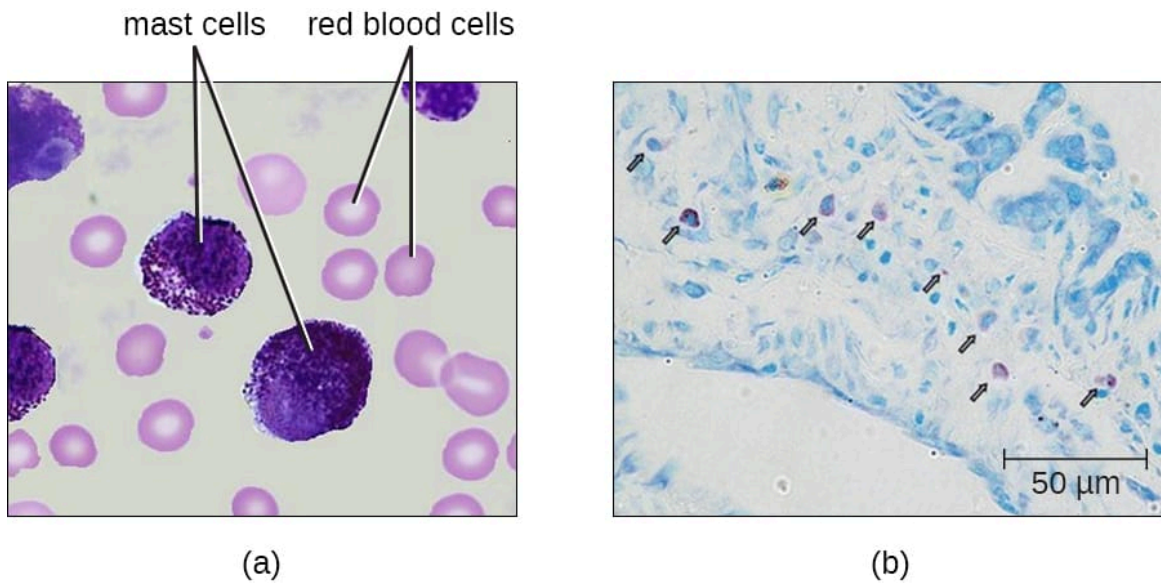


Figure 21.2.4: Mast cells function similarly to basophils by inducing and promoting inflammatory responses. (a) This figure shows mast cells in blood. In a blood smear, they are difficult to differentiate from basophils (b). Unlike basophils, mast cells migrate from the blood into various tissues. (credit right: modification of work by Greenland JR, Xu X, Sayah DM, Liu FC, Jones KD, Looney MR, Caughey GH)

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Key Concepts and Summary

- **Granulocytes** are leukocytes characterized by a lobed nucleus and granules in the cytoplasm. These include **neutrophils (PMNs)**, **eosinophils**, and **basophils**.
- Neutrophils are the leukocytes found in the largest numbers in the bloodstream and they primarily fight bacterial infections.
- Eosinophils target parasitic infections. Eosinophils and basophils are involved in allergic reactions. Both release histamine and other proinflammatory compounds from their granules upon stimulation.
- **Mast cells** function similarly to basophils but can be found in tissues outside the bloodstream.

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21.3: Cellular Defenses - Agranulocytes

Learning Objectives

- Describe the signals that activate natural killer cells
- Identify the mechanisms that NK cells use to kill target cells
- Differentiate monocytes from macrophages

Agranulocytes

As their name suggests, **agranulocytes** lack visible granules in the cytoplasm. **Agranulocytes can be categorized as lymphocytes or monocytes** (Figure 21.3.2). Among the lymphocytes are natural killer cells, which play an important role in nonspecific innate immune defenses. Lymphocytes also include the B cells and T cells, which are discussed in the next chapter because they are central players in the specific adaptive immune defenses. The monocytes differentiate into macrophages and dendritic cells, which are collectively referred to as the mononuclear phagocyte system.

Natural Killer Cells

Most lymphocytes are primarily involved in the specific adaptive immune response, and thus will be discussed in the following chapter. An exception is the **natural killer cells (NK cells)**; these mononuclear lymphocytes use nonspecific mechanisms to recognize and destroy cells that are abnormal in some way. Cancer cells and cells infected with viruses are two examples of cellular abnormalities that are targeted by NK cells. Recognition of such cells involves a complex process of identifying inhibitory and activating molecular markers on the surface of the target cell. Molecular markers that make up the major histocompatibility complex (MHC) are expressed by healthy cells as an indication of “self.” This will be covered in more detail in next chapter. NK cells are able to recognize normal MHC markers on the surface of healthy cells, and these MHC markers serve as an inhibitory signal preventing NK cell activation. However, cancer cells and virus-infected cells actively diminish or eliminate expression of MHC markers on their surface. When these MHC markers are diminished or absent, the NK cell interprets this as an abnormality and a cell in distress. This is one part of the NK cell activation process (Figure 21.3.5). NK cells are also activated by binding to activating molecular molecules on the target cell. These activating molecular molecules include “altered self” or “nonself” molecules. When a NK cell recognizes a decrease in inhibitory normal MHC molecules and an increase in activating molecules on the surface of a cell, the NK cell will be activated to eliminate the cell in distress.

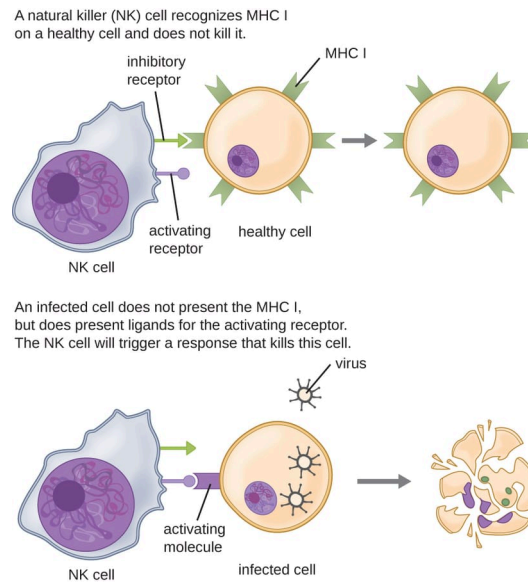


Figure 21.3.5: Natural killer (NK) cells are inhibited by the presence of the major histocompatibility cell (MHC) receptor on healthy cells. Cancer cells and virus-infected cells have reduced expression of MHC and increased expression of activating molecules. When a NK cell recognizes decreased MHC and increased activating molecules, it will kill the abnormal cell.

Once a cell has been recognized as a target, the NK cell can use several different mechanisms to kill its target. For example, it may express cytotoxic membrane proteins and cytokines that stimulate the target cell to undergo apoptosis, or controlled cell suicide. NK cells may also use **perforin-mediated cytotoxicity** to induce apoptosis in target cells. This mechanism relies on two toxins released from granules in the cytoplasm of the NK cell: **perforin**, a protein that creates pores in the target cell, and **granzymes**, proteases that enter through the pores into the target cell's cytoplasm, where they trigger a cascade of protein activation that leads to apoptosis. The NK cell binds to the abnormal target cell, releases its destructive payload, and detaches from the target cell. While the target cell undergoes apoptosis, the NK cell synthesizes more perforin and proteases to use on its next target.

NK cells contain these toxic compounds in granules in their cytoplasm. When stained, the granules are azurophilic and can be visualized under a light microscope (Figure 21.3.6). Even though they have granules, NK cells are not considered granulocytes because their granules are far less numerous than those found in true granulocytes. Furthermore, NK cells have a different lineage than granulocytes, arising from lymphoid rather than myeloid stem cells (Figure 21.3.1).

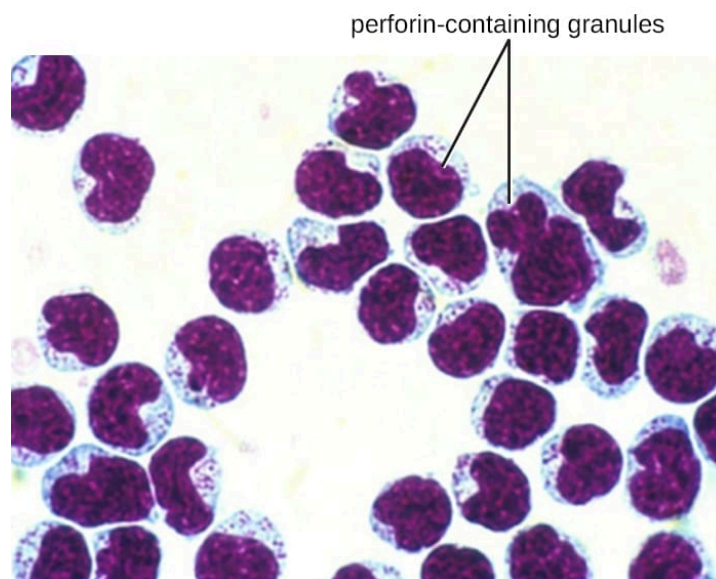


Figure 21.3.6: Natural killer cell with perforin-containing granules. (credit: modification of work by Rolstad B)

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Monocytes

The largest of the white blood cells, **monocytes** have a nucleus that lacks lobes, and they also lack granules in the cytoplasm (Figure 21.3.7). Nevertheless, they are **effective phagocytes**, engulfing pathogens and apoptotic cells to help fight infection.

When monocytes leave the bloodstream and enter a specific body tissue, they differentiate into tissue-specific phagocytes called **macrophages** and **dendritic cells**. They are particularly important residents of lymphoid tissue, as well as nonlymphoid sites and organs. Macrophages and dendritic cells can reside in body tissues for significant lengths of time. Macrophages in specific body tissues develop characteristics suited to the particular tissue. Not only do they provide immune protection for the tissue in which they reside but they also support normal function of their neighboring tissue cells through the production of cytokines. Macrophages are given tissue-specific names, and a few examples of tissue-specific macrophages are listed in Table 21.3.1. Dendritic cells are important sentinels residing in the skin and mucous membranes, which are portals of entry for many pathogens. Monocytes, macrophages, and dendritic cells are all highly phagocytic and important promoters of the immune response through their production and release of cytokines. These cells provide an essential bridge between innate and adaptive immune responses, as discussed in the next section as well as the next chapter.

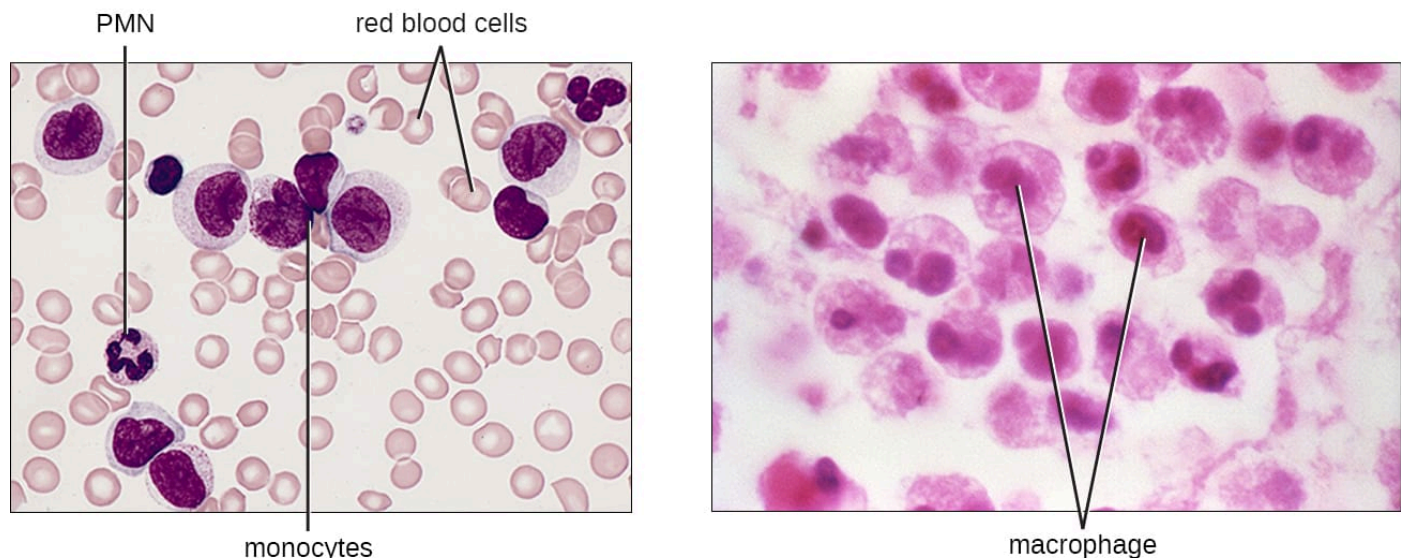


Figure 21.3.7: Monocytes are large, agranular white blood cells with a nucleus that lacks lobes. When monocytes leave the bloodstream, they differentiate and become macrophages with tissue-specific properties. (credit left: modification of work by Armed Forces Institute of Pathology; credit right: modification of work by Centers for Disease Control and Prevention)

Table 21.3.1: Macrophages Found in Various Body Tissues

Tissue	Macrophage
Brain and central nervous system	Microglial cells
Liver	Kupffer cells
Lungs	Alveolar macrophages (dust cells)
Peritoneal cavity	Peritoneal macrophages

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Key Concepts and Summary

- **Natural killer (NK)** cells are lymphocytes that recognize and kill abnormal or infected cells by releasing proteins that trigger apoptosis.
- **Monocytes** are large, mononuclear leukocytes that circulate in the bloodstream. They may leave the bloodstream and take up residence in body tissues, where they differentiate and become tissue-specific **macrophages** and **dendritic cells**.

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21.4: Phagocytosis

Learning Objectives

- Identify the steps in the process of phagocytosis
- Distinguish a phagosome from a lysosome
- Describe the mechanisms of how phagocytes kill pathogens

Pathogen Degradation

Once pathogen recognition and attachment occurs, the pathogen is engulfed in a vesicle and brought into the internal compartment of the phagocyte in a process called **phagocytosis** (Figure 21.4.3). PRRs can aid in phagocytosis by first binding to the pathogen's surface, but phagocytes are also capable of engulfing nearby items even if they are not bound to specific receptors. To engulf the pathogen, the phagocyte forms a pseudopod that wraps around the pathogen and then pinches it off into a membrane vesicle called a **phagosome**. Acidification of the phagosome (pH decreases to the range of 4–5) provides an important early antibacterial mechanism. The phagosome containing the pathogen fuses with one or more lysosomes, forming a **phagolysosome**. Formation of the phagolysosome enhances the acidification, which is essential for activation of pH-dependent digestive lysosomal enzymes and production of hydrogen peroxide and toxic reactive oxygen species. Lysosomal enzymes such as lysozyme, phospholipase, and proteases digest the pathogen. Other enzymes are involved a respiratory burst. During the respiratory burst, phagocytes will increase their uptake and consumption of oxygen, but not for energy production. The increased oxygen consumption is focused on the production of superoxide anion, hydrogen peroxide, hydroxyl radicals, and other reactive oxygen species that are antibacterial.

In addition to the reactive oxygen species produced by the respiratory burst, reactive nitrogen compounds with cytotoxic (cell-killing) potential can also form. For example, nitric oxide can react with superoxide to form peroxynitrite, a highly reactive nitrogen compound with degrading capabilities similar to those of the reactive oxygen species. Some phagocytes even contain an internal storehouse of microbicidal defensin proteins (e.g., neutrophil granules). These destructive forces can be released into the area around the cell to degrade microbes externally. Neutrophils, especially, can be quite efficient at this secondary antimicrobial mechanism.

Once degradation is complete, leftover waste products are excreted from the cell in an exocytic vesicle. However, it is important to note that not all remains of the pathogen are excreted as waste. Macrophages and dendritic cells are also antigen-presenting cells involved in the specific adaptive immune response. These cells further process the remains of the degraded pathogen and present key antigens (specific pathogen proteins) on their cellular surface. This is an important step for stimulation of some adaptive immune responses, as will be discussed in more detail in the next chapter.

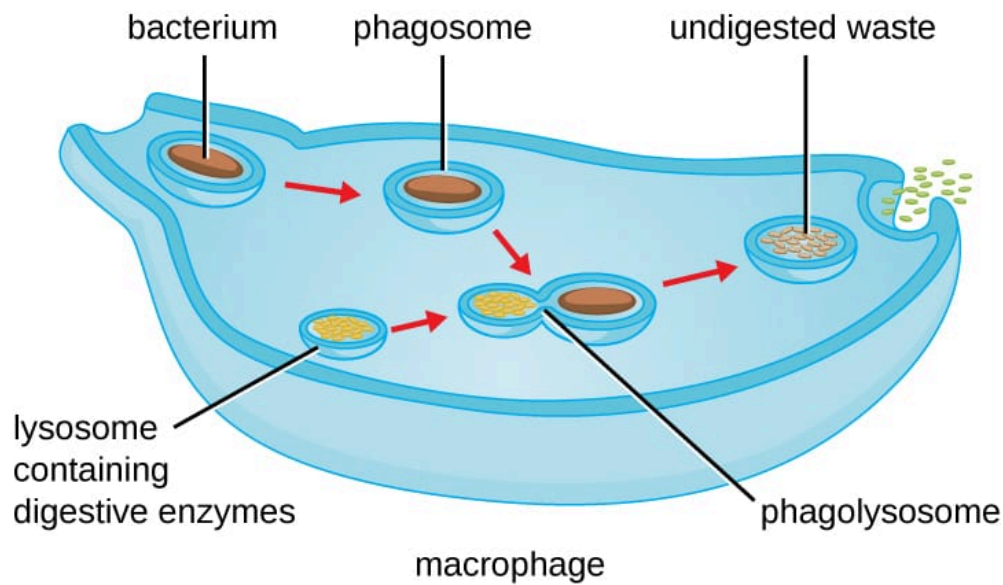


Figure 21.4.3: The stages of phagocytosis include the engulfment of a pathogen, the formation of a phagosome, the digestion of the pathogenic particle in the phagolysosome, and the expulsion of undigested materials from the cell.

Link to Learning

Visit this [link](#) to view a phagocyte chasing and engulfing a pathogen.

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When Phagocytosis Fails

Although phagocytosis successfully destroys many pathogens, some are able to survive and even exploit this defense mechanism to multiply in the body and cause widespread infection. Protozoans of the genus *Leishmania* are one example. These obligate intracellular parasites are flagellates transmitted to humans by the bite of a sand fly. Infections cause serious and sometimes disfiguring sores and ulcers in the skin and other tissues (Figure 21.4.4). Worldwide, an estimated 1.3 million people are newly infected with leishmaniasis annually.

Salivary peptides from the sand fly activate host macrophages at the site of their bite. The classic or alternate pathway for complement activation ensues with C3b opsonization of the parasite. *Leishmania* cells are phagocytosed, lose their flagella, and multiply in a form known as an amastigote (Leishman-Donovan body) within the phagolysosome. Although many other pathogens are destroyed in the phagolysosome, survival of the *Leishmania* amastigotes is maintained by the presence of surface lipophosphoglycan and acid phosphatase. These substances inhibit the macrophage respiratory burst and lysosomal enzymes. The parasite then multiplies inside the cell and lyses the infected macrophage, releasing the amastigotes to infect other macrophages within the same host. Should another sand fly bite an infected person, it might ingest amastigotes and then transmit them to another individual through another bite.

There are several different forms of leishmaniasis. The most common is a localized cutaneous form of the illness caused by *L. tropica*, which typically resolves spontaneously over time but with some significant lymphocyte infiltration and permanent scarring. A mucocutaneous form of the disease, caused by *L. viannia brasiliensis*, produces lesions in the tissue of the nose and mouth and can be life threatening. A visceral form of the illness can be caused by several of the different *Leishmania* species. It affects various organ systems and causes abnormal enlargement of the liver and spleen. Irregular fevers, anemia, liver dysfunction, and weight loss are all signs and symptoms of visceral leishmaniasis. If left untreated, it is typically fatal.



Figure 21.4.4: (a) Cutaneous leishmaniasis is a disfiguring disease caused by the intracellular flagellate *Leishmania tropica*, transmitted by the bite of a sand fly. (b) This light micrograph of a sample taken from a skin lesion shows a large cell, which is a macrophage infected with *L. tropica* amastigotes (arrows). The amastigotes have lost their flagella but their nuclei are visible. Soon the amastigotes will lyse the macrophage and be engulfed by other phagocytes, spreading the infection. (credit a: modification of work by Otis Historical Archives of "National Museum of Health & Medicine"; credit b: modification of work by Centers for Disease Control and Prevention)

Key Concepts and Summary

- Phagocytes degrade pathogens through **phagocytosis**, which involves engulfing the pathogen, killing and digesting it within a **phagolysosome**, and then excreting undigested matter.

Footnotes

- 1 World Health Organization. "Leishmaniasis." 2016. <http://www.who.int/mediacentre/factsheets/fs375/en/>.

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21.5: Inflammation

Learning Objectives

- Identify the signs of inflammation
- 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 21.5.1 illustrates a typical case of acute inflammation at the site of a skin wound.

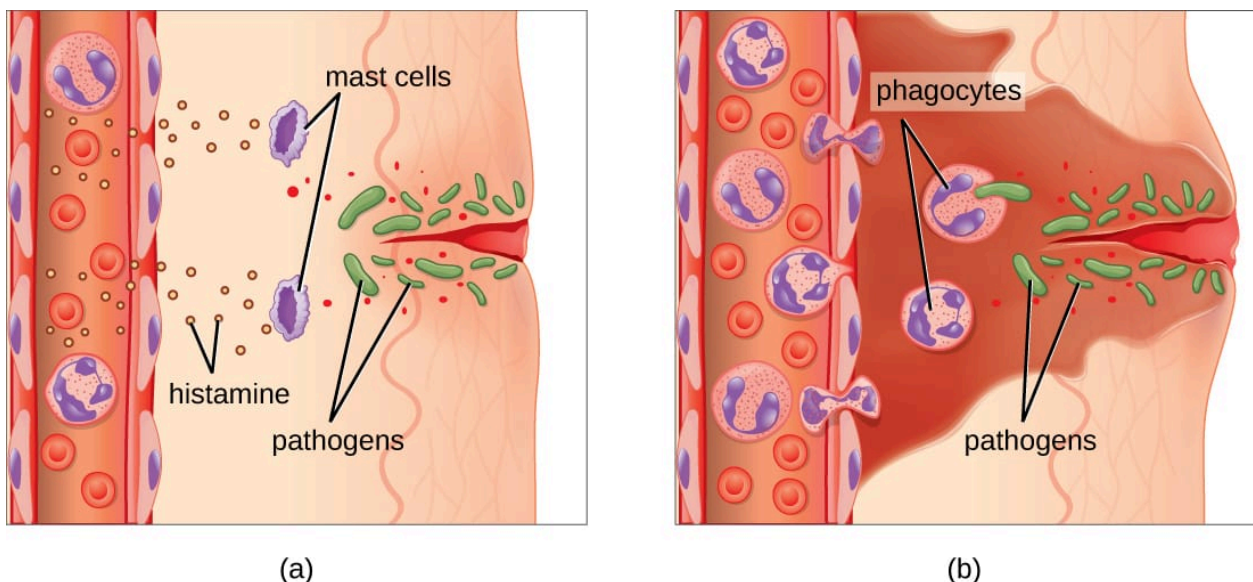


Figure 21.5.1: (a) Mast cells detect injury to nearby cells and release histamine, initiating an inflammatory response. (b) Histamine increases blood flow to the wound site, and increased vascular permeability allows fluid, proteins, phagocytes, and other immune cells to enter infected tissue. These 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.

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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 21.5.2). 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.

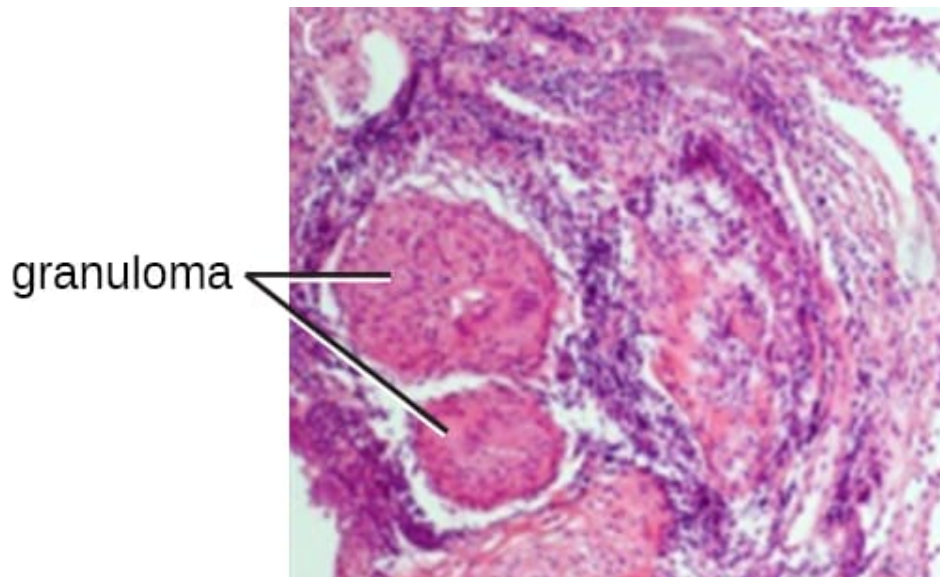


Figure 21.5.2: A tubercle is a granuloma in the lung tissue of a patient with tuberculosis. In this micrograph, white blood cells (stained purple) have walled off a pocket of tissue infected with *Mycobacterium tuberculosis*. Granulomas also occur in many other forms of disease. (credit: modification of work by Piotrowski WJ, Górski P, Duda-Szymańska J, Kwiatkowska S)

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Chronic Edema

In addition to granulomas, chronic inflammation can also result in long-term edema. A condition known as lymphatic filariasis (also known as elephantiasis) provides an extreme example. Lymphatic filariasis is caused by microscopic nematodes (parasitic worms) whose larvae are transmitted between human hosts by mosquitoes. Adult worms live in the lymphatic vessels, where their presence stimulates infiltration by lymphocytes, plasma cells, eosinophils, and thrombocytes (a condition known as lymphangitis). Because of the chronic nature of the illness, granulomas, fibrosis, and blocking of the lymphatic system may eventually occur. Over time, these blockages may worsen with repeated infections over decades, leading to skin thickened with edema and fibrosis. Lymph (extracellular tissue fluid) may spill out of the lymphatic areas and back into tissues, causing extreme swelling (Figure 21.5.3). Secondary bacterial infections commonly follow. Because it is a disease caused by a parasite, eosinophilia (a dramatic rise in the number of eosinophils in the blood) is characteristic of acute infection. However, this increase in antiparasite granulocytes is not sufficient to clear the infection in many cases.

Lymphatic filariasis affects an estimated 120 million people worldwide, mostly concentrated in Africa and Asia.  Improved sanitation and mosquito control can reduce transmission rates.



Figure 21.5.3: Elephantiasis (chronic edema) of the legs due to filariasis. (credit: modification of work by Centers for Disease Control and Prevention)

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.

Footnotes

- 1 Centers for Disease Control and Prevention. "Parasites–Lymphatic Filariasis." 2016. <http://www.cdc.gov/parasites/lymphat...info/faqs.html>.
- 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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21.6: Fever

Learning Objectives

- Explain the difference between exogenous and endogenous pyrogens
- Identify the mechanisms of how fever helps with immune defense

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 (PGE₂) from other cells, resetting the hypothalamus to initiate fever (Figure 21.6.4).

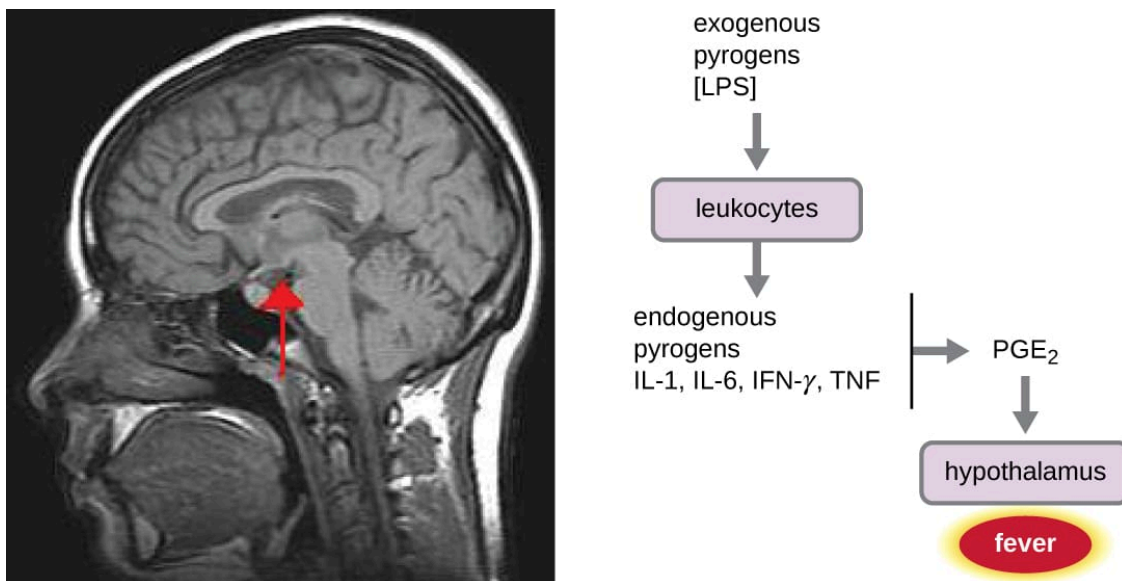


Figure 21.6.4: The role of the hypothalamus in the inflammatory response. Macrophages recognize pathogens in an area and release cytokines that trigger inflammation. The cytokines also send a signal up the vagus nerve to the hypothalamus.

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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).

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Key Concepts and Summary

- **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. 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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21.7: Pathogen Recognition

Learning Objectives

- Explain how leukocytes migrate from peripheral blood into infected tissues
- Recognize the role of adhesion molecules in the process of extravasation
- Explain the mechanisms by which leukocytes recognize pathogens
- Describe the process of phagocyte activation

Several of the cell types discussed in the previous section can be described as phagocytes—cells whose main function is to seek, ingest, and kill pathogens. This process, called phagocytosis, was first observed in starfish in the 1880s by Nobel Prize-winning zoologist Ilya Metchnikoff (1845–1916), who made the connection to white blood cells (WBCs) in humans and other animals. At the time, Pasteur and other scientists believed that WBCs were spreading pathogens rather than killing them (which is true for some diseases, such as tuberculosis). But in most cases, phagocytes provide a strong, swift, and effective defense against a broad range of microbes, making them a critical component of innate nonspecific immunity. This section will focus on the mechanisms by which phagocytes are able to seek, recognize, and destroy pathogens.

Extravasation (Diapedesis) of Leukocytes

Some phagocytes are leukocytes (WBCs) that normally circulate in the bloodstream. To reach pathogens located in infected tissue, leukocytes must pass through the walls of small capillary blood vessels within tissues. This process, called **extravasation**, or **diapedesis**, is initiated by complement factor C5a, as well as cytokines released into the immediate vicinity by resident macrophages and tissue cells responding to the presence of the infectious agent (Figure 21.7.1). Similar to C5a, many of these cytokines are proinflammatory and chemotactic, and they bind to cells of small capillary blood vessels, initiating a response in the endothelial cells lining the inside of the blood vessel walls. This response involves the upregulation and expression of various cellular adhesion molecules and receptors. Leukocytes passing through will stick slightly to the adhesion molecules, slowing down and rolling along the blood vessel walls near the infected area. When they reach a cellular junction, they will bind to even more of these adhesion molecules, flattening out and squeezing through the cellular junction in a process known as **transendothelial migration**. This mechanism of **“rolling adhesion”** allows leukocytes to exit the bloodstream and enter the infected areas, where they can begin phagocytosing the invading pathogens.

Note that extravasation does not occur in arteries or veins. These blood vessels are surrounded by thicker, multilayer protective walls, in contrast to the thin single-cell-layer walls of capillaries. Furthermore, the blood flow in arteries is too turbulent to allow for rolling adhesion. Also, some leukocytes tend to respond to an infection more quickly than others. The first to arrive typically are neutrophils, often within hours of a bacterial infection. By contrast, monocytes may take several days to leave the bloodstream and differentiate into macrophages.

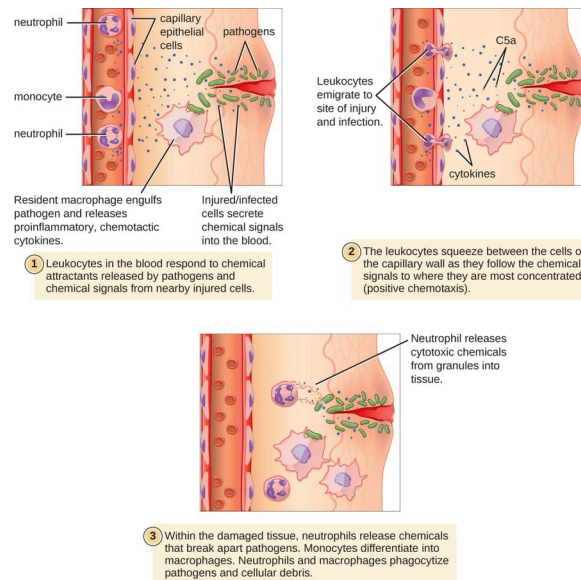


Figure 21.7.1: Damaged cells and macrophages that have ingested pathogens release cytokines that are proinflammatory and chemotactic for leukocytes. In addition, activation of complement at the site of infection results in production of the chemotactic and proinflammatory C5a. Leukocytes exit the blood vessel and follow the chemoattractant signal of cytokines and C5a to the site of infection. Granulocytes such as neutrophils release chemicals that destroy pathogens. They are also capable of phagocytosis and intracellular killing of bacterial pathogens.

Link to Learning

Watch the following videos on [leukocyte extravasation](#) and [leukocyte rolling](#) to learn more.

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Pathogen Recognition

As described in the previous section, opsonization of pathogens by antibody; complement factors C1q, C3b, and C4b; and lectins can assist phagocytic cells in recognition of pathogens and attachment to initiate phagocytosis. However, not all pathogen recognition is opsonin dependent. Phagocytes can also recognize molecular structures that are common to many groups of pathogenic microbes. Such structures are called **pathogen-associated molecular patterns (PAMPs)**. Common PAMPs include the following:

- peptidoglycan, found in bacterial cell walls;
- flagellin, a protein found in bacterial flagella;
- lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria;
- lipopeptides, molecules expressed by most bacteria; and
- nucleic acids such as viral DNA or RNA.

Like numerous other PAMPs, these substances are integral to the structure of broad classes of microbes.

The structures that allow phagocytic cells to detect PAMPs are called **pattern recognition receptors (PRRs)**. One group of PRRs is the **toll-like receptors (TLRs)**, which bind to various PAMPs and communicate with the nucleus of the phagocyte to elicit

a response. Many TLRs (and other PRRs) are located on the surface of a phagocyte, but some can also be found embedded in the membranes of interior compartments and organelles (Figure 21.7.2). These interior PRRs can be useful for the binding and recognition of intracellular pathogens that may have gained access to the inside of the cell before phagocytosis could take place. Viral nucleic acids, for example, might encounter an interior PRR, triggering production of the antiviral cytokine interferon.

In addition to providing the first step of pathogen recognition, the interaction between PAMPs and PRRs on macrophages provides an intracellular signal that activates the phagocyte, causing it to transition from a dormant state of readiness and slow proliferation to a state of hyperactivity, proliferation, production/secretion of cytokines, and enhanced intracellular killing. PRRs on macrophages also respond to chemical distress signals from damaged or stressed cells. This allows macrophages to extend their responses beyond protection from infectious diseases to a broader role in the inflammatory response initiated from injuries or other diseases.

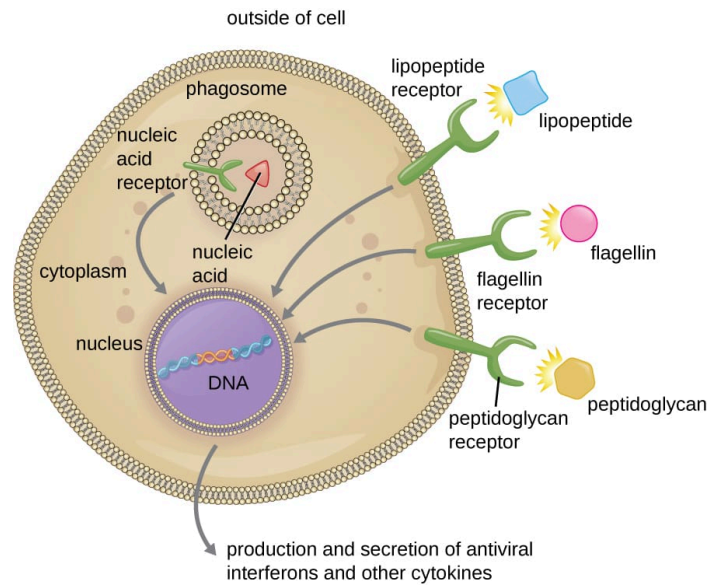


Figure 21.7.2: Phagocytic cells contain pattern recognition receptors (PRRs) capable of recognizing various pathogen-associated molecular patterns (PAMPs). These PRRs can be found on the plasma membrane or in internal phagosomes. When a PRR recognizes a PAMP, it sends a signal to the nucleus that activates genes involved in phagocytosis, cellular proliferation, production and secretion of antiviral interferons and proinflammatory cytokines, and enhanced intracellular killing.

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Key Concepts and Summary

- Phagocytes are cells that recognize pathogens and destroy them through phagocytosis.
- Recognition often takes place by the use of phagocyte receptors that bind molecules commonly found on pathogens, known as **pathogen-associated molecular patterns (PAMPs)**.
- The receptors that bind PAMPs are called **pattern recognition receptors**, or **PRRs**. **Toll-like receptors (TLRs)** are one type of PRR found on phagocytes.
- **Extravasation** of white blood cells from the bloodstream into infected tissue occurs through the process of **transendothelial migration**.

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21.E: Innate Immune Defenses II (Exercises)

21.1: Cellular Defenses

The formed elements of the blood include red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). Of these, leukocytes are primarily involved in the immune response. All formed elements originate in the bone marrow as stem cells (HSCs) that differentiate through hematopoiesis. Granulocytes are leukocytes characterized by a lobed nucleus and granules in the cytoplasm. These include neutrophils (PMNs), eosinophils, and basophils.

Multiple Choice

White blood cells are also referred to as which of the following?

- A. platelets
- B. erythrocytes
- C. leukocytes
- D. megakaryocytes

Answer

C

Hematopoiesis occurs in which of the following?

- A. liver
- B. bone marrow
- C. kidneys
- D. central nervous system

Answer

B

Granulocytes are which type of cell?

- A. lymphocyte
- B. erythrocyte
- C. megakaryocyte
- D. leukocyte

Answer

D

Matching

Match each cell type with its description.

___natural killer cell	A. stains with basic dye methylene blue, has large amounts of histamine in granules, and facilitates allergic responses and inflammation
___basophil	B. stains with acidic dye eosin, has histamine and major basic protein in granules, and facilitates responses to protozoa and helminths

___macrophage	C. recognizes abnormal cells, binds to them, and releases perforin and granzyme molecules, which induce apoptosis
___eosinophil	D. large agranular phagocyte that resides in tissues such as the brain and lungs

Answer

C, A, D, B

Match each cellular defense with the infection it would most likely target.

___natural killer cell	A. virus-infected cell
___neutrophil	B. tapeworm in the intestines
___eosinophil	C. bacteria in a skin lesion

Answer

A, C, B

Fill in the Blank

Platelets are also called _____.

Answer

thrombocytes

The cell in the bone marrow that gives rise to all other blood cell types is the _____.

Answer

pluripotent hematopoietic stem cell (HSC)

PMNs are another name for _____.

Answer

neutrophils

Kupffer cells residing in the liver are a type of _____.

Answer

macrophage

_____ are similar to basophils, but reside in tissues rather than circulating in the blood.

Answer

mast cells

Short Answer

Explain the difference between plasma and the formed elements of the blood.

List three ways that a neutrophil can destroy an infectious bacterium.

Critical Thinking

Neutrophils can sometimes kill human cells along with pathogens when they release the toxic contents of their granules into the surrounding tissue. Likewise, natural killer cells target human cells for destruction. Explain why it is advantageous for the immune system to have cells that can kill human cells as well as pathogens.

Refer to [Figure 17.3.2](#). In a blood smear taken from a healthy patient, which type of leukocyte would you expect to observe in the highest numbers?

21.2: Pathogen Recognition and Phagocytosis

Phagocytes are cells that recognize pathogens and destroy them through phagocytosis. Recognition often takes place by the use of phagocyte receptors that bind molecules commonly found on pathogens, known as pathogen-associated molecular patterns (PAMPs). The receptors that bind PAMPs are called pattern recognition receptors, or PRRs. Toll-like receptors (TLRs) are one type of PRR found on phagocytes.

Multiple Choice

PAMPs would be found on the surface of which of the following?

- A. pathogen
- B. phagocyte
- C. skin cell
- D. blood vessel wall

Answer

A

_____ on phagocytes bind to PAMPs on bacteria, which triggers the uptake and destruction of the bacterial pathogens?

- A. PRRs
- B. AMPs
- C. PAMPs
- D. PMNs

Answer

A

Which of the following best characterizes the mode of pathogen recognition for opsonin-dependent phagocytosis?

- A. Opsonins produced by a pathogen attract phagocytes through chemotaxis.
- B. A PAMP on the pathogen's surface is recognized by a phagocyte's toll-like receptors.
- C. A pathogen is first coated with a molecule such as a complement protein, which allows it to be recognized by phagocytes.
- D. A pathogen is coated with a molecule such as a complement protein that immediately lyses the cell.

Answer

C

Fill in the Blank

_____, also known as diapedesis, refers to the exit from the bloodstream of neutrophils and other circulating leukocytes.

Answer

extravasation

Toll-like receptors are examples of _____.

Answer

pattern-recognition receptors (PRRs)

Short Answer

Briefly summarize the events leading up to and including the process of transendothelial migration.

21.3: Inflammation and Fever

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).

Multiple Choice

Which refers to swelling as a result of inflammation?

- A. erythema
- B. edema
- C. granuloma
- D. vasodilation

Answer

B

Which type of inflammation occurs at the site of an injury or infection?

- A. acute
- B. chronic
- C. endogenous
- D. exogenous

Answer

A

Fill in the Blank

A(n) _____ is a walled-off area of infected tissue that exhibits chronic inflammation.

Answer

granuloma

The _____ is the part of the body responsible for regulating body temperature.

Answer

hypothalamus

Heat and redness, or _____, occur when the small blood vessels in an inflamed area dilate (open up), bringing more blood much closer to the surface of the skin.

Answer

erythema

Short Answer

Differentiate exogenous and endogenous pyrogens, and provide an example of each.

Critical Thinking

If a gram-negative bacterial infection reaches the bloodstream, large quantities of LPS can be released into the blood, resulting in a syndrome called septic shock. Death due to septic shock is a real danger. The overwhelming immune and inflammatory responses that occur with septic shock can cause a perilous drop in blood pressure; intravascular blood clotting; development of thrombi and emboli that block blood vessels, leading to tissue death; failure of multiple organs; and death of the patient. Identify and characterize two to three therapies that might be useful in stopping the dangerous events and outcomes of septic shock once it has begun, given what you have learned about inflammation and innate immunity in this chapter.

In Lubeck, Germany, in 1930, a group of 251 infants was accidentally administered a tainted vaccine for tuberculosis that contained live *Mycobacterium tuberculosis*. This vaccine was administered orally, directly exposing the infants to the deadly bacterium. Many of these infants contracted tuberculosis, and some died. However, 44 of the infants never contracted tuberculosis. Based on your knowledge of the innate immune system, what innate defenses might have inhibited *M. tuberculosis* enough to prevent these infants from contracting the disease?

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22: ADAPTIVE IMMUNITY I

People living in developed nations and born in the 1960s or later may have difficulty understanding the once heavy burden of devastating infectious diseases. For example, smallpox, a deadly viral disease, once destroyed entire civilizations but has since been eradicated. Thanks to the vaccination efforts by multiple groups, including the World Health Organization, Rotary International, and the United Nations Children's Fund (UNICEF), smallpox has not been diagnosed in a patient since 1977. Polio is another excellent example. This crippling viral disease paralyzed patients, who were often kept alive in "iron lung wards" as recently as the 1950s (Figure 22.1). Today, vaccination against polio has nearly eradicated the disease. Vaccines have also reduced the prevalence of once-common infectious diseases such as chickenpox, German measles, measles, mumps, and whooping cough. The success of these and other vaccines is due to the very specific and adaptive host defenses that are the focus of this chapter.

[Innate Nonspecific Host Defenses](#) described innate immunity against microbial pathogens. Higher animals, such as humans, also possess an adaptive immune defense, which is highly specific for individual microbial pathogens. This specific adaptive immunity is acquired through active infection or vaccination and serves as an important defense against pathogens that evade the defenses of innate immunity.

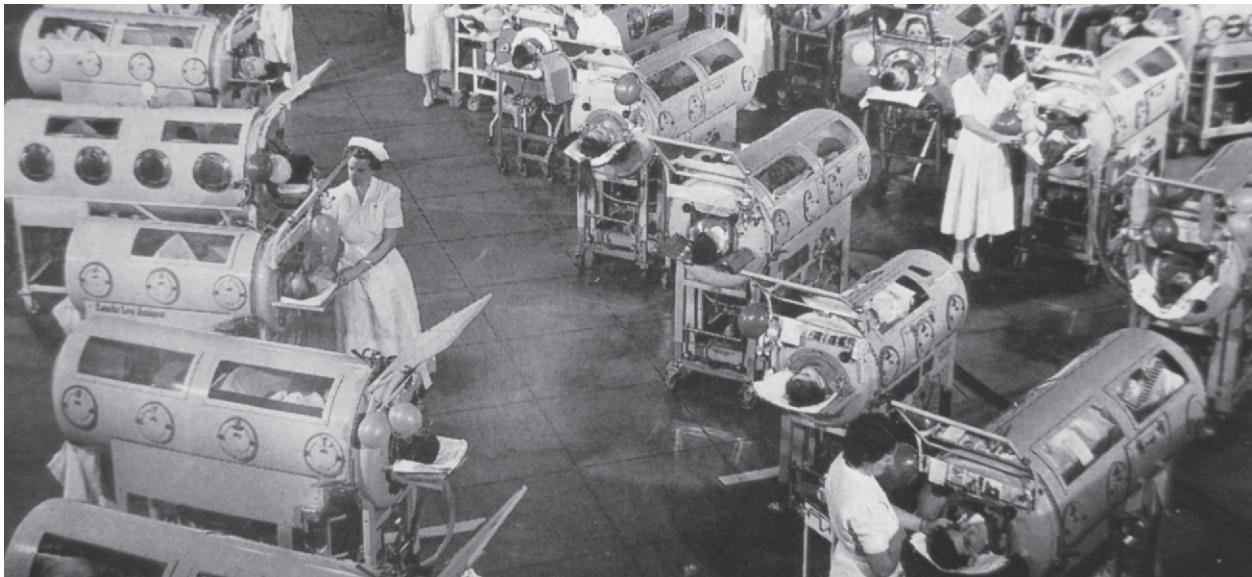


Figure 22.1: Polio was once a common disease with potentially serious consequences, including paralysis. Vaccination has all but eliminated the disease from most countries around the world. An iron-lung ward, such as the one shown in this 1953 photograph, housed patients paralyzed from polio and unable to breathe for themselves.

[22.1: Adaptive Immune Defenses - Overview](#)

[22.2: Antigens](#)

[22.3: Antibodies](#)

[22.4: Antigen-Antibody Interactions](#)

[22.5: Major Histocompatibility Complexes \(MHC\)](#)

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[22.E: Adaptive Immunity I \(Exercises\)](#)

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22.1: Adaptive Immune Defenses - Overview

Learning Objectives

- List the defining characteristics of adaptive immunity
- Explain the difference between a primary and a secondary immune response
- Distinguish between humoral and cellular immunity

Adaptive Immunity

Adaptive immunity is defined by two important characteristics: **specificity** and **memory**. Specificity refers to the adaptive immune system's ability to target specific pathogens, and memory refers to its ability to quickly respond to pathogens to which it has previously been exposed. For example, when an individual recovers from chickenpox, the body develops a *memory* of the infection that will *specifically* protect it from the causative agent, the varicella-zoster virus, if it is exposed to the virus again later.

Specificity and memory are achieved by essentially programming certain cells involved in the immune response to respond rapidly to subsequent exposures of the pathogen. This programming occurs as a result of the first exposure to a pathogen or vaccine, which triggers a **primary response**. Subsequent exposures result in a **secondary response** that is faster and stronger as a result of the body's memory of the first exposure (Figure 22.1.1). This secondary response, however, is specific to the pathogen in question. For example, exposure to one virus (e.g., varicella-zoster virus) will not provide protection against other viral diseases (e.g., measles, mumps, or polio).

Adaptive specific immunity involves the actions of two distinct cell types: **B lymphocytes (B cells)** and **T lymphocytes (T cells)**. Although B cells and T cells arise from a common hematopoietic stem cell differentiation pathway, their sites of maturation and their roles in adaptive immunity are very different.

B cells mature in the **bone marrow** and are responsible for the production of glycoproteins called **antibodies**, or **immunoglobulins**. Antibodies are involved in the body's defense against pathogens and toxins in the extracellular environment. Mechanisms of adaptive specific immunity that involve B cells and antibody production are referred to as **humoral immunity**. The maturation of **T cells** occurs in the **thymus**. T cells function as the central orchestrator of both innate and adaptive immune responses. They are also responsible for destruction of cells infected with intracellular pathogens. The targeting and destruction of intracellular pathogens by T cells is called **cell-mediated immunity**, or **cellular immunity**.

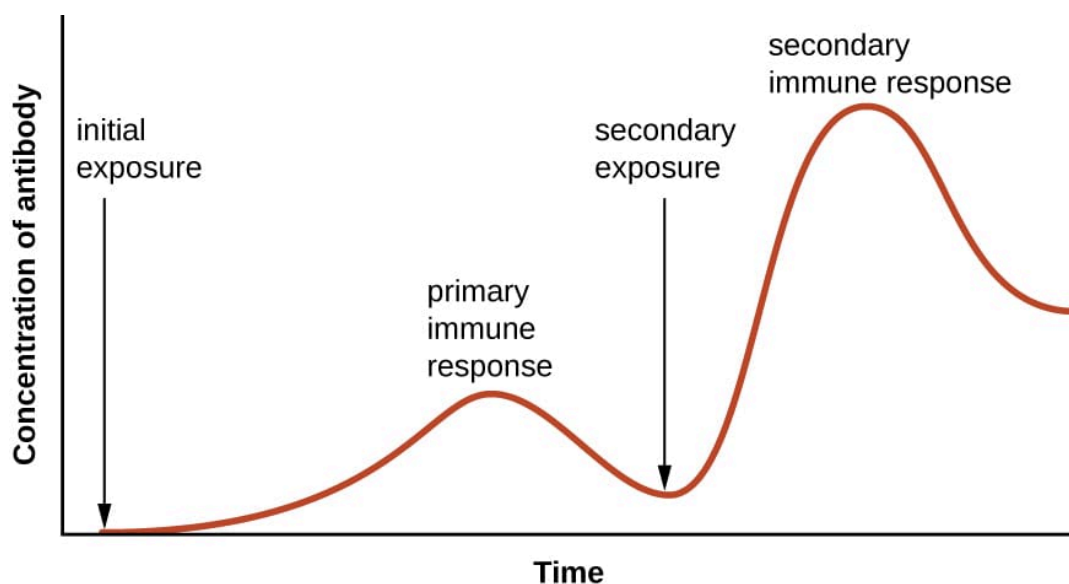


Figure 22.1.1: This graph illustrates the primary and secondary immune responses related to antibody production after an initial and secondary exposure to an antigen. Notice that the secondary response is faster and provides a much higher concentration of antibody.

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Interactive Element

Case Study Preview: “The Light Hurts: A Meningitis Close Call”

One-year-old Olivia arrives at the ER with a high-pitched cry, light sensitivity, neck pain, and swollen lymph nodes - worrying signs of a serious infection. When her CSF turns out cloudy, with low glucose and sky-high white cell counts, doctors act fast. Before test results are even finalized, they begin life-saving treatment for suspected bacterial meningitis. Days later, lab tests confirm *Neisseria meningitidis*, a fast-acting pathogen that can overwhelm young immune systems lacking memory cells or maternal antibodies.

In this case, you'll explore the roles of innate and adaptive immunity, analyze CSF and blood findings, and understand how age, vaccination schedules, and bacterial virulence factors like the **polysaccharide capsule** shape disease outcomes.

When every hour matters, what signs point to meningitis - and what kind of immunity could've stopped it?

[Chapter 18 Case Study - The Light Hurts: A Meningitis Close Call](#)

Key Concepts and Summary

- **Adaptive immunity** is an acquired defense against foreign pathogens that is characterized by **specificity** and **memory**. The first exposure to an antigen stimulates a **primary response**, and subsequent exposures stimulate a faster and strong **secondary response**.
- Adaptive immunity is a dual system involving **humoral immunity** (antibodies produced by B cells) and **cellular immunity** (T cells directed against intracellular pathogens).

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22.2: Antigens

Learning Objectives

- Compare an antigen with an epitope
- Identify factors that affect an antigen's antigenic potential
- Describe haptens and why they are not antigenic

Antigens

Antigen Definition

Activation of the adaptive immune defenses is triggered by pathogen-specific molecular structures called **antigens**. Antigens are similar to the pathogen-associated molecular patterns (PAMPs) discussed in [Pathogen Recognition and Phagocytosis](#); however, whereas PAMPs are molecular structures found on numerous pathogens, antigens are unique to a specific pathogen. The antigens that stimulate adaptive immunity to chickenpox, for example, are unique to the varicella-zoster virus but significantly different from the antigens associated with other viral pathogens.

The term *antigen* was initially used to describe molecules that stimulate the production of antibodies; in fact, the term comes from a combination of the words antibody and geneerator, and a molecule that stimulates antibody production is said to be **antigenic**. However, the role of antigens is not limited to humoral immunity and the production of antibodies; antigens also play an essential role in stimulating cellular immunity, and for this reason antigens are sometimes more accurately referred to as **immunogens**. In this text, however, we will typically refer to them as antigens.

Pathogens possess a variety of structures that may contain antigens. For example, antigens from bacterial cells may be associated with their capsules, cell walls, fimbriae, flagella, or pili. Bacterial antigens may also be associated with extracellular toxins and enzymes that they secrete. Viruses possess a variety of antigens associated with their capsids, envelopes, and the spike structures they use for attachment to cells.

Antigen Classes

Antigens may belong to any number of molecular classes, including carbohydrates, lipids, nucleic acids, proteins, and combinations of these molecules. Antigens of different classes vary in their ability to stimulate adaptive immune defenses as well as in the type of response they stimulate (humoral or cellular). The **structural complexity** of an antigenic molecule is an important factor in its antigenic potential. In general, more complex molecules are more effective as antigens. For example, the three-dimensional complex structure of **proteins** make them the most effective and potent antigens, capable of stimulating both humoral and cellular immunity. In comparison, **carbohydrates** are less complex in structure and therefore less effective as antigens; they can only stimulate humoral immune defenses. **Lipids and nucleic acids** are the least antigenic molecules, and in some cases may only become antigenic when combined with proteins or carbohydrates to form glycolipids, lipoproteins, or nucleoproteins.

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One reason the three-dimensional complexity of antigens is so important is that antibodies and T cells do not recognize and

interact with an entire antigen but with smaller exposed regions on the surface of antigens called **epitopes**. A single antigen may possess several different epitopes (Figure 22.2.2), and different antibodies may bind to different epitopes on the same antigen (Figure 22.2.3). For example, the bacterial flagellum is a large, complex protein structure that can possess hundreds or even thousands of epitopes with unique three-dimensional structures. Moreover, flagella from different bacterial species (or even strains of the same species) contain unique epitopes that can only be bound by specific antibodies.

An antigen's **size** is another important factor in its antigenic potential. Whereas large antigenic structures like flagella possess multiple epitopes, some molecules are too small to be antigenic by themselves. Such molecules, called haptens, are essentially free epitopes that are not part of the complex three-dimensional structure of a larger antigen. For a hapten to become antigenic, it must first attach to a larger carrier molecule (usually a protein) to produce a conjugate antigen. The hapten-specific antibodies produced in response to the conjugate antigen are then able to interact with unconjugated free hapten molecules. Haptens are not known to be associated with any specific pathogens, but they are responsible for some allergic responses. For example, the hapten urushiol, a molecule found in the oil of plants that cause poison ivy, causes an immune response that can result in a severe rash (called contact dermatitis). Similarly, the hapten penicillin can cause allergic reactions to drugs in the penicillin class.

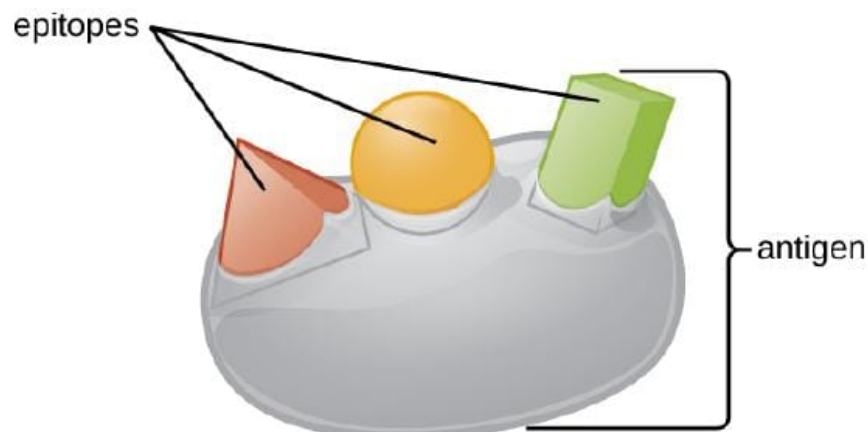


Figure 22.2.2: An antigen is a macromolecule that reacts with components of the immune system. A given antigen may contain several motifs that are recognized by immune cells.

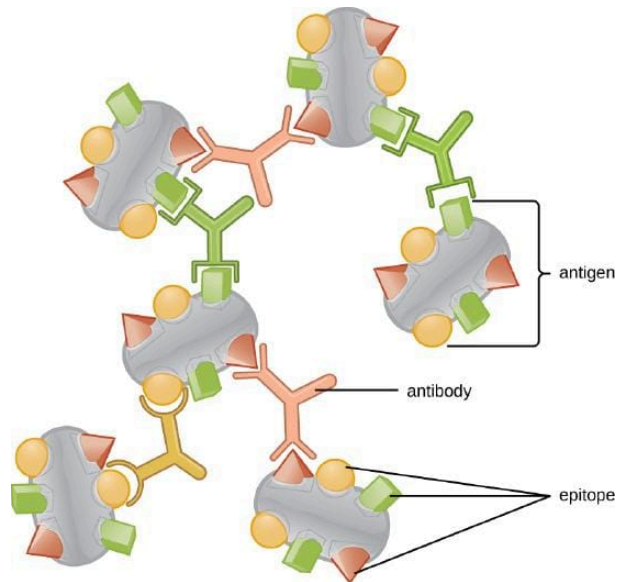


Figure 22.2.3: A typical protein antigen has multiple epitopes, shown by the ability of three different antibodies to bind to different epitopes of the same antigen.

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Key Concepts and Summary

- **Antigens**, also called **immunogens**, are molecules that activate adaptive immunity. A single antigen possesses smaller **epitopes**, each capable of inducing a specific adaptive immune response.
- An antigen's ability to stimulate an immune response depends on several factors, including its molecular class, molecular complexity, and size.

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22.3: Antibodies

Learning Objectives

- Describe the structure and function of antibodies (immunoglobulins)
- Distinguish the functions of the Fab region and the Fc region
- Identify the five classes of antibodies
- Compare and contrast IgM and IgG in terms of their structure, function and abundance

Antibodies (Immunoglobulins)

Antibodies (also called **immunoglobulins**) are glycoproteins that are present in both the blood and tissue fluids. The basic structure of an antibody monomer consists of four protein chains held together by disulfide bonds (Figure 22.3.4). A disulfide bond is a covalent bond between the sulfhydryl *R* groups found on two cysteine amino acids. The two largest chains are identical to each other and are called the **heavy chains**. The two smaller chains are also identical to each other and are called the **light chains**. Joined together, the heavy and light chains form a basic Y-shaped structure.

The two 'arms' of the Y-shaped antibody molecule are known as the **Fab region**, for "fragment of antigen binding." The far end of the Fab region is the **variable region**, which serves as the site of antigen binding. The amino acid sequence in the variable region dictates the three-dimensional structure, and thus the specific three-dimensional epitope to which the Fab region is capable of binding. Although the epitope specificity of the Fab regions is identical for each arm of a single antibody molecule, this region displays a high degree of variability between antibodies with different epitope specificities. Binding to the Fab region is necessary for neutralization of pathogens, agglutination or aggregation of pathogens, and antibody-dependent cell-mediated cytotoxicity.

The **constant region** of the antibody molecule includes the trunk of the Y and lower portion of each arm of the Y. The trunk of the Y is also called the **Fc region**, for "fragment of crystallization," and is the site of complement factor binding and binding to phagocytic cells during antibody-mediated opsonization.

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Figure 22.3.5: The five Immunoglobulin classes

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22.4: Antigen-Antibody Interactions

Learning Objectives

- Summarize the functions of antibodies
- Compare the mechanisms of opsonization and antibody-dependent cell-mediated cytotoxicity
- Relate the structure and location of antibodies to their functions

Antigen-Antibody Interactions

Different classes of antibody play important roles in the body's defense against pathogens. These functions include neutralization of pathogens, opsonization for phagocytosis, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity. For most of these functions, antibodies also provide an important link between adaptive specific immunity and innate nonspecific immunity.

Neutralization

Neutralization involves the binding of certain antibodies (IgG, IgM, or IgA) to epitopes on the surface of pathogens or toxins, preventing their attachment to cells. For example, Secretory IgA can bind to specific pathogens and block initial attachment to intestinal mucosal cells. Similarly, specific antibodies can bind to certain toxins, blocking them from attaching to target cells and thus neutralizing their toxic effects. Viruses can be neutralized and prevented from infecting a cell by the same mechanism (Figure 22.4.6).

Opsonization

As described in [Chemical Defenses](#), **opsonization** is the coating of a pathogen with molecules, such as complement factors, C-reactive protein, and serum amyloid A, to assist in phagocyte binding to facilitate phagocytosis. IgG antibodies also serve as excellent opsonins, binding their Fab sites to specific epitopes on the surface of pathogens. Phagocytic cells such as macrophages, dendritic cells, and neutrophils have receptors on their surfaces that recognize and bind to the Fc portion of the IgG molecules; thus, IgG helps such phagocytes attach to and engulf the pathogens they have bound (Figure 22.4.7).

Agglutination

Agglutination or **aggregation** involves the cross-linking of pathogens by antibodies to create large aggregates (Figure 22.4.8). IgG has two Fab antigen-binding sites, which can bind to two separate pathogen cells, clumping them together. When multiple IgG antibodies are involved, large aggregates can develop; these aggregates are easier for the kidneys and spleen to filter from the blood and easier for phagocytes to ingest for destruction. The pentameric structure of IgM provides ten Fab binding sites per molecule, making it the most efficient antibody for agglutination.

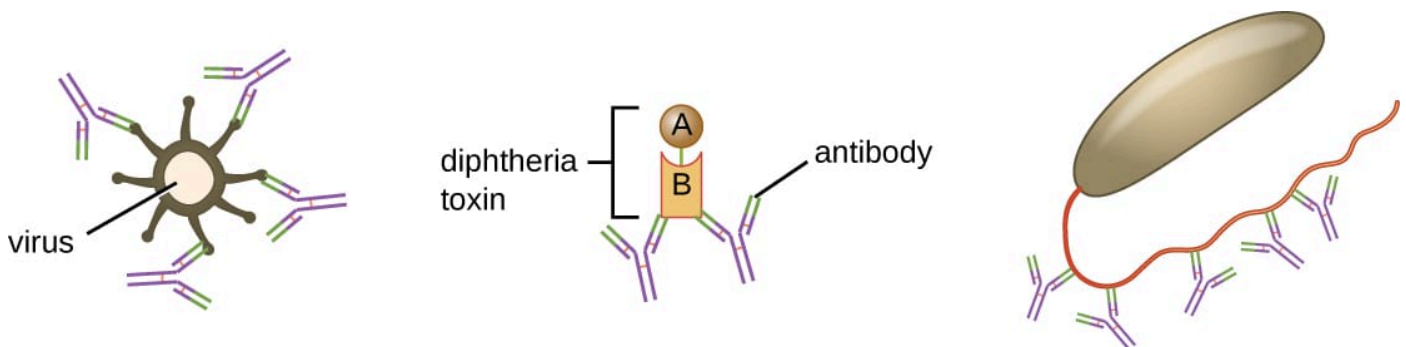


Figure 22.4.6: Neutralization involves the binding of specific antibodies to antigens found on bacteria, viruses, and toxins, preventing them from attaching to target cells.

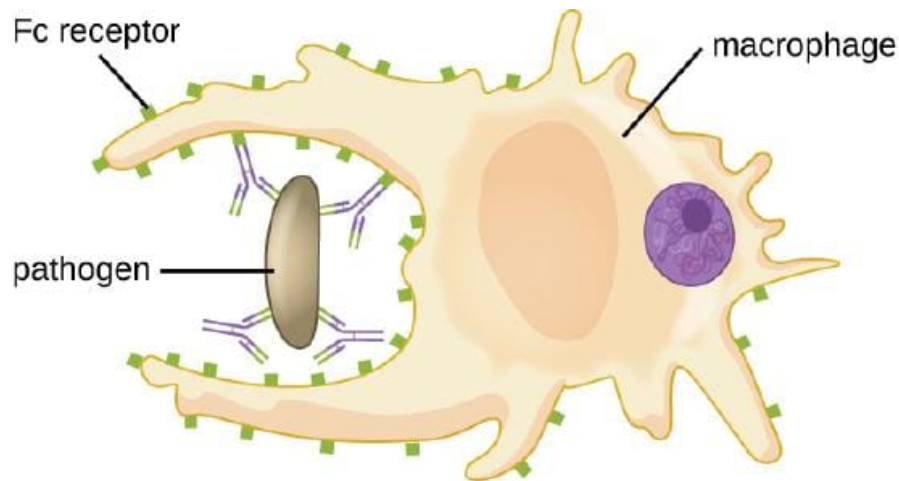


Figure 22.4.7: Antibodies serve as opsonins and inhibit infection by tagging pathogens for destruction by macrophages, dendritic cells, and neutrophils. These phagocytic cells use Fc receptors to bind to IgG-opsonized pathogens and initiate the first step of attachment before phagocytosis.

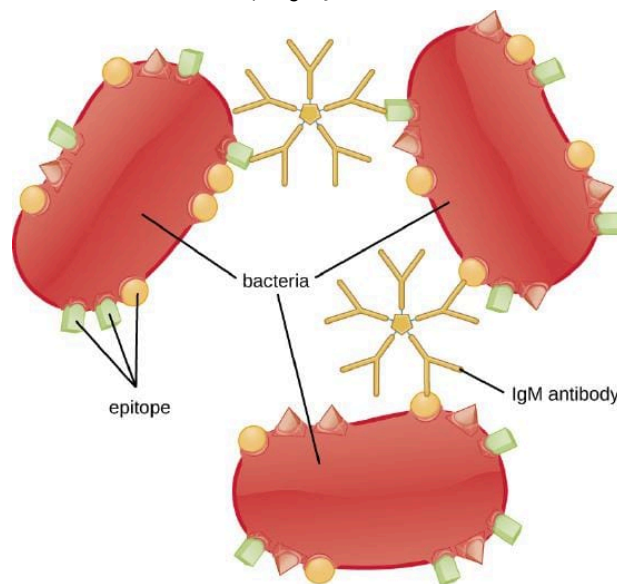


Figure 22.4.8: Antibodies, especially IgM antibodies, agglutinate bacteria by binding to epitopes on two or more bacteria simultaneously. When multiple pathogens and antibodies are present, aggregates form when the binding sites of antibodies bind with separate pathogens.

Activation of the Complement System

Another important function of antibodies is **activation of the complement cascade**. As discussed in the previous chapter, the complement system is an important component of the innate defenses, promoting the inflammatory response, recruiting phagocytes to site of infection, enhancing phagocytosis by opsonization, and killing gram-negative bacterial pathogens with the membrane attack complex (MAC). Complement activation can occur through three different pathways (see [Figure 17.2.2](#)), but the most efficient is the classical pathway, which requires the initial binding of IgG or IgM antibodies to the surface of a pathogen cell, allowing for recruitment and activation of the C1 complex.

Antibody-Dependent Cell-mediated Cytotoxicity (ADCC)

Yet another important function of antibodies is **antibody-dependent cell-mediated cytotoxicity (ADCC)**, which enhances killing of pathogens that are too large to be phagocytosed. This process is best characterized for natural killer cells (NK cells), as shown in [Figure 22.4.9](#), but it can also involve macrophages and eosinophils. ADCC occurs when the Fab region of an IgG antibody binds to a large pathogen; Fc receptors on effector cells (e.g., NK cells) then bind to the Fc region of the antibody, bringing them into close proximity with the target pathogen. The effector cell then secretes powerful cytotoxins (e.g., perforin and granzymes) that kill the pathogen.

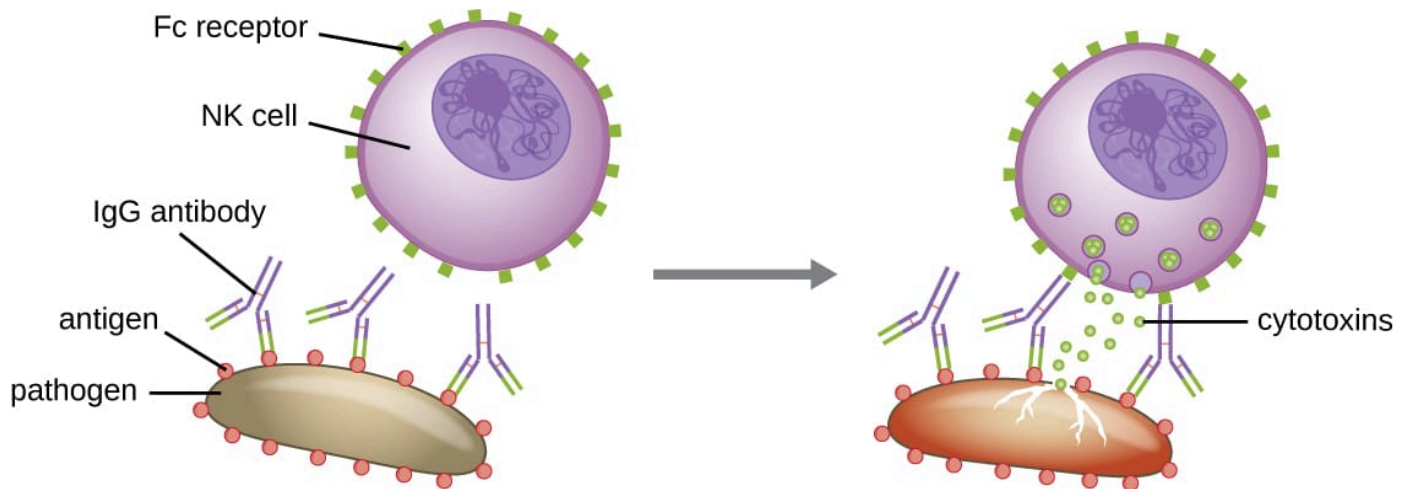


Figure 22.4.9: In this example of ADCC, antibodies bind to a large pathogenic cell that is too big for phagocytosis and then bind to Fc receptors on the membrane of a natural killer cell. This interaction brings the NK cell into close proximity, where it can kill the pathogen through release of lethal extracellular cytotoxins.

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Key Concepts and Summary

- The five classes of antibody are **IgM**, **IgG**, **IgA**, **IgE**, and **IgD**, each differing in size, arrangement, location within the body, and function. The five primary functions of antibodies are neutralization, opsonization, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity (ADCC).

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22.5: Major Histocompatibility Complexes (MHC)

Learning Objectives

- Identify cells that express MHC I and/or MHC II molecules
- Describe the structures and cellular location of MHC I and MHC II molecules

As discussed in [Cellular Defenses](#), major histocompatibility complex (MHC) molecules are expressed on the surface of healthy cells, identifying them as normal and “self” to natural killer (NK) cells. MHC molecules also play an important role in the presentation of foreign antigens, which is a critical step in the activation of T cells and thus an important mechanism of the adaptive immune system.

Major Histocompatibility Complex Molecules

The **major histocompatibility complex (MHC)** is a collection of genes coding for MHC molecules found on the surface of all nucleated cells of the body. In humans, the MHC genes are also referred to as **human leukocyte antigen (HLA)** genes. Mature red blood cells, which lack a nucleus, are the only cells that do not express MHC molecules on their surface.

There are two classes of MHC molecules involved in adaptive immunity, MHC I and MHC II (Figure 22.5.1). **MHC I** molecules are found on **all nucleated cells**; they present normal self-antigens as well as abnormal or nonself pathogens to the effector T cells involved in cellular immunity. In contrast, **MHC II** molecules are only found on **macrophages, dendritic cells, and B cells**; they present abnormal or nonself pathogen antigens for the initial activation of T cells.

Both types of MHC molecules are transmembrane glycoproteins that assemble as dimers in the cytoplasmic membrane of cells, but their structures are quite different. MHC I molecules are composed of a longer α protein chain coupled with a smaller β_2 microglobulin protein, and only the α chain spans the cytoplasmic membrane. The α chain of the MHC I molecule folds into three separate domains: α_1 , α_2 and α_3 . MHC II molecules are composed of two protein chains (an α and a β chain) that are approximately similar in length. Both chains of the MHC II molecule possess portions that span the plasma membrane, and each chain folds into two separate domains: α_1 and α_2 , and β_1 , and β_2 . In order to present abnormal or non-self-antigens to T cells, MHC molecules have a cleft that serves as the antigen-binding site near the “top” (or outermost) portion of the MHC-I or MHC-II dimer. For MHC I, the antigen-binding cleft is formed by the α_1 and α_2 domains, whereas for MHC II, the cleft is formed by the α_1 and β_1 domains (Figure 22.5.1).

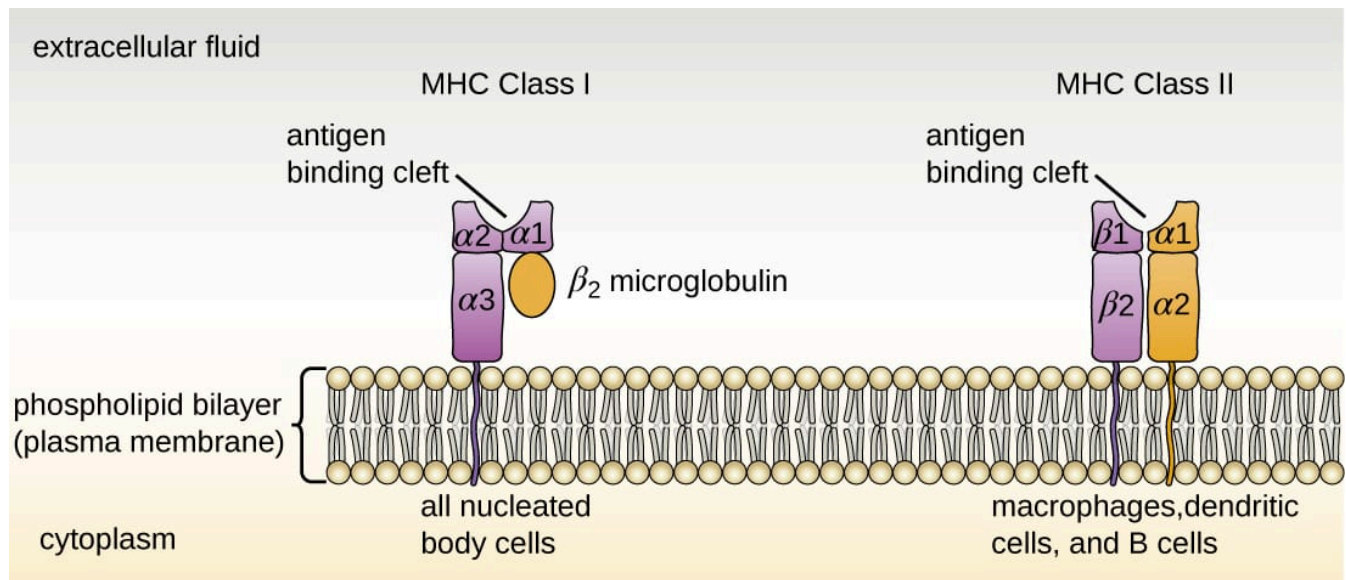


Figure 22.5.1: MHC I are found on all nucleated body cells, and MHC II are found on macrophages, dendritic cells, and B cells (along with MHC I). The antigen-binding cleft of MHC I is formed by domains α_1 and α_2 . The antigen-binding cleft of MHC II is formed by domains α_1 and β_1 .

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Key Concepts and Summary

- **Major histocompatibility complex (MHC)** is a collection of genes coding for glycoprotein molecules expressed on the surface of all nucleated cells.
- **MHC I** molecules are expressed on all nucleated cells and are essential for presentation of normal “self” antigens. Cells that become infected by intracellular pathogens can present foreign antigens on MHC I as well, marking the infected cell for destruction.
- **MHC II** molecules are expressed only on the surface of **antigen-presenting cells** (macrophages, dendritic cells, and B cells). Antigen presentation with MHC II is essential for the activation of T cells.

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22.6: Antigen Presenting Cells (APC)

Learning Objectives

- Identify the cells that are antigen-presenting cells (APCs)
- Describe the process of antigen processing and presentation with MHC I and MHC II
- Explain the role of antigen-presentation in adaptive immunity

Antigen-Presenting Cells (APCs)

All nucleated cells in the body have mechanisms for processing and presenting antigens in association with MHC molecules. This signals the immune system, indicating whether the cell is normal and healthy or infected with an intracellular pathogen. However, only macrophages, dendritic cells, and B cells have the ability to present antigens specifically for the purpose of activating T cells; for this reason, these types of cells are sometimes referred to as **antigen-presenting cells (APCs)**.

While all APCs play a similar role in adaptive immunity, there are some important differences to consider. Macrophages and dendritic cells are phagocytes that ingest and kill pathogens that penetrate the first-line barriers (i.e., skin and mucous membranes). B cells, on the other hand, do not function as phagocytes but play a primary role in the production and secretion of antibodies. In addition, whereas macrophages and dendritic cells recognize pathogens through nonspecific receptor interactions (e.g., PAMPs, toll-like receptors, and receptors for opsonizing complement or antibody), B cells interact with foreign pathogens or their free antigens using antigen-specific immunoglobulin as receptors (monomeric IgD and IgM). When the immunoglobulin receptors bind to an antigen, the B cell internalizes the antigen by endocytosis before processing and presenting the antigen to T cells.

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Antigen Presentation with MHC II Molecules

MHC II molecules are only found on the surface of APCs. Macrophages and dendritic cells use similar mechanisms for processing and presentation of antigens and their epitopes in association with MHC II; B cells use somewhat different mechanisms that will be described further in [B Lymphocytes and Humoral Immunity](#). For now, we will focus on the steps of the process as they pertain to dendritic cells.

After a dendritic cell recognizes and attaches to a pathogen cell, the pathogen is internalized by phagocytosis and is initially contained within a phagosome. Lysosomes containing antimicrobial enzymes and chemicals fuse with the phagosome to create a phagolysosome, where degradation of the pathogen for antigen processing begins. Proteases (protein-degrading) are especially important in antigen processing because only protein antigen epitopes are presented to T cells by MHC II (Figure 22.6.2).

APCs do not present all possible epitopes to T cells; only a selection of the most antigenic or immunodominant epitopes are presented. The mechanism by which epitopes are selected for processing and presentation by an APC is complicated and not well understood; however, once the most antigenic, immunodominant epitopes have been processed, they associate within the antigen-binding cleft of MHC II molecules and are translocated to the cell surface of the dendritic cell for presentation to T cells.

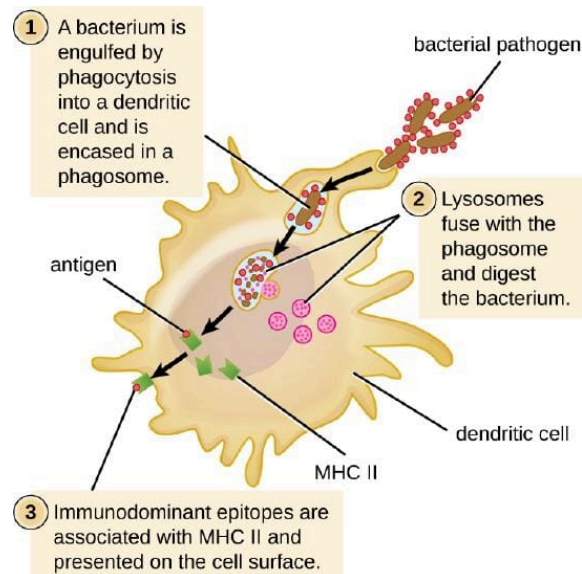


Figure 22.6.2: A dendritic cell phagocytoses a bacterial cell and brings it into a phagosome. Lysosomes fuse with the phagosome to create a phagolysosome, where antimicrobial chemicals and enzymes degrade the bacterial cell. Proteases process bacterial antigens, and the most antigenic epitopes are selected and presented on the cell's surface in conjunction with MHC II molecules. T cells recognize the presented antigens and are thus activated.

Antigen Presentation with MHC I Molecules

MHC I molecules, found on all normal, healthy, nucleated cells, signal to the immune system that the cell is a normal “self” cell. In a healthy cell, proteins normally found in the cytoplasm are degraded by proteasomes (enzyme complexes responsible for degradation and processing of proteins) and processed into self-antigen epitopes; these self-antigen epitopes bind within the MHC I antigen-binding cleft and are then presented on the cell surface. Immune cells, such as NK cells, recognize these self-antigens and do not target the cell for destruction. However, if a cell becomes infected with an intracellular pathogen (e.g., a virus), protein antigens specific to the pathogen are processed in the proteasomes and bind with MHC I molecules for presentation on the cell surface. This presentation of pathogen-specific antigens with MHC I signals that the infected cell must be targeted for destruction along with the pathogen.

Before elimination of infected cells can begin, APCs must first activate the T cells involved in cellular immunity. If an intracellular pathogen directly infects the cytoplasm of an APC, then the processing and presentation of antigens can occur as described (in proteasomes and on the cell surface with MHC I). However, if the intracellular pathogen does not directly infect APCs, an alternative strategy called **cross-presentation** is utilized. In cross-presentation, antigens are brought into the APC by mechanisms normally leading to presentation with MHC II (i.e., through phagocytosis), but the antigen is presented on an MHC I molecule for CD8 T cells. The exact mechanisms by which cross-presentation occur are not yet well understood, but it appears that cross-presentation is primarily a function of dendritic cells and not macrophages or B cells.

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Key Concepts and Summary

- **Antigen-presenting cells (APCs)** primarily ingest pathogens by phagocytosis, destroy them in the phagolysosomes, process the protein antigens, and select the most antigenic/immunodominant epitopes with MHC II for presentation to T

cells.

- **Cross-presentation** is a mechanism of antigen presentation and T-cell activation used by dendritic cells not directly infected by the pathogen; it involves phagocytosis of the pathogen but presentation on MHC I rather than MHC II.

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- [18.2: Antigens, Antigen Presenting Cells, and Major Histocompatibility Complexes](#) by OpenStax is licensed [CC BY 4.0](#). Original source: <https://openstax.org/details/books/microbiology>.

22.E: Adaptive Immunity I (Exercises)

22.1: Architecture of the Immune System

Adaptive immunity is defined by two important characteristics: specificity and memory. Specificity refers to the adaptive immune system's ability to target specific pathogens, and memory refers to its ability to quickly respond to pathogens to which it has previously been exposed. For example, when an individual recovers from chickenpox, the body develops a memory of the infection that will specifically protect it from the causative agent if it is exposed to the virus again later.

Multiple Choice

Antibodies are produced by _____.

- A. plasma cells
- B. T cells
- C. bone marrow
- D. B cells

Answer

A

Cellular adaptive immunity is carried out by _____.

- A. B cells
- B. T cells
- C. bone marrow
- D. neutrophils

Answer

B

A single antigen molecule may be composed of many individual _____.

- A. T-cell receptors
- B. B-cell receptors
- C. MHC II
- D. epitopes

Answer

D

Which class of molecules is the most antigenic?

- A. polysaccharides
- B. lipids
- C. proteins
- D. carbohydrates

Answer

C

Matching

Match the antibody class with its description.

___IgA	A. This class of antibody is the only one that can cross the placenta.
___IgD	B. This class of antibody is the first to appear after activation of B cells.
___IgE	C. This class of antibody is involved in the defense against parasitic infections and involved in allergic responses.
___IgG	D. This class of antibody is found in very large amounts in mucus secretions.
___IgM	E. This class of antibody is not secreted by B cells but is expressed on the surface of naïve B cells.

Answer

d, e, c, a, b

Fill in the Blank

There are two critically important aspects of adaptive immunity. The first is specificity, while the second is _____.

Answer

memory

_____ immunity involves the production of antibody molecules that bind to specific antigens.

Answer

Humoral

The heavy chains of an antibody molecule contain _____ region segments, which help to determine its class or isotype.

Answer

constant

The variable regions of the heavy and light chains form the _____ sites of an antibody.

Answer

antigen-binding

Short Answer

What is the difference between humoral and cellular adaptive immunity?

What is the difference between an antigen and a hapten?

Describe the mechanism of antibody-dependent cell-mediated cytotoxicity.

22.2: Antigens, Antigen Presenting Cells, and Major Histocompatibility Complexes

Major histocompatibility complex (MHC) molecules are expressed on the surface of healthy cells, identifying them as normal and “self” to natural killer (NK) cells. MHC molecules also play an important role in the presentation of foreign antigens, which is a critical step in the activation of T cells and thus an important mechanism of the adaptive immune system.

Multiple Choice

MHC I molecules present

- A. processed foreign antigens from proteasomes.
- B. processed self-antigens from phagolysosome.
- C. antibodies.
- D. T cell antigens.

Answer

A

MHC II molecules present

- A. processed self-antigens from proteasomes.
- B. processed foreign antigens from phagolysosomes.
- C. antibodies.
- D. T cell receptors.

Answer

B

Which type of antigen-presenting molecule is found on all nucleated cells?

- A. MHC II
- B. MHC I
- C. antibodies
- D. B-cell receptors

Answer

B

Which type of antigen-presenting molecule is found only on macrophages, dendritic cells, and B cells?

- A. MHC I
- B. MHC II
- C. T-cell receptors
- D. B-cell receptors

Answer

B

Fill in the Blank

MHC molecules are used for antigen _____ to T cells.

Answer

presentation

MHC II molecules are made up of two subunits (α and β) of approximately equal size, whereas MHC I molecules consist of a larger α subunit and a smaller subunit called _____.

Answer

β_2 microglobulin

Critical Thinking

Which mechanism of antigen presentation would be used to present antigens from a cell infected with a virus?

Which pathway of antigen presentation would be used to present antigens from an extracellular bacterial infection?

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23: ADAPTIVE IMMUNITY II

Adaptive immunity provides specific, long-lasting defense against pathogens through the coordinated actions of B cells and T cells. B cells recognize antigens directly with their B cell receptors (BCRs) and, with help from helper T cells (Th cells), become activated. Activated B cells differentiate into plasma cells that secrete antibodies to neutralize pathogens, enhance phagocytosis, or activate complement pathways. Some B cells become memory cells for faster responses upon re-exposure. T cells, on the other hand, recognize antigens presented by major histocompatibility complex (MHC) molecules. Helper T cells (CD4 $\diamond$) amplify immune responses by releasing cytokines, while cytotoxic T cells (CD8 $\diamond$) kill infected cells directly. Both T and B cells generate memory populations to ensure quicker, stronger responses during future infections.

Vaccines work by safely exposing the immune system to weakened, inactivated, or partial components of pathogens, prompting the development of memory B and T cells without causing disease. Types of vaccines include live attenuated, inactivated, subunit, conjugate, and mRNA vaccines. By training the adaptive immune system, vaccines provide effective, long-term protection against specific diseases, often preventing illness entirely or reducing its severity upon exposure.

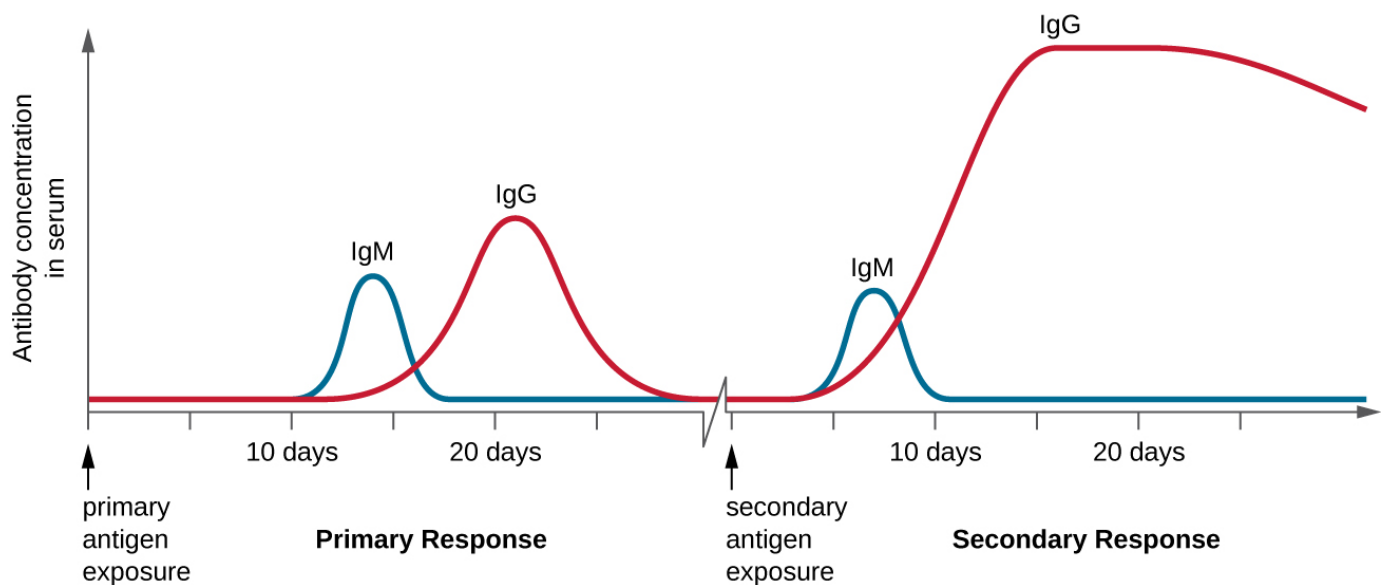


Figure 23.1: Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. The secondary response mostly involves IgG.

[23.1: T Lymphocytes - Overview](#)

[23.2: T-Cell Receptors \(TCR\) and Helper T Cells](#)

[23.3: Cytotoxic T Cells and Superantigens](#)

[23.4: B Lymphocytes and Antibodies](#)

[23.5: B Cell Activation](#)

[23.6: Vaccines](#)

[23.7: Variolation and Vaccination](#)

[23.8: Classes of Vaccines](#)

[23.E: Adaptive Immunity II \(Exercises\)](#)

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23.1: T Lymphocytes - Overview

Learning Objectives

- Describe the process of T-cell production and maturation
- Summarize the steps involved in thymic selection
- Compare and contrast central tolerance versus peripheral tolerance
- Summarize the unique functions of the three classes of T cells

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 23.1.1). 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.

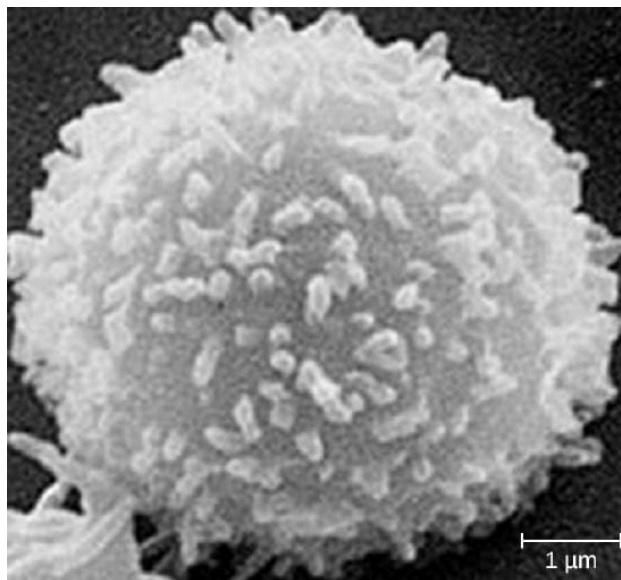


Figure 23.1.1: This scanning electron micrograph shows a T lymphocyte, which is responsible for the cell-mediated immune response. The spike-like membrane structures increase surface area, allowing for greater interaction with other cell types and their signals. (credit: modification of work by NCI)

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 23.1.2](#)), after which immature T lymphocytes enter the bloodstream and travel to the thymus for the final steps of maturation ([Figure 23.1.3](#)). Once in the thymus, the immature T lymphocytes are referred to as thymocytes.

The maturation of thymocytes within the thymus can be divided into three 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 23.1.3), where they await activation through the presentation of specific antigens by APCs. Until they are activated, they are known as **mature naïve T cells**.

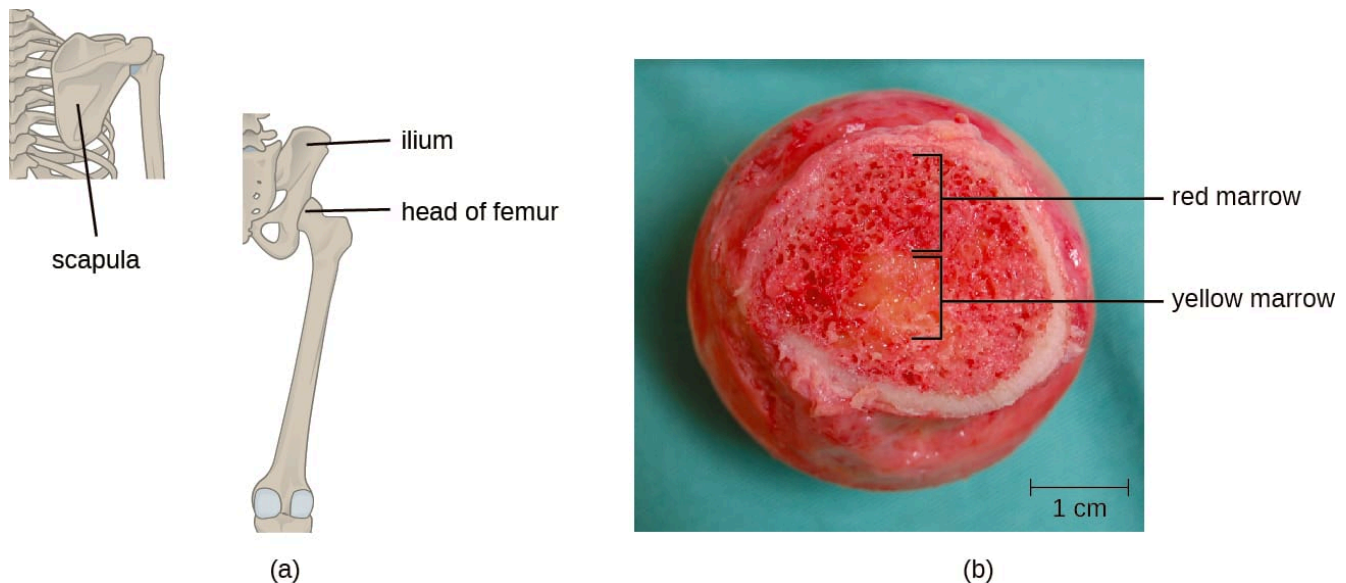


Figure 23.1.2: (a) Red bone marrow can be found in the head of the femur (thighbone) and is also present in the flat bones of the body, such as the ilium and the scapula. (b) Red bone marrow is the site of production and differentiation of many formed elements of blood, including erythrocytes, leukocytes, and platelets. The yellow bone marrow is populated primarily with adipose cells.

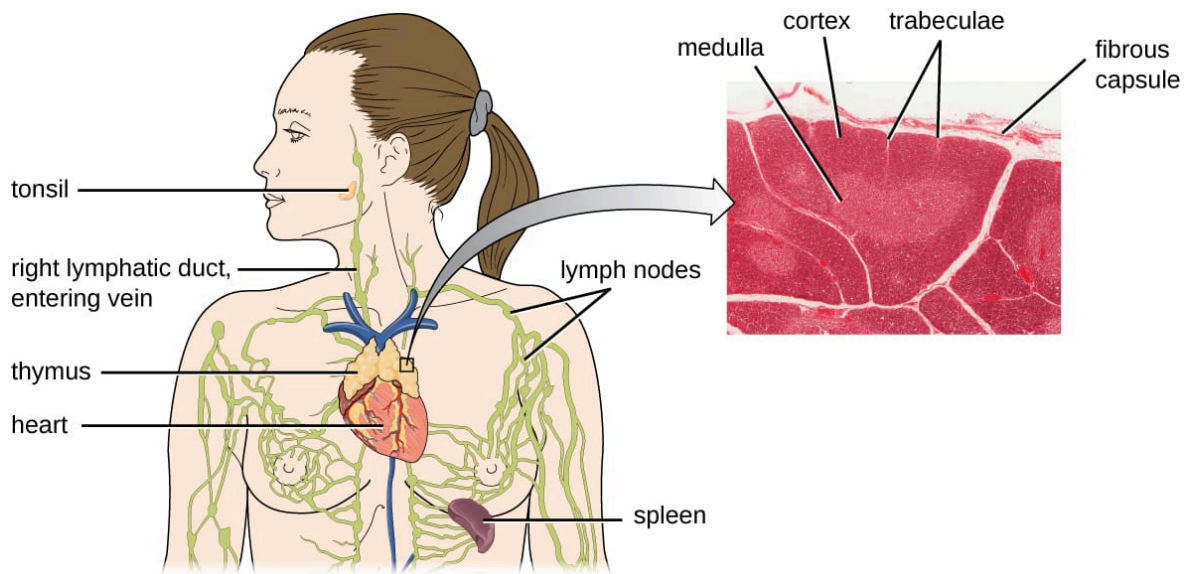


Figure 23.1.3: The thymus is a bi-lobed, H-shaped glandular organ that is located just above the heart. It is surrounded by a fibrous capsule of connective tissue. The darkly staining cortex and the lighter staining medulla of individual lobules are clearly visible in the light micrograph of the thymus of a newborn (top right, LM $\times 100$). (credit micrograph: modification of micrograph provided by the Regents of University of Michigan Medical School © 2012)

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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 23.1.1).

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, CD4 and 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 23.1.1: 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

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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.

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23.2: T-Cell Receptors (TCR) and Helper T Cells

Learning Objectives

- Explain the genetic events that lead to diversity of T-cell receptors
- Recognize how the genetic rearrangement (VDJ recombination) process can produce millions of unique TCR binding sites
- Compare and contrast the structures of TCR and antibodies / immunoglobulins (Ab / Ig)
- Describe the activation of helper T cells
- Identify the subtypes of helper T cells and their functions

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 region** and a **constant region**, and the variable region provides the antigen-binding site (Figure 23.2.4). 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 the J_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.

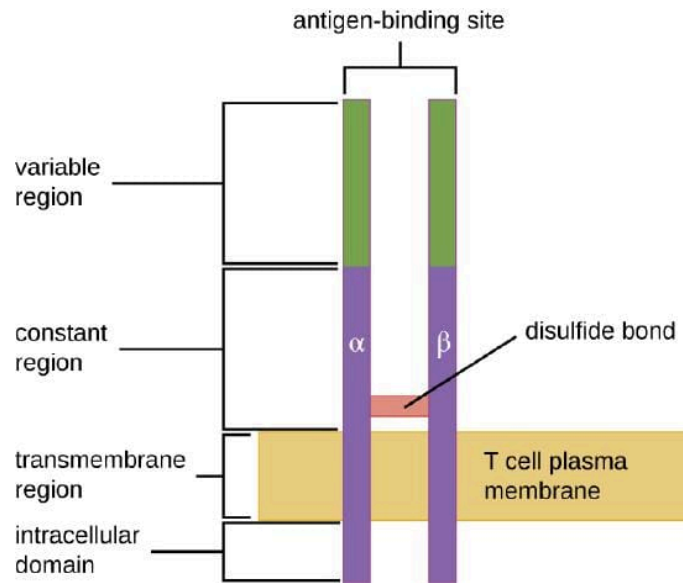


Figure 23.2.4: A T-cell receptor spans the cytoplasmic membrane and projects variable binding regions into the extracellular space to bind processed antigens associated with MHC I or MHC II molecules.

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Activation and Differentiation of Helper T Cells

Helper T Cell Activation

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.

Clonal Expansion and Clonal Differentiation

The activated helper T cell then proliferates, dividing by **mitosis** (a phenomenon called **clonal expansion**) to produce clonal naïve helper T cells. These naïve helper T cells then differentiate into subtypes with different functions (Figure 23.2.5).

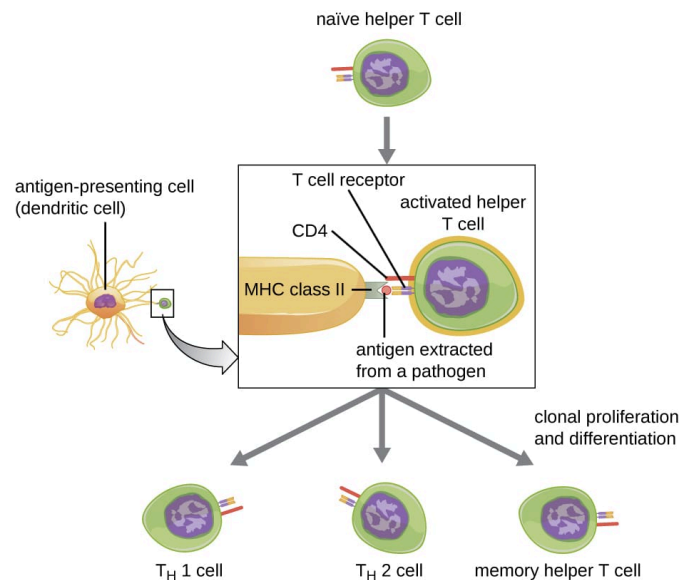


Figure 23.2.5: This illustration depicts the activation of a naïve (unactivated) helper T cell by an antigen-presenting cell and the subsequent proliferation and differentiation of the activated T cell into different subtypes.

Subtypes of Helper T Cells

Activated helper T cells can differentiate into one of **four distinct subtypes**, summarized in Table 23.2.2. 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 23.2.2: Subtypes of Helper T Cells

Subtype	Functions
T _H 1 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 _H 2 cells	Stimulate B cell activation and differentiation into plasma cells and memory B cells
	Direct antibody class switching in B cells

Subtype	Functions
T _H 17 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

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Key Concepts and Summary

- 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.

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23.3: Cytotoxic T Cells and Superantigens

Learning Objectives

- Compare and contrast the activation of helper T cells and cytotoxic T cells (CTLs)
- Describe the mechanism of CTL-mediated destruction of infected cells
- Define superantigens and give some examples

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 23.3.6). 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.

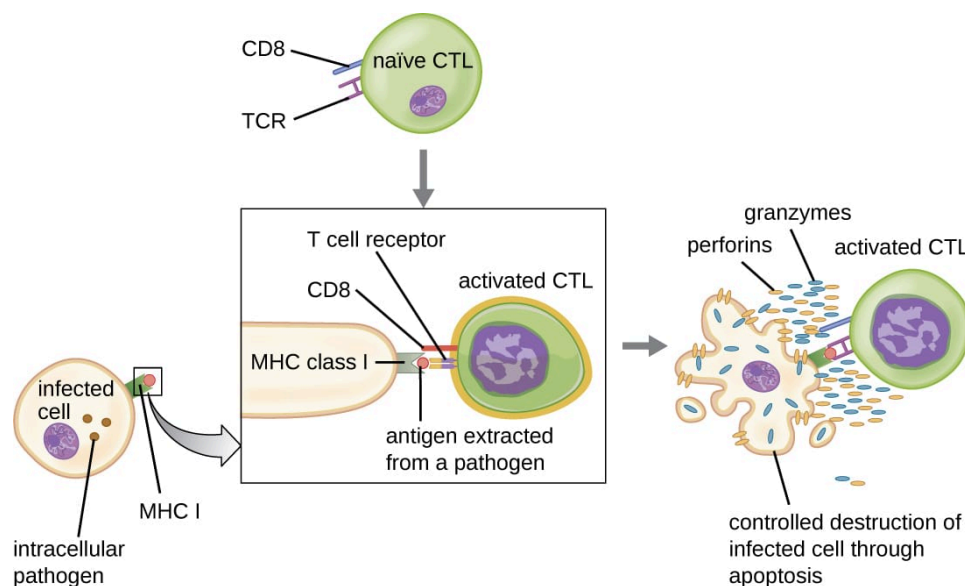


Figure 23.3.6: This figure illustrates the activation of a naïve (unactivated) cytotoxic T cell (CTL) by an antigen-presenting MHC I molecule on an infected body cell. Once activated, the CTL releases perforin and granzymes that invade the infected cell and induce controlled cell death, or apoptosis.

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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 23.3.7). 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.

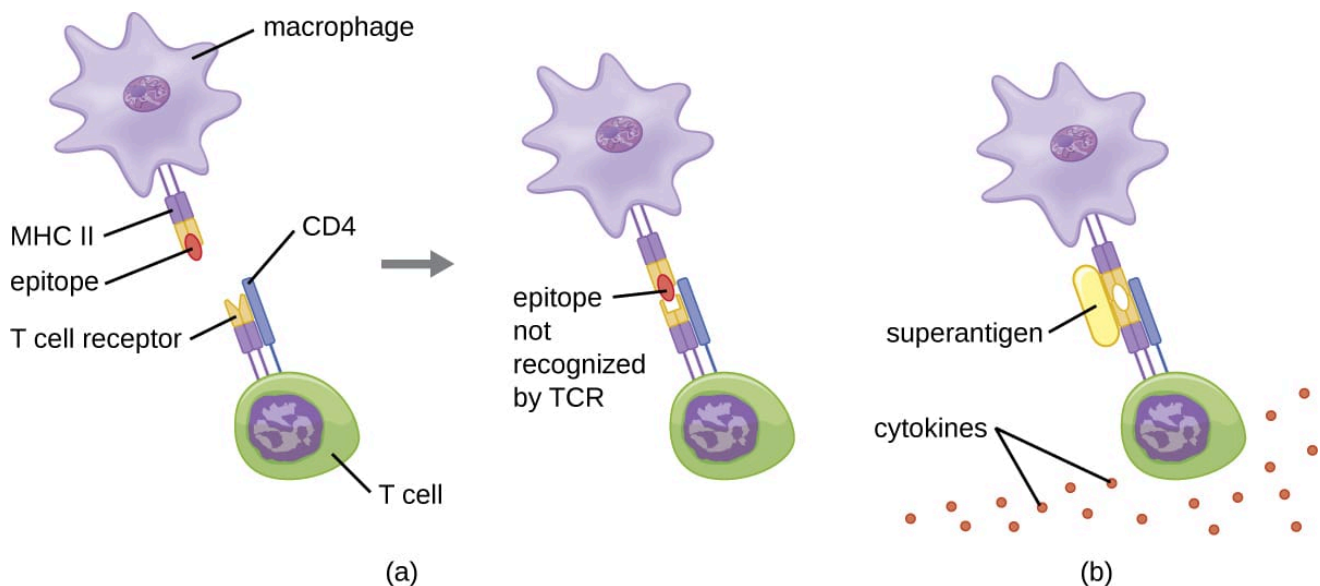


Figure 23.3.7: (a) The macrophage in this figure is presenting a foreign epitope that does not match the TCR of the T cell. Because the T cell does not recognize the epitope, it is not activated. (b) The macrophage in this figure is presenting a superantigen that is not recognized by the TCR of the T 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)

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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 23.3.6

In what way would antibiotic therapy help to combat a superantigen?

Key Concepts and Summary

- 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.

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23.4: 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 the maturation of B cells with the maturation of T cells

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 spleen for their final stages of maturation. There they become **naïve mature B cells**, i.e., mature B cells that have not yet been activated.

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B Cell Receptor (BCR)

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 23.4.1). 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 rearrangement of 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 epitopes displayed 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.

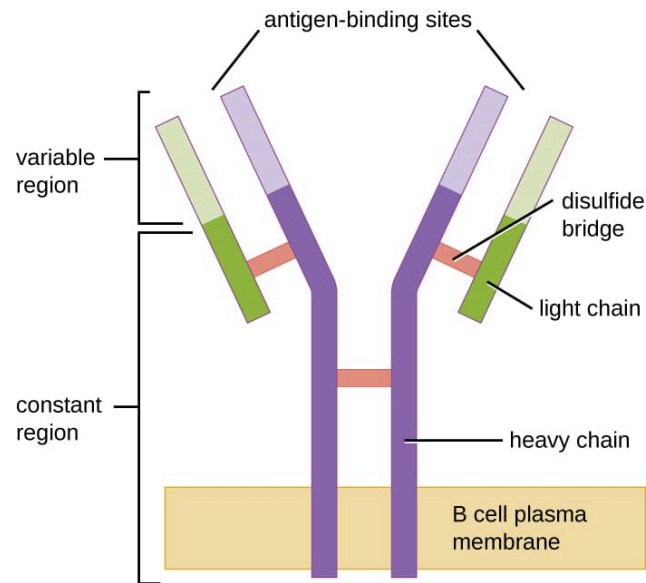


Figure 23.4.1: B-cell receptors are embedded in the membranes of B cells. The variable regions of all of the receptors on a single cell bind the same specific antigen.

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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**.

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23.5: B Cell Activation

Learning Objectives

- Compare T-dependent and T-independent activation of B cells
- Identify the two signals required for T-cell independent activation of B cells
- Describe class switching and its importance
- Compare the primary and secondary antibody responses

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-linkage of multiple BCRs, providing the first signal for activation (Figure 23.5.2). 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 (clonal expansion)** 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 23.5.2).

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.

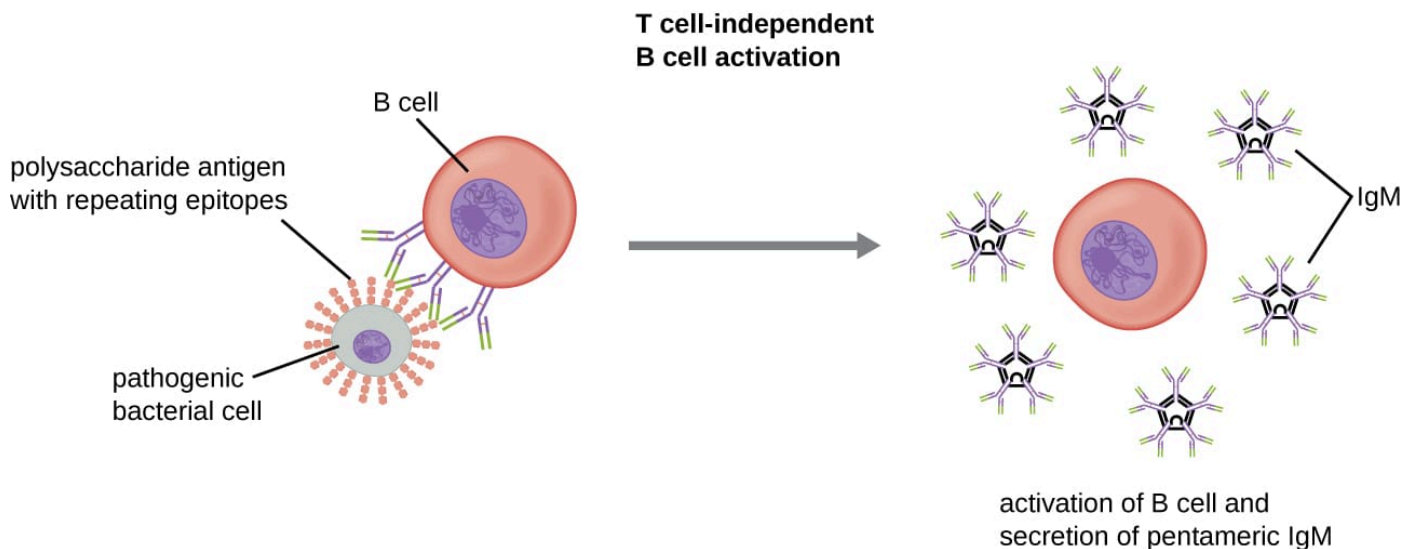


Figure 23.5.2: T-independent antigens have repeating epitopes that can induce B cell recognition and activation without involvement from T cells. A second signal, such as interaction of TLRs with PAMPs (not shown), is also required for activation of the B cell. Once activated, the B cell proliferates and differentiates into antibody-secreting plasma cells.

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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 23.5.3).

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 cells cloned 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.

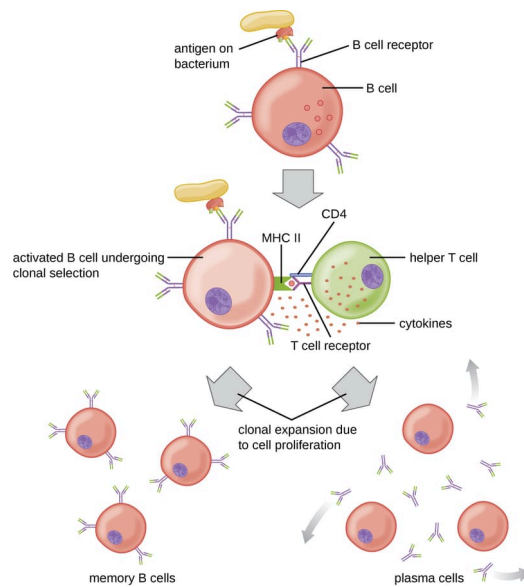


Figure 23.5.3: In T cell-dependent activation of B cells, the B cell recognizes and internalizes an antigen and presents it to a helper T cell that is specific to the same antigen. The helper T cell interacts with the antigen presented by the B cell, which activates the T cell and stimulates the release of cytokines that then activate the B cell. Activation of the B cell triggers proliferation and differentiation into B cells and plasma cells.

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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 response occurs. 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 23.5.4).

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 23.5.4). 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.

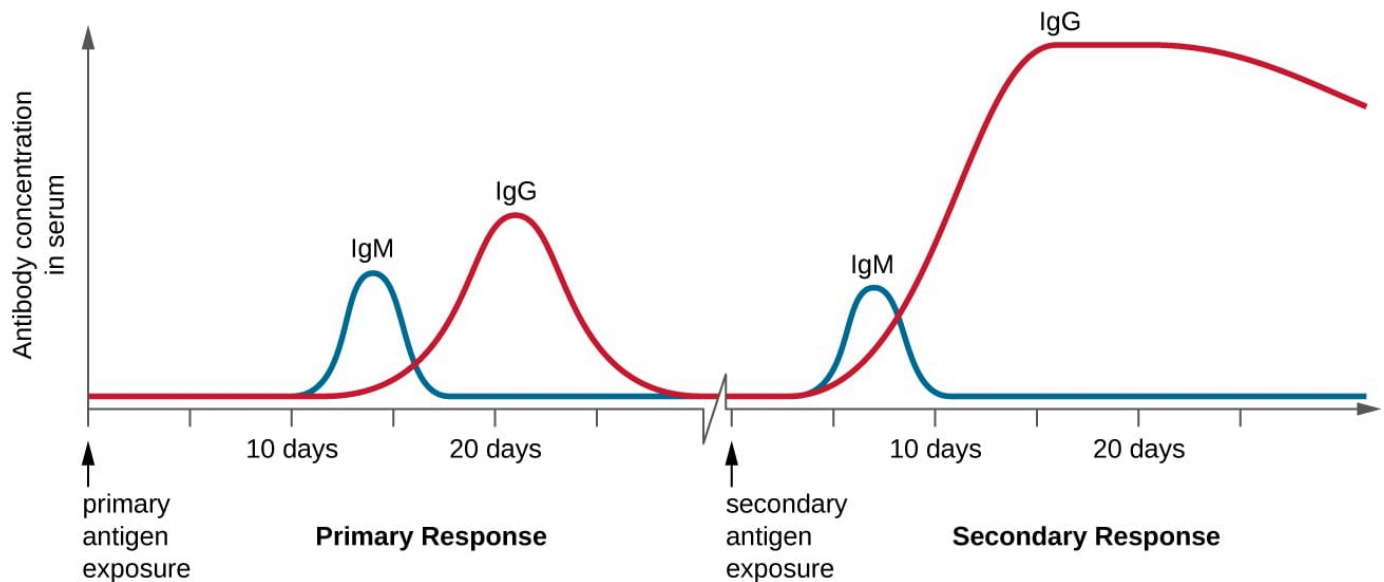


Figure 23.5.4: Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. The secondary response mostly involves IgG.

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Key Concepts and Summary

- **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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23.6: Vaccines

Learning Objectives

- Compare the various kinds of artificial immunity
- Differentiate between active and passive immunity

For many diseases, prevention is the best form of treatment, and few strategies for disease prevention are as effective as vaccination. Vaccination is a form of artificial immunity. By artificially stimulating the adaptive immune defenses, a vaccine triggers memory cell production similar to that which would occur during a primary response. In so doing, the patient is able to mount a strong secondary response upon exposure to the pathogen—but without having to first suffer through an initial infection. In this section, we will explore several different kinds of artificial immunity along with various types of vaccines and the mechanisms by which they induce artificial immunity.

Classifications of Adaptive Immunity

All forms of adaptive immunity can be described as either active or passive. Active immunity refers to the activation of an individual's own adaptive immune defenses, whereas passive immunity refers to the transfer of adaptive immune defenses from another individual or animal. Active and passive immunity can be further subdivided based on whether the protection is acquired naturally or artificially.

Natural active immunity is adaptive immunity that develops after natural exposure to a pathogen (Figure 23.6.1). Examples would include the lifelong immunity that develops after recovery from a chickenpox or measles infection (although an acute infection is not always necessary to activate adaptive immunity). The length of time that an individual is protected can vary substantially depending upon the pathogen and antigens involved. For example, activation of adaptive immunity by protein spike structures during an intracellular viral infection can activate lifelong immunity, whereas activation by carbohydrate capsule antigens during an extracellular bacterial infection may activate shorter-term immunity.

Natural passive immunity involves the natural passage of antibodies from a mother to her child before and after birth. IgG is the only antibody class that can cross the placenta from mother's blood to the fetal blood supply. Placental transfer of IgG is an important passive immune defense for the infant, lasting up to six months after birth. Secretory IgA can also be transferred from mother to infant through breast milk.

Artificial passive immunity refers to the transfer of antibodies produced by a donor (human or animal) to another individual. This transfer of antibodies may be done as a prophylactic measure (i.e., to prevent disease after exposure to a pathogen) or as a strategy for treating an active infection. For example, artificial passive immunity is commonly used for post-exposure prophylaxis against rabies, hepatitis A, hepatitis B, and chickenpox (in high risk individuals). Active infections treated by artificial passive immunity include cytomegalovirus infections in immunocompromised patients and Ebola virus infections. In 1995, eight patients in the Democratic Republic of the Congo with active Ebola infections were treated with blood transfusions from patients who were recovering from Ebola. Only one of the eight patients died (a 12.5% mortality rate), which was much lower than the expected 80% mortality rate for Ebola in untreated patients.¹ Artificial passive immunity is also used for the treatment of diseases caused by bacterial toxins, including tetanus, botulism, and diphtheria.

Artificial active immunity is the foundation for vaccination. It involves the activation of adaptive immunity through the deliberate exposure of an individual to weakened or inactivated pathogens, or preparations consisting of key pathogen antigens.

Mechanisms of Acquisition of Immunity		
	Natural acquired	Artificial acquired
Passive	Immunity acquired from antibodies passed in breast milk or through placenta 	Immunity gained through antibodies harvested from another person or an animal 
Active	Immunity gained through illness and recovery 	Immunity acquired through a vaccine 

Figure 23.6.1: The four classifications of immunity. (credit top left photo: modification of work by USDA; credit top right photo: modification of work by "Michaelberry"/Wikimedia; credit bottom left photo: modification of work by Centers for Disease Control and Prevention; credit bottom right photo: modification of work by Friskila Silitonga, Indonesia, Centers for Disease Control and Prevention)

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Herd Immunity

The four kinds of immunity just described result from an individual's adaptive immune system. For any given disease, an individual may be considered immune or susceptible depending on his or her ability to mount an effective immune response upon exposure. Thus, any given population is likely to have some individuals who are immune and other individuals who are susceptible. If a population has very few susceptible individuals, even those susceptible individuals will be protected by a phenomenon called **herd immunity**. Herd immunity has nothing to do with an individual's ability to mount an effective immune response; rather, it occurs because there are too few susceptible individuals in a population for the disease to spread effectively.

Vaccination programs create herd immunity by greatly reducing the number of susceptible individuals in a population. Even if some individuals in the population are not vaccinated, as long as a certain percentage is immune (either naturally or artificially), the few susceptible individuals are unlikely to be exposed to the pathogen. However, because new individuals are constantly entering populations (for example, through birth or relocation), vaccination programs are necessary to maintain herd immunity.

Vaccination: Obligation or Choice

A growing number of parents are choosing not to vaccinate their children. They are dubbed “antivaxxers,” and the majority of them believe that vaccines are a cause of autism (or other disease conditions), a link that has now been thoroughly disproven. Others object to vaccines on religious or moral grounds (e.g., the argument that Gardasil vaccination against HPV may promote sexual promiscuity), on personal ethical grounds (e.g., a conscientious objection to any medical intervention), or on political grounds (e.g., the notion that mandatory vaccinations are a violation of individual liberties).²

It is believed that this growing number of unvaccinated individuals has led to new outbreaks of whooping cough and measles. We would expect that herd immunity would protect those unvaccinated in our population, but herd immunity can only be maintained if enough individuals are being vaccinated.

Vaccination is clearly beneficial for public health. But from the individual parent's perspective the view can be murkier. Vaccines, like all medical interventions, have associated risks, and while the risks of vaccination may be extremely low compared to the risks of infection, parents may not always understand or accept the consensus of the medical community. Do such parents have a right to withhold vaccination from their children? Should they be allowed to put their children—and society at large—at risk?

Many governments insist on childhood vaccinations as a condition for entering public school, but it has become easy in most states to opt out of the requirement or to keep children out of the public system. Since the 1970s, West Virginia and Mississippi have had in place a stringent requirement for childhood vaccination, without exceptions, and neither state has had a case of measles since the early 1990s. California lawmakers recently passed a similar law in response to a measles outbreak in 2015, making it much more difficult for parents to opt out of vaccines if their children are attending public schools. Given this track record and renewed legislative efforts, should other states adopt similarly strict requirements?

What role should health-care providers play in promoting or enforcing universal vaccination? Studies have shown that many parents' minds can be changed in response to information delivered by health-care workers, but is it the place of health-care workers to try to persuade parents to have their children vaccinated? Some health-care providers are understandably reluctant to treat unvaccinated patients. Do they have the right to refuse service to patients who decline vaccines? Do insurance companies have the right to deny coverage to antivaxxers? These are all ethical questions that policymakers may be forced to address as more parents skirt vaccination norms.

Key Concepts and Summary

- Adaptive immunity can be divided into four distinct classifications: **natural active immunity**, **natural passive immunity**, **artificial passive immunity**, and **artificial active immunity**.
- Artificial active immunity is the foundation for **vaccination** and vaccine development. Vaccination programs not only confer artificial immunity on individuals, but also foster **herd immunity** in populations.

Footnotes

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23.7: Variolation and Vaccination

Learning Objectives

- Differentiate between variolation and vaccination
- Explain why vaccination is less risky than variolation

Variolation and Vaccination

Thousands of years ago, it was first recognized that individuals who survived a smallpox infection were immune to subsequent infections. The practice of inoculating individuals to actively protect them from smallpox appears to have originated in the 10th century in China, when the practice of **variolation** was described (Figure 23.7.2). Variolation refers to the deliberate inoculation of individuals with infectious material from scabs or pustules of smallpox victims. Infectious materials were either injected into the skin or introduced through the nasal route. The infection that developed was usually milder than naturally acquired smallpox, and recovery from the milder infection provided protection against the more serious disease.

Although the majority of individuals treated by variolation developed only mild infections, the practice was not without risks. More serious and sometimes fatal infections did occur, and because smallpox was contagious, infections resulting from variolation could lead to epidemics. Even so, the practice of variolation for smallpox prevention spread to other regions, including India, Africa, and Europe.



Figure 23.7.2: Variolation for smallpox originated in the Far East and the practice later spread to Europe and Africa. This Japanese relief depicts a patient receiving a smallpox variolation from the physician Ogata Shunsaku (1748–1810).

Although variolation had been practiced for centuries, the English physician Edward Jenner (1749–1823) is generally credited with developing the modern process of vaccination. Jenner observed that milkmaids who developed cowpox, a disease similar to smallpox but milder, were immune to the more serious smallpox. This led Jenner to hypothesize that exposure to a less virulent pathogen could provide immune protection against a more virulent pathogen, providing a safer alternative to variolation. In 1796, Jenner tested his hypothesis by obtaining infectious samples from a milkmaid's active cowpox lesion and injecting the materials into a young boy (Figure 23.7.3). The boy developed a mild infection that included a low-grade fever, discomfort in his axillae (armpit) and loss of appetite. When the boy was later infected with infectious samples from smallpox lesions, he did not contract smallpox.³ This new approach was termed **vaccination**, a name deriving from the use of cowpox (Latin *vacca* meaning "cow") to protect against smallpox. Today, we know that Jenner's vaccine worked because the cowpox virus is genetically and antigenically related to the *Variola* viruses that caused smallpox. Exposure to cowpox antigens resulted in a primary response and

the production of memory cells that identical or related epitopes of Variola virus upon a later exposure to smallpox.

The success of Jenner's smallpox vaccination led other scientists to develop vaccines for other diseases. Perhaps the most notable was Louis Pasteur, who developed vaccines for rabies, cholera, and anthrax. During the 20th and 21st centuries, effective vaccines were developed to prevent a wide range of diseases caused by viruses (e.g., chickenpox and shingles, hepatitis, measles, mumps, polio, and yellow fever) and bacteria (e.g., diphtheria, pneumococcal pneumonia, tetanus, and whooping cough).



(a)



(b)

Figure 23.7.3: (a) A painting of Edward Jenner depicts a cow and a milkmaid in the background. (b) Lesions on a patient infected with cowpox, a zoonotic disease caused by a virus closely related to the one that causes smallpox. (credit b: modification of work by the Centers for Disease Control and Prevention)

Query 23.7.1

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Key Concepts and Summary

- **Variolation** against smallpox originated in the 10th century in China, but the procedure was risky because it could cause the disease it was intended to prevent. Modern vaccination was developed by Edward Jenner, who developed the practice of inoculating patients with infectious materials from cowpox lesions to prevent smallpox.

Footnotes

1. M. Alonso and J. C. Leong. "Licensed DNA Vaccines Against Infectious Hematopoietic Necrosis Virus (IHNV)." *Recent Patents on DNA & Gene Sequences (Discontinued)* 7 no. 1 (2013): 62–65, issn 1872-2156/2212-3431. doi 10.2174/1872215611307010009.

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23.8: Classes of Vaccines

Learning Objectives

- Describe different types of vaccines
- Explain their respective advantages and disadvantages
- Identify the risk associated with a live attenuated vaccine

Classes of Vaccines

For a vaccine to provide protection against a disease, it must expose an individual to pathogen-specific antigens that will stimulate a protective adaptive immune response. By its very nature, this entails some risk. As with any pharmaceutical drug, vaccines have the potential to cause adverse effects. However, the ideal vaccine causes no severe adverse effects and poses no risk of contracting the disease that it is intended to prevent. Various types of vaccines have been developed with these goals in mind. These different classes of vaccines are described in the next section and summarized in Table 23.8.1.

Live Attenuated Vaccines

Live attenuated vaccines expose an individual to a weakened strain of a pathogen with the goal of establishing a subclinical infection that will activate the adaptive immune defenses. Pathogens are attenuated to decrease their virulence using methods such as genetic manipulation (to eliminate key virulence factors) or long-term culturing in an unnatural host or environment (to promote mutations and decrease virulence).

By establishing an active infection, live attenuated vaccines stimulate a more comprehensive immune response than some other types of vaccines. Live attenuated vaccines activate both cellular and humoral immunity and stimulate the development of memory for long-lasting immunity. In some cases, vaccination of one individual with a live attenuated pathogen can even lead to natural transmission of the attenuated pathogen to other individuals. This can cause the other individuals to also develop an active, subclinical infection that activates their adaptive immune defenses.

Disadvantages associated with live attenuated vaccines include the challenges associated with long-term storage and transport as well as the potential for a patient to develop signs and symptoms of disease during the active infection (particularly in immunocompromised patients). There is also a risk of the attenuated pathogen reverting back to full virulence. Table 23.8.1 lists examples of live attenuated vaccines.

Inactivated Vaccines

Inactivated vaccines contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of key antigens on the pathogen.

Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. Typically the response involves only humoral immunity, and the pathogen cannot be transmitted to other individuals. In addition, inactivated vaccines usually require higher doses and multiple boosters, possibly causing inflammatory reactions at the site of injection.

Despite these disadvantages, inactivated vaccines do have the advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. However, inactivated vaccines are not without their side effects. Table 23.8.1 lists examples of inactivated vaccines.

Subunit Vaccines

Whereas live attenuated and inactive vaccines expose an individual to a weakened or dead pathogen, **subunit vaccines** only expose the patient to the key antigens of a pathogen—not whole cells or viruses. Subunit vaccines can be produced either

by chemically degrading a pathogen and isolating its key antigens or by producing the antigens through genetic engineering. Because these vaccines contain only the essential antigens of a pathogen, the risk of side effects is relatively low. Table 23.8.1 lists examples of subunit vaccines.

Toxoid Vaccines

Like subunit vaccines, **toxoid vaccines** do not introduce a whole pathogen to the patient; they contain inactivated bacterial toxins, called toxoids. Toxoid vaccines are used to prevent diseases in which bacterial toxins play an important role in pathogenesis. These vaccines activate humoral immunity that neutralizes the toxins. Table 23.8.1 lists examples of toxoid vaccines.

Conjugate Vaccines

A **conjugate vaccine** is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. Conjugate vaccines have been developed to enhance the efficacy of subunit vaccines against pathogens that have protective polysaccharide capsules that help them evade phagocytosis, causing invasive infections that can lead to meningitis and other serious conditions. The subunit vaccines against these pathogens introduce T-independent capsular polysaccharide antigens that result in the production of antibodies that can opsonize the capsule and thus combat the infection; however, children under the age of two years do not respond effectively to these vaccines. Children do respond effectively when vaccinated with the conjugate vaccine, in which a protein with T-dependent antigens is conjugated to the capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide. Table 23.8.1 lists examples of conjugate vaccines.

Table 23.8.1: Classes of Vaccines

Class	Description	Advantages	Disadvantages	Examples
Live attenuated	Weakened strain of whole pathogen	Cellular and humoral immunity	Difficult to store and transport	Chickenpox, German measles, measles, mumps, tuberculosis, typhoid fever, yellow fever
		Long-lasting immunity	Risk of infection in immunocompromised patients	
		Transmission to contacts	Risk of reversion	
Inactivated	Whole pathogen killed or inactivated with heat, chemicals, or radiation	Ease of storage and transport	Weaker immunity (humoral only)	Cholera, hepatitis A, influenza, plague, rabies
		No risk of severe active infection	Higher doses and more boosters required	
Subunit	Immunogenic antigens	Lower risk of side effects	Limited longevity	Anthrax, hepatitis B, influenza, meningitis, papillomavirus, pneumococcal pneumonia, whooping cough
			Multiple doses required	
			No protection against antigenic variation	
Toxoid	Inactivated bacterial toxin	Humoral immunity to neutralize toxin	Does not prevent infection	Botulism, diphtheria, pertussis, tetanus

Class	Description	Advantages	Disadvantages	Examples
Conjugate	Capsule polysaccharide conjugated to protein	T-dependent response to capsule	Costly to produce	Meningitis (<i>Haemophilus influenzae</i> , <i>Streptococcus pneumoniae</i> , <i>Neisseria meningitidis</i>)
		Better response in young children	No protection against antigenic variation May interfere with other vaccines	

DNA Vaccines

DNA vaccines represent a relatively new and promising approach to vaccination. A DNA vaccine is produced by incorporating genes for antigens into a recombinant plasmid vaccine. Introduction of the DNA vaccine into a patient leads to uptake of the recombinant plasmid by some of the patient's cells, followed by transcription and translation of antigens and presentation of these antigens with MHC I to activate adaptive immunity. This results in the stimulation of both humoral and cellular immunity without the risk of active disease associated with live attenuated vaccines.

Although most DNA vaccines for humans are still in development, it is likely that they will become more prevalent in the near future as researchers are working on engineering DNA vaccines that will activate adaptive immunity against several different pathogens at once. First-generation DNA vaccines tested in the 1990s looked promising in animal models but were disappointing when tested in human subjects. Poor cellular uptake of the DNA plasmids was one of the major problems impacting their efficacy. Trials of second-generation DNA vaccines have been more promising thanks to new techniques for enhancing cellular uptake and optimizing antigens. DNA vaccines for various cancers and viral pathogens such as HIV, HPV, and hepatitis B and C are currently in development.

Some DNA vaccines are already in use. In 2005, a DNA vaccine against West Nile virus was approved for use in horses in the United States. Canada has also approved a DNA vaccine to protect fish from infectious hematopoietic necrosis virus.⁴ A DNA vaccine against Japanese encephalitis virus was approved for use in humans in 2010 in Australia.

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Key Concepts and Summary

- **Live attenuated vaccines** and **inactivated vaccines** contain whole pathogens that are weak, killed, or inactivated. **Subunit vaccines**, **toxoid vaccines**, and **conjugate vaccines** contain acellular components with antigens that stimulate an immune response.

Footnotes

1. S.B. Halstead and S. J. Thomas. "New Japanese Encephalitis Vaccines: Alternatives to Production in Mouse Brain." *Expert Review of Vaccines* 10 no. 3 (2011): 355–64.

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23.E: Adaptive Immunity II (Exercises)

23.1: B Lymphocytes and Antibodies

Humoral immunity refers to mechanisms of the adaptive immune defenses that are mediated by antibodies secreted by B lymphocytes, or B cells. This section focuses on B cells and discusses their production and maturation, receptors, and mechanisms of activation.

Multiple Choice

Which of the following would be a T-dependent antigen?

- A. lipopolysaccharide
- B. glycolipid
- C. protein
- D. carbohydrate

Answer

C

Which of the following would be a BCR?

- A. CD4
- B. MHC II
- C. MHC I
- D. IgD

Answer

D

Which of the following does not occur during the lag period of the primary antibody response?

- A. activation of helper T cells
- B. class switching to IgG
- C. presentation of antigen with MHC II
- D. binding of antigen to BCRs

Answer

B

Fill in the Blank

_____ antigens can stimulate B cells to become activated but require cytokine assistance delivered by helper T cells.

Answer

T-dependent

T-independent antigens can stimulate B cells to become activated and secrete antibodies without assistance from helper T cells. These antigens possess _____ antigenic epitopes that cross-link BCRs.

Answer

repetitive

Critical Thinking

A patient lacks the ability to make functioning T cells because of a genetic disorder. Would this patient's B cells be able to produce antibodies in response to an infection? Explain your answer.

23.2: Vaccines

By artificially stimulating the adaptive immune defenses, a vaccine triggers memory cell production similar to that which would occur during a primary response. In so doing, the patient is able to mount a strong secondary response upon exposure to the pathogen—but without having to first suffer through an initial infection. In this section, we explore several different kinds of artificial immunity along with various types of vaccines and their mechanisms for inducing artificial immunity.

Multiple Choice

A patient is bitten by a dog with confirmed rabies infection. After treating the bite wound, the physician injects the patient with antibodies that are specific for the rabies virus to prevent the development of an active infection. This is an example of:

- A. Natural active immunity
- B. Artificial active immunity
- C. Natural passive immunity
- D. Artificial passive immunity

Answer

D

A patient gets a cold, and recovers a few days later. The patient's classmates come down with the same cold roughly a week later, but the original patient does not get the same cold again. This is an example of:

- A. Natural active immunity
- B. Artificial active immunity
- C. Natural passive immunity
- D. Artificial passive immunity

Answer

A

Matching

Match each type of vaccine with the corresponding example.

___inactivated vaccine	A. Weakened influenza virions that can only replicate in the slightly lower temperatures of the nasal passages are sprayed into the nose. They do not cause serious flu symptoms, but still produce an active infection that induces a protective adaptive immune response.
___live attenuated vaccine	B. Tetanus toxin molecules are harvested and chemically treated to render them harmless. They are then injected into a patient's arm.
___toxoid vaccine	C. Influenza virus particles grown in chicken eggs are harvested and chemically treated to render them noninfectious. These immunogenic particles are then purified and packaged and administered as an injection.

___subunit vaccine	D. The gene for hepatitis B virus surface antigen is inserted into a yeast genome. The modified yeast is grown and the virus protein is produced, harvested, purified, and used in a vaccine.
--------------------	---

Answer

C, A, B, D

Fill in the Blank

A(n) _____ pathogen is in a weakened state; it is still capable of stimulating an immune response but does not cause a disease.

Answer

attenuated

_____ immunity occurs when antibodies from one individual are harvested and given to another to protect against disease or treat active disease.

Answer

Artificial passive

In the practice of _____, scabs from smallpox victims were used to immunize susceptible individuals against smallpox.

Answer

variolation

Short answer

Briefly compare the pros and cons of inactivated versus live attenuated vaccines.

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24: HYPERSENSITIVITIES

An allergic reaction is an immune response to a type of antigen called an allergen. Allergens can be found in many different items, from peanuts and insect stings to latex and some drugs. Unlike other kinds of antigens, allergens are not necessarily associated with pathogenic microbes, and many allergens provoke no immune response at all in most people.

Allergic responses vary in severity. Some are mild and localized, like hay fever or hives, but others can result in systemic, life-threatening reactions. Anaphylaxis, for example, is a rapidly developing allergic reaction that can cause a dangerous drop in blood pressure and severe swelling of the throat that may close off the airway.

Allergies are just one example of how the immune system—the system normally responsible for preventing disease—can actually cause or mediate disease symptoms. In this chapter, we will further explore allergies and other hypersensitivity reactions.



Figure 24.1: Bee stings and other allergens can cause life-threatening, systemic allergic reactions. Sensitive individuals may need to carry an epinephrine auto-injector (e.g., EpiPen) in case of a sting. A bee-sting allergy is an example of an immune response that is harmful to the host rather than protective; epinephrine counteracts the severe drop in blood pressure that can result from the immune response. (credit right: modification of work by Carol Bleistine)

24.1: Type I Hypersensitivities

24.2: Type II Hypersensitivities

24.3: Type III Hypersensitivities

24.4: Type IV Hypersensitivities

24.5: Hypersensitivity Pneumonitis and Summary

24.6: Diagnosis and Treatment of Hypersensitivities

24.E: Diseases of the Immune System (Exercises)

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24.1: Type I Hypersensitivities

Learning Objectives

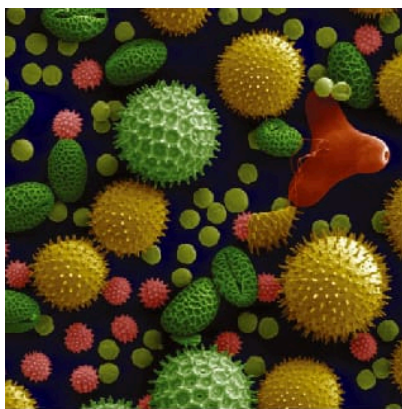
- Compare the distinguishing characteristics, mechanisms, and major examples of type I, II, III, and IV hypersensitivities
- Identify the cells that cause a type I hypersensitivity reaction
- Describe the differences between immediate and late-phase type I hypersensitivity reactions
- List the signs and symptoms of anaphylaxis

In [Adaptive Specific Host Defenses](#), we discussed the mechanisms by which adaptive immune defenses, both humoral and cellular, protect us from infectious diseases. However, these same protective immune defenses can also be responsible for undesirable reactions called **hypersensitivity** reactions. Hypersensitivity reactions are classified by their immune mechanism.

- Type I hypersensitivity reactions involve immunoglobulin E (IgE) antibody against soluble antigen, triggering mast cell degranulation.
- Type II hypersensitivity reactions involve IgG and IgM antibodies directed against cellular antigens, leading to cell damage mediated by other immune system effectors.
- Type III hypersensitivity reactions involve the interactions of IgG, IgM, and, occasionally, IgA¹ antibodies with antigen to form immune complexes. Accumulation of immune complexes in tissue leads to tissue damage mediated by other immune system effectors.
- Type IV hypersensitivity reactions are T-cell-mediated reactions that can involve tissue damage mediated by activated macrophages and cytotoxic T cells.

Type I Hypersensitivities

When a presensitized individual is exposed to an **allergen**, it can lead to a rapid immune response that occurs almost immediately. Such a response is called an **allergy** and is classified as a **type I hypersensitivity**. Allergens may be seemingly harmless substances such as animal dander, molds, or pollen. Allergens may also be substances considered innately more hazardous, such as insect venom or therapeutic drugs. Food intolerances can also yield allergic reactions as individuals become sensitized to foods such as peanuts or shellfish (Figure 24.1.1). Regardless of the allergen, the first exposure activates a primary IgE antibody response that sensitizes an individual to type I hypersensitivity reaction upon subsequent exposure.



(a)



(b)



(c)

Figure 24.1.1: (a) Allergens in plant pollen, shown here in a colorized electron micrograph, may trigger allergic rhinitis or hay fever in sensitive individuals. (b) Skin rashes are often associated with allergic reactions. (c) Peanuts can be eaten safely by most people but can provoke severe allergic reactions in sensitive individuals.

Mechanism of Type I Hypersensitivity

For susceptible individuals, a first exposure to an allergen activates a strong T_H2 cell response (Figure 24.1.2). Cytokines interleukin (IL)-4 and IL-13 from the T_H2 cells activate B cells specific to the same allergen, resulting in clonal proliferation, differentiation into plasma cells, and antibody-class switch from production of IgM to production of IgE. The fragment crystallizable (Fc) regions of the IgE antibodies bind to specific receptors on the surface of mast cells throughout the body. It is estimated that each mast cell can bind up to 500,000 IgE molecules, with each IgE molecule having two allergen-specific fragment antigen-binding (Fab) sites available for binding allergen on subsequent exposures. By the time this occurs, the allergen is often no longer present and there is no allergic reaction, but the mast cells are primed for a subsequent exposure and the individual is sensitized to the allergen.

On subsequent exposure, allergens bind to multiple IgE molecules on mast cells, cross-linking the IgE molecules. Within minutes, this cross-linking of IgE activates the mast cells and triggers **degranulation**, a reaction in which the contents of the granules in the mast cell are released into the extracellular environment. Preformed components that are released from granules include histamine, serotonin, and bradykinin (Table 24.1.1). The activated mast cells also release newly formed lipid mediators (leukotrienes and prostaglandins from membrane arachadonic acid metabolism) and cytokines such as tumor necrosis factor (Table 24.1.2).

Table 24.1.1: Selected Preformed Components of Mast Cell Granules

Granule Component	Activity
Heparin	Stimulates the generation of bradykinin, which causes increased vascular permeability, vasodilation, bronchiole constriction, and increased mucus secretion
Histamine	Causes smooth-muscle contraction, increases vascular permeability, increases mucus and tear formation
Serotonin	Increases vascular permeability, causes vasodilation and smooth-muscle contraction

The chemical mediators released by mast cells collectively cause the inflammation and signs and symptoms associated with type I hypersensitivity reactions. Histamine stimulates mucus secretion in nasal passages and tear formation from lacrimal glands, promoting the runny nose and watery eyes of allergies. Interaction of histamine with nerve endings causes itching and sneezing. The vasodilation caused by several of the mediators can result in hives, headaches, angioedema (swelling that often affects the lips, throat, and tongue), and hypotension (low blood pressure). Bronchiole constriction caused by some of the chemical mediators leads to wheezing, dyspnea (difficulty breathing), coughing, and, in more severe cases, cyanosis (bluish color to the skin or mucous membranes). Vomiting can result from stimulation of the vomiting center in the cerebellum by histamine and serotonin. Histamine can also cause relaxation of intestinal smooth muscles and diarrhea.

Table 24.1.2: Selected Newly Formed Chemical Mediators of Inflammation and Allergic Response

Chemical Mediator	Activity
Leukotriene	Causes smooth-muscle contraction and mucus secretion, increases vascular permeability
Prostaglandin	Causes smooth-muscle contraction and vasodilation
TNF- α (cytokine)	Causes inflammation and stimulates cytokine production by other cell types

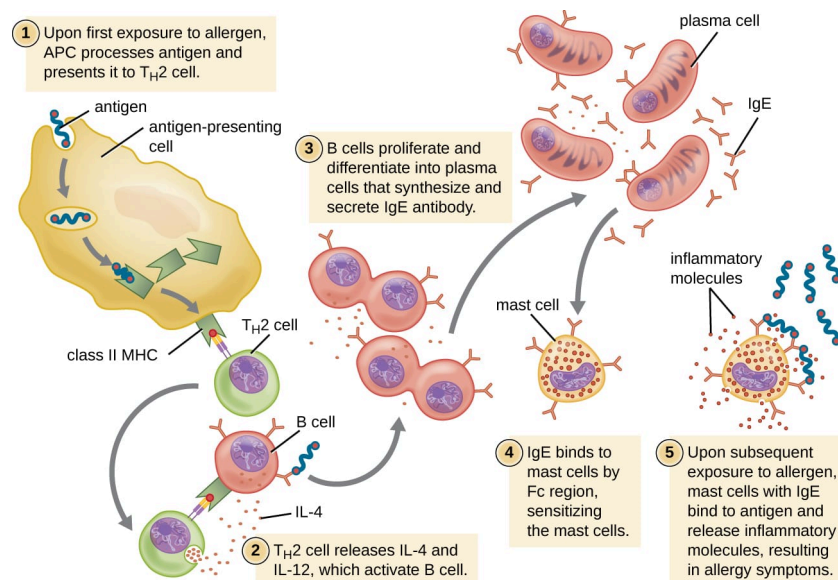


Figure 24.1.2: On first exposure to an allergen in a susceptible individual, antigen-presenting cells process and present allergen epitopes with major histocompatibility complex (MHC) II to T helper cells. B cells also process and present the same allergen epitope to T_H2 cells, which release cytokines IL-4 and IL-13 to stimulate proliferation and differentiation into IgE-secreting plasma cells. The IgE molecules bind to mast cells with their Fc region, sensitizing the mast cells for activation with subsequent exposure to the allergen. With each subsequent exposure, the allergen cross-links IgE molecules on the mast cells, activating the mast cells and causing the release of preformed chemical mediators from granules (degranulation), as well as newly formed chemical mediators that collectively cause the signs and symptoms of type I hypersensitivity reactions.

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Types of Type I Hypersensitivity

Type I hypersensitivity reactions can be either localized or systemic. **Localized type I hypersensitivity** reactions include hay fever rhinitis, hives, and asthma (Table 24.1.3). **Systemic type I hypersensitivity** reactions are referred to as **anaphylaxis** or **anaphylactic shock**. Although anaphylaxis shares many symptoms common with the localized type I hypersensitivity reactions, the swelling of the tongue and trachea, blockage of airways, dangerous drop in blood pressure, and development of shock can make anaphylaxis especially severe and life-threatening. In fact, death can occur within minutes of onset of signs and symptoms.

Late-phase reactions in type I hypersensitivities may develop 4–12 hours after the early phase and are mediated by eosinophils, neutrophils, and lymphocytes that have been recruited by chemotactic factors released from mast cells. Activation of these recruited cells leads to the release of more chemical mediators that cause tissue damage and late-phase symptoms of swelling and redness of the skin, coughing, wheezing, and nasal discharge.

Individuals who possess genes for maladaptive traits, such as intense type I hypersensitivity reactions to otherwise harmless components of the environment, would be expected to suffer reduced reproductive success. With this kind of evolutionary selective pressure, such traits would not be expected to persist in a population. This suggests that type I hypersensitivities may have an adaptive function. There is evidence that the IgE produced during type I hypersensitivity reactions is actually meant to counter helminth infections². Helminths are one of few organisms that possess proteins that are targeted by IgE. In addition, there is evidence that helminth infections at a young age reduce the likelihood of type I hypersensitivities to innocuous substances later in life. Thus it may be that allergies are an unfortunate consequence of strong selection in the mammalian lineage or earlier for a defense against parasitic worms.

Table 24.1.3: Type I Hypersensitivities

Common Name	Cause	Signs and Symptoms
Allergy-induced asthma	Inhalation of allergens	Constriction of bronchi, labored breathing, coughing, chills, body aches
Anaphylaxis	Systemic reaction to allergens	Hives, itching, swelling of tongue and throat, nausea, vomiting, low blood pressure, shock
Hay fever	Inhalation of mold or pollen	Runny nose, watery eyes, sneezing
Hives (urticaria)	Food or drug allergens, insect stings	Raised, bumpy skin rash with itching; bumps may converge into large raised areas

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The Hygiene Hypothesis

In most modern societies, good hygiene is associated with regular bathing, and good health with cleanliness. But some recent studies suggest that the association between health and clean living may be a faulty one. Some go so far as to suggest that children should be encouraged to play in the dirt—or even eat dirt³—for the benefit of their health. This recommendation is based on the so-called hygiene hypothesis, which proposes that childhood exposure to antigens from a diverse range of microbes leads to a better-functioning immune system later in life.

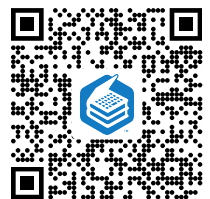
The hygiene hypothesis was first suggested in 1989 by David Strachan⁴, who observed an inverse relationship between the number of older children in a family and the incidence of hay fever. Although hay fever in children had increased dramatically during the mid-20th century, incidence was significantly lower in families with more children. Strachan proposed that the lower incidence of allergies in large families could be linked to infections acquired from older siblings, suggesting that these infections made children less susceptible to allergies. Strachan also argued that trends toward smaller families and a greater emphasis on cleanliness in the 20th century had decreased exposure to pathogens and thus led to higher overall rates of allergies, asthma, and other immune disorders.

Other researchers have observed an inverse relationship between the incidence of immune disorders and infectious diseases that are now rare in industrialized countries but still common in less industrialized countries.⁵ In developed nations, children under the age of 5 years are not exposed to many of the microbes, molecules, and antigens they almost certainly would have encountered a century ago. The lack of early challenges to the immune system by organisms with which humans and their ancestors evolved may result in failures in immune system functioning later in life.

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Key Concepts and Summary

- An **allergy** is an adaptive immune response, sometimes life-threatening, to an **allergen**.
- **Type I hypersensitivity** requires sensitization of mast cells with IgE, involving an initial IgE antibody response and IgE attachment to mast cells. On second exposure to an allergen, cross-linking of IgE molecules on mast cells triggers degranulation and release of preformed and newly formed chemical mediators of inflammation. Type I hypersensitivity may be localized and relatively minor (hives and hay fever) or system-wide and dangerous (systemic **anaphylaxis**).

Footnotes

1. D.S. Strayer et al (eds). *Rubin's Pathology: Clinicopathologic Foundations of Medicine*. 7th ed. 2Philadelphia, PA: Lippincott, Williams & Wilkins, 2014.
2. C.M. Fitzsimmons et al. "Helminth Allergens, Parasite-Specific IgE, and Its Protective Role in Human Immunity." *Frontier in Immunology* 5 (2015):47.
3. S.T. Weiss. "Eat Dirt—The Hygiene Hypothesis and Allergic Diseases." *New England Journal of Medicine* 347 no. 12 (2002):930–931.
4. D.P. Strachan "Hay Fever, Hygiene, and Household Size." *British Medical Journal* 299 no. 6710 (1989):1259.
5. H. Okada et al. "The 'Hygiene Hypothesis' for Autoimmune and Allergic Diseases: An Update." *Clinical & Experimental Immunology* 160 no. 1 (2010):1–9.

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24.2: Type II Hypersensitivities

Learning Objectives

- Summarize the ABO blood group system
- Explain how hemolytic transfusion reaction (HTR) occurs
- Describe hemolytic disease of the newborn (HDN)
- Explain how HDN can be prevented

Type II (Cytotoxic) Hypersensitivities

Immune reactions categorized as **type II hypersensitivities**, or **cytotoxic hypersensitivities**, are mediated by IgG and IgM antibodies binding to cell-surface antigens or matrix-associated antigens on basement membranes. These antibodies can either activate complement, resulting in an inflammatory response and lysis of the targeted cells, or they can be involved in antibody-dependent cell-mediated cytotoxicity (ADCC) with cytotoxic T cells.

In some cases, the antigen may be a self-antigen, in which case the reaction would also be described as an autoimmune disease. (Autoimmune diseases are described in [Autoimmune Disorders](#)). In other cases, antibodies may bind to naturally occurring, but exogenous, cell-surface molecules such as antigens associated with blood typing found on red blood cells (RBCs). This leads to the coating of the RBCs by antibodies, activation of the complement cascade, and complement-mediated lysis of RBCs, as well as opsonization of RBCs for phagocytosis. Two examples of type II hypersensitivity reactions involving RBCs are hemolytic transfusion reaction (HTR) and hemolytic disease of the newborn (HDN). These type II hypersensitivity reactions, which will be discussed in greater detail, are summarized in Table 24.2.4.

Immunohematology is the study of blood and blood-forming tissue in relation to the immune response. Antibody-initiated responses against blood cells are type II hypersensitivities, thus falling into the field of immunohematology. For students first learning about immunohematology, understanding the immunological mechanisms involved is made even more challenging by the complex nomenclature system used to identify different blood-group antigens, often called blood types. The first blood-group antigens either used alphabetical names or were named for the first person known to produce antibodies to the red blood cell antigen (e.g., Kell, Duffy, or Diego). However, in 1980, the International Society of Blood Transfusion (ISBT) Working Party on Terminology created a standard for blood-group terminology in an attempt to more consistently identify newly discovered blood group antigens. New antigens are now given a number and assigned to a blood-group system, collection, or series. However, even with this effort, blood-group nomenclature is still inconsistent.

Table 24.2.4: Common Type II Hypersensitivities

Common Name	Cause	Signs and Symptoms
Hemolytic disease of the newborn (HDN)	IgG from mother crosses the placenta, targeting the fetus' RBCs for destruction	Anemia, edema, enlarged liver or spleen, hydrops (fluid in body cavity), leading to death of newborn in severe cases
Hemolytic transfusion reactions (HTR)	IgG and IgM bind to antigens on transfused RBCs, targeting donor RBCs for destruction	Fever, jaundice, hypotension, disseminated intravascular coagulation, possibly leading to kidney failure and death

ABO Blood Group Incompatibility

The recognition that individuals have different blood types was first described by Karl Landsteiner (1868–1943) in the early 1900s, based on his observation that serum from one person could cause a clumping of RBCs from another. These studies led Landsteiner to the identification of four distinct blood types. Subsequent research by other scientists determined that the four blood types were based on the presence or absence of surface glycoproteins “A” and “B,” and this provided the foundation for the **ABO blood group system** that is still in use today (Figure 24.2.3). The functions of these antigens are unknown, but some have been associated with normal biochemical functions of the cell. Furthermore, ABO blood types are inherited as alleles (one

from each parent), and they display patterns of dominant and codominant inheritance. The alleles for A and B blood types are codominant to each other, and both are dominant over blood type O. Therefore, individuals with genotypes of AA or AO have type A blood and express the A glycoprotein antigen on the surface of their RBCs. People with genotypes of BB or BO have type B blood and express the B glycoprotein antigen on the surface of their RBCs. Those with a genotype of AB have type AB blood and express both A and B glycoprotein antigens on the surface of their RBCs. Finally, individuals with a genotype of OO have type O blood and lack A and B glycoproteins on the surface of their RBCs.

It is important to note that the RBCs of all four ABO blood types share a common protein receptor molecule, and it is the addition of specific carbohydrates to the protein receptors that determines A, B, and AB blood types. The genes that are inherited for the A, B, and AB blood types encode enzymes that add the carbohydrate component to the protein receptor. Individuals with O blood type still have the protein receptor but lack the enzymes that would add carbohydrates that would make their red blood cell type A, B, or AB.

IgM antibodies in plasma that cross-react with blood group antigens not present on an individual's own RBCs are called **isoheamagglutinins** (Figure 24.2.3). Isoheamagglutinins are produced within the first few weeks after birth and persist throughout life. These antibodies are produced in response to exposure to environmental antigens from food and microorganisms. A person with type A blood has A antigens on the surface of their RBCs and will produce anti-B antibodies to environmental antigens that resemble the carbohydrate component of B antigens. A person with type B blood has B antigens on the surface of their RBCs and will produce anti-A antibodies to environmental antigens that are similar to the carbohydrate component of A antigens. People with blood type O lack both A and B antigens on their RBCs and, therefore, produce both anti-A and anti-B antibodies. Conversely, people with AB blood type have both A and B antigens on their RBCs and, therefore, lack anti-A and anti-B antibodies.

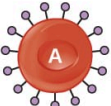
	Blood Type			
	A	B	AB	O
Red blood cell type				
Isoheamagglutinins	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens on red blood cell	 A antigen	 B antigen	 A and B antigens	None

Figure 24.2.3: Isoheamagglutinins

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A patient may require a blood transfusion because they lack sufficient RBCs (anemia) or because they have experienced significant loss of blood volume through trauma or disease. Although the blood transfusion is given to help the patient, it is essential that the patient receive a transfusion with matching ABO blood type. A transfusion with an incompatible ABO blood type may lead to a strong, potentially lethal type II hypersensitivity cytotoxic response called **hemolytic transfusion reaction (HTR)** (Figure 24.2.4).

For instance, if a person with type B blood receives a transfusion of type A blood, their anti-A antibodies will bind to and agglutinate the transfused RBCs. In addition, activation of the classical complement cascade will lead to a strong inflammatory response, and the complement membrane attack complex (MAC) will mediate massive hemolysis of the transfused RBCs. The debris from damaged and destroyed RBCs can occlude blood vessels in the alveoli of the lungs and the glomeruli of the kidneys. Within 1 to 24 hours of an incompatible transfusion, the patient experiences fever, chills, pruritus (itching), urticaria (hives), dyspnea, hemoglobinuria (hemoglobin in the urine), and hypotension (low blood pressure). In the most serious reactions, dangerously low blood pressure can lead to shock, multi-organ failure, and death of the patient.

Hospitals, medical centers, and associated clinical laboratories typically use hemovigilance systems to minimize the risk of HTRs due to clerical error. Hemovigilance systems are procedures that track transfusion information from the donor source and blood products obtained to the follow-up of recipient patients. Hemovigilance systems used in many countries identify HTRs and their outcomes through mandatory reporting (e.g., to the Food and Drug Administration in the United States), and this information is valuable to help prevent such occurrences in the future. For example, if an HTR is found to be the result of laboratory or clerical error, additional blood products collected from the donor at that time can be located and labeled correctly to avoid additional HTRs. As a result of these measures, HTR-associated deaths in the United States occur in about one per 2 million transfused units.⁶

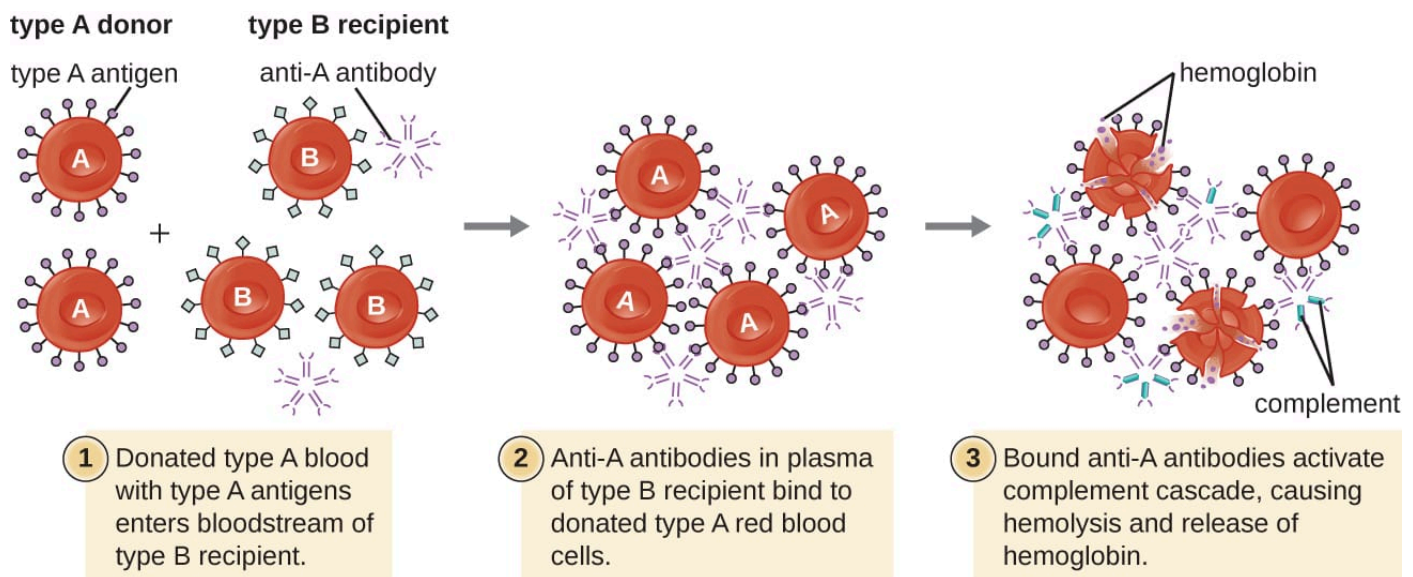


Figure 24.2.4: A type II hypersensitivity hemolytic transfusion reaction (HTR) leading to hemolytic anemia. Blood from a type A donor is administered to a patient with type B blood. The anti-A isohemagglutinin IgM antibodies in the recipient bind to and agglutinate the incoming donor type A red blood cells. The bound anti-A antibodies activate the classical complement cascade, resulting in destruction of the donor red blood cells.

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Rh Factors

Many different types of erythrocyte antigens have been discovered since the description of the ABO red cell antigens. The second most frequently described RBC antigens are **Rh factors**, named after the rhesus macaque (*Macaca mulatta*) factors identified by Karl Landsteiner and Alexander Weiner in 1940. The Rh system of RBC antigens is the most complex and immunogenic blood group system, with more than 50 specificities identified to date. Of all the Rh antigens, the one designated Rho (Weiner) or D (Fisher-Race) is the most immunogenic. Cells are classified as Rh positive (Rh+) if the Rho/D antigen is present or as Rh negative

(Rh–) if the Rho/D antigen is absent. In contrast to the carbohydrate molecules that distinguish the ABO blood groups and are the targets of IgM isohemagglutinins in HTRs, the Rh factor antigens are proteins. As discussed in [B Lymphocytes and Humoral Immunity](#), protein antigens activate B cells and antibody production through a T-cell-dependent mechanism, and the T_H2 cells stimulate class switching from IgM to other antibody classes. In the case of Rh factor antigens, T_H2 cells stimulate class switching to IgG, and this has important implications for the mechanism of HDN.

Like ABO incompatibilities, blood transfusions from a donor with the wrong Rh factor antigens can cause a type II hypersensitivity HTR. However, in contrast to the IgM isohemagglutinins produced early in life through exposure to environmental antigens, production of anti-Rh factor antibodies requires the exposure of an individual with Rh– blood to Rh+ positive RBCs and activation of a primary antibody response. Although this primary antibody response can cause an HTR in the transfusion patient, the hemolytic reaction would be delayed up to 2 weeks during the extended lag period of a primary antibody response ([B Lymphocytes and Humoral Immunity](#)). However, if the patient receives a subsequent transfusion with Rh+ RBCs, a more rapid HTR would occur with anti-Rh factor antibody already present in the blood. Furthermore, the rapid secondary antibody response would provide even more anti-Rh factor antibodies for the HTR.

Rh factor incompatibility between mother and fetus can also cause a type II hypersensitivity hemolytic reaction, referred to as **hemolytic disease of the newborn (HDN)** (Figure 24.2.5). If an Rh– woman carries an Rh+ baby to term, the mother's immune system can be exposed to Rh+ fetal red blood cells. This exposure will usually occur during the last trimester of pregnancy and during the delivery process. If this exposure occurs, the Rh+ fetal RBCs will activate a primary adaptive immune response in the mother, and anti-Rh factor IgG antibodies will be produced. IgG antibodies are the only class of antibody that can cross the placenta from mother to fetus; however, in most cases, the first Rh+ baby is unaffected by these antibodies because the first exposure typically occurs late enough in the pregnancy that the mother does not have time to mount a sufficient primary antibody response before the baby is born.

If a subsequent pregnancy with an Rh+ fetus occurs, however, the mother's second exposure to the Rh factor antigens causes a strong secondary antibody response that produces larger quantities of anti-Rh factor IgG. These antibodies can cross the placenta from mother to fetus and cause HDN, a potentially lethal condition for the baby (Figure 24.2.5).

Prior to the development of techniques for diagnosis and prevention, Rh factor incompatibility was the most common cause of HDN, resulting in thousands of infant deaths each year worldwide.⁷ For this reason, the Rh factors of prospective parents are regularly screened, and treatments have been developed to prevent HDN caused by Rh incompatibility. To prevent Rh factor-mediated HDN, human Rho(D) immune globulin (e.g., RhoGAM) is injected intravenously or intramuscularly into the mother during the 28th week of pregnancy and within 72 hours after delivery. Additional doses may be administered after events that may result in transplacental hemorrhage (e.g., umbilical blood sampling, chorionic villus sampling, abdominal trauma, amniocentesis). This treatment is initiated during the first pregnancy with an Rh+ fetus. The anti-Rh antibodies in Rho(D) immune globulin will bind to the Rh factor of any fetal RBCs that gain access to the mother's bloodstream, preventing these Rh+ cells from activating the mother's primary antibody response. Without a primary anti-Rh factor antibody response, the next pregnancy with an Rh+ will have minimal risk of HDN. However, the mother will need to be retreated with Rho(D) immune globulin during that pregnancy to prevent a primary anti-Rh antibody response that could threaten subsequent pregnancies.

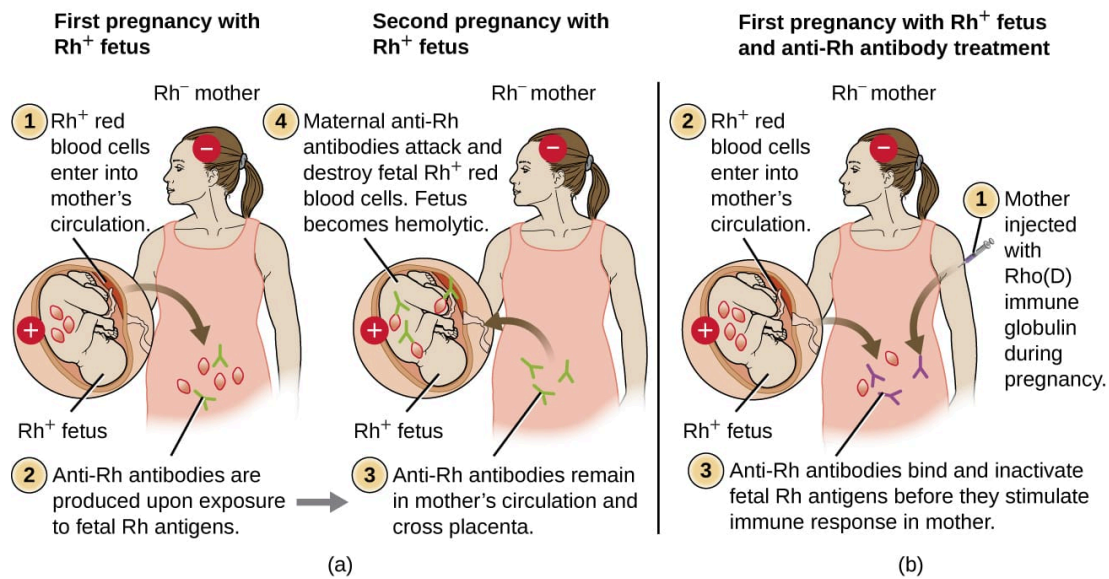


Figure 24.2.5: (a) When an Rh⁻ mother has an Rh⁺ fetus, fetal erythrocytes are introduced into the mother's circulatory system before or during birth, leading to production of anti-Rh IgG antibodies. These antibodies remain in the mother and, if she becomes pregnant with a second Rh⁺ baby, they can cross the placenta and attach to fetal Rh⁺ erythrocytes. Complement-mediated hemolysis of fetal erythrocytes results in a lack of sufficient cells for proper oxygenation of the fetus. (b) HDN can be prevented by administering Rho(D) immune globulin during and after each pregnancy with an Rh⁺ fetus. The immune globulin binds fetal Rh⁺ RBCs that gain access to the mother's bloodstream, preventing activation of her primary immune response.

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Link to Learning

Use this interactive [Blood Typing Game](#) to reinforce your knowledge of blood typing.

Key Concepts and Summary

- **Type II hypersensitivities** result from antibodies binding to antigens on cells and initiating cytotoxic responses. Examples include **hemolytic transfusion reaction** and **hemolytic disease of the newborn**

Footnotes

1. E.C. Vamvakas, M.A. Blajchman. "Transfusion-Related Mortality: The Ongoing Risks of Allogeneic Blood Transfusion and the Available Strategies for Their Prevention." *Blood* 113 no. 15 (2009):3406–3417.
2. G. Reali. "Forty Years of Anti-D Immunoprophylaxis." *Blood Transfusion* 5 no. 1 (2007):3–6.

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24.3: Type III Hypersensitivities

Learning Objectives

- Describe type III hypersensitivity and the different types
- Explain why antibody excess is important in type III hypersensitivity
- Compare and contrast Arthus reaction versus serum sickness

Type III Hypersensitivities

Type III hypersensitivities are immune-complex reactions that were first characterized by Nicolas Maurice Arthus (1862–1945) in 1903. To produce antibodies for experimental procedures, Arthus immunized rabbits by injecting them with serum from horses. However, while immunizing rabbits repeatedly with horse serum, Arthus noticed a previously unreported and unexpected localized subcutaneous hemorrhage with edema at the site of injection. This reaction developed within 3 to 10 hours after injection. This localized reaction to non-self serum proteins was called an **Arthus reaction**. An Arthus reaction occurs when soluble antigens bind with IgG in a ratio that results in the accumulation of antigen-antibody aggregates called **immune complexes**.

A unique characteristic of type III hypersensitivity is antibody excess (primarily IgG), coupled with a relatively low concentration of antigen, resulting in the formation of small immune complexes that deposit on the surface of the epithelial cells lining the inner lumen of small blood vessels or on the surfaces of tissues (Figure 24.3.6). This immune complex accumulation leads to a cascade of inflammatory events that include the following:

1. IgG binding to antibody receptors on localized mast cells, resulting in mast-cell degranulation
2. Complement activation with production of pro-inflammatory C3a and C5a (see [Chemical Defenses](#))
3. Increased blood-vessel permeability with chemotactic recruitment of neutrophils and macrophages

Because these immune complexes are not an optimal size and are deposited on cell surfaces, they cannot be phagocytosed in the usual way by neutrophils and macrophages, which, in turn, are often described as “frustrated.” Although phagocytosis does not occur, neutrophil degranulation results in the release of lysosomal enzymes that cause extracellular destruction of the immune complex, damaging localized cells in the process. Activation of coagulation pathways also occurs, resulting in thrombi (blood clots) that occlude blood vessels and cause ischemia that can lead to vascular necrosis and localized hemorrhage.

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Systemic type III hypersensitivity (serum sickness) occurs when immune complexes deposit in various body sites, resulting in a more generalized systemic inflammatory response. These immune complexes involve non-self proteins such as antibodies produced in animals for artificial passive immunity (see [Vaccines](#)), certain drugs, or microbial antigens that are continuously released over time during chronic infections (e.g., subacute bacterial endocarditis, chronic viral hepatitis). The mechanisms of serum sickness are similar to those described in localized type III hypersensitivity but involve widespread activation of mast cells, complement, neutrophils, and macrophages, which causes tissue destruction in areas such as the kidneys, joints, and blood vessels. As a result of tissue destruction, symptoms of serum sickness include chills, fever, rash, vasculitis, and arthritis. Development of glomerulonephritis or hepatitis is also possible.

Autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis can also involve damaging type III hypersensitivity reactions when auto-antibodies form immune complexes with self antigens. These conditions are discussed in

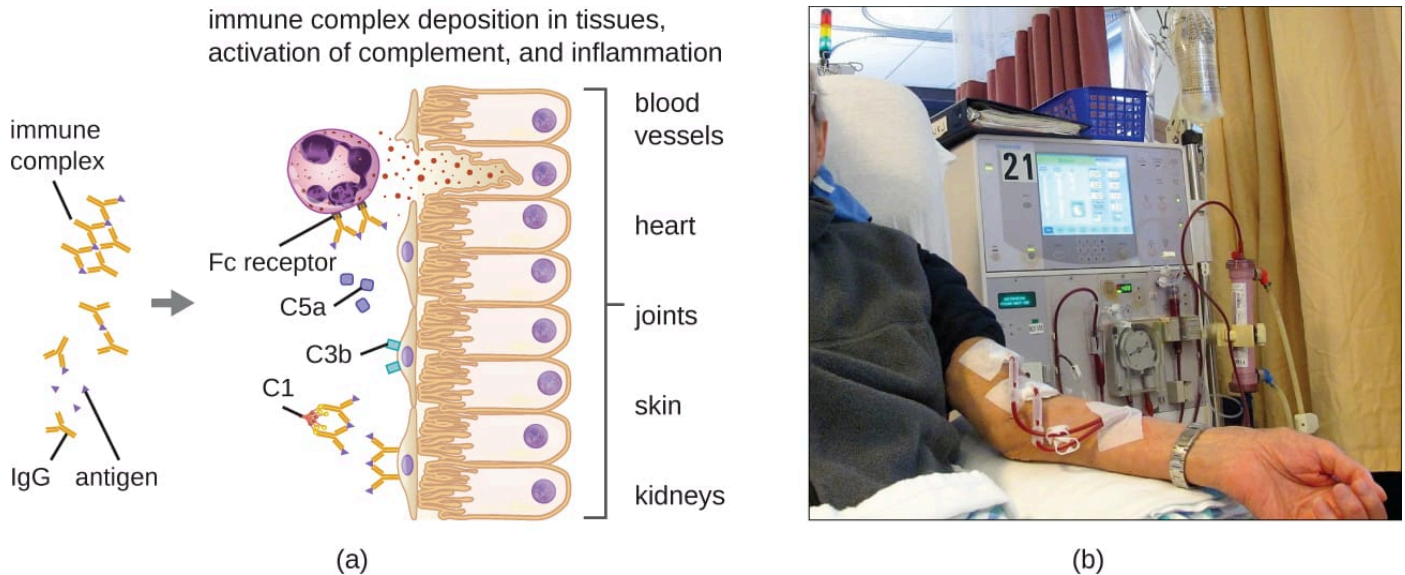


Figure 24.3.6: Type III hypersensitivities and the systems they affect. (a) Immune complexes form and deposit in tissue. Complement activation, stimulation of an inflammatory response, and recruitment and activation of neutrophils result in damage to blood vessels, heart tissue, joints, skin, and/or kidneys. (b) If the kidneys are damaged by a type III hypersensitivity reaction, dialysis may be required.

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Diphtheria Antitoxin

Antibacterial sera are much less commonly used now than in the past, having been replaced by toxoid vaccines. However, a diphtheria antitoxin produced in horses is one example of such a treatment that is still used in some parts of the world. Although it is not licensed by the FDA for use in the United States, diphtheria antitoxin can be used to treat cases of diphtheria, which are caused by the bacterium *Corynebacterium diphtheriae*. The treatment is not without risks, however. Serum sickness can occur when the patient develops an immune response to non-self horse proteins. Immune complexes are formed between the horse proteins and circulating antibodies when the two exist in certain proportions. These immune complexes can deposit in organs, causing damage such as arthritis, nephritis, rash, and fever. Serum sickness is usually transient with no permanent damage unless the patient is chronically exposed to the antigen, which can then result in irreversible damage to body sites such as joints and kidneys. Over time, phagocytic cells such as macrophages are able to clear the horse serum antigens, which results in improvement of the patient's condition and a decrease in symptoms as the immune response dissipates.

Key Concepts and Summary

- **Type III hypersensitivities** result from formation and accumulation of **immune complexes** in tissues, stimulating damaging inflammatory responses.

Footnotes

1. Centers for Disease Control and Prevention. "Diphtheria Antitoxin." <http://www.cdc.gov/diphtheria/dat.html>. Accessed March 25, 2016.

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24.4: Type IV Hypersensitivities

Learning Objectives

- Describe the three subtypes of type IV hypersensitivity
- Explain how T cells contribute to tissue damage in type IV hypersensitivity

Type IV Hypersensitivities

Type IV hypersensitivities are not mediated by antibodies like the other three types of hypersensitivities. Rather, type IV hypersensitivities are regulated by **T cells** and involve the action of effector cells. These types of hypersensitivities can be organized into three subcategories based on T-cell subtype, type of antigen, and the resulting effector mechanism (Table 24.4.5).

In the first type IV subcategory, CD4 T_H1 -mediated reactions are described as delayed-type hypersensitivities (DTH). The sensitization step involves the introduction of antigen into the skin and phagocytosis by local antigen presenting cells (APCs). The APCs activate helper T cells, stimulating clonal proliferation and differentiation into memory T_H1 cells. Upon subsequent exposure to the antigen, these sensitized memory T_H1 cells release cytokines that activate macrophages, and activated macrophages are responsible for much of the tissue damage. Examples of this T_H1 -mediated hypersensitivity are observed in tuberculin the Mantoux skin test and **contact dermatitis**, such as occurs in latex allergy reactions.

In the second type IV subcategory, CD4 T_H2 -mediated reactions result in chronic asthma or chronic allergic rhinitis. In these cases, the soluble antigen is first inhaled, resulting in eosinophil recruitment and activation with the release of cytokines and inflammatory mediators.

In the third type IV subcategory, CD8 cytotoxic T lymphocyte (CTL)-mediated reactions are associated with tissue transplant rejection and contact dermatitis (Figure 24.4.7). For this form of cell-mediated hypersensitivity, APCs process and present the antigen with MHC I to naïve CD8 T cells. When these naïve CD8 T cells are activated, they proliferate and differentiate into CTLs. Activated T_H1 cells can also enhance the activation of the CTLs. The activated CTLs then target and induce granzyme-mediated apoptosis in cells presenting the same antigen with MHC I. These target cells could be “self” cells that have absorbed the foreign antigen (such as with contact dermatitis due to poison ivy), or they could be transplanted tissue cells displaying foreign antigen from the donor.

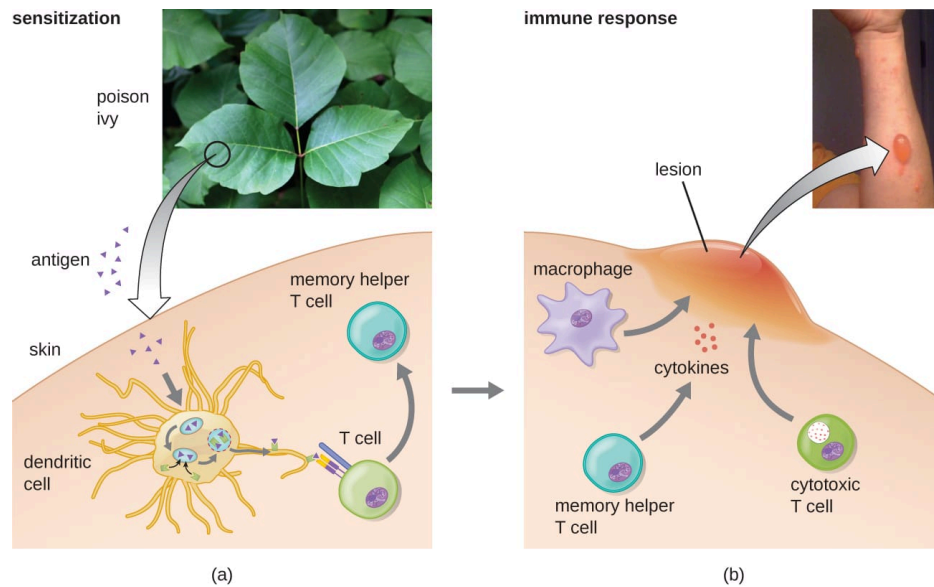


Figure 24.4.7: Exposure to hapten antigens in poison ivy can cause contact dermatitis, a type IV hypersensitivity. (a) The first exposure to poison ivy does not result in a reaction. However, sensitization stimulates helper T cells, leading to production of memory helper T cells that can become reactivated on future exposures. (b) Upon secondary exposure, the memory helper T cells become reactivated, producing inflammatory cytokines that stimulate macrophages and cytotoxic T cells to induce an inflammatory lesion at the exposed site. This lesion, which will persist until the allergen is removed, can inflict significant tissue damage if it continues long enough.

Table 24.4.5: Type IV Hypersensitivities

Subcategory	Antigen	Effector Mechanism	Examples
1	Soluble antigen	Activated macrophages damage tissue and promote inflammatory response	Contact dermatitis (e.g., exposure to latex) and delayed-type hypersensitivity (e.g., tuberculin reaction)
2	Soluble antigen	Eosinophil recruitment and activation release cytokines and pro-inflammatory chemicals	Chronic asthma and chronic allergic rhinitis
3	Cell-associated antigen	CTL-mediated cytotoxicity	Contact dermatitis (e.g., contact with poison ivy) and tissue-transplant rejection

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Using Delayed Hypersensitivity to Test for TB

Austrian pediatrician Clemens von Pirquet (1874–1929) first described allergy mechanisms, including type III serum sickness.⁹ His interest led to the development of a test for tuberculosis (TB), using the tuberculin antigen, based on earlier work identifying the TB pathogen performed by Robert Koch. Pirquet's method involved scarification, which results in simultaneous multiple punctures, using a device with an array of needles to break the skin numerous times in a small area. The device Pirquet used was similar to the tine test device with four needles seen in Figure 24.4.8.

The tips of all the needles in the array are coated with tuberculin, a protein extract of TB bacteria, effectively introducing the tuberculin into the skin. One to 3 days later, the area can be examined for a delayed hypersensitivity reaction, signs of which include swelling and redness.

As you can imagine, scarification was not a pleasant experience,¹⁰ and the numerous skin punctures put the patient at risk of developing bacterial infection of the skin. Mantoux modified Pirquet's test to use a single subcutaneous injection of purified tuberculin material. A positive test, which is indicated by a delayed localized swelling at the injection site, does not necessarily mean that the patient is currently infected with active TB. Because type IV (delayed-type) hypersensitivity is mediated by reactivation of memory T cells, such cells may have been created recently (due to an active current infection) or years prior (if a patient had TB and had spontaneously cleared it, or if it had gone into latency). However, the test can be used to confirm infection in cases in which symptoms in the patient or findings on a radiograph suggest its presence.



Figure 24.4.8: The modern version of Pirquet's scarification is the tine test, which uses devices like this to administer tuberculin antigen into the skin, usually on the inside of the forearm. The tine test is considered less reliable than the Mantoux test. (credit: modification of work by the Centers for Disease Control and Prevention)

Key Concepts and Summary

- **Type IV hypersensitivities** are not mediated by antibodies, but by helper T-cell activation of macrophages, eosinophils, and cytotoxic T cells.

Footnotes

1. B. Huber "100 Jahre Allergie: Clemens von Pirquet—sein Allergiebegriff und das ihm zugrunde liegende Krankheitsverständnis." *Wiener Klinische Wochenschrift* 118 no. 19–20 (2006):573–579.
2. C.A. Stewart. "The Pirquet Test: Comparison of the Scarification and the Puncture Methods of Application." *Archives of Pediatrics & Adolescent Medicine* 35 no. 3 (1928):388–391.

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24.5: Hypersensitivity Pneumonitis and Summary

Learning Objectives

- Explain why hypersensitivity pneumonitis is considered an occupational disease.
- Summarize the similarities and differences among different types of hypersensitivity.

Hypersensitivity Pneumonitis

Some disease caused by hypersensitivities are not caused exclusively by one type. For example, hypersensitivity pneumonitis (HP), which is often an occupational or environmental disease, occurs when the lungs become inflamed due to an allergic reaction to inhaled dust, endospores, bird feathers, bird droppings, molds, or chemicals. HP goes by many different names associated with various forms of exposure (Figure 24.5.9). HP associated with bird droppings is sometimes called pigeon fancier's lung or poultry worker's lung—both common in bird breeders and handlers. Cheese handler's disease, farmer's lung, sauna takers' disease, and hot-tub lung are other names for HP associated with exposure to molds in various environments.

Pathology associated with HP can be due to both type III (mediated by immune complexes) and type IV (mediated by T_H1 cells and macrophages) hypersensitivities. Repeated exposure to allergens can cause alveolitis due to the formation of immune complexes in the alveolar wall of the lung accompanied by fluid accumulation, and the formation of granulomas and other lesions in the lung as a result of T_H1 -mediated macrophage activation. Alveolitis with fluid and granuloma formation results in poor oxygen perfusion in the alveoli, which, in turn, can cause symptoms such as coughing, dyspnea, chills, fever, sweating, myalgias, headache, and nausea. Symptoms may occur as quickly as 2 hours after exposure and can persist for weeks if left untreated.



(a)



(b)

Figure 24.5.9: Occupational exposure to dust, mold, and other allergens can result in hypersensitivity pneumonitis. (a) People exposed daily to large numbers of birds may be susceptible to poultry worker's lung. (b) Workers in a cheese factory may become sensitized to different types of molds and develop cheese handler's disease. (credit a: modification of work by The Global Orphan Project)

Figure 24.5.10 summarizes the mechanisms and effects of each type of hypersensitivity discussed in this section.

Hypersensitivity Types and Their Mechanisms				
	Type I	Type II	Type III	Type IV
Immune reactant	IgE	IgG or IgM	IgG and IgM	T cells
Antigen form	Soluble antigen	Cell-bound antigen	Soluble antigen	Soluble or cell-bound antigen
Mechanism of activation	Allergen-specific IgE antibodies bind to mast cells via their Fc receptor. When the specific allergen binds to the IgE, cross-linking of IgE induces degranulation of mast cells.	IgG or IgM antibody binds to cellular antigen, leading to complement activation and cell lysis. IgG can also mediate ADCC with cytotoxic T cells, natural killer cells, macrophages, and neutrophils.	Antigen-antibody complexes are deposited in tissues. Complement activation provides inflammatory mediators and recruits neutrophils. Enzymes released from neutrophils damage tissue.	T _H 1 cells secrete cytokines, which activate macrophages and cytotoxic T cells.
Examples of hypersensitivity reactions	Local and systemic anaphylaxis, seasonal hay fever, food allergies, and drug allergies	Red blood cell destruction after transfusion with mismatched blood types or during hemolytic disease of the newborn.	Post-streptococcal glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus	Contact dermatitis, type I diabetes mellitus, and multiple sclerosis

Figure 24.5.10: Components of the immune system cause four types of hypersensitivities. Notice that types I-III are B-cell/antibody-mediated hypersensitivities, whereas type IV hypersensitivity is exclusively a T-cell phenomenon.

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24.6: Diagnosis and Treatment of Hypersensitivities

Learning Objectives

- Describe the prick puncture skin test
- Explain why type III hypersensitivities can be difficult to diagnose
- Describe desensitization therapy
- Explain the role of epinephrine in treatment of hypersensitivity reactions

Diagnosis of Hypersensitivities

Diagnosis of type I hypersensitivities is a complex process requiring several diagnostic tests in addition to a well-documented patient history. Serum IgE levels can be measured, but elevated IgE alone does not confirm allergic disease. As part of the process to identify the antigens responsible for a type I reaction allergy, testing through a prick puncture skin test (PPST) or an intradermal test can be performed. PPST is carried out with the introduction of allergens in a series of superficial skin pricks on the patient's back or arms (Figure 24.6.11). PPSTs are considered to be the most convenient and least expensive way to diagnose allergies, according to the US Joint Council of Allergy and the European Academy of Allergy and Immunology. The second type of testing, the intradermal test, requires injection into the dermis with a small needle. This needle, also known as a tuberculin needle, is attached to a syringe containing a small amount of allergen. Both the PPST and the intradermal tests are observed for 15–20 minutes for a wheal-flare reaction to the allergens. Measurement of any wheal (a raised, itchy bump) and flare (redness) within minutes indicates a type I hypersensitivity, and the larger the wheal-flare reaction, the greater the patient's sensitivity to the allergen.

Type III hypersensitivities can often be misdiagnosed because of their nonspecific inflammatory nature. The symptoms are easily visible, but they may be associated with any of a number of other diseases. A strong, comprehensive patient history is crucial to proper and accurate diagnosis. Tests used to establish the diagnosis of hypersensitivity pneumonitis (resulting from type III hypersensitivity) include bronchoalveolar lavage (BAL), pulmonary function tests, and high-resolution computed tomography (HRCT).



Figure 24.6.11: Results of an allergy skin-prick test to test for type I hypersensitivity to a group of potential allergens. A positive result is indicated by a raised area (wheal) and surrounding redness (flare). (credit: modification of work by "OakleyOriginals"/Flickr)

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Treatments of Hypersensitivities

Allergic reactions can be treated in various ways. Prevention of allergic reactions can be achieved by desensitization(hyposensitization) therapy, which can be used to reduce the hypersensitivity reaction through repeated injections of allergens. Extremely dilute concentrations of known allergens (determined from the allergen tests) are injected into the patient at prescribed intervals (e.g., weekly). The quantity of allergen delivered by the shots is slowly increased over a buildup period until an effective dose is determined and that dose is maintained for the duration of treatment, which can last years. Patients are usually encouraged to remain in the doctor's office for 30 minutes after receiving the injection in case the allergens administered cause a severe systemic reaction. Doctors' offices that administer desensitization therapy must be prepared to provide resuscitation and drug treatment in the case of such an event.

Desensitization therapy is used for insect sting allergies and environmental allergies. The allergy shots elicit the production of different interleukins and IgG antibody responses instead of IgE. When excess allergen-specific IgG antibodies are produced and bind to the allergen, they can act as blocking antibodies to neutralize the allergen before it can bind IgE on mast cells. There are early studies using oral therapy for desensitization of food allergies that are promising.¹¹¹² These studies involve feeding children who have allergies tiny amounts of the allergen (e.g., peanut flour) or related proteins over time. Many of the subjects show reduced severity of reaction to the food allergen after the therapy.

There are also therapies designed to treat severe allergic reactions. Emergency systemic anaphylaxis is treated initially with an epinephrine injection, which can counteract the drop in blood pressure. Individuals with known severe allergies often carry a self-administering auto-injector that can be used in case of exposure to the allergen (e.g., an insect sting or accidental ingestion of a food that causes a severe reaction). By self-administering an epinephrine shot (or sometimes two), the patient can stem the reaction long enough to seek medical attention. Follow-up treatment generally involves giving the patient antihistamines and slow-acting corticosteroids for several days after the reaction to prevent potential late-phase reactions. However, the effects of antihistamine and corticosteroid treatment are not well studied and are used based on theoretical considerations.

Treatment of milder allergic reactions typically involves antihistamines and other anti-inflammatory drugs. A variety of antihistamine drugs are available, in both prescription and over-the-counter strengths. There are also antileukotriene and antiprostaglandin drugs that can be used in tandem with antihistamine drugs in a combined (and more effective) therapy regime.

Treatments of type III hypersensitivities include preventing further exposure to the antigen and the use of anti-inflammatory drugs. Some conditions can be resolved when exposure to the antigen is prevented. Anti-inflammatory corticosteroid inhalers can also be used to diminish inflammation to allow lung lesions to heal. Systemic corticosteroid treatment, oral or intravenous, is also common for type III hypersensitivities affecting body systems. Treatment of hypersensitivity pneumonitis includes avoiding the allergen, along with the possible addition of prescription steroids such as prednisone to reduce inflammation.

Treatment of type IV hypersensitivities includes antihistamines, anti-inflammatory drugs, analgesics, and, if possible, eliminating further exposure to the antigen.

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Key Concepts and Summary

- An **allergy** is an adaptive immune response, sometimes life-threatening, to an **allergen**.
- **Type I hypersensitivity** requires sensitization of mast cells with IgE, involving an initial IgE antibody response and IgE attachment to mast cells. On second exposure to an allergen, cross-linking of IgE molecules on mast cells triggers degranulation and release of preformed and newly formed chemical mediators of inflammation. Type I hypersensitivity may be localized and relatively minor (hives and hay fever) or system-wide and dangerous (systemic **anaphylaxis**).
- **Type II hypersensitivities** result from antibodies binding to antigens on cells and initiating cytotoxic responses. Examples include **hemolytic transfusion reaction** and **hemolytic disease of the newborn**.
- **Type III hypersensitivities** result from formation and accumulation of **immune complexes** in tissues, stimulating damaging inflammatory responses.
- **Type IV hypersensitivities** are not mediated by antibodies, but by helper T-cell activation of macrophages, eosinophils, and cytotoxic T cells.

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24.E: Diseases of the Immune System (Exercises)

24.1: Hypersensitivities

An allergy is an adaptive immune response, sometimes life-threatening, to an allergen. Hypersensitivity reactions are classified by their immune mechanism.

Multiple Choice

Which of the following is the type of cell largely responsible for type I hypersensitivity responses?

- A. erythrocyte
- B. mast cell
- C. T lymphocyte
- D. antibody

Answer

B

Type I hypersensitivities require which of the following initial priming events to occur?

- A. sensitization
- B. secondary immune response
- C. cellular trauma
- D. degranulation

Answer

A

Which of the following are the main mediators/initiators of type II hypersensitivity reactions?

- A. antibodies
- B. mast cells
- C. erythrocytes
- D. histamines

Answer

A

Inflammatory molecules are released by mast cells in type I hypersensitivities; type II hypersensitivities, however, are characterized by which of the following?

- A. cell lysis (cytotoxicity)
- B. strong antibody reactions against antigens
- C. leukotriene release upon stimulation
- D. localized tissue reactions, such as hives

Answer

A

An immune complex is an aggregate of which of the following?

- A. antibody molecules
- B. antigen molecules
- C. antibody and antigen molecules
- D. histamine molecules

Answer

C

Which of the following is a common treatment for type III hypersensitivity reactions?

- A. anti-inflammatory steroid treatments
- B. antihistamine treatments
- C. hyposensitization injections of allergens
- D. RhoGAM injections

Answer

A

Which of the following induces a type III hypersensitivity?

- A. release of inflammatory molecules from mast cells
- B. accumulation of immune complexes in tissues and small blood vessels
- C. destruction of cells bound by antigens
- D. destruction of cells bound by antibodies

Answer

B

Which one of the following is not an example of a type IV hypersensitivity?

- A. latex allergy
- B. Contact dermatitis (e.g., contact with poison ivy)
- C. a positive tuberculin skin test
- D. hemolytic disease of the newborn

Answer

D

Fill in the Blank

Antibodies involved in type I hypersensitivities are of the _____ class.

Answer

IgE

Allergy shots work by shifting antibody responses to produce _____ antibodies.

Answer

IgG

A person who is blood type A would have IgM hemagglutinin antibodies against type _____ red blood cells in their plasma.

Answer

B

The itchy and blistering rash that develops with contact to poison ivy is caused by a type _____ hypersensitivity reaction.

Answer

IV

Short Answer

Although both type I and type II hypersensitivities involve antibodies as immune effectors, different mechanisms are involved with these different hypersensitivities. Differentiate the two.

What types of antibodies are most common in type III hypersensitivities, and why?

Critical Thinking

Patients are frequently given instructions to avoid allergy medications for a period of time prior to allergy testing. Why would this be important?

In some areas of the world, a tuberculosis vaccine known as bacillus Calmette-Guérin (BCG) is used. It is not used in the United States. Every person who has received this vaccine and mounted a protective response will have a positive reaction in a tuberculin skin test. Why? What does this mean for the usefulness of this skin test in those countries where this vaccine is used?

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25: DISEASES OF THE IMMUNE SYSTEM

The immune system is designed to protect the body from harmful pathogens and maintain homeostasis. However, in some cases, this vital defense system malfunctions, leading to immune responses that can harm the body rather than protect it. Autoimmune disorders occur when the immune system mistakenly targets the body's own tissues, resulting in chronic inflammation and damage. Conditions such as rheumatoid arthritis, type 1 diabetes, and systemic lupus erythematosus are examples of autoimmune diseases that arise from this self-reactivity.

In addition to autoimmune conditions, this chapter will examine the challenges of organ transplantation, where the immune system recognizes transplanted tissues as foreign and may attack them, leading to graft rejection.

Finally, we will explore immunodeficiencies, where the immune system's ability to fight infections is weakened or absent, leaving individuals vulnerable to opportunistic pathogens. Immunodeficiency can be inherited, as in primary immunodeficiency disorders, or acquired, as in HIV/AIDS. These diverse topics highlight the delicate balance of the immune system and the profound consequences when that balance is disrupted.

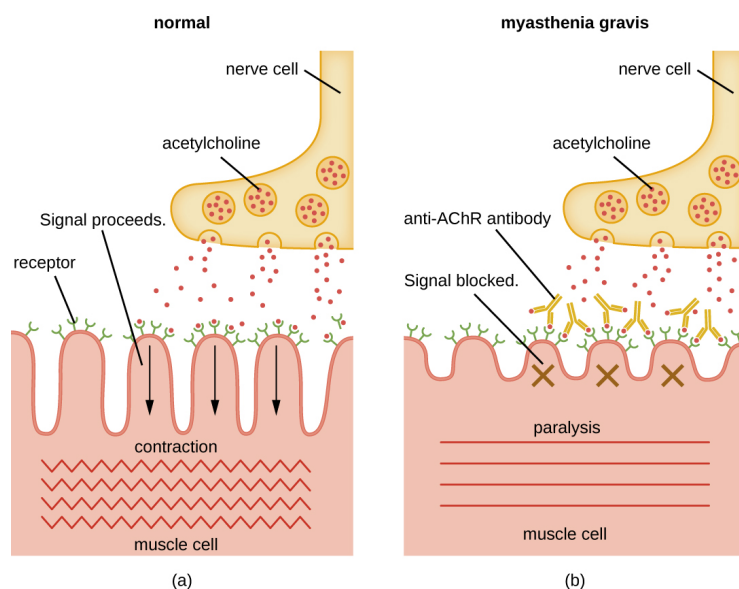


Figure 25.1: Myasthenia gravis and impaired muscle contraction. (a) Normal release of the neurotransmitter acetylcholine stimulates muscle contraction. (b) In myasthenia gravis, autoantibodies block the receptors for acetylcholine (AChR) on muscle cells, resulting in paralysis.

25.1: Organ-Specific Autoimmune Disorders

25.2: Systemic Autoimmune Disorders

25.3: Organ Transplantation and Rejection

25.4: Immunodeficiency

25.E: Diseases of the Immune System (Exercises)

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25.1: Organ-Specific Autoimmune Disorders

Learning Objectives

- Explain why autoimmune disorders develop
- Name the organ-specific autoimmune disorders and the organs that they affect
- Describe how the mechanisms of Graves disease and Hashimoto thyroiditis differ
- Name the cells that are destroyed in type 1 diabetes mellitus and describe the result

In 1970, artist Walt Kelly developed a poster promoting Earth Day, featuring a character from *Pogo*, his daily newspaper comic strip. In the poster, Pogo looks out across a litter-strewn forest and says wryly, “We have met the enemy and he is us.” Pogo was not talking about the human immune system, but he very well could have been. Although the immune system protects the body by attacking invading “enemies” (pathogens), in some cases, the immune system can mistakenly identify the body’s own cells as the enemy, resulting in **autoimmune disease**.

Autoimmune diseases are those in which the body is attacked by its own specific adaptive immune response. In normal, healthy states, the immune system induces **tolerance**, which is a lack of an anti-self immune response. However, with autoimmunity, there is a loss of immune tolerance, and the mechanisms responsible for autoimmune diseases include type II, III, and IV hypersensitivity reactions. Autoimmune diseases can have a variety of mixed symptoms that flare up and disappear, making diagnosis difficult.

The causes of autoimmune disease are a combination of the individual’s genetic makeup and the effect of environmental influences, such as sunlight, infections, medications, and environmental chemicals. However, the vagueness of this list reflects our poor understanding of the etiology of these diseases. Except in a very few specific diseases, the initiation event(s) of most autoimmune states has not been fully characterized.

There are several possible causes for the origin of autoimmune diseases and autoimmunity is likely due to several factors. Evidence now suggests that regulatory T and B cells play an essential role in the maintenance of tolerance and prevention of autoimmune responses. The regulatory T cells are especially important for inhibiting autoreactive T cells that are not eliminated during thymic selection and escape the thymus (see [T Lymphocytes and Cellular Immunity](#)). In addition, antigen mimicry between pathogen antigens and our own self antigens can lead to cross-reactivity and autoimmunity. Hidden self-antigens may become exposed because of trauma, drug interactions, or disease states, and trigger an autoimmune response. All of these factors could contribute to autoimmunity. Ultimately, damage to tissues and organs in the autoimmune disease state comes as a result of inflammatory responses that are inappropriate; therefore, treatment often includes immunosuppressive drugs and corticosteroids.

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Organ-Specific Autoimmune Diseases

Some autoimmune diseases are considered organ specific, meaning that the immune system targets specific organs or tissues. Examples of organ-specific autoimmune diseases include celiac disease, Graves disease, Hashimoto thyroiditis, type I diabetes mellitus, and Addison disease.

Celiac Disease

Celiac disease is largely a disease of the small intestine, although other organs may be affected. People in their 30s and 40s, and children are most commonly affected, but celiac disease can begin at any age. It results from a reaction to proteins, commonly called gluten, found mainly in wheat, barley, rye, and some other grains. The disease has several genetic causes (predispositions) and poorly understood environmental influences. On exposure to gluten, the body produces various autoantibodies and an inflammatory response. The inflammatory response in the small intestine leads to a reduction in the depth of the microvilli of the mucosa, which hinders absorption and can lead to weight loss and anemia. The disease is also characterized by diarrhea and abdominal pain, symptoms that are often misdiagnosed as irritable bowel syndrome.

Diagnosis of celiac disease is accomplished from serological tests for the presence of primarily IgA antibodies to components of gluten, the transglutaminase enzyme, and autoantibodies to endomysium, a connective tissue surrounding muscle fibers. Serological tests are typically followed up with endoscopy and biopsy of the duodenal mucosa. Serological screening surveys have found about 1% of individuals in the United Kingdom are positive even though they do not all display symptoms.¹ This early recognition allows for more careful monitoring and prevention of severe disease.

Celiac disease is treated with complete removal of gluten-containing foods from the diet, which results in improved symptoms and reduced risk of complications. Other theoretical approaches include breeding grains that do not contain the immunologically reactive components or developing dietary supplements that contain enzymes that break down the protein components that cause the immune response.²

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Disorders of the Thyroid

Graves disease is the most common cause of hyperthyroidism in the United States. Symptoms of Graves disease result from the production of thyroid-stimulating immunoglobulin (TSI) also called TSH-receptor antibody. TSI targets and binds to the receptor for thyroid stimulating hormone (TSH), which is naturally produced by the pituitary gland. TSI may cause conflicting symptoms because it may stimulate the thyroid to make too much thyroid hormone or block thyroid hormone production entirely, making diagnosis more difficult. Signs and symptoms of Graves disease include heat intolerance, rapid and irregular heartbeat, weight loss, goiter (a swollen thyroid gland, protruding under the skin of the throat [Figure 25.1.1]) and exophthalmia (bulging eyes) often referred to as Graves ophthalmopathy (Figure 25.1.2).



Figure 25.1.1: Goiter, a hypertrophy of the thyroid, is a symptom of Graves disease and Hashimoto thyroiditis.

The most common cause of hypothyroidism in the United States is **Hashimoto thyroiditis**, also called chronic lymphocytic thyroiditis. Patients with Hashimoto thyroiditis often develop a spectrum of different diseases because they are more likely to develop additional autoimmune diseases such as Addison disease (discussed later in this section), type 1 diabetes, rheumatoid arthritis, and celiac disease. Hashimoto thyroiditis is a T_H1 cell-mediated disease that occurs when the thyroid gland is attacked by cytotoxic lymphocytes, macrophages, and autoantibodies. This autoimmune response leads to numerous symptoms that include goiter (Figure 25.1.1), cold intolerance, muscle weakness, painful and stiff joints, depression, and memory loss.



Figure 25.1.2: Exophthalmia, or Graves ophthalmopathy, is a sign of Graves disease. (credit: modification of work by Jonathan Trobe, University of Michigan Kellogg Eye Center)

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Type 1 Diabetes

Juvenile diabetes, or **type 1 diabetes mellitus**, is usually diagnosed in children and young adults. It is a T-cell-dependent autoimmune disease characterized by the selective destruction of the β cells of the islets of Langerhans in the pancreas by CD4 T_H1 -mediated CD8 T cells, anti- β -cell antibodies, and macrophage activity. There is also evidence that viral infections can have either a potentiating or inhibitory role in the development of type 1 diabetes (T1D) mellitus. The destruction of the β cells causes a lack of insulin production by the pancreas. In T1D, β -cell destruction may take place over several years, but symptoms of hyperglycemia, extreme increase in thirst and urination, weight loss, and extreme fatigue usually have a sudden onset, and diagnosis usually does not occur until most β cells have already been destroyed.

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Autoimmune Addison Disease

Destruction of the adrenal glands (the glands lying above the kidneys that produce glucocorticoids, mineralocorticoids, and sex steroids) is the cause of **Addison disease**, also called primary adrenal insufficiency (PAI). Today, up to 80% of Addison disease cases are diagnosed as autoimmune Addison disease (AAD), which is caused by an autoimmune response to adrenal tissues disrupting adrenal function. Disruption of adrenal function causes impaired metabolic processes that require normal steroid hormone levels, causing signs and symptoms throughout the body. There is evidence that both humoral and CD4 T_H1 -driven CD8 T-cell-mediated immune mechanisms are directed at the adrenal cortex in AAD. There is also evidence that the autoimmune response is associated with autoimmune destruction of other endocrine glands as well, such as the pancreas and thyroid, conditions collectively referred to as autoimmune polyendocrine syndromes (APS). In up to 80% of patients with AAD, antibodies are produced to three enzymes involved in steroid synthesis: 21-hydroxylase (21-OH), 17 α -hydroxylase, and cholesterol side-chain-cleaving enzyme.³ The most common autoantibody found in AAD is to 21-OH, and antibodies to any of the key enzymes for steroid production are diagnostic for AAD. The adrenal cortex cells are targeted, destroyed, and replaced with fibrous tissue by immune-mediated inflammation. In some patients, at least 90% of the adrenal cortex is destroyed before symptoms become diagnostic.

Symptoms of AAD include weakness, nausea, decreased appetite, weight loss, hyperpigmentation (Figure 25.1.3), hyperkalemia (elevated blood potassium levels), hyponatremia (decreased blood sodium levels), hypoglycemia (decreased levels of blood sugar), hypotension (decreased blood pressure), anemia, lymphocytosis (decreased levels of white blood cells), and fatigue. Under extreme stress, such as surgery, accidental trauma, or infection, patients with AAD may experience an adrenal crisis that causes the patient to vomit, experience abdominal pain, back or leg cramps, and even severe hypotension leading to shock.



Figure 25.1.3: Hyperpigmentation is a sign of Addison disease. (credit: modification of work by Petros Perros)

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Interactive Element

Case Study Preview: “Sun-Kissed and Misdiagnosed: Kerry’s Autoimmune Awakening”

Kerry, a 40-year-old pilot, notices a strange rash that flares up after sun exposure - not quite a sunburn, but red, raised, and persistent. Initially puzzled, her doctor orders lab tests that uncover protein and hemoglobin in her urine, along with signs of hemolytic anemia. Before she can follow up with a specialist, Kerry has a seizure, leading to urgent testing for autoantibodies. The diagnosis: systemic lupus erythematosus (SLE), an autoimmune disease that affects multiple organs through type III hypersensitivity reactions.

This case explores the challenges of diagnosing autoimmune disorders like SLE, the role of antinuclear and anti-Sm antibodies, and why symptoms like photosensitivity, butterfly rash, and seizures are classic red flags. You'll also learn how treatments like corticosteroids and hydroxychloroquine help control flare-ups and reduce inflammation - even though there's no cure.

When the immune system turns on the self, how do you read the signs? And why doesn't sunscreen help when it's lupus, not sunburn?

[Chapter 19 Case Study - Sun-Kissed and Misdiagnosed: Kerry's Autoimmune Awakening](#)

Key Concepts and Summary

- **Autoimmune diseases** result from a breakdown in immunological tolerance. The actual induction event(s) for autoimmune states are largely unknown.
- Some autoimmune diseases attack specific organs, whereas others are more systemic.
- Organ-specific autoimmune diseases include **celiac disease**, **Graves disease**, **Hashimoto thyroiditis**, **type I diabetes mellitus**, and **Addison disease**.

Footnotes

1. [D.A. Van Heel, J. West. "Recent Advances in Coeliac Disease." *Gut* 55 no. 7 \(2006\):1037–1046.](#)
2. [ibid.](#)
3. [P. Martorell et al. "Autoimmunity in Addison's Disease." *Netherlands Journal of Medicine* 60 no. 7 \(2002\):269–275.](#)

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25.2: Systemic Autoimmune Disorders

Learning Objectives

- List the ways antibodies contribute to the pathogenesis of myasthenia gravis
- Explain why rheumatoid arthritis is considered a type III hypersensitivity
- Describe the symptoms of systemic lupus erythematosus and explain why they affect so many different parts of the body

Systemic Autoimmune Diseases

Whereas organ-specific autoimmune diseases target specific organs or tissues, systemic autoimmune diseases are more generalized, targeting multiple organs or tissues throughout the body. Examples of **systemic autoimmune diseases** include multiple sclerosis, myasthenia gravis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune central nervous system disease that affects the brain and spinal cord. Lesions in multiple locations within the central nervous system are a hallmark of multiple sclerosis and are caused by infiltration of immune cells across the blood-brain barrier. The immune cells include T cells that promote inflammation, demyelination, and neuron degeneration, all of which disrupt neuronal signaling. Symptoms of MS include visual disturbances; muscle weakness; difficulty with coordination and balance; sensations such as numbness, prickling, or “pins and needles”; and cognitive and memory problems.

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Myasthenia Gravis

Autoantibodies directed against acetylcholine receptors (AChRs) in the synaptic cleft of neuromuscular junctions lead to **myasthenia gravis** (Figure 25.2.4). Anti-AChR antibodies are high-affinity IgGs and their synthesis requires activated CD4 T cells to interact with and stimulate B cells. Once produced, the anti-AChR antibodies affect neuromuscular transmission by at least three mechanisms:

- Complement binding and activation at the neuromuscular junction
- Accelerated AChR endocytosis of molecules cross-linked by antibodies
- Functional AChR blocking, which prevents normal acetylcholine attachment to, and activation of, AChR

Regardless of the mechanism, the effect of anti-AChR is extreme muscle weakness and potentially death through respiratory arrest in severe cases.

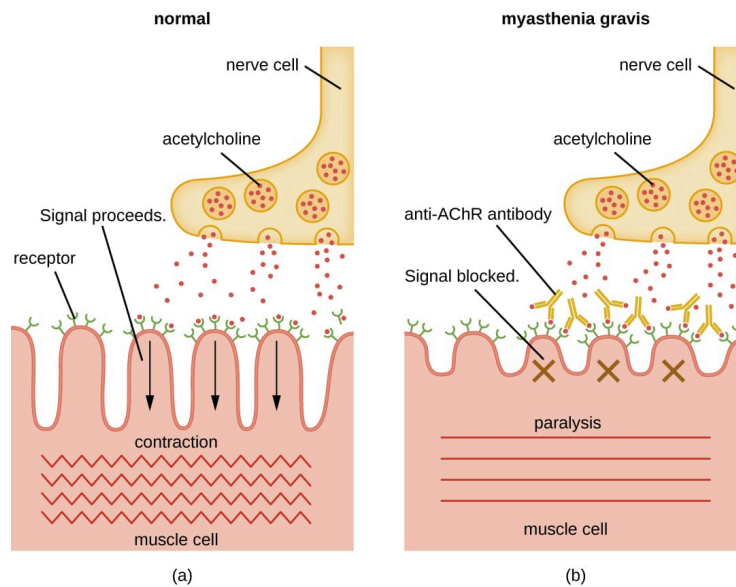


Figure 25.2.4: Myasthenia gravis and impaired muscle contraction. (a) Normal release of the neurotransmitter acetylcholine stimulates muscle contraction. (b) In myasthenia gravis, autoantibodies block the receptors for acetylcholine (AChR) on muscle cells, resulting in paralysis.

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Psoriasis

Psoriasis is a skin disease that causes itchy or sore patches of thick, red skin with silvery scales on elbows, knees, scalp, back, face, palms, feet, and sometimes other areas. Some individuals with **psoriasis** also get a form of arthritis called psoriatic arthritis, in which the joints can become inflamed. Psoriasis results from the complex interplay between keratinocytes, dendritic cells, and T cells, and the cytokines produced by these various cells. In a process called cell turnover, skin cells that grow deep in the skin rise to the surface. Normally, this process takes a month. In psoriasis, as a result of cytokine activation, cell turnover happens in just a few days. The thick inflamed patches of skin that are characteristic of psoriasis develop because the skin cells rise too fast.

Rheumatoid Arthritis

The most common chronic inflammatory joint disease is **rheumatoid arthritis (RA)** (Figure 25.2.5) and it is still a major medical challenge because of unsolved questions related to the environmental and genetic causes of the disease. RA involves type III hypersensitivity reactions and the activation of CD4 T cells, resulting in chronic release of the inflammatory cytokines IL-1, IL-6, and tumor necrosis factor- α (TNF- α). The activated CD4 T cells also stimulate the production of rheumatoid factor (RF) antibodies and anticyclic citrullinated peptide antibodies (anti-CCP) that form immune complexes. Increased levels of acute-phase proteins, such as C-reactive protein (CRP), are also produced as part of the inflammatory process and participate in complement fixation with the antibodies on the immune complexes. The formation of immune complexes and reaction to the immune factors cause an inflammatory process in joints, particularly in the hands, feet, and legs. Diagnosis of RA is based on elevated levels of RF, anti-CCP, quantitative CRP, and the erythrocyte sedimentation rate (ESR) (modified Westergren). In addition, radiographs, ultrasound, or magnetic resonance imaging scans can identify joint damage, such as erosions, a loss of bone within the joint, and narrowing of joint space.



Figure 25.2.5: The radiograph (left) and photograph (right) show damage to the hands typical of rheumatoid arthritis. (credit right: modification of work by "handarmdoc"/Flickr)

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Systemic Lupus Erythematosus

The damage and pathology of **systemic lupus erythematosus (SLE)** is caused by type III hypersensitivity reactions. Autoantibodies produced in SLE are directed against nuclear and cytoplasmic proteins. Anti-nuclear antibodies (ANAs) are present in more than 95% of patients with SLE,⁴ with additional autoantibodies including anti-double-stranded DNA (ds-DNA) and anti-Sm antibodies (antibodies to small nuclear ribonucleoprotein). Anti-ds-DNA and anti-Sm antibodies are unique to patients with SLE; thus, their presence is included in the classification criteria of SLE. Cellular interaction with autoantibodies leads to nuclear and cellular destruction, with components released after cell death leading to the formation of immune complexes.

Because autoantibodies in SLE can target a wide variety of cells, symptoms of SLE can occur in many body locations. However, the most common symptoms include fatigue, fever with no other cause, hair loss, and a sunlight-sensitive "butterfly" or wolf-mask (lupus) rash that is found in about 50% of people with SLE (Figure 25.2.6). The rash is most often seen over the cheeks and bridge of the nose, but can be widespread. Other symptoms may appear depending on affected areas. The joints may be affected, leading to arthritis of the fingers, hands, wrists, and knees. Effects on the brain and nervous system can lead to headaches, numbness, tingling, seizures, vision problems, and personality changes. There may also be abdominal pain, nausea, vomiting, arrhythmias, shortness of breath, and blood in the sputum. Effects on the skin can lead to additional areas of skin lesions, and vasoconstriction can cause color changes in the fingers when they are cold (Raynaud phenomenon). Effects on the kidneys can lead to edema in the legs and weight gain. A diagnosis of SLE depends on identification of four of 11 of the most common symptoms and confirmed production of an array of autoantibodies unique to SLE. A positive test for ANAs alone is not diagnostic.

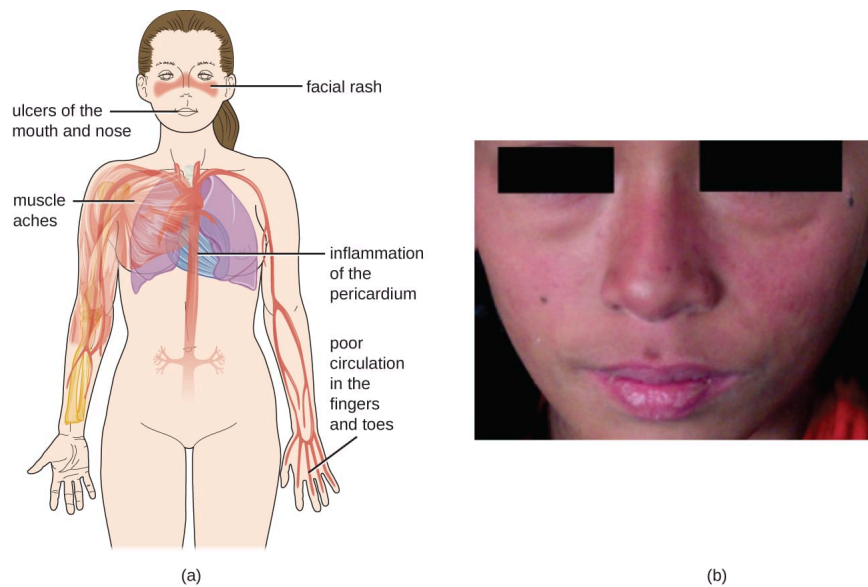


Figure 25.2.6: (a) Systemic lupus erythematosus is characterized by autoimmunity to the individual's own DNA and/or proteins. (b) This patient is presenting with a butterfly rash, one of the characteristic signs of lupus. (credit a: modification of work by Mikael Häggström; credit b: modification of work by Shrestha D, Dhakal AK, Shiva RK, Shakya A, Shah SC, Shakya H)

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Table 25.2.1 summarizes the causes, signs, and symptoms of select autoimmune diseases.

Table 25.2.1: Select Autoimmune Diseases

Disease	Cause	Signs and Symptoms
Addison disease	Destruction of adrenal gland cells by cytotoxic T cells	Weakness, nausea, hypotension, fatigue; adrenal crisis with severe pain in abdomen, lower back, and legs; circulatory system collapse, kidney failure
Celiac disease	Antibodies to gluten become autoantibodies that target cells of the small intestine	Severe diarrhea, abdominal pain, anemia, malnutrition
Diabetes mellitus (type I)	Cytotoxic T-cell destruction of the insulin-producing β cells of the pancreas	Hyperglycemia, extreme increase in thirst and urination, weight loss, extreme fatigue
Graves disease	Autoantibodies target thyroid-stimulating hormone receptors, resulting in overstimulation of the thyroid	Hyperthyroidism with rapid and irregular heartbeat, heat intolerance, weight loss, goiter, exophthalmia
Hashimoto thyroiditis	Thyroid gland is attacked by cytotoxic T cells, lymphocytes, macrophages, and autoantibodies	Thyroiditis with goiter, cold intolerance, muscle weakness, painful and stiff joints, depression, memory loss
Multiple sclerosis (MS)	Cytotoxic T-cell destruction of the myelin sheath surrounding nerve axons in the central nervous system	Visual disturbances, muscle weakness, impaired coordination and balance, numbness, prickling or “pins and needles” sensations, impaired cognitive function and memory

Disease	Cause	Signs and Symptoms
Myasthenia gravis	Autoantibodies directed against acetylcholine receptors within the neuromuscular junction	Extreme muscle weakness eventually leading to fatal respiratory arrest
Psoriasis	Cytokine activation of keratinocytes causes rapid and excessive epidermal cell turnover	Itchy or sore patches of thick, red skin with silvery scales; commonly affects elbows, knees, scalp, back, face, palms, feet
Rheumatoid arthritis	Autoantibodies, immune complexes, complement activation, phagocytes, and T cells damage membranes and bone in joints	Joint inflammation, pain and disfigurement, chronic systemic inflammation
Systemic lupus erythematosus (SLE)	Autoantibodies directed against nuclear and cytoplasmic molecules form immune complexes that deposit in tissues. Phagocytic cells and complement activation cause tissue damage and inflammation	Fatigue, fever, joint pain and swelling, hair loss, anemia, clotting, a sunlight-sensitive "butterfly" rash, skin lesions, photosensitivity, decreased kidney function, memory loss, confusion, depression

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Key Concepts and Summary

- Systemic autoimmune diseases include **multiple sclerosis**, **myasthenia gravis**, **psoriasis**, **rheumatoid arthritis**, and **systemic lupus erythematosus**.
- Treatments for autoimmune diseases generally involve anti-inflammatory and immunosuppressive drugs.

Footnotes

1. ⁴ C.C. Mok, C.S. Lau. "Pathogenesis of Systemic Lupus Erythematosus." *Journal of Clinical Pathology* 56 no. 7 (2003):481–490.

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25.3: Organ Transplantation and Rejection

Learning Objectives

- Explain why human leukocyte antigens (HLAs) are important in tissue transplantation
- Explain the types of grafts possible and their potential for interaction with the immune system
- Describe the role of immunosuppression in transplantation
- Describe what occurs during graft-versus-host disease (GVHD)

A **graft** is the transplantation of an organ or tissue to a different location, with the goal of replacing a missing or damaged organ or tissue. Grafts are typically moved without their attachments to the circulatory system and must reestablish these, in addition to the other connections and interactions with their new surrounding tissues. There are different types of grafts depending on the source of the new tissue or organ. Tissues that are transplanted from one genetically distinct individual to another within the same species are called **allografts**. An interesting variant of the allograft is an **isograft**, in which tissue from one twin is transplanted to another. As long as the twins are monozygotic (therefore, essentially genetically identical), the transplanted tissue is virtually never rejected. If tissues are transplanted from one area on an individual to another area on the same individual (e.g., a skin graft on a burn patient), it is known as an **autograft**. If tissues from an animal are transplanted into a human, this is called a **xenograft**.

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Transplant Rejection

The different types of grafts described above have varying risks for rejection (Table 25.3.1). Rejection occurs when the recipient's immune system recognizes the donor tissue as foreign (non-self), triggering an immune response. The major histocompatibility complex markers MHC I and MHC II, more specifically identified as **human leukocyte antigens (HLAs)**, play a role in transplant rejection. The HLAs expressed in tissue transplanted from a genetically different individual or species may be recognized as non-self molecules by the host's dendritic cells. If this occurs, the dendritic cells will process and present the foreign HLAs to the host's helper T cells and cytotoxic T cells, thereby activating them. Cytotoxic T cells then target and kill the grafted cells through the same mechanism they use to kill virus-infected cells; helper T cells may also release cytokines that activate macrophages to kill graft cells.

Table 25.3.1: Types of Tissue and Organ Grafts and Their Complications

Graft	Procedure	Complications
Autograft	From self to self	No rejection concerns
Isograft	From identical twin to twin	Little concern of rejection
Allograft	From relative or nonrelative to individual	Rejection possible
Xenograft	From animal to human	Rejection possible

With the three highly polymorphic MHC I genes in humans (*HLA-A*, *HLA-B*, and *HLA-C*) determining compatibility, each with many alleles segregating in a population, odds are extremely low that a randomly chosen donor will match a recipient's six-allele genotype (the two alleles at each locus are expressed codominantly). This is why a parent or a sibling may be the best donor in

many situations—a genetic match between the MHC genes is much more likely and the organ is much less likely to be rejected.

Although matching all of the MHC genes can lower the risk for rejection, there are a number of additional gene products that also play a role in stimulating responses against grafted tissue. Because of this, no non-self grafted tissue is likely to completely avoid rejection. However, the more similar the MHC gene match, the more likely the graft is to be tolerated for a longer time. Most transplant recipients, even those with tissues well matched to their MHC genes, require treatment with immunosuppressant drugs for the rest of their lives. This can make them more vulnerable than the general population to complications from infectious diseases. It can also result in transplant-related malignancies because the body's normal defenses against cancer cells are being suppressed.

Graft-versus-Host Disease

A form of rejection called graft-versus-host disease (GVHD) primarily occurs in recipients of bone marrow transplants and peripheral blood stem cells. GVHD presents a unique situation because the transplanted tissue is capable of producing immune cells; APCs in the donated bone marrow may recognize the host cells as non-self, leading to activation of the donor cytotoxic T cells. Once activated, the donor's T cells attack the recipient cells, causing acute GVHD.

Acute GVHD typically develops within weeks after a bone marrow transplant, causing tissue damage affecting the skin, gastrointestinal tract, liver, and eyes. In addition, acute GVHD may also lead to a cytokine storm, an unregulated secretion of cytokines that may be fatal. In addition to acute GVHD, there is also the risk for chronic GVHD developing months after the bone marrow transplant. The mechanisms responsible for chronic GVHD are not well understood.

To minimize the risk of GVHD, it is critically important to match the HLAs of the host and donor as closely as possible in bone marrow transplants. In addition, the donated bone marrow is processed before grafting to remove as many donor APCs and T cells as possible, leaving mostly *hematopoietic* stem cells.

The Future of Transplantation

Historically speaking, the practice of transplanting tissues—and the complications that can accompany such procedures—is a relatively recent development. It was not until 1954 that the first successful organ transplantation between two humans was achieved. Yet the field of organ transplantation has progressed rapidly since that time.

Nonetheless, the practice of transplanting non-self tissues may soon become obsolete. Scientists are now attempting to develop methods by which new organs may be grown *in vitro* from an individual's own harvested cells to replace damaged or abnormal ones. Because organs produced in this way would contain the individual's own cells, they could be transplanted into the individual without risk for rejection.

An alternative approach that is gaining renewed research interest is genetic modification of donor animals, such as pigs, to provide transplantable organs that do not elicit an immune response in the recipient. The approach involves excising the genes in the pig (in the embryo) that are most responsible for the rejection reaction after transplantation. Finding these genes and effectively removing them is a challenge, however. So too is identifying and neutralizing risks from viral sequences that might be embedded in the pig genome, posing a risk for infection in the human recipient.

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Key Concepts and Summary

- Grafts and transplants can be classified as **autografts**, **isografts**, **allografts**, or **xenografts** based on the genetic differences

between the donor's and recipient's tissues.

- Genetic differences, especially among the MHC (HLA) genes, will dictate the likelihood that **rejection** of the transplanted tissue will occur.
- Transplant recipients usually require immunosuppressive therapy to avoid rejection, even with good genetic matching. This can create additional problems when immune responses are needed to fight off infectious agents and prevent cancer.
- **Graft-versus-host disease** can occur in bone marrow transplants, as the mature T cells in the transplant itself recognize the recipient's tissues as foreign.
- Transplantation methods and technology have improved greatly in recent decades and may move into new areas with the use of stem cell technology to avoid the need for genetic matching of MHC molecules.

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25.4: Immunodeficiency

Learning Objectives

- Compare the causes of primary and secondary immunodeficiencies
- Explain why patients with chronic granulomatous disease are especially susceptible to bacterial infections
- Explain why individuals with IgA deficiency are susceptible to respiratory and GI infections
- Describe treatments for primary and secondary immunodeficiencies
- Explain why secondary immunodeficiencies can sometimes be reversed

Immunodeficiencies are inherited (primary) or acquired (secondary) disorders in which elements of host immune defenses are either absent or functionally defective. In developed countries, most immunodeficiencies are inherited, and they are usually first seen in the clinic as recurrent or overwhelming infections in infants. However, on a global scale, malnutrition is the most common cause of immunodeficiency and would be categorized as an acquired immunodeficiency. Acquired immunodeficiencies are more likely to develop later in life, and the pathogenic mechanisms of many remain obscure.

Primary Immunodeficiency

Primary immunodeficiencies, which number more than 250, are caused by inherited defects of either nonspecific innate or specific adaptive immune defenses. In general, patients born with primary immunodeficiency (PI) commonly have an increased susceptibility to infection. This susceptibility can become apparent shortly after birth or in early childhood for some individuals, whereas other patients develop symptoms later in life. Some primary immunodeficiencies are due to a defect of a single cellular or humoral component of the immune system; others may result from defects of more than one component. Examples of primary immunodeficiencies include chronic granulomatous disease, X-linked agammaglobulinemia, selective IgA deficiency, and severe combined immunodeficiency disease.

Chronic Granulomatous Disease

The causes of **chronic granulomatous disease (CGD)** are defects in the NADPH oxidase system of phagocytic cells, including neutrophils and macrophages, that prevent the production of superoxide radicals in phagolysosomes. The inability to produce superoxide radicals impairs the antibacterial activity of phagocytes. As a result, infections in patients with CGD persist longer, leading to a chronic local inflammation called a granuloma. Microorganisms that are the most common causes of infections in patients with CGD include *Aspergillus* spp., *Staphylococcus aureus*, *Chromobacterium violaceum*, *Serratia marcescens*, and *Salmonella typhimurium*.

X-Linked Agammaglobulinemia

Deficiencies in B cells due to defective differentiation lead to a lack of specific antibody production known as **X-linked agammaglobulinemia**. In 1952, Ogden C. Bruton (1908–2003) described the first immunodeficiency in a boy whose immune system failed to produce antibodies. This defect is inherited on the X chromosome and is characterized by the absence of immunoglobulin in the serum; it is called Bruton X-linked agammaglobulinemia (XLA). The defective gene, *BTK*, in XLA is now known to encode a tyrosine kinase called Bruton tyrosine kinase (Btk). In patients whose B cells are unable to produce sufficient amounts of Btk, the B-cell maturation and differentiation halts at the pre-B-cell stage of growth. B-cell maturation and differentiation beyond the pre-B-cell stage of growth is required for immunoglobulin production. Patients who lack antibody production suffer from recurrent infections almost exclusively due to extracellular pathogens that cause pyogenic infections: *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. pyogenes*, and *S. aureus*. Because cell-mediated immunity is not impaired, these patients are not particularly vulnerable to infections caused by viruses or intracellular pathogens.

Selective IgA Deficiency

The most common inherited form of immunoglobulin deficiency is selective IgA deficiency, affecting about one in 800 people. Individuals with **selective IgA deficiency** produce normal levels of IgG and IgM, but are not able to produce secretory IgA. IgA

deficiency predisposes these individuals to lung and gastrointestinal infections for which secretory IgA is normally an important defense mechanism. Infections in the lungs and gastrointestinal tract can involve a variety of pathogens, including *H. influenzae*, *S. pneumoniae*, *Moraxella catarrhalis*, *S. aureus*, *Giardia lamblia*, or pathogenic strains of *Escherichia coli*.

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Severe Combined Immunodeficiency

Patients who suffer from **severe combined immunodeficiency (SCID)** have B-cell and T-cell defects that impair T-cell dependent antibody responses as well as cell-mediated immune responses. Patients with SCID also cannot develop immunological memory, so vaccines provide them no protection, and live attenuated vaccines (e.g., for varicella-zoster, measles virus, rotavirus, poliovirus) can actually cause the infection they are intended to prevent. The most common form is X-linked SCID, which accounts for nearly 50% of all cases and occurs primarily in males. Patients with SCID are typically diagnosed within the first few months of life after developing severe, often life-threatening, opportunistic infection by *Candida* spp., *Pneumocystis jirovecii*, or pathogenic strains of *E. coli*.

Without treatment, babies with SCID do not typically survive infancy. In some cases, a bone marrow transplant may successfully correct the defects in lymphocyte development that lead to the SCID phenotype, by replacing the defective component. However, this treatment approach is not without risks, as demonstrated by the famous case of David Vetter (1971–1984), better known as “Bubble Boy” (Figure 25.4.1). Vetter, a patient with SCID who lived in a protective plastic bubble to prevent exposure to opportunistic microbes, received a bone marrow transplant from his sister. Because of a latent Epstein-Barr virus infection in her bone marrow, however, he developed mononucleosis and died of Burkitt lymphoma at the age of 12 years.



Figure 25.4.1: David Vetter, popularly known as “The Bubble Boy,” was born with SCID and lived most of his life isolated inside a plastic bubble. Here he is shown outside the bubble in a suit specially built for him by NASA. (credit: NASA Johnson Space Center)

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Secondary Immunodeficiency

A secondary immunodeficiency occurs as a result of an acquired impairment of function of B cells, T cells, or both. Secondary immunodeficiencies can be caused by:

- Systemic disorders such as diabetes mellitus, malnutrition, hepatitis, or HIV infection
- Immunosuppressive treatments such as cytotoxic chemotherapy, bone marrow ablation before transplantation, or radiation therapy
- Prolonged critical illness due to infection, surgery, or trauma in the very young, elderly, or hospitalized patients

Unlike primary immunodeficiencies, which have a genetic basis, secondary immunodeficiencies are often reversible if the underlying cause is resolved. Patients with secondary immunodeficiencies develop an increased susceptibility to an otherwise benign infection by opportunistic pathogens such as *Candida* spp., *P. jirovecii*, and *Cryptosporidium*.

HIV infection and the associated acquired immunodeficiency syndrome (AIDS) are the best-known secondary immunodeficiencies. AIDS is characterized by profound CD4 T-cell lymphopenia (decrease in lymphocytes). The decrease in CD4 T cells is the result of various mechanisms, including HIV-induced pyroptosis (a type of apoptosis that stimulates an inflammatory response), viral cytopathic effect, and cytotoxicity to HIV-infected cells.

The most common cause of secondary immunodeficiency worldwide is severe malnutrition, which affects both innate and adaptive immunity. More research and information are needed for the more common causes of secondary immunodeficiency; however, the number of new discoveries in AIDS research far exceeds that of any other single cause of secondary immunodeficiency. AIDS research has paid off extremely well in terms of discoveries and treatments; increased research into the most common cause of immunodeficiency, malnutrition, would likely be as beneficial.

An Immunocompromised Host

Benjamin, a 50-year-old male patient who has been receiving chemotherapy to treat his chronic myelogenous leukemia (CML), a disease characterized by massive overproduction of nonfunctional, malignant myelocytic leukocytes that crowd out other, healthy leukocytes, is seen in the emergency department. He is complaining of a productive, wet cough, dyspnea, and fatigue. On examination, his pulse is 120 beats per minute (bpm) (normal range is 60–100 bpm) and weak, and his blood pressure is 90/60 mm Hg (normal is 120/80 mm Hg). During auscultation, a distinct crackling can be heard in his lungs as he breathes, and his pulse-oximeter level (a measurement of blood-oxygen saturation) is 80% (normal is 95%–100%). He has a fever; his temperature is 38.9 °C (102 °F). Sputum cultures and blood samples are obtained and sent to the lab, but Benjamin goes into respiratory distress and dies before the results can be obtained.

Benjamin's death was a result of a combination of his immune system being compromised by his leukemia and his chemotherapy treatment further weakening his ability to mount an immune response. CML (and leukemia in general) and corresponding chemotherapy cause a decrease in the number of leukocytes capable of normal function, leading to secondary immunodeficiency. This increases the risk for opportunistic bacterial, viral, protozoal, and fungal infections that could include *Staphylococcus*, enteroviruses, *Pneumocystis*, *Giardia*, or *Candida*. Benjamin's symptoms were suggestive of bacterial pneumonia, but his leukemia and chemotherapy likely complicated and contributed to the severity of the pneumonia, resulting in his death. Because his leukemia was overproducing certain white blood cells, and those overproduced white blood cells were largely nonfunctional or abnormal in their function, he did not have the proper immune system blood cells to help him fight off the infection.

Table 25.4.1 summarizes primary and secondary immunodeficiencies, their effects on immune function, and typical outcomes.

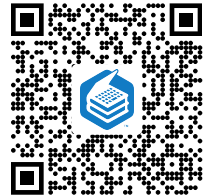
Table 25.4.1: Primary and Secondary Immunodeficiencies

Disease		Effect on Immune Function	Outcomes
Primary immunodeficiencies	Chronic granulomatous disease	Impaired killing of bacteria within the phagolysosome of neutrophils and macrophages	Chronic infections and granulomas
	Selective IgA deficiency	Inability to produce secretory IgA	Predisposition to lung and gastrointestinal infections
	Severe combined immunodeficiency disease (SCID)	Deficient humoral and cell-mediated immune responses	Early development of severe and life-threatening opportunistic infections
	X-linked agammaglobulinemia	Flawed differentiation of B cells and absence of specific antibodies	Recurrent infections almost exclusively due to pathogens that cause pyogenic infections
Secondary immunodeficiencies	Immunosuppressive therapies (e.g., chemotherapy, radiotherapy)	Impaired humoral and/or cell-mediated immune responses	Opportunistic infections, rare cancers
	Malnutrition	Impaired humoral and/or cell-mediated immune responses	Opportunistic infections, rare cancers
	Viral infection (e.g., HIV)	Impaired cell-mediated immune responses due to CD4 T-cell lymphopenia	Opportunistic infections, rare cancers

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Key Concepts and Summary

- **Primary immunodeficiencies** are caused by genetic abnormalities; **secondary immunodeficiencies** are acquired through disease, diet, or environmental exposures.
- Primary immunodeficiencies may result from flaws in phagocyte killing of innate immunity, or impairment of T cells and B cells.
- Primary immunodeficiencies include chronic granulomatous disease, X-linked agammaglobulinemia, selective IgA deficiency, and severe combined immunodeficiency disease.
- Secondary immunodeficiencies result from environmentally induced defects in B cells and/or T cells.
- Causes for secondary immunodeficiencies include malnutrition, viral infection, diabetes, prolonged infections, and chemical or radiation exposure.

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25.E: Diseases of the Immune System (Exercises)

25.1: Organ Transplantation and Rejection

Grafts and transplants can be classified as autografts, isografts, allografts, or xenografts based on the genetic differences between the donor's and recipient's tissues. Genetic differences, especially among the MHC (HLA) genes, will dictate the likelihood that rejection of the transplanted tissue will occur. Transplant recipients usually require immunosuppressive therapy to avoid rejection, even with good genetic matching.

Matching

Match the graft with its description.

___autograft	A. donor is a different species than the recipient
___allograft	B. donor and recipient are the same individual
___xenograft	C. donor is an identical twin of the recipient
___isograft	D. donor is the same species as the recipient, but genetically different

Answer

B, D, A, C

Fill in the Blank

For a transplant to have the best chances of avoiding rejection, the genes coding for the _____ molecules should be closely matched between donor and recipient.

Answer

MHC

Because it is a “transplant” that can include APCs and T cells from the donor, a bone marrow transplant may induce a very specific type of rejection known as _____ disease.

Answer

graft-versus-host

Short Answer

Why is a parent usually a better match for transplanted tissue to a donor than a random individual of the same species?

25.2: Immunodeficiency

Primary immunodeficiencies are caused by genetic abnormalities; secondary immunodeficiencies are acquired through disease, diet, or environmental exposures. Primary immunodeficiencies may result from flaws in phagocyte killing of innate immunity, or impairment of T cells and B cells. Primary immunodeficiencies include chronic granulomatous disease, X-linked agammaglobulinemia, selective IgA deficiency, and severe combined immunodeficiency disease.

Multiple Choice

Which of the following is a genetic disease that results in lack of production of antibodies?

- A. agammaglobulinemia
- B. myasthenia gravis
- C. HIV/AIDS
- D. chronic granulomatous disease

Answer

A

Which of the following is a genetic disease that results in almost no adaptive immunity due to lack of B and/ or T cells?

- A. agammaglobulinemia
- B. severe combined immunodeficiency
- C. HIV/AIDS
- D. chronic granulomatous disease

Answer

B

All but which one of the following are examples of secondary immunodeficiencies?

- A. HIV/AIDS
- B. malnutrition
- C. chronic granulomatous disease
- D. immunosuppression due to measles infection

Answer

C

Fill in the Blank

Diseases due to _____ abnormalities are termed primary immunodeficiencies.

Answer

genetic

A secondary immunodeficiency is _____, rather than genetic.

Answer

acquired

Short Answer

Compare the treatments for primary and secondary immunodeficiencies.

25.3: Cancer Immunobiology and Immunotherapy

When control of the cell cycle is lost, the affected cells rapidly divide and often lose the ability to differentiate into the cell type appropriate for their location in the body. In addition, they lose contact inhibition and can start to grow on top of each other. This

can result in formation of a tumor. It is important to make a distinction here: The term “cancer” is used to describe the diseases resulting from loss of cell-cycle regulation and subsequent cell proliferation.

Multiple Choice

Cancer results when a mutation leads to which of the following?

- A. cell death
- B. apoptosis
- C. loss of cell-cycle control
- D. shutdown of the cell cycle

Answer

C

Tumor antigens are _____ that are inappropriately expressed and found on abnormal cells.

- A. self antigens
- B. foreign antigens
- C. antibodies
- D. T-cell receptors

Answer

A

Fill in the Blank

A _____ cancer vaccine is one that stops the disease from occurring in the first place.

Answer

preventive

A _____ cancer vaccine is one that will help to treat the disease after it has occurred.

Answer

therapeutic

Short Answer

How can tumor antigens be effectively targeted without inducing an autoimmune (anti-self) response?

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26: DIAGNOSING INFECTIONS

Many laboratory tests are designed to confirm a presumptive diagnosis by detecting antibodies specific to a suspected pathogen. Unfortunately, many such tests are time-consuming and expensive. That is now changing, however, with the development of new, miniaturized technologies that are fast and inexpensive. For example, researchers at Columbia University are developing a “lab-on-a-chip” technology that will test a single drop of blood for 15 different infectious diseases, including HIV and syphilis, in a matter of minutes.¹ The blood is pulled through tiny capillaries into reaction chambers where the patient’s antibodies mix with reagents. A chip reader that attaches to a cell phone analyzes the results and sends them to the patient’s healthcare provider. Currently the device is being field tested in Rwanda to check pregnant women for chronic diseases. Researchers estimate that the chip readers will sell for about \$100 and individual chips for \$1.²

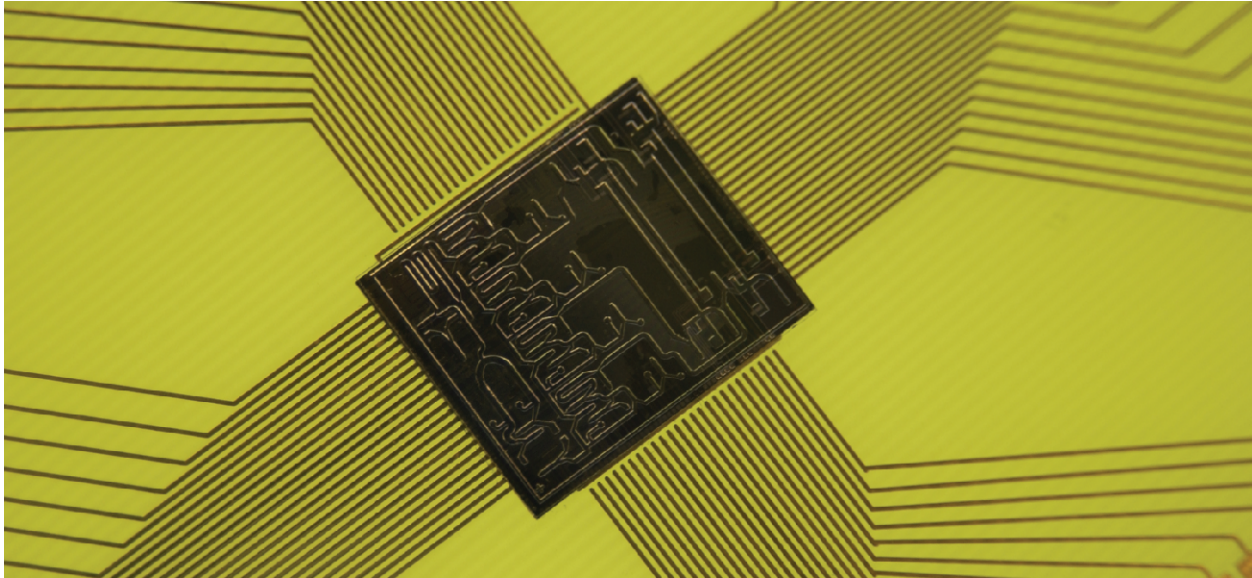


Figure 26.1: Lab-on-a-chip technology allows immunological assays to be miniaturized so tests can be done rapidly with minimum quantities of expensive reagents. The chips contain tiny flow tubes to allow movement of fluids by capillary action, reactions sites with embedded reagents, and data output through electronic sensors. (credit: modification of work by Maggie Bartlett, NHGRI)

26.1: Polyclonal Antibodies

26.2: Monoclonal Antibodies

26.3: In vitro Assays

26.4: Agglutination Assays

26.5: Enzyme Immunoassays (EIA) and Enzyme-Linked Immunosorbent Assays (ELISA)

26.6: Fluorescent Antibody Techniques

26.E: Laboratory Analysis of the Immune Response (Exercises)

Footnotes

- 1 Chin, Curtis D. et al., “Mobile Device for Disease Diagnosis and Data Tracking in Resource-Limited Settings,” *Clinical Chemistry* 59, no. 4 (2013): 629-40.
- 2 Evarts, H., “Fast, Low-Cost Device Uses the Cloud to Speed Up Testing for HIV and More,” January 24, 2013. Accessed July 14, 2016. <http://engineering.columbia.edu/fast...g-hiv-and-more>.

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26.1: Polyclonal Antibodies

Learning Objectives

- Compare the method of development, use, and characteristics of monoclonal and polyclonal antibodies
- Explain the nature of antibody cross-reactivity and why it occurs
- Compare and contrast false-positive vs. false-negative test results

In addition to being crucial for our normal immune response, antibodies provide powerful tools for research and diagnostic purposes. The high specificity of antibodies makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms. With *in vitro* assays, antibodies can be used to precipitate soluble antigens, agglutinate (clump) cells, opsonize and kill bacteria with the assistance of complement, and neutralize drugs, toxins, and viruses.

An antibody's **specificity** results from the antigen-binding site formed within the variable regions—regions of the antibody that have unique patterns of amino acids that can only bind to target antigens with a molecular sequence that provides complementary charges and noncovalent bonds. There are limitations to antibody specificity, however. Some antigens are so chemically similar that cross-reactivity occurs; in other words, antibodies raised against one antigen bind to a chemically similar but different antigen. Consider an antigen that consists of a single protein with multiple epitopes (Figure 26.1.1). This single protein may stimulate the production of many different antibodies, some of which may bind to chemically identical epitopes on other proteins.

Cross-reactivity is more likely to occur between antibodies and antigens that have low **affinity** or **avidity**. Affinity, which can be determined experimentally, is a measure of the binding strength between an antibody's binding site and an epitope, whereas avidity is the total strength of all the interactions in an antibody-antigen complex (which may have more than one bonding site). Avidity is influenced by affinity as well as the structural arrangements of the epitope and the variable regions of the antibody. If an antibody has a high affinity/avidity for a specific antigen, it is less likely to cross-react with an antigen for which it has a lower affinity/avidity.

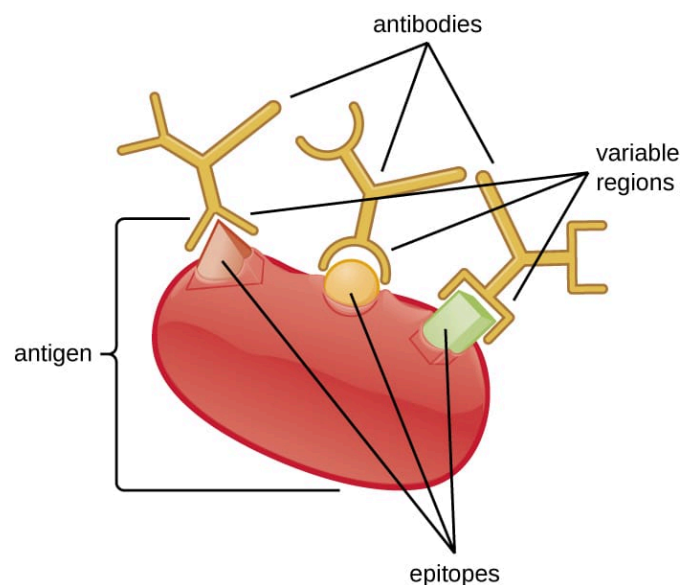


Figure 26.1.1: An antibody binds to a specific region on an antigen called an epitope. A single antigen can have multiple epitopes for different, specific antibodies.

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Producing Polyclonal Antibodies

Antibodies used for research and diagnostic purposes are often obtained by injecting a lab animal such as a rabbit or a goat with a specific antigen. Within a few weeks, the animal's immune system will produce high levels of antibodies specific for the antigen. These antibodies can be harvested in an **antiserum**, which is whole serum collected from an animal following exposure to an antigen. Because most antigens are complex structures with multiple epitopes, they result in the production of multiple antibodies in the lab animal. This so-called **polyclonal antibody** response is also typical of the response to infection by the human immune system. Antiserum drawn from an animal will thus contain antibodies from multiple clones of B cells, with each B cell responding to a specific epitope on the antigen (Figure 26.1.2).

Lab animals are usually injected at least twice with antigen when being used to produce antiserum. The second injection will activate memory cells that make class IgG antibodies against the antigen. The memory cells also undergo **affinity maturation**, resulting in a pool of antibodies with higher average affinity. Affinity maturation occurs because of mutations in the immunoglobulin gene variable regions, resulting in B cells with slightly altered antigen-binding sites. On re-exposure to the antigen, those B cells capable of producing antibody with higher affinity antigen-binding sites will be stimulated to proliferate and produce more antibody than their lower-affinity peers. An adjuvant, which is a chemical that provokes a generalized activation of the immune system that stimulates greater antibody production, is often mixed with the antigen prior to injection.

Antiserum obtained from animals will not only contain antibodies against the antigen artificially introduced in the laboratory, but it will also contain antibodies to any other antigens to which the animal has been exposed during its lifetime. For this reason, antisera must first be "purified" to remove other antibodies before using the antibodies for research or diagnostic assays.

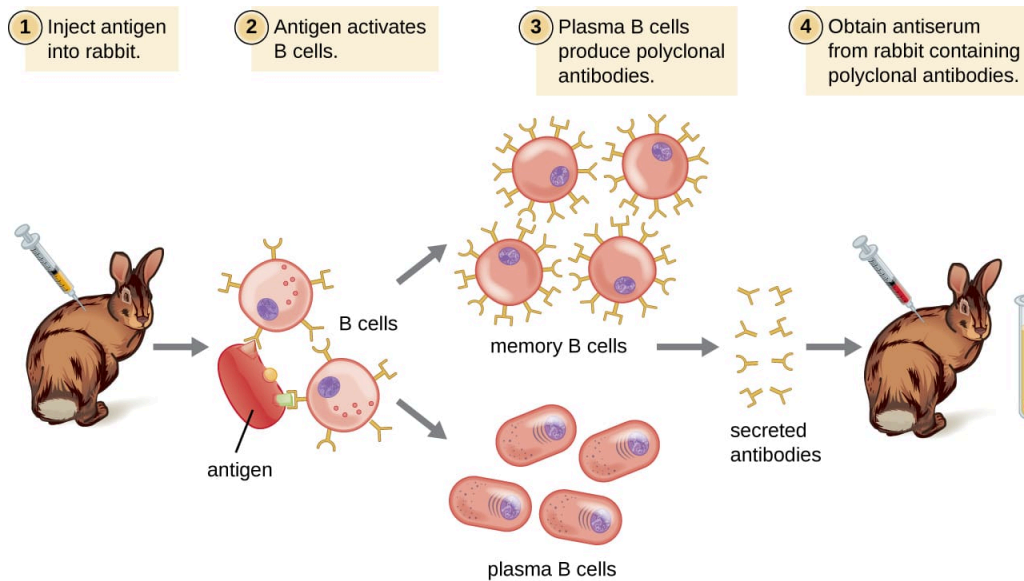


Figure 26.1.2: This diagram illustrates the process for harvesting polyclonal antibodies produced in response to an antigen.

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Clinical Uses of Polyclonal Antisera

Polyclonal antisera are used in many clinical tests that are designed to determine whether a patient is producing antibodies in response to a particular pathogen. While these tests are certainly powerful diagnostic tools, they have their limitations, because they are an indirect means of determining whether a particular pathogen is present. Tests based on a polyclonal response can sometimes lead to a **false-positive** result—in other words, a test that confirms the presence of an antigen that is, in fact, not present. Antibody-based tests can also result in a **false-negative** result, which occurs when the test fails to detect an antibody that is, in fact, present.

The accuracy of antibody tests can be described in terms of **test sensitivity** and **test specificity**. Test sensitivity is the probability of getting a positive test result when the patient is indeed infected. If a test has high sensitivity, the probability of a false negative is low. Test specificity, on the other hand, is the probability of getting a negative test result when the patient is not infected. If a test has high specificity, the probability of a false positive is low.

False positives often occur due to cross-reactivity, which can occur when epitopes from a different pathogen are similar to those found on the pathogen being tested for. For this reason, antibody-based tests are often used only as screening tests; if the results are positive, other confirmatory tests are used to make sure that the results were not a false positive.

For example, a blood sample from a patient suspected of having hepatitis C can be screened for the virus using antibodies that bind to antigens on hepatitis C virus. If the patient is indeed infected with hepatitis C virus, the antibodies will bind to the antigens, yielding a positive test result. If the patient is not infected with hepatitis C virus, the antibodies will generally not bind to anything and the test should be negative; however, a false positive may occur if the patient has been previously infected by any of a variety of pathogens that elicit antibodies that cross-react with the hepatitis C virus antigens. Antibody tests for hepatitis C have high sensitivity (a low probability of a false negative) but low specificity (a high probability of a false positive). Thus, patients who test positive must have a second, confirmatory test to rule out the possibility of a false positive. The confirmatory test is a more expensive and time-consuming test that directly tests for the presence of hepatitis C viral RNA in the blood. Only after the

confirmatory test comes back positive can the patient be definitively diagnosed with a hepatitis C infection. Antibody-based tests can result in a false negative if, for any reason, the patient's immune system has not produced detectable levels of antibodies. For some diseases, it may take several weeks following infection before the immune system produces enough antibodies to cross the detection threshold of the assay. In immunocompromised patients, the immune system may not be capable of producing a detectable level of antibodies.

Another limitation of using antibody production as an indicator of disease is that antibodies in the blood will persist long after the infection has been cleared. Depending on the type of infection, antibodies will be present for many months; sometimes, they may be present for the remainder of the patient's life. Thus, a positive antibody-based test only means that the patient was infected at some point in time; it does not prove that the infection is active.

In addition to their role in diagnosis, polyclonal antisera can activate complement, detect the presence of bacteria in clinical and food industry settings, and perform a wide array of precipitation reactions that can detect and quantify serum proteins, viruses, or other antigens. However, with the many specificities of antibody present in a polyclonal antiserum, there is a significant likelihood that the antiserum will cross-react with antigens to which the individual was never exposed. Therefore, we must always account for the possibility of false-positive results when working with a polyclonal antiserum.

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Interactive Element

Case Study Preview: "The Syringe Scandal: A Test of Trust and Immunity"

A hospital reels from a public health crisis when a healthcare worker battling addiction is caught swapping patient syringes - leading to possible HIV exposure for over 1,300 patients. You'll follow the hospital's urgent epidemiological response: contact tracing, ELISA testing, confirmatory western blots, and PCR diagnostics. Along the way, you'll unpack the strengths and pitfalls of each diagnostic method, including false positives, indeterminate results, and the seroconversion window.

This case puts you in the shoes of public health teams navigating patient risk, legal liability, and the science of HIV detection and confirmatory testing. You'll also explore how flow cytometry, CD4 counts, and PCR are used to monitor immune status and guide treatment - reminding us that behind every test result is a person seeking answers.

Can an early test provide peace of mind - or false hope? And what happens when confirmatory results fall into the gray zone?

[Chapter 20 Case Study - The Syringe Scandal: A Test of Trust and Immunity](#)

Key Concepts and Summary

- Antibodies bind with high **specificity** to antigens used to challenge the immune system, but they may also show **cross-reactivity** by binding to other antigens that share chemical properties with the original antigen.
- Injection of an antigen into an animal will result in a **polyclonal antibody** response in which different antibodies are produced that react with the various epitopes on the antigen.
- **Polyclonal antisera** are useful for some types of laboratory assays, but other assays require more specificity. Diagnostic tests that use polyclonal antisera are typically only used for screening because of the possibility of **false-positive** and **false-negative** results.

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26.2: Monoclonal Antibodies

Learning Objectives

- Describe the steps of monoclonal antibody production
- Describe how humanized monoclonal antibody is produced
- Explain why it is important to use humanized monoclonal antibodies for therapy

Producing Monoclonal Antibodies

Some types of assays require better antibody specificity and affinity than can be obtained using a polyclonal antiserum. To attain this high specificity, all of the antibodies must bind with high affinity to a single epitope. This high specificity can be provided by **monoclonal antibodies (mAbs)**. Table 26.2.1 compares some of the important characteristics of monoclonal and polyclonal antibodies.

Unlike polyclonal antibodies, which are produced in live animals, monoclonal antibodies are produced *in vitro* using tissue-culture techniques. mAbs are produced by immunizing an animal, often a mouse, multiple times with a specific antigen. B cells from the spleen of the immunized animal are then removed. Since normal B cells are unable to proliferate forever, they are fused with immortal, cancerous B cells called myeloma cells, to yield **hybridoma** cells. All of the cells are then placed in a selective medium that allows only the hybridomas to grow; unfused myeloma cells cannot grow, and any unfused B cells die off. The hybridomas, which are capable of growing continuously in culture while producing antibodies, are then screened for the desired mAb. Those producing the desired mAb are grown in tissue culture; the culture medium is harvested periodically and mAbs are purified from the medium. This is a very expensive and time-consuming process. It may take weeks of culturing and many liters of media to provide enough mAbs for an experiment or to treat a single patient. mAbs are expensive (Figure 26.2.3).

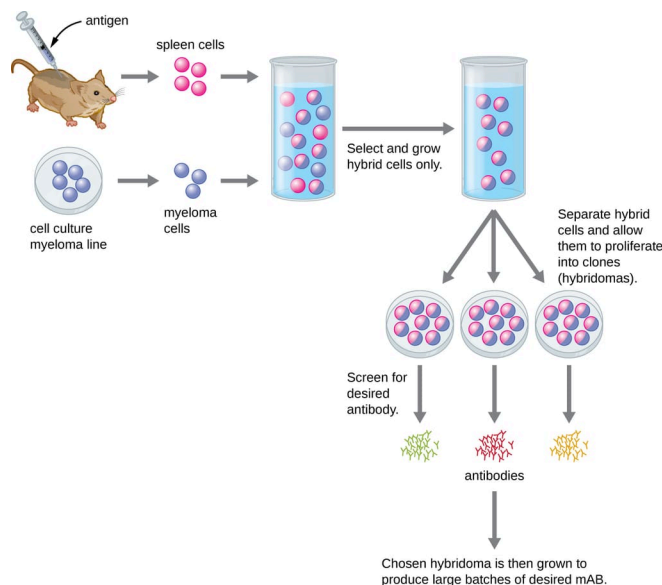


Figure 26.2.3: Monoclonal antibodies (mAbs) are produced by introducing an antigen to a mouse and then fusing polyclonal B cells from the mouse's spleen to myeloma cells. The resulting hybridoma cells are cultured and continue to produce antibodies to the antigen. Hybridomas producing the desired mAb are then grown in large numbers on a selective medium that is periodically harvested to obtain the desired mAbs.

Table 26.2.1: Characteristics of Polyclonal and Monoclonal Antibodies

Monoclonal Antibodies	Polyclonal Antibodies
Expensive production	Inexpensive production
Long production time	Rapid production

Monoclonal Antibodies	Polyclonal Antibodies
Large quantities of specific antibodies	Large quantities of nonspecific antibodies
Recognize a single epitope on an antigen	Recognize multiple epitopes on an antigen
Production is continuous and uniform once the hybridoma is made	Different batches vary in composition

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Clinical Uses of Monoclonal Antibodies

Since the most common methods for producing monoclonal antibodies use mouse cells, it is necessary to create **humanized monoclonal antibodies** for human clinical use. Mouse antibodies cannot be injected repeatedly into humans, because the immune system will recognize them as being foreign and will respond to them with neutralizing antibodies. This problem can be minimized by genetically engineering the antibody in the mouse B cell. The variable regions of the mouse light and heavy chain genes are ligated to human constant regions, and the chimeric gene is then transferred into a host cell. This allows production of a mAb that is mostly “human” with only the antigen-binding site being of mouse origin.

Humanized mAbs have been successfully used to treat cancer with minimal side effects. For example, the humanized monoclonal antibody drug Herceptin has been helpful for the treatment of some types of breast cancer. There have also been a few preliminary trials of humanized mAb for the treatment of infectious diseases, but none of these treatments are currently in use. In some cases, mAbs have proven too specific to treat infectious diseases, because they recognize some serovars of a pathogen but not others. Using a cocktail of multiple mAbs that target different strains of the pathogen can address this problem. However, the great cost associated with mAb production is another challenge that has prevented mAbs from becoming practical for use in treating microbial infections.¹

One promising technology for inexpensive mAbs is the use of genetically engineered plants to produce antibodies (or **plantibodies**). This technology transforms plant cells into antibody factories rather than relying on tissue culture cells, which are expensive and technically demanding. In some cases, it may even be possible to deliver these antibodies by having patients eat the plants rather than by extracting and injecting the antibodies. For example, in 2013, a research group cloned antibody genes into plants that had the ability to neutralize an important toxin from bacteria that can cause severe gastrointestinal disease.² Eating the plants could potentially deliver the antibodies directly to the toxin.

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Using Monoclonal Antibodies to Combat Ebola

During the 2014–2015 Ebola outbreak in West Africa, a few Ebola-infected patients were treated with ZMapp, a drug that had been shown to be effective in trials done in rhesus macaques only a few months before.³ ZMapp is a combination of three mAbs produced by incorporating the antibody genes into tobacco plants using a viral vector. By using three mAbs, the drug is effective across multiple strains of the virus. Unfortunately, there was only enough ZMapp to treat a tiny number of patients.

While the current technology is not adequate for producing large quantities of ZMapp, it does show that plantibodies—plant-produced mAbs—are feasible for clinical use, potentially cost effective, and worth further development. The last several years have seen an explosion in the number of new mAb-based drugs for the treatment of cancer and infectious diseases; however, the widespread use of such drugs is currently inhibited by their exorbitant cost, especially in underdeveloped parts of the world, where a single dose might cost more than the patient's lifetime income. Developing methods for cloning antibody genes into plants could reduce costs dramatically.

Key Concepts and Summary

- **Monoclonal antibodies** provide higher specificity than polyclonal antisera because they bind to a single epitope and usually have high **affinity**.
- Monoclonal antibodies are typically produced by culturing antibody-secreting **hybridomas** derived from mice. mAbs are currently used to treat cancer, but their exorbitant cost has prevented them from being used more widely to treat infectious diseases. Still, their potential for laboratory and clinical use is driving the development of new, cost-effective solutions such as **plantibodies**.

Footnotes

- 1 Saylor, Carolyn, Ekaterina Dadachova and Arturo Casadevall, "Monoclonal Antibody-Based Therapies for Microbial Diseases," *Vaccine* 27 (2009): G38-G46.
- 2 Nakanishi, Katsuhiro et al., "Production of Hybrid-IgG/IgA Plantibodies with Neutralizing Activity against Shiga Toxin 1," *PloS One* 8, no. 11 (2013): e80712.
- 3 Qiu, Xiangguo et al., "Reversion of Advanced Ebola Virus Disease in Nonhuman Primates with ZMapp," *Nature* 514 (2014): 47–53.

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26.3: In vitro Assays

Learning Objectives

- Describe various types of assays used to find antigen-antibody complexes
- Explain how antibodies in patient serum can be used to diagnose disease
- Describe the steps of Western blot
- Explain the function of the enzyme or the fluorophore in immuno assays

Laboratory tests to detect antibodies and antigens outside of the body (e.g., in a test tube) are called ***in vitro* assays**. When both antibodies and their corresponding antigens are present in a solution, we can often observe a precipitation reaction in which large complexes (lattices) form and settle out of solution. In the next several sections, we will discuss several common *in vitro* assays.

Neutralization Assay

To cause infection, viruses must bind to receptors on host cells. Antiviral antibodies can neutralize viral infections by coating the virions, blocking the binding ([Figure 18.1.6](#)). This activity neutralizes virions and can result in the formation of large antibody-virus complexes (which are readily removed by phagocytosis) or by antibody binding to the virus and blocking its binding to host cell receptors. This neutralization activity is the basis of **neutralization assays**, sensitive assays used for diagnoses of viral infections.

When viruses infect cells, they often cause damage (cytopathic effects) that may include lysis of the host cells. Cytopathic effects can be visualized by growing host cells in a petri dish, covering the cells with a thin layer of agar, and then adding virus (see [Isolation, Culture, and Identification of Viruses](#)). The virus will diffuse very slowly through the agar. A virus will enter a host cell, proliferate (causing cell damage), be released from the dead host cell, and then move to neighboring cells. As more and more cells die, plaques of dead cells will form ([Figure 26.3.6](#)).

During the course of a viral infection, the patient will mount an antibody response to the virus, and we can quantify those antibodies using a **plaque reduction assay**. To perform the assay, a serial dilution is carried out on a serum sample. Each dilution is then mixed with a standardized amount of the suspect virus. Any virus-specific antibodies in the serum will neutralize some of the virus. The suspensions are then added to host cells in culture to allow any nonneutralized virus to infect the cells and form plaques after several days. The titer is defined as the reciprocal of the highest dilution showing a 50% reduction in plaques. Titer is always expressed as a whole number. For example, if a 1/64 dilution was the highest dilution to show 50% plaque reduction, then the titer is 64.

The presence of antibodies in the patient's serum does not tell us whether the patient is currently infected or was infected in the past. Current infections can be identified by waiting two weeks and testing another serum sample. A four-fold increase in neutralizing titer in this second sample indicates a new infection.

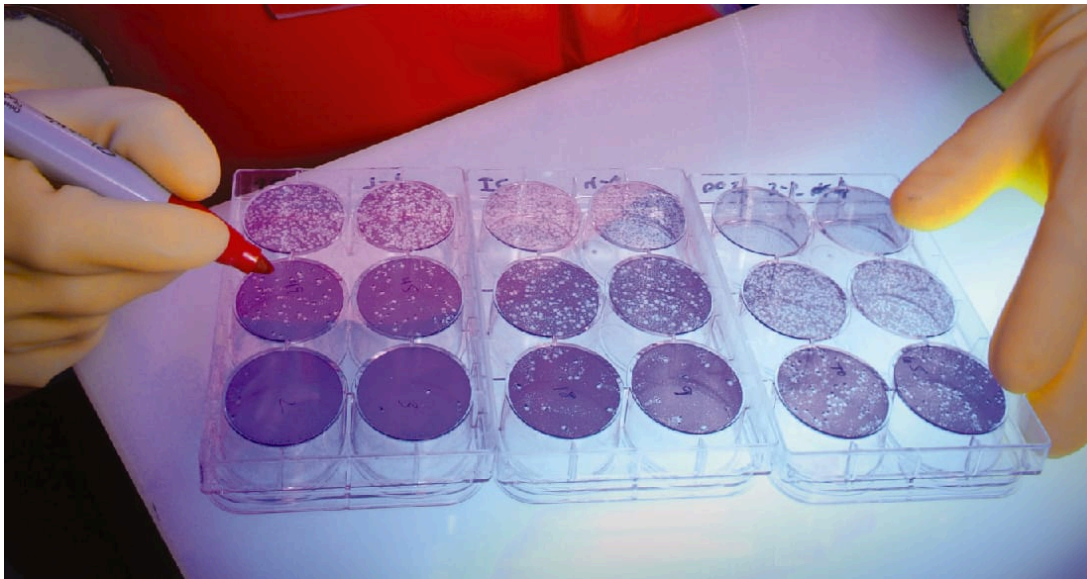


Figure 26.3.6: In a neutralization assay, antibodies in patient serum neutralize viruses added to the wells, preventing the formation of plaques. In the assay pictured, the wells with numerous plaques (white patches) contain a low concentration of antibodies. The wells with relatively few plaques have a high concentration of antibodies. (credit: modification of work by Centers for Disease Control and Prevention)

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Immunoelectrophoresis

When a patient has elevated protein levels in the blood or is losing protein in the urine, a clinician will often order a **polyacrylamide gel electrophoresis (PAGE) assay** (see [Visualizing and Characterizing DNA, RNA, and Protein](#)). This assay compares the relative abundance of the various types of serum proteins. Abnormal protein electrophoresis patterns can be further studied using **immunoelectrophoresis (IEP)**. The IEP begins by running a PAGE. Antisera against selected serum proteins are added to troughs running parallel to the electrophoresis track, forming precipitin arcs similar to those seen in an Ouchterlony assay (Figure 26.3.7). This allows the identification of abnormal immunoglobulin proteins in the sample.

IEP is particularly useful in the diagnosis of multiple myeloma, a cancer of antibody-secreting cells. Patients with multiple myeloma cannot produce healthy antibodies; instead they produce abnormal antibodies that are monoclonal proteins (M proteins). Thus, patients with multiple myeloma will present with elevated serum protein levels that show a distinct band in the gamma globulin region of a protein electrophoresis gel and a sharp spike (in M protein) on the densitometer scan rather than the normal broad smear (Figure 26.3.7). When antibodies against the various types of antibody heavy and light chains are used to

form precipitin arcs, the M protein will cause distinctly skewed arcs against one class of heavy chain and one class of light chain as seen in Figure 26.3.7.

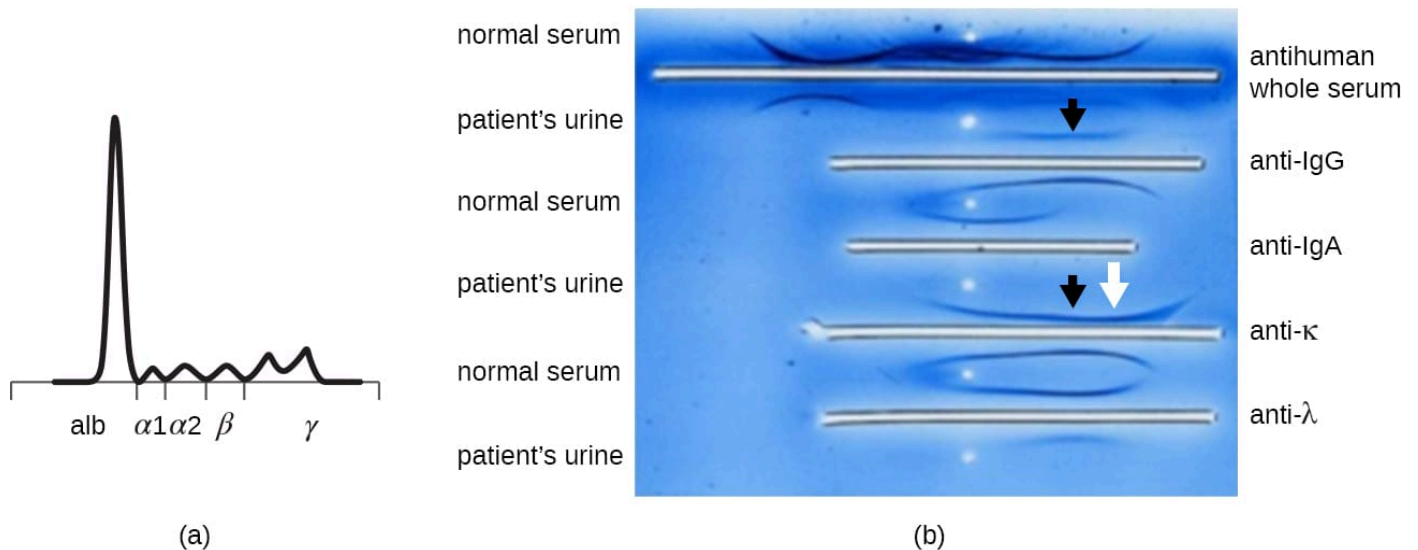


Figure 26.3.7: (a) This graph shows normal measurements of serum proteins. (b) This photograph shows an immunoelectrophoresis of urine. After electrophoresis, antisera were added to the troughs and the precipitin arcs formed, illustrating the distribution of specific proteins. The skewed arcs (arrows) help to diagnose multiple myeloma. (credit a, b: modification of work by Izawa S, Akimoto T, Ikeuchi H, Kusano E, Nagata D)

Protein Electrophoresis and the Characterization of Immunoglobulin Structure

The advent of electrophoresis ultimately led to researching and understanding the structure of antibodies. When Swedish biochemist Arne Tiselius (1902–1971) published the first protein electrophoresis results in 1937,² he could identify the protein albumin (the smallest and most abundant serum protein) by the sharp band it produced in the gel. The other serum proteins could not be resolved in a simple protein electrophoresis, so he named the three broad bands, with many proteins in each band, alpha, beta, and gamma globulins. Two years later, American immunologist Elvin Kabat (1914–2000) traveled to Sweden to work with Tiselius using this new technique and showed that antibodies migrated as gamma globulins.³ With this new understanding in hand, researchers soon learned that multiple myeloma, because it is a cancer of antibody-secreting cells, could be tentatively diagnosed by the presence of a large M spike in the gamma-globulin region by protein electrophoresis. Prior to this discovery, studies on immunoglobulin structure had been minimal, because of the difficulty of obtaining pure samples to study. Sera from multiple myeloma patients proved to be an excellent source of highly enriched monoclonal immunoglobulin, providing the raw material for studies over the next 20-plus years that resulted in the elucidation of the structure of immunoglobulin.

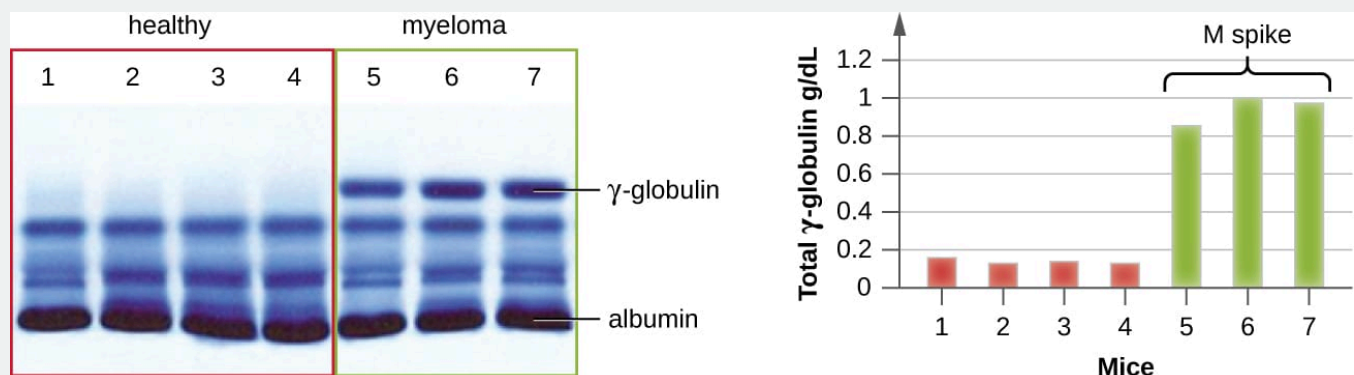


Figure 26.3.8: Electrophoresis patterns of myeloma (right) and normal sera (left). The proteins have been stained; when the density of each band is quantified by densitometry, the data produce the bar graph on the right. Both gels show the expected dense band of albumin at the bottom and an abnormal spike in the gamma-globulin region. (credit: modification of work by Soodgupta D, Hurchla MA, Jiang M, Zheleznyak A, Weilbaecher KN, Anderson CJ, Tomasson MH, Shokeen M)

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Immunoblot Assay: The Western Blot

After performing protein gel electrophoresis, specific proteins can be identified in the gel using antibodies. This technique is known as the western blot. Following separation of proteins by PAGE, the protein antigens in the gel are transferred to and immobilized on a nitrocellulose membrane. This membrane can then be exposed to a primary antibody produced to specifically bind to the protein of interest. A second antibody equipped with a molecular beacon will then bind to the first. These secondary antibodies are coupled to another molecule such as an enzyme or a fluorophore (a molecule that fluoresces when excited by light). When using antibodies coupled to enzymes, a chromogenic substrate for the enzyme is added. This substrate is usually colorless but will develop color in the presence of the antibody. The fluorescence or substrate coloring identifies the location of the specific protein in the membrane to which the antibodies are bound (Figure 26.3.9).

Typically, polyclonal antibodies are used for western blot assays. They are more sensitive than mAbs because of their ability to bind to various epitopes of the primary antigen, and the signal from polyclonal antibodies is typically stronger than that from mAbs. Monoclonal antibodies can also be used; however, they are much more expensive to produce and are less sensitive, since they are only able to recognize one specific epitope.

Several variations of the western blot are useful in research. In a southwestern blot, proteins are separated by SDS-PAGE, blotted onto a nitrocellulose membrane, allowed to renature, and then probed with a fluorescently or radioactively labeled DNA probe; the purpose of the southwestern is to identify specific DNA-protein interactions. Far-western blots are carried out to determine protein-protein interactions between immobilized proteins (separated by SDS-PAGE, blotted onto a nitrocellulose membrane, and allowed to renature) and non-antibody protein probes. The bound non-antibody proteins that interact with the immobilized proteins in a far-western blot may be detected by radiolabeling, fluorescence, or the use of an antibody with an enzymatic molecular beacon.

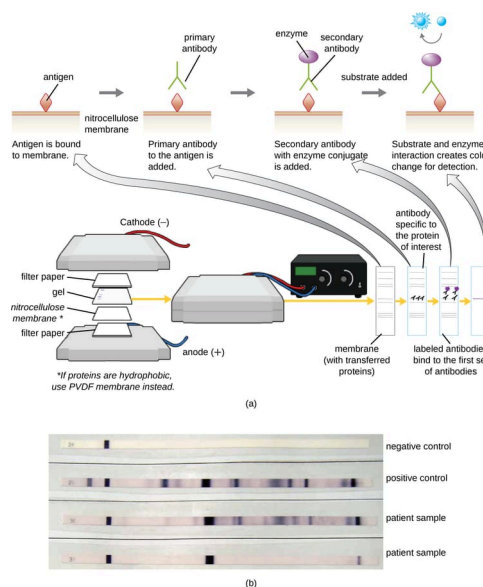


Figure 26.3.9: (a) This diagram summarizes the process of western blotting. Antibodies are used to identify specific bands on the protein gel. (b) A western blot test for antibodies against HIV. The top strip is the negative control; the next strip is the positive control. The bottom two strips are patient serum samples containing antibodies. (credit a: modification of work by "Bensaccount"/Wikimedia Commons)

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Key Concepts and Summary

- Viral infections can be detected by quantifying virus-neutralizing antibodies in a patient's serum.
- Different antibody classes in plasma or serum are identified by using **immunoelectrophoresis**.
- The presence of specific antigens (e.g., bacterial or viral proteins) in serum can be demonstrated by **western blot** assays, in which the proteins are transferred to a nitrocellulose membrane and identified using labeled antibodies.

Footnotes

1. 1 Ouchterlony, Örjan, "In Vitro Method for Testing the Toxin-Producing Capacity of Diphtheria Bacteria," *Acta Pathologica Microbiologica Scandinavica* 26, no. 4 (1949): 516-24.
2. 2 Tiselius, Arne, "Electrophoresis of Serum Globulin: Electrophoretic Analysis of Normal and Immune Sera," *Biochemical Journal* 31, no. 9 (1937): 1464.
3. 3 Tiselius, Arne and Elvin A. Kabat. "An Electrophoretic Study of Immune Sera and Purified Antibody Preparations," *The Journal of Experimental Medicine* 69, no. 1 (1939): 119-31.

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26.4: Agglutination Assays

Learning Objectives

- Compare direct and indirect agglutination
- Identify various uses of hemagglutination in the diagnosis of disease
- Explain how blood types are determined
- Explain the steps used to cross-match blood to be used in a transfusion

In addition to causing precipitation of soluble molecules and flocculation of molecules in suspension, antibodies can also clump together cells or particles (e.g., antigen-coated latex beads) in a process called **agglutination** (Figure 18.1.8). Agglutination can be used as an indicator of the presence of antibodies against bacteria or red blood cells. Agglutination assays are usually quick and easy to perform on a glass slide or microtiter plate (Figure 26.4.1). Microtiter plates have an array of wells to hold small volumes of reagents and to observe reactions (e.g., agglutination) either visually or using a specially designed spectrophotometer. The wells come in many different sizes for assays involving different volumes of reagents.

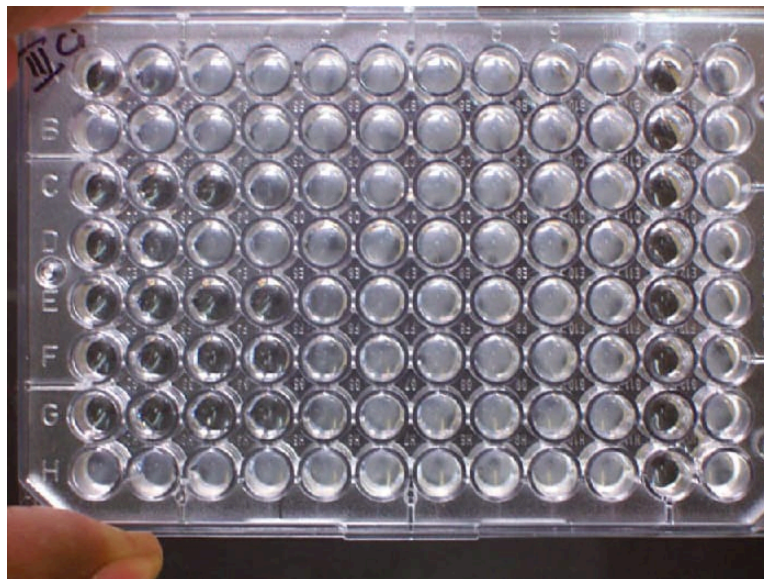


Figure 26.4.1: Microtiter plates are used for conducting numerous reactions simultaneously in an array of wells. (credit: modification of work by "Microrao"/Wikimedia)

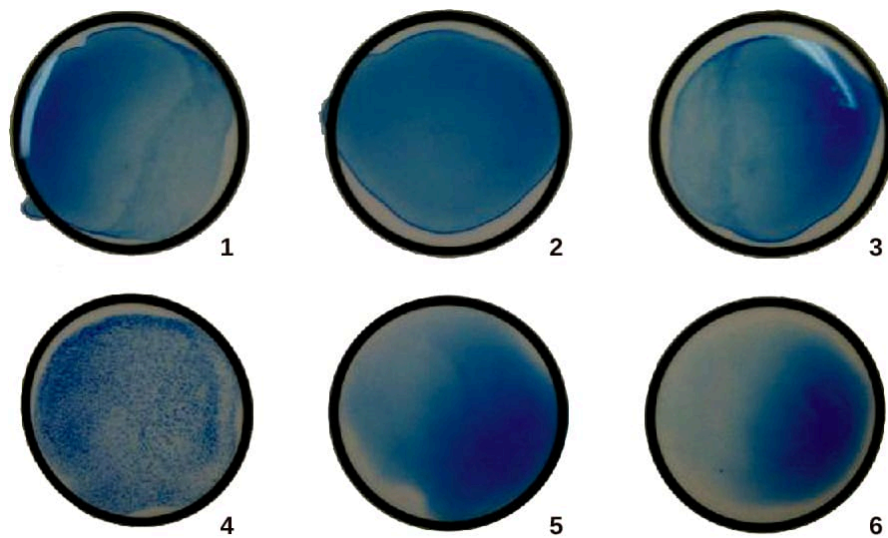
Agglutination of Bacteria and Viruses

The use of agglutination tests to identify streptococcal bacteria was developed in the 1920s by Rebecca Lancefield working with her colleagues A.R. Dochez and Oswald Avery.¹ She used antibodies to identify M protein, a virulence factor on streptococci that is necessary for the bacteria's ability to cause strep throat. Production of antibodies against M protein is crucial in mounting a protective response against the bacteria.

Lancefield used antisera to show that different strains of the same species of streptococci express different versions of M protein, which explains why children can come down with strep throat repeatedly. Lancefield classified beta-hemolytic streptococci into many groups based on antigenic differences in group-specific polysaccharides located in the bacterial cell wall. The strains are called **serovars** because they are differentiated using antisera. Identifying the serovars present in a disease outbreak is important because some serovars may cause more severe disease than others.

The method developed by Lancefield is a **direct agglutination assay**, since the bacterial cells themselves agglutinate. A similar strategy is more commonly used today when identifying serovars of bacteria and viruses; however, to improve visualization of the agglutination, the antibodies may be attached to inert latex beads. This technique is called an **indirect agglutination assay** (or

latex fixation assay), because the agglutination of the beads is a marker for antibody binding to some other antigen (Figure 26.4.2). Indirect assays can be used to detect the presence of either antibodies or specific antigens.



ASM MicrobeLibrary © Hare

Figure 26.4.2: Antibodies against six different serovars of Group A strep were attached to latex beads. Each of the six antibody preparations was mixed with bacteria isolated from a patient. The tiny clumps seen in well 4 are indicative of agglutination, which is absent from all other wells. This indicates that the serovar associated with well 4 is present in the patient sample. (credit: modification of work by American Society for Microbiology)

To identify antibodies in a patient's serum, the antigen of interest is attached to latex beads. When mixed with patient serum, the antibodies will bind the antigen, cross-linking the latex beads and causing the beads to agglutinate indirectly; this indicates the presence of the antibody (Figure 26.4.3). This technique is most often used when looking for IgM antibodies, because their structure provides maximum cross-linking. One widely used example of this assay is a test for rheumatoid factor (RF) to confirm a diagnosis of rheumatoid arthritis. RF is, in fact, the presence of IgM antibodies that bind to the patient's own IgG. RF will agglutinate IgG-coated latex beads.

In the reverse test, soluble antigens can be detected in a patient's serum by attaching specific antibodies (commonly mAbs) to the latex beads and mixing this complex with the serum (Figure 26.4.3).

Agglutination tests are widely used in underdeveloped countries that may lack appropriate facilities for culturing bacteria. For example, the Widal test, used for the diagnosis of typhoid fever, looks for agglutination of *Salmonella enterica* subspecies *typhi* in patient sera. The Widal test is rapid, inexpensive, and useful for monitoring the extent of an outbreak; however, it is not as accurate as tests that involve culturing of the bacteria. The Widal test frequently produces false positives in patients with previous infections with other subspecies of *Salmonella*, as well as false negatives in patients with hyperproteinemia or immune deficiencies.

In addition, agglutination tests are limited by the fact that patients generally do not produce detectable levels of antibody during the first week (or longer) of an infection. A patient is said to have undergone seroconversion when antibody levels reach the threshold for detection. Typically, seroconversion coincides with the onset of signs and symptoms of disease. However, in an HIV infection, for example, it generally takes 3 weeks for seroconversion to take place, and in some instances, it may take much longer.

Similar to techniques for the precipitin ring test and plaque assays, it is routine to prepare serial two-fold dilutions of the patient's serum and determine the titer of agglutinating antibody present. Since antibody levels change over time in both primary and secondary immune responses, by checking samples over time, changes in antibody titer can be detected. For example, a comparison of the titer during the acute phase of an infection versus the titer from the convalescent phase will distinguish whether an infection is current or has occurred in the past. It is also possible to monitor how well the patient's immune system is responding to the pathogen.

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Blood Typing and Cross-Matching

In addition to antibodies against bacteria and viruses to which they have previously been exposed, most individuals also carry antibodies against blood types other than their own. There are presently 33 immunologically important blood-type systems, many of which are restricted within various ethnic groups or rarely result in the production of antibodies. The most important and perhaps best known are the ABO and Rh blood groups (see [Figure 19.1.3](#)).

When units of blood are being considered for transfusion, pretransfusion blood testing must be performed. For the blood unit, commercially prepared antibodies against the A, B, and Rh antigens are mixed with red blood cells from the units to initially confirm that the blood type on the unit is accurate. Once a unit of blood has been requested for transfusion, it is vitally important to make sure the donor (unit of blood) and recipient (patient) are compatible for these crucial antigens. In addition to confirming the blood type of the unit, the patient's blood type is also confirmed using the same commercially prepared antibodies to A, B, and Rh. For example, as shown in [Figure 26.4.7](#), if the donor blood is A-positive, it will agglutinate with the anti-A antiserum and with the anti-Rh antiserum. If no agglutination is observed with any of the sera, then the blood type would be O-negative.

Following determination of the blood type, immediately prior to releasing the blood for transfusion, a **cross-match** is performed in which a small aliquot of the donor red blood cells are mixed with serum from the patient awaiting transfusion. If the patient does have antibodies against the donor red blood cells, hemagglutination will occur. To confirm any negative test results and check for sensitized red blood cells, Coombs' reagent may be added to the mix to facilitate visualization of the antibody-red blood cell interaction.

Under some circumstances, a minor cross-match may be performed as well. In this assay, a small aliquot of donor serum is mixed with patient red blood cells. This allows the detection of agglutinating antibodies in the donor serum. This test is rarely necessary because transfusions generally use packed red blood cells with most of the plasma removed by centrifugation.

Red blood cells have many other antigens in addition to ABO and Rh. While most people are unlikely to have antibodies against these antigens, women who have had multiple pregnancies or patients who have had multiple transfusions may have them because of repeated exposure. For this reason, an **antibody screen** test is used to determine if such antibodies are present. Patient serum is checked against commercially prepared, pooled, type O red blood cells that express these antigens. If agglutination occurs, the antigen to which the patient is responding must be identified and determined not to be present in the donor unit.



Figure 26.4.7: This sample of a commercially produced “bedside” card enables quick typing of both a recipient’s and donor’s blood before transfusion. The card contains three reaction sites or wells. One is coated with an anti-A antibody, one with an anti-B antibody, and one with an anti-Rh antibody. Agglutination of red blood cells in a given site indicates a positive identification of the blood antigens: in this case, A and Rh antigens for blood type A-positive.

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Table 26.4.1 summarizes the various kinds of agglutination assays discussed in this section.

Table 26.4.1: Mechanisms of Select Antibody-Antigen Assays

Type of Assay	Mechanism	Example
Agglutination	Direct: Antibody is used to clump bacterial cells or other large structures	Serotyping bacteria
	Indirect: Latex beads are coupled with antigen or antibody to look for antibody or antigen, respectively, in patient serum	Confirming the presence of rheumatoid factor (IgM-binding Ig) in patient serum
Hemagglutination	Direct: Some bacteria and viruses cross-link red blood cells and clump them together	Diagnosing influenza, mumps, and measles
	Direct Coombs’ test (DAT): Detects nonagglutinating antibodies or complement proteins on red blood cells <i>in vivo</i>	Checking for maternal antibodies binding to neonatal red blood cells
	Indirect Coombs’ test (IAT): Screens an individual for antibodies against red blood cell antigens (other than the A and B antigens) that are unbound in a patient’s serum <i>in vitro</i>	Performing pretransfusion blood testing
	Viral hemagglutination inhibition: Uses antibodies from a patient to inhibit viral agglutination	Diagnosing various viral diseases by the presence of patient antibodies against the virus
	Blood typing and cross-matching: Detects ABO, Rh, and minor antigens in the blood	Matches donor blood to recipient immune requirements

Key Concepts and Summary

- Antibodies can agglutinate cells or large particles into a visible matrix. **Agglutination** tests are often done on cards or in **microtiter plates** that allow multiple reactions to take place side by side using small volumes of reagents.
- Using antisera against certain proteins allows identification of **serovars** within species of bacteria.
- Detecting antibodies against a pathogen can be a powerful tool for diagnosing disease, but there is a period of time before patients go through **seroconversion** and the level of antibodies becomes detectable.
- Agglutination of latex beads in **indirect agglutination assays** can be used to detect the presence of specific antigens or specific antibodies in patient serum.
- **Neutralization assays** quantify the level of virus-specific antibody by measuring the decrease in hemagglutination observed after mixing patient serum with a standardized amount of virus.
- Hemagglutination assays are also used to screen and **cross-match** donor and recipient blood to ensure that the transfusion recipient does not have antibodies to antigens in the donated blood.

Footnotes

1. 1 Lancefield, Rebecca C., "The Antigenic Complex of *Streptococcus haemolyticus*. I. Demonstration of a Type-Specific Substance in Extracts of *Streptococcus haemolyticus*," *The Journal of Experimental Medicine* 47, no. 1 (1928): 91-103.

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26.5: Enzyme Immunoassays (EIA) and Enzyme-Linked Immunosorbent Assays (ELISA)

Learning Objectives

- Explain the differences and similarities between EIA, FEIA, and ELISA
- Describe the difference and similarities between immunohistochemistry and immunocytochemistry
- Describe the different purposes of direct and indirect ELISA

Similar to the western blot, **enzyme immunoassays (EIAs)** use antibodies to detect the presence of antigens. However, EIAs differ from western blots in that the assays are conducted in microtiter plates or *in vivo* rather than on an absorbent membrane. There are many different types of EIAs, but they all involve an antibody molecule whose constant region binds an enzyme, leaving the variable region free to bind its specific antigen. The addition of a substrate for the enzyme allows the antigen to be visualized or quantified (Figure 26.5.1).

In EIAs, the substrate for the enzyme is most often a chromogen, a colorless molecule that is converted into a colored end product. The most widely used enzymes are **alkaline phosphatase** and **horseradish peroxidase** for which appropriate substrates are readily available. In some EIAs, the substrate is a **fluorogen**, a nonfluorescent molecule that the enzyme converts into a fluorescent form. EIAs that utilize a fluorogen are called **fluorescent enzyme immunoassays (FEIAs)**. Fluorescence can be detected by either a fluorescence microscope or a spectrophotometer.

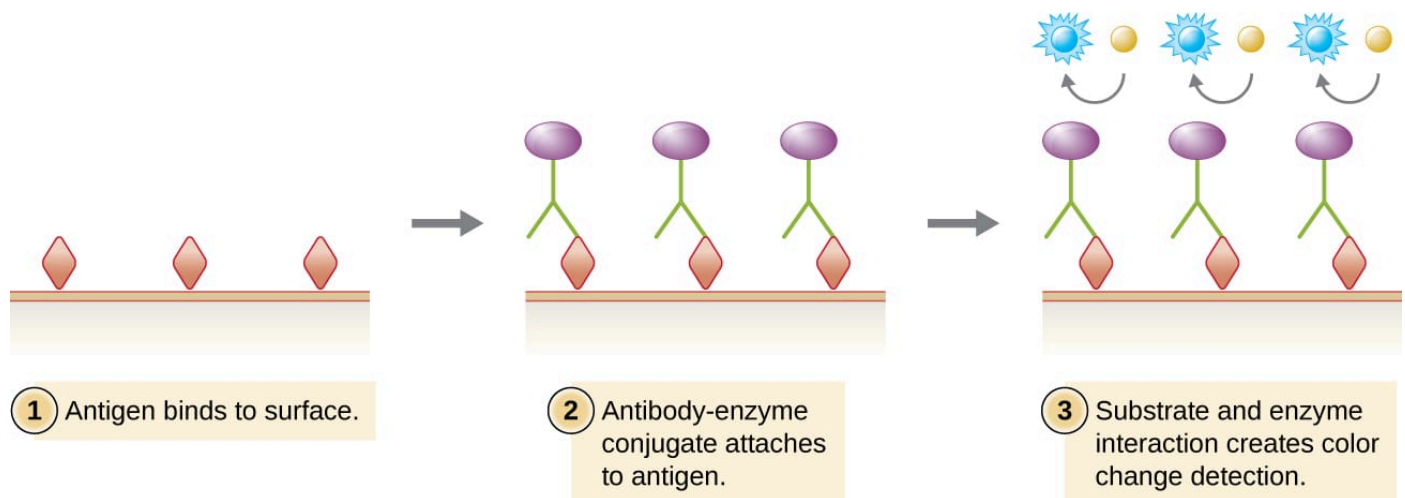


Figure 26.5.1: Enzyme immunoassays, such as the direct ELISA shown here, use an enzyme-antibody conjugate to deliver a detectable substrate to the site of an antigen. The substrate may be a colorless molecule that is converted into a colored end product or an inactive fluorescent molecule that fluoresces after enzyme activation. (credit: modification of work by "Cavetri"/Wikimedia Commons)

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The MMR Titer

The MMR vaccine is a combination vaccine that provides protection against measles, mumps, and rubella (German measles). Most people receive the MMR vaccine as children and thus have antibodies against these diseases. However, for various reasons, even vaccinated individuals may become susceptible to these diseases again later in life. For example, some children may receive only one round of the MMR vaccine instead of the recommended two. In addition, the titer of protective antibodies in an individual's body may begin to decline with age or as the result of some medical conditions.

To determine whether the titer of antibody in an individual's bloodstream is sufficient to provide protection, an MMR titer test can be performed. The test is a simple immunoassay that can be done quickly with a blood sample. The results of the test will indicate whether the individual still has immunity or needs another dose of the MMR vaccine.

Submitting to an MMR titer is often a pre-employment requirement for healthcare workers, especially those who will frequently be in contact with young children or immunocompromised patients. Were a healthcare worker to become infected with measles, mumps, or rubella, the individual could easily pass these diseases on to susceptible patients, leading to an outbreak. Depending on the results of the MMR titer, healthcare workers might need to be revaccinated prior to beginning work.

Immunostaining

One powerful use of EIA is **immunostaining**, in which antibody-enzyme conjugates enhance microscopy. **Immunohistochemistry (IHC)** is used for examining whole tissues. As seen in Figure 26.5.2, a section of tissue can be stained to visualize the various cell types. In this example, a mAb against CD8 was used to stain CD8 cells in a section of tonsil tissue. It is now possible to count the number of CD8 cells, determine their relative numbers versus the other cell types present, and determine the location of these cells within this tissue. Such data would be useful for studying diseases such as AIDS, in which the normal function of CD8 cells is crucial for slowing disease progression.

Immunocytochemistry (ICC) is another valuable form of immunostaining. While similar to IHC, in ICC, extracellular matrix material is stripped away, and the cell membrane is etched with alcohol to make it permeable to antibodies. This allows antibodies to pass through the cell membrane and bind to specific targets inside the cell. Organelles, cytoskeletal components, and other intracellular structures can be visualized in this way. While some ICC techniques use EIA, the enzyme can be replaced with a fluorescent molecule, making it a fluorescent immunoassay.

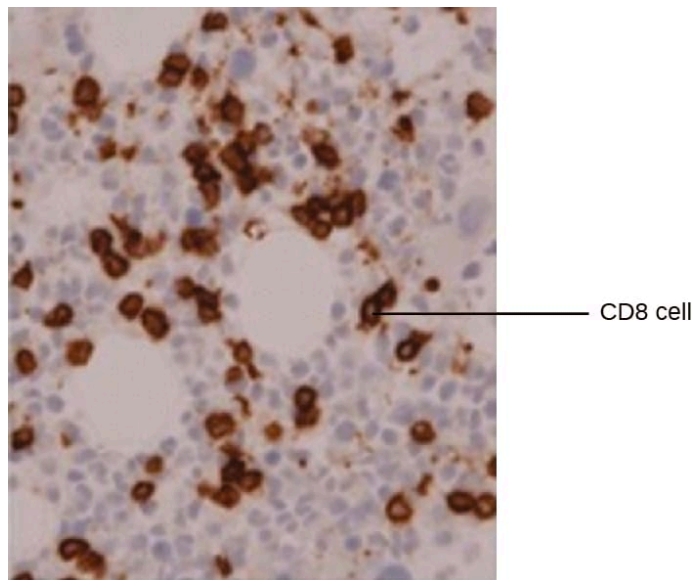


Figure 26.5.2: Enzyme-linked antibodies against CD8 were used to stain the CD8 cells in this preparation of bone marrow using a chromogen. (credit: modification of work by Yamashita M, Fujii Y, Ozaki K, Urano Y, Iwasa M, Nakamura S, Fujii S, Abe M, Sato Y, Yoshino T)

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Enzyme-linked Immunosorbent Assays (ELISAs)

The **enzyme-linked immunosorbent assays (ELISAs)** are widely used EIAs. In the **direct ELISA**, antigens are immobilized in the well of a microtiter plate. An antibody that is specific for a particular antigen and is conjugated to an enzyme is added to each well. If the antigen is present, then the antibody will bind. After washing to remove any unbound antibodies, a colorless substrate (chromogen) is added. The presence of the enzyme converts the substrate into a colored end product (Figure 26.5.1). While this technique is faster because it only requires the use of one antibody, it has the disadvantage that the signal from a direct ELISA is lower (lower sensitivity).

In a **sandwich ELISA**, the goal is to use antibodies to precisely quantify specific antigen present in a solution, such as antigen from a pathogen, a serum protein, or a hormone from the blood or urine to list just a few examples. The first step of a sandwich ELISA is to add the **primary antibody** to all the wells of a microtiter plate (Figure 26.5.3). The antibody sticks to the plastic by hydrophobic interactions. After an appropriate incubation time, any unbound antibody is washed away. Comparable washes are used between each of the subsequent steps to ensure that only specifically bound molecules remain attached to the plate. A blocking protein is then added (e.g., albumin or the milk protein casein) to bind the remaining nonspecific protein-binding sites in the well. Some of the wells will receive known amounts of antigen to allow the construction of a standard curve, and unknown antigen solutions are added to the other wells. The primary antibody captures the antigen and, following a wash, the **secondary antibody** is added, which is a polyclonal antibody that is conjugated to an enzyme. After a final wash, a colorless substrate (chromogen) is added, and the enzyme converts it into a colored end product. The color intensity of the sample caused by the end product is measured with a spectrophotometer. The amount of color produced (measured as absorbance) is directly proportional to the amount of enzyme, which in turn is directly proportional to the captured antigen. ELISAs are extremely sensitive, allowing antigen to be quantified in the nanogram (10^{-9} g) per mL range.

In an **indirect ELISA**, we quantify antigen-specific antibody rather than antigen. We can use indirect ELISA to detect antibodies against many types of pathogens, including *Borrelia burgdorferi* (Lyme disease) and HIV. There are three important differences

between indirect and direct ELISAs as shown in Figure 26.5.4. Rather than using antibody to capture antigen, the indirect ELISA starts with attaching known antigen (e.g., peptides from HIV) to the bottom of the microtiter plate wells. After blocking the unbound sites on the plate, patient serum is added; if antibodies are present (primary antibody), they will bind the antigen. After washing away any unbound proteins, the secondary antibody with its conjugated enzyme is directed against the primary antibody (e.g., antihuman immunoglobulin). The secondary antibody allows us to quantify how much antigen-specific antibody is present in the patient's serum by the intensity of the color produced from the conjugated enzyme-chromogen reaction.

As with several other tests for antibodies discussed in this chapter, there is always concern about cross-reactivity with antibodies directed against some other antigen, which can lead to false-positive results. Thus, we cannot definitively diagnose an HIV infection (or any other type of infection) based on a single indirect ELISA assay. We must confirm any suspected positive test, which is most often done using either an immunoblot that actually identifies the presence of specific peptides from the pathogen or a test to identify the nucleic acids associated with the pathogen, such as reverse transcriptase PCR (RT-PCR) or a nucleic acid antigen test.

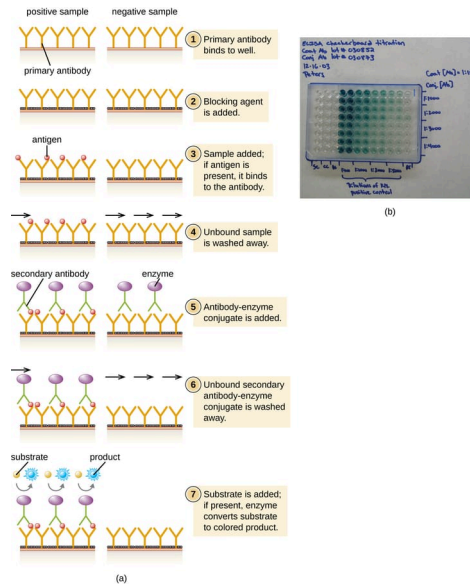


Figure 26.5.3: (a) In a sandwich ELISA, a primary antibody is used to first capture an antigen with the primary antibody. A secondary antibody conjugated to an enzyme that also recognizes epitopes on the antigen is added. After the addition of the chromogen, a spectrophotometer measures the absorbance of end product, which is directly proportional to the amount of captured antigen. (b) An ELISA plate shows dilutions of antibodies (left) and antigens (bottom). Higher concentrations result in a darker final color. (credit b: modification of work by U.S. Fish and Wildlife Service Pacific Region)

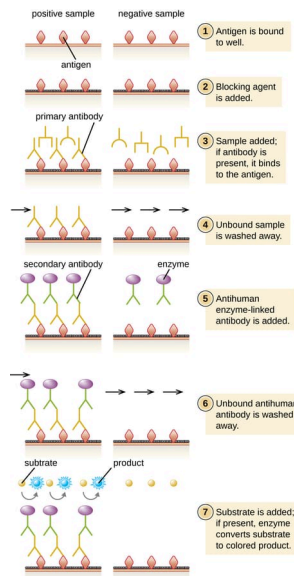


Figure 26.5.4: The indirect ELISA is used to quantify antigen-specific antibodies in patient serum for disease diagnosis. Antigen from the suspected disease agent is attached to microtiter plates. The primary antibody comes from the patient's serum, which is subsequently bound by the enzyme-conjugated secondary antibody. Measuring the production of end product allows us to detect or quantify the amount of antigen-specific antibody present in the patient's serum.

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Immunofiltration and Immunochromatographic Assays

For some situations, it may be necessary to detect or quantify antigens or antibodies that are present at very low concentration in solution. Immunofiltration techniques have been developed to make this possible. In **immunofiltration**, a large volume of fluid is passed through a porous membrane into an absorbent pad. An antigen attached to the porous membrane will capture antibody as it passes; alternatively, we can also attach an antibody to the membrane to capture antigen.

The method of immunofiltration has been adapted in the development of **immunochromatographic assays**, commonly known as **lateral flow tests** or strip tests. These tests are quick and easy to perform, making them popular for point-of-care use (i.e., in the doctor's office) or in-home use. One example is the TORCH test that allows doctors to screen pregnant women or newborns for infection by an array of viruses and other pathogens (*Toxoplasma*, other viruses, rubella, cytomegalovirus, herpes simplex). In-home pregnancy tests are another widely used example of a lateral flow test (Figure 26.5.5). Immunofiltration tests are also popular in developing countries, because they are inexpensive and do not require constant refrigeration of the dried reagents. However, the technology is also built into some sophisticated laboratory equipment.

In lateral flow tests (Figure 26.5.6), fluids such as urine are applied to an absorbent pad on the test strip. The fluid flows by capillary action and moves through a stripe of beads with antibodies attached to their surfaces. The fluid in the sample actually hydrates the reagents, which are present in a dried state in the stripe. Antibody-coated beads made of latex or tiny gold particles will bind antigens in the test fluid. The antibody-antigen complexes then flow over a second stripe that has immobilized antibody against the antigen; this stripe will retain the beads that have bound antigen. A third control stripe binds any beads. A red color (from gold particles) or blue (from latex beads) developing at the test line indicates a positive test. If the color only develops at the control line, the test is negative.

Like ELISA techniques, lateral flow tests take advantage of antibody sandwiches, providing sensitivity and specificity. While not as

quantitative as ELISA, these tests have the advantage of being fast, inexpensive, and not dependent on special equipment. Thus, they can be performed anywhere by anyone. There are some concerns about putting such powerful diagnostic tests into the hands of people who may not understand the tests' limitations, such as the possibility of false-positive results. While home pregnancy tests have become widely accepted, at-home antibody-detection tests for diseases like HIV have raised some concerns in the medical community. Some have questioned whether self-administration of such tests should be allowed in the absence of medical personnel who can explain the test results and order appropriate confirmatory tests. However, with growing numbers of lateral flow tests becoming available, and the rapid development of lab-on-a-chip technology (Figure 20.1), home medical tests are likely to become even more commonplace in the future.

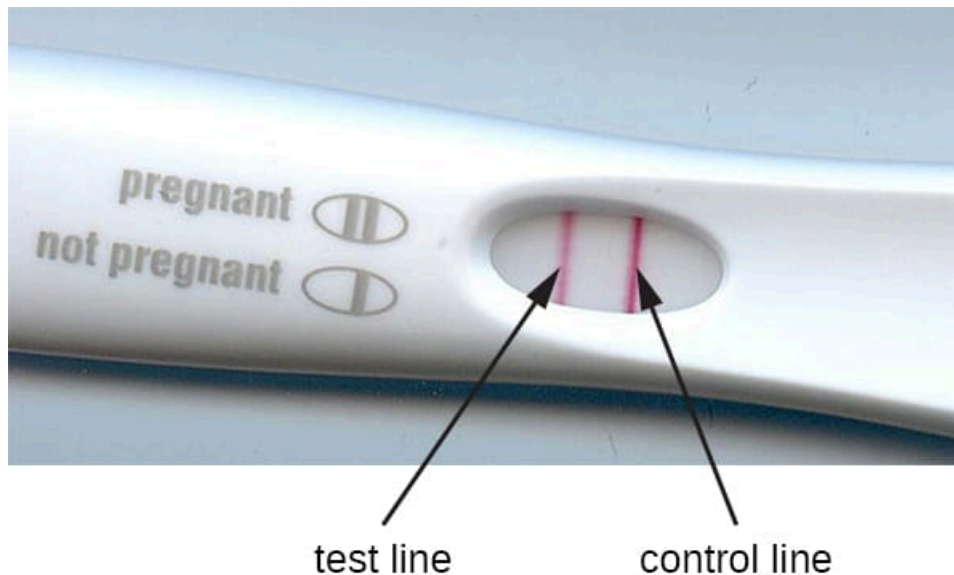


Figure 26.5.5: A lateral flow test detecting pregnancy-related hormones in urine. The control stripe verifies the validity of the test and the test line determines the presence of pregnancy-related hormones in the urine. (credit: modification of work by Klaus Hoffmeier)

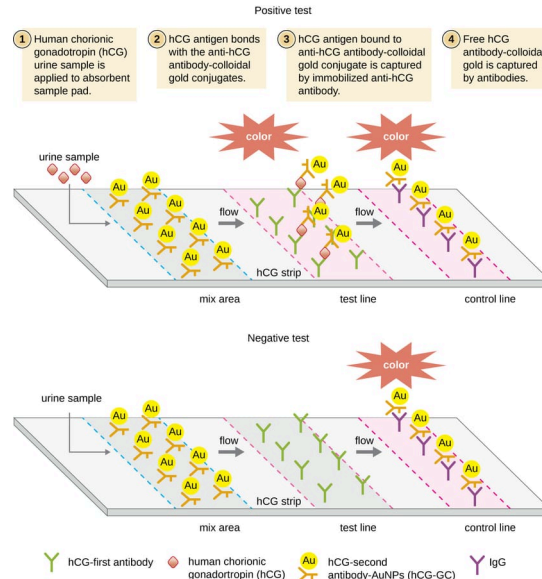


Figure 26.5.6: Immunochromatographic assays, or lateral flow tests, allow the testing of antigen in a dilute solution. As the fluid flows through the test strip, it rehydrates the reagents. Antibodies conjugated to small particles bind the antigen in the first stripe and then flow onto the second stripe where they are bound by a second, fixed antibody. This produces a line of color, depending on the color of the beads. The third, control stripe binds beads as well to indicate that the test is working properly. (credit: modification of work by Yeh CH, Zhao ZQ, Shen PL, Lin YC)

Table 26.5.1 compares some of the key mechanisms and examples of some of the EIAs discussed in this section as well as immunoblots, which were discussed in [Detecting Antigen-Antibody Complexes](#).

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Table 26.5.1: Immunoblots & Enzyme Immunoassays

Type of Assay	Mechanism	Specific Procedures	Examples
Immunoblots	Uses enzyme-antibody conjugates to identify specific proteins that have been transferred to an absorbent membrane	Western blot: Detects the presence of a particular protein	Detecting the presence of HIV peptides (or peptides from other infectious agents) in patient sera
Immunostaining	Uses enzyme-antibody conjugates to stain specific molecules on or in cells	Immunohistochemistry: Used to stain specific cells in a tissue	Stain for presence of CD8 cells in host tissue
Enzyme-linked immunosorbent assay (ELISA)	Uses enzyme-antibody conjugates to quantify target molecules	Direct ELISA: Uses a single antibody to detect the presence of an antigen	Detection of HIV antigen p24 up to one month after being infected
		Indirect ELISA: Measures the amount of antibody produced against an antigen	Detection of HIV antibodies in serum
Immunochromatographic (lateral flow) assays	Techniques use the capture of flowing, color-labeled antigen-antibody complexes by fixed antibody for disease diagnosis	Sandwich ELISA: Measures the amount of antigen bound by the antibody	Detection of antibodies for various pathogens in patient sera (e.g., rapid strep, malaria dipstick)
			Pregnancy test detecting human chorionic gonadotrophin in urine

Key Concepts and Summary

- **Enzyme immunoassays (EIA)** are used to visualize and quantify antigens. They use an antibody conjugated to an enzyme to bind the antigen, and the enzyme converts a substrate into an observable end product. The substrate may be either a chromogen or a fluorogen.
- **Immunostaining** is an EIA technique for visualizing cells in a tissue (**immunohistochemistry**) or examining intracellular structures (**immunocytochemistry**).
- **Direct ELISA** is used to quantify an antigen in solution. The primary antibody captures the antigen, and the secondary

antibody delivers an enzyme. Production of end product from the chromogenic substrate is directly proportional to the amount of captured antigen.

- **Indirect ELISA** is used to detect antibodies in patient serum by attaching antigen to the well of a microtiter plate, allowing the patient (primary) antibody to bind the antigen and an enzyme-conjugated secondary antibody to detect the primary antibody.
- **Immunofiltration and immunochromatographic assays** are used in **lateral flow tests**, which can be used to diagnose pregnancy and various diseases by detecting color-labeled antigen-antibody complexes in urine or other fluid samples

Footnotes

1. 1 Thomas, Justin G., Victor Jaffe, Judith Shaffer, and Jose Abreu, "HIV Testing: US Recommendations 2014," *Osteopathic Family Physician* 6, no. 6 (2014).

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26.6: Fluorescent Antibody Techniques

Learning Objectives

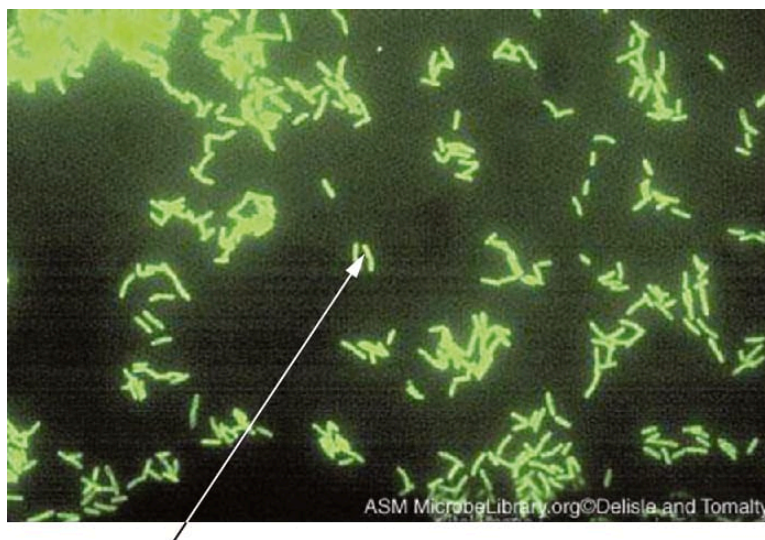
- Describe the benefits of immunofluorescent antibody assays in comparison to nonfluorescent assays
- Compare direct and indirect fluorescent antibody assays
- Explain how a flow cytometer can be used to quantify specific subsets of cells present in a complex mixture of cell types
- Explain how a fluorescence-activated cell sorter can be used to separate unique types of cells

Rapid visualization of bacteria from a clinical sample such as a throat swab or sputum can be achieved through **fluorescent antibody (FA) techniques** that attach a fluorescent marker (fluorogen) to the constant region of an antibody, resulting in a reporter molecule that is quick to use, easy to see or measure, and able to bind to target markers with high specificity. We can also label cells, allowing us to precisely quantify particular subsets of cells or even purify these subsets for further research.

As with the enzyme assays, FA methods may be direct, in which a labeled mAb binds an antigen, or indirect, in which secondary polyclonal antibodies bind patient antibodies that react to a prepared antigen. Applications of these two methods were demonstrated in [Figure 2.3.8](#). FA methods are also used in automated cell counting and sorting systems to enumerate or segregate labeled subpopulations of cells in a sample.

Direct Fluorescent Antibody Techniques

Direct fluorescent antibody (DFA) tests use a fluorescently labeled mAb to bind and illuminate a target antigen. DFA tests are particularly useful for the rapid diagnosis of bacterial diseases. For example, fluorescence-labeled antibodies against *Streptococcus pyogenes* (group A strep) can be used to obtain a diagnosis of strep throat from a throat swab. The diagnosis is ready in a matter of minutes, and the patient can be started on antibiotics before even leaving the clinic. DFA techniques may also be used to diagnose pneumonia caused by *Mycoplasma pneumoniae* or *Legionella pneumophila* from sputum samples ([Figure 26.6.1](#)). The fluorescent antibodies bind to the bacteria on a microscope slide, allowing ready detection of the bacteria using a fluorescence microscope. Thus, the DFA technique is valuable for visualizing certain bacteria that are difficult to isolate or culture from patient samples.



Fluorecein-labeled antibody attached to *Legionella* bacilli

Figure 26.6.1: A green fluorescent mAb against *L. pneumophila* is used here to visualize and identify bacteria from a smear of a sample from the respiratory tract of a pneumonia patient. (credit: modification of work by American Society for Microbiology)

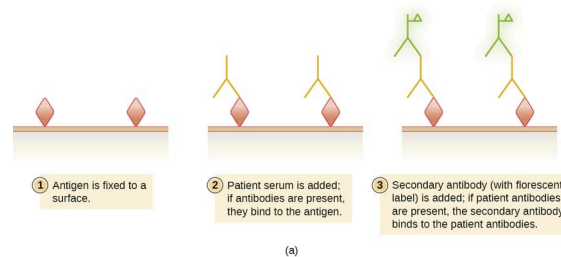
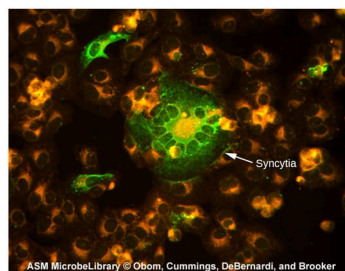
Indirect Fluorescent Antibody Techniques

Indirect fluorescent antibody (IFA) tests (Figure 26.6.2) are used to look for antibodies in patient serum. For example, an IFA test for the diagnosis of syphilis uses *T. pallidum* cells isolated from a lab animal (the bacteria cannot be grown on lab media) and a smear prepared on a glass slide. Patient serum is spread over the smear and anti-treponemal antibodies, if present, are allowed to bind. The serum is washed off and a secondary antibody added. The secondary antibody is an antihuman immunoglobulin conjugated to a fluorogen. On examination, the *T. pallidum* bacteria will only be visible if they have been bound by the antibodies from the patient's serum.

The IFA test for syphilis provides an important complement to the VDRL test discussed in [Detecting Antigen-Antibody Complexes](#). The VDRL is more likely to generate false-positive reactions than the IFA test; however, the VDRL is a better test for determining whether an infection is currently active.

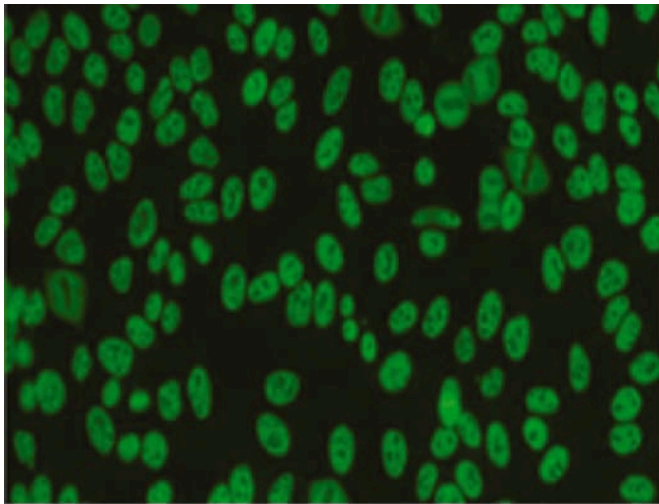
IFA tests are also useful for the diagnosis of autoimmune diseases. For example, systemic lupus erythematosus (SLE) (see [Autoimmune Disorders](#)) is characterized by elevated expression levels of antinuclear antibodies (ANA). These autoantibodies can be expressed against a variety of DNA-binding proteins and even against DNA itself. Because autoimmunity is often difficult to diagnose, especially early in disease progression, testing for ANA can be a valuable clue in making a diagnosis and starting appropriate treatment.

The IFA for ANA begins by fixing cells grown in culture to a glass slide and making them permeable to antibody. The slides are then incubated with serial dilutions of serum from the patient. After incubation, the slide is washed to remove unbound proteins, and the fluorescent antibody (antihuman IgG conjugated to a fluorogen) added. After an incubation and wash, the cells can be examined for fluorescence evident around the nucleus (Figure 26.6.3). The titer of ANA in the serum is determined by the highest dilution showing fluorescence. Because many healthy people express ANA, the American College of Rheumatology recommends that the titer must be at least 1:40 in the presence of symptoms involving two or more organ systems to be considered indicative of SLE.¹



(b)

Figure 26.6.2: (a) The IFA test is used to detect antigen-specific antibodies by allowing them to bind to antigen fixed to a surface and then illuminating these complexes with a secondary antibody-fluorogen conjugate. (b) In this example of a micrograph of an indirect fluorescent antibody test, a patient's antibodies to the measles virus bind to viral antigens present on inactivated measles-infected cells affixed to a slide. Secondary antibodies bind the patient's antibodies and carry a fluorescent molecule. (credit b: modification of work by American Society for Microbiology)



diseased



healthy control

Figure 26.6.3: In this test for antinuclear antibodies (ANA), cells are exposed to serum from a patient suspected of making ANA and then to a fluorescent mAb specific for human immunoglobulin. As a control, serum from a healthy patient is also used. Visible fluorescence around the nucleus demonstrates the presence of ANA in the patient's serum. In the healthy control where lower levels of ANA are produced, very faint green is detected. (credit left, right: modification of work by Al-Hussaini AA, Alzahrani MD, Alenizi AS, Suliman NM, Khan MA, Alharbi SA, Chentoufi AA)

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Flow Cytometry

Fluorescently labeled antibodies can be used to quantify cells of a specific type in a complex mixture using **flow cytometry** (Figure 26.6.4), an automated, cell-counting system that detects fluorescing cells as they pass through a narrow tube one cell at a time. For example, in HIV infections, it is important to know the level of CD4 T cells in the patient's blood; if the numbers fall below 500 per μL of blood, the patient becomes more likely to acquire opportunistic infections; below 200 per μL , the patient can no longer mount a useful adaptive immune response at all. The analysis begins by incubating a mixed-cell population (e.g., white blood cells from a donor) with a fluorescently labeled mAb specific for a subpopulation of cells (e.g., anti-CD4). Some experiments look at two cell markers simultaneously by adding a different fluorogen to the appropriate mAb. The cells are then introduced to the flow cytometer through a narrow capillary that forces the cells to pass in single file. A laser is used to activate the fluorogen. The fluorescent light radiates out in all directions, so the fluorescence detector can be positioned at an angle from the incident laser light.

Figure 26.6.4 shows the obscuration bar in front of the forward-scatter detector that prevents laser light from hitting the detector. As a cell passes through the laser bar, the forward-scatter detector detects light scattered around the obscuration bar. The scattered light is transformed into a voltage pulse, and the cytometer counts a cell. The fluorescence from a labeled cell is detected by the side-scatter detectors. The light passes through various dichroic mirrors such that the light emitted from the fluorophore is received by the correct detector.

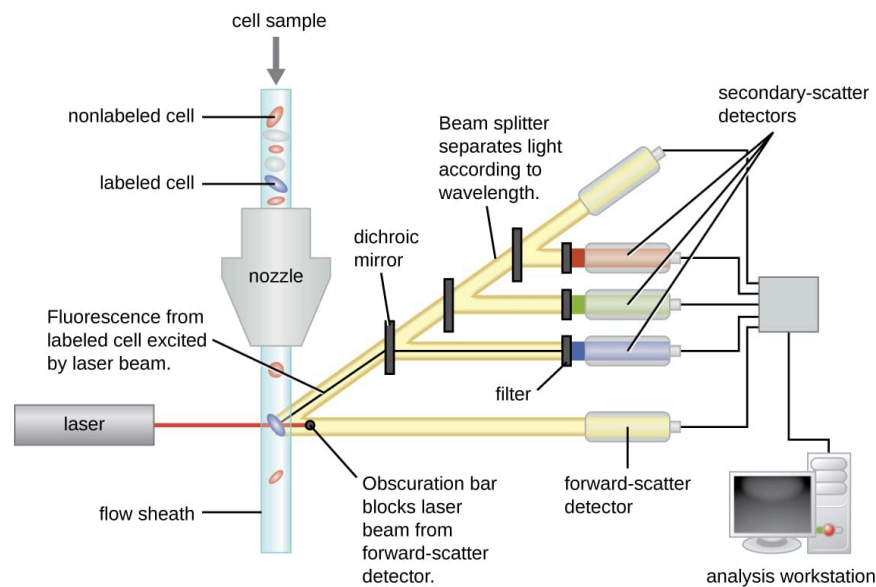


Figure 26.6.4: In flow cytometry, a mixture of fluorescently labeled and unlabeled cells passes through a narrow capillary. A laser excites the fluorogen, and the fluorescence intensity of each cell is measured by a detector. (credit: modification of work by "Kierano"/Wikimedia Commons)

Data are collected from both the forward- and side-scatter detectors. One way these data can be presented is in the form of a histogram. The forward scatter is placed on the y-axis (to represent the number of cells), and the side scatter is placed on the x-axis (to represent the fluorescence of each cell). The scaling for the x-axis is logarithmic, so fluorescence intensity increases by a factor of 10 with each unit increase along the axis. Figure 26.6.5 depicts an example in which a culture of cells is combined with an antibody attached to a fluorophore to detect CD8 cells and then analyzed by flow cytometry. The histogram has two peaks. The peak on the left has lower fluorescence readings, representing the subset of the cell population (approximately 30 cells) that does not fluoresce; hence, they are not bound by antibody and therefore do not express CD8. The peak on the right has higher fluorescence readings, representing the subset of the cell population (approximately 100 cells) that show fluorescence; hence, they are bound by the antibody and therefore do express CD8.

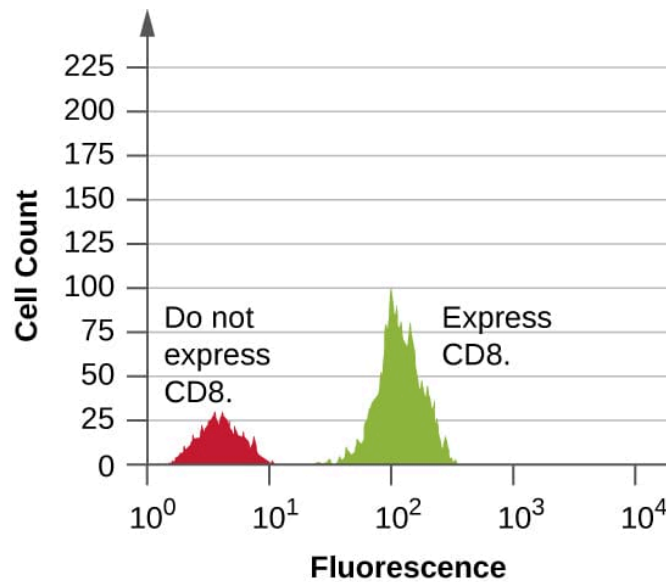


Figure 26.6.5: Flow cytometry data are often compiled as a histogram. In the histogram, the area under each peak is proportional to the number of cells in each population. The x-axis is the relative fluorescence expressed by the cells (on a log scale), and the y-axis represents the number of cells at a particular level of fluorescence.

Cell Sorting Using Immunofluorescence

The flow cytometer and immunofluorescence can also be modified to sort cells from a single sample into purified subpopulations of cells for research purposes. This modification of the flow cytometer is called a **fluorescence-activated cell sorter (FACS)**. In a FACS, fluorescence by a cell induces the device to put a charge on a droplet of the transporting fluid containing that cell. The charge is specific to the wavelength of the fluorescent light, which allows for differential sorting by those different charges. The sorting is accomplished by an electrostatic deflector that moves the charged droplet containing the cell into one collecting vessel or another. The process results in highly purified subpopulations of cells.

One limitation of a FACS is that it only works on isolated cells. Thus, the method would work in sorting white blood cells, since they exist as isolated cells. But for cells in a tissue, flow cytometry can only be applied if we can excise the tissue and separate it into single cells (using proteases to cleave cell-cell adhesion molecules) without disrupting cell integrity. This method may be used on tumors, but more often, immunohistochemistry and immunocytochemistry are used to study cells in tissues.

Link to Learning

Watch videos to learn more about how [flow cytometry](#) and a [FACS](#) work.

Query 26.6.1

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

[View this Interactive Content online](#)



Table 26.6.1 compares the mechanisms of the fluorescent antibody techniques discussed in this section.

Table 26.6.1: Fluorescent Antibody Techniques

Type of Assay	Mechanism	Examples
Direct fluorescent antibody (DFA)	Uses fluorogen-antibody conjugates to label bacteria from patient samples	Visualizing <i>Legionella pneumophila</i> from a throat swab
Indirect fluorescent antibody (IFA)	Detects disease-specific antibodies in patient serum	Diagnosing syphilis; detecting antinuclear antibodies (ANA) for lupus and other autoimmune diseases
Flow cytometry	Labels cell membranes with fluorogen-antibody conjugate markers excited by a laser; machine counts the cell and records the relative fluorescence	Counting the number of fluorescently labeled CD4 or CD8 cells in a sample
Fluorescence activated cell sorter (FACS)	Form of flow cytometry that both counts cells and physically separates them into pools of high and low fluorescence cells	Sorting cancer cells

Key Concepts and Summary

- **Immunofluorescence** assays use antibody-fluorogen conjugates to illuminate antigens for easy, rapid detection.
- **Direct immunofluorescence** can be used to detect the presence of bacteria in clinical samples such as sputum.
- **Indirect immunofluorescence** detects the presence of antigen-specific antibodies in patient sera. The fluorescent antibody binds to the antigen-specific antibody rather than the antigen.
- The use of indirect immunofluorescence assays to detect **antinuclear antibodies** is an important tool in the diagnosis of several autoimmune diseases.
- **Flow cytometry** uses fluorescent mAbs against cell-membrane proteins to quantify specific subsets of cells in complex mixtures.
- **Fluorescence-activated cell sorters** are an extension of flow cytometry in which fluorescence intensity is used to physically separate cells into high and low fluorescence populations.

Footnotes

- 1 Gill, James M., ANNA M. Quisel, PETER V. Rocca, and DENE T. Walters. "Diagnosis of systemic lupus erythematosus." *American family physician* 68, no. 11 (2003): 2179-2186.

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26.E: Laboratory Analysis of the Immune Response (Exercises)

26.1: Practical Applications of Monoclonal and Polyclonal Antibodies

In addition to being crucial for our normal immune response, antibodies provide powerful tools for research and diagnostic purposes. The high specificity of antibodies makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms. With in vitro assays, antibodies can be used to precipitate soluble antigens, agglutinate cells, and neutralize drugs, toxins, and viruses.

Multiple Choice

For many uses in the laboratory, polyclonal antibodies work well, but for some types of assays, they lack sufficient _____ because they cross-react with inappropriate antigens.

- A. specificity
- B. sensitivity
- C. accuracy
- D. reactivity

Answer

A

How are monoclonal antibodies produced?

- A. Antibody-producing B cells from a mouse are fused with myeloma cells and then the cells are grown in tissue culture.
- B. A mouse is injected with an antigen and then antibodies are harvested from its serum.
- C. They are produced by the human immune system as a natural response to an infection.
- D. They are produced by a mouse's immune system as a natural response to an infection.

Answer

A

Fill in the Blank

When we inject an animal with the same antigen a second time a few weeks after the first, _____ takes place, which means the antibodies produced after the second injection will on average bind the antigen more tightly.

Answer

affinity maturation

When using mAbs to treat disease in humans, the mAbs must first be _____ by replacing the mouse constant region DNA with human constant region DNA.

Answer

humanized

If we used normal mouse mAbs to treat human disease, multiple doses would cause the patient to respond with _____ against the mouse antibodies.

Answer

neutralizing antibodies

A polyclonal response to an infection occurs because most antigens have multiple _____,

Answer

epitopes

Short Answer

Describe two reasons why polyclonal antibodies are more likely to exhibit cross-reactivity than monoclonal antibodies.

Critical Thinking

Suppose you were screening produce in a grocery store for the presence of *E. coli* contamination. Would it be better to use a polyclonal anti-*E. coli* antiserum or a mAb against an *E. coli* membrane protein? Explain.

26.2: Detecting Antigen-Antibody Complexes in vitro

Laboratory tests to detect antibodies and antigens outside of the body (e.g., in a test tube) are called *in vitro* assays. When both antibodies and their corresponding antigens are present in a solution, we can often observe a precipitation reaction in which large complexes (lattices) form and settle out of solution.

Multiple Choice

The formation of _____ is a positive result in the VDRL test.

- A. flocculant
- B. precipitin
- C. coagulation
- D. a bright pink color

Answer

A

The titer of a virus neutralization test is the highest dilution of patient serum

- A. in which there is no detectable viral DNA.
- B. in which there is no detectable viral protein.
- C. that completely blocks plaque formation.
- D. that reduces plaque formation by at least 50%.

Answer

D

In the Ouchterlony assay, we see a sharp precipitin arc form between antigen and antiserum. Why does this arc remain visible for a long time?

- A. The antibody molecules are too large to diffuse through the agar.
- B. The precipitin lattice is too large to diffuse through the agar.
- C. Methanol, added once the arc forms, denatures the protein and blocks diffusion.
- D. The antigen molecules are chemically coupled to the gel matrix.

Answer

B

Fill in the Blank

When slowly adding antigen to an antiserum, the amount of precipitin would gradually increase until reaching the _____; addition of more antigen after this point would actually decrease the amount of precipitin.

Answer

equivalence zone or zone of equivalence

The radial immunodiffusion test quantifies antigen by mixing _____ into a gel and then allowing antigen to diffuse out from a well cut in the gel.

Answer

antiserum

Short Answer

Explain why hemolysis in the complement fixation test is a negative test for infection.

What is meant by the term “neutralizing antibodies,” and how can we quantify this effect using the viral neutralization assay?

Critical Thinking

Both IgM and IgG antibodies can be used in precipitation reactions. However, one of these immunoglobulin classes will form precipitates at much lower concentrations than the other. Which class is this, and why is it so much more efficient in this regard?

26.3: Agglutination Assays

In addition to causing precipitation of soluble molecules and flocculation of molecules in suspension, antibodies can also clump together cells or particles (e.g., antigen-coated latex beads) in a process called agglutination. Agglutination can be used as an indicator of the presence of antibodies against bacteria or red blood cells. Agglutination assays are usually quick and easy to perform on a glass slide or microtiter plate.

Multiple Choice

We use antisera to distinguish between various _____ within a species of bacteria.

- A. isotypes
- B. serovars
- C. subspecies
- D. lines

Answer

B

When using antisera to characterize bacteria, we will often link the antibodies to _____ to better visualize the agglutination.

- A. latex beads
- B. red blood cells
- C. other bacteria

D. white blood cells

Answer

A

The antibody screening test that is done along with pretransfusion blood typing is used to ensure that the recipient

- A. does not have a previously undetected bacterial or viral infection.
- B. is not immunocompromised.
- C. actually does have the blood type stated in the online chart.
- D. is not making antibodies against antigens outside the ABO or Rh systems.

Answer

D

The direct Coombs' test is designed to detect when people have a disease that causes them to

- A. have an excessively high fever.
- B. quit making antibodies.
- C. make too many red blood cells.
- D. produce antibodies that bind to their own red blood cells.

Answer

D

Viral hemagglutination assays only work with certain types of viruses because

- A. the virus must be able to cross-link red blood cells directly.
- B. the virus must be able to lyse red blood cells.
- C. the virus must not be able to lyse red blood cells.
- D. other viruses are too dangerous to work with in a clinical lab setting.

Answer

A

Fill in the Blank

In the major cross-match, we mix _____ with the donor red blood cells and look for agglutination.

Answer

patient serum

Coombs' reagent is an antiserum with antibodies that bind to human _____.

Answer

immunoglobulins/antibodies and/or complement

Short Answer

Explain why the titer of a direct hemagglutination assay is the highest dilution that still causes hemagglutination, whereas in the viral hemagglutination inhibition assay, the titer is the highest dilution at which hemagglutination is not observed.

Why would a doctor order a direct Coombs' test when a baby is born with jaundice?

Critical Thinking

When shortages of donated blood occur, O-negative blood may be given to patients, even if they have a different blood type. Why is this the case? If O-negative blood supplies were depleted, what would be the next-best choice for a patient with a different blood type in critical need of a transfusion? Explain your answers.

26.4: Enzyme Immunoassays (EIA) and Enzyme-Linked Immunosorbent Assays (ELISA)

Enzyme immunoassays (EIA) are used to visualize and quantify antigens. They use an antibody conjugated to an enzyme to bind the antigen, and the enzyme converts a substrate into an observable end product. The substrate may be either a chromogen or a fluorogen. Immunostaining is an EIA technique for visualizing cells in a tissue (immunohistochemistry) or examining intracellular structures (immunocytochemistry). Direct ELISA is used to quantify an antigen in solution.

Multiple Choice

In an enzyme immunoassay, the enzyme

- A. is bound by the antibody's antigen-binding site.
- B. is attached to the well of a microtiter plate.
- C. is conjugated to the suspect antigen.
- D. is bound to the constant region of the secondary antibody.

Answer

D

When using an EIA to study microtubules or other structures inside a cell, we first chemically fix the cell and then treat the cells with alcohol. What is the purpose of this alcohol treatment?

- A. It makes holes in the cell membrane large enough for antibodies to pass.
- B. It makes the membrane sticky so antibodies will bind and be taken up by receptor-mediated endocytosis.
- C. It removes negative charges from the membrane, which would otherwise repulse the antibodies.
- D. It prevents nonspecific binding of the antibodies to the cell membrane.

Answer

A

In a lateral-flow pregnancy test, you see a blue band form on the control line and no band form on the test line. This is probably a _____ test for pregnancy.

- A. positive
- B. false-positive
- C. false-negative
- D. negative

Answer

D

When performing an FEIA, the fluorogen replaces the _____ that is used in an EIA.

- A. antigen
- B. chromogenic substrate
- C. enzyme
- D. secondary antibody

Answer

B

Fill in the Blank

To detect antibodies against bacteria in the bloodstream using an EIA, we would run a(n) _____, which we would start by attaching antigen from the bacteria to the wells of a microtiter plate.

Answer

indirect ELISA

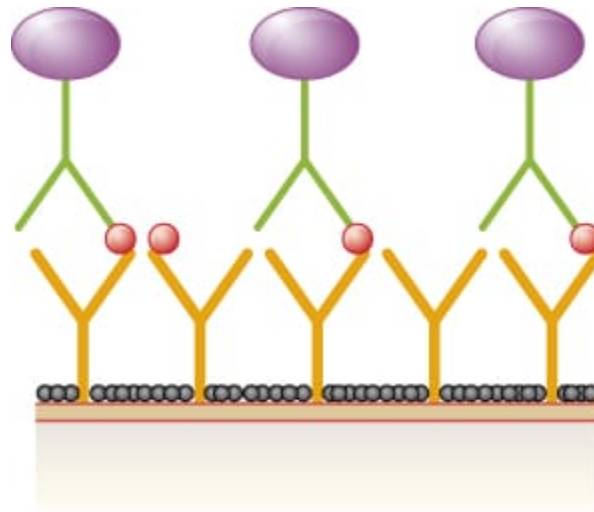
Short Answer

Why is it important in a sandwich ELISA that the antigen has multiple epitopes? And why might it be advantageous to use polyclonal antisera rather than mAb in this assay?

The pregnancy test strip detects the presence of human chorionic gonadotrophin in urine. This hormone is initially produced by the fetus and later by the placenta. Why is the test strip preferred for this test rather than using either a direct or indirect ELISA with their more quantifiable results?

Critical Thinking

Label the primary and secondary antibodies, and discuss why the production of end product will be proportional to the amount of antigen.



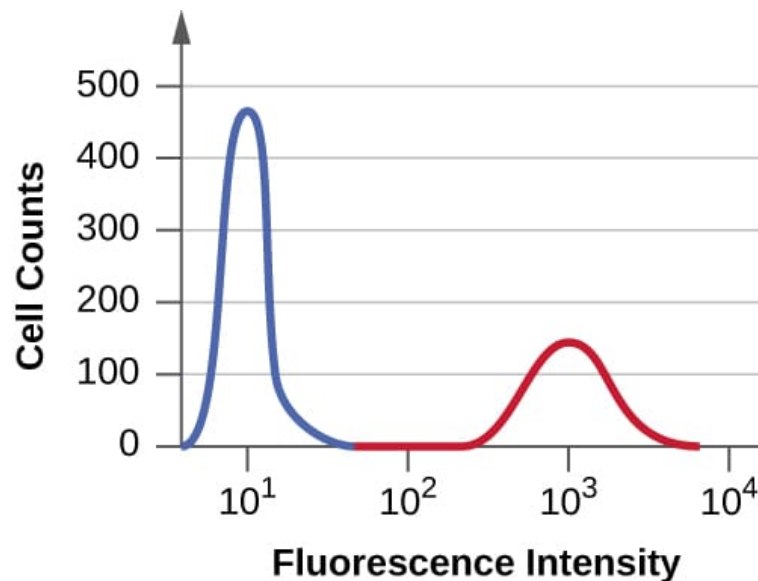
26.5: Fluorescent Auto-Antibody Techniques

Rapid visualization of bacteria from a clinical sample such as a throat swab or sputum can be achieved through fluorescent antibody (FA) techniques that attach a fluorescent marker (fluorogen) to the constant region of an antibody, resulting in a reporter molecule that is quick to use, easy to see or measure, and able to bind to target markers with high specificity. We can also label cells, allowing us to precisely quantify particular subsets of cells or even purify them for further research.

Multiple Choice

Suppose you need to quantify the level of CD8 T cells in the blood of a patient recovering from influenza. You treat a sample of

the patient's white blood cells using a fluorescent mAb against CD8, pass the cells through a flow cytometer, and produce the histogram shown below. The area under the peak to the left (blue) is three times greater than the area of the peak on the right (red). What can you determine from these data?



- A. There are no detectable CD8 cells.
- B. There are three times as many CD4 cells than CD8 cells.
- C. There are three times as many CD8 cells than CD4 cells.
- D. CD8 cells make up about one-fourth of the total number of cells.

Answer

D

In the data described in the previous question, the average fluorescence intensity of cells in the second (red) peak is about _____ that in the first (blue) peak.

- A. three times
- B. 100 times
- C. one-third
- D. 1000 times

Answer

B

In a direct fluorescent antibody test, which of the following would we most likely be looking for using a fluorescently-labeled mAb?

- A. bacteria in a patient sample
- B. bacteria isolated from a patient and grown on agar plates
- C. antiserum from a patient smeared onto a glass slide
- D. antiserum from a patient that had bound to antigen-coated beads

Answer

A

Fill in the Blank

In flow cytometry, cell subsets are labeled using a fluorescent antibody to a membrane protein. The fluorogen is activated by a(n)

_____ as the cells pass by the detectors.

Answer

laser

Fluorescence in a flow cytometer is measured by a detector set at an angle to the light source. There is also an in-line detector that can detect cell clumps or _____.

Answer

fragments

Critical Thinking

A patient suspected of having syphilis is tested using both the VDRL test and IFA. The IFA test comes back positive, but the VDRL test is negative. What is the most likely reason for these results?

A clinician suspects that a patient with pneumonia may be infected by *Legionella pneumophila*. Briefly describe two reasons why a DFA test might be better for detecting this pathogen than standard bacteriology techniques.

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