MKBN211: INTRODUCTORY MICROBIOLOGY



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MKBN211 Introductory Microbiology

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SECTION OVERVIEW

1.1: Introduction to Microbiology

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1.1.1: Defining Microbes

LEARNING OBJECTIVES

- Explain the roles of microorganisms in ecosystems and biotechnology.

A microbe, or microorganism, is a microscopic organism that comprises either a single cell (unicellular); cell clusters; or multicellular, relatively complex organisms. The study of microorganisms is called microbiology, a subject that began with Anton van Leeuwenhoek's discovery of microorganisms in 1675, using a microscope of his own design.

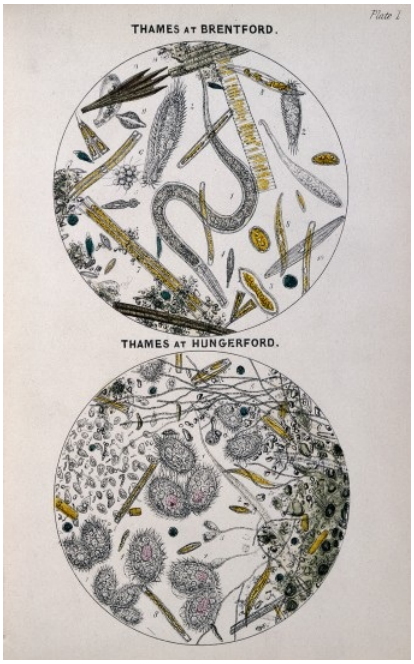


Figure: ***A Drawing of Microbes****: This is a drawing of what Arthur Hill Hassall saw under a microscope in a sample of water taken from the River Thames at two locations. Hassall was able to identify many microscopic organisms not perceptible to the unaided eye,.*

Microorganisms are very diverse; they include bacteria, fungi, algae, and protozoa; microscopic plants (green algae); and animals such as rotifers and planarians. Some microbiologists also include viruses, but others consider these as nonliving. Most microorganisms are unicellular, but this is not universal, since some multicellular organisms are microscopic. Some unicellular protists and bacteria, like *Thiomargarita namibiensis*, are macroscopic and visible to the naked eye.

Microorganisms live in all parts of the biosphere where there is liquid water, including soil, hot springs, on the ocean floor, high in the atmosphere, and deep inside rocks within the Earth's crust. Most importantly, these organisms are vital to humans and the environment, as they participate in the Earth's element cycles, such as the carbon cycle and the nitrogen cycle.

Microorganisms also fulfill other vital roles in virtually all ecosystems, such as recycling other organisms' dead remains and waste products through decomposition. Microbes have an important place in most higher-order multicellular organisms as symbionts, and they are also exploited by people in biotechnology, both in traditional food and beverage preparation, and in modern technologies based on genetic engineering. Pathogenic microbes are harmful, however, since they invade and grow within other organisms, causing diseases that kill humans, animals, and plants.

The Pathogenic Ecology of Microbes

Although many microorganisms are beneficial, many others are the cause of infectious diseases. The organisms involved include pathogenic bacteria, which cause diseases such as plague, tuberculosis, and anthrax. Biofilms —microbial communities that are very difficult to destroy—are considered responsible for diseases like bacterial infections in patients with cystic fibrosis,

Legionnaires' disease, and otitis media (middle ear infection). They produce dental plaque; colonize catheters, prostheses, transcutaneous, and orthopedic devices; and infect contact lenses, open wounds, and burned tissue.

Biofilms also produce foodborne diseases because they colonize the surfaces of food and food-processing equipment. Biofilms are a large threat because they are resistant to most of the methods used to control microbial growth. Moreover, the excessive use of antibiotics has resulted in a major global problem since resistant forms of bacteria have been selected over time. A very dangerous strain, methicillin-resistant *Staphylococcus aureus* (MRSA), has wreaked havoc recently.

In addition, protozoans are known to cause diseases such as malaria, sleeping sickness, and toxoplasmosis, while fungi can cause diseases such as ringworm, candidiasis, or histoplasmosis. Other diseases such as influenza, yellow fever, and AIDS are caused by viruses.

Food-borne diseases result from the consumption of contaminated food, pathogenic bacteria, viruses, or parasites that contaminate food. " Hygiene " is the avoidance of infection or food spoiling by eliminating microorganisms from the surroundings. As microorganisms (bacteria, in particular) are found virtually everywhere, the levels of harmful microorganisms can be reduced to acceptable levels with proper hygiene techniques. In some cases, however, it is required that an object or substance be completely sterile (i.e., devoid of all living entities and viruses). A good example of this is a hypodermic needle.

Key Points

- While most microbes are unicellular, some multicellular animals and plants are also microscopic and are therefore broadly defined as "microbes."
- Microbes serve many functions in almost any ecosystem on Earth, including decomposition and nitrogen fixation.
- Many microbes are either pathogens or parasitic organisms, both of which can harm humans.

Key Terms

- **symbiote**: An organism in a partnership with another, such that each profits from the other.
- **pathogenic**: Able to cause a harmful disease.
- **ecosystem**: The interconnectedness of plants, animals, and microbes, not only with each other but also with their environment.

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1.1.2: History of Microbiology - Hooke, van Leeuwenhoek, and Cohn

LEARNING OBJECTIVES

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

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

The Microscope and Discovery of Microorganisms

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



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

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

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

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



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

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

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

Key Points

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

Key Terms

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

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1.1.3: Pasteur and Spontaneous Generation

LEARNING OBJECTIVES

- Explain the concept of spontaneous generation

Spontaneous generation is an obsolete body of thought on the ordinary formation of living organisms without descent from similar organisms. Typically, the idea was that certain forms such as fleas could arise from inanimate matter such as dust or that maggots could arise from dead flesh. A variant idea was that of equivocal generation, in which species such as tapeworms arose from unrelated living organisms, now understood to be their hosts.

Doctrines held that these processes were commonplace and regular. Such ideas were in contradiction to that of univocal generation: effectively exclusive reproduction from genetically related parent(s), generally of the same species. The doctrine of spontaneous generation was coherently synthesized by Aristotle, who compiled and expanded the work of prior natural philosophers and the various ancient explanations of the appearance of organisms; it held sway for two millennia.

Today spontaneous generation is generally accepted to have been decisively dispelled during the 19th century by the experiments of Louis Pasteur. He expanded upon the investigations of predecessors, such as Francesco Redi who, in the 17th century, had performed experiments based on the same principles.

Louis Pasteur's 1859 experiment is widely seen as having settled the question. In summary, Pasteur boiled a meat broth in a flask that had a long neck that curved downward, like a goose. The idea was that the bend in the neck prevented falling particles from reaching the broth, while still allowing the free flow of air. The flask remained free of growth for an extended period. When the flask was turned so that particles could fall down the bends, the broth quickly became clouded. In detail, Pasteur exposed boiled broths to air in vessels that contained a filter to prevent all particles from passing through to the growth medium, and even in vessels with no filter at all, with air being admitted via a long tortuous tube that would not allow dust particles to pass. Nothing grew in the broths unless the flasks were broken open, showing that the living organisms that grew in such broths came from outside, as spores on dust, rather than spontaneously generated within the broth. This was one of the last and most important experiments disproving the theory of spontaneous generation.

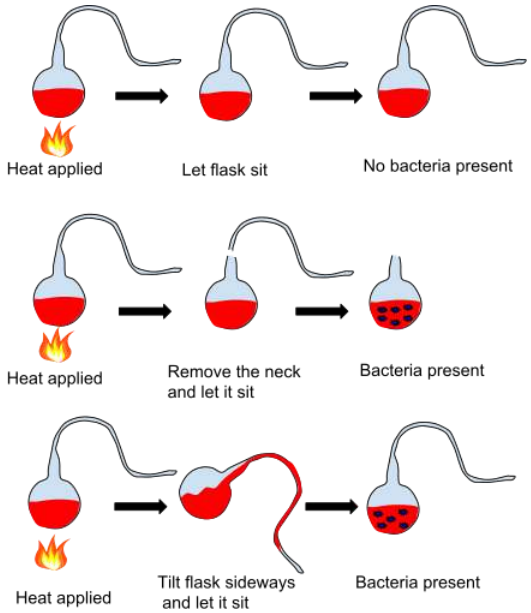


Figure: ***Pasteur's test of spontaneous generation***: *By sterilizing a food source and keeping it isolated from the outside, Pasteur observed no putrefaction of the food source (top panel). Upon exposure to the outside environment, Pasteur observed the putrefaction of the food source (bottom panel). This strongly suggested that the components needed to create life do not spontaneously arise. Louis Pasteur's pasteurization experiment illustrates the fact that the spoilage of liquid was caused by particles in the air rather than the air itself. These experiments were important pieces of evidence supporting the idea of germ theory of disease. (CC BY-SA 4.0; Kgerow16).*

Despite his experiment, objections from persons holding the traditional views persisted. Many of these residual objections were routed by the work of John Tyndall, succeeding the work of Pasteur. Ultimately, the ideas of spontaneous generation were displaced by advances in germ theory and cell theory. Disproof of the traditional ideas of spontaneous generation is no longer controversial among professional biologists. Objections and doubts have been dispelled by studies and documentation of the life cycles of various life forms. However, the principles of the very different matter of the original abiogenesis on this planet — of living from nonliving material — are still under investigation

Key Points

- Before the discovery of microbes, it was widely thought that life, as in the case of rotting food, arose from nothing. This idea was referred to as spontaneous generation.
- By sterilizing cultures and keeping them isolated from the open air, Pasteur found that contamination of the media only occurred upon exposure to the outside environment, showing that some element was needed to give rise to life. In other words, life does not arise spontaneously.
- Despite Pasteur's work and the work of others, it still took a better understanding of germ theory and cell theory to finally displace the concept of spontaneous generation.

Key Terms

- **abiogenesis**: The origination of living organisms from lifeless matter; such genesis as does not involve the action of living parents; spontaneous generation.
- **germ theory**: The germ theory of disease, also called the pathogenic theory of medicine, is a theory that proposes that microorganisms are the cause of many diseases. Although highly controversial when first proposed, germ theory was validated in the late 19th century and is now a fundamental part of modern medicine and clinical microbiology, leading to such important innovations as antibiotics and hygienic practices.

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1.1.4: Koch and Pure Culture

LEARNING OBJECTIVES

- Explain Robert Koch's postulates

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



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

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

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

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

Key Points

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

Key Terms

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

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1.1.4.1: Koch's Postulates

LEARNING OBJECTIVES

- List Koch's postulates

Koch's postulates are four criteria designed to establish a causal relationship between a causative microbe and a disease. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 and refined and published by Koch in 1890. Koch applied the postulates to establish the etiology of anthrax and tuberculosis, but they have been generalized to other diseases.



Figure: ***Robert Koch****: Robert Koch circa 1900. Koch's postulates are four criteria designed in the 1880's to establish a causal relationship between a causative microbe and a disease.*

Koch's postulates were developed in the 19th century as general guidelines to identify pathogens that could be isolated with the techniques of the day. Even in Koch's time, it was recognized that some infectious agents were clearly responsible for disease even though they did not fulfill all of the postulates. Attempts to rigidly apply Koch's postulates to the diagnosis of viral diseases in the late 19th century, at a time when viruses could not be seen or isolated in culture, may have impeded the early development of the field of virology. Currently, a number of infectious agents are accepted as the cause of disease despite their not fulfilling all of Koch's postulates. Therefore, while Koch's postulates retain historical importance and continue to inform the approach to microbiologic diagnosis, fulfillment of all four postulates is not required to demonstrate causality.

Koch's postulates

Koch's postulates are the following:

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Koch's postulates have also influenced scientists who examine microbial pathogenesis from a molecular point of view. In the 1980s, a molecular version of Koch's postulates was developed to guide the identification of microbial genes encoding virulence factors.

Key Points

- The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 and refined and published by Koch in 1890.
- Postulate 1: The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
- Postulate 2: The microorganism must be isolated from a diseased organism and grown in pure culture.
- Postulate 3: The cultured microorganism should cause disease when introduced into a healthy organism.
- Postulate 4: The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Key Terms

- **Koch's postulates**: four criteria designed to establish a causal relationship between a causative microbe and a disease
- **postulate**: A fundamental element; a basic principle.

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1.1.4.2: Exceptions to Koch's Postulates

LEARNING OBJECTIVES

- Recognize the exception to Koch's postulates

Koch's postulates were developed in the 19th century as general guidelines to identify pathogens that could be isolated with the techniques of the day. Even in Koch's time, it was recognized that some infectious agents were clearly responsible for disease, even though they did not fulfill all of the postulates. Currently, a number of infectious agents are accepted as the cause of diseases despite their not fulfilling all of Koch's postulates. Therefore, while Koch's postulates retain historical importance and continue to inform the approach to microbiologic diagnosis, fulfillment of all four postulates is not required to demonstrate causality.

Koch abandoned the requirement of the first postulate altogether when he discovered asymptomatic carriers of cholera and, later, of typhoid fever. Asymptomatic or subclinical infection carriers are now known to be a common feature of many infectious diseases, especially viruses such as polio, herpes simplex, HIV, and hepatitis C. Specifically, all doctors and virologists agree that the poliovirus causes paralysis in just a few infected subjects, and the success of the polio vaccine in preventing disease supports the conviction that the poliovirus is the causative agent.

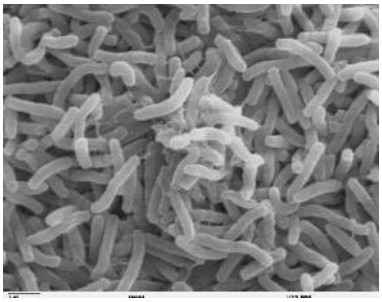


Figure: ***Cholera bacteria****: Scanning electron microscope image of Vibrio cholerae bacteria, which infect the digestive system.*

The second postulate may also be suspended for certain microorganisms or entities that cannot (at the present time) be grown in pure culture, such as prions responsible for Creutzfeldt–Jakob disease.

The third postulate specifies "should", not "must", because as Koch himself proved in regard to both tuberculosis and cholera, that not all organisms exposed to an infectious agent will acquire the infection. Noninfection may be due to such factors as general health and proper immune functioning; acquired immunity from previous exposure or vaccination; or genetic immunity, as with the resistance to malaria conferred by possessing at least one sickle cell allele.

In summary, a body of evidence that satisfies Koch's postulates is sufficient but not necessary to establish causation.

Key Points

- Koch abandoned the requirement of the first postulate altogether when he discovered asymptomatic carriers of cholera.
- The second postulate may also be suspended for certain microorganisms or entities that cannot (at the present time) be grown in pure culture, such as prions responsible for Creutzfeldt–Jakob disease.
- The third postulate specifies "should", not "must", because as Koch himself proved in regard to both tuberculosis and cholera, not all organisms exposed to an infectious agent will acquire the infection.

Key Terms

- **asymptomatic**: not exhibiting any symptoms of disease.

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SECTION OVERVIEW

1.2: Microbes and the World

Topic hierarchy

1.2.1: Types of Microorganisms

1.2.2: Classification of Microorganisms

1.2.3: Microbes and the Origin of Life on Earth

1.2.4: Environmental Diversity of Microbes

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1.2.1: Types of Microorganisms

Microorganisms make up a large part of the planet's living material and play a major role in maintaining the Earth's ecosystem.

Learning Objectives

- Define the differences between microbial organisms.

Key Points

- Microorganisms are divided into seven types: bacteria, archaea, protozoa, algae, fungi, viruses, and multicellular animal parasites (helminths).
- Each type has a characteristic cellular composition, morphology, mean of locomotion, and reproduction.
- Microorganisms are beneficial in producing oxygen, decomposing organic material, providing nutrients for plants, and maintaining human health, but some can be pathogenic and cause diseases in plants and humans.

Key Terms

- **Gram stain**: A method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative).
- **peptidoglycan**: A polymer of glycan and peptides found in bacterial cell walls.

Microorganisms or microbes are microscopic organisms that exist as unicellular, multicellular, or cell clusters. Microorganims are widespread in nature and are beneficial to life, but some can cause serious harm. They can be divided into six major types: bacteria, archaea, fungi, protozoa, algae, and viruses.

Bacteria

Bacteria are unicellular organisms. The cells are described as prokaryotic because they lack a nucleus. They exist in four major shapes: bacillus (rod shape), coccus (spherical shape), spirilla (spiral shape), and vibrio (curved shape). Most bacteria have a peptidoglycan cell wall; they divide by binary fission; and they may possess flagella for motility. The difference in their cell wall structure is a major feature used in classifying these organisms.

According to the way their cell wall structure stains, bacteria can be classified as either Gram-positive or Gram-negative when using the Gram staining. Bacteria can be further divided based on their response to gaseous oxygen into the following groups: aerobic (living in the presence of oxygen), anaerobic (living without oxygen), and facultative anaerobes (can live in both environments).

According to the way they obtain energy, bacteria are classified as heterotrophs or autotrophs. Autotrophs make their own food by using the energy of sunlight or chemical reactions, in which case they are called chemoautotrophs. Heterotrophs obtain their energy by consuming other organisms. Bacteria that use decaying life forms as a source of energy are called saprophytes.

Archaea

Archaea or Archaebacteria differ from true bacteria in their cell wall structure and lack peptidoglycans. They are prokaryotic cells with avidity to extreme environmental conditions. Based on their habitat, all Archaeans can be divided into the following groups: methanogens (methane-producing organisms), halophiles (archaeans that live in salty environments), thermophiles (archaeans that live at extremely hot temperatures), and psychrophiles (cold-temperature Archaeans). Archaeans use different energy sources like hydrogen gas, carbon dioxide, and sulphur. Some of them use sunlight to make energy, but not the same way plants do. They absorb sunlight using their membrane pigment, bacteriorhodopsin. This reacts with light, leading to the formation of the energy molecule adenosine triphosphate (ATP).

Fungi

Fungi (mushroom, molds, and yeasts) are eukaryotic cells (with a true nucleus). Most fungi are multicellular and their cell wall is composed of chitin. They obtain nutrients by absorbing organic material from their environment (decomposers), through symbiotic relationships with plants (symbionts), or harmful relationships with a host (parasites). They form characteristic filamentous tubes called hyphae that help absorb material. The collection of hyphae is called mycelium. Fungi reproduce by releasing spores.

Protozoa

Protozoa are unicellular aerobic eukaryotes. They have a nucleus, complex organelles, and obtain nourishment by absorption or ingestion through specialized structures. They make up the largest group of organisms in the world in terms of numbers, biomass, and diversity. Their cell walls are made up of cellulose. Protozoa have been traditionally divided based on their mode of locomotion: flagellates produce their own food and use their whip-like structure to propel forward, ciliates have tiny hair that beat to produce movement, amoeboids have false feet or pseudopodia used for feeding and locomotion, and sporozoans are non-motile. They also have different means of nutrition, which groups them as autotrophs or heterotrophs.

Algae

Algae, also called cyanobacteria or blue-green algae, are unicellular or multicellular eukaryotes that obtain nourishment by photosynthesis. They live in water, damp soil, and rocks and produce oxygen and carbohydrates used by other organisms. It is believed that cyanobacteria are the origins of green land plants.

Viruses

Viruses are noncellular entities that consist of a nucleic acid core (DNA or RNA) surrounded by a protein coat. Although viruses are classified as microorganisms, they are not considered living organisms. Viruses cannot reproduce outside a host cell and cannot metabolize on their own. Viruses often infest prokaryotic and eukaryotic cells causing diseases.

Multicellular Animal Parasites

A group of eukaryotic organisms consisting of the flatworms and roundworms, which are collectively referred to as the helminths. Although they are not microorganisms by definition, since they are large enough to be easily seen with the naked eye, they live a part of their life cycle in microscopic form. Since the parasitic helminths are of clinical importance, they are often discussed along with the other groups of microbes.

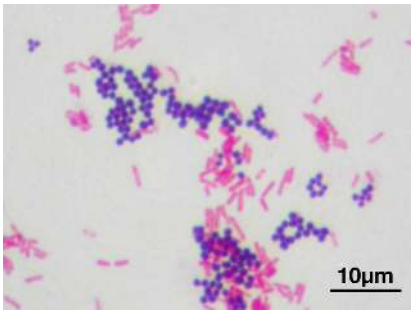


Figure: **Gram Stain**: This is a microscopic image of a Gram stain of mixed Gram-positive cocci (Staphylococcus aureus, purple) and Gram-negative bacilli (Escherichia coli, red).

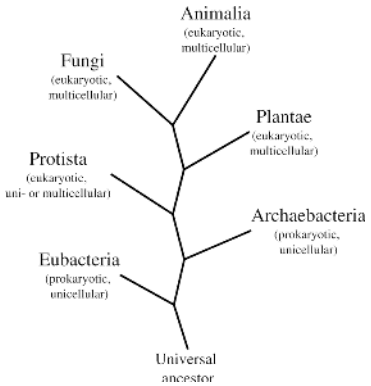


Figure: **Types of microorganisms**: This tree of life shows the different types of microorganisms.

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1.2.2: Classification of Microorganisms

Microorganisms are classified into taxonomic categories to facilitate research and communication.

Learning Objectives

- Assess how early life changed the earth

Key Points

- The classification system is constantly changing with the advancement of technology.
- The most recent classification system includes five kingdoms that are further split into phylum, class, order, family, genus, and species.
- Microorganisms are assigned a scientific name using binomial nomenclature.

Key Terms

- **DNA fingerprinting**: A method of isolating and mapping sequences of a cell's DNA to identify it.

Life on Earth is famous for its diversity. Throughout the world we can find many millions of different forms of life. Biologic classification helps identify each form according to common properties (similarities) using a set of rules and an estimate as to how closely related it is to a common ancestor (evolutionary relationship) in a way to create an order. By learning to recognize certain patterns and classify them into specific groups, biologists are better able to understand the relationships that exist among a variety of living forms that inhabit the planet.

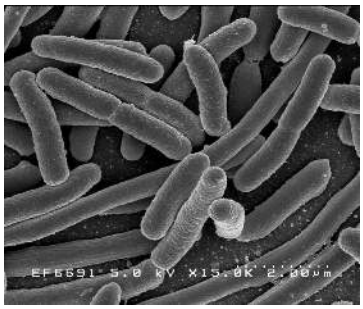


Figure: **Classification of E. coli**: Domain: Bacteria, Kingdom: Eubacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Enterobacteriales, Family: Enterobacteriaceae, Genus: Escherichia, Species: E. coli.

The first, largest, and most inclusive group under which organisms are classified is called a domain and has three subgroups: bacteria, archae, and eukarya. This first group defines whether an organism is a prokaryote or a eukaryote. The domain was proposed by the microbiologist and physicist Carl Woese in 1978 and is based on identifying similarities in ribosomal RNA sequences of microorganisms.

The second largest group is called a kingdom. Five major kingdoms have been described and include prokaryota (e.g. archae and bacteria), protoctista (e.g. protozoa and algae), fungi, plantae, and animalia. A kingdom is further split into phylum or division, class, order, family, genus, and species, which is the smallest group.

The science of classifying organisms is called taxonomy and the groups making up the classification hierarchy are called taxa. Taxonomy consists of classifying new organisms or reclassifying existing ones. Microorganisms are scientifically recognized using a binomial nomenclature using two words that refer to the genus and the species. The names assigned to microorganisms are in Latin. The first letter of the genus name is always capitalized. Classification of microorganisms has been largely aided by studies of fossils and recently by DNA sequencing. Methods of classifications are constantly changing. The most widely employed methods for classifying microbes are morphological characteristics, differential staining, biochemical testing, DNA fingerprinting or DNA base composition, polymerase chain reaction, and DNA chips.

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1.2.3: Microbes and the Origin of Life on Earth

Life on Earth is thought to have originated from the oldest single-cell archaea and bacteria.

Learning Objectives

- Assess the characteristics of pre-life earth and which adaptations allowed early microbial life to flourish.

Key Points

- The proposed mechanisms for the origin of life on Earth include endosymbiosis and panspermia. Both are debatable theories.
- In these two theories, bacteria and extremophile archaea are thought to have initiated an oxygenated atmosphere creating new forms of life.
- Evolutionary processes over billions of years gave rise to the biodiversity of life on Earth.

Key Terms

- **endosymbiosis**: A condition of living within the body or cells of another organism.
- **panspermia**: The hypothesis that microorganisms may transmit life from outer space to habitable bodies; or the process of such transmission.

Scientific evidence suggests that life began on Earth some 3.5 billion years ago. Since then, life has evolved into a wide variety of forms, which biologists have classified into a hierarchy of taxa. Some of the oldest cells on Earth are single-cell organisms called archaea and bacteria. Fossil records indicate that mounds of bacteria once covered young Earth. Some began making their own food using carbon dioxide in the atmosphere and energy they harvested from the sun. This process (called photosynthesis) produced enough oxygen to change Earth's atmosphere.

Soon afterward, new oxygen-breathing life forms came onto the scene. With a population of increasingly diverse bacterial life, the stage was set for more life to form. There is compelling evidence that mitochondria and chloroplasts were once primitive bacterial cells. This evidence is described in the endosymbiotic theory. Symbiosis occurs when two different species benefit from living and working together. When one organism actually lives inside the other it's called endosymbiosis. The endosymbiotic theory describes how a large host cell and ingested bacteria could easily become dependent on one another for survival, resulting in a permanent relationship.

Over millions of years of evolution, mitochondria and chloroplasts have become more specialized and today they cannot live outside the cell. Mitochondria and chloroplasts have striking similarities to bacteria cells. They have their own DNA, which is separate from the DNA found in the nucleus of the cell. And both organelles use their DNA to produce many proteins and enzymes required for their function. A double membrane surrounding both mitochondria and chloroplasts is further evidence that each was ingested by a primitive host. The two organelles also reproduce like bacteria, replicating their own DNA and directing their own division.

Mitochondrial DNA (mtDNA) has a unique pattern of inheritance. It is passed down directly from mother to child, and it accumulates changes much more slowly than other types of DNA. Because of its unique characteristics, mtDNA has provided important clues about evolutionary history. For example, differences in mtDNA are examined to estimate how closely related one species is to another.



Figure: **Extremophiles**: Photosynthetic fossilized cyanobacteria in a billion year old rock formation of Glacier National Park, Montana, USA.

Conditions on Earth 4 billion years ago were very different than they are today. The atmosphere lacked oxygen, and an ozone layer did not yet protect Earth from harmful radiation. Heavy rains, lightning, and volcanic activity were common. Yet the earliest cells originated in this extreme environment. Extremophiles archaea still thrive in extreme habitats. Astrobiologists are now using archaea to study the origins of life on Earth and other planets. Because archaea inhabit places previously considered incompatible with life, they may provide clues that will improve our ability to detect extraterrestrial life. Interestingly, current research suggests archaea may be capable of space travel by meteorite. Such an event termed panspermia could have seeded life on Earth or elsewhere.

The presence of archaea and bacteria changed Earth dramatically. They helped establish a stable atmosphere and produced oxygen in such quantities that eventually life forms could evolve that needed oxygen. The new atmospheric conditions calmed the weather so that the extremes were less severe. Life had created the conditions for new life to be formed. This process is one of the great wonders of nature.

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1.2.4: Environmental Diversity of Microbes

LEARNING OBJECTIVES

- Summarize how microbial diversity contributes to microbial occupation of diverse geographical niches.

The microbial world encompasses most of the phylogenetic diversity on Earth, as all Bacteria, all Archaea, and most lineages of the Eukarya are microorganisms. Microbes live in every kind of habitat (terrestrial, aquatic, atmospheric, or living host) and their presence invariably affects the environment in which they grow. Their diversity enables them to thrive in extremely cold or extremely hot environments. Their diversity also makes them tolerant of many other conditions, such as limited water availability, high salt content, and low oxygen levels.



Figure: **Microorganisms in a cold environment**: Ice algae in Antartica.



Figure: **Microorganisms in a hot environment**: Algae growing in a hot pool in New Zealand.

Not every microbe can survive in all habitats, though. Each type of microbe has evolved to live within a narrow range of conditions. Although the vast majority of microbial diversity remains undetermined, it is globally understood that the effects of microorganisms on their environment can be beneficial. The beneficial effects of microbes derive from their metabolic activities in the environment, their associations with plants and animals, and from their use in food production and biotechnological processes.

In turn, the environment and the recent temperature anomalies play a crucial role in driving changes to the microbial communities. For instance, the assemblage of microbes that exists on the surface of seawater is thought to have undergone tremendous change with respect to composition, abundance, diversity, and virulence as a result of climate-driving sea surface warming.

For microbiologists, it is critical to study microbial adaptation to different environments and their function in those environments to understand global microbial diversity, ecology, and evolution. They rely on specific physical and chemical factors such as measuring temperature, pH, and salinity within a certain geography to formulate a comparison among microbial communities and the environment different species can tolerate. Researchers collect samples from geographical areas with different environmental conditions and between seasons to determine how dispersal patterns shape microbial communities and understand why organisms live where they do. As such, microbial communities from coastal and open oceans, polar regions, rivers, lakes, soils, atmosphere, and the human body can be tested. These samplings create a starting point to understand how the abundance and composition of microbial communities correlate with climatic perturbations, interact to effect ecosystem processes, and influence human health. Interfering with natural microbial biomass disrupts the balance of nature and the ecosystem and leads to loss of biodiversity.

Key Points

- Different microbial species thrive under different environmental conditions.
- Microbial communities occupy aquatic and terrestrial habitats and constitute the majority of biodiversity on Earth.
- Microbial diversities sustain the ecosystem in which they grow.

Key Terms

- **biodiversity**: The diversity (number and variety of species) of plant and animal life within a region.
- **biomass**: The total mass of all living things within a specific area or habitat.

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SECTION OVERVIEW

1.3: The Science of Microbiology

Topic hierarchy

1.3.1: Basic Microbiology

1.3.2: Applied Microbiology

1.3.3: Immunization, Antiseptics, and Antibiotics

1.3.4: Modern Microbiology

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1.3.1: Basic Microbiology

Microbiology is the study of microscopic organisms and how they interact with humans and the environment.

Learning Objectives

- Evaluate the science of basic microbiology; understand the fundamental aspects of microbiology.

Key Points

- Microbiology focuses on organisms that are very small using various tools, which is a process done by microbiologists.
- As microbes are essential for human life and as microbes can cause human diseases, microbiology is therefore very important.
- The numbers of individual microbes and the number of microbes in and on the earth is staggering in proportions.

Key Terms

- **quantitation**: The process of quantitating.
- **immunology**: The branch of medicine that studies the body's immune system.
- **culturable**: Able to be cultured (grown in a suitable environment).

Microbiology is the study of microscopic organisms (microbes), which are defined as any living organism that is either a single cell (unicellular), a cell cluster, or has no cells at all (acellular). This includes eukaryotes, such as fungi and protists, and prokaryotes. Viruses and prions, though not strictly classed as living organisms, are also studied.

Microbiology typically includes the study of the immune system, or immunology. Generally, immune systems interact with pathogenic microbes; these two disciplines often intersect which is why many colleges offer a paired degree such as "Microbiology and Immunology. "



Figure: **Microbiologist**: A microbiology officer aboard a US naval ship examines wound cultures in the ship's microbiology laboratory.

Microbiology is a broad term which includes virology, mycology, parasitology, bacteriology, immunology, and other branches. A microbiologist is a specialist in microbiology and these related topics. Microbiological procedures usually must be aseptic and use a variety of tools such as light microscopes with a combination of stains and dyes. As microbes are absolutely required for most facets of human life (including the air we breathe and the food we eat) and are potential causes of many human diseases, microbiology is paramount for human society.

Research in the microbiology field is expanding, and in the coming years, we should see the demand for microbiologists in the workforce increase. It is estimated that only about one percent of the microorganisms present in a given environmental sample are culturable and the number of bacterial cells and species on Earth is still not possible to be determined. Recent estimates indicate that this number might be extremely high at five to the power of thirty. Although microbes were directly observed over three hundred years ago, the precise determination, quantitation, and description of its functions is far from complete, given the overwhelming diversity detected by genetic and culture-independent means.

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1.3.2: Applied Microbiology

The information gained by microbiologists can be applied to many medicinal and commercial endeavors.

Learning Objectives

- Explain applied microbiology

Key Points

- Using knowledge gained by microbiologists studying microbes, several fields of applied microbiology have formed.
- While food and medicinal applications are a big portion of applied microbiology, the study of microbes has lead to entire commercial industries which affect almost all aspects of human life.
- There are a myriad of practical applications that microbiology contributes to, including several parts of food production and medicinal applications.

Key Terms

- **rhizosphere**: The soil region subject to the influence of plant roots and their associated microorganisms.
- **biotechnology**: The use of living organisms (especially microorganisms) in industrial, agricultural, medical, and other technological applications.
- **pathogenic**: Able to cause harmful disease.

Microbiology is the study of microbes, which affect almost every aspect of life on the earth. In addition, there are huge commercial and medicinal benefits in understanding microbes. The application of this understanding is known as applied microbiology. There are many different types of applied microbiology which can be briefly defined as follows:

Medical Microbiology

Medical microbiology is the study of the pathogenic microbes and the role of microbes in human illness. This includes the study of microbial pathogenesis and epidemiology and is related to the study of disease pathology and immunology.

Pharmaceutical Microbiology

The study of microorganisms that are related to the production of antibiotics, enzymes, vitamins, vaccines, and other pharmaceutical products. Pharmaceutical microbiology also studies the causes of pharmaceutical contamination and spoil.

Industrial Microbiology

The exploitation of microbes for use in industrial processes. Examples include industrial fermentation and waste-water treatment. Closely linked to the biotechnology industry. This field also includes brewing, an important application of microbiology.

Microbial Biotechnology

The manipulation of microorganisms at the genetic and molecular level to generate useful products.

Food Microbiology and Dairy Microbiology

The study of microorganisms causing food spoilage and food-borne illness. Microorganisms can produce foods, for example by fermentation.



Figure: **Applied microbiology – Fermentation**: One of the oldest and well-known examples of applied microbiology is fermentation. In this picture the large tanks are being used for the fermentation of grapes to make wine.

Agricultural Microbiology

The study of agriculturally relevant microorganisms. This field can be further classified into the following subfields:

- Plant microbiology and plant pathology – The study of the interactions between microorganisms and plants and plant pathogens.
- Soil microbiology – The study of those microorganisms that are found in soil.
- Veterinary microbiology – The study of the role in microbes in veterinary medicine or animal taxonomy.
- Environmental microbiology – The study of the function and diversity of microbes in their natural environments. This involves the characterization of key bacterial habitats such as the rhizosphere and phyllosphere, soil and groundwater ecosystems, open oceans or extreme environments (extremophiles). This field includes other branches of microbiology such as: microbial ecology (microbially-mediated nutrient cycling), geomicrobiology, (microbial diversity), water microbiology (the study of those microorganisms that are found in water), aeromicrobiology (the study of airborne microorganisms) and epidemiology (the study of the incidence, spread, and control of disease).

This is by no means an exhaustive list of the different types of applied microbiology, but gives an indication of the expansive variety of the field and some of the benefits these studies entail.

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1.3.3: Immunization, Antiseptics, and Antibiotics

Understanding microbes gives us the ability to fight pathogens using immunization, antiseptics, and antibiotics.

Learning Objectives

- Compare immunization, antiseptics and antibiotics, and how they are used to combat human pathogens

Key Points

- Immunization is the fortification of our own immune system, priming it against potential future infections by specific microbes.
- Antiseptics are broadly defined as substances we can use on our body or surfaces around us to slow or kill microbes that could potentially harm us.
- Antibiotics, like antiseptics, can slow or kill microbes. However, unlike antiseptics, antibiotics can circulate in the human blood system and be used to fight microbial infections.

Key Terms

- **anaphylactic shock**: A severe and rapid systemic allergic reaction to an allergen, constricting the trachea and preventing breathing.
- **immunogen**: any substance that elicits a immune response; an antigen

Surprisingly, most microbes are not harmful to humans. In fact, they are all around us and even a part of us. However, some microbes are human pathogens; to combat these, we use immunization, antiseptics, and antibiotics.

Immunization is the process by which an individual's immune system becomes fortified against an agent (known as the immunogen).



Figure: **Flu Immunization**: A naval officer self-administers a nasal spray that immunizes him against the flu.

When the immune system is exposed to molecules that are foreign to the body, it will orchestrate an immune response. It will also develop the ability to respond quickly to subsequent encounters with the same substance, a phenomenon known as immunological memory. Therefore, by exposing a person to an immunogen in a controlled way, the body can learn to protect itself: this is called active immunization.

Vaccines against microorganisms that cause diseases can prepare the body's immune system, thus helping it fight or prevent an infection. The most important elements of the immune system that are improved by immunization are the T cells, the B cells, and the antibodies B cells produce. Memory B cells and memory T cells are responsible for the swift response to a second encounter with a foreign molecule. Through the use of immunizations, some infections and diseases have been almost completely eradicated throughout the United States and the world. For example, polio was eliminated in the U.S. in 1979. Active immunization and vaccination has been named one of the "Ten Great Public Health Achievements in the 20th Century. "

By contrast, in *passive* immunization, pre-synthesized elements of the immune system are transferred to a human body so it does not need to produce these elements itself. Currently, antibodies can be used for passive immunization. This method of immunization starts to work very quickly; however, it is short-lasting because the antibodies are naturally broken down and will disappear altogether if there are no B cells to produce more of them. Passive immunization occurs physiologically, when antibodies are transferred from mother to fetus during pregnancy, to protect the fetus before and shortly after birth. The antibodies can be

produced in animals, called ” serum therapy,” although there is a high chance of anaphylactic shock because of immunity against animal serum itself. Thus, humanized antibodies produced *in vitro* by cell culture are used instead if available.

In early inquiries before there was an understanding of microbes, much emphasis was given to the prevention of putrefaction. Procedures were carried out to determine the amount of agent that needed to be added to a given solution in order to prevent the development of pus and putrefaction. However, due to a lack of understanding of germ theory, this method was inaccurate. Today, an antiseptic is judged by its effect on pure cultures of a defined microbe or on their vegetative and spore forms.

Antiseptics are antimicrobial substances that are applied to living tissue to reduce the possibility of infection, sepsis, or putrefaction. Their earliest known systematic use was in the ancient practice of embalming the dead. Antiseptics are generally distinguished from antibiotics by the latter’s ability to be transported through the lymphatic system to destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non-living objects. Some antiseptics are true germicides, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and only prevent or inhibit bacterial growth. Microbicides that destroy virus particles are called viricides or antivirals.

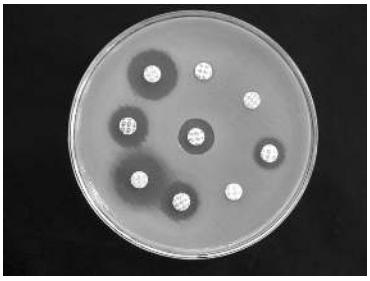


Figure: **Antibiotic Testing**: Discs soaked with various compounds are put onto a lawn of bacteria. If the compound on the disc kills or slows bacteria growth, a “halo” of clear media is seen.

An antibacterial is a compound or substance that kills or slows down the growth of bacteria. The term is often used synonymously with the term antibiotic; today, however, with increased knowledge of the causative agents of various infectious diseases, the term “antibiotic” has come to denote a broader range of antimicrobial compounds, including anti-fungal and other compounds.

The word “antibiotic” was first used in 1942 by Selman Waksman and his collaborators to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. This definition excluded substances that kill bacteria but are not produced by microorganisms (such as gastric juices and hydrogen peroxide). It also excluded synthetic antibacterial compounds, such as the sulfonamides. Many antibacterial compounds are relatively small molecules with a molecular weight of less than 2000 amu. With advances in medicinal chemistry, most of today’s antibacterials are semisynthetic modifications of various natural compounds.

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1.3.4: Modern Microbiology

LEARNING OBJECTIVES

- Discuss the fundamental aspects of microbiology

Modern microbiolgy began with the discovery of microbes, and the scope and scale of the field continues to expand today. While there is some debate, modern microbiology is accepted by most to begin with observations by the Dutch draper and haberdasher, Antonie van Leeuwenhoek, who lived for most of his life in Delft, Holland. In 1676, van Leeuwenhoek observed bacteria and other microorganisms, using a single-lens microscope of his own design. While van Leeuwenhoek is often cited as the first to observe microbes, Robert Hooke made the first recorded microscopic observation, of the fruiting bodies of molds, in 1665.

It has been suggested that a Jesuit priest called Athanasius Kircher was the first to observe microorganisms. One of his books contains a chapter in Latin, which reads in translation – "Concerning the wonderful structure of things in nature, investigated by Microscope." Here, he wrote "who would believe that vinegar and milk abound with an innumerable multitude of worms. " He noted that putrid material is full of innumerable creeping animalcule. These observations antedate Robert Hooke's Micrographia by nearly 20 years and were published some 29 years before van Leeuwenhoek saw protozoa.



Figure: ***Athanasius Kirche****: A portrait of the 17th century Jesuit priest Athanasius Kirche, who arguably discovered microbes.*

The field of bacteriology (later a subdiscipline of microbiology) was founded in the 19th century by Ferdinand Cohn, a botanist whose studies on algae and photosynthetic bacteria led him to describe several bacteria including Bacillus and Beggiatoa. Cohn was also the first to formulate a scheme for the taxonomic classification of bacteria and discover spores. Louis Pasteur and Robert Koch were contemporaries of Cohn's and are often considered to be the father of microbiology and medical microbiology, respectively. Pasteur is most famous for his series of experiments designed to disprove the then widely held theory of spontaneous generation, thereby solidifying microbiology's identity as a biological science. Pasteur also designed methods for food preservation (pasteurization) and vaccines against several diseases such as anthrax, fowl cholera, and rabies.

Koch is best known for his contributions to the germ theory of disease, proving that specific diseases were caused by specific pathogenic microorganisms. He developed a series of criteria that have become known as the Koch's postulates. Koch was one of the first scientists to focus on the isolation of bacteria in pure culture resulting in his description of several novel bacteria including *Mycobacterium tuberculosis*, the causative agent of tuberculosis. While Pasteur and Koch are often considered the founders of microbiology, their work did not accurately reflect the true diversity of the microbial world because of their exclusive focus on microorganisms having direct medical relevance.

It was not until the late 19th century and the work of Martinus Beijerinck and Sergei Winogradsky, the founders of general microbiology (an older term encompassing aspects of microbial physiology, diversity, and ecology), that the true breadth of

microbiology was revealed. Beijerinck made two major contributions to microbiology: the discovery of viruses and the development of enrichment culture techniques. While his work on the tobacco mosaic virus (TMV) established the basic principles of virology, it was his development of enrichment culturing that had the most immediate impact on microbiology by allowing for the cultivation of a wide range of microbes with wildly different physiologies. Winogradsky was the first to develop the concept of chemoautotrophy and to thereby reveal the essential role microorganisms played in geochemical processes. Specifically, he was responsible for the first isolation and description of both nitrifying and nitrogen-fixing bacteria.



Figure: ***Sergei Winogradsky****: An image of Sergei Winogradsky,who discovered nitrogen-fixing bacteria.*

Key Points

- There is some debate as to who was exactly first, but Antonie van Leeuwenhoek, Athanasius Kircher, and Robert Hooke were the first people to view microbes using some of the first self-built microscopes.
- Ferdinand Cohn, Louis Pasteur, and Robert Koch were pioneers in bacteriology, the discovery and understanding of the subset of microbes that are bacteria. This had a direct and immediate impact on food storage and disease causality.
- Martinus Beijerinck and Sergei Winogradsky are credited with the discovery of general microbiology, which laid the ground work for our understanding of microbial physiology, diversity, and ecology.

Key Terms

- **chemoautotrophy**: When a simple organism, such as a protozoan, derives its energy from chemical processes rather than photosynthesis.
- **pasteurization**: heat-treatment of a perishable food to destroy heat-sensitive vegetative cells followed by immediate cooling to limit growth of the surviving cells and germination of spores
- **rabies**: a viral disease that causes acute encephalitis in warm-blooded animals and people, characterised by abnormal behaviour such as excitement, aggressiveness, and dementia, followed by paralysis and death
- **animalcule**: An older term for a minute or microscopic animal or protozoan.

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CHAPTER OVERVIEW

2: Microscopy

2.1: Looking at Microbes

2.1.1: Microbe Size

2.1.2: Units of Measurement for Microbes

2.1.3: Refraction and Magnification

2.1.4: Magnification and Resolution

2.2: General Staining Methods

2.3: Microscopy

2.4: Other Types of Microscopy

2.4.1: Dark-Field Microscopy

2.4.2: Phase-Contrast Microscopy

2.4.3: Interference Microscopy

2.4.4: Fluorescence Microscopy

2.4.5: Confocal Micropscopy

2.4.6: Electron Microscopy

2.4.7: Scanned-Probe Microscopy

2.4.8: X-Ray Diffraction Analysis

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SECTION OVERVIEW

2.1: Looking at Microbes

Topic hierarchy

2.1.1: Microbe Size

2.1.2: Units of Measurement for Microbes

2.1.3: Refraction and Magnification

2.1.4: Magnification and Resolution

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2.1.1: Microbe Size

Microbes are often very small, even in comparison to microscopic cells from animals.

Learning Objectives

- Recall the size of microbes in comparison to human cells and viruses

Key Points

- Microbes are generally described as being microscopic in size. Therefore, they are smaller than a human eye can see.
- The size of microbes can be hard to imagine because they are so small. In comparison to animal cells, microbes tend to be smaller. They are about 1/10th the size of a typical human cell.
- Microbes are generally measured in the scale of one millionth of a meter, which is known as a micrometer.

Key Terms

- **protozoan**: Any of the diverse group of eukaryotes, of the phylum Protozoa, that are primarily unicellular, existing singly or aggregating into colonies, are usually nonphotosynthetic, and are often classified further into phyla according to their capacity for and means of motility, as by pseudopods, flagella, or cilia.
- **macroscopic**: Visible to the unassisted eye.

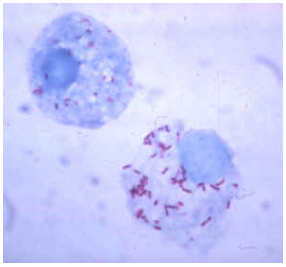


Figure: **A Microbe versus Animal Cell**: The large spheres are tick cells. The purple bars and dots are the bacterium Rickettsia rickettsii, which is the causative agent of Rocky Mountain spotted fever. Rickettsia rickettsii is a small bacterium that grows inside the cells of its hosts. These bacteria range in size from 0.2 x 0.5 micrometers to 0.3 x 2.0 micrometers.

Microbiology is the study of microbes. The name of the field is driven by the tool that largely determines if something is a microbe. Basically, microbiology is the study of organisms that one needs to use a microscope to visualize. Of course, there are exceptions. There are types of microscopes that visualize to the atomic level, which is significantly smaller than microbes. Alternatively, there are single cell organisms, such as some types of green algae and some protozoans that are generally studied by microbiologists. These are macroscopic or view-able without a microscope.



Figure: **Ventricaria ventricosa**: Ventricaria ventricosa is one of the largest known unicellular organisms. They about the size of a ping pong ball.

The size of microbes can be hard to imagine because they are so small in comparison to what most people see day to day. Even in comparison to animal cells, microbes tend to be smaller. They are about 1/10th the size of a typical human cell. So, a microbe such as a bacteria cell would be the size of a cat or small dog in comparison to a human-sized animal-cell. Viruses are about 1/10th the size of other microbes such as bacteria. Therefore, if a bacteria is the size of cat, then a virus would be about the size of a mouse.

To put a numerical value on microbial size, most measurements of microbes are done with the unit of measure of micrometer, which is one millionth of a meter (one 2,500th of an inch). In relation to something more tangible a period or ". " is about 0.5 millimeters or 0.5/1,000th of a meter. A typical microbe would be about 1/500th of a period.

Of course there are exceptions. Some unicellular organisms studied by microbiologists are macroscopic. This includes *Valonia ventricosa*, which can be up to 5 cm in length. It is a member of the Chlorophyta phylum which are a sub-group of green algae. Many types of green algae are not microscopic, but they are often studied by microbiologists.

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2.1.2: Units of Measurement for Microbes

Measuring microbes presents challenges because they are very small, requiring indirect measures of microbes to understand them better.

Learning Objectives

- Recognize the methods used to measure microbial growth

Key Points

- Understanding microbiological life means quantifying it. Since microbes are so small, this is a challenge.
- The size of a microbe is usually measured in micrometers, or one millionth of a meter.
- There are many aspects of microbes that can be measured in addition to size, including metric like genome size and growth rates.

Key Terms

- **flow cytometry**: A technique used to sort and classify cells by using fluorescent markers on their surface.
- **genome**: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of basepairs.

Microbes are broadly defined as organisms that are microscopic. As a result, measuring them can be very difficult. The units used to describe objects on a microscopic length scale are most commonly the Micrometer (oi) – one millionth of 1 meter and smaller units. Most microbes are around 1 micrometer in size. Viruses are typically $1/10^{th}$ that size. Animal cells are typically around 10 micrometers in size. However, length is not the only measurement that pertains to microbes. Microbes have genomes and these are typically smaller than the genomes of macroscopic organisms such as humans. DNA is measured in base pairs of DNA. For example, the human genome is about 3.4 billion base pairs while the common intestinal bacteria *Escherichia coli* is 4.6 million base pairs. Additionally, microbes are usually not weighed individually, but can be as an aggregate for various experiments. An estimate of the weight of an individual microbe can be made by estimating the number of microbes. This is especially important for biomass studies where the units of measurement are in units like picog, 10^{-12} of a kilogram (Kg), nanogram 10^{-9} of a Kg, and microgram, 10^{-6} of a Kg (a kilogram is a little over 2 pounds).

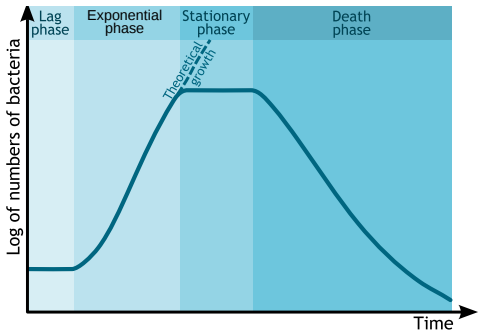


Figure: **Bacterial Growth Curve**: This chart shows the logarithmic growth of bacteria. Note the Y-axis scale is logarithmic meaning that the number represents doubling. The phases of growth are labelled on top.

Microbial growth is an important measure in understanding microbes. Microbial growth is the division of one microbe into two daughter cells in a process called binary fission. As a result, “local doubling” of the microbial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the microbial population undergoes exponential growth. The measurement of an exponential microbial growth curve in batch culture was traditionally a part of the training of all microbiologists; The basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Since there are limits on space, food, and other factors, actual growth never matches actual measured growth.

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2.1.3: Refraction and Magnification

LEARNING OBJECTIVES

- Describe refraction and distinguish between convex and concave lenses

The underlying principal of a microscope is that lenses refract light which allows for magnification. Refraction occurs when light travels through an area of space that has a changing index of refraction. The simplest case of refraction occurs when there is an interface between a uniform medium with an index of refraction and another medium with an index of refraction.



Figure: ***Refraction****: As the light is reflected off the pencil we see that, due to the different refraction indexes of water and air, the pencil appears to bend in the water. However, the pencil is straight. It is actually the water acting much like a lens in a microscope that gives it the appearance of bending.*

Some media have an index of refraction that varies gradually with position. Therefore, light rays curve through the medium rather than travelling in straight lines. This effect is what is responsible for mirages seen on hot days where the changing index of refraction of the air causes the light rays to bend creating the appearance of *specular reflections* in the distance (as if on the surface of a pool of water).

Taking advantage of the principle of refraction, devices can be built that can focus light. A device that produces converging or diverging light rays due to refraction is known as a lens. In general, two types of lenses exist: *convex lenses*, which cause parallel light rays to converge, and *concave lenses*, which cause parallel light rays to diverge. The former property of convex lenses is of special interest to microbiologists. In essence, a convex lens allows magnification. Light reflecting off an object is focused to a point. The simplest example of this that most people know is a magnifying glass. A magnifying glass is one convex lens, and this by itself allows the magnification of objects.

A microscope is basically a series of lenses that take advantage of the nature of refraction. Due to the nature of light, and the maximum amount of refraction that can be possible by a material, there are limits to the amount of magnification that can be done by a light microscope.

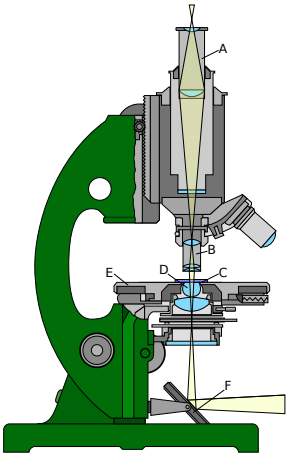


Figure: ***The Microscope****: Notice the blue areas, which represent lenses. Note also that many of the lenses are convex, thus the light that goes through a specimen is focused and therefore magnified. Labels: A) Ocular, B) Objective, C) slide holder, D) Illumination Lenses, E) Slide Stage, and F) Illumination Mirror*

Summary

- Convex lenses allow light to converge.
- Concave lenses spread light that travels through it.
- There are limits to the amount of refraction that can be done by a material, and therefore, limits to the amount a microscope can magnify a sample.

Key Terms

- **concave**: Curved like the inner surface of a sphere or bowl.
- **convex**: Curved or bowed outward like the outside of a bowl or sphere or circle.
- **specular**: Pertaining to mirrors; mirror-like, reflective.

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2.1.4: Magnification and Resolution

Magnification is the enlargement of an image; resolution is the ability to tell two objects apart.

Learning Objectives

- Define magnification and resolution

Key Points

- Magnification is the ability to make small objects seem larger, such as making a microscopic organism visible.
- Resolution is the ability to distinguish two objects from each other.
- Light microscopy has limits to both its resolution and its magnification.

Key Terms

- **airy disks**: In optics, the Airy disk (or Airy disc) and Airy pattern are descriptions of the best-focused spot of light that a perfect lens with a circular aperture can make, limited by the diffraction of light.
- **diffraction**: the breaking up of an electromagnetic wave as it passes a geometric structure (e.g., a slit), followed by reconstruction of the wave by interference

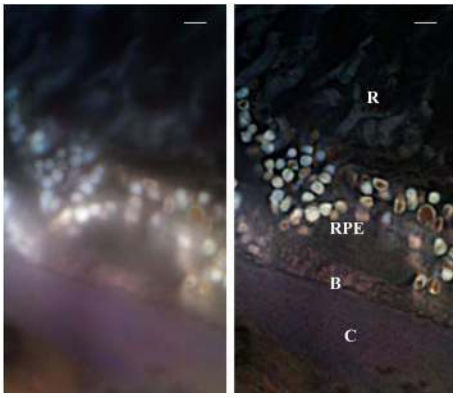


Figure: **Aging Tissue and Vision Loss**: These are micrographs of a section of a human eye. Using computer algorithms and other technology the panel on the right has a higher resolution and is therefore clearer. It should be noted that both panels are at the same magnification, yet the panel on the right has a higher resolution and gives more information on the sample. The labels represent various parts of the human eye: Bruch membrane (B); choroid (C); retinal pigment epithelium (RPE); and retinal rod cells (R). The scale bar is 2um.

Magnification is the process of enlarging something only in appearance, not in physical size. This enlargement is quantified by a calculated number also called “magnification. ” The term magnification is often confused with the term “resolution,” which describes the ability of an imaging system to show detail in the object that is being imaged. While high magnification without high resolution may make very small microbes visible, it will not allow the observer to distinguish*between* microbes or sub-cellular parts of a microbe. In reality, therefore, microbiologists depend more on resolution, as they want to be able to determine differences between microbes or parts of microbes. However, to be able to distinguish between two objects under a microscope, a viewer must first magnify to a point at which resolution becomes relevant.

Resolution depends on the distance between two distinguishable radiating points. A microscopic imaging system may have many individual components, including a lens and recording and display components. Each of these contributes to the optical resolution of the system, as will the environment in which the imaging is performed. Real optical systems are complex, and practical difficulties often increase the distance between distinguishable point sources.

At very high magnifications with transmitted light, point objects are seen as fuzzy discs surrounded by diffraction rings. These are called Airy disks. The resolving power of a microscope is taken as the ability to distinguish between two closely spaced Airy disks (or, in other words, the ability of the microscope to distinctly reveal adjacent structural detail). It is this effect of diffraction that limits a microscope’s ability to resolve fine details. The extent and magnitude of the diffraction patterns are affected by the wavelength of light (λ), the refractive materials used to manufacture the objective lens, and the numerical aperture (NA) of the

objective lens. There is therefore a finite limit beyond which it is impossible to resolve separate points in the objective field. This is known as the diffraction limit.

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2.2: General Staining Methods

LEARNING OBJECTIVES

- Compare and contrast in vitro and in vivo staining

Staining is a technique used in microscopy to enhance contrast in a microscopic image. Stains and dyes are frequently used to highlight structures in microbes for viewing, often with the aid of different microscopes. Stains may be used to define and examine different types of microbes, various stages of cellular life (e.g., the mitotic cycle), and even organelles within individual cells (e.g., mitochondria or chloroplasts).

In-vivo staining is the process of dyeing living tissue — *in vivo* means "in life" (as contrasted to *in-vitro* staining). When a certain cell or structure takes on contrasting color(s), its form (morphology) or position within a cell or tissue can be readily seen and studied. The usual purpose is to reveal cytological details that might otherwise not be apparent; however, staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells. *In-vitro* staining involves coloring cells or structures that have been removed from their biological context. Certain stains are often combined to reveal more details and features than a single stain could reveal alone, and a counterstain is a stain that increases visibility of cells or structures when the principal stain is not sufficient. Scientists and physicians can combine staining with specific protocols for fixation and sample preparation and can use these standard techniques as consistent, repeatable diagnostic tools.

There are an incredible number of stains that can be used in a variety of different methods. What follows here are some common aspects of the process of preparing for *in-vitro* staining.

- Fixation: This can itself consist of several steps. Fixation aims to preserve the shape of the cells (in this case, microbes) as much as possible. Sometimes heat fixation is used to kill, adhere, and alter the cells so they will accept stains. Most chemical fixatives generate chemical bonds between proteins and other substances within the sample, increasing their rigidity. Common fixatives include formaldehyde, ethanol, methanol, and picric acid.
- Permeabilization: This involves treatment of the cells with (usually) a mild surfactant. This treatment dissolves cell membranes, allowing larger dye molecules to enter the cell's interior.
- Mounting: This step usually involves attaching the samples to a glass microscope slide for observation and analysis. In some cases, cells may be grown directly on a slide. For samples of loose cells the sample can be directly applied to a slide.

At its simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation. Many dyes, however, require the use of a mordant — a chemical compound that reacts with the stain to form an insoluble colored precipitate. When the excess dye solution is washed away, the mordanted stain remains. There is an incredible array of stains that can be used at this step, from those that stain specific microbial types (see the figure below) to those that highlight sub-compartments or organelles of a cell, such as the nucleus or endoplasmic reticulum. Alternatively, negative staining can be employed. This is a simple staining method for bacteria, performed by smearing the cells onto the slide and then applying nigrosin (a black synthetic dye) or Indian ink (an aqueous suspension of carbon particles). After drying, the microorganisms may be viewed in bright field microscopy as lighter inclusions contrast well against the dark environment surrounding them

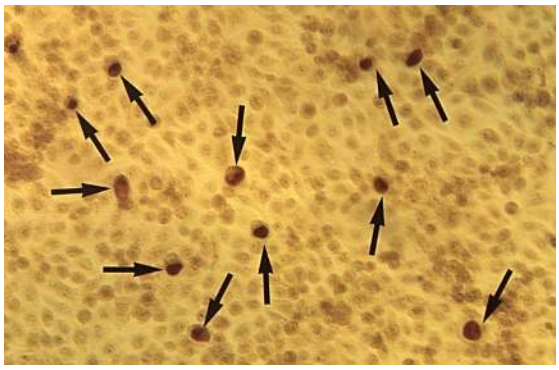


Figure: **Chlamydia Stain**: Cells of the bacterial pathogen chlamydia (indicated by arrows) are highlighted by a stain called "geimsa. "

Live, *in-vivo* staining microscopy shares many of these steps, with the exception of fixation, which invariably kills the microbe to be examined.

Key Points

- In-vivo staining, which visualizes cells that are alive, and in-vitro staining, which visualizes fixed cells, both have important uses.
- There is a vast array of stains that can be used on microbes that can highlight almost any characteristic of a cell, even organelles within a cell.
- Staining protocols can be complex, but they share some basic steps: preparation, fixation, staining, and mounting.

Key Terms

- **surfactant**: a surface active agent, or wetting agent, capable of reducing the surface tension of a liquid; typically organic compounds having a hydrophilic "head" and a hydrophobic "tail"
- **organelle**: a specialized structure found inside cells that carries out a specific life process (e.g., ribosomes, vacuoles)

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2.3: Microscopy

LEARNING OBJECTIVES

- Compare and contrast light and electron microscopy.

Cells vary in size. With few exceptions, individual cells cannot be seen with the naked eye, so scientists use microscopes (micro- = “small”; -scope = “to look at”) to study them. A microscope is an instrument that magnifies an object. Most photographs of cells are taken with a microscope; these images can also be called micrographs.

The optics of a microscope’s lenses change the orientation of the image that the user sees. A specimen that is right-side up and facing right on the microscope slide will appear upside-down and facing left when viewed through a microscope, and vice versa. Similarly, if the slide is moved left while looking through the microscope, it will appear to move right, and if moved down, it will seem to move up. This occurs because microscopes use two sets of lenses to magnify the image. Because of the manner by which light travels through the lenses, this system of two lenses produces an inverted image (binocular, or dissecting microscopes, work in a similar manner, but they include an additional magnification system that makes the final image appear to be upright).

Light Microscopes

To give you a sense of cell size, a typical human red blood cell is about eight millionths of a meter or eight micrometers (abbreviated as eight μm) in diameter; the head of a pin of is about two thousandths of a meter (two mm) in diameter. That means about 250 red blood cells could fit on the head of a pin.

Most student microscopes are classified as light microscopes. Visible light passes and is bent through the lens system to enable the user to see the specimen. Light microscopes are advantageous for viewing living organisms, but since individual cells are generally transparent, their components are not distinguishable unless they are colored with special stains. Staining, however, usually kills the cells.



(a) (b)

Figure: **Light and Electron Microscopes**: (a) Most light microscopes used in a college biology lab can magnify cells up to approximately 400 times and have a resolution of about 200 nanometers. (b) Electron microscopes provide a much higher magnification, 100,000x, and a have a resolution of 50 picometers.

Light microscopes, commonly used in undergraduate college laboratories, magnify up to approximately 400 times. Two parameters that are important in microscopy are magnification and resolving power. Magnification is the process of enlarging an object in appearance. Resolving power is the ability of a microscope to distinguish two adjacent structures as separate: the higher the resolution, the better the clarity and detail of the image. When oil immersion lenses are used for the study of small objects, magnification is usually increased to 1,000 times. In order to gain a better understanding of cellular structure and function, scientists typically use electron microscopes.

Electron Microscopes

In contrast to light microscopes, electron microscopes use a beam of electrons instead of a beam of light. Not only does this allow for higher magnification and, thus, more detail, it also provides higher resolving power. The method used to prepare the specimen

for viewing with an electron microscope kills the specimen. Electrons have short wavelengths (shorter than photons) that move best in a vacuum, so living cells cannot be viewed with an electron microscope.

In a scanning electron microscope, a beam of electrons moves back and forth across a cell's surface, creating details of cell surface characteristics. In a transmission electron microscope, the electron beam penetrates the cell and provides details of a cell's internal structures. As you might imagine, electron microscopes are significantly more bulky and expensive than light microscopes.

Key Points

- Light microscopes allow for magnification of an object approximately up to 400-1000 times depending on whether the high power or oil immersion objective is used.
- Light microscopes use visible light which passes and bends through the lens system.
- Electron microscopes use a beam of electrons, opposed to visible light, for magnification.
- Electron microscopes allow for higher magnification in comparison to a light microscope thus, allowing for visualization of cell internal structures.

Key Terms

- **resolution**: The degree of fineness with which an image can be recorded or produced, often expressed as the number of pixels per unit of length (typically an inch).
- **electron**: The subatomic particle having a negative charge and orbiting the nucleus; the flow of electrons in a conductor constitutes electricity.

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SECTION OVERVIEW

2.4: Other Types of Microscopy

Topic hierarchy

2.4.1: Dark-Field Microscopy

2.4.2: Phase-Contrast Microscopy

2.4.3: Interference Microscopy

2.4.4: Fluorescence Microscopy

2.4.5: Confocal Micropscopy

2.4.6: Electron Microscopy

2.4.7: Scanned-Probe Microscopy

2.4.8: X-Ray Diffraction Analysis

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2.4.1: Dark-Field Microscopy

LEARNING OBJECTIVES

- Generalize the process of dark-field microscopy

Radiance Against a Dark Background

Dark-field microscopy is ideally used to illuminate unstained samples causing them to appear brightly lit against a dark background. This type of microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. Rather than illuminating the sample with a filled cone of light, the condenser is designed to form a hollow cone of light. The light at the apex of the cone is focused at the plane of the specimen; as this light moves past the specimen plane it spreads again into a hollow cone. The objective lens sits in the dark hollow of this cone; although the light travels around and past the objective lens, no rays enter it.

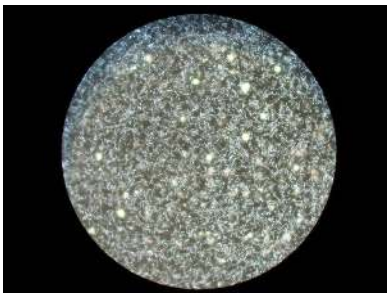


Figure: **Visualization of live bacteria**: Spirochetes bacteria observed under dark field microscopy.

The entire field appears dark when there is no sample on the microscope stage; thus the name dark-field microscopy. When a sample is on the stage, the light at the apex of the cone strikes it. The rays scattered by the sample and captured in the objective lens thus make the image.

Samples observed under dark-field microscopy should be carefully prepared since dust and other particles also scatter the light and are easily detected. Glass slides need to be thoroughly cleaned of extraneous dust and dirt. It may be necessary to filter sample media (agar, water, saline) to exclude confusing contaminants. Sample materials need to be spread thinly; too much material on the slide creates many overlapping layers and edges, making it difficult to interpret structures.

Dark-field microscopy has many applications in microbiology. It allows the visualization of live bacteria, and distinguishes some structure (rods, curved rods, spirals, or cocci) and movement.

Key Points

- In dark-field microscopy, the light reaches the specimen from an angle with the help of an opaque disk.
- The specimen appears lit up agains a dark background.
- Dark-field microscopy is most useful for extremely small living organisms that are invisible in the light microscope.

Key Terms

- **condenser**: A lens (or combination of lenses) designed to gather light and focus it onto a specimen or part of a mechanism.

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2.4.2: Phase-Contrast Microscopy

Phase-contrast microscopy visualizes differences in the refractive indexes of different parts of a specimen relative to unaltered light.

Learning Objectives

- Describe the mechanics, advantages, and disadvantages of phase-contrast microscopy

Key Points

- A phase-contrast microscope splits a beam of light into 2 types of light, direct and refracted (reflected) and brings them together to form an image of the specimen.
- Where the lights are “in-phase” the image is brighter, where the lights are “out of phase” the image is darker, and by amplifying these differences in the light, it enhances contrast.
- Phase-contrast microscopy allows for the detailed observation of living organisms, especially the internal structures.

Key Terms

- **refractive index**: the ratio of the speed of light in air or vacuum to that in another medium.

Phase-contrast microscopy is a method of manipulating light paths through the use of strategically placed rings in order to illuminate transparent objects. Dutch physicist Fritz Zernike developed the technique in the 1930s; for his efforts he was awarded the Nobel Prize in 1953.

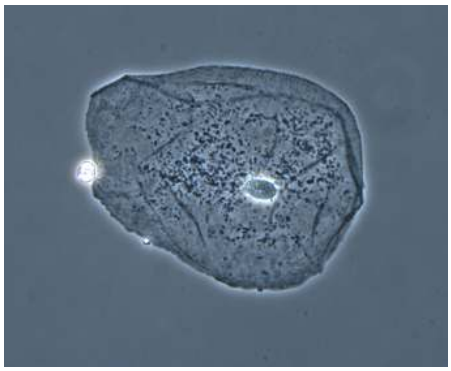


Figure: **Phase-contrast microscopy**: Phase-contrast image of a cheek epithelial cell.

In phase-contrast microscopy, parallel beams of light are passed through objects of different densities. The microscope contains special condensers that throw light “out of phase” causing it to pass through the object at different speeds. Internal details and organelles of live, unstained organisms (e.g. mitochondria, lysosomes, and the Golgi body) can be seen clearly with this microscope.

A phase ring in condenser allows a cylinder of light to pass through it while still in phase. Unaltered light hits the phase ring in the lens and is excluded. Light that is slightly altered by passing through a different refractive index is allowed to pass through. Light passing through cellular structures, such as chromosomes or mitochondria is retarded because they have a higher refractive index than the surrounding medium. Elements of lower refractive index advance the wave. Much of the background light is removed and light that constructively or destructively interfered is let through with enhanced contrast.

Phase-contrast microscopy allows the visualization of living cells in their natural state with high contrast and high resolution. This tool works best with a thin specimen and is not ideal for a thick specimen. Phase-contrast images have a characteristic grey background with light and dark features found across the sample. One disadvantage of phase-contrast microscopy is halo formation called halo-light ring.

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2.4.3: Interference Microscopy

LEARNING OBJECTIVES

- Describe the principles and different types of interference microscopy

Stereo Light Source

Interference microscopy uses a prism to split light into two slightly diverging beams that then pass through the specimen. It is thus based on measuring the differences in refractive index upon recombining the two beams. Interference occurs when a light beam is retarded or advanced relative to the other.

There are three types of interference microscopy: classical, differential contrast, and fluorescence contrast. Since its introduction in the late 1960s differential interference contrast microscopy (DIC) has been popular in biomedical research because it produces high-resolution images of fine structures by enhancing the contrasted interfaces. The image produced is of a thin optical section and appears three-dimensional, with a shadow around it. This creates a contrast across the specimen that is bright on one side and darker on the other.

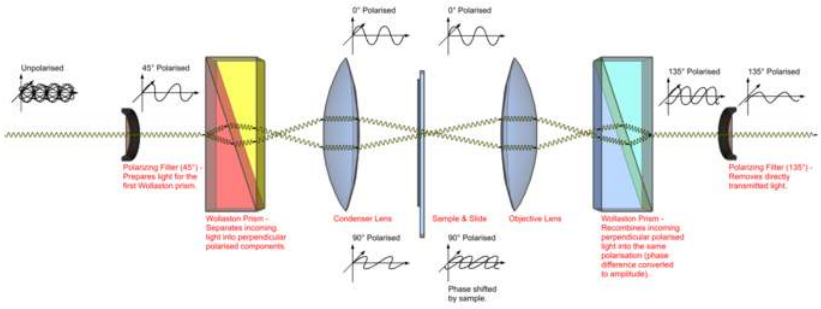


Figure: **Path of light in differential interference contrast microscopy (DIC)**: Two parallel light beams pass through the specimen and combine to produce an image.

The Interference Microscope

The microscope is a bright field light microscope with the addition of the following elements: a polarizer between the light source and the condenser, a DIC beam-splitting prism, a DIC beam-combining prism, and an analyzer. Manipulating the prism changes the beam separation, which alters the contrast of the image. When the two beams pass through the same material across the specimen they produce no interference. When the two beams pass through different material across the specimen such as on the edges, they produce alteration when combined.

Fluorescence differential interference contrast (FLIC) microscopy was developed by combining fluorescence microscopy with DIC to minimize the effects of photobleaching on fluorochromes bound to the stained specimen. The same microscope is equipped to simulataneously image a specimen using DIC and fluorescence illumination.

Key Points

- Interference microscopy is superior to phase-contrast microscopy in its ability to eliminate halos and extra light.
- In differential interference contrast microscopy (DIC), the optical path difference is determined by the product of the refractive index difference (between the specimen and its surrounding medium) and the thickness traversed by a light beam between two points on the optical path.
- Images produced by DIC have a distinctive shadow-cast appearance.

Key Terms

- **photobleaching**: The destruction of a photochemical fluorescence by high-intensity light
- **fluorochrome**: Any of various fluorescent dyes used to stain biological material before microscopic examination

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2.4.4: Fluorescence Microscopy

LEARNING OBJECTIVES

- Describe the techniques, advantages, and disadvantages of fluorescence microscopy

The fluorescent microscope uses a high-pressure mercury, halogen, or xenon vapor lamp that emits a shorter wavelength than that emitted by traditional brightfield microscopy. These light sources produce ultraviolet light. When ultraviolet light hits an object, it excites the electrons of the object, and they give off light in various shades of color. Since ultraviolet light is used a larger amount of information can be gathered; thus, the resolution of the object increases.

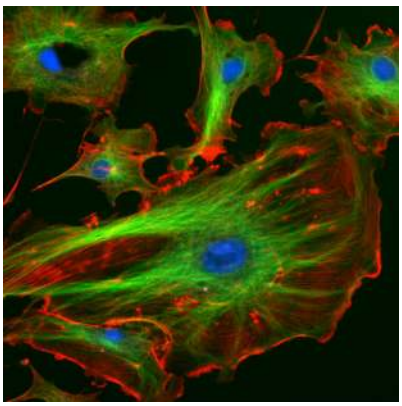


Figure: **Fluorescent cells.**: Fixed endothelial cells stained with fluorescent dyes. Nuclei are stained blue with DAPI, microtubules are stained green by an antibody bound to FITC and actin filaments are labelled red with phalloidin bound to TRITC.

Fluorescent-Antibody Technique and Dyes

This laboratory technique employs fluorescent dyes chemically linked to antibodies to help identify unknown microorganisms. This method uses the specificity of an antibody to its antigen to deliver a fluorescent dye to a target molecule. A filter is used to block the heat generated from the lamp and to match the fluorescent dye labeling the specimen. An additional barrier filter between the objective and the detector can filter out the remaining excitation light from fluorescent light.

Fluorescent dyes—molecules that absorb light of one wavelength and then re-emit it at a longer visible wavelength—can be used alone or in combination to gain specificity of the stained structure being visualized. The light emitted from the fluorophore is magnified through traditional objectives and ocular lenses. Staining organisms with these special dyes reduces the non-specific autofluorescence that some organisms can emit. Cells or organisms stained with fluorochromes appear colored against a dark background when fixed on a glass slide. Fluorescence microscopy does not allow examination of live microorganisms as it requires them to be fixed and permeabilized for the antibody to penetrate inside the cells.

Key Points

- In fluorescence microscopy, specimens are first stained with fluorochromes and then viewed through a compound microscope by using an ultraviolet (or near-ultraviolet) light source.
- Microorganisms appear as bright objects against a dark background.
- Fluorescence microscopy is used primarily in a procedure called fluorescent- antibody (FA) technique, or immunofluorescence.

Key Terms

- **autofluorescence**: Self-induced fluorescence
- **halogen**: any element of group 7, i.e. fluorine, chlorine, bromine, iodine and astatine, which form a salt by direct union with a metal

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2.4.5: Confocal Micropscopy

LEARNING OBJECTIVES

- Compare and contrast confocal and fluorescence microscopy

Confocal microscopy is a non-invasive fluorescent imaging technique that uses lasers of various colors to scan across a specimen with the aid of scanning mirrors. The point of illumination is brought to focus in the specimen by the objective lens. The scanning process uses a device that is under computer control. The sequences of points of light from the specimen are detected by a photomultiplier tube through a pinhole. The output is built into an image and transferred onto a digital computer screen for further analysis. The technique employs optical sectioning to take serial slices of the image. The slices are then stacked (Z-stack) to reconstruct the three-dimensional image of the biological sample. Optical sectioning is useful in determining cellular localization of targets. The biological sample to be studied is stained with antibodies chemically bound to fluorescent dyes similar to the method employed in fluorescence microscopy. Unlike in conventional fluorescence microscopy where the fluorescence is emitted along the entire illuminated cone creating a hazy image, in confocal microscopy the pinhole is added to allow passing of light that comes from a specific focal point on the sample and not the other. The light detected creates an image that is in focus with the original sample. Confocal microscopy has multiple applications in microbiology such as the study of biofilms and antibiotic-resistant strains of bacteria. Development of modern confocal microscopes has been accelerated by new advances in computer and storage technology, laser systems, detectors, interference filters, and fluorophores for highly specific targets.



Figure: ***Confocal Microscopy****: Tetrahymena cell, visualized using GFP-labeled anti-beta tubulin antibodies under confocal microscopy.*

Key Points

- Confocal microscopy requires immunoflurescence staining of biological samples.
- Confocal microscopy serves to control depth of field, eliminate background, and collect optical sections.
- The use of confocal microscopy has expanded to study both fixed and live cells with the ability to quantify targets.

Key Terms

- **photomultiplier tube**: A vacuum tube that detects ultraviolet, visible, and near infrared light and multiplies it 100 million times.

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2.4.6: Electron Microscopy

LEARNING OBJECTIVES

- Describe the technique employed for electron microscopy, distinguishing between different types

Electron microscopy uses a beam of electrons as an energy source. An electron beam has an exceptionally short wavelength and can hit most objects in its path, increasing the resolution of the final image captured. The electron beam is designed to travel in a vacuum to limit interference by air molecules. Magnets are used to focus the electrons on the object viewed.



Figure: ***Electron microscope****: A modern electron microscope*

There are two types of electron microscopes. The more traditional form is the transmission electron microscope (TEM). To use this instrument, ultra-thin slices of microorganisms or viruses are placed on a wire grid and then stained with gold or palladium before viewing, to create contrast. The densely coated parts of the specimen deflect the electron beam and both dark and light areas show up on the image. TEM can project images in a much higher resolution—up to the atomic level of thinner objects.

The second and most contemporary form is the scanning electron microscope (SEM). It allows the visualization of microorganisms in three dimensions as the electrons are reflected when passed over the specimen. The same gold or palladium staining is employed.

Electron microscopy has multiple applications. It is ideal to:

- explore the in vivo molecular mechanisms of disease;
- visualize the three dimensional architecture of tissues and cells;
- determine the conformation of flexible protein structures and complexes;
- observe individual viruses and macromolecular complexes in their natural biological context.

Sample preparation can be critical to generate a successful image because the choice of reagents and methods used to process a sample largely depends on the nature of the sample and the analysis required.

Key Points

- A beam of electrons, instead of light, is used with an electron microscope.
- Electron microscopes have a greater magnification because the wavelengths of electrons are much smaller than those of visible light (0.005nm as opposed to 500nm respectively–one hundred thousand times smaller).
- There are two types of electron microscopes, scanning and transmission.
- The best compound light microscopes can magnify 2000x, electron microscopes can magnify up to 100,000x.

Key Terms

- **electron beam**: a stream of electrons observed in vacuum tubes.

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2.4.7: Scanned-Probe Microscopy

LEARNING OBJECTIVES

- Describe the different types of scanning probe techniques and their advantages over other types of microscopy

3-D Images

Scanned-probe microscopy (SPM) produces highly magnified and three-dimensional-shaped images of specimens in real time. SPM employs a delicate probe to scan the surface of the specimen, eliminating the limitations that are found in electron and light microscopy. SPM covers several related technologies for imaging and measuring surfaces on a fine scale, down to the level of molecules and groups of atoms.

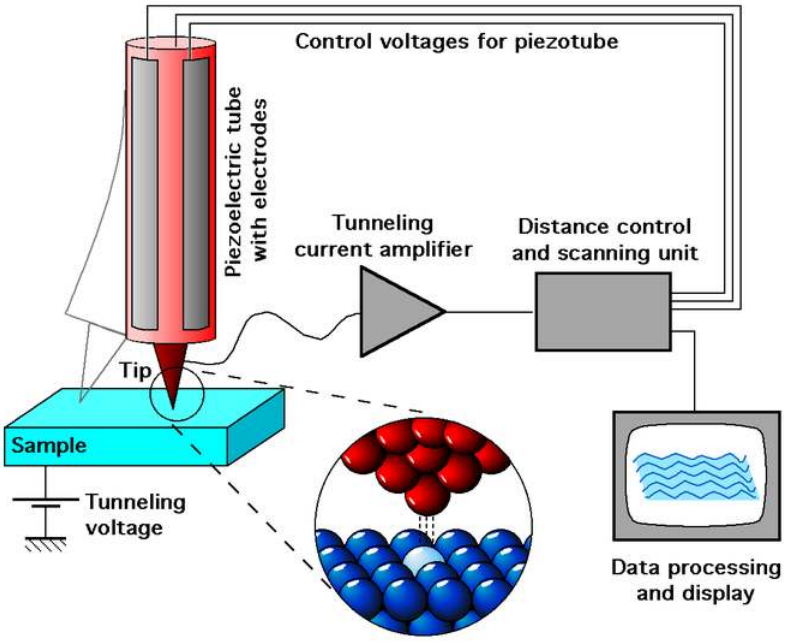


Figure: **Scanning tunneling microscopy**: Schematic diagram of a scanning tunneling microscope.

A scan may cover a distance of over 100 micrometers in the x and y directions and 4 micrometers in the z direction. SPM technologies share the concept of scanning a sharp probe tip with a small radius of curvature across the object surface. The tip is mounted on a flexible cantilever, allowing the tip to follow the surface profile. When the tip moves in proximity to the investigated object, forces of interaction between the tip and the surface influence the movement of the cantilever. Selective sensors detect these movements. Various interactions can be studied depending on the mechanics of the probe.

There are three common scanning probe techniques: *atomic force microscopy* (AFM) measures the *interaction force* between the tip and surface. The tip may be dragged across the surface, or may vibrate as it moves. The interaction force will depend on the nature of the sample, the probe tip and the distance between them. S*canning tunneling microscopy* (STM) measures a *weak electrical current* flowing between tip and sample as they are held apart. *Near-field scanning optical microscopy* (NSOM) scans a very *small light source* very close to the sample. Detection of this light energy forms the image.

Key Points

- Scanned-probe microscopy has enabled researchers to create images of surfaces at the nanometer scale with a probe.
- The probe has an extremely sharp tip that interacts with the surface of the specimen.
- There are several variations of scanned-probe microscopy of which atomic force microscopy, scanning tunneling microscopy, and near-field scanning optical microscopy are most commonly used.

Key Terms

- **micrometer**: An SI/MKS unit of measure, the length of one one-millionth of a meter. Symbols: µm, um, rm

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2.4.8: X-Ray Diffraction Analysis

LEARNING OBJECTIVES

- Summarize the methods used for x-ray diffraction analysis and the contributions they have made to science

X-ray diffraction (XRD) is a tool for characterizing the arrangement of atoms in crystals and the distances between crystal faces. The technique reveals detailed information about the chemical composition, crystallography, and microstructure of all types of natural and manufactured materials, which is key in understanding the properties of the material being studied.

Since many materials can form crystals—such as salts, metals, minerals, semiconductors, as well as various inorganic, organic, and biological molecules —X-ray crystallography has been fundamental in the development of many scientific fields. The method determined the size of atoms, the lengths and types of chemical bonds, and the atomic-scale differences among various materials, especially minerals and alloys. The method also revealed the structure and function of many biological molecules, including vitamins, drugs, proteins, and nucleic acids such as DNA.

Samples are commonly analyzed in a crystal form. X-ray diffraction is caused by constructive interference of x-ray waves that reflect off internal crystal planes. A thin film or layer of powder is fixed in the path of monochromatic x-rays. A detector measures x-rays from the sample over a range of angles. The powder consists of tiny crystals randomly oriented. At certain angles of the sensor, populations of crystals have the correct angle so that Bragg's equation is satisfied for one of the crystal planes, resulting in a spike in X-rays.

The output graph displays x-ray intensity over 2 theta, the angle of the detector. The data generated with this technique requires extensive mathematical analysis that is now made easier by available computer algorithms. The analysis consists of indexing, merging, and phasing variations in electron density. It begins with the identification of molecules using the international center for diffraction database (ICDD). This is an organization dedicated to collecting, editing, publishing, and distributing powder diffraction data for the identification of crystalline materials. Further analysis involves structure refinement and quantitative phase using the general structure analysis system (GSAS), which ultimately leads to the identification of the amorphous or crystalline phase of a matter and helps construct its three dimensional atomic model.

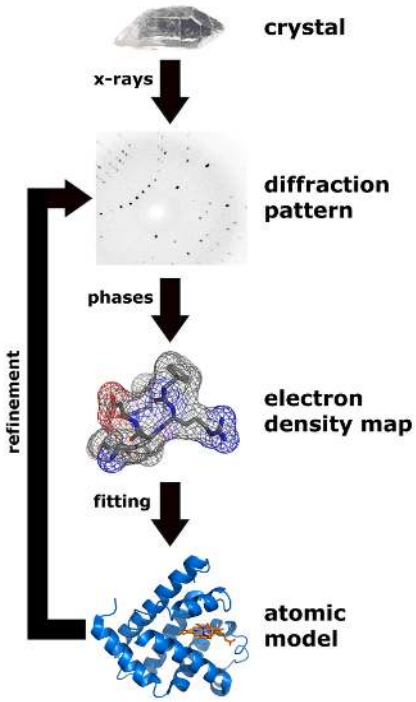


Figure: ***Structure determination by X-ray crystallography****: X-ray diffraction analysis workflow. In an X-ray diffraction measurement, a crystal is mounted on a goniometer and gradually rotated while being bombarded with X-rays, producing a diffraction pattern of regularly spaced spots known as reflections. The two-dimensional images taken at different rotations are converted into a three-dimensional model of the density of electrons within the crystal using the mathematical method of Fourier transforms, combined with chemical data known for the sample.*

Key Points

- X-ray diffraction utilizes x-ray beams targeted to hit crystallized matter and generates a diffraction pattern.
- Data collected using this method undergo a systematic analytical process that employes mathematical models and computer algorithms to obtain the final 3D atom model of a matter.
- X-ray diffraction analysis identifies composition and chemical bonds between atoms of crystal, liquid, powder, or amorphous samples.

Key Terms

- **Bragg's equation**: Gives the angles for coherent and incoherent scattering from a crystal lattice.
- **crystallography**: The experimental science of determining the arrangement of atoms in solids.

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SECTION OVERVIEW

3.1: Overview of Prokaryotic and Eukaryotic Cells

Topic hierarchy

3.1.1: Characteristics of Prokaryotic Cells

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3.1.1: Characteristics of Prokaryotic Cells

A prokaryote is a simple, unicellular organism that lacks an organized nucleus or other membrane-bound organelle.

Learning Objectives

- Describe the structure of prokaryotic cells

Key Points

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

Key Terms

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

Components of Prokaryotic Cells

All cells share four common components:

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

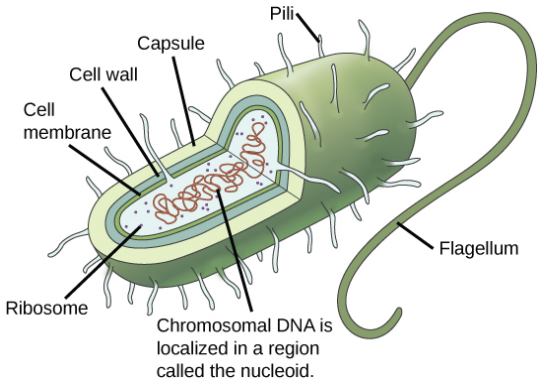


Figure: **General Structure of a Prokaryotic Cell**: This figure shows the generalized structure of a prokaryotic cell.All prokaryotes have chromosomal DNA localized in a nucleoid, ribosomes, a cell membrane, and a cell wall.The other structures shown are present in some, but not all, bacteria.

However, prokaryotes differ from eukaryotic cells in several ways.

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

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

Cell Size

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

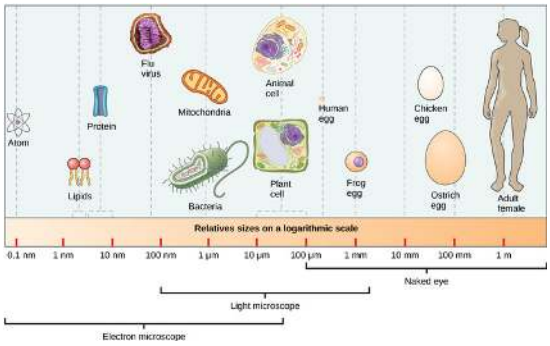


Figure: **Microbial Size**: This figure shows relative sizes of microbes on a logarithmic scale (recall that each unit of increase in a logarithmic scale represents a 10-fold increase in the quantity being measured).

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

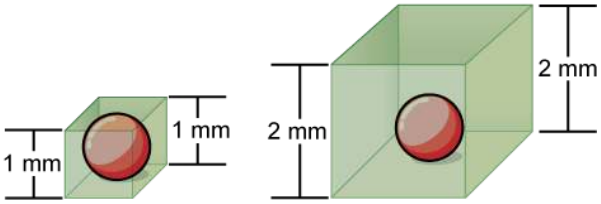


Figure: **Cell Surface Size**: Notice that as a cell increases in size, its surface area-to-volume ratio decreases.When there is insufficient surface area to support a cell's increasing volume, a cell will either divide or die.The cell on the left has a volume of 1 mm3 and a surface area of 6 mm2, with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

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SECTION OVERVIEW

3.2: The Cytoplasmic Membrane of Prokaryotic and Eukaryotic Cells

Topic hierarchy

3.2.1: Components of Plasma Membranes

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3.2.1: Components of Plasma Membranes

The plasma membrane protects the cell from its external environment, mediates cellular transport, and transmits cellular signals.

Learning Objectives

- Describe the function and components of the plasma membrane

Key Points

- The principal components of the plasma membrane are lipids (phospholipids and cholesterol), proteins, and carbohydrates.
- The plasma membrane protects intracellular components from the extracellular environment.
- The plasma membrane mediates cellular processes by regulating the materials that enter and exit the cell.
- The plasma membrane carries markers that allow cells to recognize one another and can transmit signals to other cells via receptors.

Key Terms

- **plasma membrane**: The semipermeable barrier that surrounds the cytoplasm of a cell.
- **receptor**: A protein on a cell wall that binds with specific molecules so that they can be absorbed into the cell.

Structure of Plasma Membranes

The plasma membrane (also known as the cell membrane or cytoplasmic membrane) is a biological membrane that separates the interior of a cell from its outside environment.

The primary function of the plasma membrane is to protect the cell from its surroundings. Composed of a phospholipid bilayer with embedded proteins, the plasma membrane is selectively permeable to ions and organic molecules and regulates the movement of substances in and out of cells. Plasma membranes must be very flexible in order to allow certain cells, such as red blood cells and white blood cells, to change shape as they pass through narrow capillaries.

The plasma membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell, and in attaching to the extracellular matrix and other cells to help group cells together to form tissues. The membrane also maintains the cell potential.

In short, if the cell is represented by a castle, the plasma membrane is the wall that provides structure for the buildings inside the wall, regulates which people leave and enter the castle, and conveys messages to and from neighboring castles. Just as a hole in the wall can be a disaster for the castle, a rupture in the plasma membrane causes the cell to lyse and die.

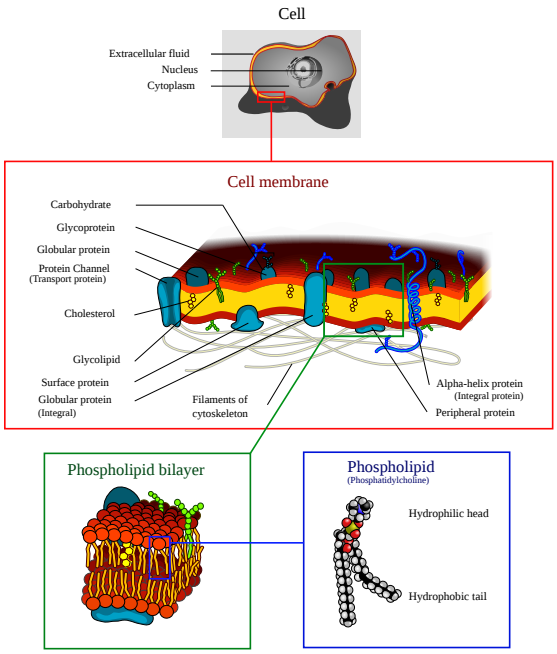


Figure: **The plasma membrane**: The plasma membrane is composed of phospholipids and proteins that provide a barrier between the external environment and the cell, regulate the transportation of molecules across the membrane, and communicate with other cells via protein receptors.

The Plasma Membrane and Cellular Transport

The movement of a substance across the selectively permeable plasma membrane can be either "passive"—i.e., occurring without the input of cellular energy —or "active"—i.e., its transport requires the cell to expend energy.

The cell employs a number of transport mechanisms that involve biological membranes:

1. Passive osmosis and diffusion: transports gases (such as O_2 and CO_2)and other small molecules and ions
2. Transmembrane protein channels and transporters: transports small organic molecules such as sugars or amino acids
3. Endocytosis: transports large molecules (or even whole cells) by engulfing them
4. Exocytosis: removes or secretes substances such as hormones or enzymes

The Plasma Membrane and Cellular Signaling

Among the most sophisticated functions of the plasma membrane is its ability to transmit signals via complex proteins. These proteins can be receptors, which work as receivers of extracellular inputs and as activators of intracellular processes, or markers, which allow cells to recognize each other.

Membrane receptors provide extracellular attachment sites for effectors like hormones and growth factors, which then trigger intracellular responses. Some viruses, such as Human Immunodeficiency Virus (HIV), can hijack these receptors to gain entry into the cells, causing infections.

Membrane markers allow cells to recognize one another, which is vital for cellular signaling processes that influence tissue and organ formation during early development. This marking function also plays a later role in the "self"-versus-"non-self" distinction of the immune response. Marker proteins on human red blood cells, for example, determine blood type (A, B, AB, or O).

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SECTION OVERVIEW

3.3: Transport Across the Cell Membrane

Topic hierarchy

3.3.1: Facilitated Transport

3.3.2: Primary Active Transport

3.3.3: ABC Transporters

3.3.4: Siderophores

3.3.5: Group Translocation

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3.3.1: Facilitated Transport

LEARNING OBJECTIVES

- Explain why and how passive transport occurs

Facilitated transport is a type of passive transport. Unlike simple diffusion where materials pass through a membrane without the help of proteins, in facilitated transport, also called facilitated diffusion, materials diffuse across the plasma membrane with the help of membrane proteins. A concentration gradient exists that would allow these materials to diffuse into the cell without expending cellular energy. However, these materials are ions or polar molecules that are repelled by the hydrophobic parts of the cell membrane. Facilitated transport proteins shield these materials from the repulsive force of the membrane, allowing them to diffuse into the cell.

The material being transported is first attached to protein or glycoprotein receptors on the exterior surface of the plasma membrane. This allows the material that is needed by the cell to be removed from the extracellular fluid. The substances are then passed to specific integral proteins that facilitate their passage. Some of these integral proteins are collections of beta-pleated sheets that form a channel through the phospholipid bilayer. Others are carrier proteins which bind with the substance and aid its diffusion through the membrane.

Channels

The integral proteins involved in facilitated transport are collectively referred to as transport proteins; they function as either channels for the material or carriers. In both cases, they are transmembrane proteins. Channels are specific for the substance that is being transported. Channel proteins have hydrophilic domains exposed to the intracellular and extracellular fluids; they additionally have a hydrophilic channel through their core that provides a hydrated opening through the membrane layers. Passage through the channel allows polar compounds to avoid the nonpolar central layer of the plasma membrane that would otherwise slow or prevent their entry into the cell. Aquaporins are channel proteins that allow water to pass through the membrane at a very high rate.

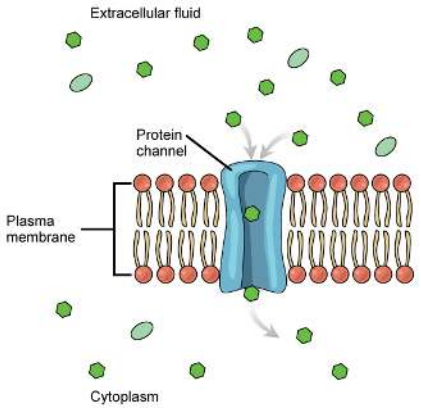


Figure: **Channel Proteins in Facilitated Transport**: Facilitated transport moves substances down their concentration gradients. They may cross the plasma membrane with the aid of channel proteins.

Channel proteins are either open at all times or they are “gated,” which controls the opening of the channel. The attachment of a particular ion to the channel protein may control the opening or other mechanisms or substances may be involved. In some tissues, sodium and chloride ions pass freely through open channels, whereas in other tissues, a gate must be opened to allow passage. An example of this occurs in the kidney, where both forms of channels are found in different parts of the renal tubules. Cells involved in the transmission of electrical impulses, such as nerve and muscle cells, have gated channels for sodium, potassium, and calcium in their membranes. Opening and closing of these channels changes the relative concentrations on opposing sides of the membrane of these ions, resulting in the facilitation of electrical transmission along membranes (in the case of nerve cells) or in muscle contraction (in the case of muscle cells).

Carrier Proteins

Another type of protein embedded in the plasma membrane is a carrier protein. This protein binds a substance and, in doing so, triggers a change of its own shape, moving the bound molecule from the outside of the cell to its interior; depending on the gradient, the material may move in the opposite direction. Carrier proteins are typically specific for a single substance. This adds to the overall selectivity of the plasma membrane. The exact mechanism for the change of shape is poorly understood. Proteins can change shape when their hydrogen bonds are affected, but this may not fully explain this mechanism. Each carrier protein is specific to one substance, and there are a finite number of these proteins in any membrane. This can cause problems in transporting enough of the material for the cell to function properly.

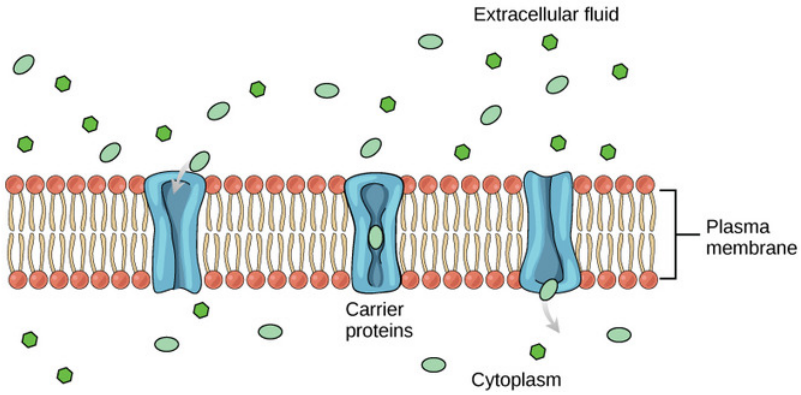


Figure: ***Carrier Proteins****: Some substances are able to move down their concentration gradient across the plasma membrane with the aid of carrier proteins. Carrier proteins change shape as they move molecules across the membrane.*

An example of this process occurs in the kidney. Glucose, water, salts, ions, and amino acids needed by the body are filtered in one part of the kidney. This filtrate, which includes glucose, is then reabsorbed in another part of the kidney. Because there are only a finite number of carrier proteins for glucose, if more glucose is present than the proteins can handle, the excess is not transported; it is excreted from the body in the urine. In a diabetic individual, this is described as “spilling glucose into the urine.” A different group of carrier proteins called glucose transport proteins, or GLUTs, are involved in transporting glucose and other hexose sugars through plasma membranes within the body.

Channel and carrier proteins transport material at different rates. Channel proteins transport much more quickly than do carrier proteins. Channel proteins facilitate diffusion at a rate of tens of millions of molecules per second, whereas carrier proteins work at a rate of a thousand to a million molecules per second.

Key Points

- A concentration gradient exists that would allow ions and polar molecules to diffuse into the cell, but these materials are repelled by the hydrophobic parts of the cell membrane.
- Facilitated diffusion uses integral membrane proteins to move polar or charged substances across the hydrophobic regions of the membrane.
- Channel proteins can aid in the facilitated diffusion of substances by forming a hydrophilic passage through the plasma membrane through which polar and charged substances can pass.
- Channel proteins can be open at all times, constantly allowing a particular substance into or out of the cell, depending on the concentration gradient; or they can be gated and can only be opened by a particular biological signal.
- Carrier proteins aid in facilitated diffusion by binding a particular substance, then altering their shape to bring that substance into or out of the cell.

Key Terms

- **facilitated diffusion**: The spontaneous passage of molecules or ions across a biological membrane passing through specific transmembrane integral proteins.
- **membrane protein**: Proteins that are attached to, or associated with the membrane of a cell or an organelle.

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3.3.2: Primary Active Transport

LEARNING OBJECTIVES

Describe how a cell moves sodium and potassium out of and into the cell against its electrochemical gradient

The sodium-potassium pump maintains the electrochemical gradient of living cells by moving sodium in and potassium out of the cell. The primary active transport that functions with the active transport of sodium and potassium allows secondary active transport to occur. The secondary transport method is still considered active because it depends on the use of energy as does primary transport.

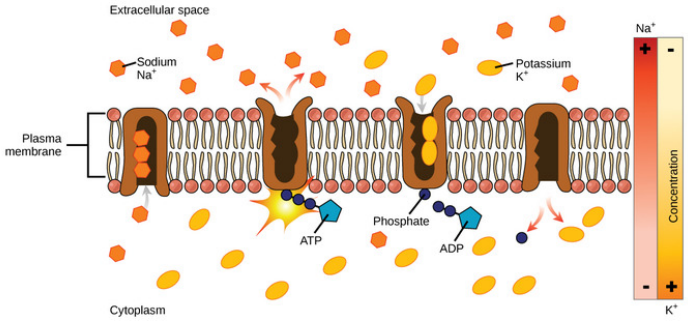


Figure: **Active Transport of Sodium and Potassium**: Primary active transport moves ions across a membrane, creating an electrochemical gradient (electrogenic transport).

One of the most important pumps in animals cells is the sodium-potassium pump (Na^+-K^+ ATPase), which maintains the electrochemical gradient (and the correct concentrations of Na^+ and K^+) in living cells. The sodium-potassium pump moves two K^+ into the cell while moving three Na^+ out of the cell. The Na+-K+ ATPase exists in two forms, depending on its orientation to the interior or exterior of the cell and its affinity for either sodium or potassium ions. The process consists of the following six steps:

- With the enzyme oriented towards the interior of the cell, the carrier has a high affinity for sodium ions. Three sodium ions bind to the protein.
- ATP is hydrolyzed by the protein carrier, and a low-energy phosphate group attaches to it.
- As a result, the carrier changes shape and re-orients itself towards the exterior of the membrane. The protein's affinity for sodium decreases, and the three sodium ions leave the carrier.
- The shape change increases the carrier's affinity for potassium ions, and two such ions attach to the protein. Subsequently, the low-energy phosphate group detaches from the carrier.
- With the phosphate group removed and potassium ions attached, the carrier protein repositions itself towards the interior of the cell.
- The carrier protein, in its new configuration, has a decreased affinity for potassium, and the two ions are released into the cytoplasm. The protein now has a higher affinity for sodium ions, and the process starts again.

Several things have happened as a result of this process. At this point, there are more sodium ions outside of the cell than inside and more potassium ions inside than out. For every three ions of sodium that move out, two ions of potassium move in. This results in the interior being slightly more negative relative to the exterior. This difference in charge is important in creating the conditions necessary for the secondary process. The sodium-potassium pump is, therefore, an electrogenic pump (a pump that creates a charge imbalance), creating an electrical imbalance across the membrane and contributing to the membrane potential.

Key Points

- The sodium-potassium pump moves K+ into the cell while moving Na+ at a ratio of three Na+ for every two K+ ions.
- When the sodium-potassium- ATPase enzyme points into the cell, it has a high affinity for sodium ions and binds three of them, hydrolyzing ATP and changing shape.
- As the enzyme changes shape, it reorients itself towards the outside of the cell, and the three sodium ions are released.

- The enzyme's new shape allows two potassium to bind and the phosphate group to detach, and the carrier protein repositions itself towards the interior of the cell.
- The enzyme changes shape again, releasing the potassium ions into the cell.
- After potassium is released into the cell, the enzyme binds three sodium ions, which starts the process over again.

Key Terms

- **electrogenic pump**: An ion pump that generates a net charge flow as a result of its activity.
- **Na+-K+ ATPase**: An enzyme located in the plasma membrane of all animal cells that pumps sodium out of cells while pumping potassium into cells.

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3.3.3: ABC Transporters

LEARNING OBJECTIVES

- Summarize the function of the three major ABC transporter categories: in prokaryotes, in gram-negative bacteria and the subgroup of ABC proteins

ATP-binding cassette transporters (ABC-transporters) are members of a protein superfamily that is one of the largest and most ancient families with representatives in all extant phyla from prokaryotes to humans. ABC transporters are transmembrane proteins that utilize the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes including translocation of various substrates across membranes and non-transport-related processes such as translation of RNA and DNA repair. They transport a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and sterols, and drugs. Proteins are classified as ABC transporters based on the sequence and organization of their ATP-binding cassette (ABC) domain(s).

ABC transporters are involved in tumor resistance, cystic fibrosis and a range of other inherited human diseases along with both bacterial (prokaryotic) and eukaryotic (including human) development of resistance to multiple drugs. Bacterial ABC transporters are essential in cell viability, virulence, and pathogenicity.

ABC transporters are divided into three main functional categories. In prokaryotes, importers mediate the uptake of nutrients into the cell. The substrates that can be transported include ions, amino acids, peptides, sugars, and other molecules that are mostly hydrophilic. The membrane-spanning region of the ABC transporter protects hydrophilic substrates from the lipids of the membrane bilayer thus providing a pathway across the cell membrane. In gram-negative bacteria, exporters transport lipids and some polysaccharides from the cytoplasm to the periplasm. Eukaryotes do not possess any importers. Exporters or effluxers, which are both present in prokaryotes and eukaryotes, function as pumps that extrude toxins and drugs out of the cell. The third subgroup of ABC proteins do not function as transporters, but rather are involved in translation and DNA repair processes.

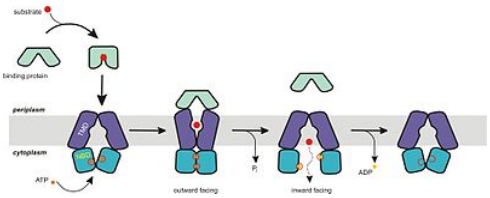


Figure: ***Mechanism of ABC transport***: *Proposed mechanism of transport for ABC importers. This alternating-access model was based on the crystal structures of ModBC-A*

In bacterial efflux systems, certain substances that need to be extruded from the cell include surface components of the bacterial cell (e.g. capsular polysaccharides, lipopolysaccharides, and teichoic acid), proteins involved in bacterial pathogenesis (e.g. hemolysis, heme-binding protein, and alkaline protease), heme, hydrolytic enzymes, S-layer proteins, competence factors, toxins, antibiotics, bacteriocins, peptide antibiotics, drugs and siderophores. They also play important roles in biosynthetic pathways, including extracellular polysaccharide biosynthesis and cytochrome biogenesis.

Key Points

- ABC transporters use the energy of ATP hydrolysis to transport substrates across cell membranes.
- Bacterial ABC transporters are essential in cell viability, virulence, and pathogenicity.
- The substrates that can be transported include ions, amino acids, peptides, sugars, and other molecules that are mostly hydrophilic.

Key Terms

- **membrane**: A flexible enclosing or separating tissue forming a plane or film and separating two environments (usually in a plant or animal).
- **hydrolysis**: A chemical process of decomposition involving the splitting of a bond and the addition of the hydrogen cation and the hydroxide anion of water.

- **ATP-binding cassette (ABC) domain**: The ATP-binding cassette (ABC) family is a group of proteins which bind and hydrolyse ATP in order to transport substances across cellular membranes.

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3.3.4: Siderophores

LEARNING OBJECTIVES

- Describe the function and variety of siderophores

Iron is essential for almost all living organisms as it is involved in a wide variety of important metabolic processes. However, iron is not always readily available; therefore, microorganisms use various iron uptake systems to secure sufficient supplies from their surroundings. There is considerable variation in the range of iron transporters and iron sources utilized by different microbial species. Pathogens, in particular, require efficient iron acquisition mechanisms to enable them to compete successfully for iron in the highly iron-restricted environment of the host's tissues and body fluids.

Siderophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi, and grasses. Siderophores are amongst the strongest soluble Fe^{3+} binding agents known. Iron is essential for almost all life, because of its vital role in processes like respiration and DNA synthesis. However, despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in many environments such as the soil or sea is limited by the very low solubility of the Fe^{3+} ion. This ion state is the predominant one of iron in aqueous, non-acidic, oxygenated environments, and accumulates in common mineral phases such as iron oxides and hydroxides (the minerals that are responsible for red and yellow soil colours). Hence, it cannot be readily utilized by organisms. Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe^{3+}complexes that can be taken up by active transport mechanisms. Many siderophores are nonribosomal peptides, although several are biosynthesised independently.

Siderophores are amongst the strongest binders to Fe^{3+} known, with enterobactin being one of the strongest of these. Because of this property, they have attracted interest from medical science in metal chelation therapy, with the siderophore desferrioxamine B gaining widespread use in treatments for iron poisoning and thalassemia.

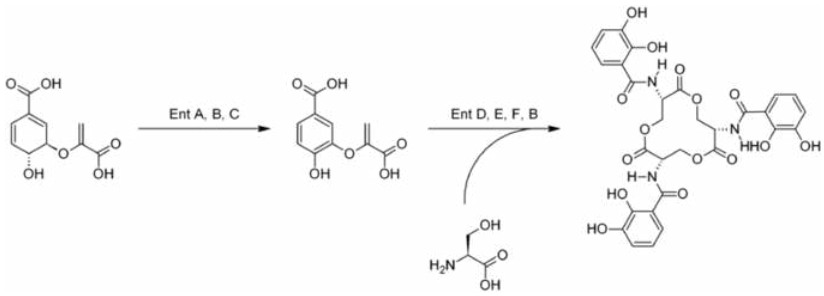


Figure: ***Synthesis of enterobactin****: Enterobactin (also known as Enterochelin) is a high affinity siderophore that acquires iron for microbial systems. It is primarily found in Gram-negative bacteria, such as Escherichia coli and Salmonella typhimurium.*

Iron is tightly bound to proteins such as hemoglobin, transferrin, lactoferrin, and ferritin. There are great evolutionary pressures put on pathogenic bacteria to obtain this metal. For example, the anthrax pathogen *Bacillus anthracis*releases two siderophores, bacillibactin and petrobactin, to scavenge ferric iron from iron proteins. While bacillibactin has been shown to bind to the immune system protein siderocalin, petrobactin is assumed to evade the immune system and has been shown to be important for virulence in mice.

Besides siderophores, some pathogenic bacteria produce *hemophores* (heme binding scavenging proteins) or have receptors that bind directly to iron/heme proteins. In eukaryotes, other strategies to enhance iron solubility and uptake are the acidification of the surrounding (e.g. used by plant roots) or the extracellular reduction of Fe^{3+} into the more soluble Fe^{2+} ions.

Siderophores usually form a stable, hexadentate, octahedral complex with Fe^{3+}preferentially compared to other naturally occurring abundant metal ions, although if there are less than six donor atoms water can also coordinate. The most effective siderophores are those that have three bidentate ligands per molecule, forming a hexadentate complex and causing a smaller entropic change than that caused by chelating a single ferric ion with separate ligands.

Siderophores are usually classified by the ligands used to chelate the ferric iron. The majors groups of siderophores include the catecholates (phenolates), hydroxamates and carboxylates (e.g. derivatives of citric acid). Citric acid can also act as a siderophore.

The wide variety of siderophores may be due to evolutionary pressures placed on microbes to produce structurally different siderophores, which cannot be transported by other microbes' specific active transport systems, or in the case of pathogens deactivated by the host organism.

Key Points

- Siderophores are important for some pathogenic bacteria for their acquisition of iron. Many siderophores are nonribosomal peptides, although several are biosynthesised independently.
- The wide variety of siderophores may be due to evolutionary pressures placed on microbes to produce structurally different siderophores which cannot be transported by other microbes' specific active transport systems, or in the case of pathogens deactivated by the host organism.
- Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms.

Key Terms

- **siderophores**: Sidereophores are small, high-affinity iron chelating compounds secreted by microorganisms such as bacteria and fungi, and also grasses. Siderophores are amongst the strongest soluble Fe^{3+} binding agents known.

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3.3.5: Group Translocation

Group translocation is a protein export or secretion pathway found in plants, bacteria, and archaea.

Learning Objectives

- Recall the following types of transport systems: PEP group translocation and the TAT pathway

Key Points

- PEP is known as a multi-component system that always involves enzymes of the plasma membrane and those in the cytoplasm. An example of this transport is found in E. coli cells.
- The Tat pathway is a protein export, or secretion pathway, that serves to actively translocate folded proteins across a lipid membrane bilayer.
- Systems for secreting proteins across the bacterial outer membrane may be quite complex and play key roles in pathogenesis.

Key Terms

- **phosphotransferase system**: A distinct method used by bacteria for sugar uptake where the source of energy is from phosphoenolpyruvate (PEP).
- **Tat pathway**: A protein export or secretion pathway found in plants, bacteria, and archaea.

With some exceptions, bacteria lack membrane-bound organelles as found in eukaryotes, but they may assemble proteins onto various types of inclusions such as gas vesicles and storage granules. Bacteria may have a single plasma membrane (Gram-positive bacteria) or an inner membrane plus an outer membrane separated by the periplasm (Gram-negative bacteria). Proteins may be incorporated into the plasma membrane. They can also be trapped in either the periplasm or secreted into the environment, according to whether or not there is an outer membrane. The basic mechanism at the plasma membrane is similar to the eukaryotic one. In addition, bacteria may target proteins into or across the outer membrane. Systems for secreting proteins across the bacterial outer membrane may be quite complex. The systems play key roles in pathogenesis. These systems may be described as type I secretion, type II secretion, etc. In most Gram-positive bacteria, certain proteins are targeted for export across the plasma membrane and subsequent covalent attachment to the bacterial cell wall.

A specialized enzyme, sortase, cleaves the target protein at a characteristic recognition site near the protein C-terminus, such as an LPXTG motif (where X can be any amino acid), then transfers the protein onto the cell wall. Several analogous systems are found that also feature a signature motif on the extracytoplasmic face, a C-terminal transmembrane domain, and cluster of basic residues on the cytosolic face at the protein's extreme C-terminus. The PEP-CTERM/exosortase system, found in many Gram-negative bacteria, seems to be related to extracellular polymeric substance production. The PGF-CTERM/archaeosortase A system in archaea is related to S-layer production. The GlyGly-CTERM/rhombosortase system, found in the Shewanella, Vibrio, and a few other genera, seems involved in the release of proteases, nucleases, and other enzymes.

PEP group translocation, also known as the phosphotransferase system or PTS, is a distinct method used by bacteria for sugar uptake where the source of energy is from phosphoenolpyruvate (PEP). It is known as a multi-component system that always involves enzymes of the plasma membrane and those in the cytoplasm. An example of this transport is found in E. coli cells. The system was discovered by Saul Roseman in 1964.

The twin-arginine translocation pathway (Tat pathway) is a protein export or secretion pathway found in plants, bacteria, and archaea. In contrast to the Sec pathway which transports proteins in an unfolded manner, the Tat pathway serves to actively translocate folded proteins across a lipid membrane bilayer. In bacteria, the Tat translocase is found in the cytoplasmic membrane and serves to export proteins to the cell envelope or to the extracellular space. In Gram-negative bacteria the Tat translocase is composed of three essential membrane proteins: TatA, TatB, and TatC. In the most widely studied Tat pathway, that of the Gram-negative bacterium Escherichia coli, these three proteins are expressed from an operon with a fourth Tat protein, TatD, which is not required for Tat function. A fifth Tat protein TatE that is homologous to the TatA protein is present at a much lower level in the cell than TatA. It is not believed to play any significant role in Tat function.

The Tat pathways of Gram-positive bacteria differ in that they do not have a TatB component. In these bacteria the Tat system is made up from a single TatA and TatC component, with the TatA protein being bifunctional and fulfilling the roles of both E. coli TatA and TatB. Not all bacteria carry the tatABC genes in their genome. However, of those that do, there seems to be no discrimination between pathogens and nonpathogens. Despite that fact, some pathogenic bacteria such as Pseudomonas aeruginosa, Legionella pneumophila, Yersinia pseudotuberculosis, and E. coli O157:H7 rely on a functioning Tat pathway for full virulence in infection models. In addition, a number of exported virulence factors have been shown to rely on the Tat pathway. One such category of virulence factors are the phospholipase C enzymes, which have been shown to be Tat-exported in Pseudomonas aeruginosa and thought to be Tat-exported in Mycobacterium tuberculosis.

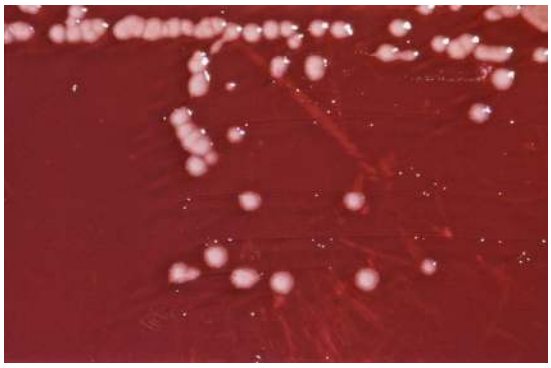


Figure: **Pseudomonas aeruginosa**: P. aeruginosa is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon-using microorganism (or “HUM bug”), causing microbial corrosion. It creates dark, gellish mats sometimes improperly called “algae” because of their appearance.

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SECTION OVERVIEW

3.4: Cell Walls of Prokaryotes

Topic hierarchy

3.4.1: The Cell Wall of Bacteria

3.4.2: Gram-Negative Outer Membrane

3.4.3: Gram-Positive Cell Envelope

3.4.4: Mycoplasmas and Other Cell-Wall-Deficient Bacteria

3.4.5: Cell Walls of Archaea

3.4.6: Damage of the Cell Wall

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3.4.1: The Cell Wall of Bacteria

Learning Objectives

- Recall the characteristics of a bacterial cell wall

Bacterial cells lack a membrane bound nucleus. Their genetic material is naked within the cytoplasm. Ribosomes are their only type of organelle. The term “nucleoid” refers to the region of the cytoplasm where chromosomal DNA is located, usually a singular, circular chromosome. Bacteria are usually single-celled, except when they exist in colonies. These ancestral cells reproduce by means of binary fission, duplicating their genetic material and then essentially splitting to form two daughter cells identical to the parent. A wall located outside the cell membrane provides the cell support, and protection against mechanical stress or damage from osmotic rupture and lysis.

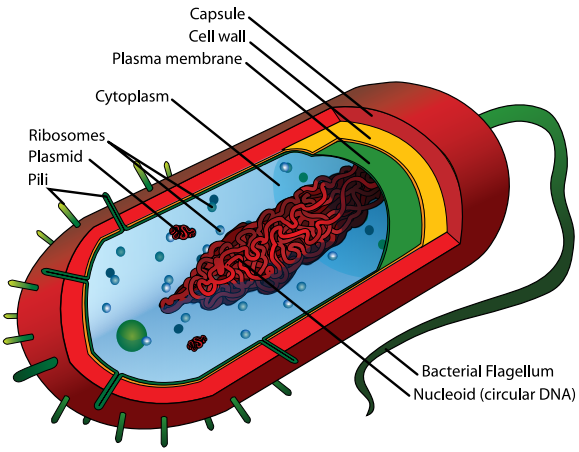


Figure **3.4.1.1**: Bacterial Cell Wall: The anatomy of bacterial cell structure. (CC BY-SA; via Wikimedia)

The major component of the bacterial cell wall is peptidoglycan or murein. This rigid structure of peptidoglycan, specific only to prokaryotes, gives the cell shape and surrounds the cytoplasmic membrane. Peptidoglycan is a huge polymer of disaccharides (glycan) cross-linked by short chains of identical amino acids (peptides) monomers. The backbone of the peptidoglycan molecule is composed of two derivatives of glucose: N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) with a pentapeptide coming off NAM and varying slightly among bacteria. The NAG and NAM strands are synthesized in the cytosol of the bacteria. They are connected by inter-peptide bridges. They are transported across the cytoplasmic membrane by a carrier molecule called bactoprenol. From the peptidoglycan inwards all bacterial cells are very similar. Going further out, the bacterial world divides into two major classes: Gram positive (Gram +) and Gram negative (Gram -). The cell wall provides important ligands for adherence and receptor sites for viruses or antibiotics.

Key Points

- A cell wall is a layer located outside the cell membrane found in plants, fungi, bacteria, algae, and archaea.
- A peptidoglycan cell wall composed of disaccharides and amino acids gives bacteria structural support.
- The bacterial cell wall is often a target for antibiotic treatment.

Key Terms

- **binary fission**: The process whereby a cell divides asexually to produce two daughter cells.

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3.4.2: Gram-Negative Outer Membrane

LEARNING OBJECTIVES

- Recognize the characteristics of a gram-negative bacteria

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

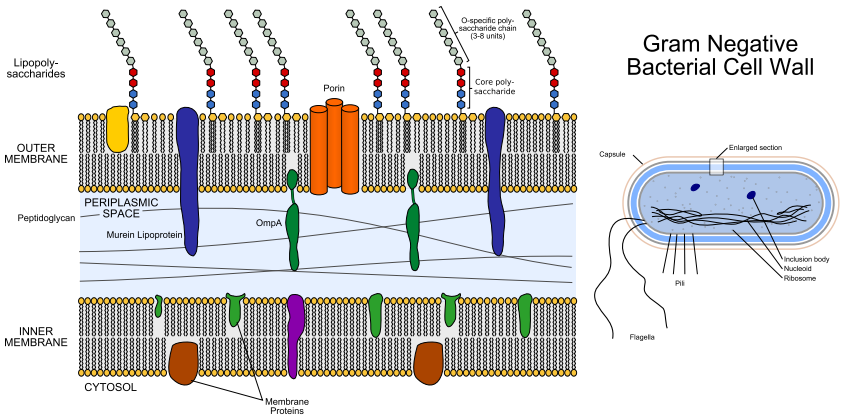


Figure: **Structure of Gram-negative cell wall**: Gram-negative outer membrane composed of lipopolysaccharides.

In the Gram-negative Bacteria the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the outer membrane. The gram-negative bacteria do not retain crystal violet but are able to retain a counterstain, commonly safranin, which is added after the crystal violet. The safranin is responsible for the red or pink color seen with a gram-negative bacteria. The Gram-negative's cell wall is thinner (10 nanometers thick) and less compact than that of Gram-positive bacteria, but remains strong, tough, and elastic to give them shape and protect them against extreme environmental conditions. The outer membrane of Gram-negative bacteria invariably contains a unique component, lipopolysaccharide (LPS) in addition to proteins and phospholipids. The LPS molecule is toxic and is classified as an endotoxin that elicits a strong immune response when the bacteria infect animals.

In Gram-negative bacteria the outer membrane is usually thought of as part of the outer leaflet of the membrane structure and is relatively permeable. It contains structures that help bacteria adhere to animal cells and cause disease. The peptidoglycan layer is non-covalently anchored to lipoprotein molecules called Braun's lipoproteins through their hydrophobic head. Sandwiched between the outer membrane and the plasma membrane, a concentrated gel-like matrix (the periplasm) is found in the periplasmic space. It is in fact an integral compartment of the gram-negative cell wall and contains binding proteins for amino acids, sugars, vitamins, iron, and enzymes essential for bacterial nutrition. The periplasm space can act as reservoir for virulence factors and a dynamic flux of macromolecules representing the cell's metabolic status and its response to environmental factors. Together, the plasma membrane and the cell wall (outer membrane, peptidoglycan layer, and periplasm) constitute the gram-negative envelope.

Key Points

- The outer membrane of Gram-negative bacteria contains lipopolysaccharides, proteins, and phospholipids.
- The lipopolysaccharide component acts as a virulence factor and causes disease in animals.
- More virulence factors are harbored in the periplasmic space between the outer membrane and the plasma membrane.

Key Terms

- **lipopolysaccharide**: any of a large class of lipids conjugated with polysaccharides
- **endotoxin**: Any toxin secreted by a microorganism and released into the surrounding environment only when it dies.

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3.4.3: Gram-Positive Cell Envelope

Gram-positive bacteria have cell envelopes made of a thick layer of peptidoglycans.

Learning Objectives

- Compare and contrast a gram-positive and negative stain

Key Points

- Gram-positive bacteria stain violet by Gram staining due the presence of peptidoglycan in their cell wall.
- Peptidoglycans are attached to negatively-charged lipoteichoic acid monomers important for cell direction and adherence.
- Lipoteichoic acids are covalently linked to lipids within the cytoplasmic membrane, thus connecting the peptidoglycans to the cell cytoplasm.

Key Terms

- **Gram stain**: A method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative).

Gram-positive bacteria are stained dark blue or violet by Gram staining. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram-variable and Gram-indeterminate groups as well.

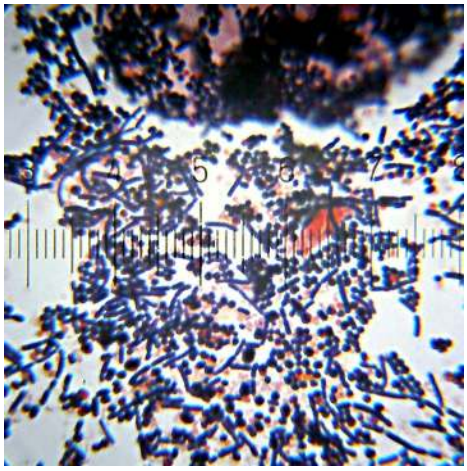


Figure: **Gram-positive bacteria**: These bacteria stain violet by Gram staining.

It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram-positive bacteria. A Gram-positive results in a purple/blue color while a Gram-negative results in a pink/red color. The Gram stain is almost always the first step in the identification of a bacterial organism, and is the default stain performed by laboratories over a sample when no specific culture is referred.

In Gram-positive bacteria, the cell wall is thick (15-80 nanometers), and consists of several layers of peptidoglycan. They lack the outer membrane envelope found in Gram-negative bacteria. Running perpendicular to the peptidoglycan sheets is a group of molecules called teichoic acids, which are unique to the Gram-positive cell wall. Teichoic acids are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars.

The teichoic acid polymers are occasionally anchored to the plasma membrane (called lipoteichoic acid, LTA), and apparently directed outward at right angles to the layers of peptidoglycan. Teichoic acids give the Gram-positive cell wall an overall negative charge due to the presence of phosphodiester bonds between teichoic acid monomers. The functions of teichoic acid are not fully known but it is believed to serve as a chelating agent and means of adherence for the bacteria. These are essential to the viability of Gram-positive bacteria in the environment and provide chemical and physical protection.

One idea is that they provide a channel of regularly-oriented, negative charges for threading positively-charged substances through the complicated peptidoglycan network. Another theory is that teichoic acids are in some way involved in the regulation and

assembly of muramic acid sub-units on the outside of the plasma membrane.

There are instances, particularly in the streptococci, wherein teichoic acids have been implicated in the adherence of the bacteria to tissue surfaces and are thought to contribute to the pathogenicity of Gram-positive bacteria.

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3.4.4: Mycoplasmas and Other Cell-Wall-Deficient Bacteria

Some bacteria lack a cell wall but retain their ability to survive by living inside another host cell.

Learning Objectives

- Distinguish between bacteria with and without cell walls

Key Points

- Examples of bacteria that lack a cell wall are Mycoplasma and L-form bacteria.
- Mycoplasma is an important cause of disease in animals and is not affected by antibiotic treatments that target cell wall synthesis.
- Mycoplasma acquire cholesterol from the environment and form sterols to build their cytoplasmic membrane.

Key Terms

- **osmotic environment**: environment with controlled net movement of molecules from a region of high solvent concentration to a region of low solvent concentration through a permeable membrane.

For most bacterial cells, the cell wall is critical to cell survival, yet there are some bacteria that do not have cell walls. *Mycoplasma* species are widespread examples and some can be intracellular pathogens that grow inside their hosts. This bacterial lifestyle is called parasitic or saprophytic. Cell walls are unnecessary here because the cells only live in the controlled osmotic environment of other cells. It is likely they had the ability to form a cell wall at some point in the past, but as their lifestyle became one of existence inside other cells, they lost the ability to form walls.

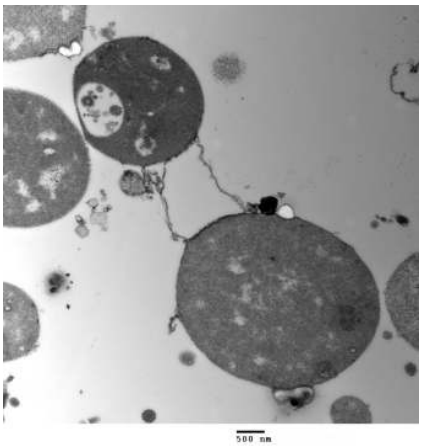


Figure: **L-form bacteria**: L-form bacterial lack a cell wall structure.

Consistent with this very limited lifestyle within other cells, these microbes also have very small genomes. They have no need for the genes for all sorts of biosynthetic enzymes, as they can steal the final components of these pathways from the host. Similarly, they have no need for genes encoding many different pathways for various carbon, nitrogen and energy sources, since their intracellular environment is completely predictable. Because of the absence of cell walls, *Mycoplasma* have a spherical shape and are quickly killed if placed in an environment with very high or very low salt concentrations. However, *Mycoplasma* do have unusually tough membranes that are more resistant to rupture than other bacteria since this cellular membrane has to contend with the host cell factors. The presence of sterols in the membrane contributes to their durability by helping to increase the forces that hold the membrane together. Other bacterial species occasionally mutate or respond to extreme nutritional conditions by forming cells lacking walls, termed L-forms. This phenomenon is observed in both gram-positive and gram-negative species. L-forms have varied shapes and are sensitive to osmotic shock.

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3.4.5: Cell Walls of Archaea

Archaeal cell walls differ from bacterial cell walls in their chemical composition and lack of peptidoglycans.

Learning Objectives

- State the similarities between the cell walls of archaea and bacteria

Key Points

- Archaea are single-celled microorganisms that lack a cell nucleus and membrane -bound organelles.
- Like other living organisms, archaea have a semi-rigid cell wall that protects them from the environment.
- The cell wall of archaea is composed of S-layers and lack peptidoglycan molecules with the exception of methanobacteria who have pseudopeptidoglycan in their cell wall.

Key Terms

- **cellulose**: A complex carbohydrate that forms the main constituent of the cell wall in most plants and is important in the manufacture of numerous products, such as paper, textiles, pharmaceuticals, and explosives.
- **chitin**: A complex polysaccharide, a polymer of N-acetylglucosamine, found in the exoskeletons of arthropods and in the cell walls of fungi; thought to be responsible for some forms of asthma in humans.
- **cytoplasm**: The contents of a cell except for the nucleus. It includes cytosol, organelles, vesicles, and the cytoskeleton.

As with other living organisms, archaeal cells have an outer cell membrane that serves as a protective barrier between the cell and its environment. Within the membrane is the cytoplasm, where the living functions of the archeon take place and where the DNA is located. Around the outside of nearly all archaeal cells is a cell wall, a semi-rigid layer that helps the cell maintain its shape and chemical equilibrium. All three of these regions may be distinguished in the cells of bacteria and most other living organisms.

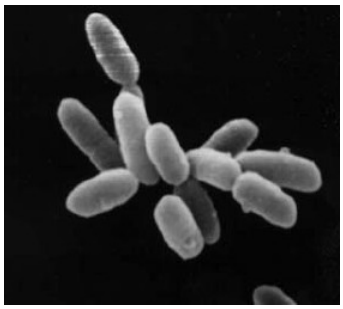


Figure: **Archaea**: Cluster of halobacterium (archaea)

A closer look at each region reveals structural similarities but major differences in chemical composition between bacterial and archaeal cell wall. Archaea builds the same structures as other organisms, but they build them from different chemical components. For instance, the cell walls of all bacteria contain the chemical peptidoglycan. Archaeal cell walls do not contain this compound, though some species contain a similar one. It is assembled from surface-layer proteins called S-layers. Likewise, archaea do not produce walls of cellulose (as do plants) or chitin (as do fungi). The cell wall of archaeans is chemically distinct. Methanogens are the only exception and possess pseudopeptidoglycan chains in their cell wall that lacks amino acids and N-acetylmuramic acid in their chemical composition. The most striking chemical differences between Archaea and other living things lie in their cell membrane. There are four fundamental differences between the archaeal membrane and those of all other cells: (1) chirality of glycerol, (2) ether linkage, (3) isoprenoid chains, and (4) branching of side chains.

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3.4.6: Damage of the Cell Wall

The cell wall is responsible for bacterial cell survival and protection against environmental factors and antimicrobial stress.

Learning Objectives

- Discuss the effects that damage to the cell wall has on the bacterial cell

Key Points

- Gram-positive and Gram-negative bacteria are protected by an external cell wall composed of varying layers of peptidoglycan.
- Damage to bacterial cell wall compromises its integrity and creates imbalance of electrolytes that trigger cell death.
- Some antibiotic classes act by inhibiting the synthesis of cell wall building blocks leading to cell lysis and death.

Key Terms

- **hydrolase**: An enzyme that catalyzes the hydrolysis of a substrate.
- **transpeptidase**: Any enzyme that catalyzes the transfer of an amino or peptide group from one molecule to another

The cell wall is the principal stress-bearing and shape-maintaining element in bacteria. Its integrity is thus of critical importance to the viability of a particular cell. In both gram-positive and gram-negative bacteria, the scaffold of the cell wall consists of a cross-linked polymer peptidoglycan. The cell wall of gram-negative bacteria is thin (approximately only 10 nanometers in thickness), and is typically comprised of only two to five layers of peptidoglycan, depending on the growth stage. In gram-positive bacteria, the cell wall is much thicker (20 to 40 nanometers thick).

While the peptidoglycan provides the structural framework of the cell wall, teichoic acids, which make up roughly 50% of the cell wall material, are thought to control the overall surface charge of the wall. This affects murein hydrolase activity, resistance to antibacterial peptides, and adherence to surfaces. Although both of these molecules are polymerized on the surface of the cytoplasmic membrane, their precursors are assembled in the cytoplasm. Any event that interferes with the assembling of the peptidoglycan precursor, and the transport of that object across the cell membrane, where it will integrate into the cell wall, would compromise the integrity of the wall. Damage to the cell wall disturbs the state of cell electrolytes, which can activate death pathways (apoptosis or programmed cell death). Regulated cell death and lysis in bacteria plays an important role in certain developmental processes, such as competence and biofilm development. They also play an important role in the elimination of damaged cells, such as those irreversibly injured by environmental or antibiotic stress. An example of an antibiotic that interferes with bacterial cell wall synthesis is Penicillin. Penicillin acts by binding to transpeptidases and inhibiting the cross-linking of peptidoglycan subunits. A bacterial cell with a damaged cell wall cannot undergo binary fission and is thus certain to die.

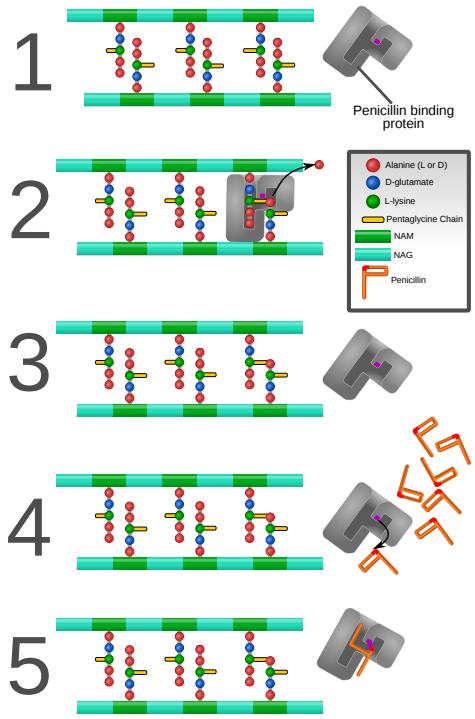


Figure: **Penicillin mechanism of action**: Penicillin acts by binding to penicillin binding proteins and inhibiting the cross-linking of peptidoglycan subunits.

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SECTION OVERVIEW

3.5: Specialized External Structures of Prokaryotes

Topic hierarchy

3.5.1: Endospores

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3.5.1: Endospores

LEARNING OBJECTIVES

- Describe the function and advantage of endospore formation, as well as the methods for viewing it.

An endospore is a dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum. Endospore formation is usually triggered by lack of nutrients, and usually occurs in Gram-positive bacteria. In endospore formation, the bacterium divides within its cell wall. One side then engulfs the other. Endospores enable bacteria to lie dormant for extended periods, even centuries. When the environment becomes more favorable, the endospore can reactivate itself to the vegetative state. Examples of bacteria that can form endospores include Bacillus and Clostridium. The endospore consists of the bacterium's DNA and part of its cytoplasm, surrounded by a very tough outer coating. Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants. They are commonly found in soil and water, where they may survive for long periods of time. Bacteria produce a single endospore internally.

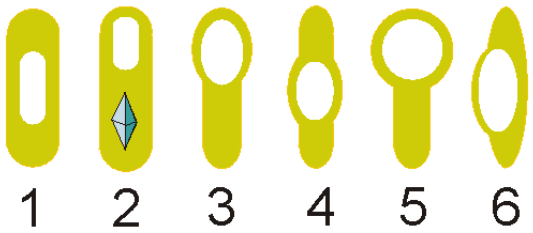


Figure: ***Endospore morphology****: Variations in endospore morphology: (1, 4) central endospore; (2, 3, 5) terminal endospore; (6) lateral endospore.*

Viewing endospores under the light microscope can be difficult due to the impermeability of the endospore wall to dyes and stains. While the rest of a bacterial cell may stain, the endospore is left colorless. To combat this, a special stain technique called a Moeller stain is used. That allows the endospore to show up as red, while the rest of the cell stains blue. Another staining technique for endospores is the Schaeffer-Fulton stain, which stains endospores green and bacterial bodies red. There are variations in endospore morphology. Examples of bacteria having terminal endospores include Clostridium tetani, the pathogen that causes the disease tetanus. Bacteria having a centrally placed endospore include Bacillus cereus, and those having a subterminal endospore include Bacillus subtilis. Sometimes the endospore can be so large that the cell can be distended around the endospore. This is typical of Clostridium tetani.

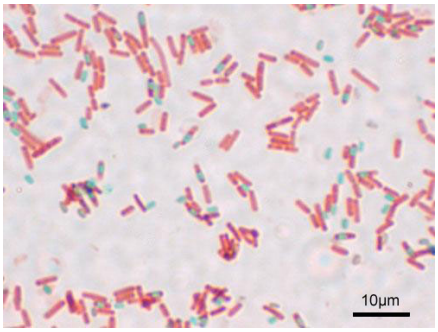


Figure: **Bacillus subtilis stained with the Schaeffer-Fulton stain.**: A stained preparation of Bacillus subtilis showing endospores as green and the vegetative cell as red.

When a bacterium detects environmental conditions are becoming unfavorable it may start the process of endosporulation, which takes about eight hours. The DNA is replicated and a membrane wall known as a spore septum begins to form between it and the rest of the cell. The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around the DNA, and the developing structure is now known as a *forespore*. Calcium dipicolinate is incorporated into the forespore during this time. Next the peptidoglycan cortex forms between the two layers and the bacterium adds a spore coat to the outside of the forespore. Sporulation is now complete, and the mature endospore will be released when the surrounding vegetative cell is degraded.

While resistant to extreme heat and radiation, endospores can be destroyed by burning or by autoclaving. Endospores are able to survive boiling at 100°C for hours, although the longer the number of hours the fewer that will survive. An indirect way to destroy them is to place them in an environment that reactivates them to their vegetative state. They will germinate within a day or two with the right environmental conditions, and then the vegetative cells can be straightforwardly destroyed. This indirect method is called Tyndallization. It was the usual method for a while in the late 19th century before the advent of inexpensive autoclaves. Prolonged exposure to ionising radiation, such as x-rays and gamma rays, will also kill most endospores.

Reactivation of the endospore occurs when conditions are more favourable and involves activation, germination, and outgrowth. Even if an endospore is located in plentiful nutrients, it may fail to germinate unless activation has taken place. This may be triggered by heating the endospore. Germination involves the dormant endospore starting metabolic activity and thus breaking hibernation. It is commonly characterised by rupture or absorption of the spore coat, swelling of the endospore, an increase in metabolic activity, and loss of resistance to environmental stress.

As a simplified model for cellular differentiation, the molecular details of endospore formation have been extensively studied, specifically in the model organism Bacillus subtilis. These studies have contributed much to our understanding of the regulation of gene expression, transcription factors, and the sigma factor subunits of RNA polymerase.

Endospores of the bacterium Bacillus anthracis were used in the 2001 anthrax attacks. The powder found in contaminated postal letters was composed of extracellular anthrax endospores. Inhalation, ingestion or skin contamination of these endospores led to a number of deaths.

Geobacillus stearothermophilus endospores are used as biological indicators when an autoclave is used in sterilization procedures. Bacillus subtilis spores are useful for the expression of recombinant proteins and in particular for the surface display of peptides and proteins as a tool for fundamental and applied research in the fields of microbiology, biotechnology and vaccination.

Key Points

- Examples of bacteria that can form endospores include Bacillus and Clostridium.
- Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants.
- While resistant to extreme heat and radiation, endospores can be destroyed by burning or by autoclaving.

Key Terms

- **endospore**: A dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum.

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SECTION OVERVIEW

3.6: Specialized Internal Structures of Prokaryotes

Topic hierarchy

3.6.1: Ribosomes

3.6.2: Cell Inclusions and Storage Granules

3.6.3: Carboxysomes

3.6.4: Magnetosomes

3.6.5: Gas Vesicles

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3.6.1: Ribosomes

LEARNING OBJECTIVES

- Compare and contrast ribosome structure and function in prokaryotes and eukaryotes

Ribosomes are tiny spherical organelles that make proteins by joining amino acids together. Many ribosomes are found free in the cytosol, while others are attached to the rough endoplasmic reticulum. The purpose of the ribosome is to translate messenger RNA (mRNA) to proteins with the aid of tRNA. In eukaryotes, ribosomes can commonly be found in the cytosol of a cell, the endoplasmic reticulum or mRNA, as well as the matrix of the mitochondria. Proteins synthesized in each of these locations serve a different role in the cell. In prokaryotes, ribosomes can be found in the cytosol as well. This protein-synthesizing organelle is the only organelle found in both prokaryotes and eukaryotes, asserting the fact that the ribosome is a trait that evolved early on, most likely present in the common ancestor of eukaryotes and prokaryotes. Ribosomes are not membrane bound.

Ribosomes are composed of two subunits, one large and one small, that only bind together during protein synthesis. The purpose of the ribosome is to take the actual message and the charged aminoacyl-tRNA complex to generate the protein. To do so, they have three binding sites. One is for the mRNA; the other two are for the tRNA. The binding sites for tRNA are the A site, which holds the aminoacyl-tRNA complex, and the P site, which binds to the tRNA attached to the growing polypeptide chain.

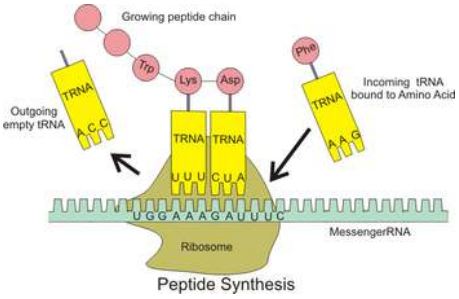


Figure: **Peptide synthesis by a ribosome.**: The ribosome assembles amino acids into a protein. The specific amino acids are controlled by the mRNA sequence. This is required by all living cells and associated viruses.

In most bacteria, the most numerous intracellular structure is the ribosome which is the site of protein synthesis in all living organisms. All prokaryotes have 70S (where S=Svedberg units) ribosomes while eukaryotes contain larger 80S ribosomes in their cytosol. The 70S ribosome is made up of a 50S and 30S subunits. The 50S subunit contains the 23S and 5S rRNA while the 30S subunit contains the 16S rRNA. These rRNA molecules differ in size in eukaryotes and are complexed with a large number of ribosomal proteins, the number and type of which can vary slightly between organisms. The ribosome is the most commonly observed intracellular multiprotein complex in bacteria.

Ribosome assembly consists of transcription, translation, the folding of rRNA and ribosomal proteins, the binding of ribosomal proteins, and the binding and release of the assembly components to make the ribosome. In vivo assembly of the 30S subunit has two intermediates (p130S and p230S) and the 50S subunit has three intermediates (p150S, p250S, and p350S). However, the reconstitution intermediates are not the same as in vitro. The intermediates of the 30S subunit yield 21S and 30S particles while the intermediates of the 50S subunit yield 32S, 43S, and 50S particles. The intermediates in the in vivo assembly are precursor rRNA which is different from in vitro which uses matured rRNA. To complete the mechanism of ribosome assembly, these precursor rRNA gets transformed in the polysomes.

Key Points

- All prokaryotes have 70S (where S=Svedberg units) ribosomes while eukaryotes contain larger 80S ribosomes in their cytosol. The 70S ribosome is made up of a 50S and 30S subunits.
- Ribosomes play a key role in the catalysis of two important and crucial biological processes. peptidyl transfer and peptidyl hydrolysis.
- Ribosomes are tiny spherical organelles that make proteins by joining amino acids together. Many ribosomes are found free in the cytosol, while others are attached to the rough endoplasmic reticulum.

Key Terms

- **ribosome**: Small organelles found in all cells; involved in the production of proteins by translating messenger RNA.
- **translation**: A process occurring in the ribosome, in which a strand of messenger RNA (mRNA) guides assembly of a sequence of amino acids to make a protein.
- **Svedberg**: The Svedberg unit (S) offers a measure of particle size based on its rate of travel in a tube subjected to high g-force.

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3.6.2: Cell Inclusions and Storage Granules

Bacteria have different methods of nutrient storage that are employed in times of plenty, for use in times of want.

Learning Objectives

- Explain the hypothesis regarding the formation of inclusion bodies and the importance of storage granules

Key Points

- Sulfur granules are especially common in bacteria that use hydrogen sulfide as an electron source.
- When genes from one organism are expressed in another, the resulting protein sometimes forms inclusion bodies.
- Many bacteria store excess carbon in the form of polyhydroxyalkanoates or glycogen.

Key Terms

- **Inclusion bodies**: Inclusion bodies are nuclear or cytoplasmic aggregates of stainable substances, usually proteins.

Cell Inclusions and Storage Granules

Bacteria, despite their simplicity, contain a well-developed cell structure responsible for many unique biological properties not found among archaea or eukaryotes. Because of the simplicity of bacteria relative to larger organisms, and the ease with which they can be manipulated experimentally, the cell structure of bacteria has been well studied, revealing many biochemical principles that have been subsequently applied to other organisms.

Most bacteria do not live in environments that contain large amounts of nutrients at all times. To accommodate these transient levels of nutrients, bacteria contain several different methods of nutrient storage that are employed in times of plenty, for use in times of want. For example, many bacteria store excess carbon in the form of polyhydroxyalkanoates or glycogen. Some microbes store soluble nutrients, such as nitrate in vacuoles. Sulfur is most often stored as elemental (S0) granules which can be deposited either intra- or extracellularly. Sulfur granules are especially common in bacteria that use hydrogen sulfide as an electron source. Most of the above mentioned examples can be viewed using a microscope, and are surrounded by a thin non-unit membrane to separate them from the cytoplasm.

Inclusion bodies are nuclear or cytoplasmic aggregates of stainable substances, usually proteins. They typically represent sites of viral multiplication in a bacterium or a eukaryotic cell, and usually consist of viral capsid proteins. Inclusion bodies have a non-unit lipid membrane. Protein inclusion bodies are classically thought to contain misfolded protein. However, this has recently been contested, as green fluorescent protein will sometimes fluoresce in inclusion bodies, which indicates some resemblance of the native structure and researchers have recovered folded protein from inclusion bodies.

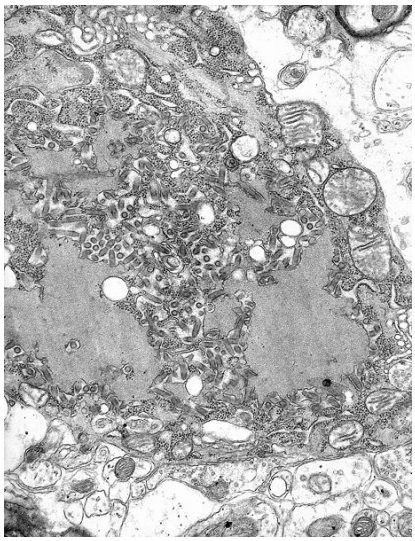


Figure: **Electron Micrograph of the Rabies Virus.**: This electron micrograph shows the rabies virus, as well as Negri bodies, or cellular inclusions.

When genes from one organism are expressed in another the resulting protein sometimes forms inclusion bodies. This is often true when large evolutionary distances are crossed; for example, a cDNA isolated from Eukarya and expressed as a recombinant gene in a prokaryote, risks the formation of the inactive aggregates of protein known as inclusion bodies. While the cDNA may properly code for a translatable mRNA, the protein that results will emerge in a foreign microenvironment. This often has fatal effects, especially if the intent of cloning is to produce a biologically active protein. For example, eukaryotic systems for carbohydrate modification and membrane transport are not found in prokaryotes.

The internal microenvironment of a prokaryotic cell (pH, osmolarity) may differ from that of the original source of the gene. Mechanisms for folding a protein may also be absent, and hydrophobic residues that normally would remain buried may be exposed and available for interaction with similar exposed sites on other ectopic proteins. Processing systems for the cleavage and removal of internal peptides would also be absent in bacteria. The initial attempts to clone insulin in a bacterium suffered all of these deficits. In addition, the fine controls that may keep the concentration of a protein low will also be missing in a prokaryotic cell, and overexpression can result in filling a cell with ectopic protein that, even if it were properly folded, would precipitate by saturating its environment.

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3.6.3: Carboxysomes

LEARNING OBJECTIVES

- Generalize the function of carboxysomes in autotrophic bacteria

Carboxysomes are intracellular structures found in many autotrophic bacteria, including Cyanobacteria, Knallgasbacteria, Nitroso- and Nitrobacteria. They are proteinaceous structures resembling phage heads in their morphology; they contain the enzymes of carbon dioxide fixation in these organisms. It is thought that the high local concentration of the enzymes, along with the fast conversion of bicarbonate to carbon dioxide by carbonic anhydrase, allows faster and more efficient carbon dioxide fixation than is possible inside the cytoplasm. Similar structures are known to harbor the B_{12}-containing coenzyme glycerol dehydratase, the key enzyme of glycerol fermentation to 1,3-propanediol, in some Enterobacteriaceae, such as Salmonella.

Carboxysomes are bacterial microcompartments that contain enzymes involved in carbon fixation. Carboxysomes are made of polyhedral protein shells about 80 to 140 nanometres in diameter. These compartments are thought to concentrate carbon dioxide to overcome the inefficiency of RuBisCo (ribulose bisphosphate carboxylase/oxygenase) – the predominant enzyme in carbon fixation and the rate limiting enzyme in the Calvin cycle. These organelles are found in all cyanobacteria and many chemotrophic bacteria that fix carbon dioxide.

Carboxysomes are an example of a wider group of protein micro-compartments that have dissimilar functions but similar structures, based on homology of the two shell protein families. Using electron microscopy the first carboxysomes were seen in 1956, in the cyanobacterium Phormidium uncinatum. In the early 1960s, similar polyhedral objects were observed in other cyanobacteria. These structures were named polyhedral bodies in 1961; over the next few years they were also discovered in some chemotrophic bacteria that fixed carbon dioxide. Among these are *Halothiobacillus*, *Acidithiobacillus*, *Nitrobacter* and *Nitrococcus*.

Carboxysomes were first purified from Thiobacillus neapolitanus in 1973, and were shown to contain RuBisCo held within a rigid outer covering.

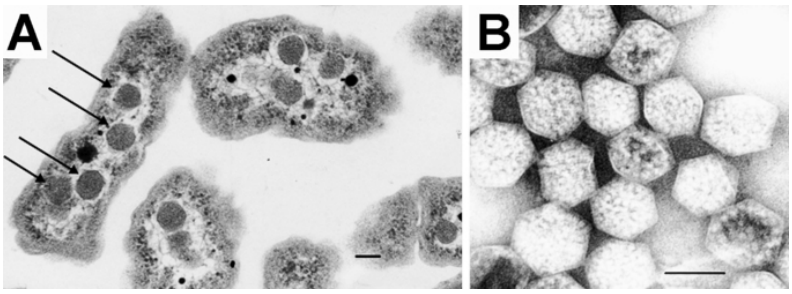


Figure: ***Electron Micrograph of a carboxysome****: (A) A thin-section electron micrograph of H. neapolitanus cells with carboxysomes inside. In one of the cells shown, arrows highlight the visible carboxysomes. (B) A negatively stained image of intact carboxysomes isolated from H. neapolitanus. The features visualized arise from the distribution of stain around proteins forming the shell as well as around the RuBisCO molecules that fill the carboxysome interior. Scale bars indicate 100 nm.*

Key Points

- Carboxysomes are proteinaceous structures resembling phage heads in their morphology and contain the enzymes of carbon dioxide fixation in these organisms.
- Carboxysomes are made of polyhedral protein shells about 80 to 140 nanometres in diameter.
- These organelles are found in all cyanobacteria and many chemotrophic bacteria that fix carbon dioxide.

Key Terms

- **carboxysome**: A bacterial organelle that contains enzymes involved in carbon fixation.

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3.6.4: Magnetosomes

LEARNING OBJECTIVES

- Illustrate the structure of magnetosomes and the advantages that they provide to magentotactic bacteria

Magnetosomes are intracellular organelles found in magnetotactic bacteria that allow them to sense and align themselves along a magnetic field (magnetotaxis). They contain 15 to 20 magnetite crystals that together act like a compass needle to orient magnetotactic bacteria in geomagnetic fields, thereby simplifying their search for their preferred microaerophilic environments. Each magnetite crystal within a magnetosome is surrounded by a lipid bilayer. Specific soluble and transmembrane proteins are sorted to the membrane. Recent research has shown that magnetosomes are invaginations of the inner membrane and not freestanding vesicles. Magnetite-bearing magnetosomes have also been found in eukaryotic magnetotactic algae, with each cell containing several thousand crystals.

Magnetotactic bacteria usually mineralize either iron oxide magnetosomes, which contain crystals of magnetite (Fe_3O_4), or iron sulfide magnetosomes, which contain crystals of greigite (Fe_3S_4). Several other iron sulfide minerals have also been identified in iron sulfide magnetosomes — including mackinawite (tetragonal FeS) and a cubic FeS — which are thought to be precursors of Fe3S4. One type of magnetotactic bacterium present at the oxic-anoxic transition zone (OATZ) of the southern basin of the Pettaquamscutt River Estuary, Narragansett, Rhode Island is known to produce both iron oxide and iron sulfide magnetosomes.



Figure: ***Magnetospirilli with black magnetosome chains faintly visible****: There is a broad range of shapes and groups of magnetic bacteria. However, cultivation of these organisms in the laboratory is often difficult. Only a few strains of magnetotactic bacteria have been isolated in pure culture, a tiny minority of the vast diversity of naturally occurring populations from largely unexplored natural habitats such as the marine environment.*

The particle morphology of magnetosome crystals varies, but is consistent within cells of a single magnetotactic bacterial species or strain. Three general crystal morphologies have been reported in magnetotactic bacteria on the basis: roughly cuboidal, elongated prismatic (roughly rectangular), and tooth-, bullet-, or arrowhead-shaped. Magnetosome crystals are typically 35–120 nm long, which makes them single- domain. Single-domain crystals have the maximum possible magnetic moment per unit volume for a given composition. Smaller crystals are superparamagnetic–that is, not permanently magnetic at ambient temperature, and domain walls would form in larger crystals. In most magnetotactic bacteria, the magnetosomes are arranged in one or more chains.

Magnetic interactions between the magnetosome crystals in a chain cause their magnetic dipole moments to orientate parallel to each other along the length of the chain. The magnetic dipole moment of the cell is usually large enough so that its interaction with Earth's magnetic field overcomes thermal forces that tend to randomize the orientation of the cell in its aqueous surroundings. Magnetotactic bacteria also use aerotaxis, a response to changes in oxygen concentration that favors swimming toward a zone of optimal oxygen concentration. In lakes or oceans the oxygen concentration is commonly dependent on depth. As long as the Earth's magnetic field has a significant downward slant, the orientation along field lines aids the search for the optimal concentration. This process is called magneto-aerotaxis.

Key Points

- Magnetosomes contain 15 to 20 magnetite crystals that together act like a compass needle to orient magnetotactic bacteria in geomagnetic fields, thereby simplifying their search for their preferred microaerophilic environments.
- The particle morphology of magnetosome crystals varies, but is consistent within cells of a single magnetotactic bacterial species or strain.

- Each magnetite crystal within a magnetosome is surrounded by a lipid bilayer. Specific soluble and transmembrane proteins are sorted to the membrane.

Key Terms

- **magnetotaxis**: The supposed ability to sense a magnetic field and coordinate movement in response, later discovered to be natural magnetism: such creatures orient themselves magnetically even after death.
- **magnetosome**: A membranous prokaryotic organelle, containing mineral crystals, present in magnetotactic bacteria.

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3.6.5: Gas Vesicles

LEARNING OBJECTIVES

- Discuss the role of a gas vesicle in regards to survival.

Gas vesicles are spindle-shaped structures found in some planktonic bacteria that provides buoyancy to these cells by decreasing their overall cell density. Positive buoyancy is needed to keep the cells in the upper reaches of the water column, so that they can continue to perform photosynthesis. They are made up of a shell of protein that has a highly hydrophobic inner surface, making it impermeable to water (and stopping water vapor from condensing inside), but permeable to most gases. Because the gas vesicle is a hollow cylinder, it is liable to collapse when the surrounding pressure becomes too great.

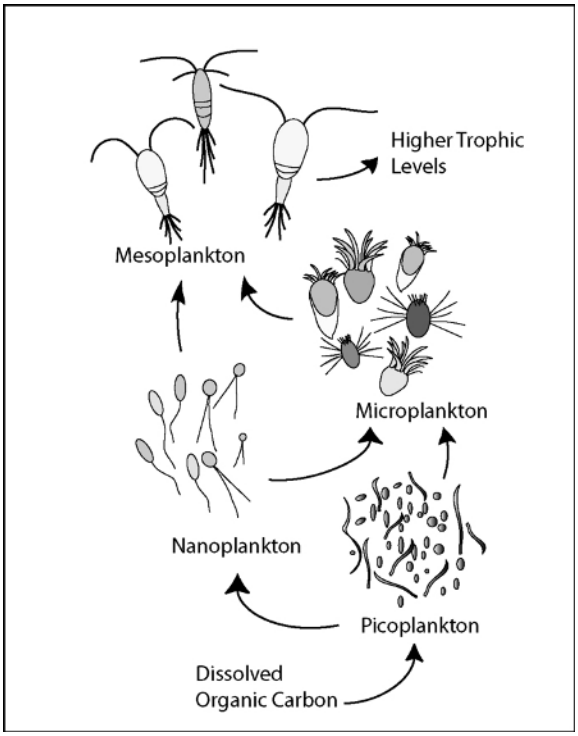


Figure: **Illustration of a microbial loop**: Gas vesicles provide bouyancy for some planktonic bacteria by decreasing their overall cell density.

Natural selection has fine-tuned the structure of the gas vesicle to maximize its resistance to buckling by including an external strengthening protein, GvpC, rather like the green thread in a braided hosepipe. There is a simple relationship between the diameter of the gas vesicle and pressure at which it will collapse – the wider the gas vesicle the weaker it becomes. However, wider gas vesicles are more efficient. They provide more buoyancy per unit of protein than narrow gas vesicles. Different species produce gas vesicles of different diameters, allowing them to colonize different depths of the water column (fast growing, highly competitive species with wide gas vesicles in the top most layers; slow growing, dark-adapted, species with strong narrow gas vesicles in the deeper layers). The diameter of the gas vesicle will also help determine which species survive in different bodies of water. Deep lakes that experience winter mixing will expose the cells to the hydrostatic pressure generated by the full water column. This will select for species with narrower, stronger gas vesicles.

Key Points

- They are made up of a shell of protein that has a highly hydrophobic inner surface, making it impermeable to water (and stopping water vapour from condensing inside), but permeable to most gases.
- Natural selection has fine tuned the structure of the gas vesicle to maximize its resistance to buckling, including an external strengthening protein, GvpC, rather like the green thread in a braided hosepipe.
- The diameter of the gas vesicle will also help determine which species survive in different bodies of water.

Key Terms

- **gas vesicle**: Gas vesicles are spindle-shaped structures found in some planktonic bacteria that provide buoyancy to these cells by decreasing their overall cell density.
- **gas gangrene**: a bacterial infection that produces gas in tissues in necrotizing or rotting tissues

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SECTION OVERVIEW

3.7: Internal Structures of Eukaryotic Cells

Topic hierarchy

3.7.1: The Nucleus and Ribosomes

3.7.2: Mitochondria

3.7.3: Comparing Plant and Animal Cells

3.7.4: The Endoplasmic Reticulum

3.7.5: The Golgi Apparatus

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3.7.1: The Nucleus and Ribosomes

Found within eukaryotic cells, the nucleus contains the genetic material that determines the entire structure and function of that cell.

Learning Objectives

- Explain the purpose of the nucleus in eukaryotic cells

Key Points

- The nucleus contains the cell ‘s DNA and directs the synthesis of ribosomes and proteins.
- Found within the nucleoplasm, the nucleolus is a condensed region of chromatin where ribosome synthesis occurs.
- Chromatin consists of DNA wrapped around histone proteins and is stored within the nucleoplasm.
- Ribosomes are large complexes of protein and ribonucleic acid (RNA) responsible for protein synthesis when DNA from the nucleus is transcribed.

Key Terms

- **histone**: any of various simple water-soluble proteins that are rich in the basic amino acids lysine and arginine and are complexed with DNA in the nucleosomes of eukaryotic chromatin
- **nucleolus**: a conspicuous, rounded, non-membrane bound body within the nucleus of a cell
- **chromatin**: a complex of DNA, RNA, and proteins within the cell nucleus out of which chromosomes condense during cell division

The Nucleus

One of the main differences between prokaryotic and eukaryotic cells is the nucleus. As previously discussed, prokaryotic cells lack an organized nucleus while eukaryotic cells contain membrane-bound nuclei (and organelles) that house the cell’s DNA and direct the synthesis of ribosomes and proteins.

The nucleus stores chromatin (DNA plus proteins) in a gel-like substance called the nucleoplasm. To understand chromatin, it is helpful to first consider chromosomes. Chromatin describes the material that makes up chromosomes, which are structures within the nucleus that are made up of DNA, the hereditary material. You may remember that in prokaryotes, DNA is organized into a single circular chromosome. In eukaryotes, chromosomes are linear structures. Every eukaryotic species has a specific number of chromosomes in the nuclei of its body’s cells. For example, in humans, the chromosome number is 46, while in fruit flies, it is eight. Chromosomes are only visible and distinguishable from one another when the cell is getting ready to divide. In order to organize the large amount of DNA within the nucleus, proteins called histones are attached to chromosomes; the DNA is wrapped around these histones to form a structure resembling beads on a string. These protein-chromosome complexes are called chromatin.

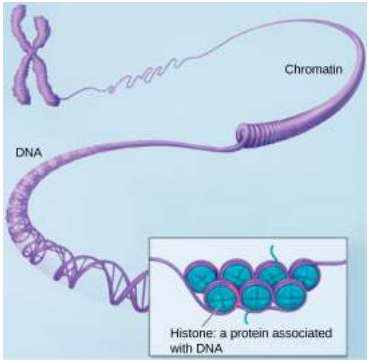


Figure: **DNA is highly organized**: This image shows various levels of the organization of chromatin (DNA and protein). Along the chromatin threads, unwound protein-chromosome complexes, we find DNA wrapped around a set of histone proteins.

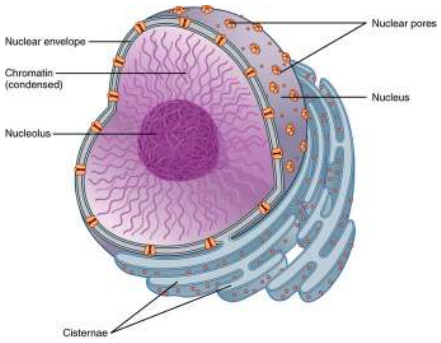


Figure: **The nucleus stores the hereditary material of the cell**: The nucleus is the control center of the cell. The nucleus of living cells contains the genetic material that determines the entire structure and function of that cell.

The nucleoplasm is also where we find the nucleolus. The nucleolus is a condensed region of chromatin where ribosome synthesis occurs. Ribosomes, large complexes of protein and ribonucleic acid (RNA), are the cellular organelles responsible for protein synthesis. They receive their "orders" for protein synthesis from the nucleus where the DNA is transcribed into messenger RNA (mRNA). This mRNA travels to the ribosomes, which translate the code provided by the sequence of the nitrogenous bases in the mRNA into a specific order of amino acids in a protein.

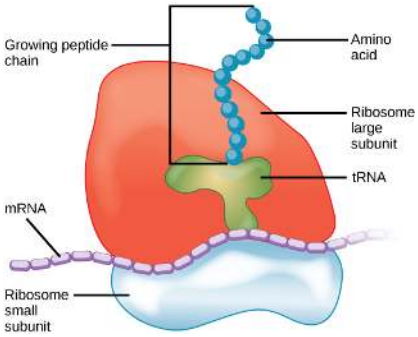


Figure: **Ribosomes are responsible for protein synthesis**: Ribosomes are made up of a large subunit (top) and a small subunit (bottom). During protein synthesis, ribosomes assemble amino acids into proteins.

Lastly, the boundary of the nucleus is called the nuclear envelope. It consists of two phospholipid bilayers: an outer membrane and an inner membrane. The nuclear membrane is continuous with the endoplasmic reticulum, while nuclear pores allow substances to enter and exit the nucleus.

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3.7.2: Mitochondria

LEARNING OBJECTIVES

- Explain the role of the mitochondria.

One of the major features distinguishing prokaryotes from eukaryotes is the presence of mitochondria. Mitochondria are double-membraned organelles that contain their own ribosomes and DNA. Each membrane is a phospholipid bilayer embedded with proteins. Eukaryotic cells may contain anywhere from one to several thousand mitochondria, depending on the cell's level of energy consumption. Each mitochondrion measures 1 to 10 micrometers (or greater) in length and exists in the cell as an organelle that can be ovoid to worm-shaped to intricately branched.

Mitochondria Structure

Most mitochondria are surrounded by two membranes, which would result when one membrane-bound organism was engulfed into a vacuole by another membrane-bound organism. The mitochondrial inner membrane is extensive and involves substantial infoldings called cristae that resemble the textured, outer surface of alpha-proteobacteria. The matrix and inner membrane are rich with the enzymes necessary for aerobic respiration.

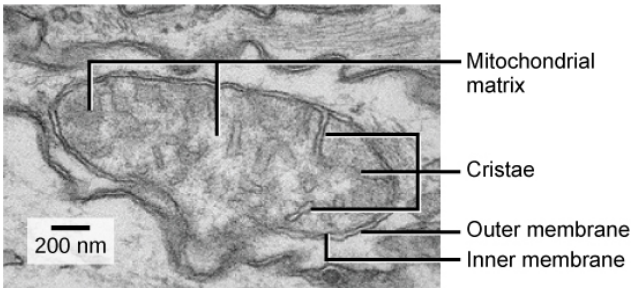


Figure: ***Mitochondrial structure****: This electron micrograph shows a mitochondrion as viewed with a transmission electron microscope. This organelle has an outer membrane and an inner membrane. The inner membrane contains folds, called cristae, which increase its surface area. The space between the two membranes is called the intermembrane space, and the space inside the inner membrane is called the mitochondrial matrix. ATP synthesis takes place on the inner membrane.*

Mitochondria have their own (usually) circular DNA chromosome that is stabilized by attachments to the inner membrane and carries genes similar to genes expressed by alpha-proteobacteria. Mitochondria also have special ribosomes and transfer RNAs that resemble these components in prokaryotes. These features all support the hypothesis that mitochondria were once free-living prokaryotes.

Mitochondria Function

Mitochondria are often called the "powerhouses" or "energy factories" of a cell because they are responsible for making adenosine triphosphate (ATP), the cell's main energy-carrying molecule. ATP represents the short-term stored energy of the cell. Cellular respiration is the process of making ATP using the chemical energy found in glucose and other nutrients. In mitochondria, this process uses oxygen and produces carbon dioxide as a waste product. In fact, the carbon dioxide that you exhale with every breath comes from the cellular reactions that produce carbon dioxide as a by-product.

It is important to point out that muscle cells have a very high concentration of mitochondria that produce ATP. Your muscle cells need a lot of energy to keep your body moving. When your cells don't get enough oxygen, they do not make a lot of ATP. Instead, the small amount of ATP they make in the absence of oxygen is accompanied by the production of lactic acid. In addition to the aerobic generation of ATP, mitochondria have several other metabolic functions. One of these functions is to generate clusters of iron and sulfur that are important cofactors of many enzymes. Such functions are often associated with the reduced mitochondrion-derived organelles of anaerobic eukaryotes.

Origins of Mitochondria

There are two hypotheses about the origin of mitochondria: *endosymbiotic* and *autogenous*, but the most accredited theory at present is endosymbiosis. The endosymbiotic hypothesis suggests mitochondria were originally prokaryotic cells, capable of implementing oxidative mechanisms. These prokaryotic cells may have been engulfed by a eukaryote and became endosymbionts living inside the eukaryote.

Key Points

- Mitochondria contain their own ribosomes and DNA; combined with their double membrane, these features suggest that they might have once been free-living prokaryotes that were engulfed by a larger cell.
- Mitochondria have an important role in cellular respiration through the production of ATP, using chemical energy found in glucose and other nutrients.
- Mitochondria are also responsible for generating clusters of iron and sulfur, which are important cofactors of many enzymes.

Key Terms

- **alpha-proteobacteria**: A taxonomic class within the phylum Proteobacteria — the phototropic proteobacteria.
- **adenosine triphosphate**: a multifunctional nucleoside triphosphate used in cells as a coenzyme, often called the “molecular unit of energy currency” in intracellular energy transfer
- **cofactor**: an inorganic molecule that is necessary for an enzyme to function

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3.7.3: Comparing Plant and Animal Cells

Although they are both eukaryotic cells, there are unique structural differences between animal and plant cells.

Learning Objectives

- Differentiate between the structures found in animal and plant cells

Key Points

- Centrosomes and lysosomes are found in animal cells, but do not exist within plant cells.
- The lysosomes are the animal cell's "garbage disposal", while in plant cells the same function takes place in vacuoles.
- Plant cells have a cell wall, chloroplasts and other specialized plastids, and a large central vacuole, which are not found within animal cells.
- The cell wall is a rigid covering that protects the cell, provides structural support, and gives shape to the cell.
- The chloroplasts, found in plant cells, contain a green pigment called chlorophyll, which captures the light energy that drives the reactions of plant photosynthesis.
- The central vacuole plays a key role in regulating a plant cell's concentration of water in changing environmental conditions.

Key Terms

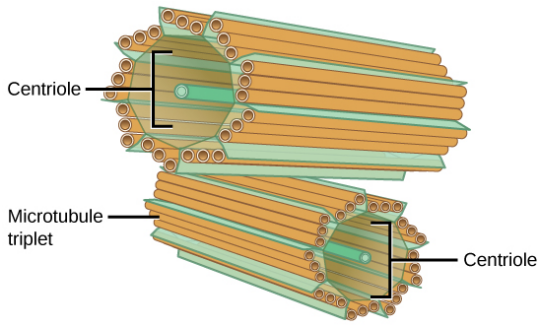
- **protist**: Any of the eukaryotic unicellular organisms including protozoans, slime molds and some algae; historically grouped into the kingdom Protoctista.
- **autotroph**: Any organism that can synthesize its food from inorganic substances, using heat or light as a source of energy
- **heterotroph**: an organism that requires an external supply of energy in the form of food, as it cannot synthesize its own

Animal Cells versus Plant Cells

Each eukaryotic cell has a plasma membrane, cytoplasm, a nucleus, ribosomes, mitochondria, peroxisomes, and in some, vacuoles; however, there are some striking differences between animal and plant cells. While both animal and plant cells have microtubule organizing centers (MTOCs), animal cells also have centrioles associated with the MTOC: a complex called the centrosome. Animal cells each have a centrosome and lysosomes, whereas plant cells do not. Plant cells have a cell wall, chloroplasts and other specialized plastids, and a large central vacuole, whereas animal cells do not.

The Centrosome

The centrosome is a microtubule-organizing center found near the nuclei of animal cells. It contains a pair of centrioles, two structures that lie perpendicular to each other. Each centriole is a cylinder of nine triplets of microtubules. The centrosome (the organelle where all microtubules originate) replicates itself before a cell divides, and the centrioles appear to have some role in pulling the duplicated chromosomes to opposite ends of the dividing cell. However, the exact function of the centrioles in cell division isn't clear, because cells that have had the centrosome removed can still divide; and plant cells, which lack centrosomes, are capable of cell division.



The Centrosome Structure: The centrosome consists of two centrioles that lie at right angles to each other. Each centriole is a cylinder made up of nine triplets of microtubules. Nontubulin proteins (indicated by the green lines) hold the microtubule triplets

together.

Lysosomes

Animal cells have another set of organelles not found in plant cells: lysosomes. The lysosomes are the cell's "garbage disposal." In plant cells, the digestive processes take place in vacuoles. Enzymes within the lysosomes aid the breakdown of proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles. These enzymes are active at a much lower pH than that of the cytoplasm. Therefore, the pH within lysosomes is more acidic than the pH of the cytoplasm. Many reactions that take place in the cytoplasm could not occur at a low pH, so the advantage of compartmentalizing the eukaryotic cell into organelles is apparent.

The Cell Wall

The cell wall is a rigid covering that protects the cell, provides structural support, and gives shape to the cell. Fungal and protistan cells also have cell walls. While the chief component of prokaryotic cell walls is peptidoglycan, the major organic molecule in the plant cell wall is cellulose, a polysaccharide comprised of glucose units. When you bite into a raw vegetable, like celery, it crunches. That's because you are tearing the rigid cell walls of the celery cells with your teeth.

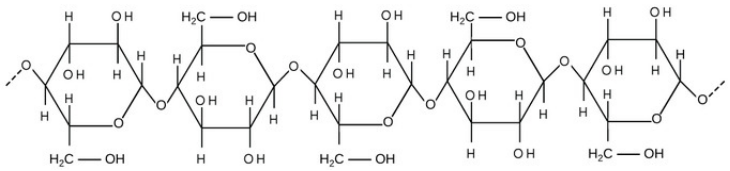


Figure: **Cellulose**: Cellulose is a long chain of β-glucose molecules connected by a 1-4 linkage. The dashed lines at each end of the figure indicate a series of many more glucose units. The size of the page makes it impossible to portray an entire cellulose molecule.

Chloroplasts

Like mitochondria, chloroplasts have their own DNA and ribosomes, but chloroplasts have an entirely different function. Chloroplasts are plant cell organelles that carry out photosynthesis. Photosynthesis is the series of reactions that use carbon dioxide, water, and light energy to make glucose and oxygen. This is a major difference between plants and animals; plants (autotrophs) are able to make their own food, like sugars, while animals (heterotrophs) must ingest their food.

Like mitochondria, chloroplasts have outer and inner membranes, but within the space enclosed by a chloroplast's inner membrane is a set of interconnected and stacked fluid-filled membrane sacs called thylakoids. Each stack of thylakoids is called a granum (plural = grana). The fluid enclosed by the inner membrane that surrounds the grana is called the stroma.

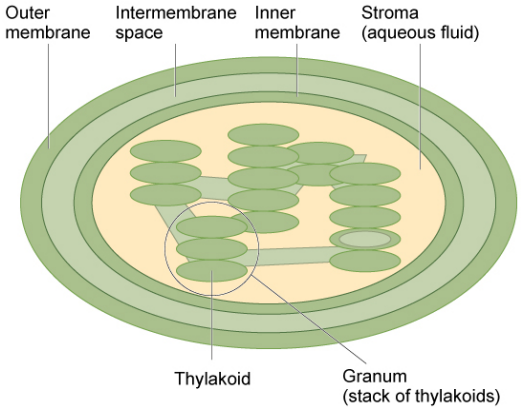


Figure: **The Chloroplast Structure**: The chloroplast has an outer membrane, an inner membrane, and membrane structures called thylakoids that are stacked into grana. The space inside the thylakoid membranes is called the thylakoid space. The light harvesting reactions take place in the thylakoid membranes, and the synthesis of sugar takes place in the fluid inside the inner membrane, which is called the stroma.

The chloroplasts contain a green pigment called chlorophyll, which captures the light energy that drives the reactions of photosynthesis. Like plant cells, photosynthetic protists also have chloroplasts. Some bacteria perform photosynthesis, but their chlorophyll is not relegated to an organelle.

The Central Vacuole

The central vacuole plays a key role in regulating the cell's concentration of water in changing environmental conditions. When you forget to water a plant for a few days, it wilts. That's because as the water concentration in the soil becomes lower than the water concentration in the plant, water moves out of the central vacuoles and cytoplasm. As the central vacuole shrinks, it leaves the cell wall unsupported. This loss of support to the cell walls of plant cells results in the wilted appearance of the plant. The central vacuole also supports the expansion of the cell. When the central vacuole holds more water, the cell gets larger without having to invest a lot of energy in synthesizing new cytoplasm.

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3.7.4: The Endoplasmic Reticulum

The endoplasmic reticulum is an organelle that is responsible for the synthesis of lipids and the modification of proteins.

Learning Objectives

- Describe the structure of the endoplasmic reticulum and its role in synthesis and metabolism

Key Points

- If the endoplasmic reticulum (ER) has ribosomes attached to it, it is called rough ER; if it does not, then it is called smooth ER.
- The proteins made by the rough endoplasmic reticulum are for use outside of the cell.
- Functions of the smooth endoplasmic reticulum include synthesis of carbohydrates, lipids, and steroid hormones; detoxification of medications and poisons; and storage of calcium ions.

Key Terms

- **lumen**: The cavity or channel within a tube or tubular organ.
- **reticulum**: A network

The Endoplasmic Reticulum

The endoplasmic reticulum (ER) is a series of interconnected membranous sacs and tubules that collectively modifies proteins and synthesizes lipids. However, these two functions are performed in separate areas of the ER: the rough ER and the smooth ER. The hollow portion of the ER tubules is called the lumen or cisternal space. The membrane of the ER, which is a phospholipid bilayer embedded with proteins, is continuous with the nuclear envelope.

Rough ER

The rough endoplasmic reticulum (RER) is so named because the ribosomes attached to its cytoplasmic surface give it a studded appearance when viewed through an electron microscope. Ribosomes transfer their newly synthesized proteins into the lumen of the RER where they undergo structural modifications, such as folding or the acquisition of side chains. These modified proteins will be incorporated into cellular membranes—the membrane of the ER or those of other organelles —or secreted from the cell (such as protein hormones, enzymes). The RER also makes phospholipids for cellular membranes. If the phospholipids or modified proteins are not destined to stay in the RER, they will reach their destinations via transport vesicles that bud from the RER's membrane. Since the RER is engaged in modifying proteins (such as enzymes, for example) that will be secreted from the cell, the RER is abundant in cells that secrete proteins. This is the case with cells of the liver, for example.

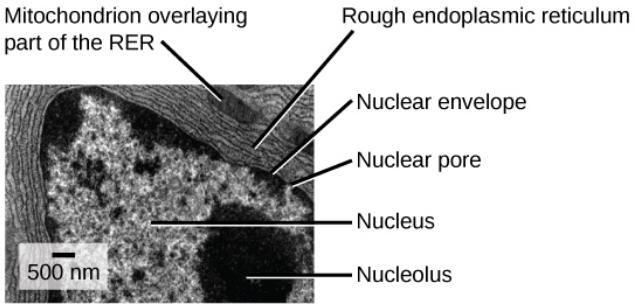


Figure: **Rough Endoplasmic Reticulum**: This transmission electron micrograph shows the rough endoplasmic reticulum and other organelles in a pancreatic cell.

Smooth ER

The smooth endoplasmic reticulum (SER) is continuous with the RER but has few or no ribosomes on its cytoplasmic surface. Functions of the SER include synthesis of carbohydrates, lipids, and steroid hormones; detoxification of medications and poisons; and storage of calcium ions. In muscle cells, a specialized SER called the sarcoplasmic reticulum is responsible for storage of the calcium ions that are needed to trigger the coordinated contractions of the muscle cells.

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3.7.5: The Golgi Apparatus

LEARNING OBJECTIVES

- Describe the structure of the Golgi apparatus and its role in protein modification and secretion

We have already mentioned that vesicles can bud from the ER and transport their contents elsewhere, but where do the vesicles go? Before reaching their final destination, the lipids or proteins within the transport vesicles still need to be sorted, packaged, and tagged so that they wind up in the right place. Sorting, tagging, packaging, and distribution of lipids and proteins takes place in the Golgi apparatus (also called the Golgi body), a series of flattened membranes.

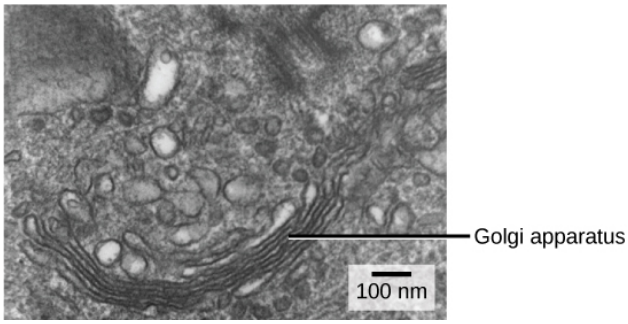


Figure: ***The Golgi apparatus sorts and packages cellular products****: The Golgi apparatus in this white blood cell is visible as a stack of semicircular, flattened rings in the lower portion of the image. Several vesicles can be seen near the Golgi apparatus.*

The receiving side of the Golgi apparatus is called the cis face. The opposite side is called the trans face. The transport vesicles that formed from the ER travel to the cis face, fuse with it, and empty their contents into the lumen of the Golgi apparatus. As the proteins and lipids travel through the Golgi, they undergo further modifications that allow them to be sorted. The most frequent modification is the addition of short chains of sugar molecules. These newly-modified proteins and lipids are then tagged with phosphate groups or other small molecules so that they can be routed to their proper destinations.

Finally, the modified and tagged proteins are packaged into secretory vesicles that bud from the trans face of the Golgi. While some of these vesicles deposit their contents into other parts of the cell where they will be used, other secretory vesicles fuse with the plasma membrane and release their contents outside the cell.

In another example of form following function, cells that engage in a great deal of secretory activity (such as cells of the salivary glands that secrete digestive enzymes or cells of the immune system that secrete antibodies) have an abundance of Golgi. In plant cells, the Golgi apparatus has the additional role of synthesizing polysaccharides, some of which are incorporated into the cell wall and some of which are used in other parts of the cell.

Key Points

- The Golgi apparatus is a series of flattened sacs that sort and package cellular materials.
- The Golgi apparatus has a cis face on the ER side and a trans face opposite of the ER.
- The *trans* face secretes the materials into vesicles, which then fuse with the cell membrane for release from the cell.

Key Terms

- **vesicle**: A membrane-bound compartment found in a cell.

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SECTION OVERVIEW

3.8: Other Eukaryotic Components

Topic hierarchy

3.8.1: Peroxisomes

3.8.2: Lysosomes

3.8.3: Intermediate Filaments and Microtubules

3.8.4: Extracellular Matrix of Animal Cells

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3.8.1: Peroxisomes

LEARNING OBJECTIVES

- Name the various functions that peroxisomes perform inside the cell

A type of organelle found in both animal cells and plant cells, a peroxisome is a membrane-bound cellular organelle that contains mostly enzymes. Peroxisomes perform important functions, including lipid metabolism and chemical detoxification. They also carry out oxidation reactions that break down fatty acids and amino acids.

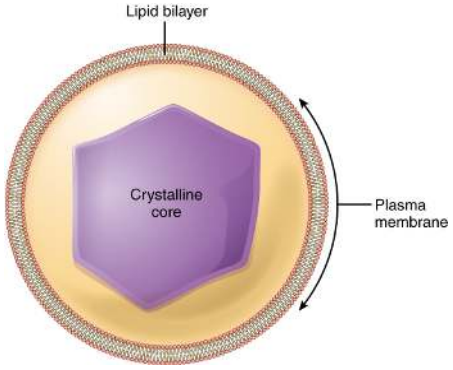


Figure: ***Peroxisomes****: Peroxisomes are membrane-bound organelles that contain an abundance of enzymes for detoxifying harmful substances and lipid metabolism.*

In contrast to the digestive enzymes found in lysosomes, the enzymes within peroxisomes serve to transfer hydrogen atoms from various molecules to oxygen, producing hydrogen peroxide (H_2O_2). In this way, peroxisomes neutralize poisons, such as alcohol, that enter the body. In order to appreciate the importance of peroxisomes, it is necessary to understand the concept of reactive oxygen species.

Reactive oxygen species (ROS), such as peroxides and free radicals, are the highly-reactive products of many normal cellular processes, including the mitochondrial reactions that produce ATP and oxygen metabolism. Examples of ROS include the hydroxyl radical OH, H_2O_2, and superoxide (O_{-2}). Some ROS are important for certain cellular functions, such as cell signaling processes and immune responses against foreign substances. Many ROS, however, are harmful to the body. Free radicals are reactive because they contain free unpaired electrons; they can easily oxidize other molecules throughout the cell, causing cellular damage and even cell death. Free radicals are thought to play a role in many destructive processes in the body, from cancer to coronary artery disease.

Peroxisomes oversee reactions that neutralize free radicals. They produce large amounts of the toxic H_2O_2 in the process, but contain enzymes that convert H_2O_2into water and oxygen. These by-products are then safely released into the cytoplasm. Like miniature sewage treatment plants, peroxisomes neutralize harmful toxins so that they do not cause damage in the cells. The liver is the organ primarily responsible for detoxifying the blood before it travels throughout the body; liver cells contain an exceptionally high number of peroxisomes.

Key Points

- Lipid metabolism and chemical detoxification are important functions of peroxisomes.
- Peroxisomes are responsible for oxidation reactions that break down fatty acids and amino acids.
- Peroxisomes oversee reactions that neutralize free radicals, which cause cellular damage and cell death.
- Peroxisomes chemically neutralize poisons through a process that produces large amounts of toxic H_2O_2, which is then converted into water and oxygen.
- The liver is the organ primarily responsible for detoxifying the blood before it travels throughout the body; as a result, liver cells contain large amounts of peroxisomes.

Key Terms

- **enzyme**: a globular protein that catalyses a biological chemical reaction
- **free radical**: Any molecule, ion or atom that has one or more unpaired electrons; they are generally highly reactive and often only occur as transient species.

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3.8.2: Lysosomes

LEARNING OBJECTIVES

- Describe how lysosomes function as the cell's waste disposal system

Lysosomes are organelles that digest macromolecules, repair cell membranes, and respond to foreign substances entering the cell.

Lysosomes

A lysosome has three main functions: the breakdown/digestion of macromolecules (carbohydrates, lipids, proteins, and nucleic acids), cell membrane repairs, and responses against foreign substances such as bacteria, viruses and other antigens. When food is eaten or absorbed by the cell, the lysosome releases its enzymes to break down complex molecules including sugars and proteins into usable energy needed by the cell to survive. If no food is provided, the lysosome's enzymes digest other organelles within the cell in order to obtain the necessary nutrients.

In addition to their role as the digestive component and organelle-recycling facility of animal cells, lysosomes are considered to be parts of the endomembrane system. Lysosomes also use their hydrolytic enzymes to destroy pathogens (disease-causing organisms) that might enter the cell. A good example of this occurs in a group of white blood cells called macrophages, which are part of your body's immune system. In a process known as phagocytosis or endocytosis, a section of the plasma membrane of the macrophage invaginates (folds in) and engulfs a pathogen. The invaginated section, with the pathogen inside, then pinches itself off from the plasma membrane and becomes a vesicle. The vesicle fuses with a lysosome. The lysosome's hydrolytic enzymes then destroy the pathogen.

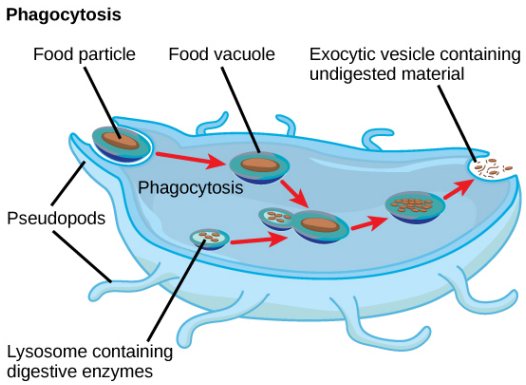


Figure: ***Lysosomes digest foreign substances that might harm the cell****: A macrophage has engulfed (phagocytized) a potentially pathogenic bacterium and then fuses with a lysosomes within the cell to destroy the pathogen. Other organelles are present in the cell but for simplicity are not shown.*

A lysosome is composed of lipids, which make up the membrane, and proteins, which make up the enzymes within the membrane. Usually, lysosomes are between 0.1 to 1.2µm, but the size varies based on the cell type. The general structure of a lysosome consists of a collection of enzymes surrounded by a single-layer membrane. The membrane is a crucial aspect of its structure because without it the enzymes within the lysosome that are used to breakdown foreign substances would leak out and digest the entire cell, causing it to die.

Lysosomes are found in nearly every animal-like eukaryotic cell. They are so common in animal cells because, when animal cells take in or absorb food, they need the enzymes found in lysosomes in order to digest and use the food for energy. On the other hand, lysosomes are not commonly-found in plant cells. Lysosomes are not needed in plant cells because they have cell walls that are tough enough to keep the large/foreign substances that lysosomes would usually digest out of the cell.

Key Points

- Lysosomes breakdown/digest macromolecules (carbohydrates, lipids, proteins, and nucleic acids), repair cell membranes, and respond against foreign substances such as bacteria, viruses and other antigens.
- Lysosomes contain enzymes that break down the macromolecules and foreign invaders.

- Lysosomes are composed of lipids and proteins, with a single membrane covering the internal enzymes to prevent the lysosome from digesting the cell itself.
- Lysosomes are found in all animal cells, but are rarely found within plant cells due to the tough cell wall surrounding a plant cell that keeps out foreign substances.

Key Terms

- **enzyme**: a globular protein that catalyses a biological chemical reaction
- **lysosome**: An organelle found in all types of animal cells which contains a large range of digestive enzymes capable of splitting most biological macromolecules.

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3.8.3: Intermediate Filaments and Microtubules

Microtubules are part of the cell's cytoskeleton, helping the cell resist compression, move vesicles, and separate chromosomes at mitosis.

Learning Objectives

- Describe the roles of microtubules as part of the cell's cytoskeleton

Key Points

- Microtubules help the cell resist compression, provide a track along which vesicles can move throughout the cell, and are the components of cilia and flagella.
- Cilia and flagella are hair-like structures that assist with locomotion in some cells, as well as line various structures to trap particles.
- The structures of cilia and flagella are a "9+2 array," meaning that a ring of nine microtubules is surrounded by two more microtubules.
- Microtubules attach to replicated chromosomes during cell division and pull them apart to opposite ends of the pole, allowing the cell to divide with a complete set of chromosomes in each daughter cell.

Key Terms

- **microtubule**: Small tubes made of protein and found in cells; part of the cytoskeleton
- **flagellum**: a flagellum is a lash-like appendage that protrudes from the cell body of certain prokaryotic and eukaryotic cells
- **cytoskeleton**: A cellular structure like a skeleton, contained within the cytoplasm.

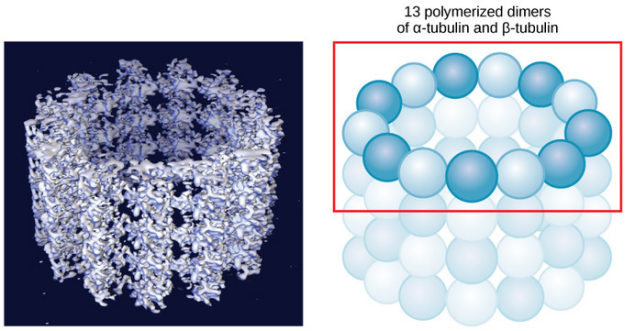


Figure: **Micrtubule Structure**: Microtubules are hollow, with walls consisting of 13 polymerized dimers of α-tubulin and β-tubulin (right image). The left image shows the molecular structure of the tube.

Microtubules

As their name implies, microtubules are small hollow tubes. Microtubules, along with microfilaments and intermediate filaments, come under the class of organelles known as the cytoskeleton. The cytoskeleton is the framework of the cell which forms the structural supporting component. Microtubules are the largest element of the cytoskeleton. The walls of the microtubule are made of polymerized dimers of α-tubulin and β-tubulin, two globular proteins. With a diameter of about 25 nm, microtubules are the widest components of the cytoskeleton. They help the cell resist compression, provide a track along which vesicles move through the cell, and pull replicated chromosomes to opposite ends of a dividing cell. Like microfilaments, microtubules can dissolve and reform quickly.

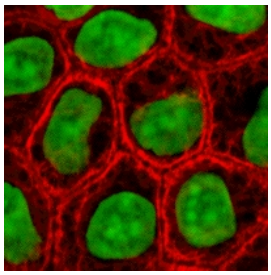


Figure: **Stained Keratin Intermediate filaments**: Keratin cytoskeletal intermediate filaments are concentrated around the edge of the cells and merge into the surface membrane. This network of intermediate filaments from cell to cell holds together tissues like skin.

Microtubules are also the structural elements of flagella, cilia, and centrioles (the latter are the two perpendicular bodies of the centrosome). In animal cells, the centrosome is the microtubule-organizing center. In eukaryotic cells, flagella and cilia are quite different structurally from their counterparts in prokaryotes.

Intermediate Filaments

Intermediate filaments (IFs) are cytoskeletal components found in animal cells. They are composed of a family of related proteins sharing common structural and sequence features. Intermediate filaments have an average diameter of 10 nanometers, which is between that of 7 nm actin (microfilaments), and that of 25 nm microtubules, although they were initially designated 'intermediate' because their average diameter is between those of narrower microfilaments (actin) and wider myosin filaments found in muscle cells. Intermediate filaments contribute to cellular structural elements and are often crucial in holding together tissues like skin.

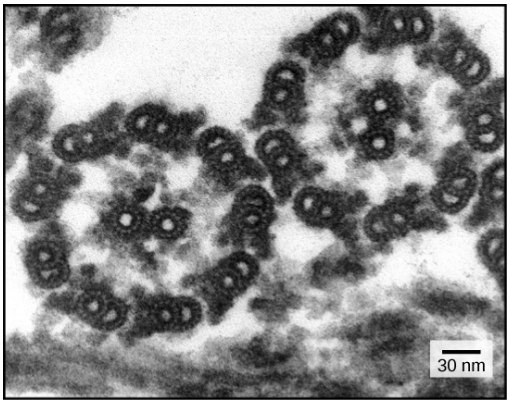


Figure: **Microtubules are the structural component of flagella**: This transmission electron micrograph of two flagella shows the 9 + 2 array of microtubules: nine microtubule doublets surround a single microtubule doublet.

Flagella and Cilia

Flagella (singular = flagellum) are long, hair-like structures that extend from the plasma membrane and are used to move an entire cell (for example, sperm, *Euglena*). When present, the cell has just one flagellum or a few flagella. When cilia (singular = cilium) are present, however, many of them extend along the entire surface of the plasma membrane. They are short, hair-like structures that are used to move entire cells (such as paramecia) or substances along the outer surface of the cell (for example, the cilia of cells lining the Fallopian tubes that move the ovum toward the uterus, or cilia lining the cells of the respiratory tract that trap particulate matter and move it toward your nostrils).

Despite their differences in length and number, flagella and cilia share a common structural arrangement of microtubules called a "9 + 2 array." This is an appropriate name because a single flagellum or cilium is made of a ring of nine microtubule doublets surrounding a single microtubule doublet in the center.

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3.8.4: Extracellular Matrix of Animal Cells

The extracellular matrix of animal cells holds cells together to form a tissue and allow tissues to communicate with each other.

LEARNING OBJECTIVES

Explain the role of the extracellular matrix in animal cells

KEY TAKEAWAYS

Key Points

- The extracellular matrix of animal cells is made up of proteins and carbohydrates.
- Cell communication within tissue and tissue formation are main functions of the extracellular matrix of animal cells.
- Tissue communication is kick-started when a molecule within the matrix binds a receptor; the end results are conformational changes that induce chemical signals that ultimately change activities within the cell.

Key Terms

- **collagen**: Any of more than 28 types of glycoprotein that forms elongated fibers, usually found in the extracellular matrix of connective tissue.
- **proteoglycan**: Any of many glycoproteins that have heteropolysaccharide side chains
- **extracellular matrix**: All the connective tissues and fibres that are not part of a cell, but rather provide support.

Extracellular Matrix of Animal Cells

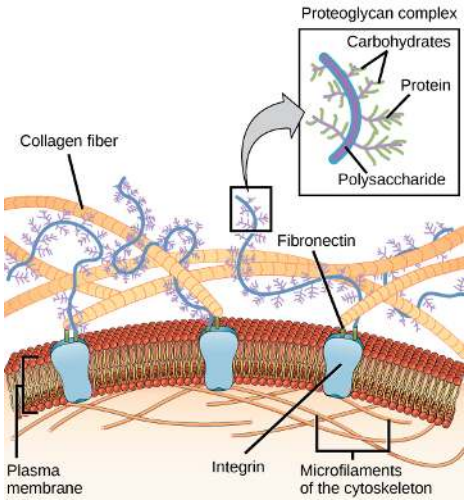


Figure: **The Extracellular Matrix**: The extracellular matrix consists of a network of proteins and carbohydrates.

Most animal cells release materials into the extracellular space. The primary components of these materials are proteins. Collagen is the most abundant of the proteins. Its fibers are interwoven with carbohydrate-containing protein molecules called proteoglycans. Collectively, these materials are called the extracellular matrix. Not only does the extracellular matrix hold the cells together to form a tissue, but it also allows the cells within the tissue to communicate with each other.

How does this cell communication occur? Cells have protein receptors on the extracellular surfaces of their plasma membranes. When a molecule within the matrix binds to the receptor, it changes the molecular structure of the receptor. The receptor, in turn, changes the conformation of the microfilaments positioned just inside the plasma membrane. These conformational changes induce chemical signals inside the cell that reach the nucleus and turn “on” or “off” the transcription of specific sections of DNA. This affects the production of associated proteins, thus changing the activities within the cell.

An example of the role of the extracellular matrix in cell communication can be seen in blood clotting. When the cells lining a blood vessel are damaged, they display a protein receptor called tissue factor. When a tissue factor binds with another factor in the extracellular matrix, it causes platelets to adhere to the wall of the damaged blood vessel and stimulates the adjacent smooth muscle

cells in the blood vessel to contract (thus constricting the blood vessel). Subsequently, a series of steps are initiated which then prompt the platelets to produce clotting factors.

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SECTION OVERVIEW

3.9: Protein Export and Secretion

Topic hierarchy

3.9.1: Endocytosis

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3.9.1: Endocytosis

LEARNING OBJECTIVES

- Describe endocytosis, including phagocytosis, pinocytosis, and receptor-mediated endocytosis.

Endocytosis is a type of active transport that moves particles, such as large molecules, parts of cells, and even whole cells, into a cell. There are different variations of endocytosis, but all share a common characteristic: the plasma membrane of the cell invaginates, forming a pocket around the target particle. The pocket pinches off, resulting in the particle being contained in a newly-created intracellular vesicle formed from the plasma membrane.

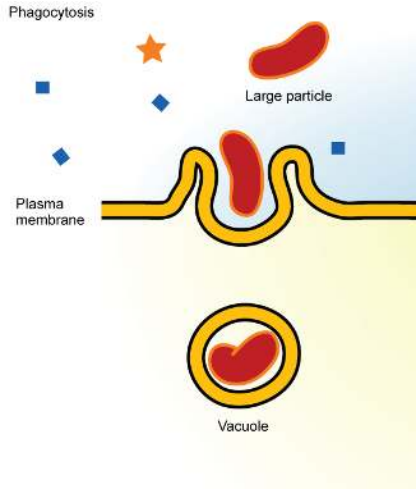


Figure: **Phagocytosis**: In phagocytosis, the cell membrane surrounds the particle and engulfs it.

Phagocytosis

Phagocytosis (the condition of “cell eating”) is the process by which large particles, such as cells or relatively large particles, are taken in by a cell. For example, when microorganisms invade the human body, a type of white blood cell called a neutrophil will remove the invaders through this process, surrounding and engulfing the microorganism, which is then destroyed by the neutrophil.

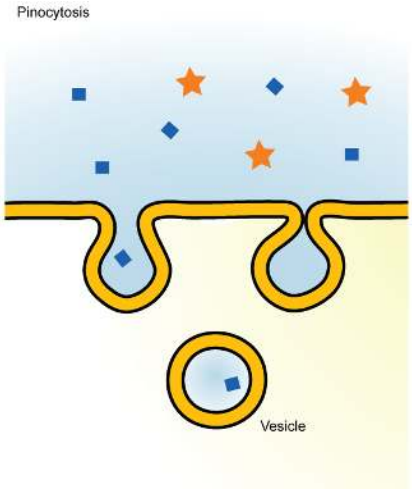


Figure: **Pinocytosis**: In pinocytosis, the cell membrane invaginates, surrounds a small volume of fluid, and pinches off.

In preparation for phagocytosis, a portion of the inward-facing surface of the plasma membrane becomes coated with a protein called clathrin, which stabilizes this section of the membrane. The coated portion of the membrane then extends from the body of the cell and surrounds the particle, eventually enclosing it. Once the vesicle containing the particle is enclosed within the cell, the clathrin disengages from the membrane and the vesicle merges with a lysosome for the breakdown of the material in the newly-

formed compartment (endosome). When accessible nutrients from the degradation of the vesicular contents have been extracted, the newly-formed endosome merges with the plasma membrane and releases its contents into the extracellular fluid. The endosomal membrane again becomes part of the plasma membrane.

Pinocytosis

A variation of endocytosis is called pinocytosis. This literally means “cell drinking” and was named at a time when the assumption was that the cell was purposefully taking in extracellular fluid. In reality, this is a process that takes in molecules, including water, which the cell needs from the extracellular fluid. Pinocytosis results in a much smaller vesicle than does phagocytosis, and the vesicle does not need to merge with a lysosome.

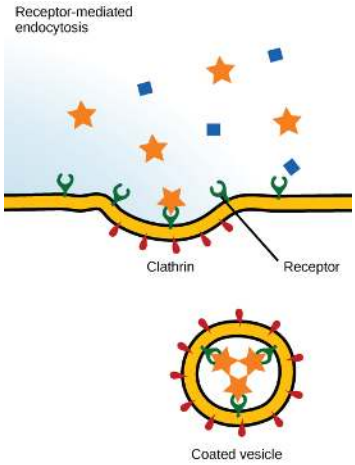


Figure: **Receptor-Mediated Endocytosis**: In receptor-mediated endocytosis, uptake of substances by the cell is targeted to a single type of substance that binds to the receptor on the external surface of the cell membrane.

Potocytosis, a variant of pinocytosis, is a process that uses a coating protein, called caveolin, on the cytoplasmic side of the plasma membrane, which performs a similar function to clathrin. The cavities in the plasma membrane that form the vacuoles have membrane receptors and lipid rafts in addition to caveolin. The vacuoles or vesicles formed in caveolae (singular caveola) are smaller than those in pinocytosis. Potocytosis is used to bring small molecules into the cell and to transport these molecules through the cell for their release on the other side of the cell, a process called transcytosis.

Receptor-mediated Endocytosis

A targeted variation of endocytosis, known as receptor-mediated endocytosis, employs receptor proteins in the plasma membrane that have a specific binding affinity for certain substances. In receptor-mediated endocytosis, as in phagocytosis, clathrin is attached to the cytoplasmic side of the plasma membrane. If uptake of a compound is dependent on receptor-mediated endocytosis and the process is ineffective, the material will not be removed from the tissue fluids or blood. Instead, it will stay in those fluids and increase in concentration. Some human diseases are caused by the failure of receptor-mediated endocytosis. For example, the form of cholesterol termed low-density lipoprotein or LDL (also referred to as “bad” cholesterol) is removed from the blood by receptor-mediated endocytosis. In the human genetic disease familial hypercholesterolemia, the LDL receptors are defective or missing entirely. People with this condition have life-threatening levels of cholesterol in their blood, because their cells cannot clear LDL particles from their blood.

Although receptor-mediated endocytosis is designed to bring specific substances that are normally found in the extracellular fluid into the cell, other substances may gain entry into the cell at the same site. Flu viruses, diphtheria, and cholera toxin all have sites that cross-react with normal receptor-binding sites and gain entry into cells.

Key Points

- Endocytosis consists of phagocytosis, pinocytosis, and receptor -mediated endocytosis.
- Endocytosis takes particles into the cell that are too large to passively cross the cell membrane.
- Phagocytosis is the taking in of large food particles, while pinocytosis takes in liquid particles.
- Receptor-mediated endocytosis uses special receptor proteins to help carry large particles across the cell membrane.

Key Terms

- **endosome**: An endocytic vacuole through which molecules internalized during endocytosis pass en route to lysosomes
- **neutrophil**: A cell, especially a white blood cell that consumes foreign invaders in the blood.

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Topic hierarchy

3.10.1: Microscopy

3.10.2: Crystallographic Analysis

3.10.3: Genetic Analysis

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3.10.1: Microscopy

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SECTION OVERVIEW

4.1: Overview of Viruses

Topic hierarchy

4.1.1: Discovery and Detection of Viruses

4.1.2: Nature of the Virion

4.1.3: Viral Genomes

4.1.4: Host Range

4.1.5: Viral Size

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4.1.1: Discovery and Detection of Viruses

Viruses are infectious particles about 100 times smaller than bacteria and can only be observed by electron microscopy.

Learning Objectives

- Describe how viruses were first discovered and how they are detected

Key Points

- Virions, single virus particles, are 20–250 nanometers in diameter.
- In the past, viruses were classified by the type of nucleic acid they contained, DNA or RNA, and whether they had single- or double-stranded nucleic acid.
- Molecular analysis of viral replicative cycles is now more routinely used to classify viruses.

Key Terms

- **virus**: a submicroscopic infectious organism, now understood to be a non-cellular structure consisting of a core of DNA or RNA surrounded by a protein coat
- **virion**: a single individual particle of a virus (the viral equivalent of a cell)

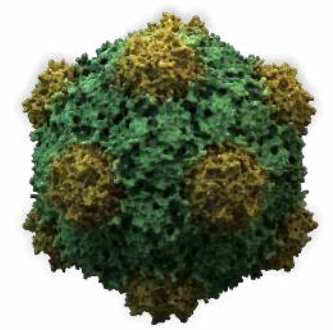


Figure: **The structure of the icosahedral cowpea mosaic virus**: In the past, viruses were classified by the type of nucleic acid they contained, DNA or RNA, and whether they had single- or double-stranded nucleic acid.

Discovery and Detection

Viruses were first discovered after the development of a porcelain filter, called the Chamberland-Pasteur filter, which could remove all bacteria visible in the microscope from any liquid sample. In 1886, Adolph Meyer demonstrated that a disease of tobacco plants, tobacco mosaic disease, could be transferred from a diseased plant to a healthy one via liquid plant extracts. In 1892, Dmitri Ivanowski showed that this disease could be transmitted in this way even after the Chamberland-Pasteur filter had removed all viable bacteria from the extract. Still, it was many years before it was proven that these “filterable” infectious agents were not simply very small bacteria, but were a new type of tiny, disease-causing particle.

Virions, single virus particles, are very small, about 20–250 nanometers in diameter. These individual virus particles are the infectious form of a virus outside the host cell. Unlike bacteria (which are about 100 times larger), we cannot see viruses with a light microscope, with the exception of some large virions of the poxvirus family. It was not until the development of the electron microscope in the late 1930s that scientists got their first good view of the structure of the tobacco mosaic virus (TMV) and other viruses. The surface structure of virions can be observed by both scanning and transmission electron microscopy, whereas the internal structures of the virus can only be observed in images from a transmission electron microscope. The use of these technologies has enabled the discovery of many viruses of all types of living organisms. They were initially grouped by shared morphology. Later, groups of viruses were classified by the type of nucleic acid they contained, DNA or RNA, and whether their nucleic acid was single- or double-stranded. More recently, molecular analysis of viral replicative cycles has further refined their classification.

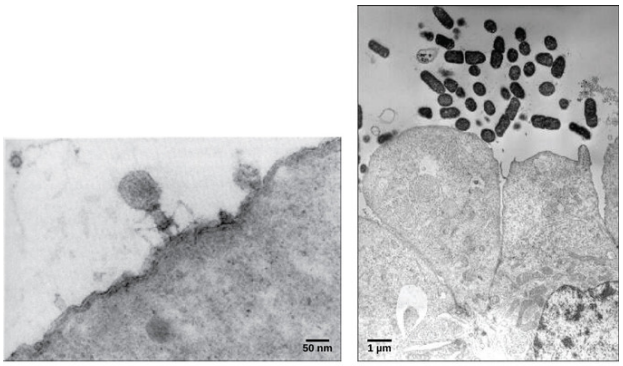


Figure: **Examples of transmission electron micrographs of viruses**: In these transmission electron micrographs, (a) a virus is dwarfed by the bacterial cell it infects, while (b) these E. coli cells are dwarfed by cultured colon cells.

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4.1.2: Nature of the Virion

A virion is a complete viral particle consisting of RNA or DNA surrounded by a protein shell, constituting the infective form of a virus.

Learning Objectives

- Illustrate the attributes of a virion

Key Points

- The virion shell or capsid protects the interior core that includes the genome and other proteins. After the virion binds to the surface of a specific host cell, its DNA or RNA is injected into the host cell and viral replication occurs, resulting in the spread of the infection to other host cells.
- A virion is the infectious particle that is designed for transmitting the nucleic acid genome among hosts or host cells.
- Virions are produced in the cytoplasm of complex viral 'factories,' the virus.

Key Terms

- **capsid**: The outer protein shell of a virus.

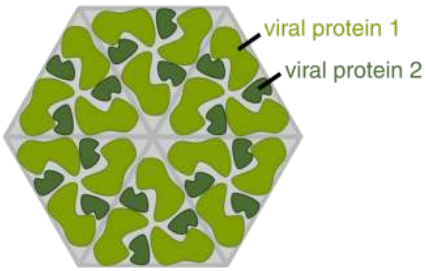


Figure: **Virion capsid**: Structure of a capsid and the organization of molecules that constitute it.

A virion is an entire virus particle consisting of an outer protein shell called a *capsid* and an inner core of nucleic acid (either ribonucleic or deoxyribonucleic acid—RNA or DNA). The core confers infectivity, and the capsid provides specificity to the virus. In some virions the capsid is further enveloped by a fatty membrane, in which case the virion can be inactivated by exposure to fat solvents such as ether and chloroform. Many virions are spheroidal—actually icosahedral (the capsid having 20 triangular faces)—with regularly arranged units called capsomeres, two to five or more along each side. The nucleic acid is densely coiled within. Other virions have a capsid consisting of an irregular number of surface spikes, with the nucleic acid loosely coiled within. Virions of most plant viruses are rod-shaped; the capsid is a naked cylinder (lacking a fatty membrane) within which lies a straight or helical rod of nucleic acid.

Virion capsids are formed from identical protein subunits called capsomeres. Viruses can have a lipid "envelope" derived from the host cell membrane. The capsid is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction. Virally coded protein subunits will self-assemble to form a capsid, in general requiring the presence of the virus genome. Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as nucleoproteins, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid. The capsid and entire virus structure can be mechanically (physically) probed through atomic force microscopy.

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4.1.3: Viral Genomes

LEARNING OBJECTIVES

- Generalize the features of viral genomes

Viral diseases have an enormous impact on human health worldwide. Genomic technologies are providing infectious disease researchers an unprecedented capability to study at a genetic level the viruses that cause disease and their interactions with infected hosts. An enormous variety of genomic structures can be seen among viral species; as a group, they contain more structural genomic diversity than plants, animals, archaea, or bacteria. There are millions of different types of viruses, although only about 5,000 of them have been described in detail.

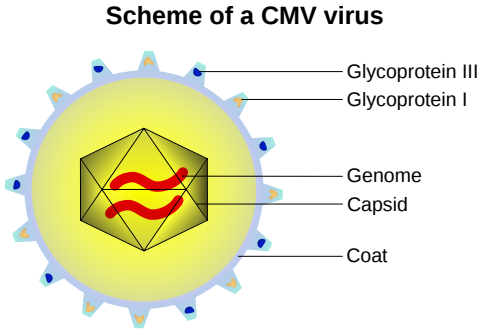


Figure: ***Diagram of a virus****: The location of the genome inside the virus.*

A virus has either DNA or RNA genes and is called a DNA virus or a RNA virus, respectively. The vast majority of viruses have RNA genomes. Plant viruses tend to have single-stranded RNA genomes and bacteriophages tend to have double-stranded DNA genomes. Viral genomes are circular, as in the polyomaviruses, or linear, as in the adenoviruses. The type of nucleic acid is irrelevant to the shape of the genome. Among RNA viruses and certain DNA viruses, the genome is often divided up into separate parts, in which case it is called segmented. For RNA viruses, each segment often codes for only one protein, and they are usually found together in one capsid. However, all segments are not required to be in the same virion for the virus to be infectious, as demonstrated by the brome mosaic virus and several other plant viruses.

A viral genome, irrespective of nucleic acid type, is almost always either single-stranded or double-stranded. Single-stranded genomes consist of an unpaired nucleic acid, analogous to one-half of a ladder split down the middle. Double-stranded genomes consist of two complementary paired nucleic acids, analogous to a ladder. The virus particles of some virus families, such as those belonging to the Hepadnaviridae, contain a genome that is partially double-stranded and partially single-stranded.

Key Points

- In modern molecular biology and genetics, the genome is the entirety of an organism ‘s hereditary information. It is encoded either in DNA or, for many types of virus, in RNA.
- A virus has either DNA or RNA genes and is called a DNA virus or a RNA virus.
- The genome includes both the genes and the non-coding sequences of the DNA/RNA.

Key Terms

- **genome**: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of basepairs.

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4.1.4: Host Range

A virus' host range is the range of cell types and host species a virus is able to infect.

Learning Objectives

- Explain factors that limit viral host range

Key Points

- The host range or host specificity of a parasite is the collection of hosts that an organism can use as a partner.
- The host range is usually a function of an inability of the virus to successfully adsorb and/or enter cells because of an incompatibility between virus capsid proteins (or virus envelope proteins) and the host receptor molecule.
- The host range is also a function of an incompatibility between the biochemistry of the virus and the biochemistry of the host. For closely related hosts, the biochemical differences can be quite subtle.

Key Terms

- **surface receptor**: Cell surface receptors (membrane receptors, transmembrane receptors) are specialized integral membrane proteins that take part in communication between the cell and the outside world.
- **commensal**: A term for a form of symbiosis in which one organism derives a benefit while the other is unaffected

Factors Limiting Viral Infection

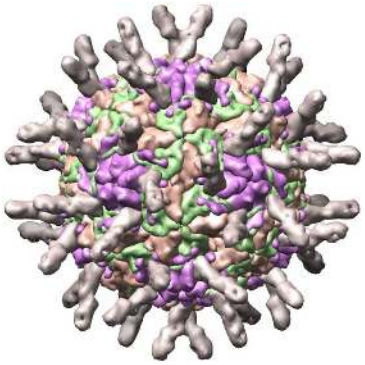


Figure: **ICAM-1**: structure of ICAM-1 molecule that enables viruses to bind to host's cell membrane.

A host is an organism that harbors a parasite or a mutual or commensal symbiont, typically providing nourishment and shelter. Resistance to and recovery from viral infections depend on the interactions that occur between the virus and the host. The defenses mounted by the host may act directly on the virus or indirectly on virus replication by altering or killing the infected cell. Non-specific host defenses function early in an encounter with a virus to prevent or limit infection, while the specific host defenses function after infection in recovery to provide immunity for subsequent challenges.

The host defense mechanisms involved in a particular viral infection will vary depending on the virus, dose, and portal of entry. The host has many barriers against infection that are inherent in the organism. These represent the first line of defense, which functions to prevent or limit infection Examples of natural barriers include but are not limited to skin, the expression of surface receptors such as CD4, complement receptors, glycophorin, intercelullar adhesion molecule 1 (ICAM-1), mucus, a ciliated epithelium, low pH, and humoral and cellular components.

The host range of the virus will depend upon the presence of the receptors described above. If a host lacks the receptor for a virus, or if the host cell lacks some component necessary for the replication of a virus, the host will inherently be resistant to that virus. For example, mice lack the receptors for polio viruses and thus are resistant to polio virus. Similarly, humans are inherently resistant to plant and many animal viruses.

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4.1.5: Viral Size

Most viruses range in size from 5 to 300 nanometers (nm), although some Paramyxoviruses can be up to 14,000 nm long.

Learning Objectives

- Recognize the cause and effect of different sizes of viruses

Key Points

- The size and shape of a virus helps us understand its class and components.
- Viruses also exist in different shapes that include helical, polyhedral, enveloped, and complex.
- Proteins and nucleic acid of viruses determine their size and shape.

Key Terms

- **bacteriophage**: A virus that specifically infects bacteria.

A virus is an infectious agent of small size and simple composition that can multiply only in living cells of animals, plants, or bacteria. They range in size from about 20 to 400 nanometres in diameter (1 nanometre = 10^{-9} meters). By contrast, the smallest bacteria are about 400 nanometres in size. A virus consists of a single- or double-stranded nucleic acid and at least one protein surrounded by a protein shell, called a capsid; some viruses also have an outer envelope composed of fatty materials (lipids) and proteins. The nucleic acid carries the virus's genome —its collection of genes—and may consist of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The protein capsid provides protection for the nucleic acid and may contain enzymes that enable the virus to enter its appropriate host cell.

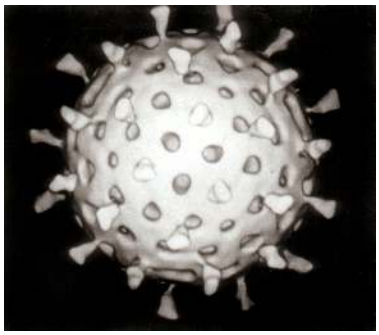


Figure: **Viral structure**: Rotavirus particles measure 76.5 nm in diameter and are not enveloped.

There exists a strong association between viral geometry and features of viral disease outbreaks. The amount and arrangement of the proteins and nucleic acid of viruses determine their size and shape. The protein and nucleic acid constituents have properties unique for each class of virus; when assembled, they determine the size and shape of the virus for that specific class.

Only the largest and most complex viruses can be seen under the light microscope at the highest resolution. Any determination of the size of a virus also must take into account its shape, since different classes of viruses have distinctive shapes. Shapes of viruses are predominantly of two kinds: rods, or filaments, so called because of the linear array of the nucleic acid and the protein subunits; and spheres, which are actually 20-sided (icosahedral) polygons. Most plant viruses are small and are either filaments or polygons, as are many bacterial viruses. The larger and more-complex bacteriophages contain double-stranded DNA as their genetic information and combine both filamentous and polygonal shapes. The classic T4 bacteriophage is composed of a polygonal head, which contains the DNA genome, and a special-function rod-shaped tail of long fibres. Structures such as these are unique to the bacteriophages.

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SECTION OVERVIEW

4.2: Structure of Viruses

Topic hierarchy

4.2.1: Viral Morphology

4.2.2: General Morphology

4.2.3: Complex and Asymmetrical Virus Particles

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4.2.1: Viral Morphology

Viruses of all shapes and sizes consist of a nucleic acid core, an outer protein coating or capsid, and sometimes an outer envelope.

Learning Objectives

- Describe the relationship between the viral genome, capsid, and envelope

Key Points

- Viruses are classified into four groups based on shape: filamentous, isometric (or icosahedral), enveloped, and head and tail.
- Many viruses attach to their host cells to facilitate penetration of the cell membrane, allowing their replication inside the cell.
- Non-enveloped viruses can be more resistant to changes in temperature, pH, and some disinfectants than are enveloped viruses.
- The virus core contains the small single- or double-stranded genome that encodes the proteins that the virus cannot get from the host cell.

Key Terms

- **capsid**: the outer protein shell of a virus
- **envelope**: an enclosing structure or cover, such as a membrane
- **filamentous**: Having the form of threads or filaments
- **isometric**: of, or being a geometric system of three equal axes lying at right angles to each other (especially in crystallography)

Viral Morphology

Viruses are acellular, meaning they are biological entities that do not have a cellular structure. Therefore, they lack most of the components of cells, such as organelles, ribosomes, and the plasma membrane. A virion consists of a nucleic acid core, an outer protein coating or capsid, and sometimes an outer envelope made of protein and phospholipid membranes derived from the host cell. The capsid is made up of protein subunits called capsomeres. Viruses may also contain additional proteins, such as enzymes. The most obvious difference between members of viral families is their morphology, which is quite diverse. An interesting feature of viral complexity is that host and virion complexity are uncorrelated. Some of the most intricate virion structures are observed in bacteriophages, viruses that infect the simplest living organisms: bacteria.

Morphology

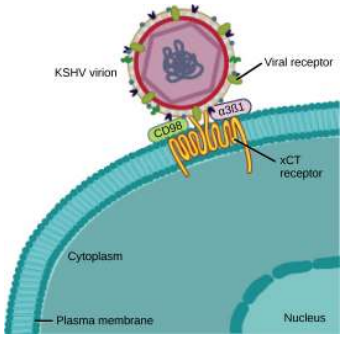


Figure: **Example of a virus attaching to its host cell**: The KSHV virus binds the xCT receptor on the surface of human cells. This attachment allows for later penetration of the cell membrane and replication inside the cell.

Viruses come in many shapes and sizes, but these are consistent and distinct for each viral family. In general, the shapes of viruses are classified into four groups: filamentous, isometric (or icosahedral), enveloped, and head and tail. Filamentous viruses are long and cylindrical. Many plant viruses are filamentous, including TMV (tobacco mosaic virus). Isometric viruses have shapes that are roughly spherical, such as poliovirus or herpesviruses. Enveloped viruses have membranes surrounding capsids. Animal viruses, such as HIV, are frequently enveloped. Head and tail viruses infect bacteria. They have a head that is similar to icosahedral viruses and a tail shape like filamentous viruses.

Many viruses use some sort of glycoprotein to attach to their host cells via molecules on the cell called viral receptors. For these viruses, attachment is a requirement for later penetration of the cell membrane, allowing them to complete their replication inside

the cell. The receptors that viruses use are molecules that are normally found on cell surfaces and have their own physiological functions. Viruses have simply evolved to make use of these molecules for their own replication.

Overall, the shape of the virion and the presence or absence of an envelope tell us little about what disease the virus may cause or what species it might infect, but they are still useful means to begin viral classification. Among the most complex virions known, the T4 bacteriophage, which infects the *Escherichia coli* bacterium, has a tail structure that the virus uses to attach to host cells and a head structure that houses its DNA. Adenovirus, a non-enveloped animal virus that causes respiratory illnesses in humans, uses glycoprotein spikes protruding from its capsomeres to attach to host cells. Non-enveloped viruses also include those that cause polio (poliovirus), plantar warts (papillomavirus), and hepatitis A (hepatitis A virus).

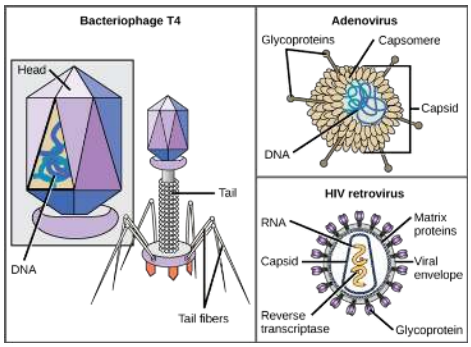


Figure: **Examples of virus shapes**: Viruses can be either complex in shape or relatively simple. This figure shows three relatively-complex virions: the bacteriophage T4, with its DNA-containing head group and tail fibers that attach to host cells; adenovirus, which uses spikes from its capsid to bind to host cells; and HIV, which uses glycoproteins embedded in its envelope to bind to host cells.

Enveloped virions like HIV consist of nucleic acid and capsid proteins surrounded by a phospholipid bilayer envelope and its associated proteins. Glycoproteins embedded in the viral envelope are used to attach to host cells. Other envelope proteins include the matrix proteins that stabilize the envelope and often play a role in the assembly of progeny virions. Chicken pox, influenza, and mumps are examples of diseases caused by viruses with envelopes. Because of the fragility of the envelope, non-enveloped viruses are more resistant to changes in temperature, pH, and some disinfectants than are enveloped viruses.

Types of Nucleic Acid

Unlike nearly all living organisms that use DNA as their genetic material, viruses may use either DNA or RNA. The virus core contains the genome or total genetic content of the virus. Viral genomes tend to be small, containing only those genes that encode proteins that the virus cannot obtain from the host cell. This genetic material may be single- or double-stranded. It may also be linear or circular. While most viruses contain a single nucleic acid, others have genomes that have several, called segments.

In DNA viruses, the viral DNA directs the host cell's replication proteins to synthesize new copies of the viral genome and to transcribe and translate that genome into viral proteins. DNA viruses cause human diseases, such as chickenpox, hepatitis B, and some venereal diseases, like herpes and genital warts.

RNA viruses contain only RNA as their genetic material. To replicate their genomes in the host cell, the RNA viruses encode enzymes that can replicate RNA into DNA, which cannot be done by the host cell. These RNA polymerase enzymes are more likely to make copying errors than DNA polymerases and, therefore, often make mistakes during transcription. For this reason, mutations in RNA viruses occur more frequently than in DNA viruses. This causes them to change and adapt more rapidly to their host. Human diseases caused by RNA viruses include hepatitis C, measles, and rabies.

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4.2.2: General Morphology

Viruses have a variety of shapes and structures.

Learning Objectives

- Distinguish between the 5 main morphological virus types

Key Points

- Viruses are very small and to reliably visualize them, stains and electron microscopy are needed.
- Each virus is a nucleic acid (RNA or DNA) surrounded by a coating, referred to as an envelope or capsid.
- Viruses encode capsid proteins which encase the nucleic acid. Sometimes, viral proteins combine with host proteins to make the envelope.
- The shape of a viral coat has implications on how a virus infects a host.

Key Terms

- **capsomere**: Any of the individual protein subunits of a viral capsid
- **capsid**: The outer protein shell of a virus.
- **icosahedral**: of, relating to, or having the shape of an icosahedron

Viruses display a wide diversity of shapes and sizes, called morphologies. In general, there are five main morphological virus types:

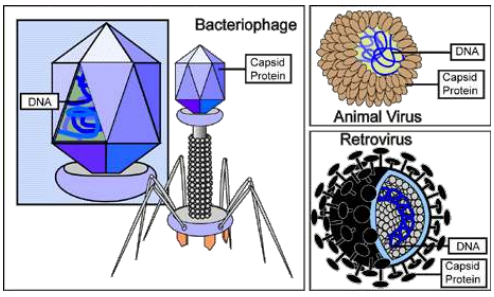


Figure: **Viral structure**: An outline of the structures of some common viral types.

1. Helical – These viruses are composed of a single type of capsomer stacked around a central axis to form a helical structure, which may have a central cavity, or hollow tube.
2. Icosahedral – Most animal viruses are icosahedral or near-spherical with icosahedral symmetry.
3. Prolate – This is an isosahedron elongated along one axis and is a common arrangement of the heads of bacteriophages.
4. Envelope – Some species of virus envelop themselves in a modified form of one of the cell membranes, either the outer membrane surrounding an infected host cell or internal membranes such as nuclear membrane or endoplasmic reticulum, thus gaining an outer lipid bilayer known as a viral envelope.
5. Complex – These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall.

A complete virus particle, known as a virion, consists of nucleic acid surrounded by a protective coat of protein called a capsid. These are formed from identical protein subunits called capsomeres. Viruses can have a lipid “envelope” derived from the host cell membrane. The capsid is made from proteins encoded by the viral genome and its shape serves as the basis for morphological distinction. Virally coded protein subunits will self-assemble to form a capsid, in general requiring the presence of the virus genome. Complex viruses code for proteins that assist in the construction of their capsid. Proteins associated with nucleic acid are known as nucleoproteins, and the association of viral capsid proteins with viral nucleic acid is called a nucleocapsid. The capsid and entire virus structure can be mechanically (physically) probed through atomic force microscopy.

viruses are much smaller than bacteria. Most viruses that have been studied have a diameter between 20 and 300 nanometers. Some filoviruses have a total length of up to 1400 nm; their diameters are only about 80 nm. Most viruses, such as virions, cannot be seen

with an optical microscope, so scanning and transmission electron microscopes are used to visualize them.

To increase the contrast between viruses and the background, electron-dense “stains” are used. These are solutions of salts of heavy metals, such as tungsten, that scatter the electrons from regions covered with the stain. When virions are coated with stain (positive staining), fine detail is obscured. Negative staining overcomes this problem by staining the background only.

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4.2.3: Complex and Asymmetrical Virus Particles

Complex viruses are often asymetrical or symetrical in combination with other structures such as a tail.

Learning Objectives

- Break down the differences between complex and asymmetrical viruses

Key Points

- Viruses can be structurally very different.
- Some complex viruses are large enough to be visible with a light microscope.
- The viruses of archaea are unique compared to other viruses.

Key Terms

- **capsid**: The outer protein shell of a virus.
- **poxvirus**: Any of the group of DNA viruses belonging to the family Poxviridae, which cause pox diseases in vertebrates.

Viruses have many structural shapes, often falling into certain categories. While some have symmetrical shapes, viruses with asymmetrical structures are referred to as “complex. ” These viruses possess a capsid that is neither purely helical nor purely icosahedral, and may possess extra structures such as protein tails or a complex outer walls.

Some bacteriophages, such as Enterobacteria phage T4, have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibers. This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell.

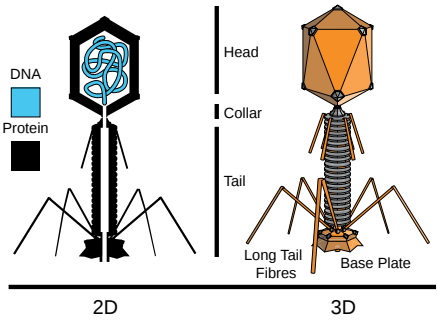


Figure: **T4 Bacteriophage**: T4 is a bacteriophage that infects E. coli and is referred to as a complex virus. Although it has an icosahedral head, its tail makes it asymmetrical, or complex in terms of structure.

The poxviruses are large, complex viruses that have an unusual morphology. The viral genome is associated with proteins within a central disk structure known as a nucleoid. The nucleoid is surrounded by a membrane and two lateral bodies of unknown function. The virus has an outer envelope with a thick layer of protein studded over its surface. The whole virion is slightly pleiomorphic, ranging from ovoid to brick shape.

Mimivirus is the largest characterized virus, with a capsid diameter of 400 nm. Protein filaments measuring 100 nm project from the surface. The capsid appears hexagonal under an electron microscope, therefore the capsid is probably icosahedral. In 2011, researchers discovered a larger virus on the ocean floor off the coast of Las Cruces, Chile. Provisionally named *Megavirus chilensis*, it can be seen with a basic optical microscope.

Some viruses that infect archaea have complex structures unrelated to any other form of virus. These include a wide variety of unusual shapes, ranging from spindle-shaped structures, to viruses that resemble hooked rods, teardrops, or even bottles. Other archaeal viruses resemble the tailed bacteriophages, and can have multiple tail structures.

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SECTION OVERVIEW

4.3: Classifying Viruses

Topic hierarchy

4.3.1: The International Committee on Taxonomy of Viruses

4.3.2: The Baltimore Virus Classification

4.3.3: Evolution of Viruses

4.3.4: Medical Importance of Viruses

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4.3.1: The International Committee on Taxonomy of Viruses

The International Committee on Taxonomy of Viruses (ICTV) establishes guidelines to maintain viral family uniformity.

Learning Objectives

- Describe the purpose and objectives of the International Committee on Taxonomy of Viruses

Key Points

- Only a small part of the total diversity of viruses has been studied. Analyses of samples from humans found that about 20% of the virus sequences recovered have not been seen before.
- A large majority of sequences of viral samples from the environment, such as from seawater and ocean sediments, are completely novel.
- The general taxonomic structure is as follows: Order (-virales); Family (-viridae); Subfamily (-virinae); Genus (-virus); Species (-virus).

Key Terms

- **taxonomy**: the academic discipline of defining groups of biological organisms on the basis of shared characteristics and giving names to those groups. Each group is given a rank and groups of a given rank can be aggregated to form a super group of higher rank and thus create a hierarchical classification.

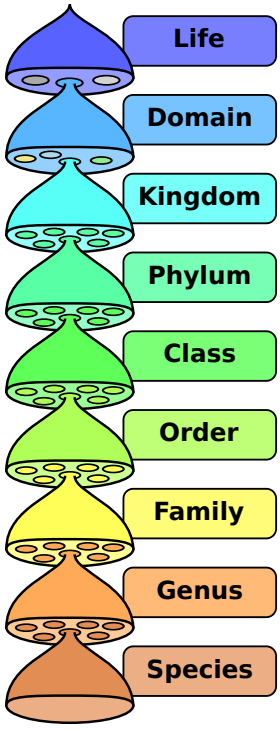


Figure: **Microbial taxonomy**: Taxonomic ranks

The International Committee on Taxonomy of Viruses (ICTV) is a committee which authorizes and organizes the taxonomic classification of viruses. They have developed a universal taxonomic scheme for viruses and aim to describe all the viruses of living organisms. Members of the committee are considered to be world experts on viruses. The committee formed from and is governed by the Virology Division of the International Union of Microbiological Societies. Detailed work such as delimiting the boundaries of species within a family is typically done by study groups, which consist of experts in the families. The committee also operates an authoritative database (ICTVdB) containing taxonomic information for 1,950 virus species, as of 2005. It is open to the public and is searchable by several different means.

The official objectives of the ICTV are:

1. To develop an internationally agreed upon taxonomy for viruses.
2. To develop internationally agreed upon names for virus taxa, including species and subviral agents.
3. To communicate taxonomic decisions to all users of virus names, in particular the international community of virologists, by publications and via the Internet.
4. To maintain an index of virus names.
5. To maintain an ICTV database on the Internet, that records the data that characterize each named viral taxon, together with the common names of each taxon in all major languages.

Proposals for new names, name changes, and the establishment and taxonomic placement of taxa are handled by the Executive Committee of the ICTV in the form of proposals. All relevant ICTV subcommittees and study groups are consulted prior to a decision being made. The name of a taxon has no status until it has been approved by ICTV, and names will only be accepted if they are linked to approved hierarchical taxa. If no suitable name is proposed for a taxon, the taxon may be approved and the name be left undecided until the adoption of an acceptable international name, when one is proposed to and accepted by ICTV. Names must not convey a meaning for the taxon which would seem to either exclude viruses which are rightfully members of that taxa, exclude members which might one day belong to that taxa, or include viruses which are members of different taxa.

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4.3.2: The Baltimore Virus Classification

The Baltimore classification groups viruses into families depending on their type of genome.

Learning Objectives

- List the characteristics of viruses that are useful for Baltimore classification

Key Points

- Viral genome ‘s nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication determine its class.
- Other classifications are determined by the disease caused by the virus or its morphology.
- Viruses can be placed in one of the seven groups.

Key Terms

- **genome**: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of basepairs.

Virus classification is the process of naming viruses and placing them into a taxonomic system. Much like the classification systems used for cellular organisms, virus classification is the subject of ongoing debate and proposals. This is mainly due to the pseudo-living nature of viruses, which is to say they are non-living particles with some chemical characteristics similar to those of life. As such, they do not fit neatly into the established biological classification system in place for cellular organisms.

Baltimore classification (first defined in 1971) is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication. Named after David Baltimore, a Nobel Prize-winning biologist, these groups are designated by Roman numerals and discriminate viruses depending on their mode of replication and genome type. Other classifications are determined by the disease caused by the virus or its morphology, neither of which are satisfactory due to different viruses either causing the same disease or looking very similar. In addition, viral structures are often difficult to determine under the microscope.

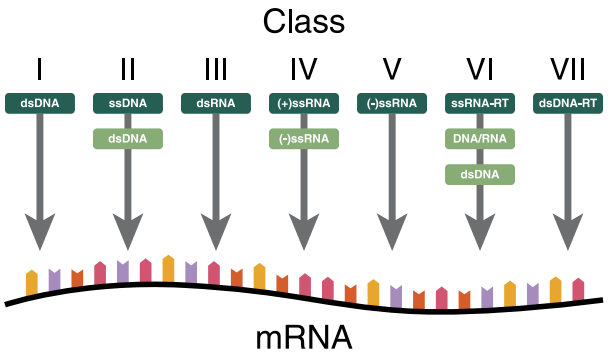


Figure: **Viral classes**: Baltimore classification of viruses

Classifying viruses according to their genome means that those in a given category will all behave in a similar fashion, offering some indication of how to proceed with further research. Viruses can be placed in one of the seven following groups:

1. I: dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)
2. II: ssDNA viruses (+)sense DNA (e.g. Parvoviruses)
3. III: dsRNA viruses (e.g. Reoviruses)
4. IV: (+)ssRNA viruses (+)sense RNA (e.g. Picornaviruses, Togaviruses)
5. V: (−)ssRNA viruses (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
6. VI: ssRNA-RT viruses (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
7. VII: dsDNA-RT viruses (e.g. Hepadnaviruses)

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4.3.3: Evolution of Viruses

The evolution of viruses is speculative as they do not fossilize; biochemical and genetic information is used to create virus histories.

Learning Objectives

- Describe the difficulties in determining the origin of viruses

Key Points

- Scientists agree that viruses don't have a single common ancestor, but have yet to agree on a single hypothesis about virus origins.
- The devolution or the regressive hypothesis suggests that viruses evolved from free-living cells.
- The escapist or the progressive hypothesis suggests that viruses originated from RNA and DNA molecules that escaped from a host cell.
- The self-replicating hypothesis posits a system of self-replication that most probably involves evolution alongside the host cells.

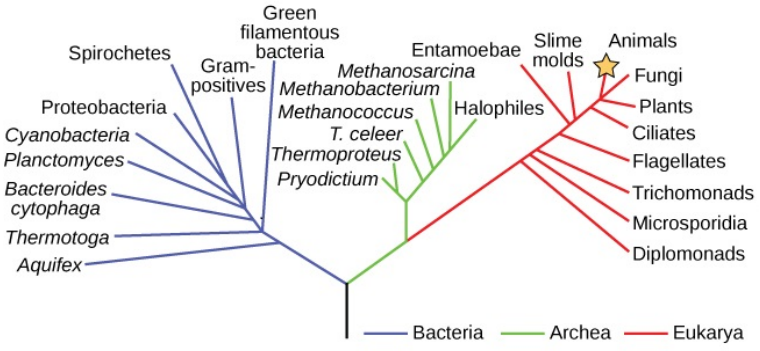
Key Terms

- **self-replicating**: able to generate a copy of itself
- **devolution**: degeneration (as opposed to evolution)

Evolution of Viruses

Although biologists have accumulated a significant amount of knowledge about how present-day viruses evolve, much less is known about how viruses originated in the first place. When exploring the evolutionary history of most organisms, scientists can look at fossil records and similar historic evidence. However, viruses do not fossilize, so researchers must conjecture by investigating how today's viruses evolve and by using biochemical and genetic information to create speculative virus histories.

While most findings agree that viruses don't have a single common ancestor, scholars have yet to find one hypothesis about virus origins that is fully accepted in the field. One possible hypothesis, called devolution or the regressive hypothesis, proposes to explain the origin of viruses by suggesting that viruses evolved from free-living cells. However, many components of how this process might have occurred are a mystery. A second hypothesis (called escapist or the progressive hypothesis) accounts for viruses having either an RNA or a DNA genome and suggests that viruses originated from RNA and DNA molecules that escaped from a host cell. A third hypothesis posits a system of self-replication similar to that of other self-replicating molecules, probably evolving alongside the cells they rely on as hosts; studies of some plant pathogens support this hypothesis.



(a) Rooted phylogenetic tree

Figure: **Common ancestor tree of life**: This phylogenetic tree of the three domains of life (Bacteria, Archaea, and Eukarya) attempts to identify when various species diverged from a common ancestor. Finding a common ancestor for viruses has proven to be far more difficult, especially since they do not fossilize.

As technology advances, scientists may develop and refine further hypotheses to explain the origin of viruses. The emerging field called virus molecular systematics attempts to do just that through comparisons of sequenced genetic material. These researchers

hope to one day better understand the origin of viruses, a discovery that could lead to advances in the treatments for the ailments they produce.

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4.3.4: Medical Importance of Viruses

Viruses are obligate intracellular parasites that hijack a host cell's machinery to replicate, thereby causing disease.

Learning Objectives

- Describe the fundamental characteristics of viruses

Key Points

- Viruses multiply by taking control of the host cell's genetic material and regulating the synthesis and assembly of new viruses.
- Viruses are able to infect a host cell and cause acute diseases or alter its genetic material to cause chronic diseases such as cancer.
- Most viral infections can resolve in weeks but others are the cause of more serious, debilitating and sometimes fatal diseases.

Key Terms

- **vaccination**: inoculation with a vaccine in order to protect a particular disease or strain of disease.
- **eradication**: the act of plucking up by the roots; a rooting out; extirpation; utter destruction.

Viruses are extremely diverse and have evolved to infect nearly all life forms. Amid this diversity, viruses with similar genome organizations exhibit major conserved themes in their replication strategies. Once inside a cell, all viruses must uncoat, replicate, and transcribe their genomes, and then repackage their genomes into viral progeny that are released from cells. RNA viruses in particular must coordinate the switch between plus and minus strand synthesis and between replication and transcription while protecting their genomes from cellular nucleases. Because of the conserved nature of a virus 's intracellular life cycle, fundamental advances in our understanding of replication have come from viruses that infect both animal and non-animal hosts.

The devastating effects of viral diseases such as AIDs, smallpox, polio, influenza, diarrhea, and hepatitis are well known, and studies of viral pathogens are easily justified from a world health perspective. Sobering examples of emerging viral diseases have occurred. Among these are the sudden emergence of the coronavirus that causes severe acute respiratory syndrome (SARS), the continued transmission of an avian influenza virus to humans ("bird flu"), and the isolation of poliovirus vaccine -wild type recombinants that have hampered poliovirus eradication efforts. In addition, the threat of bioterrorism became a reality on U.S. soil, creating an obligation for scientists to respond with aggressive countermeasures. Vaccination remains the preferred strategy for controlling viral diseases because the intimate association of viruses with the host cellular machinery complicates the development of safe drugs. However, certain viruses have proven difficult targets for vaccines, and antiviral drugs provide the only option for controlling disease.

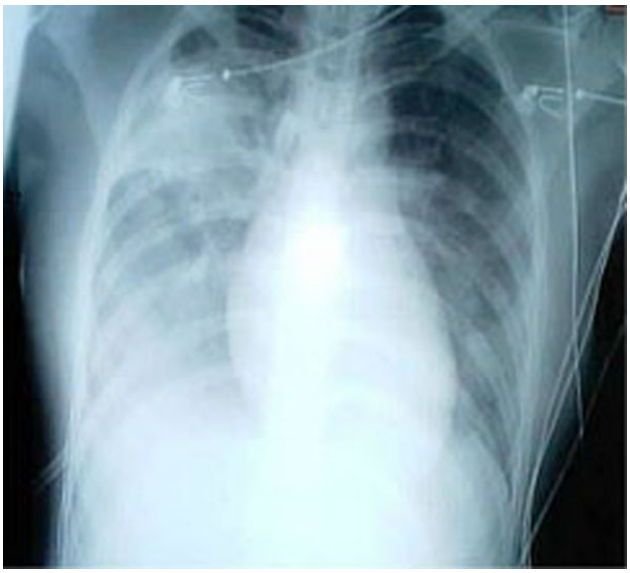


Figure: **Medical importance of SARS**: A chest x-ray showing increased opacity in both lungs, indicative of pneumonia, in a patient with SARS.

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SECTION OVERVIEW

4.4: Culturing Viruses

Topic hierarchy

4.4.1: Batch Culture of Bacteriophages

4.4.2: Tissue Culture of Animal Viruses

4.4.3: Inoculation of Live Animals

4.4.4: Viral Identification

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4.4.1: Batch Culture of Bacteriophages

Bacteriophage cultures require host cells in which the virus or phage multiply.

Learning Objectives

- Define the reasons for, and ways to batch culture bacteriophages

Key Points

- A bacteriophage is a type of virus that infects bacteria. It does so by injecting genetic material – either DNA or RNA – which it carries enclosed in an outer protein capsid.
- To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella.
- Phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

Key Terms

- **bacteriophage**: A virus that specifically infects bacteria.

Strategies of Replication

Virus or phage cultures require host cells in which to multiply. For bacteriophages, cultures are grown by infecting bacterial cells. The phage can then be isolated from the resulting plaques in a lawn of bacteria on a plate.

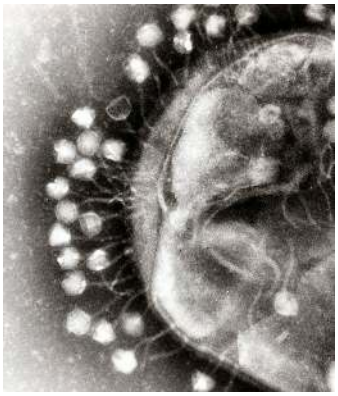


Figure: **Bacteriophages infecting a bacteria**: Virus or phage cultures require host cells in which to multiply. For bacteriophages, cultures are grown by infecting bacterial cells. The phage can then be isolated from the resulting plaques in a lawn of bacteria on a plate.

A bacteriophage is any one of a number of viruses that infect bacteria. They do this by injecting genetic material, which they carry enclosed in an outer protein capsid, into a host bacterial cell. The genetic material can be ssRNA, dsRNA, ssDNA, or dsDNA (‘ss-‘ or ‘ds-‘ prefix denotes single-strand or double-strand), along with either circular or linear arrangements.

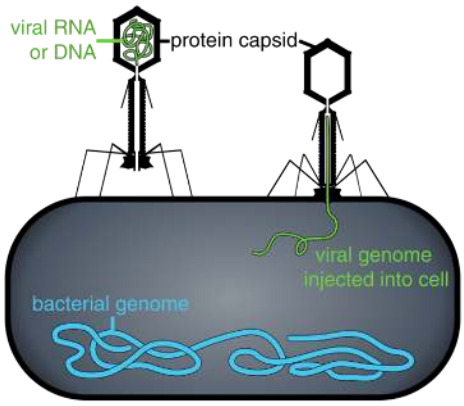


Figure: **Bacteriophage**: Diagram of how some bacteriophages infect bacterial cells.

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only those bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them. As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution within blood, lymphatic circulation, irrigation, soil water, or other environments..

Phages may be released via cell lysis, by extrusion, or, in a few cases, by budding. Lysis, by tailed phages, is achieved by an enzyme called endolysin, which attacks and breaks down the cell wall peptidoglycan. An altogether different phage type, the filamentous phages, make the host cell continually secrete new virus particles. Released virions are described as free, and, unless defective, are capable of infecting a new bacterium. Budding is associated with certain Mycoplasma phages. In contrast to virion release, phages displaying a lysogenic cycle do not kill the host but, rather, become long-term residents as prophage.

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4.4.2: Tissue Culture of Animal Viruses

Viruses cannot be grown in standard microbiological broths or on agar plates, instead they have be to cultured inside suitable host cells.

Learning Objectives

- Discover the use of, and reasons for, culturing animal viruses in host cells

Key Points

- Tissue culture is a useful method for cultivating clinical samples suspected of harboring a virus. This method helps with the detection, identification, and characterization of viruses in the laboratory.
- Tissue culture of animal viruses involves growing animal cells in flasks using various broth media and then infecting these cells with virus.
- Transfection can be carried out using calcium phosphate, by electroporation, or by mixing a cationic lipid with the material to produce liposomes, which fuse with the cell membrane and deposit their cargo inside.
- Cytopathic effect is a non-lytic damage that viruses cause to cells. These vary in their manifestation and damaging effect.

Key Terms

- **cell culture**: The complex process by which cells are grown under controlled conditions, generally outside of their natural environment.
- **cytopathic effect**: Refers to degenerative changes in cells, especially in tissue culture, and may be associated with the multiplication of certain viruses.

Cell culture is the complex process by which cells are grown under controlled conditions, generally outside of their natural environment. In practice, the term “cell culture” now refers to the culturing of cells derived from multi-cellular eukaryotes, especially animal cells. However, there are also cultures of plants, fungi, and microbes, including viruses, bacteria, and protists. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture. Animal cell culture became a common laboratory technique in the mid-1900’s, but the concept of maintaining live cell lines separated from their original tissue source was discovered in the 19th century.

Viruses are obligate intracellular parasites that require living cells in order to replicate. Cultured cells, eggs, and laboratory animals may be used for virus isolation. Although embroyonated eggs and laboratory animals are very useful for the isolation of certain viruses, cell cultures are the sole system for virus isolation in most laboratories. The development of methods for cultivating animal cells has been essential to the progress of animal virology. To prepare cell cultures, tissue fragments are first dissociated, usually with the aid of trypsin or collagenase. The cell suspension is then placed in a flat-bottomed glass or plastic container (petri dish, a flask, a bottle, test tube) together with a suitable liquid medium. e.g. Eagle’s, and an animal serum. After a variable lag, the cells will attach and spread on the bottom of the container and then start dividing, giving rise to a primary culture. Attachment to a solid support is essential for the growth of normal cells.

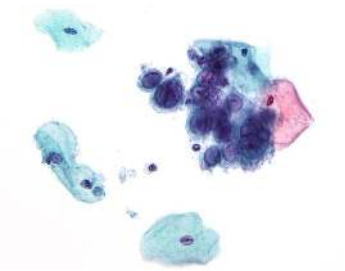


Figure: **Cytopathic effect**: Viral cytopathic effect of herpes simplex virus.

Cell cultures vary greatly in their susceptibility to different viruses. It is of utmost importance that the most sensitive cell cultures are used for a particular suspected virus. Specimens for cell culture should be transported to the laboratory as soon as possible upon being taken. Swabs should be put in a vial containing virus transport medium. Bodily fluids and tissues should be placed in a sterile

container. Upon receipt, the specimen is inoculated into several different types of cell culture depending on the nature of the specimen and the clinical presentation. The maintenance media should be changed after one hour or the next morning. The inoculated tubes should be incubated at 35-37°C in a rotating drum. Rotation is optimal for the isolation of respiratory viruses and result in an earlier appearance of the cytopathic effects (CPE) for many viruses. If stationary tubes are used, it is critical that the culture tubes be positioned so that the cell monolayer is bathed in nutrient medium.

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4.4.3: Inoculation of Live Animals

Live animal inoculation is a method used to cultivate viruses.

Learning Objectives

- Describe live animal innoculation

Key Points

- Live inoculation was first used on human volunteers for the study of yellow fever virus.
- Animals of choice for cultivating viruses include monkeys, rabbits, guinea pigs, rats, hamsters, and mice.
- Live animal inoculation requires an experienced personnel to perform various inoculation techniques.

Key Terms

- **latent**: Existing or present but concealed or inactive.
- **oncogenesis**: The formation and development of tumors
- **inoculation**: The introduction of an antigenic substance or vaccine into the body to produce immunity to a specific disease.

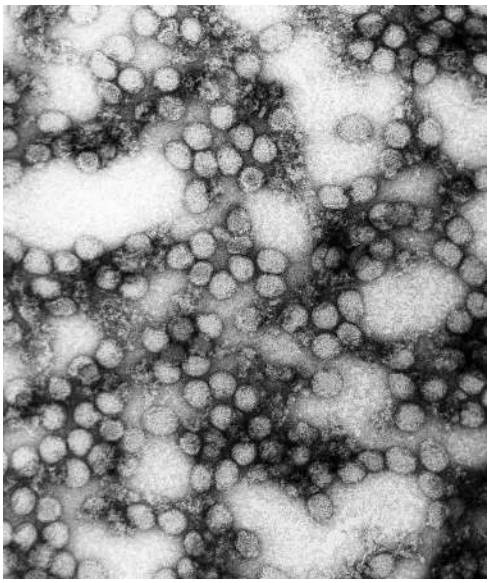


Figure: **Yellow fever virus**: A micrograph of the yellow fever virus.

Viruses are obligate intracellular parasites and cannot grow on inanimate media. They need living cells for replication, which can be provided by inoculation in live animals among other methods used to culture viruses (cell culture or inoculation of embryonated eggs). Inoculation of human volunteers was the only known method of cultivation of viruses and understanding viral disease. In 1900, Reed and his colleagues used human volunteers for their work on yellow fever. Due to serious risk involved, human volunteers are recruited only when no other method is available and the virus is relatively harmless. Smallpox was likely the first disease people tried to prevent by purposely inoculating themselves with other infections and was the first disease for which a vaccine was produced. Today, studying viruses via the inoculation of humans would require a stringent study of ethical practices by an institutional review board.

In the past few decades, animal inoculation has been employed for virus isolation. The laboratory animals used include monkeys, rabbits, guinea pigs, rats, hamsters, and mice. The choice of animals and route of inoculation (intracerebral, intraperitoneal, subcutaneous, intradermal, or intraocular) depends largely on the type of virus to be isolated. Handling of animals and inoculation into various routes requires special experience and training. In addition to virus isolation, animal inoculation can also be used to observe pathogenesis, immune response, epidemiology, and oncogenesis. Growth of the virus in inoculated animals may be indicated by visible lesions, disease, or death. Sometimes, serial passage into animals may be required to obtain visible evidence of

viral growth. Animal inoculation has several disadvantages as immunity may interfere with viral growth, and the animal may harbor latent viruses.

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4.4.4: Viral Identification

The genetic material within virus particles varies considerably between different types of viruses.

Learning Objectives

- Compare the replication of DNA, RNA, and reverse transcribing viruses

Key Points

- The genome replication of most DNA viruses takes place in the cell's nucleus.
- RNA viruses can be placed into four different groups depending on their modes of replication.
- Reverse transcribing viruses have ssRNA (Retroviridae, Metaviridae, Pseudoviridae) or dsDNA (Caulimoviridae, and Hepadnaviridae) in their particles.

Key Terms

- **genome**: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of basepairs.

Replication of Viruses

The genetic material within virus particles and the method by which the material is replicated vary considerably between different types of viruses.

TYPES

DNA viruses: The genome replication of most DNA viruses takes place in the cell's nucleus. If the cell has the appropriate receptor on its surface, these viruses sometimes enter the cell by direct fusion with the cell membrane (e.g., herpesviruses) or, more usually, by receptor-mediated endocytosis. Most DNA viruses are entirely dependent on the host cell's DNA and RNA synthesizing machinery and RNA processing machinery; however, viruses with larger genomes may encode much of this machinery themselves. In eukaryotes the viral genome must cross the cell's nuclear membrane to access this machinery, while in bacteria it need only enter the cell.

RNA viruses: Replication usually takes place in the cytoplasm. RNA viruses can be placed into four different groups, depending on their modes of replication. The polarity of single-stranded RNA viruses largely determines the replicative mechanism, depending on whether or not it can be used directly by ribosomes to make proteins. The other major criterion is whether the genetic material is single-stranded or double-stranded. All RNA viruses use their own RNA replicase enzymes to create copies of their genomes.

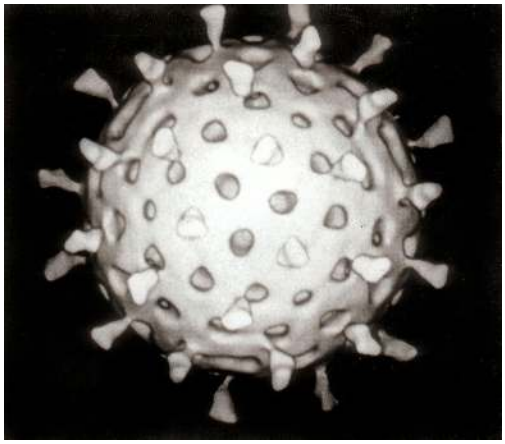


Figure: **Computer assisted reconstruction of a rotavirus particle**: An example of Baltimore Virus classification I: dsDNA virusesII: ssDNA virusesIII: dsRNA virusesIV: (+)ssRNA virusesV: (−)ssRNA virusesVI: ssRNA-RT virusesVII: dsDNA-RT viruses

Reverse transcribing viruses: These have ssRNA (Retroviridae, Metaviridae, Pseudoviridae) or dsDNA (Caulimoviridae, and Hepadnaviridae) in their particles. Reverse transcribing viruses with RNA genomes (retroviruses), use a DNA intermediate to replicate, whereas those with DNA genomes (pararetroviruses) use an RNA intermediate during genome replication. Both types use a reverse transcriptase, or RNA-dependent DNA polymerase enzyme, to carry out the nucleic acid conversion. Retroviruses integrate the DNA produced by reverse transcription into the host genome as a provirus as a part of the replication process. Pararetroviruses do not, although integrated genome copies, usually of plant pararetroviruses, can give rise to infectious virus. They are susceptible to antiviral drugs that inhibit the reverse transcriptase enzyme, e.g. zidovudine and lamivudine. An example of the first type is HIV, which is a retrovirus. Examples of the second type are the Hepadnaviridae, which includes Hepatitis B virus.

The *Baltimore classification* developed by David Baltimore is a virus classification system that groups viruses into families, depending on their type of genome (DNA, RNA, single-stranded (ss), double-stranded (ds), etc.) and their method of replication. Classifying viruses according to their genome means that those in a given category will all behave in much the same way, which offers some indication of how to proceed with further research.

In summary:

- I: dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: ssDNA viruses (+)sense DNA (e.g. Parvoviruses)
- III: dsRNA viruses (e.g. Reoviruses)IV: (+)ssRNA viruses (+)sense RNA (e.g. Picornaviruses, Togaviruses)V: (−)ssRNA viruses (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: ssRNA-RT viruses (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: dsDNA-RT viruses (e.g. Hepadnaviruses)

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SECTION OVERVIEW

4.5: Viral Replication

Topic hierarchy

4.5.1: General Features of Virus Replication

4.5.2: Steps of Virus Infections

4.5.3: Tissue Tropism in Animal Viruses

4.5.4: Animal Viruses

4.5.5: Plant Virus Life Cycles

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4.5.1: General Features of Virus Replication

Virologists describe the formation of viruses during the infection process in target host cells as viral replication.

Learning Objectives

- Outline the features of viral replication

Key Points

- Viral populations do not grow through cell division, because they are acellular. Instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble in the cell.
- The life cycle of viruses differs greatly between species but there are six basic stages in the life cycle of viruses: attachment, penetration (viral entry), uncoating, replication, and lysis.
- Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome.

Key Terms

- **lysis**: The disintegration or destruction of cells
- **leukocyte**: A white blood cell.
- **uncoating**: A process in which the viral capsid of a virus is removed, leading to the release of the viral genomic nucleic acid.
- **attachment**: specific binding between viral capsid proteins and specific receptors on the host cellular surface

Multiplication Within the Host Cell

Viral replication is the term used indicate the formation of biological viruses during the infection process in the target host cells. Viruses must first penetrate and enter the cell before viral replication can occur. From the perspective of the virus, the purpose of viral replication is to allow reproduction and survival of its kind. By generating abundant copies of its genome and packaging these copies into viruses, the virus is able to continue infecting new hosts.

Replication between viruses is varied and depends on the type of genes involved. Most DNA viruses assemble in the nucleus; most RNA viruses develop solely in cytoplasm. Viral populations do not grow through cell division, because they are acellular. Instead, they hijack the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble inside the cell.

The life cycle of viruses differs greatly between species but there are six common basic stages:

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV can infect only a limited range of human leukocytes. Its surface protein, gp120, specifically interacts only with the CD4 molecule – a chemokine receptor – which is most commonly found on the surface of CD4+ T-Cells. This mechanism has evolved to favor those viruses that infect only cells within which they are capable of replication. Attachment to the receptor can fore the viral envelope protein to undergo either changes that result in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

Penetration follows attachment. Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called *viral entry*. The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall. However, nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called plasmodesmata. Bacteria, like plants, have strong cell walls that a virus must breach to infect the cell. However, since bacterial cell walls are much less thick than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside.

Uncoating is a process in which the viral capsid is removed: This may be by degradation by viral or host enzymes or by simple dissociation. In either case the end-result is the release of the viral genomic nucleic acid.

Replication of viruses depends on the multiplication of the genome. This is accomplished through synthesis of viral messenger RNA (mRNA) from “early” genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: “late” gene expression is, in general, of structural or virion proteins.

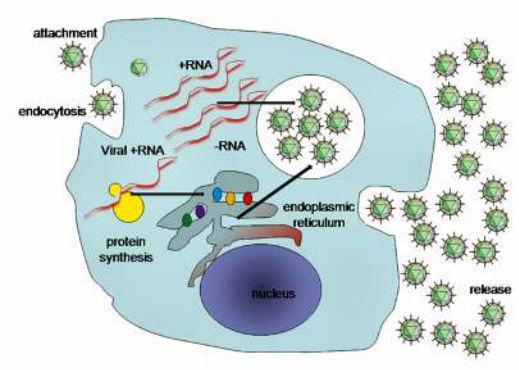


Figure: **Hepatitis C virus**: A simplified diagram of the Hepatitis C virus replication cycle.

Following the structure-mediated self-assembly of the virus particles, some modification of the proteins often occurs. In viruses such as HIV, this modification (sometimes called maturation) occurs after the virus has been released from the host cell.

Viruses can be released from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present. This is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host’s chromosome. The viral genome is then known as a *provirus* or, in the case of bacteriophages a *prophage*. Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host; however, at some point the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host’s plasma or other internal membrane. The genetic material within virus particles, and the method by which the material is replicated, varies considerably between different types of viruses.

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4.5.2: Steps of Virus Infections

Viral infection involves the incorporation of viral DNA into a host cell, replication of that material, and the release of the new viruses.

Learning Objectives

- List the steps of viral replication and explain what occurs at each step

Key Points

- Viral replication involves six steps: attachment, penetration, uncoating, replication, assembly, and release.
- During attachment and penetration, the virus attaches itself to a host cell and injects its genetic material into it.
- During uncoating, replication, and assembly, the viral DNA or RNA incorporates itself into the host cell's genetic material and induces it to replicate the viral genome.
- During release, the newly-created viruses are released from the host cell, either by causing the cell to break apart, waiting for the cell to die, or by budding off through the cell membrane.

Key Terms

- **virion**: a single individual particle of a virus (the viral equivalent of a cell)
- **glycoprotein**: a protein with covalently-bonded carbohydrates
- **retrovirus**: a virus that has a genome consisting of RNA

Steps of Virus Infections

A virus must use cell processes to replicate. The viral replication cycle can produce dramatic biochemical and structural changes in the host cell, which may cause cell damage. These changes, called cytopathic (causing cell damage) effects, can change cell functions or even destroy the cell. Some infected cells, such as those infected by the common cold virus known as rhinovirus, die through lysis (bursting) or apoptosis (programmed cell death or "cell suicide"), releasing all progeny virions at once. The symptoms of viral diseases result from the immune response to the virus, which attempts to control and eliminate the virus from the body and from cell damage caused by the virus. Many animal viruses, such as HIV (Human Immunodeficiency Virus), leave the infected cells of the immune system by a process known as budding, where virions leave the cell individually. During the budding process, the cell does not undergo lysis and is not immediately killed. However, the damage to the cells that the virus infects may make it impossible for the cells to function normally, even though the cells remain alive for a period of time. Most productive viral infections follow similar steps in the virus replication cycle: attachment, penetration, uncoating, replication, assembly, and release.

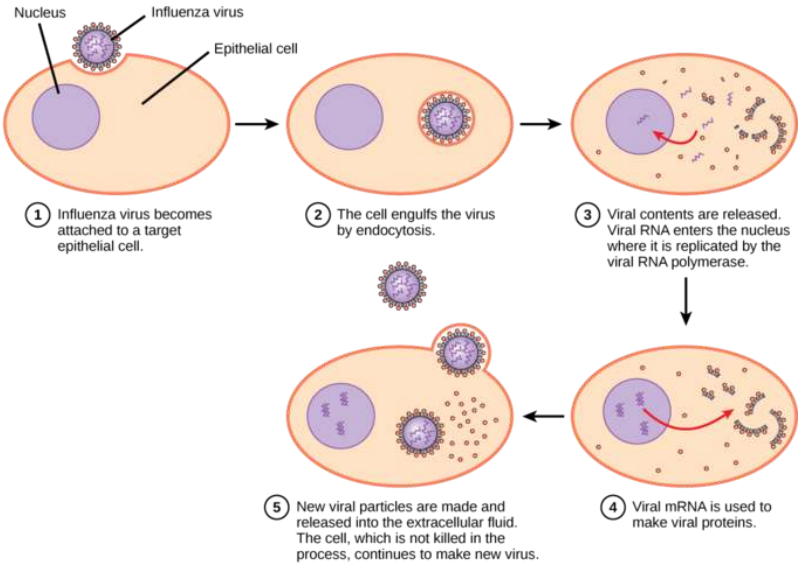


Figure: **Pathway to viral infection**: In influenza virus infection, glycoproteins attach to a host epithelial cell. As a result, the virus is engulfed. RNA and proteins are made and assembled into new virions.

Attachment

A virus attaches to a specific receptor site on the host cell membrane through attachment proteins in the capsid or via glycoproteins embedded in the viral envelope. The specificity of this interaction determines the host (and the cells within the host) that can be infected by a particular virus. This can be illustrated by thinking of several keys and several locks where each key will fit only one specific lock.

Entry

The nucleic acid of bacteriophages enters the host cell naked, leaving the capsid outside the cell. Plant and animal viruses can enter through endocytosis, in which the cell membrane surrounds and engulfs the entire virus. Some enveloped viruses enter the cell when the viral envelope fuses directly with the cell membrane. Once inside the cell, the viral capsid is degraded and the viral nucleic acid is released, which then becomes available for replication and transcription.

Replication and Assembly

The replication mechanism depends on the viral genome. DNA viruses usually use host cell proteins and enzymes to make additional DNA that is transcribed to messenger RNA (mRNA), which is then used to direct protein synthesis. RNA viruses usually use the RNA core as a template for synthesis of viral genomic RNA and mRNA. The viral mRNA directs the host cell to synthesize viral enzymes and capsid proteins, and to assemble new virions. Of course, there are exceptions to this pattern. If a host cell does not provide the enzymes necessary for viral replication, viral genes supply the information to direct synthesis of the missing proteins. Retroviruses, such as HIV, have an RNA genome that must be reverse transcribed into DNA, which then is incorporated into the host cell genome.

To convert RNA into DNA, retroviruses must contain genes that encode the virus-specific enzyme reverse transcriptase, which transcribes an RNA template to DNA. Reverse transcription never occurs in uninfected host cells; the needed enzyme, reverse transcriptase, is only derived from the expression of viral genes within the infected host cells. The fact that HIV produces some of its own enzymes not found in the host has allowed researchers to develop drugs that inhibit these enzymes. These drugs, including the reverse transcriptase inhibitor AZT, inhibit HIV replication by reducing the activity of the enzyme without affecting the host's metabolism. This approach has led to the development of a variety of drugs used to treat HIV and has been effective at reducing the number of infectious virions (copies of viral RNA) in the blood to non-detectable levels in many HIV-infected individuals.

Egress

The last stage of viral replication is the release of the new virions produced in the host organism. They are then able to infect adjacent cells and repeat the replication cycle. As you have learned, some viruses are released when the host cell dies, while other

viruses can leave infected cells by budding through the membrane without directly killing the cell.

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4.5.3: Tissue Tropism in Animal Viruses

Host tropism refers to the way in which viruses/pathogens determine which cells become infected by a given pathogen.

Learning Objectives

- Explain viral tropism

Key Points

- Viruses must bind to specific cell surface receptors in order to enter a cell.
- If a cell does not express these receptors then the virus cannot normally infect it.
- In virology, Tissue tropism is the cells and tissues of a host which support growth of a particular virus or bacteria. Some viruses have a broad tissue tropism and can infect many types of cells and tissues. Other viruses may infect primarily a single tissue.

Key Terms

- **dendritic cell**: Any cell, having branching processes, that forms part of the mammalian immune system.
- **macrophage**: A white blood cell that phagocytizes necrotic cell debris and foreign material, including viruses, bacteria, and tattoo ink. It presents foreign antigens on MHC II to lymphocytes. Part of the innate immune system.

A tropism is a biological phenomenon, indicating growth or turning movement of a biological organism in response to an environmental stimulus. In tropisms, this response is dependent on the direction of the stimulus (as opposed to nastic movements which are non-directional responses). Viruses and other pathogens also affect what is called “host tropism” or “cell tropism. ” Case tropism refers to the way in which different viruses/pathogens have evolved to preferentially target specific host species or specific cell types within those species.

Host tropism is the name given to a process of tropism that determines which cells can become infected by a given pathogen. Host tropism is determined by the biochemical receptor complexes on cell surfaces that are permissive or non-permissive to the docking or attachment of various viruses.

Various factors determine the ability of a pathogen to infect a particular cell. For example, viruses must bind to specific cell surface receptors to enter a cell. If a cell does not express these receptors then the virus cannot normally infect it. Viral tropism is determined by a combination of susceptibility and permissiveness: a host cell must be both permissive (allow viral entry) and susceptible (possess the receptor complement needed for viral entry) for a virus to establish infection. An example of this is the HIV virus, which exhibits tropism for CD4 related immune cells (e.g. T helper cells, macrophages or dendritic cells). These cells express a CD4 receptor, to which the HIV virus can bind, through the gp120 and gp41 proteins on its surface.

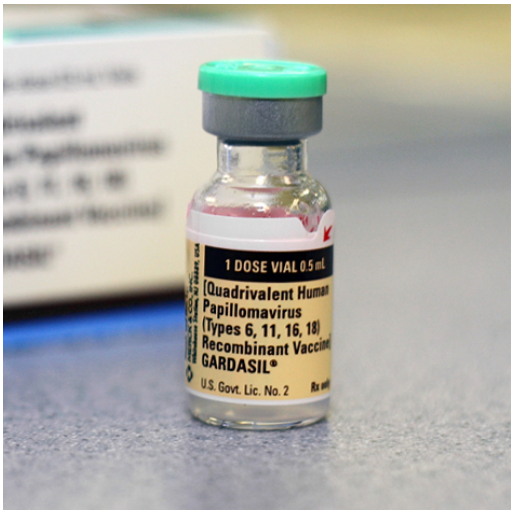


Figure: **Human papillomavirus vaccine**: Gardasil is a human papillomavirus vaccine on the market and it protects against HPV-16 and HPV-18 which cause 70% of cervical cancers, 80% of anal cancers, 60% of vaginal cancers, and 40% of vulvar cancers.

In virology, Tissue tropism is the cells and tissues of a host that support growth of a particular virus or bacteria. Some viruses have a broad tissue tropism and can infect many types of cells and tissues. Other viruses may infect primarily a single tissue.

Factors influencing viral tissue tropism include: 1) the presence of cellular receptors permitting viral entry, 2) availability of transcription factors involved in viral replication, 3) the molecular nature of the viral tropogen, and 4) the cellular receptors are the proteins found on a cell or viral surface.

These receptors are like keys allowing the viral cell to fuse with a cell or attach itself to a cell. The way that these proteins are acquired is through similar process to that of an infection cycle.

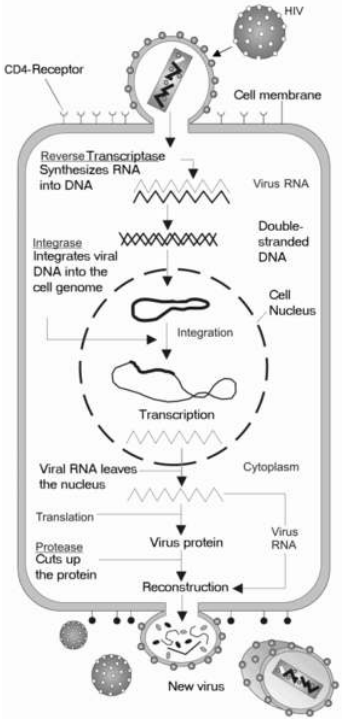


Figure: **HIV life cycle**: HIV has a gp120 which is precisely what the CD4 marker is on the surface of the macrophages and T cells. Therefore, HIV can enter T cells and macrophages.

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4.5.4: Animal Viruses

Animal viruses have their genetic material copied by a host cell after which they are released into the environment to cause disease.

Learning Objectives

- Describe various animal viruses and the diseases they cause

Key Points

- Animal viruses may enter a host cell by either receptor -mediated endocytosis or by changing shape and entering the cell through the cell membrane.
- Viruses cause diseases in humans and other animals; they often have to run their course before symptoms disappear.
- Examples of viral animal diseases include hepatitis C, chicken pox, and shingles.

Key Terms

- **receptor-mediated endocytosis**: a process by which cells internalize molecules (endocytosis) by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being internalized

Animal Viruses

Animal viruses, unlike the viruses of plants and bacteria, do not have to penetrate a cell wall to gain access to the host cell. Non-enveloped or “naked” animal viruses may enter cells in two different ways. When a protein in the viral capsid binds to its receptor on the host cell, the virus may be taken inside the cell via a vesicle during the normal cell process of receptor-mediated endocytosis. An alternative method of cell penetration used by non-enveloped viruses is for capsid proteins to undergo shape changes after binding to the receptor, creating channels in the host cell membrane. The viral genome is then “injected” into the host cell through these channels in a manner analogous to that used by many bacteriophages. Enveloped viruses also have two ways of entering cells after binding to their receptors: receptor-mediated endocytosis and fusion. Many enveloped viruses enter the cell by receptor-mediated endocytosis in a fashion similar to some non-enveloped viruses. On the other hand, fusion only occurs with enveloped virions. These viruses, which include HIV among others, use special fusion proteins in their envelopes to cause the envelope to fuse with the plasma membrane of the cell, thus releasing the genome and capsid of the virus into the cell cytoplasm.

After making their proteins and copying their genomes, animal viruses complete the assembly of new virions and exit the cell. Using the example of HIV, enveloped animal viruses may bud from the cell membrane as they assemble themselves, taking a piece of the cell’s plasma membrane in the process. On the other hand, non-enveloped viral progeny, such as rhinoviruses, accumulate in infected cells until there is a signal for lysis or apoptosis, and all virions are released together.

Animal viruses are associated with a variety of human diseases. Some of them follow the classic pattern of acute disease, where symptoms worsen for a short period followed by the elimination of the virus from the body by the immune system with eventual recovery from the infection. Examples of acute viral diseases are the common cold and influenza. Other viruses cause long-term chronic infections, such as the virus causing hepatitis C, whereas others, like herpes simplex virus, cause only intermittent symptoms. Still other viruses, such as human herpes viruses 6 and 7, which in some cases can cause the minor childhood disease roseola, often successfully cause productive infections without causing any symptoms at all in the host; these patients have an asymptomatic infection.

In hepatitis C infections, the virus grows and reproduces in liver cells, causing low levels of liver damage. The damage is so low that infected individuals are often unaware that they are infected, with many infections only detected by routine blood work on patients with risk factors such as intravenous drug use. Since many of the symptoms of viral diseases are caused by immune responses, a lack of symptoms is an indication of a weak immune response to the virus. This allows the virus to escape elimination by the immune system and persist in individuals for years, while continuing to produce low levels of progeny virions in what is known as a chronic viral disease. Chronic infection of the liver by this virus leads to a much greater chance of developing liver cancer, sometimes as much as 30 years after the initial infection.

As mentioned, herpes simplex virus can remain in a state of latency in nervous tissue for months, even years. As the virus “hides” in the tissue and makes few if any viral proteins, there is nothing for the immune response to act against; immunity to the virus

slowly declines. Under certain conditions, including various types of physical and psychological stress, the latent herpes simplex virus may be reactivated and undergo a lytic replication cycle in the skin, causing the lesions associated with the disease. Once virions are produced in the skin and viral proteins are synthesized, the immune response is again stimulated and resolves the skin lesions in a few days by destroying viruses in the skin. As a result of this type of replicative cycle, appearances of cold sores and genital herpes outbreaks only occur intermittently, even though the viruses remain in the nervous tissue for life. Latent infections are common with other herpes viruses as well, including the varicella-zoster virus that causes chickenpox. After having a chickenpox infection in childhood, the varicella-zoster virus can remain latent for many years and reactivate in adults to cause the painful condition known as “shingles”.

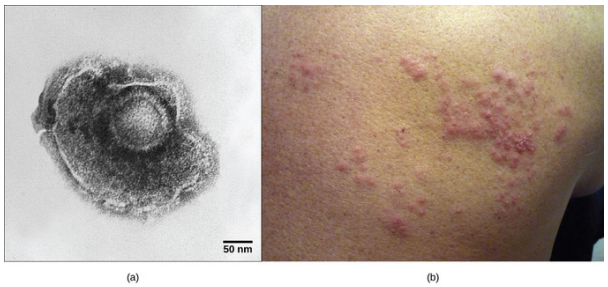


Figure: **Chicken pox virus**: (a) Varicella-zoster, the virus that causes chickenpox, has an enveloped icosahedral capsid visible in this transmission electron micrograph. Its double-stranded DNA genome incorporates into the host DNA and reactivates after latency in the form of (b) shingles, often exhibiting a rash.

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4.5.5: Plant Virus Life Cycles

Plant viruses are often spread from plant to plant by organisms known as vectors.

Learning Objectives

- Outline plant virus life cycles

Key Points

- Plant viruses are harmless to humans and other animals because they can only reproduce in living plant cells.
- For the virus to reproduce and thereby establish infection, it must enter cells of the host organism and use those cells' materials.
- A virus must take control of the host cell's replication mechanisms. At this stage a distinction between susceptibility and permissibility of a host cell is made.
- After control is established and the environment is set for the virus to begin making copies of itself, replication occurs quickly by the millions.

Key Terms

- **vector**: A carrier of a disease-causing agent.

Plant viruses are viruses that affect plants. Like all other viruses, plant viruses are obligate intracellular parasites that do not have the molecular machinery to replicate without a host. Plant viruses are pathogenic to higher plants.

There are many types of plant virus, but often they only cause a loss of yield, and it is not economically viable to try to control them. Plant viruses are often spread from plant to plant by organisms (vectors). These are normally insects, but some fungi, nematode worms and single-celled organisms have been shown to be vectors. When control of plant virus infections is considered economical, (for perennial fruits for example), efforts are concentrated on killing the vectors and removing alternate hosts such as weeds. Plant viruses are harmless to humans and other animals because they can only reproduce in living plant cells.

Viral Life Cycle

For the virus to reproduce and thereby establish infection, it must enter cells of the host organism and use those cells' materials. To enter the cells, proteins on the surface of the virus interact with proteins of the cell. Attachment, or adsorption, occurs between the viral particle and the host cell membrane. A hole forms in the cell membrane, then the virus particle or its genetic contents are released into the host cell, where viral reproduction may commence. Next, a virus must take control of the host cell's replication mechanisms. At this stage, a distinction between susceptibility and permissibility of a host cell is made. Permissibility determines the outcome of the infection. After control is established and the environment is set for the virus to begin making copies of itself, replication occurs quickly by the millions. After a virus has made many copies of itself, it usually has exhausted the cell of its resources. The host cell is now no longer useful to the virus, therefore the cell often dies and the newly produced viruses must find a new host. The process by which virus progeny are released to find new hosts, is called shedding. This is the final stage in the viral life cycle. Some viruses can "hide" within a cell, either to evade the host cell defenses or immune system, or simply because it is not in the best interest of the virus to continually replicate. This hiding is deemed latency. During this time, the virus does not produce any progeny, it remains inactive until external stimuli—such as light or stress—prompts it to activate.

Viruses can be spread by direct transfer of sap, and by contact of a wounded plant with a healthy one. Such contact may occur during agricultural practices, when damage is caused by tools or hands, or naturally, when an animal feeds on the plant. Generally Tobacco mosaic virus (TMV), potato viruses, and cucumber mosaic viruses are transmitted via sap.

Tobacco mosaic virus and Cauliflower mosaic virus (CaMV) are frequently used in plant molecular biology. Of special interest is the CaMV 35S promoter, which is a very strong promoter most frequently used in plant transformations.

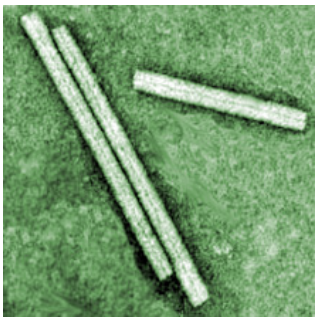


Figure: **Tobacco Mosaic Virus**: Tobacco mosaic virus (TMV) is a positive-sense single stranded RNA virus that infects plants, especially tobacco and other members of the family Solanaceae. The infection causes characteristic patterns (mottling and discoloration) on the leaves (hence the name). TMV was the first virus to be discovered. Although it was known from the late 19th century that an infectious disease was damaging tobacco crops, it was not until 1930 that the infectious agent was determined to be a virus.

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SECTION OVERVIEW

4.6: Subviral Entities

Topic hierarchy

4.6.1: Defective Viruses

4.6.2: Viroids

4.6.3: Prions

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4.6.1: Defective Viruses

LEARNING OBJECTIVES

- Distinguish between defective viruses, satellite viruses, and helper viruses

Virologists also study subviral particles, infectious entities notably smaller and simpler than viruses:

- viroids (naked circular RNA molecules infecting plants)
- satellites (nucleic acid molecules with or without a capsid that require a helper virus for infection and reproduction)
- prions (proteins that can exist in a pathological conformation that induces other prion molecules to assume that same conformation)

Not all viruses can reproduce in a host cell by themselves. Since viruses are so small, the size of their genome is limited. For example, some viruses have coded instructions for only making a few different proteins for the viruses' capsid. On the other hand, the human genome codes for over 30,000 different proteins. Therefore, the lack of coded instructions causes some viruses to need the presence of other viruses to help them reproduce themselves. Such viruses are called replication defective.

Satellites depend on co-infection of a host cell with a helper virus for productive multiplication. Their nucleic acids have substantially distinct nucleotide sequences from either their helper virus or host. When a satellite subviral agent encodes the coat protein in which it is encapsulated, it is then called a satellite virus. Satellite viral particles should not be confused with satellite DNA.

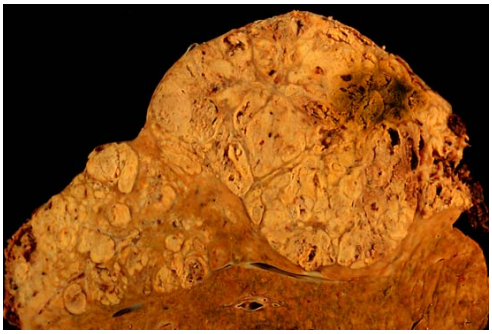


Figure: ***Hepatocellular Carcinoma****: Cirrhosis leading to hepatocellular carcinoma (autopsy specimen). The photo shows a view of a longitudinal slice taken through the full length of the liver.*

The hepatitis delta virus of humans has an RNA genome similar to viroids, but has a protein coat derived from hepatitis B virus and cannot produce one of its own. Therefore, it is a defective virus and cannot replicate without the help of hepatitis B virus. In similar manner, the sputnik virophage is dependent on mimivirus, which infects the protozoan Acanthamoeba castellanii. These viruses that are dependent on the presence of other virus species in the host cell are called satellites. They may represent evolutionary intermediates of viroids and viruses.

Hepatitis D, also referred to as hepatitis D virus (HDV) and classified as Hepatitis delta virus, is a disease caused by a small circular enveloped RNA virus. It is one of five known hepatitis viruses: A, B, C, D, and E. HDV is considered to be a subviral satellite because it can only propagate in the presence of the hepatitis B virus (HBV). Transmission of HDV can occur either via simultaneous infection with HBV (coinfection) or superimposed on chronic hepatitis B or hepatitis B carrier state (superinfection). Both superinfection and coinfection with HDV results in more severe complications compared to infection with HBV alone. These complications include a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased chance of developing liver cancer in chronic infections. In combination with hepatitis B virus, hepatitis D has the highest mortality rate of all the hepatitis infections of 20%.

Key Points

- Not all viruses can reproduce in a host cell by themselves. Since viruses are so small, the size of their genome is limited. The lack of coded instructions causes some viruses to need the presence of other viruses to help them reproduce themselves. Such viruses are called replication defective.

- A satellite is a subviral agent composed of nucleic acid that depends on the co- infection of a host cell with a helper or master virus for its multiplication. When a satellite encodes the coat protein in which its nucleic acid is encapsidated it is referred to as a satellite virus.
- These viruses that are dependent on the presence of other virus species in the host cell are called satellites and may represent evolutionary intermediates of viroids and viruses.

Key Terms

- **Helper virus**: A helper virus is a virus used when producing copies of a helper dependent viral vector which does not have the ability to replicate on its own. The helper virus is used to coinfect cells alongside the viral vector and provides the necessary enzymes for replication of the genome of the viral vector.
- **Satellite**: A subviral agent composed of nucleic acid that depends on the co-infection of a host cell with a helper or master virus for its multiplication.

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4.6.2: Viroids

LEARNING OBJECTIVES

- Relate the structure and replication of a viroid to its ability to cause diseases in plants

Viroids are plant pathogens that consist of a short stretch (a few hundred nucleobases) of highly complementary, circular, single-stranded RNA without the protein coat that is typical for viruses. In comparison, the genome of the smallest known viruses capable of causing an infection by themselves is around 2 kilobases in size. The human pathogen Hepatitis D virus is similar to viroids. Viroids are extremely small in size, ranging from 246 to 467 nucleotide (nt) long genome and consisting of fewer than 10,000 atoms. Viroids were discovered and given this name by Theodor Otto Diener, a plant pathologist at the Agricultural Research Service in Maryland, in 1971.

Viroid RNA does not code for any protein. The replication mechanism involves RNA polymerase II, an enzyme normally associated with synthesis of messenger RNA from DNA, which instead catalyzes “rolling circle” synthesis of new RNA using the viroid’s RNA as template. Some viroids are ribozymes, having catalytic properties which allow self-cleavage and ligation of unit-size genomes from larger replication intermediates. The first viroid to be identified was the potato spindle tuber viroid (PSTVd). Some 33 species have been identified.



Figure: ***Putative secondary structure of the potato spindle tuber viroid.****: black – secondary structure of the viroid red – GAAAC sequence common to all viroids yellow – central conservative sequence blue – nucleotide numbers*

There has long been confusion over how viroids are able to induce symptoms in plants without encoding any protein products within their sequences. Evidence now suggests that RNA silencing is involved in the process. First, changes to the viroid genome can dramatically alter its virulence. This reflects the fact that any siRNAs produced would have less complementary base pairing with target messenger RNA. Secondly, siRNAs corresponding to sequences from viroid genomes have been isolated from infected plants. Finally, transgenic expression of the noninfectious hpRNA of potato spindle tuber viroid develops all the corresponding viroid like symptoms. This evidence indicates that when viroids replicate via a double stranded intermediate RNA, they are targeted by a dicer enzyme and cleaved into siRNAs that are then loaded onto the RNA-induced silencing complex. The viroid siRNAs actually contain sequences capable of complementary base pairing with the plant’s own messenger RNAs and induction of degradation or inhibition of translation is what causes the classic viroid symptoms.

Virusoids are circular single-stranded RNAs dependent on plant viruses for replication and encapsidation. The genome of virusoids consists of several hundred nucleotides and only encodes structural proteins. Virusoids are similar to viroids in size, structure, and means of replication. Virusoids, while being studied in virology, are not considered as viruses but as subviral particles. Since they depend on helper viruses, they are classified as satellites.

The Pospiviroidae are a family of viroids, including the first viroid to be discovered, PSTVd. Their secondary structure is key to their biological activity. The classification of this family is based on differences in the conserved central region sequence. Pospiviroidae replication occurs in an asymmetric fashion via host cell RNA polymerase, RNase, and RNA ligase.

The Avsunviroidae are a family of viroids. At present three members are known. They consist of RNA genomes between 246-375 nucleotides in length. They are single stranded covalent circles and have intramolecular base pairing. All members lack a central conserved region. Replication occurs in the chloroplasts of plant cells. Key features of replication include no helper virus required and no proteins are encoded for. Unlike the other family of viroids, Pospiviroidae, Avsunviroidae are thought to replicate via a symmetrical rolling mechanism. It is thought the positive RNA strand acts as a template to form negative strands with the help of an enzyme thought to be RNA polymerase II. The negative RNA strands are then cleaved by ribozyme activity and circularizes. A second rolling circle mechanism forms a positive strand which is also cleaved by ribozyme activity and then ligated to become circular. The site of replication is unknown, but it is thought to be in the chloroplast and in the presence of Mg2+ ions.

Avocado sunblotch viroid (ASBV) is an important disease affecting avocado trees. Infections result in lower yields and poorer quality fruit. ASBV is the smallest known viroid that infects plants and is transmitted by pollen and infected seeds or budwood. Trees infected with the viroid often show no symptoms other than a reduction in yield. However, they are still carriers and can pass

the disease onto other plants. Symptoms in more serious infections include depressed longitudinal streaks of yellow in the fruit. The fruit may also become red or white in color. Symptoms in the leaf are uncommon, but include bleached veins and petioles. Rectangular cracking patterns also occur in the bark of older branches. Infected but symptomless trees have a higher concentration of viroid particles than those showing symptoms. Symptomless trees also represent a greater danger in terms of spread of the viroid.

Key Points

- Viroid RNA does not code for any protein. The replication mechanism involves RNA polymerase II, an enzyme normally associated with synthesis of messenger RNA from DNA, which instead catalyzes “rolling circle” synthesis of new RNA using the viroid’s RNA as template.
- The first viroid to be identified was potato spindle tuber viroid (PSTVd). Some 33 species have been identified.
- There has long been confusion over how viroids are able to induce symptoms in plants without encoding any protein products within their sequences. Evidence now suggests that RNA silencing is involved in the process.
- Virusoids are circular single-stranded RNAs dependent on plant viruses for replication and encapsidation. Since they depend on helper viruses, they are classified as satellites.

Key Terms

- **Virusoid**: Circular single-stranded RNAs dependent on plant viruses for replication and encapsidation. The genome of virusoids consist of several hundred nucleotides and only encodes structural proteins.
- **viroid**: plant pathogens, of the order Viroidales, that consist of just a short section of RNA but without the protein coat typical of viruses

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4.6.3: Prions

LEARNING OBJECTIVES

- Compare the protein-only hypothesis of prion diseases with the virion hypothesis, as well as the heterodimer model and the fibril model of prion replication

A prion is an infectious agent composed of protein in a misfolded form. This is the central idea of the Prion Hypothesis, which remains debated. This is in contrast to all other known infectious agents (virus /bacteria/fungus/parasite) which must contain nucleic acids (either DNA, RNA, or both). The word *prion*, coined in 1982 by Stanley B. Prusiner, is derived from the words protein and infection. Prions are responsible for the transmissible spongiform encephalopathies in a variety of mammals, including bovine spongiform encephalopathy (BSE, also known as “mad cow disease”) in cattle and Creutzfeldt–Jakob disease (CJD) in humans. All known prion diseases affect the structure of the brain or other neural tissue, are currently untreatable and universally fatal.

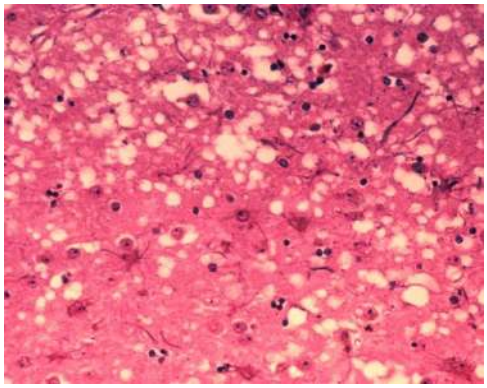


Figure: ***Prion-affected tissue****: This micrograph of brain tissue reveals the cytoarchitectural histopathologic changes found in bovine spongiform encephalopathy. The presence of vacuoles, i.e. microscopic “holes” in the gray matter, gives the brain of BSE-affected cows a sponge-like appearance when tissue sections are examined in the lab.*

Prions propagate by transmitting a misfolded protein state. When a prion enters a healthy organism, it induces existing, properly folded proteins to convert into the disease-associated prion form; it acts as a template to guide the misfolding of more proteins into prion form. These newly-formed prions can then go on to convert more proteins themselves; triggering a chain reaction. All known prions induce the formation of an amyloid fold, in which the protein polymerises into an aggregate consisting of tightly-packed beta sheets. Amyloid aggregates are fibrils, growing at their ends, and replicating when breakage causes two growing ends to become four growing ends.

The incubation period of prion diseases is determined by the exponential growth rate associated with prion replication, which is a balance between the linear growth and the breakage of aggregates. Propagation of the prion depends on the presence of normally-folded protein in which the prion can induce misfolding; animals which do not express the normal form of the prion protein cannot develop nor transmit the disease.

All known mammalian prion diseases are caused by the so-called prion protein, PrP. The endogenous, properly-folded form is denoted PrPC (for Common or Cellular) while the disease-linked, misfolded form is denoted PrPSc (for Scrapie, after one of the diseases first linked to prions and neurodegeneration.) The precise structure of the prion is not known, though they can be formed by combining PrPC, polyadenylic acid, and lipids in a Protein Misfolding Cyclic Amplification (PMCA) reaction.

Proteins showing prion-type behavior are also found in some fungi, which has been useful in helping to understand mammalian prions. Fungal prions do not appear to cause disease in their hosts.

The first hypothesis that tried to explain how prions replicate in a protein-only manner was the heterodimer model. This model assumed that a single PrPSc molecule binds to a single PrPC molecule and catalyzes its conversion into PrPSc. The two PrPSc molecules then come apart and can go on to convert more PrPC. However, a model of prion replication must explain both how prions propagate, and why their spontaneous appearance is so rare. Manfred Eigen showed that the heterodimer model requires

PrPSc to be an extraordinarily effective catalyst, increasing the rate of the conversion reaction by a factor of around 10^{15}. This problem does not arise if PrPSc exists only in aggregated forms such as amyloid, where cooperativity may act as a barrier to spontaneous conversion. What is more, despite considerable effort, infectious monomeric PrPSc has never been isolated.

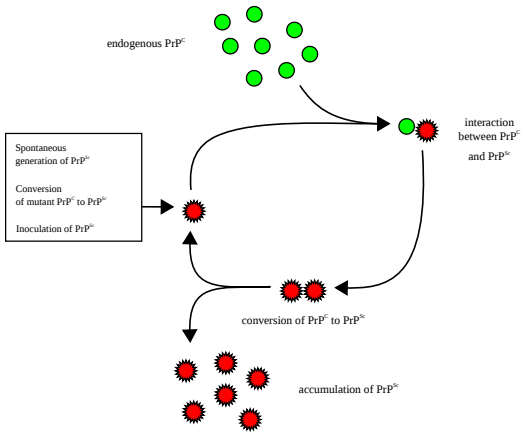


Figure: ***Diagram demonstrating how prion numbers increase****: Heterodimer model of prion propagation.*

An alternative model assumes that PrPSc exists only as fibrils, and that fibril ends bind PrPC and convert it into PrPSc. If this were all, then the quantity of prions would increase linearly, forming ever longer fibrils. But exponential growth of both PrPSc and of the quantity of infectious particles is observed during prion disease. This can be explained by taking into account fibril breakage. A mathematical solution for the exponential growth rate resulting from the combination of fibril growth and fibril breakage has been found.

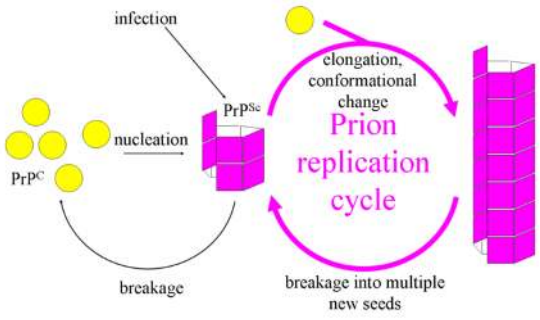


Figure: **The Fibril Model**: Illustrating prion propagation.

The protein-only hypothesis has been criticised by those who feel that the simplest explanation of the evidence to date is viral. For more than a decade, Yale University neuropathologist Laura Manuelidis has been proposing that prion diseases are caused instead by an unidentified slow virus. In January 2007, she and her colleagues published an article reporting to have found a virus in 10%, or less, of their scrapie-infected cells in culture.

The virion hypothesis states that TSEs are caused by a replicable informational molecule (likely to be a nucleic acid) bound to PrP. Many TSEs, including scrapie and BSE, show strains with specific and distinct biological properties, a feature which supporters of the virion hypothesis feel is not explained by prions.

Recent studies propagating TSE infectivity in cell-free reactions and in purified component chemical reactions strongly suggest against TSE's viral nature. Using a similar defined recipe of multiple components (PrP, POPG lipid, RNA), Jiyan Ma and colleagues generated infectious prions from recombinant PrP expressed from E. coli, casting further doubt on this hypothesis.

Key Points

- Prions are responsible for the transmissible spongiform encephalopathies in a variety of mammals, including bovine spongiform encephalopathy and Creutzfeldt–Jakob disease in humans. All known prion diseases affect the structure of neural tissue, are currently untreatable and universally fatal.
- Prions propagate by transmitting a misfolded protein state. When a prion enters a healthy organism, it induces existing, properly-folded proteins to convert into the disease-associated, prion form; it then acts as a template to guide the misfolding of more proteins into prion form.
- All known mammalian prion diseases are caused by the so-called prion protein, PrP. The endogenous, properly folded, form is denoted PrPC while the disease-linked, misfolded form is denoted PrPSc.The precise structure of the prion is not known.
- The first hypothesis to explain how prions replicate in a protein-only manner was the heterodimer model, which assumed that a single PrPSc molecule binds to a single PrPC molecule and catalyzes its conversion into PrPSc. The two PrPSc molecules then come apart and can go on to convert more PrPC.
- An alternative model assumes that PrPSc exists only as fibrils and that fibril ends bind PrPC and convert it into PrPSc. The exponential growth of both PrPSc and of the quantity of infectious particles observed during prion disease can be explained by taking fibril breakage into account.
- The protein-only hypothesis has been criticised by those who feel that the simplest explanation of the evidence to date is viral. However, recent studies propagating TSE infectivity in cell-free reactions and in purified component chemical reactions strongly suggest against TSE's viral nature.

Key Terms

- **Amyloid**: Insoluble fibrous protein aggregates sharing specific structural traits. They arise from at least 18 inappropriately folded versions of proteins and polypeptides present naturally in the body. These misfolded structures alter their proper configuration such that they erroneously interact with one another or other cell components forming insoluble fibrils. They have been associated with the pathology of more than 20 serious human diseases in that, abnormal accumulation of amyloid fibrils in organs may lead to amyloidosis, and may play a role in various neurodegenerative disorders.
- **Creutzfeldt–Jakob disease**: A degenerative neurological disorder (brain disease) that is incurable and invariably fatal. In CJD, the brain tissue develops holes and takes on a sponge-like texture, due to a type of infectious protein called a prion.
- **prion**: A self-propagating misfolded conformer of a protein that is responsible for a number of diseases that affect the brain and other neural tissue.

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SECTION OVERVIEW

4.7: Viral Diversity

Topic hierarchy

4.7.1: Overview of Bacterial Viruses

4.7.2: RNA Bacteriophages

4.7.3: Single-Stranded DNA Bacteriophages

4.7.4: Double-Stranded DNA Bacteriophages

4.7.5: Mu- A Double-Stranded Transposable DNA Bacteriophage

4.7.6: Virulent Bacteriophages and T4

4.7.7: Temperate Bacteriophages - Lambda and P1

4.7.8: Viruses of Archaea

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4.7.1: Overview of Bacterial Viruses

Bacteriophages are viruses that infect bacteria and are among the most common and diverse entities in the biosphere.

Learning Objectives

- Evaluate the complexity of bacteriophages

Key Points

- Phages are obligate intracellular parasites that are able to reproduce only while infecting bacteria. Bacteriophages are comprised of proteins that encapsulate a DNA or RNA genome.
- Bacteriophages occur in over 140 bacterial or archaeal genera. They arose repeatedly in different hosts and there are at least 11 separate lines of descent. Nineteen families are currently recognised that infect bacteria and archaea.
- Phages are widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water.
- Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both.
- To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria. This specificity means a bacteriophage can infect only certain bacteria bearing receptors to which they can bind, which in turn determines the phage's host range.

Key Terms

- **bacteriophage**: A virus that specifically infects bacteria.
- **lysogeny**: the process by which a bacteriophage incorporates its nucleic acids into a host bacterium

Bacteriophages

Bacteriophages (phages) are potentially the most numerous "organisms" on Earth. They are among the most common and diverse entities in the biosphere. They are the viruses of bacteria (more generally, of prokaryotes). Phages are obligate intracellular parasites, meaning that they are able to reproduce only while infecting bacteria. Bacteriophages are comprised of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Their genomes may encode as few as four genes, and as many as hundreds of genes. Phages replicate within bacteria following the injection of their genome into the cytoplasm.

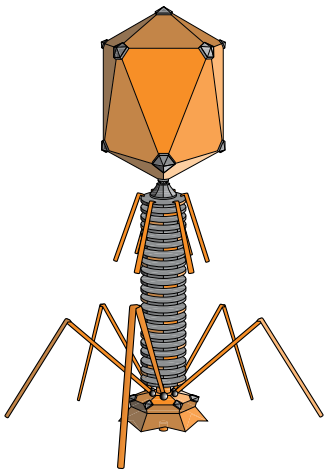


Figure: **Artistic rendering of a T4 bacteriophage**: The structure of a typical myovirus bacteriophage

Phage-ecological interactions are quantitatively vast. Bacteria (along with archaea) are highly diverse, with possibly millions of species. Phage-ecological interactions are also qualitatively diverse. There are huge numbers of environment types, bacterial-host types, and also individual phage types. Bacteriophages occur in over 140 bacterial or archaeal genera. They arose repeatedly in different hosts and there are at least 11 separate lines of descent. Over 5100 bacteriophages have been examined in the electron

microscope since 1959. Of these, at least 4950 phages (96%) have tails. Of the tailed phages 61% have long, noncontractile tails (Siphoviridae). Tailed phages appear to be monophyletic and are the oldest known virus group.

Phages are widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×108 virions per milliliter have been found in microbial mats at the surface. Up to 70% of marine bacteria may be infected by phages.

The dsDNA tailed phages, or Caudovirales, account for 95% of all the phages reported in the scientific literature, and possibly make up the majority of phages on the planet. However, other phages occur abundantly in the biosphere, with different virions, genomes and lifestyles. Phages are classified by the International Committee on Taxonomy of Viruses (ICTV) according to morphology and nucleic acid.

Nineteen families are currently recognised that infect bacteria and archaea. Of these, only two families have RNA genomes and only five families are enveloped. Of the viral families with DNA genomes, only two have single-stranded genomes. Eight of the viral families with DNA genomes have circular genomes, while nine have linear genomes. Nine families infect bacteria only, nine infect archaea only, and one (Tectiviridae) infects both bacteria and archaea.

Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both. With lytic phages such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect.

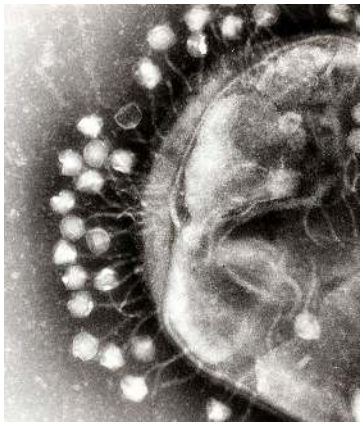


Figure: **Electron micrograph of Bacteriophages**: In this electron micrograph of bacteriophages attached to a bacterial cell, the viruses are the size and shape of coliphage T1.

In contrast, the lysogenic cycle does not result in immediate lysing of the host cell. Those phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with host DNA and replicate along with it fairly harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients; then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is reproduced in all of the cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and the lytic cycle is the phage lambda of E. coli.

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only certain bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them.

Phages may be released via cell lysis, by extrusion, or, in a few cases, by budding. Lysis, by tailed phages, is achieved by an enzyme called endolysin, which attacks and breaks down the cell wall peptidoglycan. An altogether different phage type, the filamentous phages, make the host cell continually secrete new virus particles. Budding is associated with certain Mycoplasma phages.

Bacteriophage genomes are especially mosaic: the genome of any one phage species appears to be composed of numerous individual modules. These modules may be found in other phage species in different arrangements. Mycobacteriophages –

bacteriophages with mycobacterial hosts – have provided excellent examples of this mosaicism. In these mycobacteriophages, genetic assortment may be the result of repeated instances of site-specific recombination and illegitimate recombination (the result of phage genome acquisition of bacterial host genetic sequences).

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4.7.2: RNA Bacteriophages

Nineteen families of bacteriophages that infect bacteria and archaea are currently recognized; of these, only two families have RNA genomes.

Learning Objectives

- Identify differences between bacterial ssRNA and dsRNA viruses

Key Points

- Cystovirus is a genus of dsRNA virus that infect certain Gram-negative bacteria. All cystoviruses are distinguished by their three strands of dsRNA and their protein and lipid outer layer. No other bacteriophage has any lipid in its outer coat.
- RNA -dependent RNA polymerases (RdRPs) are critical components in the life cycle of double-stranded RNA (dsRNA) viruses. However, it is not fully understood how these important enzymes function during viral replication.
- Bacteriophage Φ6 is a member of the Cystoviridae family that infects Pseudomonas bacteria (typically plant-pathogenic P. syringae). It is a lytic phage, though under certain circumstances has been observed to display a delay in lysis that may be described as a "carrier state".

Key Terms

- **RNA genome**: Like DNA, RNA can carry genetic information. RNA viruses have genomes composed of RNA that encodes a number of proteins.

Nineteen families of bacteriophages that infect bacteria and archaea are currently recognized. Of these, only two families have RNA genomes: Cystoviridae (segmented dsRNA) and Leviviridae (linear ssRNA).

The Leviviridae include the genera Allolevivirus (type species: *Enterobacteria phage Qβ*) and Levivirus (type species: *Enterobacteria phage MS2*).

Cystovirus is a genus of dsRNA virus that infect certain Gram-negative bacteria. All cystoviruses are distinguished by their three strands (analogous to chromosomes) of dsRNA, totalling ~14 kb in length, and by their protein and lipid outer layer. No other bacteriophage has any lipid in its outer coat, though the Tectiviridae and the Corticoviridae have lipids within their capsids.

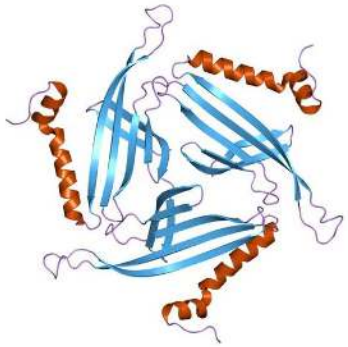


Figure: **Structure of bacteriophage PP7 from Pseudomonas aeruginosa**: Members of this protein family form the capsid of Pseudomonas phage PP7. They adopt a secondary structure consisting of a six-stranded beta sheet and an alpha helix.

Most identified cystoviruses infect Pseudomonas species, but this is likely biased due to the method of screening and enrichment. The type species is *Pseudomonas phage Φ6*, but there are many other members of this family: Φ7, Φ8, Φ9, Φ10, Φ11, Φ12, and Φ13 have been identified and named, but other cystoviruses have also been isolated.

Members of the Cystoviridae appear to be most closely related to the Reoviridae, but also share homology with the Totiviridae. Cystoviruses are the only bacteriophage that are more closely related to viruses of eukaryotes than to other phage.

Bacteriophage Φ6 is a member of the Cystoviridae family. It infects Pseudomonas bacteria (typically plant-pathogenic *P. syringae*). It has a three-part, segmented, double-stranded RNA genome, totalling ~13.5 kb in length. Φ6 and its relatives have a lipid

membrane around their nucleocapsid, a rare trait among bacteriophages. It is a lytic phage, though under certain circumstances has been observed to display a delay in lysis that may be described as a “carrier state. ”

Φ6 typically attaches to the Type IV pilus of *P. syringae* with its attachment protein, P3. It is thought that the cell then retracts its pilus, pulling the phage toward the bacterium. Fusion of the viral envelope with the bacterial outer membrane is facilitated by the phage protein, P6. The muralytic (peptidoglycan-digesting) enzyme, P5, then digests a portion of the cell wall, and the nucleocapsid enters the cell coated with the bacterial outer membrane.

RNA-dependent RNA polymerases (RdRPs) are critical components in the life cycle of double-stranded RNA (dsRNA) viruses. However, it is not fully understood how these important enzymes function during viral replication. Expression and characterization of the purified recombinant RdRP of Φ6 is the first direct demonstration of RdRP activity catalyzed by a single protein from a dsRNA virus. The recombinant Φ6 RdRP is highly active in vitro, possesses RNA replication and transcription activities, and is capable of using both homologous and heterologous RNA molecules as templates.

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4.7.3: Single-Stranded DNA Bacteriophages

Of the viral families with DNA genomes, only two have single-stranded genomes, the Inoviridae and the Microviridae.

Learning Objectives

- Illustrate the characteristics and life cycle of ssDNA bacteriophages

Key Points

- The Inoviridae are a family of filamentous bacteriophages. The virons are non-enveloped, rod-shaped, and filamentous. Viron release may involve host lysis, but alternatively productive infection may occur by budding from the host membrane.
- The Microviridae are a family of bacteriophages with a single-stranded DNA genome. Their genomes are among the smallest of the DNA viruses. Although the majority of species in this family have lytic life cycles, a few may have temperate life cycles.
- The Microviridae are divided into two subfamilies, Gokushovirinae and Microvirinae. These groups differ in their hosts, genome structure, and viron composition.

Key Terms

- **microviridae**: The Microviridae are a family of bacteriophages with a single-stranded DNA genome.
- **virion**: A single individual particle of a virus (the viral equivalent of a cell).

Nineteen viral families are currently recognized that infect bacteria and archaea. Of these, only two families have RNA genomes and only five are enveloped. Of the viral families with DNA genomes, only two have single-stranded genomes.

Inoviridae

The Inoviridae are a family of filamentous bacteriophages. The name of the family is derived from the Greek *nos*, meaning “muscle. ”

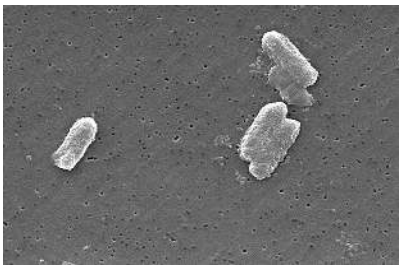


Figure: **Citrobacter freundii**: Citrobacter freundii, one member of the family Enterobacteriaceae. The genus Inovirus infect species of Enterobacteriaceae.

Taxonomy

There are two genera in this family: Inovirus and Plectrovirus. These genera differ in their host range: Plectovirii infect hosts of the class Mollicutes, while Inovirii infect species of the Enterobacteriaceae, Pseudomonadaceae, Spirillaceae, Xanthomonadaceae, Clostridium, and Propionibacterium classes.

Physical and Genomic Properties

Inoviridae are non-enveloped, rod-shaped, and filamentous. The capsid has a helical symmetry, and in general has a length of 85-280 nm or 760-1950 nm and a width of 10-16 nm or 6-8 nm, respectively. These morphological differences depend on the species.

The genomes are non-segmented, circular, positive-sense, single-stranded DNA, 4.4-8.5 kilobases in length. They encode 4 to 11 proteins. Replication of the genome occurs via a dsDNA intermediate and the rolling circle mechanism. Gene transcription is by the host’s cellular machinery, as each gene has a specific promoter.

Life Cycle

There are six steps in the life cycle of Inoviridae:

1. Adsorbion to the host via specific receptor(s)
2. Movement of the viral DNA into the host cell
3. Conversion of the single-strand form to a double-stranded intermediate
4. Replication of the viral genome
5. Synthesis of the new virions
6. Release of the new virions from the host

Action in Host

Conversion from single-stranded to double-stranded form is carried out by the host's own DNA polymerase. The host's RNA polymerase binds to the viral genome and syntheses RNA. Some of this RNA is translated and the remainder is used to initiate DNA replication.

Virion release may involve host lysis, but alternatively productive infection may occur by budding from the host membrane. This pattern is typically seen in the Plectivirus genus. A number of exceptions to this life cycle are known. Lysogenic species, which encode integrases, exist within this family.

Microviridae

The Microviridae are a family of bacteriophages with a single-stranded DNA genome. The name of this family is derived from the Greek *micro*, meaning "small. " This refers to the size of their genomes, which are among the smallest of the DNA viruses.

Taxonomy

This family is divided into two subfamilies, Gokushovirinae (derived from the Japanese for "very small") and Microvirinae. These groups differ in their hosts, genome structure, and virion composition.

Gokushoviruses are currently known to infect only obligate intra-cellular parasites. These species are members of the genera Bdellovibrio, Chlamydia, and Spiroplasma. Subfamily Microvirinae are all of the genus Microvirus. All seven such members infect Enterobacteria.

Physical and Genomic Properties

Members of the subfamily Gokushovirus have only two structural proteins: capsid proteins F (Virus Protein 1) and DNA pilot protein H (Virus Protein 2) and do not use scaffolding proteins. They also possess 'mushroom-like' protrusions positioned at the three-fold axes of symmetry of their icosahedral capsids. These are formed by large insertion loops within the protein F of gokushoviruses and are absent in the microviruses. They lack both the external scaffolding protein D and the major spike protein G of the species in the genus Microvirus. The genomes of this group tend to be smaller, about 4.5 kb in length. This subfamily includes the genera Bdellomicrovirus, Chlamydiamicrovirus, and Spiromicrovirus.

Microviridae are non-enveloped and round with an icosahedral symmetry. They have a diameter between 25-27 nanometers and lack tails. Each virion has 60 copies each of the F, G, and J proteins and 12 copies of the H protein. Viruses in this family replicate their genomes via a rolling circle mechanism and encode dedicated RCR initiation proteins. Although the majority of species in this family have lytic life cycles, a few may have temperate life cycles.

Life Cycle

There are a number of steps in the life cycle:

1. Adsorbion to the host via specific receptor(s)
2. Movement of the viral DNA into the host cell
3. Conversion of the single-strand form to a double-stranded intermediate, known as the replicative form I
4. Transcription of early genes
5. Replication of the viral genome
6. Late genes are now transcribed by the host's RNA polymerase

7. Synthesis of the new virions
8. Maturation of the virions in the host cytoplasm
9. Release from the host

Action in Host

Cell lysis is mediated by the phiX174-encoded protein E, which inhibits the peptidoglycan synthesis, leading to the eventual bursting of the infected cell.

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4.7.4: Double-Stranded DNA Bacteriophages

The dsDNA tailed phages, or Caudovirales, account for 95% of all known phages and possibly make up the majority of phages on the planet.

Learning Objectives

- Describe dsDNA bacteriophages

Key Points

- The Caudovirales are an order of viruses also known as the tailed bacteriophages. The virus particles have a distinct shape; each virion has an icosohedral head that contains the viral genome, and is attached to a flexible tail by a connector protein.
- The order encompasses a wide range of viruses, many of which contain genes of similar nucleotide sequence and function. Some tailed bacteriophage genomes can vary quite significantly in nucleotide sequence, even among the same genus. There are at least 350 recognized species in this order.
- Because of the lack of homology between the amino acid and DNA sequences of these viruses, the three families here are defined based on morphology: the Myoviridae have long contractile tails, the Podoviridae have short noncontractile tails, and the Siphoviridae have long non-contractile tails.

Key Terms

- **Caudovirales**: A taxonomic order within the kingdom Virus—the bacteriophages that have tails.

The Double-Stranded DNA (dsDNA) tailed phages, or Caudovirales, account for 95% of all the phages reported in the scientific literature, and possibly make up the majority of phages on the planet. Nineteen families that infect bacteria and archaea are currently recognized; of these, 15 have double-stranded DNA genomes.

Under the Baltimore classification scheme, the Caudovirales are group I viruses as they have double-stranded DNA (dsDNA) genomes, which can be anywhere from 18,000 base pairs to 500,000 base pairs in length. The virus particles have a distinct shape; each virion has an icosohedral head that contains the viral genome, and is attached to a flexible tail by a connector protein. The order encompasses a wide range of viruses, many of which contain genes of similar nucleotide sequence and function. Some tailed bacteriophage genomes can vary quite significantly in nucleotide sequence, however, even among the same genus. Due to their characteristic structure and possession of potentially homologous genes, it is believed these bacteriophages possess a common origin. There are at least 350 recognized species in this order.

Upon encountering a host bacterium, the tail section of the virion binds to receptors on the cell surface and delivers the DNA into the cell by use of an injectisome-like mechanism (an *injectisome* is a nanomachine that evolved for the delivery of proteins by type III secretion). The tail section of the virus punches a hole through the bacterial cell wall and plasma membrane and the genome passes down the tail into the cell. Once inside, the genes are expressed from transcripts made by the host machinery, using host ribosomes. Typically, the genome is replicated by use of concatemers, in which overlapping segments of DNA are made, and then put together to form the whole genome.

Viral capsid proteins come together to form a precursor prohead, into which the genome enters. Once this has occurred, the prohead undergoes maturation by cleavage of capsid subunits to form an icosohedral phage head with 5-fold symmetry. After the head maturation, the tail is joined in one of two ways: either the tail is constructed separately and joined with the connector, or the tail is constructed directly onto the phage head. The tails consist of helix-based proteins with 6-fold symmetry. After maturation of virus particles, the cell is lysed by lysins, holins, or a combination of the two.

Because the lack of homology between the amino acid and DNA sequences of these viruses precludes these from being used as taxonomic markers (as is common for other organisms), the three families here are defined on the basis of morphology. This classification scheme was originated by Bradley in 1969 and has since been extended. All viruses in this order have icosahedral or oblate heads, but differ in the length and contractile abilities of their tails. The Myoviridae have long tails that are contractile, the Podoviridae have short noncontractile tails, and the Siphoviridae have long non-contractile tails. Siphoviridae constitute the majority of the known tailed viruses.

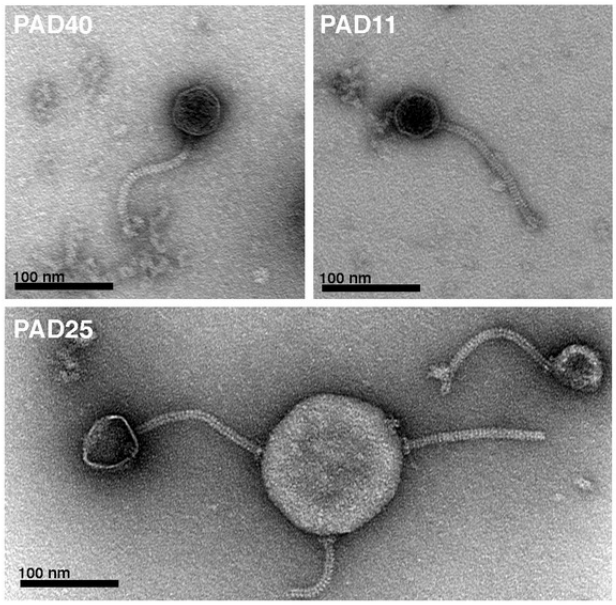


Figure: **Siphovirus phages**: Electron micrographs of bacteriophages from P. acnes. Phages were negatively stained with 0.75% uranyl formate and subjected to transmission electron microscopy. The phages have a head of approximately 55 nm in diameter, loaded with genetic material. Their tails have a size of 150 × 10 nm and are flexible and non-contractile. In the lower micrograph, PAD25 is adhering to bacterial cell debris, and two phages have lost their heads. At the attachment site between the phage and the cell debris, a base plate with attached spikes can be observed. All phages were classified as Siphoviruses based on their morphology.

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4.7.5: Mu- A Double-Stranded Transposable DNA Bacteriophage

Bacteriophage Mu is a temperate bacteriophage that uses DNA-based transposition in its lysogenic cycle.

Learning Objectives

- Outline the life cycle of Mu phages

Key Points

- All of the known temperate phages employ one of only three different systems for their lysogenic cycle: lambda-like integration/excision, Mu-like transposition, or the plasmid-like partitioning of phage N15.
- Phage Mu uses DNA -based transposition to integrate its genome into the genome of the host cell that it is infecting. It can then use transposition to initiate its viral DNA replication.
- Mu phage transposition is the best-known example of replicative transposition. Its transposition mechanism is somewhat similar to a homologous recombination.

Key Terms

- **replicative transposition**: A mechanism of transposition in which the transposable element is duplicated during the reaction, so that the transposing entity is a copy of the original element.

Bacteriophage Mu, or phage Mu, is a temperate bacteriophage, a type of virus that infects bacteria. It belongs to the family Myoviridae, and consists of an icosahedral head, a contractile tail, and six tail fibers.

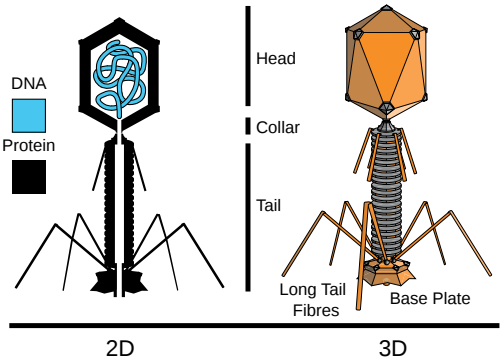


Figure: **Myoviridae**: Structural overview of the T4 phage, from the same family (Myoviridae) as Mu bacteriophage.

All of the known temperate phages employ one of only three different systems for their lysogenic cycle: lambda-like integration/excision, Mu-like transposition, or the plasmid-like partitioning of phage N15.

Mu bacteriophage uses DNA-based transposition to integrate its genome into the genome of the host cell that it is infecting. It can then use transposition to initiate its viral DNA replication. Once the viral DNA is inserted into the bacteria, the Mu's transposase protein/enzyme in the cell recognizes the recombination sites at the ends of the viral DNA (gix-L and gix-R sites) and binds to them, allowing the process of replicating the viral DNA or embedding it into the host genome. A transposable element (TE) is a DNA sequence that can change its relative position (self-transpose) within the genome of a single cell. The mechanism of transposition can be either "copy and paste" or "cut and paste. " Transposition can create phenotypically significant mutations and alter the cell's genome size.

Mu phage transposition is the best known example of replicative transposition. Its transposition mechanism is somewhat similar to a homologous recombination. Replicative transposition is a mechanism of transposition in molecular biology, proposed by James A. Shapiro in 1979, in which the transposable element is duplicated during the reaction, so that the transposing entity is a copy of the original element. In this mechanism, the donor and receptor DNA sequences form a characteristic intermediate "theta" configuration, sometimes called a "Shapiro intermediate. " Replicative transposition is characteristic to retrotransposons and occurs from time to time in class II transposons.

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4.7.6: Virulent Bacteriophages and T4

T-4 bacteriophage is a virulent bacteriophage that infects E. coli bacteria; virulent bacteriophages have a lytic life cycle.

Learning Objectives

- Summarize how the T4 life cycle serves as a model for viral virulence

Key Points

- Virulence is the degree of pathogenicity within a group or species of parasites as indicated by case fatality rates and/or the ability of the organism to invade the tissues of the host. The pathogenicity of an organism is determined by its virulence factors.
- A key difference between the lytic and lysogenic phage cycles is that in the lytic phage, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterial DNA.
- The T-4's tail fibres allow attachment to a host cell, and the T4's tail is hollow so that it can pass its nucleic acid to the cell it is infecting during attachment. T4 is capable of undergoing only a lytic lifecycle and not the lysogenic lifecycle.

Key Terms

- **lytic cycle**: The normal process of viral reproduction involving penetration of the cell membrane, nucleic acid synthesis, and lysis of the host cell.
- **virulence**: the degree of pathogenicity within a group or species of parasites as indicated by case fatality rates and/or the ability of the organism to invade the tissues of the host.

Virulence is the degree of pathogenicity within a group or species of parasites as indicated by case fatality rates and/or the ability of the organism to invade the tissues of the host. The pathogenicity of an organism is determined by its virulence factors. Virus virulence factors determine whether an infection will occur and how severe the resulting viral disease symptoms are. Viruses often require receptor proteins on host cells to which they specifically bind. Typically, these host cell proteins are endocytosed and the bound virus then enters the host cell. Virulent viruses such as HIV, which causes AIDS, have mechanisms for evading host defenses.

Some viral virulence factors confer ability to replicate during the defensive inflammation responses of the host such as during virus-induced fever. Many viruses can exist inside a host for long periods during which little damage is done. Extremely virulent strains can eventually evolve by mutation and natural selection within the virus population inside a host. The term "neurovirulent" is used for viruses such as rabies and herpes simplex which can invade the nervous system and cause disease there.

Model organisms of virulent viruses that have been extensively studied include virus T4 and other T-even bacteriophages which infect Escherichia coli and a number of related Bacteria.

The lytic cycle is one of the two cycles of viral reproduction, the other being the lysogenic cycle. The lytic cycle is typically considered the main method of viral replication, since it results in the destruction of the infected cell. A key difference between the lytic and lysogenic phage cycles is that in the lytic phage, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterial DNA. The location of viral DNA in the lysogenic phage cycle is within the host DNA, therefore in both cases the virus/phage replicates using the host DNA machinery, but in the lytic phage cycle, the phage is a free floating separate molecule to the host DNA.

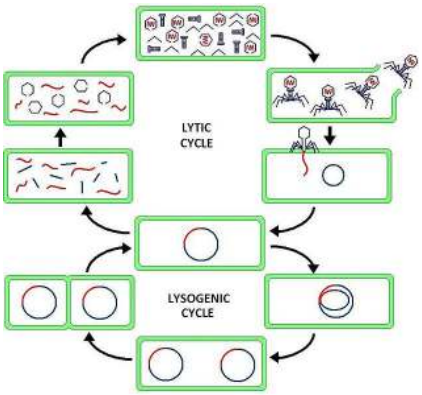


Figure: **Cycles of viral reproduction**: Comparison of the bacteriophage lysogenic and lytic cycles.

The lytic cycle is a six-stage cycle. In the first stage, called “penetration,” the virus injects its own nucleic acids into a host cell. Then the viral acids form a circle in the center of the cell. The cell then mistakenly copies the viral acids instead of its own nucleic acids. Then the viral DNA organize themselves as viruses inside the cell. When the number of viruses inside becomes too much for the cell to hold, the membrane splits and the viruses are free to infect other cells. Some viruses escape the host cell without bursting the cell membrane; instead, they bud off from it by taking a portion of the membrane with them. Because it otherwise is characteristic of the lytic cycle in other steps, it still belongs to this category, although it is sometimes named the Productive Cycle. HIV, influenza and other viruses that infect eukaryotic organisms generally use this method.

T-4 bacteriophage is a bacteriophage that infects E. coli bacteria. Its double-stranded DNA genome is about 169 kbp long and is held in an icosahedral head, also known as a capsid. T4 is a relatively large phage, at approximately 90 nm wide and 200 nm long (most phages range from 25 to 200 nm in length). Its tail fibres allow attachment to a host cell, and the T4’s tail is hollow so that it can pass its nucleic acid to the cell it is infecting during attachment. T4 is capable of undergoing only a lytic lifecycle and not the lysogenic lifecycle.

The T4 Phage initiates an E. coli infection by recognizing cell surface receptors of the host with its long tail fibers (LTF). A recognition signal is sent through the LTFs to the baseplate. This unravels the short tail fibers (STF) that bind irreversibly to the E. coli cell surface. The baseplate changes conformation and the tail sheath contracts causing GP5 at the end of the tail tube to puncture the outer membrane of the cell. The lysozyme domain of GP5 is activated and degrades the periplasmic peptidoglycan layer. The remaining part of the membrane is degraded and then DNA from the head of the phage can travel through the tail tube and enter the E. coli.

The lytic lifecycle (from entering a bacterium to its destruction) takes approximately 30 minutes (at 37 °C) and consists of:

- Adsorption and penetration (starting immediately)
- Arrest of host gene expression (starting immediately)
- Enzyme synthesis (starting after 5 minutes)
- DNA replication (starting after 10 minutes)
- Formation of new virus particles (starting after 12 minutes)

After the life cycle is complete, the host cell bursts open and ejects the newly built viruses into the environment, destroying the host cell. T4 has a burst size of approximately 100-150 viral particles per infected host. Complementation, deletion, and recombination tests can be used to map out the rII gene locus by using T4. These bacteriophage infect a host cell with their information and then blow up the host cell, thereby propagating themselves.

The T4 phage has some unique features, including:

- Eukaryote-like introns
- High speed DNA copying mechanism, with only 1 error in 300 copies
- Special DNA repair mechanisms
- It infects E. coli O157:H7

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4.7.7: Temperate Bacteriophages - Lambda and P1

In virology, temperate refers to the ability of some bacteriophages to display a lysogenic life cycle.

Learning Objectives

- Evaluate the differences between the temperate phages, P1, and lambda

Key Points

- Many temperate phages can integrate their genomes into their host bacterium ‘s chromosome, together becoming a lysogen as the phage genome becomes a prophage. A temperate phage is also able to undergo a productive, typically-lytic life cycle.
- P1 is a temperate bacteriophage (phage) that infects Escherichia coli and some other bacteria. A unique feature of phage P1 is that during lysogeny its genome is not incorporated into the bacterial chromosome, as is commonly observed during lysogeny of other bacteriophage.
- Enterobacteria phage λ (lambda phage, coliphage λ) is a bacterial virus, or bacteriophage, that infects the bacterial species Escherichia coli.
- With the infection of a bacteria by phage, a lytic cycle usually ensues where the lambda DNA is replicated many times and the genes for head, tail, and lysis proteins are expressed. Under certain conditions the phage DNA may integrate itself into the host cell chromosome in the lysogenic pathway.

Key Terms

- **lytic life cycle**: One of the two cycles of viral reproduction (the other being the lysogenic cycle). The lytic cycle is typically considered the main method of viral replication and it results in the destruction of the infected cell.
- **temperate bacteriophage**: Phages able to undergo lysogeny.

In virology, temperate refers to the ability of some bacteriophages (notable coliphage λ) to display a lysogenic life cycle. Many (but not all) temperate phages can integrate their genomes into their host bacterium’s chromosome, together becoming a lysogen as the phage genome becomes a prophage. A temperate phage is also able to undergo a productive, typically-lytic life cycle, where the prophage is expressed, replicates the phage genome, and produces phage progeny, which then leave the bacterium. With phage the term *virulent* is often used as an antonym to *temperate*, but more strictly a virulent phage is one that has lost its ability to display lysogeny through mutation, rather than a phage lineage with no genetic potential to ever display lysogeny (which more properly would be described as an obligately lytic phage).

P1 is a temperate bacteriophage (phage) that infects *Escherichia coli* and some other bacteria. When undergoing a lysogenic cycle, the phage genome exists as a plasmid in the bacterium, unlike other phages (e.g., the lambda phage) that integrate into the host DNA. P1 has an icosahedral “head” containing the DNA, attached to a contractile tail with six tail fibers.

The virion is similar in structure to the T4 phage, but simpler. It has an icosahedral head containing the genome attached at one vertex to the tail. The tail has a tube surrounded by a contractile sheath, and ends in a base plate with six tail fibers. The tail fibers are involved in attaching to the host and providing specificity.

At around 93Kbp in length, the genome of the P1 phage is moderately large compared to the genomes of others, like T4 (169Kbp), lambda (48Kbp), and Ff (6.4Kbp). In the viral particle it is in the form of a linear double-stranded DNA molecule. Once inserted into the host, it circularizes and replicates as a plasmid.

Temperate phage, such as P1, have the ability to exist within the bacterial cell they infect in two different ways. In lysogeny, P1 can exist within a bacterial cell as a circular DNA, in that it exists by replicating as if it were a plasmid and does not cause cell death. Alternatively, in its lytic phase, P1 can promote cell lysis during growth, resulting in host cell death. During lysogeny, new phage particles are not produced. In contrast, during lytic growth many new phage particles are assembled and released from the cell. By alternating between these two modes of infection, P1 can survive during extreme nutritional conditions that may be imposed upon the bacterial host in which it exists.

A unique feature of phage P1 is that during lysogeny its genome is not incorporated into the bacterial chromosome, as is commonly observed during lysogeny of other bacteriophage. Instead, P1 exists independently within the bacterial cell, much like a plasmid

would. P1 replicates as a 90 kilobase (kb) plasmid in the lysogenic state and is partitioned equally into two new daughter cells during normal cell division.

Enterobacteria phage λ (lambda phage, coliphage λ) is a bacterial virus, or bacteriophage, that infects the bacterial species *Escherichia coli*. This virus is temperate and may reside within the genome of its host through lysogeny.

Lambda phage consists of a virus particle including a head (also known as a capsid), a tail, and tail fibers. The head contains the phage's double-stranded circular DNA genome. The phage particle recognizes and binds to its host, *E. coli*, causing DNA in the head of the phage to be ejected through the tail into the cytoplasm of the bacterial cell. Usually, a "lytic cycle" ensues, where the lambda DNA is replicated many times and the genes for head, tail, and lysis proteins are expressed. This leads to assembly of multiple new phage particles within the cell and subsequent cell lysis, releasing the cell contents, including virions that have been assembled, into the environment. However, under certain conditions the phage DNA may integrate itself into the host cell chromosome in the lysogenic pathway. In this state, the λ DNA is called a prophage and stays resident within the host's genome without apparent harm to the host. The host can be termed a lysogen when a prophage is present.

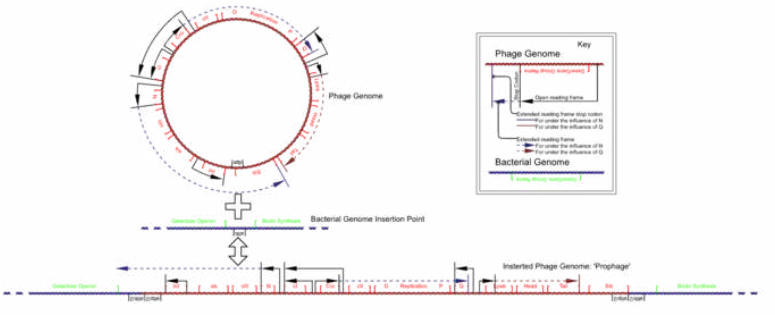


Figure: **Insertion of the bacteriophage lambda**: Schematic representation of the insertion of the bacteriophage lambda. Note how sib is displaced by the recombination event from the N extended PL promoter open reading frame.

The virus particle consists of a head and a tail that can have tail fibers. The head contains 48,490 base pairs of double-stranded, linear DNA, with 12-base single-stranded segments at both 5′ ends. These two single-stranded segments are the "sticky ends" of what is called the cos site. The cos site circularizes the DNA in the host cytoplasm. In its circular form, the phage genome therefore is 48,502 base pairs in length. The prophage exists as a linear section of DNA inserted into the host chromosome.

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4.7.8: Viruses of Archaea

LEARNING OBJECTIVES

- Illustrate the typical characteristics of archaea-infecting viruses

A virus infecting archaea was first described in 1974. Several others have been described since then. Most have head-tail morphologies and linear double-stranded DNA genomes. Other morphologies have also been described including spindle shaped, rod shaped, filamentous, icosahedral, and spherical. Additional morphological types may exist.

Archaea can be infected by double-stranded DNA viruses that are unrelated to any other form of virus and have a variety of unusual shapes. These viruses have been studied in the most detail in thermophilics, particularly the orders Sulfolobales and Thermoproteales. Two groups of single-stranded DNA viruses that infect archaea have been recently isolated. One group is exemplified by the Halorubrum pleomorphic virus 1 ("Pleolipoviridae") infecting halophilic archaea and the other one by the Aeropyrum coil-shaped virus.

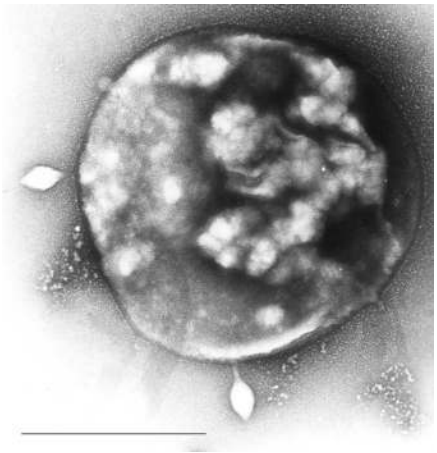


Figure: **Archaeal viral infection**: Cell of Sulfolobus infected by virus STSV1 observed under microscopy. Two spindle-shaped viruses were being released from the host cell. The strain of Sulfolobus and STSV1 (Sulfolobus tengchongensis Spindle-shaped Virus 1) were isolated by Xiaoyu Xiang and his colleagues in an acidic hot spring in Yunnan Province, China. At present, STSV1 is the largest archaeal virus to have been isolated and studied. Its genome sequence has been sequenced.

Double-stranded DNA viruses infecting archaea:

- Bacteriophages (viruses infecting bacteria) belonging to the families Tectiviridae and Corticoviridae have a lipid bilayer membrane inside the icosahedral protein capsid and the membrane surrounds the genome. The crenarchaeal virus Sulfolobus turreted icosahedral virus has a similar structure.
- Species of the order Ligamenvirales and the families Ampullaviridae, Bicaudaviridae, Clavaviridae, Fuselloviridae, Globuloviridae, and Guttaviridae infect hyperthermophilic archaea species of the Crenarchaeota.
- Species of the genus Salterprovirus infect halophilic archaea species of the Euryarchaeota.

Single-stranded DNA viruses infecting archaea:

Although around 50 archaeal viruses are known, all but two have double stranded genomes. The first archaeal ssDNA virus to be isolated is the Halorubrum pleomorphic virus 1, which has a pleomorphic enveloped virion and a circular genome. Defenses against these viruses may involve RNA interference from repetitive DNA sequences that are related to the genes of the viruses.

The second single stranded DNA virus infecting Archaea is Aeropyrum coil-shaped virus (ACV). The genome is circular and with 24,893 nucleotides is currently the largest known ssDNA genome. The viron is nonenveloped, hollow, cylindrical, and formed from a coiling fiber. The morphology and the genome appear to be unique. ACV has been suggested to represent a new viral family tentatively called "Spiraviridae" (from Latin spira, "a coil"). The Aeropyrum coil-shaped virus infects a hyperthermophilic (optimal growth at 90-95°C) host. Notably, the latter virus has the largest currently reported ssDNA genome.

Key Points

- Archaea can be infected by double-stranded DNA viruses that are unrelated to any other form of virus and have a variety of unusual shapes. These viruses have been studied in most detail in thermophilics, particularly the orders Sulfolobales and Thermoproteales.
- Although around 50 archaeal viruses are known, all but two have double stranded genomes; two groups of single-stranded DNA viruses that infect archaea have been recently isolated.
- Defenses against these ssDNA viruses may involve RNA interference from repetitive DNA sequences that are related to the genes of the viruses.

Key Terms

- **DNA virus**: A DNA virus is a virus that has DNA as its genetic material and replicates using a DNA-dependent DNA polymerase. The nucleic acid is usually double-stranded DNA (dsDNA) but may also be single-stranded DNA (ssDNA).

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SECTION OVERVIEW

4.8: Positive-Strand RNA Viruses in Animals

Topic hierarchy

4.8.1: Positive-Strand RNA Viruses of Animals

4.8.2: Virus Attachment and Genome Entry

4.8.3: Viral Replication and Gene Expression

4.8.4: Viral Exit

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4.8.1: Positive-Strand RNA Viruses of Animals

Positive-Strand RNA Viruses of Animals

Positive strand RNA viruses are the single largest group of RNA viruses with 30 families.

LEARNING OBJECTIVES

Categorize the characteristics of positive-strand viruses

KEY TAKEAWAYS

Key Points

- Nucleic acid is usually single-stranded RNA (ssRNA), but may be double-stranded RNA (dsRNA).
- An RNA virus is a virus that has RNA (ribonucleic acid) as its genetic material.
- Notable human diseases caused by RNA viruses include SARS, influenza, hepatitis C, West Nile fever, and polio.

Key Terms

- **virus**: A submicroscopic infectious organism, now understood to be a non-cellular structure consisting of a core of DNA or RNA surrounded by a protein coat. It requires a living cell to replicate, and often causes disease in the host organism.
- **genetic**: Relating to genetics or genes.
- **RNA**: Ribonucleic acid (RNA) is a ubiquitous family of large biological molecules that performs multiple vital roles in the coding, decoding, regulation, and expression of genes.

Single stranded RNA viruses can be classified according to the sense or polarity of their RNA into negative-sense and positive-sense, or ambisense RNA viruses. Positive-sense viral RNA is similar to mRNA and thus can be immediately translated by the host cell. Negative-sense viral RNA is complementary to mRNA and thus must be converted to positive-sense RNA by an RNA polymerase before translation. As such, purified RNA of a positive-sense virus can directly cause infection though it may be less infectious than the whole virus particle. Purified RNA of a negative-sense virus is not infectious by itself as it needs to be transcribed into positive-sense RNA; each virion can be transcribed to several positive-sense RNAs. Ambisense RNA viruses resemble negative-sense RNA viruses, except they also translate genes from the positive strand. A common viral positive-strand RNA viruses that infect humans are the picornaviruses.



Figure: **Foot and Mouth Disease**: Foot and Mouth Disease is caused by the Aphthovirus virus which positive-strand RNA virus, of the Picornaviridae family of animal viruses.

A picornavirus is a virus belonging to the family Picornaviridae. Picornaviruses are non-enveloped, positive-stranded RNA viruses with an icosahedral capsid. The genome RNA is unusual because it has a protein on the 5′ end that is used as a primer for transcription by RNA polymerase. The name is derived from pico, meaning small, and RNA, referring to the ribonucleic acid genome, so “picornavirus” literally means small RNA virus. Picornaviruses are separated into a number of genera and include many important pathogens of humans and animals. The diseases they cause are varied, ranging from acute “common-cold”-like illnesses, to poliomyelitis, to chronic infections in livestock. Additional species not belonging to any of the recognized genera continue to be described.

Picornaviruses are separated into a number of genera. Contained within the picornavirus family are many organisms of importance as vertebrate and human pathogens, shown in the table below.Enteroviruses infect the enteric tract, which is reflected in their name. On the other hand, rhinoviruses infect primarily the nose and the throat. Enteroviruses replicate at 37°C, whereas rhinoviruses grow better at 33°C, as this is the lower temperature of the nose. Enteroviruses are stable under acid conditions and thus they are able to

survive exposure to gastric acid. In contrast, rhinoviruses are acid-labile (inactivated or destroyed by low pH conditions) and that is the reason why rhinovirus infections are restricted to the nose and throat.

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4.8.2: Virus Attachment and Genome Entry

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface.

Learning Objectives

- Differentiate virus attachment and genome entry

Key Points

- Viral entry is the earliest stage of infection in the viral life cycle, as the virus comes into contact with the host cell and introduces viral material into the cell.
- Attachment to the receptor can induce the viral envelope protein to undergo changes that results in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.
- Virions enter the host cell through receptor-mediated endocytosis or membrane fusion.

Key Terms

- **capsid**: The outer protein shell of a virus.
- **receptors**: In the field of biochemistry, a receptor is a molecule most often found on the surface of a cell, which receives chemical signals originating externally from the cell.
- **virions**: An entire virus particle, consisting of an outer protein shell called a capsid and an inner core of nucleic acid.

Viral populations do not grow through cell division, because they are acellular. Instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble in the cell. The life cycle of viruses differs greatly between species, but they all share the same basic life cycle stages.

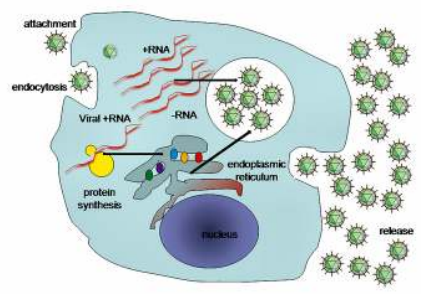


Figure: **A typical virus replication cycle**: There are six basic stages in the life cycle of viruses: attachment, penetration, uncoating, replication, assembly of viral particles, and release.

Attachment is a specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus. For example, HIV infects a limited range of human leukocytes. This is because its surface protein, gp120, specifically interacts with the CD4 molecule, a chemokine receptor, which is most commonly found on the surface of CD4+ T-Cells. This mechanism has evolved to favor those viruses that infect only cells in which they are capable of replication. Attachment to the receptor can induce the viral envelope protein to undergo changes that results in the fusion of viral and cellular membranes, or changes of non-enveloped virus surface proteins that allow the virus to enter.

Penetration follows attachment: Virions enter the host cell through receptor-mediated endocytosis or membrane fusion. This is often called “viral entry. ” The infection of plant and fungal cells is different from that of animal cells. Plants have a rigid cell wall made of cellulose, and fungi one of chitin, so most viruses can get inside these cells only after trauma to the cell wall. However, nearly all plant viruses (such as tobacco mosaic virus) can also move directly from cell to cell, in the form of single-stranded nucleoprotein complexes, through pores called “plasmodesmata”. Bacteria, such as plants, have strong cell walls that a virus must breach to infect the cell. However, given that bacterial cell walls are less thick than plant cell walls due to their much smaller size, some viruses have evolved mechanisms that inject their genome into the bacterial cell across the cell wall, while the viral capsid remains outside

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4.8.3: Viral Replication and Gene Expression

RNA viruses are classified into distinct groups depending on their genome and mode of replication.

Learning Objectives

- Examine viral gene expression during virus replication

Key Points

- Positive sense, negative sense, double stranded viruses, and retroviruses are RNA viruses with different modes of replication.
- Positive-sense ssRNA viruses (Group IV) have their genome directly utilized as if it were mRNA.
- Replication of viruses involves primarily multiplication of the genome.
- The polarity of single-stranded RNA viruses largely determines the replicative mechanism.

Key Terms

- **genome**: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of basepairs.
- **virus**: A submicroscopic infectious organism, now understood to be a non-cellular structure consisting of a core of DNA or RNA surrounded by a protein coat. It requires a living cell to replicate, and often causes disease in the host organism.
- **replication**: Process by which an object, person, place or idea may be copied mimicked or reproduced.

Replication of viruses primarily involves the multiplication of the viral genome. Replication also involves synthesis of viral messenger RNA (mRNA) from “early” genes (with exceptions for positive sense RNA viruses), viral protein synthesis, possible assembly of viral proteins, then viral genome replication mediated by early or regulatory protein expression. This may be followed, for complex viruses with larger genomes, by one or more further rounds of mRNA synthesis: “late” gene expression is, in general, necessary for structural or virion proteins. Viral replication usually takes place in the cytoplasm.

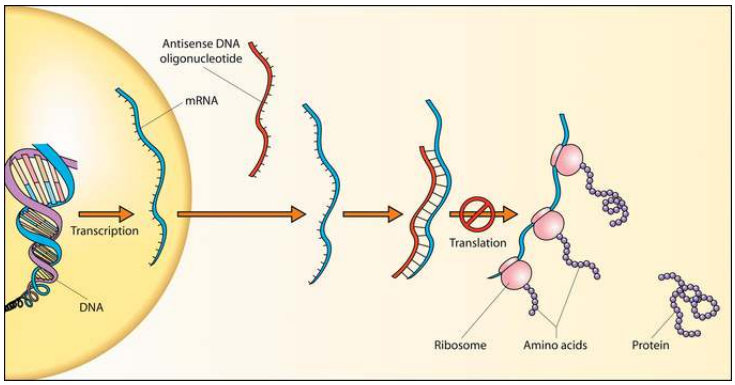


Figure: **DNA to RNA to protein**: Schematic showing how antisense DNA strands can interfere with protein translation.

Viruses that replicate via RNA intermediates need an RNA-dependent RNA- polymerase to replicate their RNA, but animal cells do not seem to possess a suitable enzyme. Therefore, this type of animal RNA virus needs to code for an RNA-dependent RNA polymerase. No viral proteins can be made until viral messenger RNA is available; thus, the nature of the RNA in the virion affects the strategy of the virus: In plus-stranded RNA viruses, the virion (genomic) RNA is the same sense as mRNA and so functions as mRNA. This mRNA can be translated immediately upon infection of the host cell. Examples: poliovirus (picornavirus), togaviruses, and flaviviruses.

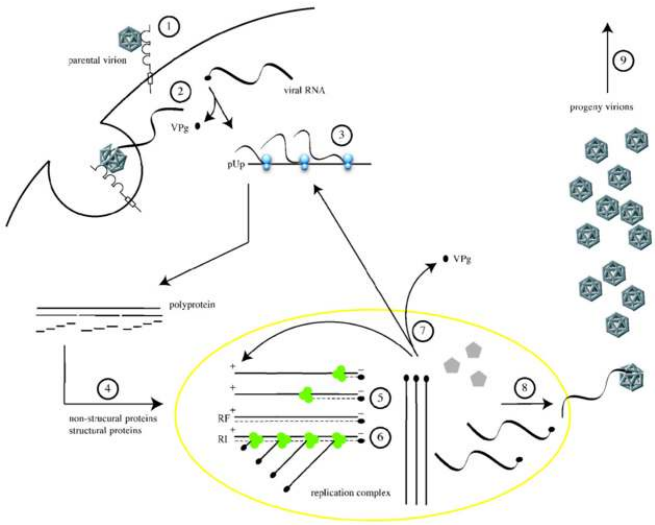


Figure: **The replication cycle of poliovirus**: The cellular life cycle of poliovirus. It is initiated by binding of a poliovirion to the cell surface macromolecule CD155, which functions as the receptor (1). Uncoating of the viral RNA is mediated by receptor-dependent destabilization of the virus capsid (2). Cleavage of the viral protein VPg is performed by a cellular phosphodiesterase, and translation of the viral RNA occurs by a cap-independent (IRES-mediated) mechanism (3). Proteolytic processing of the viral polyprotein yields mature structural and non-structural proteins (4). The positive-sense RNA serves as template for complementary negative-strand synthesis, thereby producing a double-stranded RNA (replicative form, RF) (5). Initiation of many positive strands from a single negative strand produces the partially single-stranded replicative intermediate (RI) (6). The newly synthesized positive-sense RNA molecules can serve as templates for translation (7) or associate with capsid precursors to undergo encapsidation and induce the maturation cleavage of VP0 (8), which ultimately generates progeny virions. Lysis of the infected cell results in release of infectious progeny virions (9).

RNA viruses are classified into distinct groups depending on their genome and mode of replication (and the numerical groups based on the older Baltimore classification).

Positive-sense ssRNA viruses (Group IV) have their genome directly utilized as if it were mRNA, with host ribosomes translating it into a single protein which is modified by host and viral proteins to form the various proteins needed for replication. One of these includes RNA-dependent RNA polymerase (RNA replicase), which copies the viral RNA to form a double-stranded replicative form, in turn this directs the formation of new virions.

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4.8.4: Viral Exit

Viruses are released from the host cell following assembly.

Learning Objectives

- Explain how viruses exit host cells

Key Points

- Once replication has been completed and the host cell is exhausted of all resources in making viral progeny, the viruses may begin to leave the cell by several methods.
- Viral exit methods include budding, exocytosis, and cell lysis.
- Budding through the cell envelope, in effect using the cell's membrane for the virus itself is most effective for viruses that need an envelope.
- This process will slowly use up the cell membrane and eventually lead to the demise of the cell.

Key Terms

- **exocytosis**: The secretion of substances through cellular membranes, either to excrete waste products or as a regulatory function
- **lysogenic**: Of, relating to, or causing lysis.
- **virions**: An entire virus particle, consisting of an outer protein shell called a capsid and an inner core of nucleic acid.

Viral populations do not grow through cell division because they are acellular. Instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble in the cell. The life cycle of viruses differs between species, but follows the same basic stages.

Viruses can be released from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present. This is a feature of many bacterial and some animal viruses. Some viruses undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a " provirus " or, in the case of bacteriophages a "prophage. " Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host. However, at some point, the provirus or prophage may give rise to active virus, which may lyse the host cells. Enveloped viruses (e.g., HIV) typically are released from the host cell by budding. During this process the virus acquires its envelope, which is a modified piece of the host's plasma or other, internal membrane.

Viral shedding refers to the successful reproduction, expulsion, and host-cell infection caused by virus progeny. Once replication has been completed and the host cell is exhausted of all resources in making viral progeny, the viruses may begin to leave the cell by several methods.

Different types of virus have varying sites of synthesis and replication. For example, synthesis and replication for DNA viruses occur in the cell's nucleus while it is usually the cytoplasm for RNA viruses. Virus assembly depends on the site of synthesis and such sites are the nucleus, endoplasmic reticulum, and the Golgi apparatus aka Golgi body. Aside from this, assembly also occurs in the viroplasm which is an inclusion body in a cell. When the virus has replicated and multiplied, they would want to leave the infected cell and infect other cells. However, they require an envelope to enclose the DNA as well as to bind with the other healthy cells so that they can infect. The viral envelope is the typical lipid bilayer, derived from the host cell itself and sources usually come from the nuclear membrane, endoplasmic reticulum, Golgi apparatus/body, and plasma membrane. It also depends on where the virus 'bud' off from the host.

Budding is a method which viruses use to exit the cell. "Budding" through the cell envelope, in effect using the cell's membrane for the virus itself is most effective for viruses that need an envelope in the first place. These include enveloped viruses such as HSV, SARS, or smallpox. Prior to budding, the virus may put its own receptor onto the surface of the cell in preparation for the virus to bud through, forming an envelope with the viral receptors already on it. This process will slowly use up the cell membrane and eventually lead to the demise of the cell. This is also how antiviral responses are able to detect virus infected cells.

Other methods for exit would be cell lysis. This method releases the virus from the infected cell by bursting its membrane and this kills the cell as well. Another method is by accumulating the virus particles in vesicles and releasing them via exocytosis.

Exocytosis is the process where vesicles containing the virus are secreted/excreted out of the infected cell.

Positive-strand RNA mature virions are infectious. Virions are released following cell lysis. Excess capsids are formed and inclusion bodies may be seen in the cytoplasm.

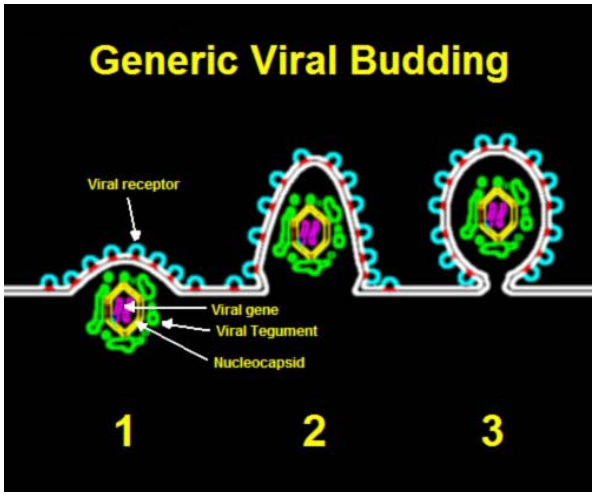


Figure: **Generic Viral Budding**: Viral budding uses the host's cell membrane eventually causing cell death.

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SECTION OVERVIEW

4.9: Negative-Strand RNA Viruses in Animals

Topic hierarchy

4.9.1: Negative-Strand RNA Viruses of Animals

4.9.2: Attachment and Entry to the Host Cell

4.9.3: Replicative Cycle of Influenza A

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4.9.1: Negative-Strand RNA Viruses of Animals

Negative-strand RNA viruses are single-stranded viruses that can infect several types of animals.

Learning Objectives

- Explain the mechanism of genome replication in negative-strand RNA viruses

Key Points

- Negative-strand RNA viruses can infect animals, but in several cases they can go from animals into humans, such as the SARS virus of the Ebola Zaire virus.
- The viron RNA is negative sense (complementary to mRNA and cannot encode proteins), which means it must be replciated over to mRNA before protein production can begin. This is carried out by an RNA-dependent RNA-polymerase.
- Negative-strand viruses can be found in many niches of Earth and are responsible for many common and very deleterious diseases of animals.

Key Terms

- **SARS**: The SARS coronavirus, sometimes shortened to SARS-CoV, is the virus that causes severe acute respiratory syndrome (SARS).
- **RNA-dependent RNA-polymerase**: (RdRP, or RNA replicase) An enzyme that catalyzes the replication of RNA from an RNA template. This is in contrast to a typical DNA-dependent RNA polymerase, which catalyzes the transcription of RNA from a DNA template.

The study of animal viruses is important from a veterinary viewpoint, but many animal viruses are also important from a human medical perspective. The emergence of the SARS virus or Ebola Zaire virus in the human population, coming from an animal source, highlights the importance of animals in bearing infectious agents. In addition, research into animal viruses has made an important contribution to our understanding of viruses in general, including their replication, molecular biology, evolution, and interaction with the host. Animal RNA viruses can be classified according to the sense or polarity of their RNA into negative-sense, positive-sense, or ambisense RNA viruses.



Figure: **Rabies**: Note the salivia dripping from the dog's mouth, a typical sign of a rabies infection. The infection of domestic animals with rabies was common until the 1960s; now most instances of rabies-infected animals are found in the wild.

The RNA found in a negative-sense virus is not infectious by itself, as it needs to be transcribed into positive-sense RNA. The complementary plus-sense mRNA must be made before proteins can be translated from the viral genome. This RNA negative-strand to positive-strand copying is carried out by an RNA-dependent RNA-polymerase. Each virion that has one negative-strand copy can be transcribed to several positive-sense RNAs. There are several different types of negative-strand RNA viruses that infect animals; two families will be discussed here in further detail.

Rhabdoviruses are a diverse family of single-stranded, negative-sense RNA viruses that can successfully utilize a myriad of ecological niches, ranging from plants and insects, to fish and mammals. This virus family includes pathogens —the rabies virus,

vesicular stomatitis virus, potato yellow dwarf virus, etc.—that are of tremendous public health, veterinary, and agricultural significance. Due to the relative simplicity of their genomes and morphology, in recent years rhabdoviruses have become powerful model systems for studying molecular virology.

Paramyxoviruses are a diverse family of non-segmented negative-strand RNA viruses that include many highly pathogenic viruses affecting humans, animals, and birds. In recent years the advent of reverse genetics has led to a greater understanding of their genomics, molecular biology, and viral pathogenesis. Paramyxoviruses cause a range of diseases in animal species: canine distemper virus (dogs), phocine distemper virus (seals), cetacean morbillivirus (dolphins and porpoises), Newcastle disease virus (birds), and rinderpest virus (cattle). Some paramyxoviruses, such as the henipaviruses, are zoonotic pathogens, occurring naturally in an animal host, but also able to infect humans.

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4.9.2: Attachment and Entry to the Host Cell

For influenza viral propagation to begin, there first must be viron attachment and entry into a host cell.

Learning Objectives

- Explain the role of hemagglutinin in the attachment and entry processes of influenza virus

Key Points

- A glycoprotein on the surface of a virus recognizes a receptor on a host, beginning the attachment process.
- After attachment, the influenza virus is brought into the host cell through an endosome. The low pH of the endosome breaks down the viral capsid and releases the viral contents into the cell.
- A well-described example of this is the influenza virus that relies on hemagglutinin, the glycoprotein that allows the influenza virus to attach to target cells, in this case cells in the human respiratory pathway.

Key Terms

- **endosome**: An endocytic vacuole through which molecules are internalized during endocytosis pass, en route to lysosomes.
- **glycoprotein**: A protein with covalently bonded carbohydrates.
- **sialic**: Of or pertaining to sialic acid or its derivatives.

One of the best understood examples of virus entry into the host cell is the influenza viral infection. The glycoprotein responsible for attachment on the surface of an influenza viral particle is hemagglutinin (HA). HA is an antigenic glycoprotein. It is responsible for binding the virus to the cell that is being infected. HA proteins bind to cells with sialic acid on the membranes, such as cells in the upper respiratory tract or erythrocytes.

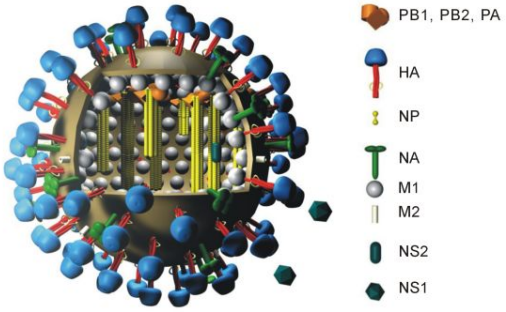


Figure: **Swine influenza**: A depiction of the different structures present on and in an influenza virus. Of special note is HA (hemagglutinin), the glycoprotein critical for influenza attachment and entry into host cells.

HA has two functions. First, it allows the recognition of target vertebrate cells, accomplished through the binding of these cells' sialic acid-containing receptors. Second, once bound, it facilitates the entry of the viral genome into the target cells by causing the fusion of the host endosomal membrane with the viral membrane. HA binds to the monosaccharide sialic acid that is present on the surface of its target cells, which causes the viral particles to stick to the cell's surface. The cell membrane then engulfs the virus and the portion of the membrane that encloses it pinches off to form a new membrane-bound compartment within the cell called an endosome, which contains the engulfed virus. The cell then attempts to begin digesting the contents of the endosome by acidifying its interior and transforming it into a lysosome.

However, as soon as the pH within the endosome drops to about 6.0, the original folded structure of the HA molecule becomes unstable, causing it to partially unfold and release a very hydrophobic portion of its peptide chain that was previously hidden within the protein. This so-called "fusion peptide" acts like a molecular grappling hook by inserting itself into the endosomal membrane and locking on. Then, when the rest of the HA molecule refolds into a new structure (which is more stable at the lower pH), it "retracts the grappling hook" and pulls the endosomal membrane right up next to the virus particle's own membrane, causing the two to fuse together. Once this has happened, the contents of the virus, including its RNA genome, are free to pour out into the cell's cytoplasm.

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4.9.3: Replicative Cycle of Influenza A

LEARNING OBJECTIVES

- Contrast the roles of hemagglutinin and neuraminidase throughout the major stages of the replicative cycle of influenza A virus

Influenza A follows the typical life cycle of most influenza viruses. The infection and replication is a multi-step process:

- Binding to and entering the cell
- Delivering the genome to a site where it can produce new copies of viral proteins and RNA
- Assembling these components into new viral particles
- Exiting the host cell

Influenza viruses bind through hemagglutinin onto sialic acid sugars on the surfaces of epithelial cells, typically in the nose, throat, and lungs of mammals, and the intestines of birds (Step 1 in infection figure). After the hemagglutinin is cleaved by a protease, the cell imports the virus by endocytosis.

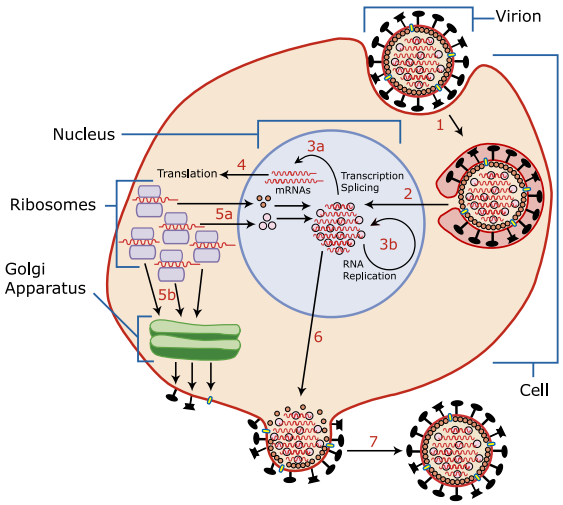


Figure: ***Influenza replication cycle****: Host invasion and replication cycle of an influenza virus. Step 1: Binding Step 2: Entry Step 3: Complex formation and transcription Step 4: Translation Step 5: Secretion Step 6: Assembly Step 7: Release*

The intracellular details are still being worked out. It is known that virions converge to the microtubule organizing center, interact with acidic endosomes, and finally enter the target endosomes for genome release. Once inside the cell, the acidic conditions in the endosome cause two events to happen:

1. The hemagglutinin protein fuses the viral envelope with the vacuole's membrane.
2. The M2 ion channel allows protons to move through the viral envelope and acidify the core of the virus, which causes the core to dissemble and release the viral RNA and core proteins.

The viral RNA (vRNA) molecules, accessory proteins, and RNA-dependent RNA polymerase are then released into the cytoplasm (Step 2 in figure). These core proteins and vRNA form a complex that is transported into the cell nucleus, where the RNA-dependent RNA polymerase begins transcribing complementary positive-sense vRNA (Steps 3a and b in figure).

The vRNA either enters into the cytoplasm and translated (Step 4) or remains in the nucleus. Newly synthesized viral proteins are either secreted through the Golgi apparatus onto the cell surface (in the case of neuraminidase and hemagglutinin, Step 5b) or transported back into the nucleus to bind vRNA and form new viral genome particles (Step 5a).

Other viral proteins have multiple actions in the host cell—including degrading cellular mRNA and using the released nucleotides for vRNA synthesis, and also inhibiting translation of host-cell mRNAs.

Negative-sense vRNAs that form the genomes of future viruses, RNA-dependent RNA polymerase, and other viral proteins are assembled into a virion. Hemagglutinin and neuraminidase molecules cluster into a bulge in the cell membrane. The vRNA and viral core proteins leave the nucleus and enter this membrane protrusion (Step 6). The mature virus buds off from the cell in a sphere of the host phospholipid membrane, acquiring hemagglutinin and neuraminidase with this membrane coat (Step 7). As before, the viruses adhere to the cell through hemagglutinin; the mature viruses detach once their neuraminidase has cleaved sialic acid residues from the host cell. Drugs that inhibit neuraminidase, such as oseltamivir, therefore prevent the release of new infectious viruses and halt viral replication. After the release of new influenza viruses, the host cell dies.

Key Points

- A component of the viral coat, hemagglutinin, binds to the surface of target cells. After binding, the virus fuses and is imported by endocytosis.
- Once the vRNA is released into the cytoplasm, it is transported into the nucleus where it is transcribed. The resulting vRNAs are then transported into the cytoplasm and new viral particles are made.
- The endosome ‘s acidic environment breaks down the viron coat, releasing the vRNA. The acidic environment also promotes the release of the vRNA to proteins that are bound to it.
- Once new viral particles are made in the host cell and bud off, the host cell dies.

Key Terms

- **neuraminidase**: An antigenic enzyme, found on the surfaces of viruses, that catalyzes the hydrolysis of terminal acylneuraminic residues from oligosaccharides, glycoproteins, and glycolipids.
- **hemagglutinin**: An antigenic glycoprotein that causes agglutination of red blood cells.
- **sialic**: Of or pertaining to sialic acid or its derivatives.

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SECTION OVERVIEW

4.10: 9. 10- Retroviruses- Double-Stranded RNA Viruses

Topic hierarchy

4.10.1: Double-Stranded RNA Viruses - Retroviruses

4.10.2: HIV Attachment and Host Cell Entry

4.10.3: Retroviral RNA Genome

4.10.4: Replicative Cycle of HIV

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4.10.1: Double-Stranded RNA Viruses - Retroviruses

Retroviruses are viruses that are able to reverse transcribe their RNA genome into DNA, which is then integrated into a host genome.

Learning Objectives

- Identify the unique features of retroviruses

Key Points

- In a double stranded RNA form, retroviruses infect a host cell with their genome, and then are reverse transcribed into double stranded DNA, with the DNA then integrated into the home cell genome.
- When integrated into a host genome, a retrovirus is hard to detect and can lay dormant for prolonged periods, having no discernible effect on the host.
- Retroviruses can be human pathogens, and cause many diseases, but have also proven to be invaluable tools when used by molecular biologists.

Key Terms

- **reverse transcriptase**: An enzyme that catalyzes the formation of DNA from RNA; found in retroviruses.
- **transposon**: A segment of DNA that can move to a different position within a genome.
- **integrase**: Any enzyme that integrates viral DNA into that of an infected cell.

A retrovirus is an RNA virus that is duplicated in a host cell using the reverse transcriptase enzyme to produce DNA from its RNA genome. The DNA is then incorporated into the host's genome by an integrase enzyme. The virus thereafter replicates as part of the host cell's DNA. Retroviruses are enveloped viruses that belong to the viral family Retroviridae. A special variant of retroviruses are endogenous retroviruses, which are integrated into the genome of the host and inherited across generations. Endogenous retroviruses are a type of transposon.

The virus itself stores its nucleic acid in the form of an mRNA genome and serves as a means of delivering that genome into cells it targets as an obligate parasite (a parasite that cannot live without its host). That process of delivering the genome into cells constitutes the infection. Once in the host's cell, the RNA strands undergo reverse transcription in the cytoplasm and are integrated into the host's genome, at which point the retroviral DNA is referred to as a provirus. It is difficult to detect the virus until it has infected the host, where the provirus can stay for months, even years, before becoming active and making new infectious viral particles.

In most viruses, DNA is transcribed into RNA, and then RNA is translated into protein. Retroviruses, however, function differently. Their RNA is reverse-transcribed into DNA, which is integrated into the host cell's genome (when it becomes a provirus), and then undergoes the usual transcription and translation processes to express the genes carried by the virus. So, the information contained in a retroviral gene is used to generate the corresponding protein via the sequence: RNA → DNA → RNA → protein. Retroviruses can be pathogens of many different hosts, including humans. A notable retrovirus is Human immunodeficiency virus (HIV), the virus responsible for acquired immunodeficiency syndrome (AIDS). As well as infecting a host, some retroviruses can cause cancer.

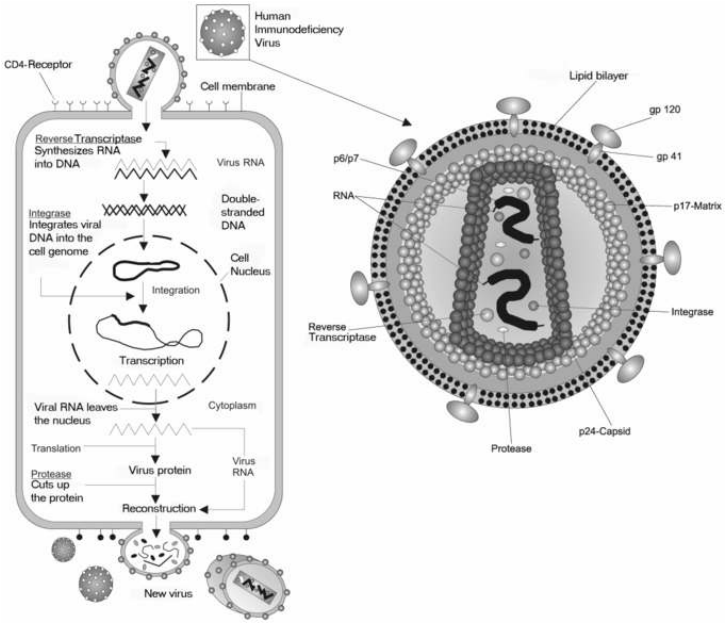


Figure: **HIV viral life cycle**: This diagram depicts the viral life cycle of HIV, from infection, integration into a host genome, reconstruction, and formation of new viral particles. The inset on the left depicts an individual HIV particle.

Finally, retroviruses are proving to be valuable research tools in molecular biology and have been successfully used in gene delivery systems.

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4.10.2: HIV Attachment and Host Cell Entry

The attachment and fusion of HIV virons to host cells are crucial to HIV infection.

Learning Objectives

- Define the unique aspects of HIV attachment and host cell entry

Key Points

- HIV proteins bind to specific receptors of host cells, most importantly HIV coat proteins gp41 and gp160.
- After attaching to the host, many of the receptors on both the cell and invading virus bind together for fusion of the virus with its host.
- Because HIV attachment is critical for the HIV replication cycle, understanding the specific mechanisms by which HIV attachment occurs has implications for potential treatments of HIV.

Key Terms

- **glycoprotein**: A protein with covalently bonded carbohydrates.
- **macrophages**: A type of white blood cell that targets foreign material, including bacteria and viruses.
- **capsid**: The outer protein shell of a virus.
- **chemokine**: Any of various cytokines, produced during inflammation, that organize the leukocytes.
- **T cells**: A lymphocyte, from the thymus, that can recognise specific antigens and can activate or deactivate other immune cells.

HIV entry is the earliest stage of infection in the HIV viral life cycle, occurring when the HIV virus comes into contact with the host cell and introduces viral material into the cell. HIV enters macrophages and CD4-positive T cells (CD4 is a glycoprotein receptor found on cells) by the adsorption of glycoproteins on its surface to receptors on the target cell, followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell.

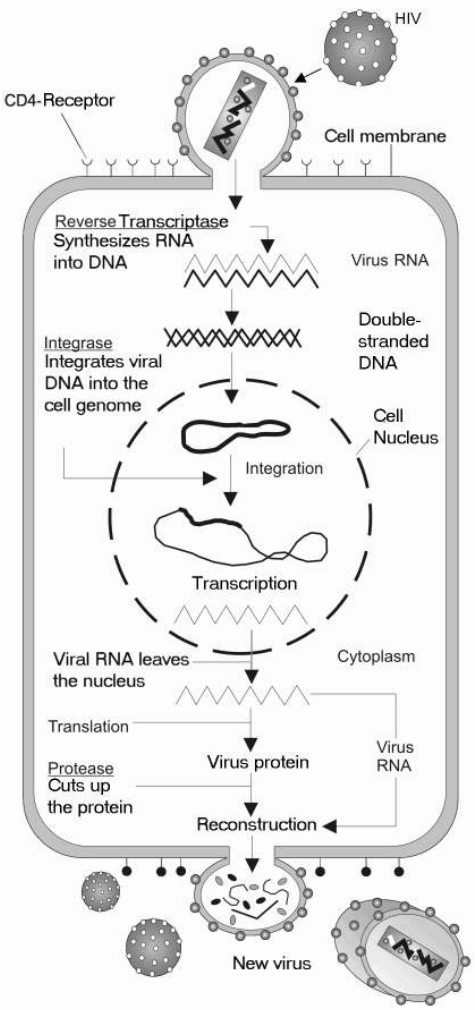


Figure: **HIV Replication**: Steps in the HIV Replication Cycle: Fusion of the HIV cell to the host cell surface.Cell Entry, HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.Viral DNA is formed by reverse transcription.Viral DNA is transported across the nucleus and integrates into the host DNA.New viral RNA is used as genomic RNA to make viral proteins.New viral RNA and proteins move to cell surface and a new, immature, HIV virus forms.Virus maturation and protease release of individual HIV proteins.

Entry to the cell begins through interaction of the trimeric envelope complex and both CD4 and a chemokine receptor on the host cell on the cell surface. The HIV coat protein, gp120, binds to integrin α4β7, activating LFA-1 (the central integrin involved in the establishment of bridges known as “virological synapses”) which facilitate efficient cell-to-cell spreading of HIV-1.

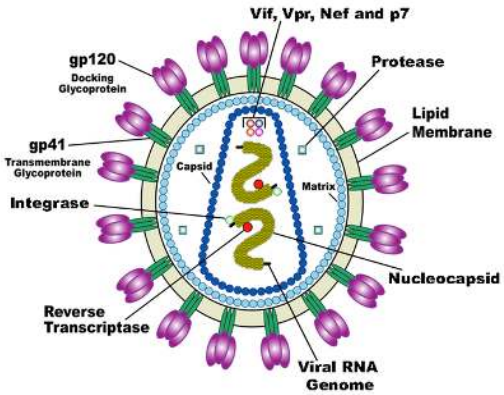


Figure: **Origin of HIV-1 M: An Example of Molecular Clock Application**: The attachment and fusion of HIV virons to host cells are crucial to allowing HIV infection to occur. Shown in purple is gp120 and in green gp41, two proteins crucial in viral docking to host cells.

After attachment, the HIV viron must next fuse with the host cell. The first step in fusion begins after the attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor. This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane. Repeat sequences in gp41, known as HR1 and HR2, then interact, causing the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membranes close together, allowing fusion of the membranes and subsequent entry of the viral capsid.

After HIV has bound to the target cell, the HIV RNA and various enzymes (including reverse transcriptase, integrase, ribonuclease, and protease) are injected into the cell. Because HIV attachment is critical for the HIV replication cycle, understanding the specific mechanisms through which HIV attachment occurs has implications for potential treatments of HIV.

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4.10.3: Retroviral RNA Genome

LEARNING OBJECTIVES

- Discover the features of retroviral genomes

The retroviral while in the viral capsid consists of a dimer RNA. It has a cap at the 5′ end and a poly(A) tail at the 3′ end. The RNA genome also has terminal noncoding regions, which are important in replication, and internal regions that encode virion proteins for gene expression.

The 5′ end includes four regions, which are R, U5, PBS, and L.

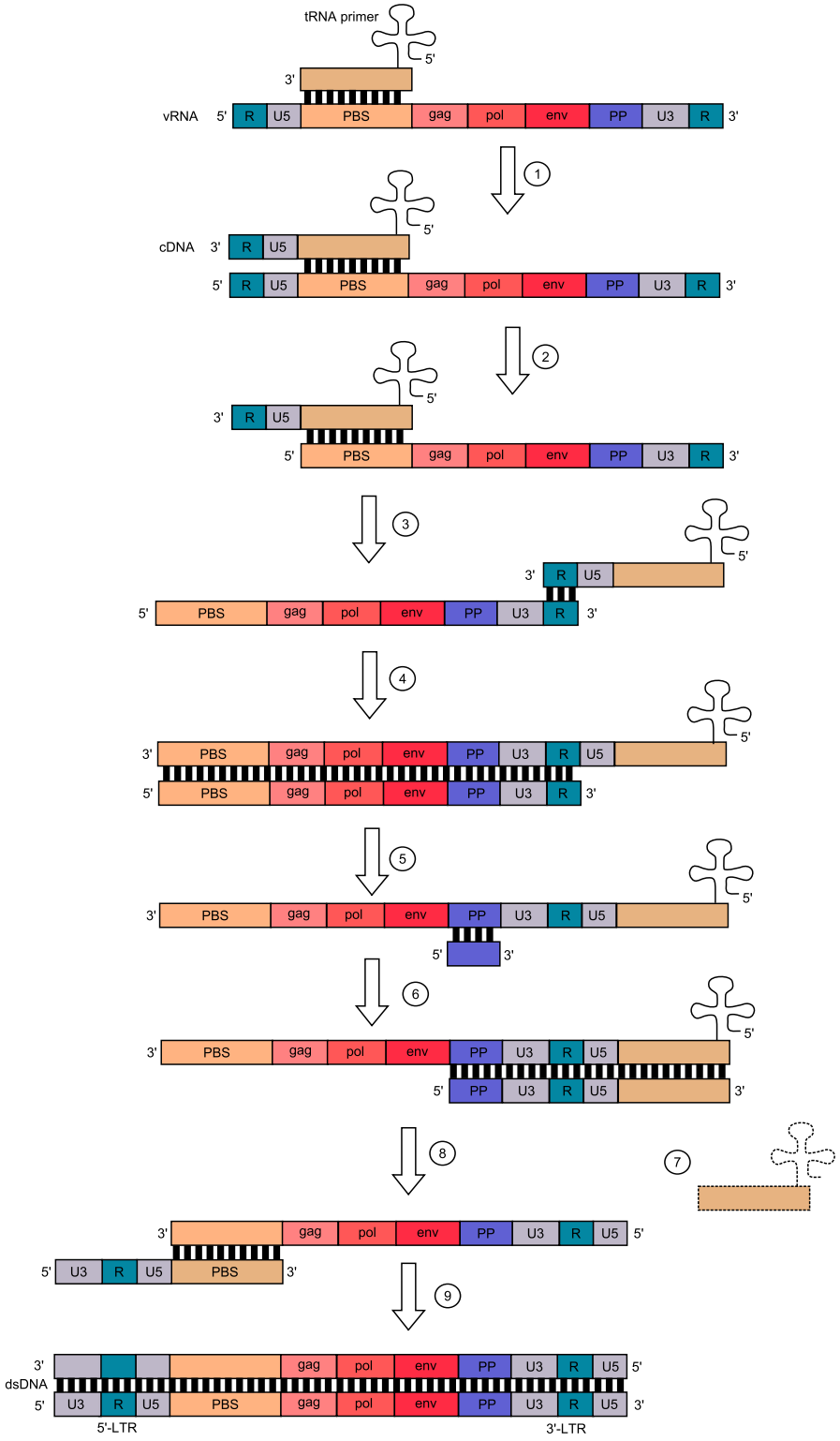
- The R region is a short repeated sequence at each end of the genome during the reverse transcription in order to ensure correct end-to-end transfer in growing chain.
- U5, on the other hand, is a short unique sequence between R and PBS.
- PBS (primer binding site) consists of 18 bases complementary to 3′ end of the tRNA primer, which supplies an ‘OH group to initiate reverse transcription.
- The L region is an untranslated leader region that give the signal for packaging of genome RNA.

The 3′ end includes 3 regions, which are PPT (polypurine tract), U3, and R.

- PPT (or PP), polypurine tract is the primer for plus-strand DNA synthesis during reverse transcription.
- U3 is a sequence between PPT and R, which has signal that provirus can use in transcription.
- R is the terminal repeated sequence at 3′ end, the same as the R (i.e., repeat region of the 5′ end).

Inbetween the 5′ and 3′ region is the protein encoding region of the retrovirus, consisting of gag proteins, protease (PR), pol proteins and env proteins. Gag proteins are major components of the viral capsid, which are about 2,000-4,000 copies per virion. Protease is expressed differently in different viruses. It functions in proteolytic cleavages during virion maturation to make mature gag and pol proteins. Pol proteins, such as the reverse transcriptase (RT), are responsible for synthesis of viral DNA and integration into host DNA after infection. Finally, env proteins play a role in association and entry of virion into the host cell. Possessing a functional copy of an env gene is what makes retroviruses distinct from retroelements. The env gene serves three distinct functions: enabling the retrovirus to enter/exit host cells through endosomal membrane trafficking, protection from the extracellular environment via the lipid bilayer, and the ability to enter cells. The ability of the retrovirus to bind to its target host cell using specific cell-surface receptors is given by the surface component (SU) of the env, while the ability of the retrovirus to enter the cell via membrane fusion is imparted by the membrane-anchored trans-membrane component (TM). Thus, the env protein is what enables the retrovirus to be infectious.

Please refer to the figure, in it you see all the elements of a retroviral genome and how they interact to contribute to retroviral reverse transcription and integration.



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Figure: ***Retroviral genome****: Through the mechanism of reverse transcription by human immunodeficiency virus (HIV), we see what the different genomic elements of a retrovirus are. Reverse transcription occurs in the cytoplasm of host cell. In this process, viral ssRNA is transcribed by the viral reverse transcriptase (RT) into double stranded DNA. Reverse transcription takes place in 5'→3' direction. tRNA ("cloverleaf") hybridizes to PBS and provides -OH group for initiation of reverse transcription. 1) Complementary DNA (cDNA) is formed. 2) Template in RNA:DNA hybrid is degraded by RNase H domain of reverse transcriptase 3) DNA:tRNA is transferred to the 3'-end of the template. 4) First strand synthesis takes place. 5) The rest of viral ssRNA is degraded by RNase H, except for the PP site. 6) Synthesis of second strand of ssDNA is initiated from the 3'-end of the template. tRNA is necessary to synthesis of complementary PBS 7) tRNA is degraded 8) PBS from the second strand hybridizes with the complementary PBS on the first strand. 9) Synthesis of both strands is completed by the DNA Polmerase function of reverse transcriptase. Both dsDNA ends have U3-R-U5 sequences, so called long terminal repeat sequences (3'LTR and 5'LTR, respectively). LTRs mediate integration of the retroviral DNA into another region of the host genome.Key: U3 – promoter region, U5 – recognition site for viral integrase; PBS – primer binding site; PP – polypurine section (polypurine tract); gag, pol, and env. Colors mark complementary sequences.*

Key Points

- The ends of a retrovirus, both the 5′ and 3′, contain many elements needed for the retroviral life cycle, including the regions referred to as R, U5, PBS, PPT, and U3.
- In between the 5′ and 3′ ends is the protein coding region which includes the gag, pol, and env encoding regions.
- Key to the unique attributes of a retrovirus is the pol region, which encodes a reverse trancriptase (RT), RT is the enzyme which takes the RNA form of the retrovirus genome and converts into DNA, the DNA form of which can integrate into the host genome.

Key Terms

- **protease**: An enzyme that cuts or cleaves proteins.
- **capsid**: The outer protein shell of a virus.

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4.10.4: Replicative Cycle of HIV

LEARNING OBJECTIVES

- Compare and contrast HIV replication to other viruses

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that causes acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. HIV can infect dendritic cells (DCs). DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs. HIV enters macrophages and T cells by the adsorption of glycoproteins on its surface to receptors on the target cell. This is followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell.

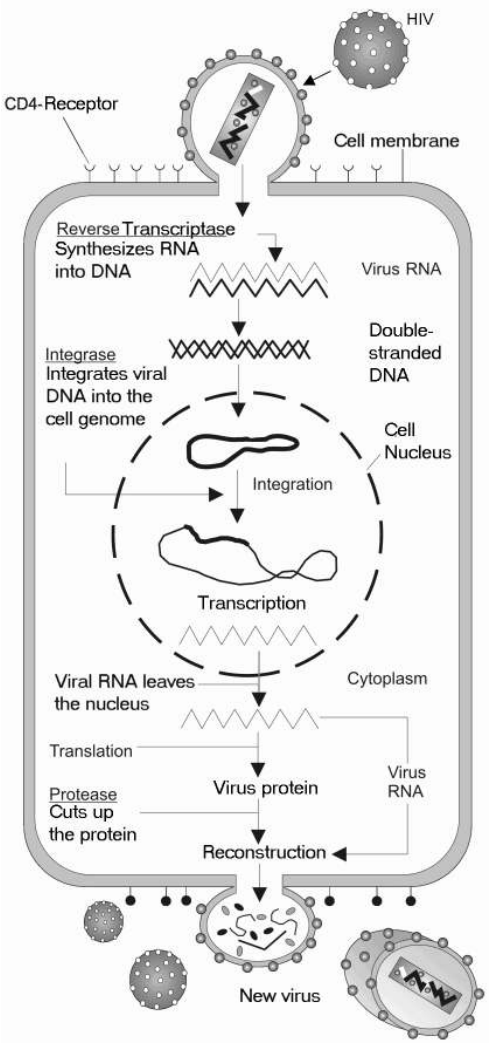


Figure: ***HIV Replication***: *Steps in the HIV Replication Cycle: Fusion of the HIV cell to the host cell surface.Cell Entry, HIV RNA, reverse transcriptase, integrase, and other viral proteins enter the host cell.Viral DNA is formed by reverse transcription.Viral DNA is transported across the nucleus and integrates into the host DNA.New viral RNA is used as genomic RNA to make viral proteins.New viral RNA and proteins move to cell surface and a new, immature, HIV virus forms.Virus maturation and protease release of individual HIV proteins.*

Shortly after the viral capsid enters the cell, an enzyme called reverse transcriptase liberates the single-stranded (+)RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule. The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune

system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the antisense cDNA. Together, the cDNA and its complement form a double-stranded viral DNA that is then transported into the cell nucleus.

This integrated viral DNA may then lie dormant, in the latent stage of HIV infection. To actively produce the virus, certain cellular transcription factors need to be present. The most important of these is NF-κB (NF kappa B), which is upregulated when T-cells become activated. This means that those cells most likely to be killed by HIV are those currently fighting infection. During viral replication, the integrated DNA provirus is transcribed into mRNA, which is then spliced into smaller pieces. These small pieces are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev.

As the newly produced Rev protein accumulates in the nucleus, it binds to viral mRNAs and allows unspliced RNAs to leave the nucleus, where they are otherwise retained until spliced. At this stage, the structural proteins Gag and Env are produced from the full-length mRNA. The full-length RNA is actually the virus genome; it binds to the Gag protein and is packaged into new virus particles. The final step of the viral cycle, assembly of new HIV-1 virions, begins at the plasma membrane of the host cell. The Env polyprotein goes through the endoplasmic reticulum and is transported to the Golgi complex. There, it is cleaved by HIV protease and processed into the two HIV envelope glycoproteins, gp41 and gp120. These are transported to the plasma membrane of the host cell where gp41 anchors gp120 to the membrane of the infected cell. The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell.

Maturation occurs either in the forming bud or in the immature virion after it buds from the host cell. During maturation, HIV proteases cleave the polyproteins into individual functional HIV proteins. This cleavage step can be inhibited by protease inhibitors. The various structural components then assemble to produce a mature HIV virion. The mature virion is then able to infect another cell.

Key Points

- First the HIV viron binds to host cell, after binding the virus and cell fuse, which releases the various enzymes HIV needs to reverse transcribe and integrate into the host genome.
- The reverse transcription of HIV viral RNA to DNA is error prone, causing HIV to have a high mutation rate. This makes it difficult to design treatments against HIV.
- The HIV provirus can stay dormant in the host genome for years. It may become active when the host T cell is itself activated by fighting an infection that the body is facing.
- Understanding the HIV life cycle will help in providing effective treatments against HIV.

Key Terms

- **provirus**: A virus genome, such as HIV, that integrates itself into the DNA of a host cell so as to be passively replicated along with the host genome.
- **reverse transcriptase**: An enzyme that catalyzes the formation of DNA from RNA; found in retroviruses.

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SECTION OVERVIEW

4.11: 9. 11- DNA Viruses in Eukaryotes

Topic hierarchy

4.11.1: Plant DNA Viruses

4.11.2: Replication of Double-Stranded DNA Viruses of Animals

4.11.3: Double-Stranded DNA Viruses - Herpesviruses

4.11.4: Attachment and Entry of Herpes Simplex

4.11.5: Replication of Herpes Simplex Virus

4.11.6: Immunodeficiency

4.11.7: Double-Stranded DNA Viruses - Pox Viruses

4.11.8: Double-Stranded DNA Viruses- Adenoviruses

4.11.9: Retroviruses and Hepadnavirus

4.11.10: Treatment of Animal Viral Infections

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4.11.1: Plant DNA Viruses

DNA viruses are relatively rare in plants, but are responsible for a significant amount of crop damage worldwide.

Learning Objectives

- Differentiate between ssDNA and dsDNA plant viruses

Key Points

- DNA viruses are relatively rare in plants, compared to their RNA counterparts.
- Like most viruses, the genomes of most single stranded DNA viruses are small, encoding only a few proteins, and are therefore dependent on host cell factors for replication.
- Double stranded DNA viruses only infect lower species of plants, such as algae. These viruses are huge dsDNA viruses with genomes ranging from 160 to 560 kb with up to 600 protein-encoding genes, making them distinctly different from viruses infecting higher plants.
- Plant viruses are generally spread through vectors, such as insects, but can also be passed from generation to generation.

Key Terms

- **Baltimore Classification System**: The Baltimore classification, developed by David Baltimore, is a virus classification system that groups viruses into families, depending on their type of genome (DNA, RNA, single-stranded (ss), double-stranded (ds), etc.) and their method of replication.
- **plasmodesmata**: Plasmodesmata (singular: plasmodesma) are microscopic channels which traverse the cell walls of plant cells and some algal cells, enabling transport and communication between them.
- **capsid**: The outer protein shell of a virus.

A DNA virus is a virus with DNA as its genetic material and replicates using a DNA-dependent DNA polymerase. DNA viruses belong to either Group I (double-stranded DNA; dsDNA) or Group II (single-stranded DNA; ssDNA) of the Baltimore classification system for viruses. Single-stranded DNA is usually expanded to double-stranded in infected cells.

DNA viruses are relatively rare in plants. Seventeen percent of plant viruses are ssDNA, while dsDNA viruses infect only lower plants, such as eukaryotic algae. The rarity of dsDNA plant viruses is notable when compared to other taxonomic kingdoms; a quarter of animal viruses and three quarters of bacterial viruses are dsDNA.

Plant viruses are transmitted through a variety of different methods, and generally require breach of protective barriers. Viruses can be spread by direct transfer of sap by contact of a wounded plant with a healthy one, most commonly resulting from agricultural practices, as by damage caused by tools or hands. More often, viruses are spread through vector intermediaries such as insects, nematodes, or protozoa which pick up viruses by feeding on infected plants, and then spread the virus to healthy plants.

Viral transmission from generation to generation occurs in about 20% of plant viruses. When viruses are transmitted by seeds, the seed is infected in the generative cells and the virus is maintained in the germ cells, or occasionally in the seed coat. Little is known about the mechanisms involved in the transmission of plant viruses via seeds, though environment is known to play a key role.

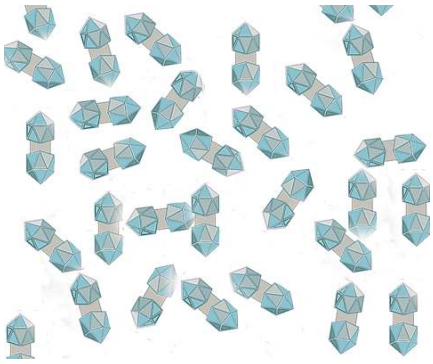


Figure: **Geminiviruses**: Drawing of geminiviruses, characterized by elongated, geminate capsids with two incomplete T=1 icosahedra joined at the missing vertex.

The *Geminiviridae* and *Nanoviridae* are the two families of ssDNA viruses known to infect plants. *Geminiviridae* is the largest known family of single-stranded DNA viruses. It contains a wide range of plant viruses including bean golden mosaic virus, beet curly top virus, maize streak virus, and tomato pseudo-curly top virus, which together are responsible for a significant amount of crop damage worldwide. The genome can either be a single component between 2500-3100 nucleotides, or, in the case of some begomoviruses, two similar-sized components each between 2600 and 2800 nucleotides. They have elongated, geminate capsids with two incomplete T=1 icosahedra joined at the missing vertex. The capsids range in size from 18-20 nm in diameter with a length of about 30 nm. Begomoviruses possess two component genomes separated into two different particles, both of which must usually be transmitted together to initiate a new infection within a suitable host cell. Like many viruses, geminivirus genomes encode only a few proteins, and are therefore dependent on host cell factors for replication. Geminiviruses replication occurs within the nucleus of an infected plant cell via a rolling circle mechanism, similar to that seen in bacteriophages, such as M13, and many plasmids. The resulting ssDNA is packaged into germinate particles in the nucleus. It is not clear if these particles can then leave the nucleus and be transmitted to surrounding cells as virions, or whether ssDNA is trafficked from cell to cell via the plasmodesmata. These viruses tend to be introduced into and initially infect differentiated plant cells, via the piercing mouthparts of the vector insect: however, these cells generally lack the host enzymes necessary for DNA replication, making it difficult for the virus to replicate. To overcome this block geminiviruses can induce plant cells to reenter the cell cycle from a quiescent state so that viral replication can occur.

The *Nanoviridae* are a family of single-stranded DNA viruses that infect plants. Their name is derived from the Greek word ‘nano’ (dwarf) because of their small genome and their stunting effect on infected plants.



Figure: **Maize Streak Virus**: The black-faced leafhopper (Graminella nigrifrons) transmits both maize fine streak virus and maize chlorotic dwarf virus.

Virions of this family have a capsid and are non-enveloped. The capsid is icosahedral with diameter of 18-20 nm. The genome is composed of 6 to 11 segments of single-stranded circular DNA each ~1 kb in length, with the exact number of segments varying depending on the genus. The segments each encode a single protein. There is a putative stem loop structure in the non-coding region of each segment which has a conserved 9-nucleotide sequence at its apex. Each member has up to 4 segments encoding replication proteins of ~33 kDa. The other segments encode products of 10-20 kDa in size and include a coat protein of ~19 kDa and a protein with a retinoblastoma binding motif.

Double-Stranded DNA Viruses

Double-stranded DNA viruses of plants are rare, and only infect lower plants, such as algae. These viruses (family *Phycodnaviridae*) are huge dsDNA viruses with genomes ranging from 160 to 560 kb with up to 600 protein-encoding genes, making them distinctly different from viruses infecting higher plants. They are found in aqueous environments throughout the world and play dynamic, albeit largely undocumented, roles in regulating algal communities such as the termination of massive algal blooms commonly referred to as red and brown tides.

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4.11.2: Replication of Double-Stranded DNA Viruses of Animals

Most double-stranded DNA viruses replicate within the host cell nucleus.

Learning Objectives

- Differentiate the ways which different classes of dsDNA viruses replicate

Key Points

- From the perspective of the virus, the purpose of viral replication is to allow production and survival of its kind.
- Most double-stranded DNA viruses replicate within the host cell nucleus, including polyomaviruses, adenoviruses, and herpesviruses—poxviruses, however, replicate in the cytoplasm.
- Adenoviruses and herpes viruses encode their own replication factors.

Key Terms

- **Okazaki fragments**: Okazaki fragments are short, newly synthesized DNA fragments that are formed on the lagging template strand during DNA replication.
- **polymerase**: Any of various enzymes that catalyze the formation of polymers of DNA or RNA using an existing strand of DNA or RNA as a template.

Viral replication is the formation of biological viruses during the infection process in the target host cells. Viruses must first get into the cell before viral replication can occur. From the perspective of the virus, the purpose of viral replication is to allow production and survival of its kind. By generating abundant copies of its genome and packaging these copies into viruses, the virus is able to continue infecting new hosts. Replication between viruses is greatly varied and depends on the type of genes involved in them. Most DNA viruses assemble in the nucleus while most RNA viruses develop solely in cytoplasm.

Double-stranded DNA viruses usually must enter the host nucleus before they are able to replicate. Some of these viruses require host cell polymerases to replicate their genome, while others, such as adenoviruses or herpes viruses, encode their own replication factors. However, in either cases, replication of the viral genome is highly dependent on a cellular state permissive to DNA replication and, thus, on the cell cycle. The virus may induce the cell to forcefully undergo cell division, which may lead to transformation of the cell and, ultimately, cancer. An example of a family within this classification is the Adenoviridae.

Polyomaviruses, adenoviruses, and herpesviruses are all nuclear-replicating DNA viruses, each with their own specific approaches to replication. There is only one well-studied example in which a double-stranded DNA virus does not replicate within the nucleus. This is the Poxvirus family, which comprises highly pathogenic viruses that infect vertebrates.

Polyomaviruses

Polyomaviridae is a family of viruses whose natural hosts are primarily mammals and birds. Most of these viruses, such as BK virus and JC virus, are very common and typically asymptomatic in most human populations studied. However, some polyomaviruses are associated with human disease, particularly in immunocompromised individuals. Some members of the family are oncoviruses, meaning they can cause tumors; they often persist as latent infections in a host without causing disease, but may produce tumors in a host of a different species, or in individuals with ineffective immune systems. The name polyoma refers to the viruses' ability to produce multiple (poly-) tumors (-oma).

Replication

Prior to genome replication, the processes of viral attachment, entry and uncoating occur. Polyomavirus virions are subsequently endocytosed and transported first to the endoplasmic reticulum where a conformational change occurs; then by an unknown mechanism the virus is exported to the nucleus. Polyomaviruses replicate in the nucleus of the host.

Adenoviruses

Adenoviruses (members of the family Adenoviridae) are medium-sized (90–100 nm), nonenveloped (without an outer lipid bilayer) viruses with an icosahedral nucleocapsid containing a double stranded DNA genome.

Adenoviruses represent the largest nonenveloped viruses. They are able to be transported through the endosome (i.e., envelope fusion is not necessary). The virion also has a unique “spike” or fiber associated with each penton base of the capsid that aids in attachment to the host cell via the receptor on the surface of the host cell.

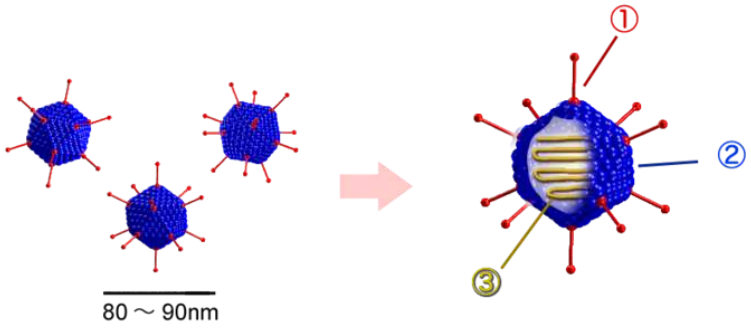


Figure: **Adenovirus structure**: Adenoviruses are non-enveloped (i.e., they have no external lipid bilayer) and are icosahedral (i.e., shaped like a polyhedron with 20 faces). They have fibers at their vertices that help them attach to host cells.

Replication

Adenoviruses possess a linear dsDNA genome and are able to replicate in the nucleus of vertebrate cells using the host’s replication machinery.

Once the virus has successfully gained entry into the host cell, the endosome acidifies, which alters virus topology by causing capsid components to disband, which in turn destroys the endosome and allows the virion entry into the cytoplasm. It is transported to the nuclear pore, disassembles, and is released into the nucleus. At this point viral gene expression can occur and new virus particles can be generated.

Herpesviruses

Herpesviridae is a large family of DNA viruses that cause diseases in animals, including humans. The members of this family are also known as herpesviruses. The family name is derived from the Greek word herpein (“to creep”), referring to the latent, recurring infections typical of this group of viruses. Herpesviridae can cause latent or lytic infections.

At least five species of Herpesviridae – HSV-1 and HSV-2 (both of which can cause orolabial herpes and genital herpes), Varicella zoster virus (which causes chicken-pox and shingles), Epstein-Barr virus (which causes mononucleosis), and Cytomegalovirus – are extremely widespread among humans. More than 90% of adults have been infected with at least one of these, and a latent form of the virus remains in most people. In total, there are 8 herpesvirus types that infect humans: herpes simplex viruses 1 and 2, varicella-zoster virus, EBV (Epstein-Barr virus), human cytomegalovirus, human herpesvirus 6, human herpesvirus 7, and Kaposi’s sarcoma-associated herpesvirus. There are more than 130 herpesviruses, and some are from mammals, birds, fish, reptiles, amphibians, and molluscs.

Replication

All herpesviruses are nuclear-replicating—the viral DNA is transcribed to mRNA within the infected cell’s nucleus. Infection is initiated when a viral particle contacts a cell with specific types of receptor molecules on the cell surface. Following binding of viral envelope glycoproteins to cell membrane receptors, the virion is internalized and dismantled, allowing viral DNA to migrate to the cell nucleus. Within the nucleus, replication of viral DNA and transcription of viral genes occurs.

Poxviruses

Poxviridae is a family of viruses. Human, vertebrates, and arthropods serve as natural hosts. There are currently 69 species in this family, divided among 28 genera, which are divided into two subfamilies. Diseases associated with this family include smallpox.

Poxviridae viral particles (virions) are generally enveloped (external enveloped virion- EEV), though the intracellular mature virion (IMV) form of the virus, which contains different envelope, is also infectious. The virion is exceptionally large—around 200 nm in diameter and 300 nm in length.

Replication

The replication of poxvirus is unusual for a virus with double-stranded DNA genome (dsDNA) because it occurs in the cytoplasm, although this is typical of other large DNA viruses. Poxvirus encodes its own machinery for genome transcription, a DNA dependent RNA polymerase, which makes replication in the cytoplasm possible. Most dsDNA viruses require the host cell's DNA-dependent RNA polymerase to perform transcription. These host DNA are found in the nucleus, and therefore most dsDNA viruses carry out a part of their infection cycle within the host cell's nucleus.

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4.11.3: Double-Stranded DNA Viruses - Herpesviruses

Herpes viruses cause a wide range of latent, recurring infections including oral and genital herpes, cytomegalovirus, and chicken pox.

Learning Objectives

- Recognize the attributes of herpes viruses

Key Points

- Herpesviridae is a large family of DNA viruses that cause diseases in animals, including humans.
- The structure of herpes viruses consists of a relatively large double-stranded, linear DNA genome encased within an icosahedral protein cage called the capsid, which is wrapped in a lipid bilayer called the envelope.
- Notable herpes viruses include herpes simplex viruses 1 and 2, Varicella zoster virus (the causative agent of shingles and chicken pox), cytomegalovirus, and Kaposi's sarcoma virus.
- There is no method to eradicate herpes virus from the body, but antiviral medications, such as acyclovir, can reduce the frequency, duration, and severity of outbreaks.

Key Terms

- **tegument**: A natural covering of the body or of a bodily organ.
- **capsid**: The outer protein shell of a virus.
- **virion**: A single individual particle of a virus (the viral equivalent of a cell).

Herpesviridae is a large family of DNA viruses that cause diseases in animals, including humans. The members of this family are also known as herpes viruses. The family name is derived from the Greek word *herpein* ("to creep"), referring to the latent, recurring infections typical of this group of viruses.

Animal herpes viruses all share some common properties. The structure of these viruses consists of a relatively large double-stranded, linear DNA genome encased within an icosahedral protein cage called the capsid, which is wrapped in a lipid bilayer called the envelope. The envelope is joined to the capsid by means of a tegument. This complete particle is known as the virion. HSV-1 and HSV-2 each contain at least 74 genes within their genomes, although speculation over gene crowding allows as many as 84 unique protein-coding genes by 94 putative pen reading frames. These genes encode a variety of proteins involved in forming the capsid, tegument and envelope of the virus, as well as controlling the replication and infectivity of the virus.

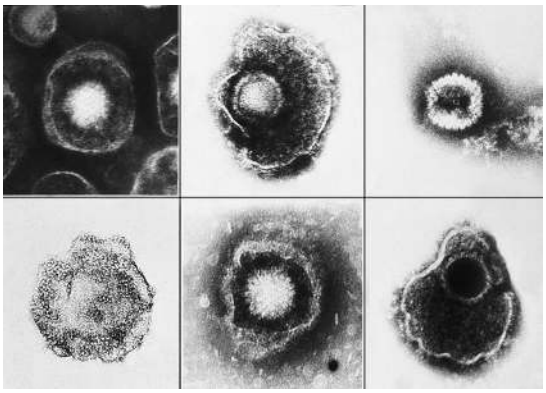


Figure: **Herpesviridae**: Various viruses from the Herpesviridae family seen using an electron micrograph Amongst these members is varicella-zoster (Chickenpox), and herpes simplex type 1 and 2 (HSV-1, HSV-2).

Types of herpes viruses

There are nine distinct herpes viruses which cause disease in humans:

- HHV-1 Herpes simplex virus-1 (HSV-1)
- HHV-2 Herpes simplex virus-2 (HSV-2)

- HHV-3 Varicella zoster virus (VZV)
- HHV-4 Epstein-Barr virus (EBV)
- HHV-5 Cytomegalovirus (CMV)
- HHV-6A/B Roseolovirus, Herpes lymphotropic virus
- HHV-7 Pityriasis Rosea
- HHV-8 Kaposi's sarcoma-associated herpesvirus

Of particular interest include HSV-1 and HSV-2, which cause oral and/or genital herpes, HSV-3 which causes chickenpox and shingles, and HHV-5 which causes mononucleosis-like symptoms, and HHV-8 which causes a Kaposi's sarcoma, a cancer of the lymphatic epithelium.

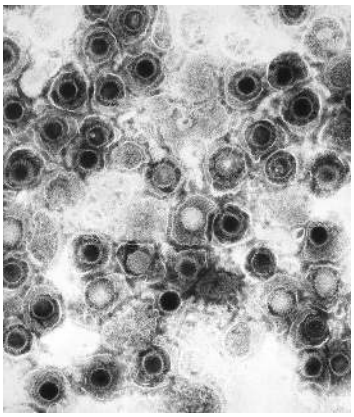


Figure: **Herpes Simplex Virions**: This negatively-stained transmission electron micrograph (TEM) revealed the presence of numerous herpes simplex virions, members of the Herpesviridae family. There are two strains of the herpes simplex virus, HSV-1, which is responsible for cold sores, and HSV-2, which is responsible for genital herpes. At the core of its icosahedral proteinaceous capsid, the HSV contains a double-stranded DNA linear genome.

Infection is caused through close contact with an infected individual. Infection is initiated when a viral particle comes in contact with the target cell specific to the individual herpes virus. Viral glycoproteins bind cell surface receptors molecules on the cell surface, followed by virion internalization and disassembly. Viral DNA then migrates to the cell nucleus where replication of viral DNA and transcription of viral genes occurs.

During symptomatic infection, infected cells transcribe lytic viral genes. In some host cells, a small number of viral genes termed *latency-associated transcripts* accumulate instead. In this fashion, the virus can persist in the cell (and thus the host) indefinitely. While primary infection is often accompanied by a self-limited period of clinical illness, long-term latency is symptom-free.

Reactivation of latent viruses

This has been implicated in a number of diseases (e.g. Shingles, Pityriasis Rosea). Following activation, transcription of viral genes transitions from latency-associated transcripts to multiple lytic genes; these lead to enhanced replication and virus production. Often, lytic activation leads to cell death. Clinically, lytic activation is often accompanied by emergence of non-specific symptoms such as low grade fever, headache, sore throat, malaise, and rash, as well as clinical signs such as swollen or tender lymph nodes, and immunological findings such as reduced levels of natural killer cells.

There is no method to eradicate the herpes virus from the body, but antiviral medications, such as *acyclovir*, can reduce the frequency, duration, and severity of outbreaks. Analgesics such as *ibuprofen* and *acetaminophen* can reduce pain and fever. Topical anesthetic treatments such as *prilocaine*, *lidocaine*, *benzocaine* or *tetracaine* can also relieve itching and pain.

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4.11.4: Attachment and Entry of Herpes Simplex

Herpes simplex virus attaches to a host's cells with viral envelope glycoproteins, which then allows entry of the viral capsid into the host cell.

Learning Objectives

- Illustrate HSV attachment to host cells

Key Points

- The genome encodes for 11 different glycoproteins, four of which, gB, gC, gD and gH, are involved in viral attachment.
- The sequential stages of HSV entry are analogous to those of other viruses.
- First, complementary viral and cell surface receptors bring the viral and host cell membranes into close proximity. Next, the two membranes begin to merge, forming a hemifusion state. Finally, a stable entry pore is formed through which the viral envelope contents are introduced to the host cell.

Key Terms

- **glycoprotein**: A protein with covalently bonded carbohydrates.
- **hemifusion**: Partial fusion, or the first stage in full fusion.
- **heparan sulfate**: A polysaccharide found, associated with protein, in all animal tissue; it has a regulatory function in several biological activities.

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) are two members of the herpes virus family, *Herpesviridae*, that infect humans. Both HSV-1 (which produces most cold sores) and HSV-2 (which produces most genital herpes) are ubiquitous and contagious. They can be spread when an infected person is producing and shedding the virus.

The sequential stages of HSV entry are analogous to those of other viruses. At first, complementary receptors on the virus and the cell surface bring the viral and cell membranes into close proximity. In an intermediate state, the two membranes begin to merge, forming a hemifusion state. Finally, a stable entry pore is formed through which the viral envelope contents are introduced to the host cell.

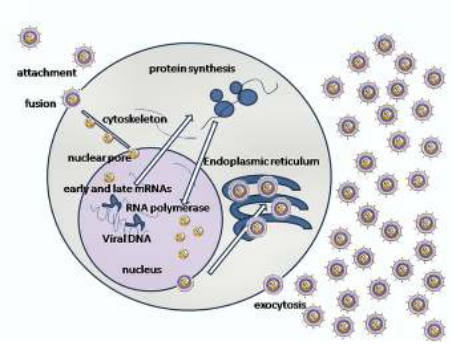


Figure: **Virus replication**: Herpes simplex virus attaches to host cell surface receptors using glycoproteins. Following attachment, the viral envelope fuses with the host cell membrane and the viral capsid gains entry into the cell.

The genome encodes for 11 different glycoproteins, four of which, gB, gC, gD and gH, are involved in viral attachment. Initial interactions occur when viral envelope glycoprotein C (gC) binds to a cell surface particle called heparan sulfate. A second glycoprotein, glycoprotein D (gD), binds specifically to at least one of three known entry receptors. These include herpesvirus entry mediator (HVEM), nectin-1 and 3-O sulfated heparan sulfate. The receptor provides a strong, fixed attachment to the host cell. These interactions bring the membrane surfaces into mutual proximity and allow for other glycoproteins embedded in the viral envelope to interact with other cell surface molecules. Once bound to the HVEM, gD changes its conformation and interacts with viral glycoproteins H (gH) and L (gL), which form a complex. The interaction of these membrane proteins results in the hemifusion state. Afterward, gB interaction with the gH/gL complex creates an entry pore for the viral capsid. Glycoprotein B interacts with glycosaminoglycans on the surface of the host cell.

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4.11.5: Replication of Herpes Simplex Virus

Interferons play pivotal roles in shaping the immune responses in mammals.

Learning Objectives

- List the treatments we have to combat viruses

Key Points

- Vaccines prime the body's immune system against specific pathogens, but are not effective for treating an infection.
- Many animal viruses are also important from a human medical perspective. The emergence of the SARS virus in the human population, coming from an animal source, highlights the importance of animals in bearing infectious agents. Avian influenza viruses can directly infect humans.
- Immune therapy using immunomodulatory factors, such as interferons, is effective for treatment of hepatitis B and C.
- Immune therapy using immunomodulatory factors, such as interferons, is effective for treatment of hepatitis B and C.

Key Terms

- **foot and mouth disease**: A highly variable and transmissible viral disease. The virus enters the body through inhalation and affects cattle worldwide.
- **interferon**: Any of a group of glycoproteins, produced by the immune system, that prevent viral replication in infected cells.

The study of animal viruses is important from a veterinary viewpoint. Many animal viruses are also important from a human medical perspective. The emergence of the SARS virus in the human population, coming from an animal source, highlights the importance of animals in bearing infectious agents. Avian influenza viruses can directly infect humans. In addition research into animal viruses has made an important contribution to our understanding of viruses in general, their replication, molecular biology, evolution, and interaction with the host.

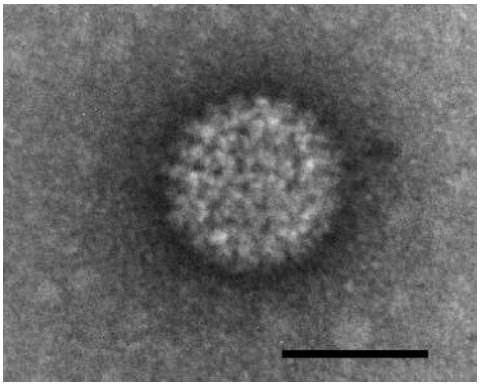


Figure: **TEM of the Bluetongue virus**: Bluetongue virus (BTV), a member of Orbivirus genus within the Reoviridae family causes serious disease in livestock (sheep, goat, cattle).

Rhabdoviruses are a diverse family of single stranded, negative sense RNA viruses that can successfully utilize a myriad of ecological niches, ranging from plants and insects, to fish and mammals. This virus family includes pathogens such as rabies virus, vesicular stomatitis virus, and potato yellow dwarf virus that are of tremendous public health, veterinary, and agricultural significance. Due to the relative simplicity of their genomes and morphology, in recent years rhabdoviruses have become powerful model systems for studying molecular virology.

Foot and mouth disease virus (FMDV) is the prototypic member of the Aphthovirus genus in the Picornaviridae family. This picornavirus is the etiological agent of an acute systemic vesicular disease that affects cattle worldwide, foot-and-mouth disease. FMDV is a highly variable and transmissible virus. It enters the body through inhalation. Soon after infection, the single stranded positive RNA that constitutes the viral genome is efficiently translated using a cap-independent mechanism driven by the internal ribosome entry site element (IRES). This process occurs concomitantly with the inhibition of cellular protein synthesis, caused by

the expression of viral proteases. In depth knowledge of the molecular basis of the viral cycle is needed to control viral pathogenesis and disease spreading.

Pestiviruses account for important diseases in animals such as Classical swine fever (CSF) and Bovine viral diarrhea / Mucosal disease (BVD/MD). The molecular biology of pestiviruses shares many similarities and peculiarities with the human hepaciviruses. Genome organization and translation strategy are highly similar for the members of both genera. One hallmark of pestiviruses is their unique strategy to establish persistent infection during pregnancy.

Coronavirus (CoV) genome replication takes place in the cytoplasm in a membrane-protected microenvironment, and starts with the translation of the genome to produce the viral replicase.

The first line of defense against viral infections is usually antiviral vaccines, which prime the body's immune system against specific pathogens. Vaccines traditionally consist of an attenuated (weakened or killed) version of the virus, although many vaccines now target specific immunogenic targets unique to a particular pathogen. Both viral and cellular proteins are required for replication and transcription. CoVs initiate translation by cap-dependent and cap-independent mechanisms. Cell macromolecular synthesis may be controlled after CoV infection by locating some virus proteins in the host cell nucleus. Infection by different coronaviruses cause in the host alteration in the transcription and translation patterns, in the cell cycle, the cytoskeleton, apoptosis and coagulation pathways, inflammation, and immune and stress responses. The balance between genes up- and down-regulated could explain the pathogenesis caused by these viruses.

Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics for bacteria, antiviral drugs are usually specific for a particular virus. Unlike most antibiotics, antiviral drugs do not destroy their target pathogen; instead they inhibit their development.

In addition to targeting viral infections directly, some therapeutics work by enhancing the immune responses necessary for viral clearance. One of the best-known of this class of drugs are interferons, which inhibit viral synthesis in infected cells. Interferons (IFNs) play pivotal roles in shaping the immune responses in mammals and are particularly important for the control of viral infections, cell growth, and immune regulation. These proteins rapidly induce an "anti-viral state" in cells that surround infected cells. In order to survive, viruses have evolved multiple strategies to evade the anti-viral effects of IFNs. Elucidating the molecular and cellular biology of the virus-interferon interaction is key to understanding issues such as viral pathogenesis, latency, and the development of novel antivirals.



Figure: **Pediatric polio vaccination in India by a Stop Transmission of Polio (STOP) teams (2002)**: Vaccinations are the best defense against a wide range of viruses, but they are not effective in treating active infections.

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4.11.6: Immunodeficiency

Immunodeficiency occurs when the immune system cannot appropriately respond to infections.

Learning Objectives

- Explain the problems associated with immunodeficiency

Key Points

- If a pathogen is allowed to proliferate to certain levels, the immune system can become overwhelmed; immunodeficiency occurs when the immune system fails to respond sufficiently to a pathogen.
- Immunodeficiency can be caused by many factors, including certain pathogens, malnutrition, chemical exposure, radiation exposure, or even extreme stress.
- HIV is a virus that causes immunodeficiency by infecting helper T cells, causing cytotoxic T cells to destroy them.

Key Terms

- **phagocyte**: a cell of the immune system, such as a neutrophil, macrophage or dendritic cell, that engulfs and destroys viruses, bacteria, and waste materials
- **lysis**: the disintegration or destruction of cells
- **immunodeficiency**: a depletion in the body's natural immune system, or in some component of it

Immunodeficiency

Failures, insufficiencies, or delays at any level of the immune response can allow pathogens or tumor cells to gain a foothold to replicate or proliferate to high enough levels that the immune system becomes overwhelmed, leading to immunodeficiency; it may be acquired or inherited. Immunodeficiency can be acquired as a result of infection with certain pathogens (such as HIV), chemical exposure (including certain medical treatments), malnutrition, or, possibly, by extreme stress. For instance, radiation exposure can destroy populations of lymphocytes, elevating an individual's susceptibility to infections and cancer. Dozens of genetic disorders result in immunodeficiencies, including Severe Combined Immunodeficiency (SCID), bare lymphocyte syndrome, and MHC II deficiencies. Rarely, primary immunodeficiencies that are present from birth may occur. Neutropenia is one form in which the immune system produces a below-average number of neutrophils, the body's most abundant phagocytes. As a result, bacterial infections may go unrestricted in the blood, causing serious complications.

HIV/AIDS

Human immunodeficiency virus infection / acquired immunodeficiency syndrome (HIV/AIDS), is a disease of the human immune system caused by infection with human immunodeficiency virus (HIV). During the initial infection, a person may experience a brief period of influenza-like illness. This is typically followed by a prolonged period without symptoms. As the illness progresses, it interferes more and more with the immune system. The person has a high probability of becoming infected, including from opportunistic infections and tumors that do not usually affect people who have working immune systems.

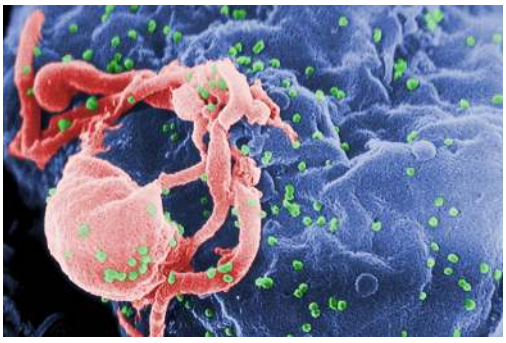


Figure: **Image of HIV: scanning electron micrograph of HIV-1 budding (in green, color added) from cultured lymphocyte**: Multiple round bumps on cell surface represent sites of assembly and budding of HIV. During primary infection, the level of HIV may reach several million virus particles per milliliter of blood.

After the virus enters the body, there is a period of rapid viral replication, leading to an abundance of virus in the peripheral blood. During primary infection, the level of HIV may reach several million virus particles per milliliter of blood. This response is accompanied by a marked drop in the number of circulating CD4+ T cells, cells that are or will become helper T cells. The acute viremia, or spreading of the virus, is almost invariably associated with activation of CD8+ T cells (which kill HIV-infected cells) and, subsequently, with antibody production. The CD8+ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the CD4+ T cell counts recover.

Ultimately, HIV causes AIDS by depleting CD4+ T cells (helper T cells). This weakens the immune system, allowing opportunistic infections. T cells are essential to the immune response; without them, the body cannot fight infections or kill cancerous cells. The mechanism of CD4+ T cell depletion differs in the acute and chronic phases. During the acute phase, HIV-induced cell lysis and killing of infected cells by cytotoxic T cells accounts for CD4+ T cell depletion, although apoptosis (programmed cell death) may also be a factor. During the chronic phase, the consequences of generalized immune activation coupled with the gradual loss of the ability of the immune system to generate new T cells appear to account for the slow decline in CD4+ T cell numbers.

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4.11.7: Double-Stranded DNA Viruses - Pox Viruses

The poxviruses are a family of large, complex, enveloped DNA viruses that infect a variety of vertebrate and invertebrate hosts.

Learning Objectives

- Examine pox viruses for their relevance to human disease and research

Key Points

- The most famous of the poxviruses was smallpox. Smallpox is one of two infectious diseases to have been eradicated, the other being rinderpest, which was declared eradicated in 2011.
- The most abundant and simplest infectious form of the poxvirus particle, the mature virion, consists of the viral DNA genome encased in a proteinaceous core and an outer lipoprotein membrane.
- Poxviruses exhibit a temporally-regulated gene expression program: early, intermediate, and late genes drive DNA replication followed by expression of structural proteins necessary for progeny virion assembly.

Key Terms

- **recombinant**: This term refers to something formed by combining existing elements in a new combination. Thus, the phrase recombinant DNA refers to an organism created in the lab by adding DNA from another species.
- **lipoprotein**: Any of a large group of complexes of protein and lipid with many biochemical functions.

The poxviruses are a family of large, complex, enveloped DNA viruses that infect a variety of vertebrate and invertebrate hosts. Poxviruses are of significance both medically and scientifically due to their wide distribution, pathogenicity, and cytoplasmic replicative life cycle. Several prominent members, including variola virus (causative agent of smallpox), molluscum contagiosum virus (cause of a common skin infection of young children and immunosuppressed adults) and monkeypox virus (agent of a smallpox-like disease in parts of Africa), are of considerable concern for public health and biodefense.

The most famous of the poxviruses was smallpox. Smallpox was an infectious disease unique to humans, caused by either of two virus variants, Variola major and Variola minor. The disease is also known by the Latin names Variola or Variola vera, which is a derivative of the Latin varius, meaning “spotted,” or varus, meaning “pimple. ” The term “smallpox” was first used in Britain in the 15th century to distinguish variola from the “great pox” (syphilis). The last naturally occurring case of smallpox (Variola minor) was diagnosed on October 26, 1977. After vaccination campaigns throughout the 19th and 20thcenturies, the World Health Organization (WHO) certified the eradication of smallpox in 1979. Smallpox is one of two infectious diseases to have been eradicated, the other being rinderpest, which was declared eradicated in 2011.

The prototypic and most studied poxvirus, vaccinia virus (VACV), serves as an effective smallpox vaccine, a platform for recombinant vaccines against other pathogens, and an efficient gene expression vector for basic research. Along its approximate 195-kbp double-stranded DNA genome, VACV encodes approximately 200 proteins, ranging in function from viral RNA and DNA synthesis and virion assembly to modulation of host immune defenses.

The most abundant and simplest infectious form of the poxvirus particle, the mature virion (MV), consists of the viral DNA genome encased in a proteinaceous core and an outer lipoprotein membrane with approximately 60 and 25 associated viral proteins, respectively. Following attachment to cell surfaces and fusion with the plasma or endosomal membrane, poxvirus replication is initiated by entry of the viral core into the cytoplasm, where all subsequent steps of the life cycle take place. Poxvirus cores harbor the viral DNA-dependent RNA polymerase and transcription factors necessary for expression of early genes, which constitute nearly half of the viral genome and encode proteins needed for DNA replication and intermediate gene transcription, as well as a large number of immunomodulators.

Poxviruses exhibit a temporally-regulated gene expression program, i.e., expression of early genes encoding DNA replication and intermediate transcription factors triggers the expression of intermediate genes encoding late gene specific transcription factors. Late gene products primarily consist of structural proteins needed for progeny virion assembly, as well as those enzymes destined for incorporation into progeny virions, and used for early gene expression during the next round of infection. Assembly of the MV involves more than 80 viral gene products. In addition, during transit through the cytoplasm, a subset of progeny MVs acquires two

additional membrane bilayers, one of which is lost during exocytosis of the particle, to yield the less abundant enveloped virion (EV). Thus, an EV is essentially an MV with an additional membrane in which at least six unique proteins are associated. EVs are antigenically distinct from MVs and are important for efficient virus dissemination in the infected host and protection against immune defenses. In contrast, MVs are released upon cell lysis and may be important for animal-to-animal transmission.



Figure: **Girl infected with smallpox. Bangladesh, 1973.**: In ordinary type smallpox the bumps are filled with a thick, opaque fluid and often have a depression or dimple in the center. This is a major distinguishing characteristic of smallpox.

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4.11.8: Double-Stranded DNA Viruses- Adenoviruses

LEARNING OBJECTIVES

- Define the characteristics of adenoviruses

Adenoviruses are medium-sized (90–100 nm), non-enveloped, icosahedral viruses composed of a nucleocapsid and a linear, double-stranded DNA (dsDNA) genome. There are 57 described serotypes in humans, which are responsible for 5–10% of upper respiratory infections in children, and many infections in adults as well.

Diversity

Viruses of the family *Adenoviridae* infect vertebrates, including humans. Among human-tropic viruses classification can be complex; there are 57 accepted human adenovirus types (HAdV-1 to 57) in seven species (Human adenovirus A to G). Different species/serotypes are associated with different conditions:

- respiratory disease (mainly species HAdV-B and C)
- conjunctivitis (HAdV-B and D)
- gastroenteritis (HAdV-F types 40, 41, HAdV-G type 52)

In addition to human viruses, *Adenoviridae* can be divided into five genera: *Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus*, and *Ichtadenovirus*.

Genome

Structurally, adenoviruses represent the largest non-enveloped viruses. They possess non-segmented dsDNA genomes between 26 and 45 Kbp, significantly larger than other dsDNA viruses. The virion also has unique “spike” or fiber associated with each penton base of the capsid that aids in attachment to the host cell via host cell surface receptors.

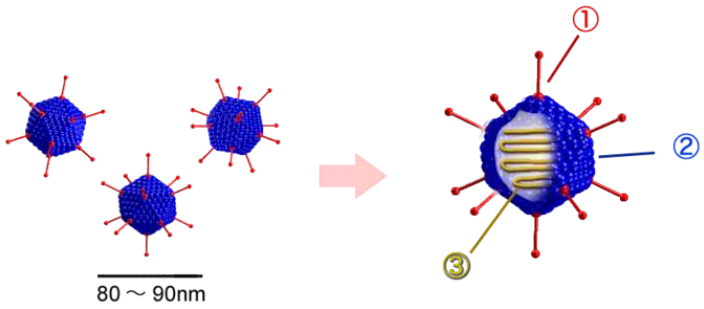


Figure: **Adenovirus Structure**: 1) Penton capsomeres 2) Hexon capsomeres 3) Viral genome (linear dsDNA)

Viral Entry and Replication

Entry of adenoviruses into the host cell involves two sets of interactions between the virus and the host cell. First, entry into the host cell is initiated by the knob domain of the fiber protein binding to a host cell receptor, either CD46 for the group B human adenovirus serotypes, or the coxsackievirus adenovirus receptor for all other serotypes. Next, a specialized motif in the penton base protein interacts with αv integrin, stimulating internalization of the adenovirus via clathrin-coated pits, resulting in entry of the virion into the host cell within an endosome.

Following internalization, the endosome acidifies, which alters virus topology, causing capsid components to disassociate. These changes, as well as the toxic nature of the pentons, result in the release of the virion into the cytoplasm. With the help of cellular microtubules, the virus is transported to the nuclear pore complex, where viral gene expression can occur.

The adenovirus life cycle is separated by the DNA replication process into two phases: an early and a late phase. In both, a primary transcript that is alternatively spliced to generate monocistronic mRNAs compatible with the host’s ribosome is generated, allowing for the products to be translated.

The early genes are responsible for expressing mainly non-structural, regulatory proteins. The goal of these proteins is threefold: to alter the expression of host proteins necessary for DNA synthesis; to activate other viral genes (such as the virus-encoded DNA polymerase); and to avoid premature death of the infected cell by the host-immune defenses (blockage of apoptosis, blockage of interferon activity, and blockage of MHC class I translocation and expression).

The late phase of the adenovirus lifecycle is focused on producing sufficient quantities of structural protein to pack all the genetic material produced by DNA replication. Once the viral components have successfully been replicated, the virus is assembled into its protein shells and released from the cell as a result of virally induced cell lysis.

Transmission

Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body and water. Adenoviruses are spread primarily via respiratory droplets; however, they can also be spread by fecal routes.

Humans infected with adenoviruses display a wide range of responses, from no symptoms at all to the severe infections typical of Adenovirus serotype 14. In the past, U.S. military recruits were vaccinated against two serotypes of adenoviruses, with a corresponding decrease in illnesses caused by those serotypes. Although the vaccine is no longer manufactured for civilians, military personnel can receive the vaccine as of 2014.

Infections

Viral transmission occurs primarily through expectorate, but can also be transmitted via contact with infected objects. Most adenovirus infections affect the upper respiratory tract. These often show up as conjunctivitis, tonsillitis, ear infection, or croup. Adenoviruses, types 40 and 41 can also cause gastroenteritis. A combination of conjunctivitis and tonsillitis is particularly common with adenovirus infections. Some children (especially small ones) can develop adenovirus bronchiolitis or pneumonia, both of which can be severe.

Utilization in Treatment of Unrelated Diseases

Adenovirus is used as a vehicle to administer targeted therapy in the form of recombinant DNA or protein. Specific modifications on fiber proteins are used to target Adenovirus to certain cell types; a major effort is made to limit hepatotoxicity and prevent multiple organ failure. Adenovirus dodecahedron serves as a potent delivery platform for foreign antigens to human myeloid dendritic cells (MDC), and is efficiently presented by MDC to M1-specific CD8+ T lymphocytes.

Key Points

- Adenoviruses are medium-sized (90–100 nm), non-enveloped, icosahedral viruses composed of a nucleocapsid and a linear double-stranded DNA (dsDNA) genome.
- There are 57 described serotypes in humans, which are responsible for 5–10% of upper respiratory infections in children, and many infections in adults as well.
- Adenoviruses bind cell surface receptors on host cells, resulting in entry of the virion into the host cell within an endosome.
- The adenovirus life cycle is separated by the DNA replication process into two phases: an early and a late phase. Early genes are responsible for expressing mainly non-structural, regulatory proteins, while late genes produce structural protein necessary for viral replication.

Key Terms

- **endosome**: An endocytic vacuole through which molecules are internalized during endocytosis pass, en route to lysosomes.
- **recombinant DNA**: DNA that has been engineered by splicing together fragments of DNA from multiple species and introduced into the cells of a host.
- **integrin**: Any of many heterodimeric transmembrane proteins that function as receptors in communication between cells.
- **penton**: A pentagonal capsomere of an adenovirus capsid.
- **capsid**: A capsid is the protein shell of a virus.

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4.11.9: Retroviruses and Hepadnavirus

Hepadnaviruses, retroviruses, use virally encoded reverse transcriptase to convert RNA into DNA.

Learning Objectives

- Differentiate between retroviruses and hepadnaviruses

Key Points

- Retrovirus RNA serves as a template for reverse transcriptase and is copied into DNA.
- Hepadnaviruses are a family of viruses which can cause liver infections in humans and animals.

Key Terms

- **endogenous**: produced, originating or growing from within
- **episome**: A segment of DNA that can exist and replicate either autonomously in the cytoplasm or as part of achromosome, mainly found in bacteria.

A well-studied family of this class of viruses includes the retroviruses. One defining feature is the use of reverse transcriptase to convert the positive-sense RNA into DNA. Instead of using the RNA for templates of proteins, they use DNA to create the templates, which is spliced into the host genome using integrase. Replication can then commence with the help of the host cell's polymerases. A well-studied example of this includes HIV.

A special variant of retroviruses are endogenous retroviruses, which are integrated into the genome of the host and inherited across generations.

The virus itself stores its nucleic acid in the form of a +mRNA (including the 5'cap and 3'PolyA inside the virion) genome. This then serves as a means of delivery of that genome into cells it targets as an obligate parasite, and constitutes the infection. Once in the host's cell, the RNA strands undergo reverse transcription in the cytoplasm and are integrated into the host's genome, at which point the retroviral DNA is referred to as a provirus. It is difficult to detect the virus until it has infected the host.

In most viruses, DNA is transcribed into RNA, and then RNA is translated into protein. However, retroviruses function differently – their RNA is reverse-transcribed into DNA, which is integrated into the host cell's genome (when it becomes a provirus), and then undergoes the usual transcription and translational processes to express the genes carried by the virus. So, the information contained in a retroviral gene is used to generate the corresponding protein via the sequence: RNA → DNA → RNA → protein. This extends the fundamental process identified by Francis Crick, in which the sequence is: DNA → RNA → protein. Retroviruses are proving to be valuable research tools in molecular biology and have been used successfully in gene delivery systems.

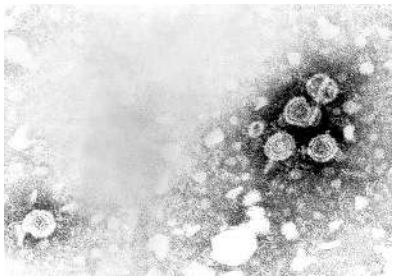


Figure: **Hepatitis B Virus**: TEM micrograph showing hepatitis B virions.

Hepadnaviruses are a family of viruses which can cause liver infections in humans and animals. There are two recognized genera:

- Genus *Orthohepadnavirus* ; type species: *Hepatitis B virus*
- Genus *Avihepadnavirus* ; type species: *Duck hepatitis B virus*

Hepadnaviruses have very small genomes of partially double-stranded, partially single stranded circular DNA. The genome consists of two uneven strands of DNA. One has a negative-sense orientation, and the other, shorter, strand has a positive-sense orientation.Hepadnaviruses replicate through an RNA intermediate (which they transcribe back into cDNA using reverse

transcriptase). The reverse transcriptase becomes covalently linked to a short 3- or 4-nucleotide primer. Most hepadnaviruses will only replicate in specific hosts, and this makes experiments using in vitro methods very difficult.

HBV infection is initiated through viral attachment to an unknown cell surface receptor. The virally encoded DNA polymerase acts upon the DNA, leaving it fully double-stranded.

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4.11.10: Treatment of Animal Viral Infections

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SECTION OVERVIEW

4.12: 9. 12- Viruses and Cancer

Topic hierarchy

4.12.1: Cancer Viruses

4.12.2: DNA Oncogenic Viruses

4.12.3: RNA Oncogenic Viruses

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4.12.1: Cancer Viruses

Viruses can cause cancer by transforming a normal cell into a malignant cell.

Learning Objectives

- Illustrate how cancer viruses turn normal cells into tumor cells

Key Points

- A direct oncogenic viral mechanism involves either the insertion of additional viral oncogenic genes into the host cell, or the enhancement of already existing oncogenic genes in the genome.
- Tumor viruses come in a variety of forms. Viruses with a DNA genome, such as adenovirus, and viruses with an RNA genome, like the Hepatitis C virus (HCV), can cause cancers. Retroviruses having both DNA and RNA genomes (Human T-lymphotropic virus and hepatitis B virus) can also cause cancers.
- Viruses can become carcinogenic when they integrate into the host cell genome as part of a biological accident, such as polyomaviruses and papillomaviruses.

Key Terms

- **oncogenic**: Tending to cause the formation of tumors.
- **transformation**: The alteration of a bacterial cell caused by the transfer of DNA from another, especially if pathogenic.

Worldwide, cancer viruses are estimated to cause 15-20% of all cancers in humans. Most viral infections, however, do not lead to tumor formation; several factors influence the progression from viral infection to cancer development. These factors include host's genetic makeup, mutation occurrence, exposure to cancer causing agents, and immune impairment.

Viruses typically initiate cancer development by suppressing the host's immune system, causing inflammation over a long period of time, or by altering host genes. Cancer cells have characteristics that differ from normal cells, such as acquiring the ability to grow uncontrollably. This can result from having control of their own growth signals, losing sensitivity to anti-growth signals, and losing the ability to undergo apoptosis, or programmed cell death.

Cancer cells don't experience biological aging, and maintain their ability to undergo cell division and growth. Transformation occurs when a virus infects and genetically alters a cell. The infected cell is regulated by the viral genes and has the ability to undergo abnormal new growth. Scientists have been able to discern some commonality among viruses that cause tumors. The tumor viruses or oncoviruses change cells by integrating their genetic material with the host cell's DNA. Unlike the integration seen in prophages, this is a permanent insertion; the genetic material is never removed.

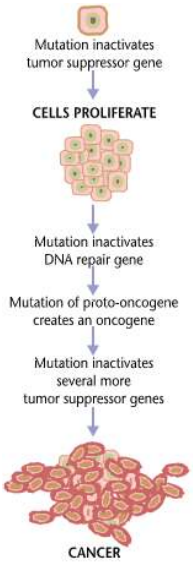


Figure: **Mutations Leading to Increased Cell Division**: Cancer is caused by a series of mutations. Viral infections contribute to the process through genetic alteration.

The insertion mechanism can differ depending on whether the nucleic acid in the virus is DNA or RNA. In DNA viruses, the genetic material can be directly inserted into the host's DNA. RNA viruses must first transcribe RNA to DNA and then insert the genetic material into the host cell's DNA.

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4.12.2: DNA Oncogenic Viruses

An estimated 15 percent of all human cancers worldwide may be attributed to viruses.

Learning Objectives

- Outline DNA oncogenic viruses

Key Points

- Both DNA and RNA viruses have been shown to be capable of causing cancer in humans.
- Epstein-Barr virus, human papilloma virus, hepatitis B virus, and human herpes virus-8 are the four DNA viruses that are capable of causing the development of human cancers.
- The presence of viral gene products in tumor cells that require them to maintain their unchecked proliferation, can provide important targets for directed therapies that specifically can distinguish tumor cells from normal cells.

Key Terms

- **oncogenic**: Tending to cause the formation of tumors.

There are two classes of cancer viruses: DNA and RNA viruses. Several viruses have been linked to certain types of cancer in humans. These viruses have varying ways of reproduction and represent several different virus families.

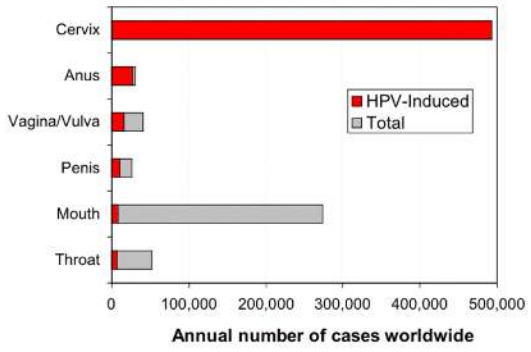


Figure: **Virus Causing Cervical Cancer**: Human papilloma virus is strongly linked to the development of cervical cancer among other types of cancers.

DNA Oncogenic Viruses include the following:

- The Epstein-Barr virus has been linked to Burkitt's lymphoma. This virus infects B cells of the immune system and epithelial cells.
- The hepatitis B virus has been linked to liver cancer in people with chronic infections.
- Human papilloma viruses have been linked to cervical cancer. They also cause warts and benign papillomas.
- Human herpes virus-8 has been linked to the development of Kaposi sarcoma. Kaposi sarcoma causes patches of abnormal tissue to develop in various area of the body including under the skin, in the lining of the mouth, nose, and throat or in other organs.

DNA tumor viruses have two life-styles. In permissive cells, all parts of the viral genome are expressed. This leads to viral replication, cell lysis and cell death. In cells that are non-permissive for replication, viral DNA is usually, but not always, integrated into the cell chromosomes at random sites. Only part of the viral genome is expressed. These are the early control functions of the virus. Viral structural proteins are not made, and no progeny virus is released.

The first DNA tumor viruses to be discovered were rabbit fibroma virus and Shope papilloma virus, both discovered by Richard Shope in the 1930s. Papillomas are benign growths, such as warts, of epithelial cells. They were discovered by making a filtered extract of a tumor from a wild rabbit and injecting the filtrate into another rabbit in which a benign papilloma grew. However, when the filtrate was injected into a domestic rabbit, the result was a carcinoma, a malignant growth. A seminal observation was that it

was no longer possible to isolate infectious virus from the malignant growth because the virus had become integrated into the chromosomes of the malignant cells.

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4.12.3: RNA Oncogenic Viruses

An estimated 15% of all human cancers worldwide may be attributed to viruses.

Learning Objectives

- Classify the viruses with oncogenic properties

Key Points

- Both DNA and RNA viruses have been shown to be capable of causing cancer in humans.
- Human T lymphotrophic virus type 1 and hepatitis C viruses are the two RNA viruses that contribute to human cancers.
- Hepatitis C virus is an enveloped RNA virus capable of causing acute and chronic hepatitis in humans by infecting liver cells. It is estimated 3% of the world's population are carriers. Chronic infection with hepatitis C virus results in cirrhosis, which in turn can lead to liver cancer.

Key Terms

- **oncogenic**: Tending to cause the formation of tumors.
- **hepatocellular**: Of or pertaining to the cells of the liver

There are two classes of cancer viruses: DNA and RNA viruses. Several viruses have been linked to certain types of cancer in humans. These viruses have varying ways of reproduction and represent several different virus families. Specifically, RNA viruses have RNA as their genetic material and can be either single-stranded RNA (ssRNA) or double-stranded (dsRNA). RNA viruses are classified based on the Baltimore classification system and do not take into account viruses with DNA intermediates in their life cycle. Viruses which contain RNA for their genetic material but do include DNA intermediates in their life cycle are called "retroviruses. " There are numerous RNA oncogenic viruses that have been linked to various cancer types. These various oncogenic viruses include:

1. Human T lymphotrophic virus type 1 (HTLV-I), a retrovirus, has been linked to T-cell leukemia. 2. The hepatitis C virus has been linked to liver cancer in people with chronic infections.

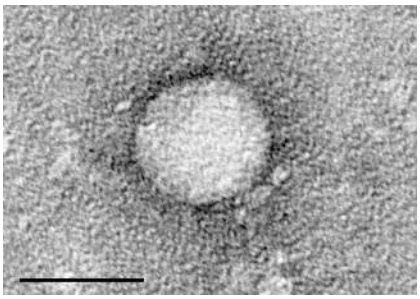


Figure: **Electron micrograph of Hepatitis C**: Hepatitis C viral infections have been linked to the development of liver cancer.

2. Hepatitis viruses includes hepatitis B and hepatitis C have been linked to hepatocellular carcinoma.

3. Human papillomaviruses (HPV) have been linked to cancer of the cervix, anus, penis, vagina/vulva, and some cancers of the head and neck.

4. Kaposi's sarcoma-associated herpesvirus (HHV-8) has been linked to Kaposi's sarcoma and primary effusion lymphoma.

5. Epstein-Barr virus (EBV) has been linked to Burkitt's lymphoma, Hodgkin's lymphoma, post-transplantation lymphoproliferative disease, and nasopharyngeal carcinoma.

RNA Retroviruses

Retroviruses are different from DNA tumor viruses in that their genome is RNA, but they are similar to many DNA tumor viruses in that the genome is integrated into host genome. Since RNA makes up the genome of the mature virus particle, it must be copied to DNA prior to integration into the host cell chromosome. This lifestyle goes against the central dogma of molecular biology in

which that DNA is copied into RNA. The outer envelope comes from the host cell plasma membrane. Coat proteins (surface antigens) are encoded by env (envelope) gene and are glycosylated. One primary gene product is made, but this is cleaved so that there are more than one surface glycoprotein in the mature virus (cleavage is by host enzyme in the Golgi apparatus). The primary protein (before cleavage) is made on ribosomes attached to the endoplasmic reticulum and is a transmembrane (type 1) protein. Inside the membrane is an icosahedral capsid containing proteins encoded by the gag gene (group-specific AntiGen). Gag-encoded proteins also coat the genomic RNA. Again, there is one primary gene product. This is cleaved by a virally-encoded protease (from the pol gene). There are two molecules of genomic RNA per virus particle with a 5′ cap and a 3′ poly A sequence. Thus, the virus is diploid. The RNA is plus sense (same sense as mRNA). About 10 copies of reverse transcriptase are present within the mature virus, these are encoded by the pol gene. Pol gene codes for several functions (again, as with gag and env, a polyprotein is made that is then cut up).

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SECTION OVERVIEW

4.13: 9. 13- Viral Ecology

Topic hierarchy

4.13.1: Emergence of Viral Pathogens

4.13.2: Viral Roles in Ecosystems

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4.13.1: Emergence of Viral Pathogens

LEARNING OBJECTIVES

- Demonstrate the ways a virus can go from benign to pathogenic

Humans or other potential viral hosts are constantly exposed to viruses, yet most viral exposure has no effect. However, many viruses that were once benign later become pathogens through a genetic change, which can occur by different mechanisms. One common evolutionary process whereby viral genes change over time is called genetic drift, where individual bases in the DNA or RNA mutate to other bases. Most of these point mutations are “silent”—they do not change the protein that the gene encodes—but others can confer evolutionary advantages such as resistance to antiviral drugs.

The transformation of viruses from benign to pathogenic occurs via two additional processes more specific to viruses. Viral genomes are constantly mutating, producing new forms of these antigens. If one of these new forms of an antigen is sufficiently different from the old antigen, it will no longer bind to the receptors and viruses with these new antigens can evade immunity to the original strain of the virus. When such a change occurs, people who have had the illness in the past will lose their immunity to the new strain and vaccines against the original virus will also become less effective. Two processes drive the antigens to change: antigenic drift and antigenic shift (antigenic drift being the more common).

Antigenic drift is a mechanism for variation by viruses that involves the accumulation of mutations within the antibody -binding sites so that the resulting viruses cannot be inhibited as well by antibodies against previous strains, making it easier for them to spread throughout a partially immune population. Antigenic drift occurs in both influenza A and influenza B viruses. The rate of antigenic drift is dependent on two characteristics: the duration of the epidemic and the strength of host immunity. A longer epidemic allows for selection pressure to continue over an extended period of time and stronger host immune responses increase selection pressure for the development of novel antigens.

Alternatively, the change can occur by antigenic shift. Antigenic shift is a specific case of reassortment or viral shift that confers a phenotypic change; it is the process by which two or more different strains of a virus, or strains of two or more different viruses, combine to form a new subtype having a mixture of the surface antigens of the two or more original strains. The term is often applied specifically to influenza, as that is the best-known example, but the process also occurs with other viruses, such as the visna virus in sheep. When this happens with influenza viruses, pandemics might result.

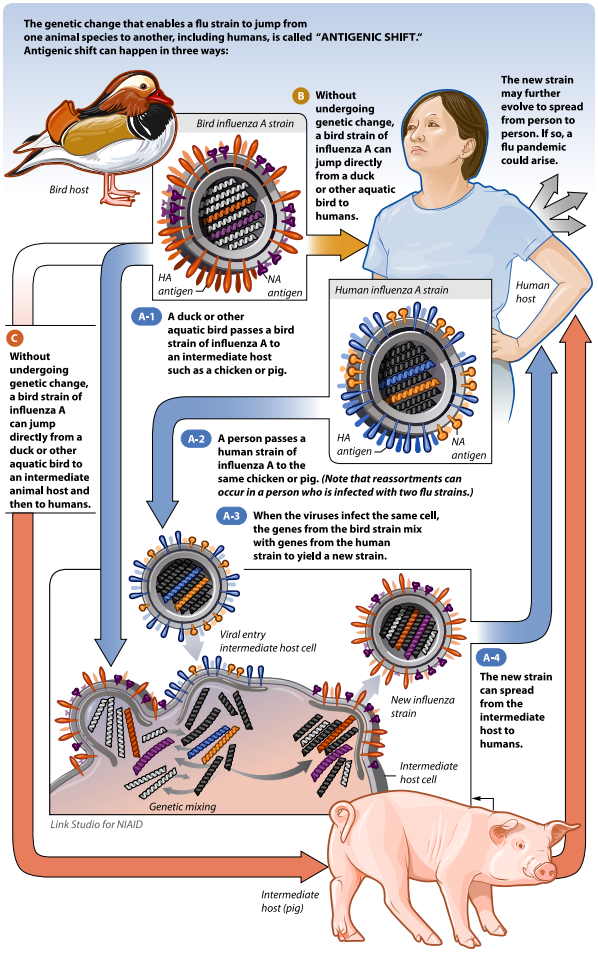


Figure: ***Pathogen emergence by antigenic shift***: *This figure describes in detail how a virus that cannot infect a human can gain the ability to infect a human.*

Antigenic shift occurs only in influenza A because it infects more than just humans. Affected species include other mammals and birds, giving influenza A the opportunity for a major reorganization of surface antigens. Influenza B and C principally infect humans, minimizing the chance that a reassortment will change its phenotype drastically.

For example, if a pig was infected with a human influenza virus and an avian influenza virus at the same time, an antigenic shift could occur, producing a new virus that had most of the genes from the human virus, but a hemagglutinin or neuraminidase from the avian virus. The resulting new virus would likely be able to infect humans and spread from person to person, but it would have surface proteins (hemagglutinin and/or neuraminidase) not previously seen in influenza viruses that infect humans, and therefore most people would have little or no immune protection. If this new virus causes illness in people and can be transmitted easily from person to person, an influenza pandemic can occur. The most recent 2009 H1N1 outbreak was a result of an antigenic shift and reassortment between human, avian, and swine viruses.

Key Points

- For a virus to become pathogenic, genetic changes must occur. These changes occur via mutations, antigenic shift, or antigenic drift.
- Antigenic drift is the natural mutation over time of known strains, thus small changes may cause a harmless virus to become dangerous.
- Antigenic shift is a specific case of reassortment or viral shift that confers a phenotypic change. It is the process by which two or more different strains of a virus, or strains of two or more different viruses, combine to form a new subtype that can be more pathogenic than the original strains.
- Antigenic shift is a major driving force behind viruses inhabiting new niches and is responsible for some of the more aggressive human viruses.

Key Terms

- **antigenic shift**: A specific case of reassortment or viral shift that confers a phenotypic change; it is the process by which two or more different strains of a virus, or strains of two or more different viruses, combine to form a new subtype having a mixture of the surface antigens of the two or more original strains.
- **antigenic drift**: A mechanism for variation by viruses that involves the accumulation of mutations within the antibody-binding sites so that the resulting viruses cannot be inhibited as well by antibodies against previous strains, making it easier for them to spread throughout a partially immune population.

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4.13.2: Viral Roles in Ecosystems

LEARNING OBJECTIVES

- Show the roles viruses play on ecosystems

Metagenomics is a relatively recent field of study that tries to understand the diversity—especially microbial—of the world around us. Through these studies it is now known that the number of viral particles and number of different viral species in almost every environment on Earth is immense. It is largely believed that viruses by species are the most numerous of any biological entity on earth. This is typified by the role of viruses in marine ecology. A teaspoon of seawater contains about one million viruses.

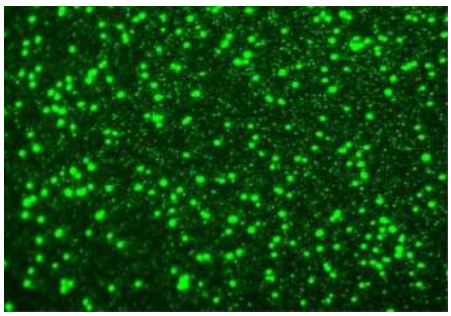


Figure: ***Viral diversity in seawater****: The large green dots are bacteria while the smaller green dots are viral particles. This represents a fraction of the viral diversity seen in teaspoon of marine water.*

Viruses are essential to the regulation of saltwater and freshwater ecosystems. Most of these viruses are bacteriophages, which are harmless to plants and animals. They infect and destroy the bacteria in aquatic microbial communities, comprising the most important mechanism of recycling carbon in the marine environment. The organic molecules released from the bacterial cells by the viruses stimulate fresh bacterial and algal growth.

Microorganisms constitute more than 90% of the biomass in the sea. It is estimated that viruses kill approximately 20% of this biomass each day, and that there are 15 times as many viruses in the oceans as there are bacteria and archaea. Viruses are the main agents responsible for the rapid destruction of harmful algal blooms, which often kill other marine life. The number of viruses in the oceans decreases further offshore and deeper into the water, where there are fewer host organisms. The effects of marine viruses are far-reaching; by increasing the amount of photosynthesis in the oceans, viruses are indirectly responsible for reducing the amount of carbon dioxide in the atmosphere by approximately 3 gigatonnes of carbon per year. Like any organism, marine mammals are susceptible to viral infections. In 1988 and 2002, thousands of harbor seals were killed in Europe by the phocine distemper virus. Many other viruses, including caliciviruses, herpesviruses, adenoviruses, and parvoviruses, circulate in marine mammal populations.

As mentioned, marine viruses are mostly bacteriophages, or phages. Phages are obligate intracellular parasites, meaning that they are able to reproduce only while infecting bacteria. Phages therefore are found only within environments that contain bacteria. Most environments contain bacteria, including our own bodies (called normal flora). Often these bacteria are found in large numbers. As a consequence, phages are found almost everywhere. As a rule of thumb, many phage biologists expect that phage population densities will exceed bacterial densities by a ratio of 10:1 or more (VBR or virus-to-bacterium ratio).

Estimates of bacterial numbers on Earth reach approximately 10^{30}; consequently, there is an expectation that 10^{31} or more individual virus (mostly phage) particles exist, making phages the most numerous category of “organisms” on our planet. Bacteria (along with archaea) appear to be highly diverse and there are possibly millions of species. Phage-ecological interactions therefore are quantitatively vast, with huge numbers of interactions. Phage-ecological interactions are also qualitatively diverse: there are huge numbers of environment types, bacterial-host types, and also individual phage types.

Key Points

- The diversity and numbers of different viruses is incredible.
- In the world’s oceans, 90% of the biomass is microbial, with viruses turning over 20% of that daily. Without the turnover of biomass driven by viruses, many sources of food would not be present for other organisms.

- As bacteria are incredibly diverse, then the viruses that infect them, phages, are even more diverse. As bacteria inhabit almost every niche of the earth, so do viruses.

Key Terms

- **ecological**: Relating to ecology, the interrelationships of organisms and their environment.
- **metagenomics**: The study of genomes recovered from environmental samples; especially the differentiation of genomes from multiple organisms or individuals, either in a symbiotic relationship, or at a crime scene.
- **intracellular**: Inside or within a cell.

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CHAPTER OVERVIEW

5: Eukaryotic Microorganisms

Eukaryote organisms have one or more cells with a nucleus and other organelles enclosed within membranes.

5.1: Protozoa

5.1.1: Characteristics of Protozoa

5.1.2: Medically Important Protozoa

5.1.3: Protozoa (Exercises)

5.2: Fungi

5.2.1: Overview of Fungi

5.2.2: Yeasts

5.2.3: Molds

5.2.4: Fungal Pathogenicity

5.2.5: Chemotherapeutic Control of Fungi

5.2.6: Fungi (Exercises)

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5.1: Protozoa

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5.1.1: Characteristics of Protozoa

Learning Objectives

After completing this section you should be able to perform the following objectives.

1. Briefly describe protozoa.
2. Briefly describe 3 ways protozoans may reproduce asexually.
3. Define the following:
   A. trophozoite
   B. protozoan cyst.

Protozoa are unicellular eukaryotic microorganisms lacking a cell wall and belonging to the Kingdom Protista. Although there are nearly 20,000 species of protozoa, relatively few cause disease; most inhabit soil and water. Protozoa reproduce asexually by the following means:

1. **fission**: One cell splits into two.
2. **schizogony**: A form of asexual reproduction characteristic of certain protozoa, including sporozoa, in which daughter cells are produced by multiple fission of the nucleus of the parasite followed by segmentation of the cytoplasm to form separate masses around each smaller nucleus.
3. **budding**: Buds form around a nucleus and pinch off of the parent cell.

Some protozoa also reproduce sexually by fusion of gametes (Figure 5.1.1.1).

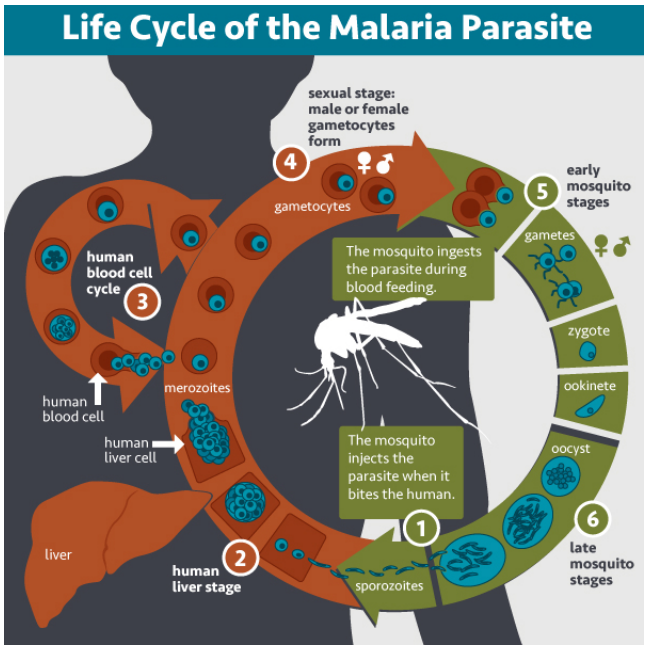


Figure **5.1.1.1:** Life Cycle of Plasmodium, the Protozoan that causes Malaria. (1) A female Anopheles mosquito carrying malaria-causing parasites feeds on a human and injects the parasites in the form of sporozoites into the bloodstream. The sporozoites travel to the liver and invade liver cells. (2) Over 5-16 days*, the sporozoites grow, divide, and produce tens of thousands of haploid forms, called merozoites, per liver cell. Some malaria parasite species also produce hypnozoites in the liver that remain dormant for extended periods, causing relapses weeks or months later. (3) The merozoites exit the liver cells and re-enter the bloodstream, beginning a cycle of invasion of red blood cells, known as asexual replication. In the red blood cells they develop into mature schizonts, which rupture, releasing newly formed merozoites that then reinvade other red blood cells. This cycle of invasion and cell rupture repeats every 1-3 days* and can result in thousands of parasite-infected red blood cells in the host bloodstream, leading to illness and complications of malaria that can last for months if not treated. (4) Some of the merozoite-infected blood cells leave the cycle of asexual replication. Instead of replicating, the merozoites in these cells develop into sexual forms of the parasite, called male and female gametocytes. In some malaria species, young gametocytes sequester in the bone marrow and some organs while late stage (stage V) gametocytes, circulate in the bloodstream. (5) When a mosquito bites an infected human, it ingests the gametocytes. In the mosquito midgut, the infected human red blood cells burst, releasing the gametocytes, which develop further into mature sexual forms called gametes. Male and female gametes fuse to form diploid zygotes, which develop into actively moving ookinetes that burrow through the mosquito midgut wall and form oocysts on the other side. (6) Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After 8-15 days*, the oocyst bursts, releasing sporozoites into the body cavity of the mosquito, from which they travel to and invade the mosquito salivary glands. The cycle of human infection re-starts when the mosquito takes a blood meal, injecting the sporozoites from its salivary glands into the human bloodstream. (7) The vegetative, reproducing, feeding form of a protozoan is called a trophozoite. Under certain conditions, some protozoa produce a protective form called a cyst that enable them to survive harsh environments. Cysts allow some pathogens to survive outside their host. *from* NIAID .

? Exercise: Think-Pair-Share Questions

1. Protozoa that cause gastrointestinal infections are capable of producing cyst forms as well as trophozoites. State why this is essential to these pathogens.

The Role of Protozoan Cytoplasmic Membrane Components in Initiating Body Defense

Initiation of Innate Immunity

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

Components of protozoa that function as PAMPs include GPI-anchored proteins (GPI = Glycosylphosphatidylinositol) and mannose-rich glycans (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar) that function as PAMPs. These mannose-rich glycans are common in microbial glycoproteins and glycolipids but rare in those of humans. These PAMPs bind to pattern-recognition receptors or PRRs on a variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

Initiation of Adaptive Immunity

Proteins associated with protozoa function as antigens and initiate adaptive immunity. An antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes. An immunogen is an antigen that is recognized by the body as non-self and stimulates an adaptive immune response. The body recognizes an antigen as foreign when epitopes of that antigen bind to B-lymphocytes and T-lymphocytes by means of epitope-specific receptor molecules having a shape complementary to that of the epitope. The epitope receptor on the surface of a B-lymphocyte is called a B-cell receptor and is actually an antibody molecule. The receptor on a T-lymphocyte is called a T-cell receptor (TCR). This will be discussed in greater detail in Unit 6.

We will now briefly look at some medically important protozoa classified into phyla based on their motility. Illustrations can be found in your Lab Manual in Lab 20.

Summary

Protozoa are unicellular eukaryotic microorganisms lacking a cell wall and belonging to the Kingdom Protista. Protozoa reproduce asexually by fission, schizogony, or budding. Some protozoa can also reproduce sexually. Relatively few protozoa cause disease. The vegetative, reproducing, feeding form of a protozoan is called a trophozoite. Under certain conditions, some protozoa produce a protective form called a cyst. Components of protozoa that function as PAMPs include GPI-anchored proteins and mannose-rich glycans. These PAMPS bind to PRRs on various defense cells and trigger innate immunity. Protozoan molecules can also trigger adaptive immunity such as the production of antibody molecules against protozoan antigens.

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5.1.2: Medically Important Protozoa

Learning Objectives

1. State a disease caused by each of the following protozoans and indicate their means of motility and how they are transmitted to humans:
    a. *Entamoeba histolytica*
    b. *Acanthamoeba*
    c. *Giardia lamblia*
    d. *Trichomonas vaginalis*
    e. *Trypanosoma brucei-gambiens*
    f. *Balantidium coli*
    g. *Plasmodium* species
    h. *Toxoplasma gondii*
    i. *Cryptosporidium*

The Sarcomastigophora (Amoeboflagellates)

The amoebas (subphylum Sarcodina) move by extending lobelike projections of their cytoplasm called pseudopodia .

- Photomicrograph of an amoeba.



Video YouTube movie an amoeba moving by forming pseudopodia (https://www.youtube.com/embed/7pR7TNzJ_pA).

a. *Entamoeba histolytica* (see photomicrograph) which causes a gastrointestinal infection called amoebic dysentery. The organism produces protective cysts which pass out of the intestines of the infected host and are ingested by the next host. It is transmitted by the fecal-oral route.

b. *Acanthamoeba* can cause rare, but severe infections of the eye, skin, and central nervous system. *Acanthamoeba* keratitis is an infection of the eye that typically occurs in healthy persons and can result in blindness or permanent visual impairment. Granulomatous amebic encephalitis (GAE) is an infection of the brain and spinal cord typically occurring in persons with a compromised immune system. *Acanthamoeba* is found in soil, dust, and a variety of water sources including lakes, tap water, swimming pools, and heating and air conditioning units. It typically enters the eyes and most cases are associated with contact lens use, but it can also enter cuts or wounds and be inhaled.

c. *Naegleria fowleri* (sometimes called the"brain-eating amoeba"), is another amoeba that can cause a rare but devastating infection of the brain called primary amebic meningoencephalitis (PAM). The amoeba is commonly found in warm freshwater rivers, lakes,

rivers, and hot springs, as well as in the soil. It typically causes infections when contaminated water enters the body through the nose where it can subsequently travel to the brain.

YouTube movie of *Acanthamoeba*

The flagellates (subphylum Mastigophora) move by means of flagella. Some also have an undulating membrane .

a. *Giardia lamblia* (see photomicrograph) can cause a gastrointestinal infection called giardiasis. Cysts pass out of the intestines of the infected host and are ingested by the next host. It is transmitted by the fecal-oral route.

YouTube animation illustrating giardiasis

- Scanning electron micrograph of *Giardia* in the intestines; courtesy of Dennis Kunkel's Microscopy.
- Scanning electron micrograph of *Giardia*;courtesy of CDC.

b. *Trichomonas vaginalis* (see photomicrograph) infects the vagina and the male urinary tract causing an infection called genitourinary trichomoniasis. It does not produce a cysts stage and is usually transmitted by sexual contact.

YouTube movie *Trichomonas vaginalis*.

YouTube movie showing motility of *Trichomonas vaginalis*.

c. *Trypanosoma brucei gambiens* (see photomicrograph) causes African sleeping sickness and is transmitted by the bite of an infected Tsetse fly. The disease primarily involves the lymphatic and nervous systems of humans.

YouTube movie of *Trypanosoma*

The Ciliophora

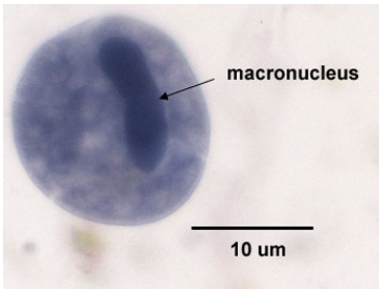
The ciliates move by means of cilia.

- Scanning electron micrograph of *Paramecium*, a ciliated protozoan; courtesy of Dennis Kunkel's Microscopy.



YouTube movie showing motility of Balantidium coli.

a. The only pathogenic ciliate is *Balantidium coli* (see photomicrograph) which causes a diarrhea-type infection called balantidiasis. Cysts pass out of the intestines of the infected host and are ingested by the next host. It is transmitted by the fecal-oral route.



Balantidium coli in a Fecal Smear

The Apicomplexans

The apicomplexans are not motile in their mature forms, reproduce both asexually and sexually, and often have complex life cycles for transmission from host to host. They possess a complex of organelles called apical complexes at their apex that contain enzymes used in penetrating host tissues.

Species of *Plasmodium* (Figure 5) cause malaria and are transmitted by the bite of an infected female *Anopheles* mosquito. They reproduces asexually by schizogony in human liver cells and red blood cells but also reproduce sexually by gametes in the mosquito (see the life cycle of *Plasmodium*). In the case of malaria caused by *P. vivax* and *P. ovale*, a dormant form or hypnozoite remains in the liver and may cause later relapses.

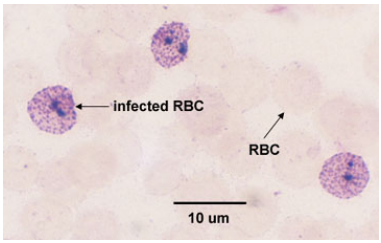


Figure 5: Plasmodium-Infected Red Blood Cells (arrows)

Toxoplasma gondii is another intracellular apicomplexan and causes toxoplasmosis (see the AIDS pathology tutorial at the University of Utah). It can infect most mammals and is contracted by inhaling or ingesting cysts from the feces of infected domestic cats, where the protozoa reproduce both asexually and sexually, or by ingesting raw meat of an infected animal. Toxoplasmosis is usually mild in people with normal immune responses but can infect the brain, heart, or lungs of people who are immunosuppressed. It can also be transmitted congenitally and infect the nervous system of the infected child.

Cryptosporidium is an intracellular parasite that causes a gastrointestinal infection called cryptosporidiosis, although in people who are immunosuppressed it can also cause respiratory and gallbladder infections. It is transmitted by the fecal-oral route.

> Movie of motile *Cryptosporidium*, courtesy of the Sibly Lab, Washington University in St. Louis School of Medicine.
>
> Movie of *Cryptosporidium* entering an epithelial cell, courtesy of the Sibly Lab, Washington University in St. Louis School of Medicine.

Virulence Factors that Promote Colonization of Protozoans

Virulence factors that promote protozoal colonization of the host include the ability to:

1. contact host cells;
2. adhere to host cells and resist physical removal;
3. invade host cells;
4. compete for nutrients;
5. resist innate immune defenses such as phagocytosis and complement; and
6. evade adaptive immune defenses.

Examples of virulence factors that promote protozoal colonization include:

1. Some protozoa, such as *Entamoeba histolytica*,*Trichomonas vaginalis*, *Giardia lamblia*, and *Balantidium coli* use pseudopodia, flagella or cilia to swim through mucus and contact host cells.
2. Protozoa use adhesins associated with their cytoplasmic membrane to adhere to host cells, colonize, and resist flushing.

3. Some protozoa, such as the apicomplexans (*Plasmodium (inf)*, *Toxoplasma gondii (inf),* and *Cryptosporidium (inf)*) possess a complex of organelles called apical complexes at their apex that contain enzymes used in penetrating host tissues and cells.
4. Protozoans such as Trypanosoma *brucei gambiens (inf)* and *Plasmodium* species *(inf)* are able to change their surface antigens during their life cycle in the human. As the protozoa change the amino acid sequence and shape of their surface antigens, antibodies and cytotoxic T-lymphocytes made against a previous shape will no longer fit and the body has to start a new round of adaptive immunity against the new antigen shape.
5. Some protozoa, such as *Entamoeba histolytica (inf)* shed their surface antigens so that antibodies made by the body against these surface antigens are tied up by the shed antigens.

To view a Quicktime movie of *Cryptosporidium* and electron micrographs of *Giardia* and *Entamoeba*, see the Parasites section of the CELL'S ALIVE web page.

Summary

Protozoan diseases include amoebic dysentery, giardiasis, balantidiasis, cryptosporidiosis African sleeping sickness, acanthamoebiasis, toxoplasmosis, and genitourinary trichomoniasis. Many of the same factors that enable bacteria to colonize a host also enable protozoans to colonize a host. Many of the same factors that enable bacteria to harm the host enable protozoans to harm the host.

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5.1.3: Protozoa (Exercises)

These are homework exercises to accompany Kaiser's "Microbiology" TextMap. Microbiology is the study of microorganisms, which are defined as any microscopic organism that comprises either a single cell (unicellular), cell clusters or no cell at all (acellular). This includes eukaryotes, such as fungi and protists, and prokaryotes. Viruses and prions, though not strictly classed as living organisms, are also studied.

9.1: Characteristics of Protozoa

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

1. Match the following:

   _____ Multiple fission. The nucleus divides many times before the cell divides. The single cell then separates into numerous daughter cells.

   _____ Division in which one cell splits in two.

   _____ Division in which a cell pinches off of the parent cell.

   _____ The vegetative, reproducing, feeding form of a protoaoan.

   _____ A protective form that enables protozoa to survive harsh environments.

   A. trophozoite
   B. cyst
   C. fission
   D. schizogony
   E. budding

9.2: Medically Important Protozoa

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

1. Matching

   _____ Moves by flagella; transmitted by ingesting cysts via the fecal-oral route; causes an intestinal infection.

   _____ Moves by cilia; transmitted by ingesting cysts via the fecal-oral route; causes an intestinal infection.

   _____ Moves by flagella; transmitted by an infected tsetse fly; causes African sleeping sickness.

   _____ Nonmotile in the body; reproduces sexually and asexually; transmitted by an infecteded *Anopheles* mosquito; causes malaria.

   _____ Moves by flagella; transmitted sexually; causes vaginitis.

   _____ Nonmotile in the body; reproduces sexually and asexually; transmitted by eating infected meat or inhaling or ingesting cysts from cat feces.

   a. *Entamoeba histolytica*
   b. *Acanthamoeba*
   c. *Giardia lamblia*
   d. *Trichomonas vaginalis*
   e. *Trypanosoma brucei-gambiens*
   f. *Balantidium coli*
   g. *Plasmodium* species

h. *Toxoplasma gondii*
i. *Cryptosporidium*

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SECTION OVERVIEW

5.2: Fungi

Yeasts are eukaryotic microorganisms classified as members of the fungus kingdom with 1,500 species currently identified and are estimated to constitute 1% of all described fungal species.

5.2.1: Overview of Fungi

5.2.2: Yeasts

5.2.3: Molds

5.2.4: Fungal Pathogenicity

5.2.5: Chemotherapeutic Control of Fungi

5.2.6: Fungi (Exercises)

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5.2.1: Overview of Fungi

Learning Objectives

1. Name 3 groups of fungi.
2. Define mycosis.

Mycology is the study of fungi. Fungi include yeasts, molds, and fleshy fungi. They:

1. are eukaryotic;
2. have a rigid cell wall;
3. are chemoheterotrophs (organisms that require organic compounds for both carbon and energy sources);
4. obtain their nutrients by absorption;
5. obtain nutrients as saprophytes, organisms that live off of decaying matter, or as parasites, organisms that live off of living matter.

Of the over 100,000 species of fungi, only about 100 species are pathogenic for animals. They play a major role in the recycling of nutrients by their ability to cause decay and are used by industry to produce a variety of useful products. However, they also cause many undesirable economic effects such as the spoilage of fruits, grains, and vegetables, as well as the destruction of unpreserved wood and leather products. We will be concerned mainly with the yeasts and molds, especially those causing mycoses (fungal infections).

Summary

Fungi include yeasts, molds, and fleshy fungi. Fungi are are eukaryotic organisms and possess a cell wall. Most fungi are saprophytes, organisms that live off of decaying matter; a few are parasites, organisms that live off of living matter. A fungal infection is called a mycosis.

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5.2.2: Yeasts

Learning Objectives

1. Briefly describe yeasts and state how they reproduce asexually.
2. Briefly describe pseodohypae, hyphae, blastoconidia (blastospores), and chlamydoconidia (chlamydospores) and name a yeast producing these structures.
3. Name three potentially pathogenic yeasts and state an infection each causes.

Yeast Morphology

1. Yeast (see Figure 1) are unicellular fungi which usually appear as oval cells 1-5 µm wide by 5-30 µm long.
2. They have typical eukaryotic structures (see Figure 2 and Figure 3).
3. They have a thick polysaccharide cell wall.
4. They are facultative anaerobes.
5. The yeast *Candida* is said to be dimorphicin that it can grow as an oval, budding yeast, but under certain culture conditions, the budding yeast may elongate and remain attached producing filament-like structures called pseudohyphae. *C. albicans* may also produce true hyphae similar to molds (see Figure 4). In this case long, branching filaments lacking complete septa form. The pseudohyphae and hyphae help the yeast to invade deeper tissues after it colonizes the epithelium. Asexual spores called blastoconidia (blastospores) develop in clusters along the hyphae, often at the points of branching. Under certain growth conditions, thick-walled survival spores called chlamydoconidia (chlamydospores) may also form at the tips or as a part of the hyphae (see Figure 5.)

For More Information: A Comparison of Prokaryotic and Eukaryotic Cells from Unit 1

The Role of Fungal Cell Wall Components in Initiating Body Defense

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

Components of the yeast cell wall that function as PAMPs include lipoteichoic acids, and zymosan. In addition, bacteria and other microorganisms also possess mannose-rich glycans (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar) that function as PAMPs. These mannose-rich glycans are common in microbial glycoproteins and glycolipids but rare in those of humans. These PAMPs bind to pattern-recognition receptors on a

variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

Flash animation showing the release of fungal mannans from the cell walls of yeast and their subsequent binding to pattern-recognition receptors on a macrophage.

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

For More Information: Review of Pattern-Recognition Receptors from Unit 5

For More Information: Review of Inflammation from Unit 5

Yeast cell wall components also activate the alternative complement pathway and the lectin pathway, defense pathways that play a variety of roles in body defense. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against bacterial cell wall antigens. An antigen is defined as a substance that reacts with antibody molecules and antigen receptors on lymphocytes. An immunogen is an antigen that is recognized by the body as non-self and stimulates an adaptive immune response.

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

Reproduction of yeasts

1. Yeasts reproduce asexually by a process called budding (see Figure 1 and Figure 6). A bud is formed on the outer surface of the parent cell as the nucleus divides. One nucleus migrates into the elongating bud. Cell wall material forms between the bud and the parent cell and the bud breaks away.

- Scanning electron micrograph of *Saccharomyces*; courtesy of Dennis Kunkel's Microscopy.
- Movie of *Saccharomyces cerevisiae* reproducing by budding. Movie of Growth and Division of Budding Yeast (*Saccharomyces cerevisiae*) . © Phillip Meaden, author. Licensed for use, ASM MicrobeLibrary.

2. A few yeasts, such as *Candida albicans*, also produce clusters of asexual reproductive spores called blastoconidia (blastospores) and thick-walled survival spores called chlamydoconidia (chlamydospores) ; see Figure 5.

3. Yeasts can also reproduce sexually by means of sexual spores called ascospores which result from the fusion of the nuclei from two cells followed by meiosis. Sexual reproduction is much less common than asexual reproduction but does allow for genetic recombination.

Yeast Infections

Candida albicans

Candida albicans is found as normal flora on the mucous membranes and in the gastrointestinal tract, but is usually held in check by normal flora bacteria and normal body defenses.

Candida can cause a variety of opportunistic infections in people who are debilitated, immunosuppressed, or have received prolonged antibacterial therapy. Women who are diabetic, pregnant, taking oral contraceptives, or having menopause are also more prone to vaginitis. These conditions alter the sugar concentration and pH of the vagina making it more favorable for the growth of *Candida*. People who are immunosuppressed frequently develop thrush, vaginitis, and sometimes disseminated infections.

Any *Candida* infection is called candidiasis. *Candida* most commonly causes vaginitis , thrush (an infection of the mouth), balantitis (an infection of the foreskin and head of the penis), onychomycosis (an infection of the nails), and dermatitis (diaper rash and other infections of moist skin). Less commonly, *Candida* can infect the lungs, blood, heart, and meninges, especially in the compromised or immunosuppressed host. *Candida* now causes about 10% of all cases of septicemia. Candidiasis of the esophagus, trachea, bronchi, or lungs, in conjunction with a positive HIV antibody test, is one of the indicator diseases for AIDS.

Candida is said to be dimorphic, that is it has two different growth forms. It can grow as an oval, budding yeast, but under certain culture conditions, the budding yeast may elongate and remain attached producing filament-like structures called pseudohyphae. *C. albicans* may also produce true hyphae similar to molds. In this case long, branching filaments lacking complete septa form. The pseudohyphae and hyphae help the yeast to invade deeper tissues after it colonizes the epithelium. Asexual spores called blastoconidia are reproductive units produced by budding in yeasts. Under certain growth conditions, thick-walled survival spores called chlamydoconidia may also form at the tips or as a part of the hyphae (see Figure 5)

The most common Candida species causing human infections is C. albicans, causing 50-60% of all Candida infections. *Candida glabrata* is second, causing 15-20% of Candida infections; *Candida parapsilosis* is third, responsible for 10-20%.

Cryptococcus neoformans

A lesser known but often more serious pathogenic yeast is C*ryptococcus*neoformans. Like many fungi, this yeast can also reproduce sexually and the name given to the sexual form of the yeast is *Filobasidiella neoformans*. It appears as an oval yeast 5-6 µm in diameter, forms buds with a thin neck, and is surrounded by a thick capsule (Figure 8.2.6). It does not produce pseudohyphae and chlamydospores. The capsule enables the yeast to resist phagocytic engulfment. The yeast is dimorphic. In its sexual form, as well as in its asexual form under certain conditions, it can produce a hyphal form.

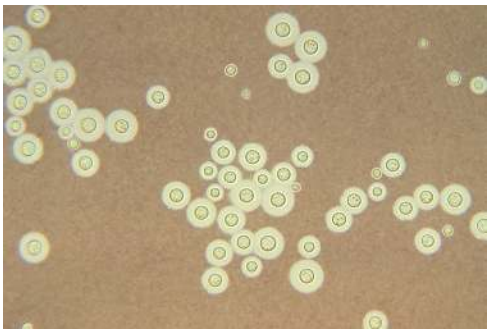


Figure 8.2.6: India ink stain of encapsulated Cryptococcus neoformans. Note encapsulated yeast.Image provided by Dr. Leanor Haley. Courtesy of the Centers for Disease Control and Prevention.

Cryptococcus infections are usually mild or subclinical but, when symptomatic, usually begin in the lungs after inhalation of the yeast in dried bird feces. It is typically associated with with pigeon and chicken droppings and soil contaminated with these droppings. *Cryptococcus*, found in soil, actively grows in the bird feces but does not grow in the bird itself. Usually the infection does not proceed beyond this pulmonary stage. In the immunosuppressed host, however, it may spread through the blood to the meninges and other body areas, often causing cryptococcal meningoencephalitis. Any disease by this yeast is usually called cryptococcosis.

Dissemination of the pulmonary infection can result in a very severe and often fatal cryptococcal meningoencephalitis. Cutaneous and visceral infections are also found. Although exposure to the organism is probably common, large outbreaks are rare, indicating that an immunosuppressed host is usually required for the development of severe disease. Extrapulmonary cryptococcosis, in conjunction with a positive HIV antibody test, is another indicator disease for AIDS.

Pneumocystis jiroveci

Pneumocystis jiroveci (formerly called *Pneumocystis carinii*) (see Figure 7 and Figure 8) is thought to be transmitted from person to person by the respiratory route and is almost always asymptomatic. However, in persons with highly depressed immune responses, such as people with leukemias or infected with the Human Immunodeficiency Virus (HIV), *P. jiroveci* can cause a severe pneumonia called PCP (*Pneumocystis* pneumonia).

P. jiroveci can be found in three distinct morphologic stages:

- The trophozoite (trophic form), a haploid amoeboid form 1-4 µm in diameter that replicates by mitosis and binary fission. The trophic forms are irregular shaped and often appears in clusters.
- A precystic form or early cyst. Haploid trophic forms conjugate and produce a zygote or sporocyte (early cyst).
- The cyst form, which contains several intracystic bodies or spores are 5-8 µm in diameter. It has been postulated that in formation of the cyst form (late phase cyst), the zygote undergoes meiosis and subsequent mitosis to typically produce eight haploid ascospores (sporozoites) See Figure 7. As the haploid ascospores are released the cysts often collapse forming crescent-shaped bodies (see Figure 8). *P. jiroveci* is usually transmitted by inhalation of the cyst form. Released ascospores then develop into replicating trophic forms that attach to the wall of the alveoli and replicate to fill the alveoli.

In biopsies from lung tissue or in tracheobronchial aspirates, both a trophic form about 1-4 µm in diameter with a distinct nucleus and a cyst form between 5-8 µm in diameter with 6-8 intracystic bodies (ascospores) can be seen.

Malassezia globosa

Malassezia globosa is a dimorphic yeast that is the most frequent cause of a superficial skin infection called tinea versicolor that commonly appears as a hypopigmentation of the infected skin. *M. globosa* is also the most common cause of dandruff and seborrheic dermatitis. The yeast is naturally found on the skin. To view additional photomicrographs of *Candida*, *Cryptococcus*, and *Pneumocystis*, see the AIDS Pathology Tutorial at the University of Utah.

Concept Map for Fungi, Part-1: Yeasts

? Exercise: Think-Pair-Share Questions

1. A woman has taken broad spectrum antibiotics for two weeks to treat a bacterial infection. She subsequently develops vaginitis.
   a. Explain what might account for this.
   b. Why didn't the antibiotics prevent the vaginitis?
2. A young child with an immunosuppressive disorder and living in an urban area routinely played in a park with a large pigeon population. The child subsequently developed a respiratory infection followed by symptoms of meningitis.
   a. What infection might be expected and why?
   b. What might the lab look for in the spinal fluid to help confirm this?

Summary

1. Yeasts are eukaryotic unicellular fungi
2. Some yeast are dimorphic in that they can grow as an oval, budding yeast, but under certain culture conditions, they may produce filament-like structures called hyphae similar to molds.
3. Components of the yeast cell wall that function as pathogen-associated molecular patterns or PAMPs include lipoteichoic acids, zymosan, and mannose-rich glycans.
4. These PAMPs bind to pattern-recognition receptors or PRRs on a variety of body defense cells and triggers innate immune defenses.
5. Cell wall molecules can also trigger adaptive immunity such as the production of antibody molecules against bacterial cell wall antigens.
6. Yeasts reproduce asexually by a process called budding.
7. Candida albicans is found as normal flora on the mucous membranes and in the gastrointestinal tract but is usually held in check by the body's normal microbiota and normal body defenses.
8. Candida can cause a variety of opportunistic infections in people who are debilitated, immunosuppressed, or have received prolonged antibacterial therapy, and infect the lungs, blood, heart, and meninges, especially in the compromised or immunosuppressed host.
9. Cryptococcus neoformans infections are usually mild or subclinical but, when symptomatic, usually begin in the lungs after inhalation of the yeast in dried bird feces.
10. Pneumocystis jiroveci can cause a severe pneumonia called PCP (Pneumocystis pneumonia).
11. Malassezia globosa is the most frequent cause of a superficial skin infection called tinea versicolor and also the most common cause of dandruff.

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5.2.3: Molds

Learning Objectives

1. Define:
   a. mold
   b. hyphae
   c. mycelium
   d. vegetative mycelium
   e. aerial mycelium.
2. Briefly describe the following fungal asexual reproductive spores:
   a. conidiospores
   b. macroconidia,
   c. microconidia
   d. sporangiospores
   e. arthrospores
3. Define dermatophyte, list 2 genera of dermatophytes, and name three dermatophytic infections.
4. Describe what is meant by the term "dimorphic fungus", name two systemic infections caused by dimorphic fungi, and state how they are initially contracted.

Mold Morphology

Molds are multinucleated, filamentous fungi composed of hyphae. A hypha is a branching tubular structure approximately 2-10 µm in diameter which is usually divided into cell-like units by crosswalls called septa. The total mass of hyphae is termed a mycelium. The portion of the mycelium that anchors the mold and absorbs nutrients is called the vegetative mycelium , composed of vegetative hyphae; the portion that produces asexual reproductive spores is the aerial mycelium , composed of aerial hyphae (Figure 1).

Molds have typical eukaryotic structures (Figure 2) and have a cell wall usually composed of chitin, sometimes cellulose, and occasionally both. Furthermore, molds are obligate aerobes *and* grow by elongation at apical tips of their hyphae and thus are able to penetrate the surfaces on which they begin growing.

For More Information: A Comparison of Prokaryotic and Eukaryotic Cells from Unit 1

Reproduction of Molds

1. Molds reproduce primarily by means of asexual reproductive spores (Figure 1). These include the following.

   a. conidiospores (conidia) See Figure 3.

   Spores borne externally on an aerial hypha called a conidiophore ; see Figure 4 and Figure 5.

   - Scanning electron micrographs of the conidiospores of *Penicillium* and of *Aspergillus*; courtesy of Dennis Kunkel's Microscopy.

   b. sporangiospores See Figure 6.

   Spores borne in a sac or sporangium on an aerial hypha called a sporangiophore ; see Figure 7.

   - Scanning electron micrograph of the conidiospores of *Rhizopus*; courtesy of Dennis Kunkel's Microscopy.

   c. arthrospores See Figure 8.

   spores produced by fragmentation of a vegetative hypha (Figure 9).

2. Molds may also reproduce sexually by sexual spores such as ascospores and zygospores, but this is not common.

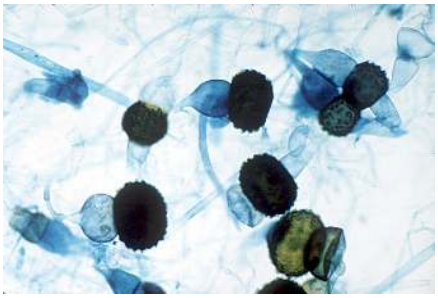


Figure 8.3.1: *Light micrograph of a whole-mount slide of zygospores of Rhizopus. from Wikipedia (Curtis Clark)*

Pathogenic Molds

Dermatophytes

The dermatophytes are a group of molds that cause superficial mycoses of the hair, skin, and nails and utilize the protein keratin, that is found in hair, skin, and nails, as a nitrogen and energy source. Infections are commonly referred to as ringworm or tinea infections and include:

- tinea capitis (infection of the skin of the scalp, eyebrows, and eyelashes)
- tinea barbae (infection of the bearded areas of the face and neck)
- tinea faciei (infection of the skin of the face)
- tinea corporis (infection of the skin regions other than the scalp, groin, palms, and soles)
- tinea cruris (infection of the groin; jock itch)
- tinea unguium (onchomycosis; infection of the fingernails and toenails)
- tinea pedis (athlete's foot; infection of the soles of the feet and between the toes).

The three most common dermatophytes are *Microsporum, Trichophyton*, and *Epidermophyton*. They produce characteristic asexual reproductive spores called macroconidia and microconidia (Figure 10 and Figure 11).

- Scanning electron micrograph of the macroconidia of *Epidermophyton*; courtesy of Dennis Kunkel's Microscopy.

Another tinea infection of the skin is tinea versicolor caused by the yeast *Malassezia globosa*. Tinea versicolor appears as a hypopigmentation of the infected skin. *M. globosa* is also the most common cause of dandruff.

Dimorphic Fungi

Dimorphic fungi may exhibit two different growth forms. Outside the body they grow as a mold, producing hyphae and asexual reproductive spores, but in the body they grow in a non-mycelial yeast form. These infections appear as systemic mycoses and usually begin by inhaling spores from the mold form. After germination in the lungs, the fungus grows as a yeast. Factors such as body temperature, osmotic stress, oxidative stress, and certain human hormones activate a dimorphism-regulating histidine kinase enzyme in dimorphic molds, causing them to switch from their avirulent mold form to their more virulent yeast form.

For example:

a. Coccidioides *immitis* causes coccidioidomycosis (Figure 12), a disease endemic to the southwestern United States. An estimated 100,000 infections occur annually in the United States, but one to two thirds of these cases are subclinical.

The mold form of the fungus grows in arid soil and produces thick-walled, barrel-shaped asexual spores called arthrospores (Figure 8) by a fragmentation of its vegetative hyphae.

After inhalation, the arthrospores germinate and develop into endosporulating spherules (Figure 13) in the terminal bronchioles of the lungs. The spherules reproduce by a process called endosporulation, where the spherule

produces numerous endospores (yeast-like particles), ruptures, and releases viable endospores that develop into new spherules.

b. *Histoplasma capsulatum* (Figure 14)is a dimorphic fungus that causes histoplasmosis, a disease commonly found in the Great Lakes region and the Mississippi and Ohio River valleys. Approximately 250,000 people are thought to be infected annually in the US, but clinical symptoms of histoplasmosis occur in less than 5% of the population. Most individuals with histoplasmosis are asymptomatic. Those who develop clinical symptoms are typically either immunocompromised or are exposed to a large quantity of fungal spores.

The mold form of the fungus often grows in bird or bat droppings or soil contaminated with these droppings and produces large tuberculate macroconidia and small microconidia (Figure 15). Although birds cannot be infected by the fungus and do not transmit the disease, bird excretions contaminate the soil and enrich it for mycelial growth. Bats, however, can become infected and transmit histoplasmosis through their droppings. After inhalation of the fungal spores and their germination in the lungs, the fungus grows as a budding, encapsulated yeast (Figure 16).

- Chest X-ray of a person with histoplasmosis.

c. Blastomycosis, caused by *Blastomyces dermatitidis*, is common around the Great Lakes region and the Mississippi and Ohio River valleys.Infection can range from an asymptomatic, self-healing pulmonary infection to widely disseminated and potentially fatal disease. Pulmonary infection may be asymptomatic in nearly 50% of patients. *Blastomyces dermatitidis* can also sometimes infect the skin.

Blastomyces dermatitidis produces a mycelium with small conidiospores (Figure 17) and grows actively in bird droppings and contaminated soil. When spores are inhaled or enter breaks in the skin, they germinate and the fungus grows as a yeast (Figure 18).having a characteristic thick cell wall. It is diagnosed by culture and by biopsy examination.

These infections usually remains localized in the lungs, but in rare cases may spread throughout the body.

As mentioned earlier, the yeast *Candida albicans* can also exhibit dimorphism.

- To view additional photomicrographs of *Coccidioides* and *Histoplasma*, see the AIDS Pathology Tutorial at the University of Utah.

Opportunistic Molds

Certain molds once considered as non-pathogenic have recently become a fairly common cause of opportunistic lung and wound infections in the debilitated or immunosuppressed host. These include the common molds *Aspergillus* (Figure 4) and *Rhizopus* (Figure 6). Although generally harmless in most healthy individuals, *Aspergillus* species do cause allergic bronchopulmonary aspergillosis (ABPA), chronic necrotizing *Aspergillus* pneumonia (or chronic necrotizing pulmonary aspergillosis [CNPA]), aspergilloma (a mycetoma or fungus ball in a body cavity such as the lung), and invasive aspergillosis. In highly immunosuppressed individuals, however, *Aspergillus* may disseminate beyond the lung via the blood.

Mucormycoses are infections caused by fungi belonging to the order of Mucorales. *Rhizopus* species are the most common causative organisms. The most common infection is a severe infection of the facial sinuses, which may extend into the brain. Other mycoses include pulmonary, cutaneous, and gastrointestinal.

? Exercise: Think-Pair-Share Questions

1. A patient infected with HIV and living in the southwestern US frequently takes walks in a dry, arid area that was once a ranch. On a particular windy and dusty day, he hikes near an area where bulldozers are excavating the area for a housing development. A couple of weeks later he develops severe respiratory symptoms. A microscopic examination of lung tissue in the lab shows spherical bodies filled with yeast like particles.
   a. What infection does he most likely have?

b. How specifically did he contract this infection?

2. A woman notices an intense itching between her toes. The skin appears red and inflamed with some cracking of the skin. A scraping of the skin is viewed under a microscope and fungal hyphae and large leaf-shaped spores are evident.

   What infection does this person most likely have and how can you tell from this information?

Summary

Molds are multinucleated, filamentous fungi composed of hyphae. Molds reproduce primarily by means of asexual reproductive spores. The dermatophytes are a group of molds that cause superficial mycoses of the hair, skin, and nails and utilize the protein keratin that is found in hair, skin, and nails, as a nitrogen and energy source.

Dimorphic fungi may exhibit two different growth forms. Outside the body they grow as a mold, producing hyphae and asexual reproductive spores, but in the body they grow in a yeast-like form. The appearance of a mold and the type of spores it produces is useful in its identification. Mold infections include tinea or ringworm, coccidioidomycosis, histoplasmosis, blastomycosis, and Aspergillosis.

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5.2.4: Fungal Pathogenicity

Learning Objectives

- Name at least three fungal virulence factors that promote fungal colonization.
- Name at least two fungal virulence factors that damage the host.

As with the bacteria, fungal virulence factors can be divided into two categories: virulence factors that promote fungal colonization of the host; and virulence factors that damage the host.

Virulence Factors that Promote Fungal Colonization

Virulence factors that promote fungal colonization of the host include the ability to:

1. adhere to host cells and resist physical removal;
2. invade host cells;
3. compete for nutrients;
4. resist innate immune defenses such as phagocytosis and complement; and
5. evade adaptive immune defenses.

Examples of virulence factors that promote fungal colonization include:

1. A compromised immune system is the primary predisposing factor for serious fungal infections. A person highly immunosuppressed, such as a person taking immunosuppressive drugs to suppress transplant rejection, or a person with advancing HIV infection, or a person with other immunosuppressive disorders, becomes very susceptible to infections by fungi generally considered not very harmful to a healthy person with normal defenses.

2. As with bacteria, the ability to adhere to host cells with cell wall adhesins seems to play a role in fungal virulence.

3. Some fungi produce capsules allowing them to resist phagocytic engulfment, such as the yeast *Cryptococcus neoformans* and the yeast form of *Histoplasma capsulatum* (Figure 1).

4. *Candida albicans* stimulates the production of a cytokine called GM-CSF and this cytokine can suppress the production of complement by monocytes and macrophages. This may decrease the production of the opsonin C3b as well as the complement proteins that enhance chemotaxis of phagocytes.

5. *C. albicans* also appears to be able to acquire iron from red blood cells.

6. *C. albicans* produces acid proteases and phospholipases that aid in the penetration and damage of host cell membranes.

7. Some fungi are more resistant to phagocytic destruction, e.g., *Candida albicans, Histoplasma capsulatum*, and *Coccidioides immitis*.

8. There is evidence that when the yeast form of *Candida* enters the blood it activates genes allowing it to switch from its budding form to its hyphal form. In addition, when engulfed by macrophages, it starts producing the tubular germ tubes which penetrate the membrane of the macrophage thus causing its death.

   A movie of *Candida* killing a macrophage from within from the Theriot Lab Website at Stanford University Medical School: *Candida albicans* killing macrophages from inside out.

9. Factors such as body temperature, osmotic stress, oxidative stress, and certain human hormones activate a dimorphism-regulating histidine kinase enzyme in dimorphic molds, such as *Histoplasma capsulatum, Blastomyces dermatitidis*, and *Coccidioides immitis,* causing them to switch from their avirulent mold form to their virulent yeast form. It also triggers the yeast *Candida albicans* to switch from its yeast form to its more virulent hyphal form.

Virulence Factors that Damage the Host

Like bacteria, fungal PAMPs binding to PRRs can trigger excessive cytokine production leading to a harmful inflammatory response that damages tissues and organs. As fungi grow in the body, they can secrete enzymes to digest cells. These include proteases, phospholipases, and elastases. In response to both the fungus and to cell injury, cytokines are released. As seen earlier under Bacterial Pathogenesis, this leads to an inflammatory response and extracellular killing by phagocytes that leads to further destruction of host tissues.

Many molds secrete mycotoxins , especially when growing on grains, nuts and beans. These toxins may cause a variety of effects in humans and animals if ingested including loss of muscle coordination, weight loss, and tremors. Some mycotoxins are mutagenic and carcinogenic. Aflatoxins, produced by certain *Aspergillus* species, are especially carcinogenic. A mold called *Stachybotrys chartarum* is a mycotoxin producer that has been implicated as a potential serious problem in homes and buildings as one of the causes of "sick building syndrome." Mycotoxin symptoms in humans include dermatitis, inflammation of mucous membranes, , cough, fever, headache, and fatigue.

Summary

Many of the same factors that enable bacteria to colonize the body also enable fungi to colonize. Many of the same factors that enable bacteria to harm the body also enable fungi to cause harm.

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5.2.5: Chemotherapeutic Control of Fungi

Learning Objectives

1. Briefly describe 3 different ways antifungal chemotherapeutic agents may affect fungi and give an example of an antibiotic for each way.

Remember that like human cells, fungal cells are eukaryotic. Since fungal cells, unlike prokaryotic bacterial cells, are not that different from human cells, it is more difficult to find a chemotherapeutic agent that is selectively toxic for fungi, that is, will inhibit or kill fungal cells without also inhibiting or killing human cells. Some of the common antifungal chemotherapeutic agents are listed below.

1. One antibiotic, griseofulvin (*Fulvicin, Grifulvin, Gris-PEG*), interferes with nuclear division by preventing the aggregation of microtubules needed for mitosis in superficial mycelial fungi. It is used only for severe dermatophyte infections.
2. The antimetabolites trimethoprim + sulfomethoxazole , trimetrexate, atovaquone, and flucytosine interfere with normal nucleic acid synthesis. Trimethoprim/sulfomethoxazole (*Septra, Bactrim*), atovaquone (*Mepron*), and trimetrexate (*Neutrexin*) are used to treat *Pneumocystis* pneumonia. Flucytosine (*Ancobon*) is used for more serious *Candida* infections.
3. Polyene antibiotics such as amphotericin B, pimaricin, and nystatin are fungicidal drugs that bind to ergosterol in the fungal cytoplasmic membrane thus altering its structure and function and causing leakage of cellular needs. Nystatin (*Mycostatin*) is used to treat superficial *Candida* infections (thrush, vaginitis, cutaneous infections), amphotericin B (*Abelcet, Fungizone*) is used for systemic *Candida* infections, *Cryptococcus* infections, and dimorphic fungal infections.
4. The azole derivative antibiotics such as clotrimazole, miconazole, itraconazole, fluconazole, and ketoconazole, are fungistatic drugs used to treat many fungal infections. They interfere with ergosterol biosynthesis and thus alter the structure of the cytoplasmic membrane as well as the function of several membrane-bound enzymes like those involved in nutrient transport and chitin synthesis. Clotrimazole (*Lotramin, Mycelex*), miconazole (*Monistat*), and econazole (*Spectazole*) are used to treat superficial *Candida* and dermatophyte infections; oxiconazole (*Oxistat*) and sulconazole (*Exelderm*) are used for dermatophyte infections; butaconazole (*Femstat-3*), terconazole (*Terazole*), and tioconazole (*Vagistat-1*) are used for *Candida* vaginitis; ketoconazole (*Nizoral*) and itraconazole (*Sporanox*) are used for systemic *Candida, Cryptococcus*, and dimorphic fungal infections; and fluconazole (*Diflucan*) is used for *Candida* infections. Voriconazole (*VFEND*) is a triazole is used to treat *Candida* infections such as candidemia, disseminated infections in skin and infections in abdomen, kidney, bladder wall, and wounds. It is also used for invasive aspergillosis.
5. Echinocandins, including caspofungin (*Cancidas*) and micafungin (*Mycamine*) are intravenous antifungals that inhibits glucan synthesis in fungal cell walls. It is used in the treatment of candidemia , *Candida* intra-abdominal abscesses, peritonitis, esophageal candidiasis, and pleural space infections.
6. Naftifine (*Naftin*) and terbinafine (*Lamisil*) are allylamines that block synthesis of ergosterol as does the topical thiocarbonate tolnaftate. They are used to treat dermatophyte infections.

Exercise: Think-Pair-Share Questions

1. Why are there so few antifungal chemotherapeutic agents compared to the number of antibacterial agents?
2. Most of the antifungal agents interfere with the synthesis of ergosterol in the fungal cytoplasmic membrane.
   a. How does this harm the fungus?
   b. Why don't these agents work on bacterial and viral infections?

For a more detailed description of any specific antimicrobial agent, see the website of RxList - The Internet Drug Index.

Summary

Because fungi, like human cells, are eukaryotic, there are far fewer chemotherapeutic agents that are selectively toxic for fungi than there are for prokaryotic bacteria. Most antifungal agents bind to or interfere with the synthesis of ergosterol, the sterol in their cytoplasmic membrane, altering membrane structure and function.

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5.2.6: Fungi (Exercises)

These are homework exercises to accompany Kaiser's "Microbiology" TextMap. Microbiology is the study of microorganisms, which are defined as any microscopic organism that comprises either a single cell (unicellular), cell clusters or no cell at all (acellular). This includes eukaryotes, such as fungi and protists, and prokaryotes. Viruses and prions, though not strictly classed as living organisms, are also studied.

8.1: Overview of Fungi

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

1. A fungal infection is termed a _____.

8.2: Yeasts

Questions

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

1. Briefly describe a typical yeast and state how it reproduces asexually.
2. Match the following:

   _____ Reproductive spores produced by yeast by budding.

   _____ Thick walled survival spores produced by the yeast *Candida*.

   _____Long, continuous fungal filaments produced by dimorphic yeast.

   a. hyphae
   b. blastoconidia (blastospores)
   c. chlamydoconidia (chlamydospores)
3. Name 3 potentially pathogenic yeasts and state an infection each causes.

   a.
   b.
   c.

8.3: Molds

Questions

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

1. Define mold.
2. Match the following:

   _____ The hyphae that grow up in the air and produce asexual reproductive spores.

   _____ Large asexual reproductive mold spores coming of of vegetative hyphae and often produced by dermatophytes.

   _____ Asexual reproductive mold spores produced inside a sac or sporangium at the end of an aerial hypha.

   _____ The hyphae that anchor a mold and absorb nutrients.

   _____ Asexual reproductive mold spores produced in chains at the end of an aerial hypha.

   _____ A branching tubular structure of a mold that is usually divided into cell-like units by crosswalls called septa.

_____ Asexual reproductive mold spores produced by fragmentation of vegetative hyphae.

A. hypha
B. macroconidia
C. vegetative mycelium
D. aerial mycelium
E. sporangiospores
F. arthrospores
G. conidiospores

3. Define dermatophyte.
4. List 2 genera of dermatophytes.
    a.
    b.
5. Name 3 dermatophytic infections.
6. Describe what is meant by the term "dimorphic fungus", name 2 systemic infections caused by dimorphic fungi, and state how they are initially contracted.

8.4: Fungal Pathogenicity

1. Name at least 3 fungal virulence factors that promote fungal colonization.
    a.
    b.
    c.
2. Name 2 fungal virulence factors that damage the host.
    a.
    b.

8.5: Chemotherapeutic Control of Fungi

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

1. Briefly describe 2 different ways antifungal chemotherapeutic agents may affect fungi and give an example of an antibiotic for each way.
    a.
    b.

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CHAPTER OVERVIEW

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SECTION OVERVIEW

6.1: Microbial Nutrition

Topic hierarchy

6.1.1: Chemical Analysis of Microbial Cytoplasm

6.1.2: Sources of Essential Nutrients

6.1.3: Limitation of Microbial Growth by Nutrient Supply

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6.1.1: Chemical Analysis of Microbial Cytoplasm

LEARNING OBJECTIVES

- Describe the ultrastructure of the microbial cytoplasm

The bacterial cytoplasmic membrane plays a role in permeability and energy conservation in microbial cell structure. The cytoplasmic membranes in bacteria are composed of a phospholipid bilayer; this layer differs from those found in eukaryotes in their lack of sterols. The membranes however, may contain compounds called hopanoids and various fatty acids as well.

The fatty acids present within the cytoplasmic membrane are categorized into classes based on the addition of functional groups; methyl or hydroxyl groups are two examples of such groups. The cytoplasm itself is enclosed within the membrane. In bacteria it lacks the structures seen in eukaryotic cells, including a mitochondria, nucleus, or chloroplasts. The components of the microbial cytoplasm include macromolecules, smaller molecules, various inorganic ions, and cytoplasmic inclusions. The macromolecules included in a bacterial cytoplasm include proteins, DNA, RNA molecules. Smaller molecules include precursors to macromolecules and vitamins. Below is an overview of the components found in microbial cytoplasm based on chemical analysis.

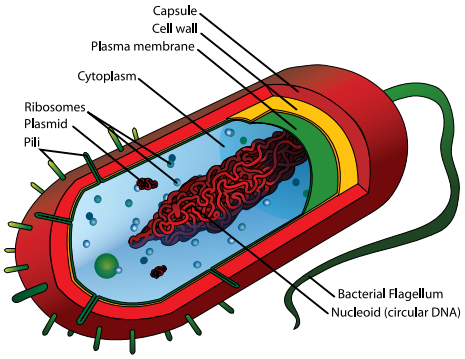


Figure: ***Typical Prokaryotic Cell****: Structure of a typical prokaryotic cell. Note the location of the cytoplasm and its components.*

The cytosol is a major component of the cytoplasm; it is the liquid portion of the cytoplasm that is not enclosed within a membrane-bound component. The cytosol is typically composed of water, salts, and organic molecules. Elements found within the cytosol include carbon, hydrogen, nitrogen, oxygen, phosphorous, and sulfur. These elements are critical to metabolic processes used by microbes.

Macromolecules found within bacterial cytoplasm include the nucleoid region, ribosomes, proteins, and enzymes. The nucleoid region is the area within the cell that houses the genetic material. Prokaryotes may sometimes contain an extra chromosomal piece of DNA referred to as the plasmid. The ribosomes, similar to ribosomes in eukaryotes, are responsible for protein synthesis. Unlike eukaryotes, prokaryotes, specifically bacteria, typically contain one cytosol-specific ribosome. Eukaryotes have multiple types of ribosomes, including the mitochondria and cytosol).

Another group of substances found within the cytoplasm include small particles referred to as inclusions. These inclusions are characterized by their granular appearance and insolubility. They are suspended in the cytosol. Inclusions vary, based on cell types. Typically, inclusions function as reserve materials. In prokaryotes, for example, lipid droplets are plentiful in cells which require lipid storage mechanisms. These lipid droplets store molecules such as fatty acids which are present in the cytoplasmic membrane of prokaryotes. *E. coli* offers another example of bacterial inclusions. These *E. coli* inclusions are composed of protein aggregates. In addition, inclusions can contain phosphate reserves, sulfur reserves, or photosynthetic pigments.

Key Points

- The cytosol contains the liquid portion of the cytoplasm and is not contained within a membrane -bound component.
- Macromolecules within the cytoplasm include DNA, ribosomes and proteins.
- Cytoplasmic inclusions within the cytoplasm are characterized by a granular appearance and vary based on cell type.

Key Terms

- **hopanoids**: a group of pentacyclic compounds that exhibit various functions in prokaryotes

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6.1.2: Sources of Essential Nutrients

LEARNING OBJECTIVES

- Describe the types of nutrients that are used by microorganisms for growth and metabolism

Nutrients are materials that are acquired from the environment and are used for growth and metabolism. Microorganisms (or microbes) vary significantly in terms of the source, chemical form, and amount of essential elements they need. Some examples of these essential nutrients are carbon, oxygen, hydrogen, phosphorus, and sulfur. There are two categories of essential nutrients: macro-nutrients (which are needed in large amounts) and micro-nutrients (which are needed in trace or small amounts). Macro-nutrients usually help maintain the cell structure and metabolism. Micro-nutrients help enzyme function and maintain protein structure.

Organic and Inorganic Nutrients

Organic nutrients contain some combination of carbon and hydrogen atoms. Inorganic nutrients are elements or simply molecules that are made of elements other than carbon and hydrogen.

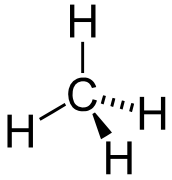


Figure: ***Methane, a simple organic molecule****: Methane is one of the simplest organic molecules.*

Essential Nutrients

The sources of common essential nutrients are carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. Organisms usually absorb carbon when it is in its organic form. Carbon in its organic form is usually a product of living things. Another essential nutrient, nitrogen, is part of the structure of protein, DNA, RNA, and ATP. Nitrogen is important for heterotroph survival, but it must first be degraded into basic building blocks, such as amino acids, in order to be used. Oxygen is an important component of both organic and inorganic compounds. It is essential to the metabolism of many organisms. Hydrogen has many important jobs including maintaining the pH of solutions and providing free energy in reactions of respiration. Phosphate is an important player in making nucleic acids and cellular energy transfers. Without sufficient phosphate, an organism will cease to grow. Lastly, sulfur is found in rocks and sediments and is found widely in mineral form.

Key Points

- Nutrients are materials that are acquired from the environment and are used for growth and metabolism. Microorganisms (or microbes) vary significantly in the source, chemical form, and amount they will need of these essential elements.
- Macro-nutrients are needed in large amounts and micro-nutrients are needed in trace or small amounts.
- Organic nutrients contain some combination of carbon and hydrogen atoms. Inorganic nutrients are elements or simply molecules that are made of elements other than carbon and hydrogen.

Key Terms

- **organic**: relating to the compounds of carbon, and relating to natural products
- **inorganic**: relating to a compound that does not contain carbon.
- **metabolism**: the complete set of chemical reactions that occur in living cells.

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6.1.3: Limitation of Microbial Growth by Nutrient Supply

LEARNING OBJECTIVES

- Describe the role of nutrients in microbial growth and their culture in the lab

Nutrients are necessary for microbial growth and play a vital role in the proper cultivation of microorganisms in the laboratory and for proper growth in their natural environments. The types of nutrients that are required include those that supply energy, carbon and additional necessary materials. The nutrients used to propagate growth are organism -specific, based on their cellular and metabolic processes..



Figure: ***Anthrax Culture****: An image of an anthrax culture grown on a petri dish. For microogranisms to be cultured in the laboratory or undergo successful growth in their natural environment, the proper nutrients are absolutely necessary.*

The common nutrients which are found to be required in all living things include carbon, nitrogen, sulfur, phosphorus, potassium, magnesium, calcium, oxygen, iron and additional trace elements. Essential nutrients are nutrients absolutely required by an organism. Two categories of essential nutrients are macro- and micro-nutrients. Macronutrients are necessary in large amounts; micronutrients tend to be needed in smaller amounts and are often trace elements.

Nutrients as Limiting Factors

In regard to required nutrients for proper growth, there are often limiting factors involved. The limiting factor or limiting nutrient affects and controls growth. The availability of specific nutrients dictates organismal growth by controlling and limiting activation of cellular and metabolic pathways necessary for progress. When all nutrients and parameters are ideal and constant during the growth phase, this is regarded as a steady state: all requirements are present and microorganisms thrive. In circumstances where there are less than ideal parameters, such as a lack of specific requirements, the growth process is affected.

In industrial microbiology this concept is critical, as microbial growth and production is dictated by proper cellular growth and metabolism. The production of necessary components if often controlled by the presence and concentration of a limiting nutrient. Hence, it is critical to identify the required nutrients and ensure these are supplied in the culturing of microorganisms.

Key Points

- The common nutrients which are found to be required in all living things include carbon, nitrogen, sulfur, phosphorus, potassium, magnesium, calcium, oxygen, iron and additional trace elements.
- Both and macro- and micro-nutrients are critical in proper organismal growth as they play important roles in cellular and metabolic processes.
- The limiting nutrient is essential for growth and based on its concentration and presence or absence can control growth.

Key Terms

- **micronutrient**: an element or nutrient required in small quantities.
- **macronutrients**: any element or nutrient required in large amounts.

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SECTION OVERVIEW

6.2: Cell Differentiation and Starvation

Topic hierarchy

6.2.1: Activation of Starvation by Survival Genes

6.2.2: Oligotrophs

6.2.3: Starvation-Induced Fruiting Bodies

6.2.4: Bacterial Differentiation

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6.2.1: Activation of Starvation by Survival Genes

Stringent response is a stress response that occurs in bacteria and plant chloroplasts in reaction to stress conditions.

Learning Objectives

- Discuss how starvation activates survival genes

Key Points

- The stringent response is signaled by the alarmone (p)ppGpp.
- In Escherichia coli (p)ppGpp, production is mediated by the ribosomal protein L11 and the ribosome-associated protein RelA with the A-site bound deacylated tRNA being the ultimate inducer.
- In other bacteria, stringent response is mediated by a variety of RelA/SpoT Homologue (RSH) proteins, some of which activate synthetically, hydrolytically, or both (Rel).

Key Terms

- **stringent response**: a stress response in bacteria in reaction to amino-acid starvation, fatty acid limitation, and other stress conditions.

Stringent Response, also called stringent control, is a stress response that occurs in bacteria and plant chloroplasts in reaction to amino-acid starvation, fatty acid limitation, iron limitation, heat shock, and other stress conditions. The stringent response is signaled by the alarmone (p)ppGpp and modulating transcription of up to 1/3 of all genes in the cell. This in turn causes the cell to divert resources away from growth and division and toward amino acid synthesis in order to promote survival until nutrient conditions improve.

In *Escherichia coli*, (p)ppGpp production is mediated by the ribosomal protein L11 and the ribosome-associated protein RelA with the A-site bound deacylated tRNA being the ultimate inducer. RelA converts GTP and ATP into pppGpp by adding the pyrophosphate from ATP onto the 3′ carbon of the ribose in GTP, releasing AMP. pppGpp is converted to ppGpp by the *gpp* gene product, releasing Pi. ppGpp is converted to GDP by the *spoT* gene product, releasing pyrophosphate (PPi). GDP is converted to GTP by the *ndk* gene product. Nucleoside triphosphate (NTP) provides the Pi, and is converted to Nucleoside diphosphate (NDP).

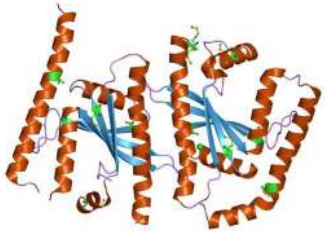


Figure: **RelA/ SpoT protein homologue**: In bacteria stringent response is mediated by a variety of RelA/SpoT Homologue (RSH) proteins.

In other bacteria, stringent response is mediated by a variety of RelA/SpoT Homologue (RSH) proteins. Some of these proteins activate synthetically, hydrolytically, or both (Rel). The disabling of the stringent response by distruption of relA and spoT in *Pseudomonas aeruginosa*, produced in infectious cells and biofilms characterized by nutrient limitation, causes greater susceptibility to antibiotics.

During the stringent response, (p)ppGpp accumulation affects the resource-consuming cell processes replication, transcription, and translation. (p)ppGpp is thought to bind RNA polymerase and alter the transcriptional profile, decreasing the synthesis of translational machinery (such as rRNA and tRNA), and increasing the transcription of biosynthetic genes. Additionally, the initiation of new rounds of replication is inhibited and the cell cycle arrests until nutrient conditions improve. Translational GTPases involved in protein biosynthesis are also affected by ppGpp, with Initiation Factor 2 (IF2) being the main target.

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6.2.2: Oligotrophs

LEARNING OBJECTIVES

- Examine oligotrophs and their adaptation to nutrient poor environments

An oligotroph is an organism that thrives in an environment that offers very low levels of nutrients. They may be contrasted with copiotrophs, which prefer nutritionally rich environments. Oligotrophs are characterized by slow growth, low rates of metabolism, and generally low population density. Oligotrophic environments include deep oceanic sediments, caves, glacial and polar ice, deep subsurface soil, aquifers, ocean waters, and leached soils. An ecosystem or environment is said to be oligotrophic if it offers little to sustain life. The term is commonly utilized to describe environments of water, ice, air, rock or soil with very low nutrient levels. Oligotrophic environments are of special interest for alternative energy sources and survival strategies upon which life could rely. An example of oligotrophic bacterium are *Caulobacter crescentus*.



Figure: ***Extremophiles****: These hot springs are an example of harsh environments that some extremophiles can grow in.*

Caulobacter crescentus is a Gram-negative, oligotrophic bacterium widely distributed in fresh water lakes and streams. The control circuitry that directs and paces Caulobacter cell cycle progression involves the entire cell operating as an integrated system. The control circuitry monitors the environment and the internal state of the cell, including the cell topology, as it orchestrates activation of cell cycle subsystems and Caulobacter crescentus asymmetric cell division. The proteins of the Caulobacter cell cycle control system and its internal organization are co-conserved across many alphaproteobacteria species, but there are great differences in the regulatory apparatus' functionality and peripheral connectivity to other cellular subsystems from species to species. The Caulobacter cell cycle control system has been exquisitely optimized by evolutionary selection as a total system for robust operation in the face of internal stochastic noise and environmental uncertainty.

The bacterial cell's control system has a hierarchical organization. The signaling and the control subsystem interfaces with the environment by means of sensory modules largely located on the cell surface. The genetic network logic responds to signals received from the environment and from internal cell status sensors to adapt the cell to current conditions.

Key Points

- Oligotrophic environments are of special interest for alternative energy sources and survival strategies upon which life could rely.

Key Terms

- **oligotroph**: An organism capable of living in an environment that offers very low levels of nutrients.

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6.2.3: Starvation-Induced Fruiting Bodies

Starvation-induced fruiting bodies can aggregate up to 500 micrometres long and contain approximately 100,000 bacterial cells.

Learning Objectives

- Explain starvation induced fruit bodies

Key Points

- In fruiting bodies, the bacteria perform separate tasks; this type of cooperation is a simple type of multicellular organisation.
- *Myxococcus xanthus* colonies exist as a self-organized, predatory single- species biofilm called a swarm.
- The fruiting process is thought to benefit *myxobacteria* by ensuring that cell growth is resumed with a group (swarm) of *myxobacteria*, rather than as isolated cells.

Key Terms

- **quorum sensing**: A proposed method of communication between bacterial cells by the release and sensing of small diffusible signal molecules.
- **stigmergy**: A mechanism of spontaneous, indirect coordination between agents or actions, where the trace left in the environment by an action stimulates the performance of a subsequent action.
- **saprotrophic**: Extra-cellular digestion involved in the processing of dead or decayed organic matter

Starvation-Induced Fruiting Bodies

When starved of amino acids, *myxobacteria*, or slime bacteria, detect surrounding cells in a process known as *quorum sensing* . Migrating towards each other, they aggregate to form fruiting bodies up to 500 micrometers long containing approximately 100,000 bacterial cells. In these fruiting bodies, the bacteria perform separate tasks; this type of cooperation is a simple type of multicellular organisation. About one in 10 cells migrate to the top of these fruiting bodies and differentiate into a specialized dormant state called *myxospore*, which is more resistant to drying and other adverse environmental conditions than ordinary cells.

The *myxobacteria* are a group of bacteria that predominantly live in the soil and feed on insoluble organic substances. The *myxobacteria* have very large genomes, relative to other bacteria e.g., 9–10 million nucleotides. *Sorangium cellulosum* has the largest known (as of 2008) bacterial genome, at 13.0 million nucleotides.

Myxobacteria are included among the delta group of *proteobacteria*, a large taxon of Gram-negative forms. They can move actively by gliding and typically travel in swarms (also known as wolf packs), containing many cells kept together by intercellular molecular signals. Individuals benefit from aggregation as it allows accumulation of extracellular enzymes which are used to digest food that increases feeding efficiency.

Myxobacteria produce a number of biomedically and industrially-useful chemicals, such as antibiotics, and export those chemicals outside of the cell. When nutrients are scarce, myxobacterial cells aggregate into fruiting bodies, a process long-thought to be mediated by chemotaxis but now considered to be a function of a form of contact-mediated signaling. These fruiting bodies can take different shapes and colors, depending on the species.



Figure: **Bacteria use cell signaling to communicate**: A swarm of M. xanthus is a distributed system: a population of millions of identical entities that communicate among themselves in a non-centralized fashion, thus behaving as a single entity.

Within the fruiting bodies, cells begin as rod-shaped vegetative cells and develop into rounded myxospores with thick cell walls. These myxospores, analogous to spores in other organisms, are more likely to survive until nutrients are more plentiful. The fruiting process is thought to benefit *myxobacteria* by ensuring that cell growth is resumed with a group (swarm) of *myxobacteria*, rather than as isolated cells. At a molecular level, initiation of fruiting body development is regulated by Pxr sRNA.

Myxococcus xanthus colonies exist as a self-organized, predatory, saprotrophic, single-species biofilm called a swarm. *Myxococcus xanthus*, which can be found almost ubiquitously in soil, are thin rod-shaped, gram-negative cells that exhibit self-organizing behavior as a response to environmental cues. The swarm modifies its environment through *stigmergy*. This behavior facilitates predatory feeding, as the concentration of extracellular digestive enzymes secreted by the bacteria increases.

M. xanthus is a model organism for studying development, the behavior in which starving bacteria self-organize to form fruiting bodies: dome shaped structures of approximately 100,000 cells. These swarms differentiate into metabolically quiescent and environmentally resistant myxospores over the course of several days. During this process of self-organizing, dense ridges of cells move in traveling waves (ripples) that grow and shrink over several hours.

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6.2.4: Bacterial Differentiation

Several bacteria alter their morphology in response to the types and concentrations of external compounds.

Learning Objectives

- Explain bacterial differentiation to eukaryotic-like sturctures

Key Points

- Bacterial morphology changes help to optimize interactions with cells and the surfaces to which they attach.
- Oxidative stress, nutrient limitation, DNA damage and antibiotics exposure are some of stress conditions to which bacteria respond, altering their DNA replication and cell division.
- The most frequent shape alteration may be filamentation triggered by a limitation in the availability of one or more nutrients.

Key Terms

- **cytoskeleton**: A cellular structure like a skeleton, contained within the cytoplasm.
- **septum**: a partition that separates the cells of a (septated) fungus
- **segrosomes**: multiprotein complexes that partition chromosomes/plasmids in bacteria.
- **cell division**: a process by which a cell divides into two cells.

Bacterial morphological plasticity refers to evolutionary changes in the shape and size of bacterial cells. As bacteria evolve, morphological changes occur to maintain the consistency of the cell. However, this consistency could be affected in some circumstances (such as environmental stress) and changes in bacterial shape and size. In bacteria, the transformation into filamentous organisms have been recently demonstrated. These are survival strategies that affect the normal physiology of the bacteria in response to factors such as innate immune response, predator sensing, quorum sensing and antimicrobial signs.

Normally, bacteria have different shapes and sizes which include coccus, rod and helical/spiral (among others less common). This forms the basis for their classification. For instance, rod shapes may allow bacteria to attach more readily in environments with shear stress (e.g., in flowing water). Cocci may have access to small pores, creating more attachment sites per cell and hiding themselves from external shear forces. Spiral bacteria combine some of the characteristics of cocci (small footprints) and of filaments (more surface area on which shear forces can act) and the ability to form an unbroken set of cells to build biofilms. Several bacteria alter their morphology in response to the types and concentrations of external compounds. Bacterial morphology changes help to optimize interactions with cells and the surfaces to which they attach. This mechanism has been described in bacteria such as Escherichia coli and Helicobacter pylori.

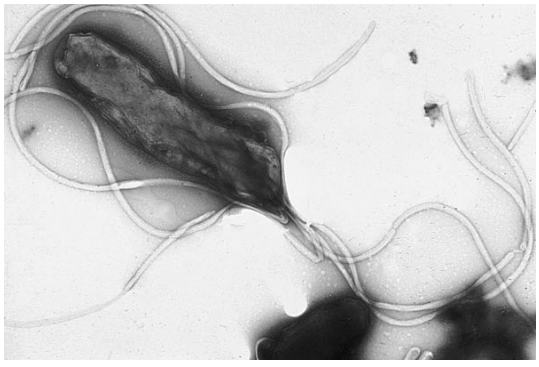


Figure: **Electron micrograph of H. pylori possessing multiple flagella (negative staining)**: Several bacteria alter their morphology in response to the types and concentrations of external compounds.This has been described in bacteria such as E. coli and H. pylori.

Oxidative stress, nutrient limitation, DNA damage and antibiotics exposure are some stress conditions to which bacteria respond, altering their DNA replication and cell division. Filamentous bacteria have been considered to be over-stressed, sick and dying members of the population. However, the filamentous members of some communities have vital roles in the population's continued existence, since the filamentous phenotype can confer protection against lethal environments.Filamentous *E. coli* can be up to 70

μm in length and has been identified as playing an important role in pathogenesis in human cystitis. There are different mechanisms identified in some bacteria that are attributable to the development of filamentous forms.

Nutritional stress can change bacterial morphology. The most frequent shape alteration may be filamentation triggered by a limitation in the availability of one or more nutrients. Since the filament can increase a cell's uptake–proficiency surface without changing its surface-to-volume ratio appreciably, this may be enough reason for cells to be filament. Moreover, the filamentation benefits bacterial cells attaching to a surface because it increases specific surface area in direct contact with the solid medium. In addition, the filamentation may allow bacterial cells to access nutrients by enhancing the possibility that the filament will be exposed to a nutrient-rich zone and pass compounds to the rest of the cell's biomass. For example, *Actinomyces israelii* grows as filamentous rods or branched in the absence of phosphate, cysteine, or glutathione. However, it returns to a regular rod-like morphology when adding back these nutrients.

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SECTION OVERVIEW

6.3: Culturing Bacteria

Topic hierarchy

6.3.1: Culture Media

6.3.2: Complex and Synthetic Media

6.3.3: Selective and Differential Media

6.3.4: Aseptic Technique, Dilution, Streaking, and Spread Plates

6.3.5: Special Culture Techniques

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6.3.1: Culture Media

LEARNING OBJECTIVES

- Classify culture media

Culture medium or growth medium is a liquid or gel designed to support the growth of microorganisms. There are different types of media suitable for growing different types of cells. Here, we will discuss microbiological cultures used for growing microbes, such as bacteria or yeast.

NUTRIENT BROTHS AND AGAR PLATES

These are the most common growth media, although specialized media are sometimes required for microorganism and cell culture growth. Some organisms, termed fastidious organisms, need specialized environments due to complex nutritional requirements. Viruses, for example, are obligate intracellular parasites and require a growth medium containing living cells. Many human microbial pathogens also require the use of human cells or cell lysates to grow on a media.

The most common growth media nutrient broths (liquid nutrient medium) or LB medium (Lysogeny Broth) are liquid. These are often mixed with agar and poured into Petri dishes to solidify. These agar plates provide a solid medium on which microbes may be cultured. They remain solid, as very few bacteria are able to decompose agar. Many microbes can also be grown in liquid cultures comprised of liquid nutrient media without agar.



Figure: **Microbial pathogen growing on blood-agar plate**: Red blood cells are used to make an agar plate. Different pathogens that can use red blood cells to grow are shown on these plates. On the left is staphylococcus and the right streptococcus.

DEFINED VS UNDEFINED MEDIA

This is an important distinction between growth media types. A *defined* medium will have known quantities of all ingredients. For microorganisms, it provides trace elements and vitamins required by the microbe and especially a defined carbon and nitrogen source. Glucose or glycerol are often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources. An *undefined* medium has some complex ingredients, such as yeast extract, which consists of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.

There are many different types of media that can be used to grow specific microbes, and even promote certain cellular processes; such as wort, the medium which is the growth media for the yeast that makes beer. Without wort in certain conditions, fermentation cannot occur and the beer will not contain alcohol or be carbonated (bubbly).

COMMON BROADLY-DEFINED CULTURE MEDIA

Nutrient media – A source of amino acids and nitrogen (e.g., beef, yeast extract). This is an undefined medium because the amino acid source contains a variety of compounds with the exact composition being unknown. These media contain all the elements that most bacteria need for growth and are non-selective, so they are used for the general cultivation and maintenance of bacteria kept in laboratory-culture collections.

Minimal media – Media that contains the minimum nutrients possible for colony growth, generally without the presence of amino acids, and are often used by microbiologists and geneticists to grow “wild type” microorganisms. These media can also be used to select for or against the growth of specific microbes. Usually a fair amount of information must be known about the microbe to determine its minimal media requirements.

Selective media – Used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing.

Differential media – Also known as indicator media, are used to distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection and identification of microorganisms.

These few examples of general media types provide some indication only; there are a myriad of different types of media that can be used to grow and control microbes.

Key Points

- Culture media contains the nutrients needed to sustain a microbe.
- Culture media can vary in many ingredients allowing the media to select for or against microbes.
- Glucose or glycerol are often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources in culture media.

Key Terms

- **culture**: The process of growing a bacterial or other biological entity in an artificial medium.
- **lysogeny broth**: Lysogeny broth (LB) is a nutritionally-rich medium; primarily used for the growth of bacteria.

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6.3.2: Complex and Synthetic Media

In defined media all the chemical compounds are known, while undefined media has partially unknown chemical constituents.

Learning Objectives

- Differentiate complex and synthetic medias

Key Points

- Defined media is made from constituents that are completely understood.
- Undefined media has some part of which is not entirely defined.
- The presence of extracts from animals or other microbes makes a media undefined as the entire chemical composition of extracts are not completely known.

Key Terms

- **recombinant**: This term refers to something formed by combining existing elements in a new combination. Thus, the phrase recombinant DNA refers to an organism created in the lab by adding DNA from another species.
- **serum**: The clear yellowish fluid obtained upon separating whole blood into its solid and liquid components after it has been allowed to clot. Also called blood serum.

There are many types of culture media, which is food that microbes can live on. Two major sub types of media are complex and synthetic medias, known as undefined and defined media.



Figure: **Undefined Media**: Luria Broth as shown here is made with yeast extract, as yeast extract is not completely chemically defined Luria Broth is therefore an undefined media. By Lilly_M [GFDL (http://www.gnu.org/copyleft/fdl.html), CC-BY-SA, via Wikimedia Commons

An undefined medium has some complex ingredients, such as yeast extract or casein hydrolysate, which consist of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.A defined medium (also known as chemically defined medium or synthetic medium) is a medium in which all the chemicals used are known, no yeast, animal, or plant tissue is present. A chemically defined medium is a growth medium suitable for the culture of microbes or animal cells (including human) of which all of the chemical components are known. The term chemically defined medium was defined by Jayme and Smith as a 'Basal formulation which may also be protein-free and is comprised solely of biochemically-defined low molecular weight constituents.

A chemically defined medium is entirely free of animal-derived components (including microbial derived components such as yeast extract) and represents the purest and most consistent cell culture environment. By definition chemically defined media cannot contain either fetal bovine serum, bovine serum albumin, or human serum albumin as these products are derived from bovine or human sources and contain complex mixes of albumins and lipids. The term ‘chemically defined media’ is often misused in the literature to refer to serum albumin-containing media. Animal serum or albumin is routinely added to culture media as a source of nutrients and other ill-defined factors, despite technical disadvantages to its inclusion and its high cost. Technical disadvantages to using serum include the undefined nature of serum, batch-to-batch variability in composition, and the risk of contamination. There are increasing concerns about animal suffering inflicted during serum collection that add an ethical imperative to move away from the use of serum wherever possible. Chemically defined media differ from serum-free media in that bovine serum albumin or human serum albumin with either a chemically defined recombinant version (which lacks the albumin associated lipids) or synthetic chemical such as the polymer polyvinyl alcohol which can reproduce some of the functions of serums.

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6.3.3: Selective and Differential Media

LEARNING OBJECTIVES

- Compare selective and differential media

There are many types of media used in the studies of microbes. Two types of media with similar implying names but very different functions, referred to as selective and differential media, are defined as follows.

Selective media are used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing. Media lacking an amino acid such as proline in conjunction with *E. coli* unable to synthesize it were commonly used by geneticists before the emergence of genomics to map bacterial chromosomes. Selective growth media are also used in cell culture to ensure the survival or proliferation of cells with certain properties, such as antibiotic resistance or the ability to synthesize a certain metabolite. Normally, the presence of a specific gene or an allele of a gene confers upon the cell the ability to grow in the selective medium. In such cases, the gene is termed a marker. Selective growth media for eukaryotic cells commonly contain neomycin to select cells that have been successfully transfected with a plasmid carrying the neomycin resistance gene as a marker. Gancyclovir is an exception to the rule as it is used to specifically kill cells that carry its respective marker, the Herpes simplex virus thymidine kinase (HSV TK). Some examples of selective media include:

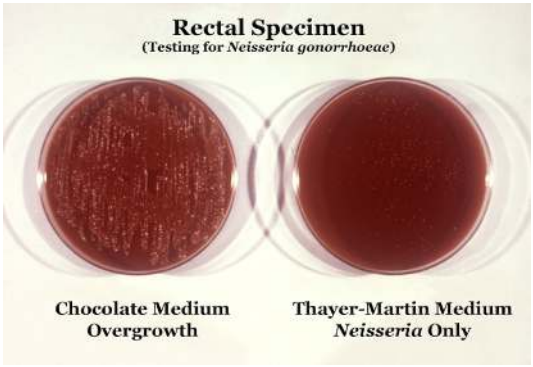


Figure: **Non-selective versus selective media.**: The non-selective media on the right allows of the growth of several different bactarial species and is overgrown with bacteria (whitish lines). While the plate on the right selectively only allows the bacteria Neisseria gonorrhoeae, to grow (white dots).

- Eosin methylene blue (EMB) that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram negative bacteria.
- YM (yeast and mold) which has a low pH, deterring bacterial growth.
- MacConkey agar for Gram-negative bacteria.
- Hektoen enteric agar (HE) which is selective for Gram-negative bacteria.
- Mannitol salt agar (MSA) which is selective for Gram-positive bacteria and differential for mannitol.
- Terrific Broth (TB) is used with glycerol in cultivating recombinant strains of Escherichia coli.
- Xylose lysine desoxyscholate (XLD), which is selective for Gram-negative bacteria buffered charcoal yeast extract agar, which is selective for certain gram-negative bacteria, especially Legionella pneumophila.

Differential media or indicator media distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria. Examples of differential media include:

- Blood agar (used in strep tests), which contains bovine heart blood that becomes transparent in the presence of hemolytic.
- Streptococcuseosin methylene blue (EMB), which is differential for lactose and sucrose fermentation.

- MacConkey (MCK), which is differential for lactose fermentationmannitol salt agar (MSA), which is differential for mannitol fermentation.
- X-gal plates, which are differential for lac operon mutants.

Key Points

- Selective media generally selects for the growth of a desired organism, stopping the growth of or altogether killing non-desired organisms.
- Differential media takes advantage of biochemical properties of target organisms, often leading to a visible change when growth of target organisms are present.
- Differential media, unlike selective media, does not kill organisms. It indicates if a target organism is present.

Key Terms

- **recombinant**: This term refers to something formed by combining existing elements in a new combination. Thus, the phrase recombinant DNA refers to an organism created in the lab by adding DNA from another species.
- **gene**: A unit of heredity; a segment of DNA or RNA that is transmitted from one generation to the next. It carries genetic information such as the sequence of amino acids for a protein.
- **allele**: One of a number of alternative forms of the same gene occupying a given position on a chromosome.

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6.3.4: Aseptic Technique, Dilution, Streaking, and Spread Plates

Microbiologists rely on aseptic technique, dilution, colony streaking and spread plates for day-to-day experiments.

Learning Objectives

- Recall aseptic technique, dilution series, streaking and spreading plates

Key Points

- Aseptic technique is basically the mindset of keeping things free of contamination, as the world we live in has so many microbes that can interfere with experiments.
- Colony streaking leads to to the isolation of individual colonies, which are a group of microbes that came from one single progenitor mircrobe.
- Spread plates allow for the even spreading of bacteria onto a petri dish; allowing for the isolation of individual colonies, for counting or further experiments.

Key Terms

- **colony**: A bacterial colony is defined as a visible cluster of bacteria growing on the surface of or within a solid medium, presumably cultured from a single cell.
- **bunsen burner**: A small laboratory gas burner whose air supply may be controlled with an adjustable hole.

Microbiologists have many tools, but four relatively simple techniques are used by microbiologists daily, these are outlined here.

Aseptic technique or sterile technique is used to avoid contamination of sterile media and equipment during cell culture. Sterile technique should always be employed when working with live cell cultures and reagents/media that will be used for such cultures. This technique involves using flame to kill contaminating organisms, and a general mode of operation that minimizes exposure of sterile media and equipment to contaminants.

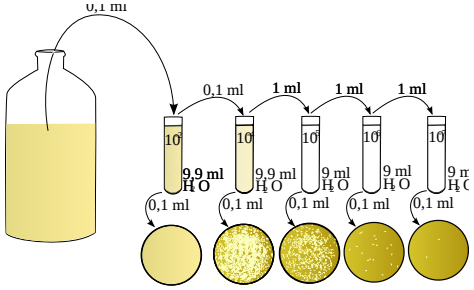


Figure: **Serial Dilution**: Example of Serial dilution of bacteria in five steps. The diluted bacteria were then spread plated. By Leberechtc (Own work) [GFDL (http://www.gnu.org/copyleft/fdl.html) and CC-BY-3.0 (http://creativecommons.org/licenses/by/3.0)], via Wikimedia Commons

When working with cultures of living organisms, it is extremely important to maintain the environments in which cells are cultured and manipulated as free of other organisms as possible. This requires that exposure of containers of sterilized culture media to outside air should be minimized, and that flame is used to “re-sterilize” container lids and rims. This means passing rims and lids through the flame produced by a Bunsen burner in order to kill microorganisms coming in contact with those surfaces.

Sterile technique, in general, is a learned state-of-being, or mantra, where every utilization of any sterile material comes with the caveat of taking every precaution to ensure it remains as free of contaminants as possible for as long as possible. Heat is an excellent means of killing microorganisms, and the Bunsen burner is the sterile technician’s best friend.

A serial dilution is the step-wise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. A ten-fold serial dilution could be 1 M, 0.1 M, 0.01 M, 0.001 M… Serial dilutions are used to accurately create highly-diluted solutions as well. A culture of microbes can be diluted in the same fashion. For a ten-fold dilution on a 1 mL scale, vials are filled with 900 microliters of water or media, and 100 microliters of the

stock microbial solution are serially transferred, with thorough mixing after every dilution step. The dilution of microbes is very important to get to microbes diluted enough to count on a spread plate (described later).

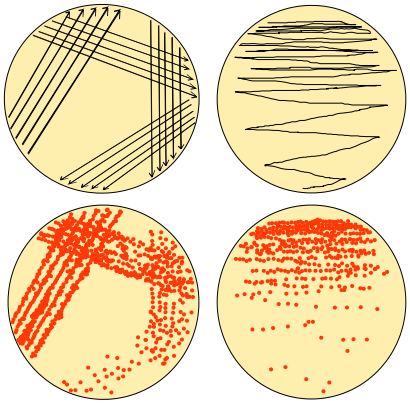


Figure: **Streak plate**: Four streak plates. Successful streaks lead to individual colonies of microbes.

In microbiology, streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested.The streaking is done using a sterile tool, such as a cotton swab or commonly an inoculation loop. This is dipped in an inoculum such as a broth or patient specimen containing many species of bacteria.The sample is spread across one quadrant of a petri dish containing a growth medium, usually an agar plate which has been sterilized in an autoclave. Choice of which growth medium is used depends on which microorganism is being cultured, or selected for. Growth media are usually forms of agar, a gelatinous substance derived from seaweed.

Spread plates are simply microbes spread on a media plate. Microbes are in a solution, and can be diluted. They are then transferred to a petri dish with media specific for the growth of the microbe of interest. The solution is then spread uniformly through a number of possible means, the most popular is the use of sterile glass beads that are shook on top of the media, spreading the microbe-containing liquid evenly on the plate. Also common is the use of a bent-glass rod, often referred to as a hockey stick, due to its similar shape. The glass rod is sterilized and used to spread the microbe-containing liquid uniformly on the plate.

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6.3.5: Special Culture Techniques

LEARNING OBJECTIVES

- Evaluate special culture techniques

Microbiologists would prefer to use well-defined media to grow a microbe, making the microbe easier to control. However, microbes are incredibly varied in what they use as a food source, the environments they live in, and the danger levels they may have for humans and other organisms they may compete with. Therefore they need special nutrient and growth environments. To grow these difficult microbes, microbiologists often turn to undefined media which is chosen based on price and more-so in this case by necessity as some microorganisms have never been cultured on defined media. Some special culture conditions are relatively simple as demonstrated by microaerophile.

A microaerophile is a microorganism that requires oxygen to survive, but requires environments containing lower levels of oxygen than are present in the atmosphere (~20% concentration). Many microphiles are also capnophiles, as they require an elevated concentration of carbon dioxide. In the laboratory they can be easily cultivated in a candle jar. A candle jar is a container into which a lit candle is introduced before sealing the container's airtight lid. The candle's flame burns until extinguished by oxygen deprivation, which creates a carbon dioxide-rich, oxygen-poor atmosphere in the jar. Many labs also have access directly to carbon dioxide and can add the desired carbon dioxide levels directly to incubators where they want to grow microaerophiles.

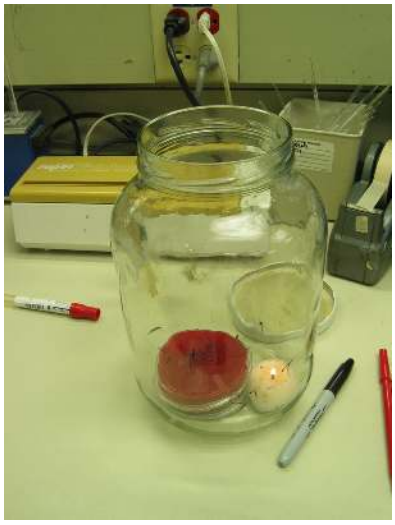


Figure: ***Candle jar****: A candle is lit in a jar with a culture plate. The lid is put on, as the burns it increases the carbon dioxide levels in the jar.*

Animals can often be used to culture microbes. For example, armadillos are often used in the study of leprosy. They are particularly susceptible due to their unusually low body temperature, which is hospitable to the leprosy bacterium, Mycobacterium leprae. The leprosy bacterium is difficult to culture and armadillos have a body temperature of 34°C, similar to human skin. Likewise, humans can acquire a leprosy infection from armadillos by handling them or consuming armadillo meat. Additionally, Syphillis which is caused by the bacteria *Treponema pallidum* is difficult to grow with defined media, so rabbits are used to culture *Treponema pallidum*. *Treponema pallidum* belongs to the Spirochaetesphylum of bacteria.

To date Spirochaetes are very difficult if not impossible to rear in a controlled laboratory environment. This also includes other human pathogens like the bacterium that causes Lyme disease. Using animals to culture human-pathogens has problems. First, the use of animals is always difficult for technical and ethical reasons. Also, a microbe growing on animal other than a human may behave very differently from how that same microbe will behave on a human. Some human pathogens are grown directly on cells cultured from humans. Exemplified by the bacteria *Chlamydia trachomatis*, the bacteria responsible for the sexually transmitted infection (STI) in humans known as Chlamydia. As *Chlamydia trachomatis* only grows in humans. The human cell culture known as McCoy cell culture is used to culture this bacteria.

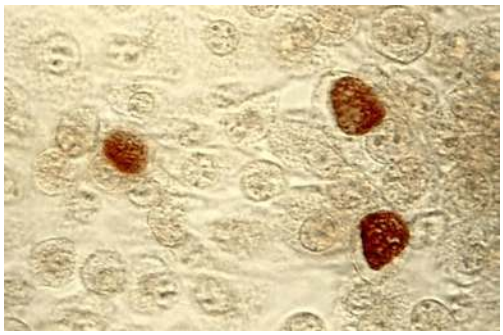


Figure: ***Chlamydias bacteria group****: Light microscope view of cells infected with chlamydiae as shown by the brown inclusion bodies.*

A large concern of microbiology is trying to find ways in which humans can avoid or get rid of microbrial infections. As typified by some of the above examples, some microbes have to be grown in the lab, and some of them can infect humans. To deal with this, microbiologists use a classification of biosafety levels. A biosafety level is the level of the biocontainment precautions required to isolate dangerous biological agents in an enclosed facility. The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest at level 4 (BSL-4). In the United States, the Centers for Disease Control and Prevention (CDC) have specified these levels.

- **Biosafety Level 1**: This level is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, with minimal potential hazard to laboratory personnel and the environment.
- **Biosafety Level 2**: This level is similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It includes various bacteria and viruses that cause only mild disease to humans or are difficult to contract via aerosol in a lab setting such as chlamydia.
- **Biosafety Level 3**: This level is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease after inhalation. It includes various bacteria, parasites, and viruses that can cause severe to fatal disease in humans, but for which treatments exist (eg. yellow fever).
- **Biosafety Level 4**: This level is reserved for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, agents that cause severe to fatal disease in humans for which vaccines or other treatments are not available, such as Bolivian and Argentine hemorrhagic fevers, Marburg virus, and the Ebola virus. Very few laboratories are biosafety level 4.



Figure: **Positive pressure suit**: A scientist puts on a positive pressure suit, something needed to work with the most dangerous human pathogens in a biosafety level 4 laboratory.

Key Points

- Microbes, often those that we know little about, have to be cultured with undefined media or growth conditions.
- The use of animals to culture animals is sometimes necessary as no simple media can be used, this presents technical and ethical issues.
- As human pathogens are often studied by microbiologists, special safety conditions know as biosafety levels are used to keep researches free of infection from the pathogens they study.

Key Terms

- **yellow fever**: An acute febrile illness of tropical regions, caused by a flavivirus and spread by mosquitoes, characterized by jaundice, black vomit, and the absence of urination.
- **Lyme disease**: Infection by a bacterium of the genus Borrelia which is transmitted by ticks. Symptoms include a rash followed by fever, joint pain, and headaches.

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SECTION OVERVIEW

6.4: Microbial Culture Methods

Topic hierarchy

6.4.1: Enrichment and Isolation

6.4.2: Pure Culture

6.4.3: Preserving Bacterial Cultures

6.4.4: The FISH Technique

6.4.5: Coupling Specific Genes to Specific Organisms Using PCR

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6.4.1: Enrichment and Isolation

LEARNING OBJECTIVES

- List the growth phases of microrganisms and the different types of growth media available to culture them

The most common growth media for microorganisms are nutrient broths and agar plates; specialized media are required for some microorganisms. Some, termed *fastidious organisms*, require specialized environments due to complex nutritional requirements. Viruses, for example, are obligate intracellular parasites and require a growth medium containing living cells.

Growth media: defined vs. undefined

An important distinction between growth media types is that of defined versus undefined media.

A defined medium will have known quantities of all ingredients. For microorganisms, this consists of providing trace elements and vitamins required by the microbe, and especially, a defined source of both carbon and nitrogen. Glucose or glycerol is often used as carbon sources, and ammonium salts or nitrates as inorganic nitrogen sources.

An undefined medium has some complex ingredients, such as yeast extract or casein hydrolysate, which consist of a mixture of many, many chemical species in unknown proportions. Undefined media are sometimes chosen based on price and sometimes by necessity – some microorganisms have never been cultured on defined media.

Types of media

Enriched media contain the nutrients required to support the growth of a wide variety of organisms, including some of the more fastidious ones. They are commonly used to harvest as many different types of microbes as are present in the specimen. Blood agar is an enriched medium in which nutritionally-rich whole blood supplements the basic nutrients. Chocolate agar is enriched with heat-treated blood (40-45°C), which turns brown and gives the medium the color for which it is named.

Selective media are used for the growth of only selected microorganisms. For example, if a microorganism is resistant to a certain antibiotic, such as *ampicillin*or *tetracycline*, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess the resistance, from growing. Media lacking an amino acid, such as proline in conjunction with E. coli unable to synthesize it, were commonly used by geneticists before the emergence of genomics to map bacterial chromosomes.



Figure: ***TSI Agar slant tubes****: The agar triple-sugar iron is one of the culture media used for the differentiation of most enterobacteria.*

Differential/indicator media distinguish one microorganism type from another growing on the same media. This type of media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators (such as neutral red, phenol red, eosin y, or methylene blue) added to the medium to visibly indicate the defining characteristics of a microorganism. This type of media is used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria. The agar triple-sugar iron (TSI) is one of the culture media used for the differentiation of most enterobacteria.

Growth in closed culture systems, such as a batch culture in LB broth, where no additional nutrients are added and waste products are not removed, the bacterial growth will follow a predicted growth curve and can be modeled.

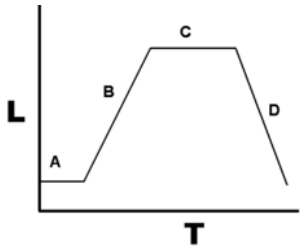


Figure: **Bacterial growth curve**: Bacterial growth in batch culture can be modeled with four different phases: lag phase (A), exponential or log phase (B), stationary phase (C), and death phase (D).

Growth phases

During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs.

Exponential phase (sometimes called the log or logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population.Under controlled conditions, cyanobacteria can double their population four times a day. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

The stationary phase is due to a growth-limiting factor; this is mostly depletion of a nutrient, and/or the formation of inhibitory products such as organic acids.

At death phase, bacteria run out of nutrients and die.

Culture

Batch culture is the most common laboratory-growth method in which bacterial growth is studied, but it is only one of many. The bacterial culture is incubated in a closed vessel with a single batch of medium.

In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a *chemostat*, also known as an open or continuous culture: a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices include *turbidostats* and *auxostats*. Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria.

In a synecological culture, a true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual.

Key Points

- The most common growth media for microorganisms are nutrient broths and agar plates.
- Open cultures allow for a replenishment of nutrients and a reduction of waste buildup in the media.
- Selective media are used for the growth of only selected microorganisms.
- Differential media or indicator media distinguish one microorganism type from another growing on the same media.

Key Terms

- **closed culture**: A closed culture has no additional nutrients added to the system, and waste products are not removed. Cultures in a closed system will follow a predicted growth curve.
- **Enriched media**: Contains nutrients required to support the growth of a wide variety of organisms.
- **open culture**: A continuous culture where periodically some of the bacterial culture is removed and added to fresh sterile medium.

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6.4.2: Pure Culture

LEARNING OBJECTIVES

- Describe how pure microbial cultures can be grown in agar-based growth medium

Microbial cultures are foundational and basic diagnostic methods used extensively as a research tool in molecular biology. It is often essential to isolate a pure culture of microorganisms. A pure (or axenic) culture is a population of cells or multicellular organisms growing in the absence of other species or types. A pure culture may originate from a single cell or single organism, in which case the cells are genetic clones of one another. For the purpose of gelling the microbial culture, the medium of agarose gel (agar) is used. Agar is a gelatinous substance derived from seaweed. A cheap substitute for agar is guar gum, which can be used for the isolation and maintenance of thermophiles.

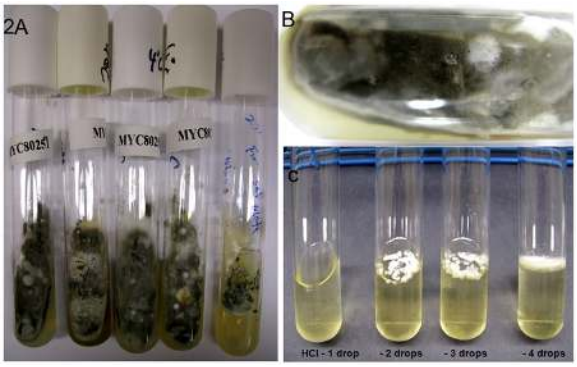


Figure: ***Selective Media**: Geomyces destructans in culture from bat tissues. (A) Original culture tubes of Sabouraud agar supplemented with nine antibiotics and incubated at 4°C for six- or eight-weeks; notice the profuse growth of G. destructans strains. (B) Some fungal contamination on individual isolates was visible as depicted in the close-up of a culture tube. (C) Enrichment and recovery of pure fungal colonies by treating a culture contaminated with bacteria with hydrochloric acid.*

Microbiological cultures can be grown in petri dishes of differing sizes that have a thin layer of agar-based growth medium. Once the growth medium in the petri dish is inoculated with the desired bacteria, the plates are incubated at the best temperature for the growing of the selected bacteria (for example, usually at 37 degrees Celsius for cultures from humans or animals or lower for environmental cultures). Another method of bacterial culture is liquid culture, in which the desired bacteria are suspended in liquid broth, a nutrient medium. These are ideal for preparation of an antimicrobial assay. The experimenter would inoculate liquid broth with bacteria and let it grow overnight (they may use a shaker for uniform growth). Then they would take aliquots of the sample to test for the antimicrobial activity of a specific drug or protein (antimicrobial peptides). As an alternative, the microbiologist may decide to use static liquid cultures. These cultures are not shaken and they provide the microbes with an oxygen gradient.

Key Points

- A pure culture may originate from a single cell or single organism, in which case the cells are genetic clones of one another.
- Microbial cultures are foundational and basic diagnostic methods used extensively as a research tool in molecular biology.
- The most common form of microbial cultures are liquid or solid (agar).

Key Terms

- **agar**: A gelatinous material obtained from the marine algae, used as a bacterial culture medium, in electrophoresis and as a food additive.

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6.4.3: Preserving Bacterial Cultures

LEARNING OBJECTIVES

- Describe how bacterial cultures can be stored for a long time at -80 °C in glycerol

Three species of bacteria, *Carnobacterium pleistocenium*, *Chryseobacterium greenlandensis*, and *Herminiimonas glaciei*, have reportedly been revived after surviving for thousands of years frozen in ice. As a practical matter, as a researcher, you will want to preserve your selected bacteria so you can go back to it if something goes wrong. Whenever you successfully transform a bacterial culture with a plasmid or whenever you obtain a new bacterial strain, you will want to make a long term stock of that bacteria. Bacteria can be stored for months and years if they are stored at -80 °C and in a high percentage of glycerol.



Figure: ***Bacteria in liquid media****: An erlenmeyer containing a bacterial culture. Bacteria that have been preserved in glycerol stocks can be grown overnight in liquid media to promote propagation.*

In order to ensure a pure culture is being preserved, pick a single colony of the bacteria off a plate, grow it overnight in the appropriate liquid media, and with shaking. Take the overnight culture and and mix an aliquot with 40% glycerol in sterile water and place in a cryogenic vial. It is important to label the vial with all the relevant information (e.g. strain, vector, date, researcher, etc.). Freeze the glycerol stock and store at -80 °C. At this point you should also record the strain information and record the location.

While it is possible to make a long term stock from cells in the stationary phase, ideally your culture should be in logarithmic growth phase. Certain antibiotics in the medium should be removed first as they are supposedly toxic over time, e.g. Tetracycline. To do this, spin the culture down and resuspend it in the same volume of straight LB medium.

Key Points

- Preserve your selected bacteria so you always have something to go back to if something goes wrong.
- While it is possible to make a long term stock from cells in stationary phase, ideally your culture should be in logarithmic growth phase.
- Ensure a pure culture is being preserved by picking a single colony of the bacteria off a plate for cryopreservation.

Key Terms

- **cryogenic**: of, relating to, or performed at low temperatures
- **logarithmic growth phase**: exponential phase (sometimes called the log phase or the logarithmic phase) is a period characterized by cell doubling.

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6.4.4: The FISH Technique

LEARNING OBJECTIVES

- Describe how fluorescent in situ hybridization (FISH) is used in clinical and biomedical studies to detect and localize the presence or absence of specific DNA sequences and to identify pathogens

FISH (fluorescence in situ hybridization) is a cytogenetic technique developed by biomedical researchers in the early 1980s. It is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes bind to those targets that show a high degree of sequence complementarity. FISH can be used to detect RNA or DNA sequences of interest. FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets, including mRNAs, in cells. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues.

Central to FISH are the use of probes. The probe must be large enough to hybridize specifically with its target but not so large as to impede the hybridization process. They are anti-sense to the target mRNA or DNA of interest, thus they hybridize to targets. The probe can be tagged directly with fluorophores, or with targets for flourescently labelled antibodies or other substrates. Different types of tags can be used, therefore different targets can be detected in the same sample simultaneously (multi-colour FISH). Tagging can be done in various ways, such as nick translation, or PCR using tagged nucleotides. Probes can vary in length from 20 to 30 nucleotides to much longer sequences.

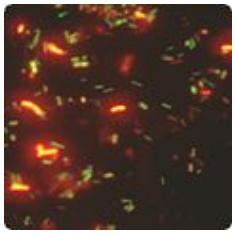


Figure: **Dual label FISH image**: Here is an example of FISH being used to differentiate Bifidobacteria (red) and other bacteria (green)

FISH is often used in clinical studies. If a patient is infected with a suspected pathogen, bacteria from the patient's tissues or fluids, are typically grown on agar to determine the identity of the pathogen. Many bacteria, however, even well-known species, do not grow well under laboratory conditions. FISH can be used to directly detect the presence of the suspect on small samples of the patient's tissue. FISH can also be used to compare the genomes of two biological species, to deduce evolutionary relationships. A similar hybridization technique is called a zoo blot. Bacterial FISH probes are often primers for the 16s rRNA region. FISH is widely used in the field of microbial ecology, to identify microorganisms. Biofilms, for example, are composed of complex (often) multi-species bacterial organizations. Preparing DNA probes for one species and performing FISH with this probe allows one to visualize the distribution of this specific species within the biofilm. Preparing probes (in two different colors) for two species allows to visualize/study co-localization of these two species in the biofilm, and can be useful in determining the fine architecture of the biofilm.

Key Points

- FISH can be used in a clinical setting to identify pathogens or DNA / RNA targets of interest.
- FISH is used to detect and localize the presence or absence of specific DNA or RNA sequences in tissue or cells.
- FISH can also be used to compare the genomes of two biological species, such as in ecological studies, where a bacteria may not be culturable, it can be identified using FISH.

Key Terms

- **fluorescence**: The emission of light (or other electromagnetic radiation) by a material when stimulated by the absorption of radiation or of a subatomic particle
- **hybridize**: To combine complementary subunits of multiple biological macromolecules.

- **FISH**: Fluorescence in situ hybridization is a cytogenetic technique used to detect and localize thespecific DNA or RNA sequences.

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6.4.5: Coupling Specific Genes to Specific Organisms Using PCR

LEARNING OBJECTIVES

- Describe how polymerase chain reaction (PCR) allows for the amplification and mutation of DNA and enables researchers to study very small samples

Polymerase chain reaction (PCR) is a useful technique for scientists, because it allows for the amplification and mutation of DNA. Through PCR, small quantities of DNA can be replicated by orders of magnitude, not only essentially preserving the sample if successful, but allowing for study on a much larger scale.. Without PCR, the studies we perform would be limited by the amount of DNA we were able to isolate from samples. Through PCR, the original DNA is essentially limitless, allowing scientists to induce various mutations in different genes for further study.

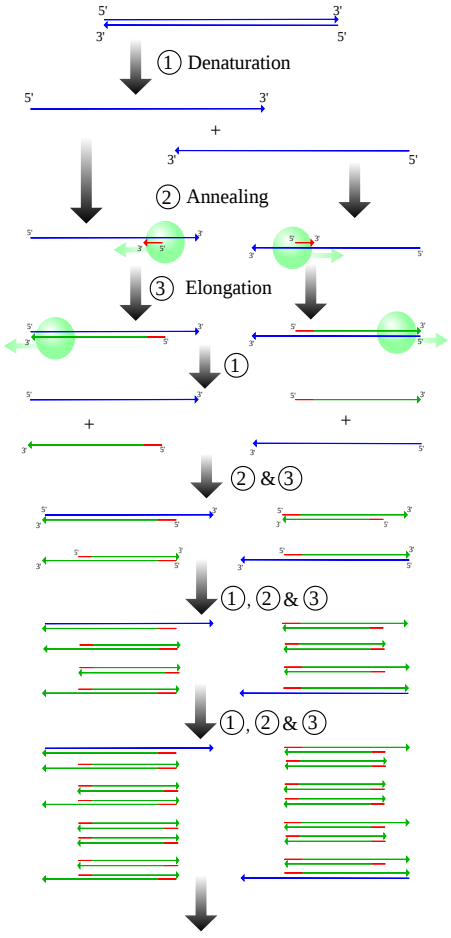


Figure: ***Polymerase chain reaction****: a schematic of the polymerase chain reaction*

Through site-directed mutagenesis or customized primers, individual mutations in DNA can be made. By changing the amino acids transcribed from DNA through individual mutations, the importance of those amino acids with respect to gene function can be analyzed. However, this process can be difficult, particularly when genes act in concert (with varying expression with respect to gene activity). The length of time it takes to run a successful PCR and perform other techniques before additional studies can be done (protein expression, isolation, and purification, for example), makes biochemical research time-consuming and difficult. However, PCR, coupled with other biochemical techniques, allows us to analyze the very core of organisms and the processes by which they function. Common PCR protocols in labs today include knockout genotyping, fluorescence genotyping and mutant

genotyping. Researchers can use PCR as a method of searching for genes by using primers that flank the target sequence of the gene along with all other necessary components for PCR. If the gene is present, the primers will bind and amplify the DNA, giving a band of amplified DNA on the agarose gel that will be run. If the gene is not present, the primers will not anneal and no amplification will occur.

The ability to identify specific genes to specific organisms has increased the use of PCR and has allowed it to be more specific and eliminate the possibility of cross contaminants. The identification of specific genes to specific organisms has important medical diagnostic value.

PCR is a reliable method to detect the presence of unwanted genetic materials, such as infections and bacteria in the clinical setting. It can even allow identification of an infectious agent without culturing. For example, in diagnosis of diseases like AIDS, PCR can be used to detect the small percentage of cells that are infected with HIV by utilizing primers that are specific for genes specialized to the HIV virus. PCR can reveal the presence of HIV in people who have not mounted an immune response to this pathogen, which may otherwise be missed with an antibody assay). Additionally, PCR is used for identifying bacterial species, such as *Mycobacterium tuberculosis* in tissue specimens. With the use of PCR, as few as 10 bacilli per million human cells can be readily detected. The bacilli are identified by using *Mycobacterium tuberculosis* specific genes.

Key Points

- PCR allows for identification of an infectious agent without the need for culturing.
- Researchers can use PCR as a method of searching for specific genes and/or mutations.
- PCR, coupled with other biochemical techniques, allows us to analyze the very core of organisms and the processes by which they function.

Key Terms

- **polymerase chain reaction**: A technique in molecular biology for creating multiple copies of DNA from a sample; used in genetic fingerprinting etc.

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SECTION OVERVIEW

6.5: Bacterial Population Growth

Topic hierarchy

6.5.1: Chemical Assays, Radioisotopic Methods, and Microelectrodes

6.5.2: Stable Isotopes

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6.5.1: Chemical Assays, Radioisotopic Methods, and Microelectrodes

There are numerous tests and assays available that are utilized to aid in bacterial identification in a variety of settings.

Learning Objectives

- Contrast the different tests that can be used in studies of microbes

Key Points

- Chemical assays used for bacterial identification utilize various components of the microorganisms including structural, cellular and metabolic indicators.
- Radioisotopic methods include the use of radioisotopes to help identify specific metabolic pathways utilized by bacteria by tracking uptake and breakdown of specific nutrients labeled with radioactivity.
- Micro-electrodes are commonly being used in bacterial identification, specifically for pathogenic bacteria, as a means to identify biological components in a variety of environments by combining with bio-sensors.

Key Terms

- **peptidoglycan**: A polymer of glycan and peptides found in bacterial cell walls.

Within the field of microbiology, there are specific tests or assays utilized to quantitatively and qualitatively measure microorganism components. These assays are often utilized to aid in bacterial identification. Three major types used for this purpose include chemical assays, radio isotopic methods and the use of micro electrodes. The following is an overview of these methodologies.

Chemical Assays

Chemical assays are utilized to identify and determine chemical components within a microorganism. Many of these assays test for specific cellular components and may have overlap with chemical analysis, which focuses on exact chemical composition.

Gram Staining

Examples of chemical assays include the classic test for Gram-positive or Gram-negative bacteria via Gram staining. Gram staining is utilized to differentiate bacteria into either of these Gram groups. The Gram staining technique is based on both chemical and physical properties of bacterial cell walls and tests for the presence of peptidoglycan.

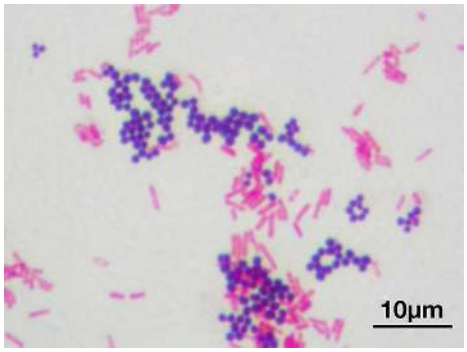


Figure: **Gram Staining**: An example of a chemical assay used for bacterial identification.

Oxidative-Fermentation Glucose Test

The O-F test is utilized to determine the way in which a bacteria is capable of metabolizing carbohydrates such as glucose. The two major mechanisms from which bacteria can obtain energy include oxidation of glucose and lactose fermentation. This specific assay identifies which method bacteria use by cultivating bacteria in various conditions.

Hydrolysis Tests

The process of hydrolysis is characterized by the ability to chemically split a molecule by the addition of water. There are numerous tests utilized in bacterial identification which involve testing for hydrolysis of specific substances. These tests include

hydrolysis of starch, lipids, casein and gelatin. The basis of these tests is to identify and determine if a microbe has the proper enzymes and molecules to breakdown and use these specific molecules as sources of energy for cellular growth.

Radioisotopic Methods

Radioisotopes are specific types of isotopes that emit radioactivity. Isotopes of an element vary in the number of neutrons within their nuclei. In the field of microbiology, radioisotopes have been used

Micro-electrodes

Electrodes are characterized by a system of electrical conductors that are used to make contact with a non-metallic portion of a circuit. In regards to microbiology and bacterial identification, micro-electrodes are commonly being utilized to identify pathogenic bacteria in numerous settings. The micro-electrodes have the capability to function as bio-sensors and detect specific biological components of microbes.

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6.5.2: Stable Isotopes

LEARNING OBJECTIVES

- Demonstrate how isotopes are used in bacterial identification

The term isotope refers to the number of neutrons a certain element contains. Elements of the same name (for example, oxygen) must always have the same number of protons, but the number of neutrons can change. Adding or subtracting neutrons from an atom does not change the elemental properties, but it can alter some of its features (like making it more radioactive).

Stable Isotopes

While the number of neutrons in a particular atom can change, there is a certain threshold where the atom is given more neutrons that its nuclear force can hold. At this point, the neutrons start to be released. The release is also known as decay. During this time, the atom is deemed “unstable. ” The atom will continue to lose neutrons until it become stable again. A stable isotope is a chemical isotope that is not radioactive. There are some cases where atoms have no stable isotopes so they continue to lose neutrons, and later protons and electrons, until they become another element entirely. Research has shown that there are 80 elements with one or more stable isotopes. Of these, 26 have only one stable isotope which is also known as being monoisotopic. The element with the most stable isotopes is tin which as 10 stable isotopes.

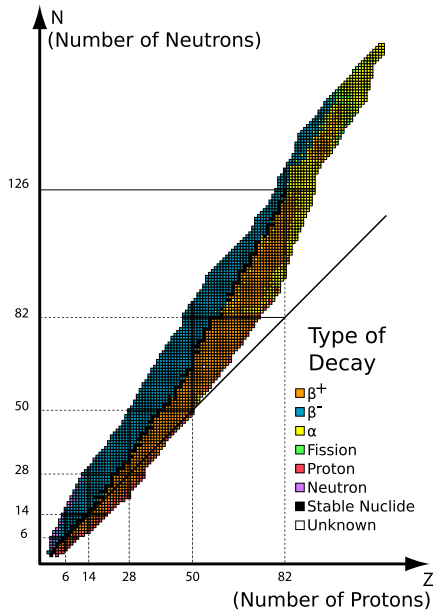


Figure: **Table of Isotopes**: This is a table that represents atom decay yielding various isotopes.

Key Points

- The term isotope refers to the number of neutrons a certain element contains.
- Elements of the same name must always have the same number of protons, but the number of neutrons can change.
- Adding or subtracting neutrons from an atom does not change the elemental properties, but it can alter some of its features (like making it more radioactive).
- While the number of neutrons in a particular atom can change, there is a certain threshold where the atom is given more neutrons that its nuclear force can hold. At this point, the atom is deemed unstable.
- The atom will continue to lose neutrons, or decay, until it become stable again. A stable isotope is a chemical isotope that is not radioactive.

Key Terms

- **isotope**: Any of two or more forms of an element where the atoms have the same number of protons, but a different number of neutrons within their nuclei. As a consequence, atoms for the same isotope will have the same atomic number but a different mass number (atomic weight).
- **atom**: The smallest possible amount of matter which still retains its identity as a chemical element, now known to consist of a nucleus surrounded by electrons.
- **Radioactive**: A particle that has spontaneous emission of ionizing radiation as a consequence of a nuclear reaction, or directly from the breakdown of an unstable nucleus.

Extensions

Knowledge about stable isotopes is important in a variety of fields. Scientists have used information on these topics in botanical and plant biological investigations as well as ecological and biological studies. Additionally, some scientists have used oxygen isotope ratios to reconstruct historical atmospheric temperatures. This work is especially important due to our current concern with climate change/global warming.

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SECTION OVERVIEW

6.6: Microbial Growth

Topic hierarchy

6.6.1: Binary Fission

6.6.2: Fts Proteins and Cell Division

6.6.3: MreB and Determinants of Cell Morphology

6.6.4: Peptidoglycan Synthesis and Cell Division

6.6.5: Generation Time

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6.6.1: Binary Fission

LEARNING OBJECTIVES

- Describe the process of binary fission in prokaryotes

Prokaryotes, such as bacteria, propagate by binary fission. For unicellular organisms, cell division is the only method used to produce new individuals. In both prokaryotic and eukaryotic cells, the outcome of cell reproduction is a pair of daughter cells that are genetically identical to the parent cell. In unicellular organisms, daughter cells are individuals.

Due to the relative simplicity of the prokaryotes, the cell division process, or binary fission, is a less complicated and much more rapid process than cell division in eukaryotes. The single, circular DNA chromosome of bacteria is not enclosed in a nucleus, but instead occupies a specific location, the nucleoid, within the cell. Although the DNA of the nucleoid is associated with proteins that aid in packaging the molecule into a compact size, there are no histone proteins and thus, no nucleosomes in prokaryotes. The packing proteins of bacteria are, however, related to the cohesin and condensin proteins involved in the chromosome compaction of eukaryotes.

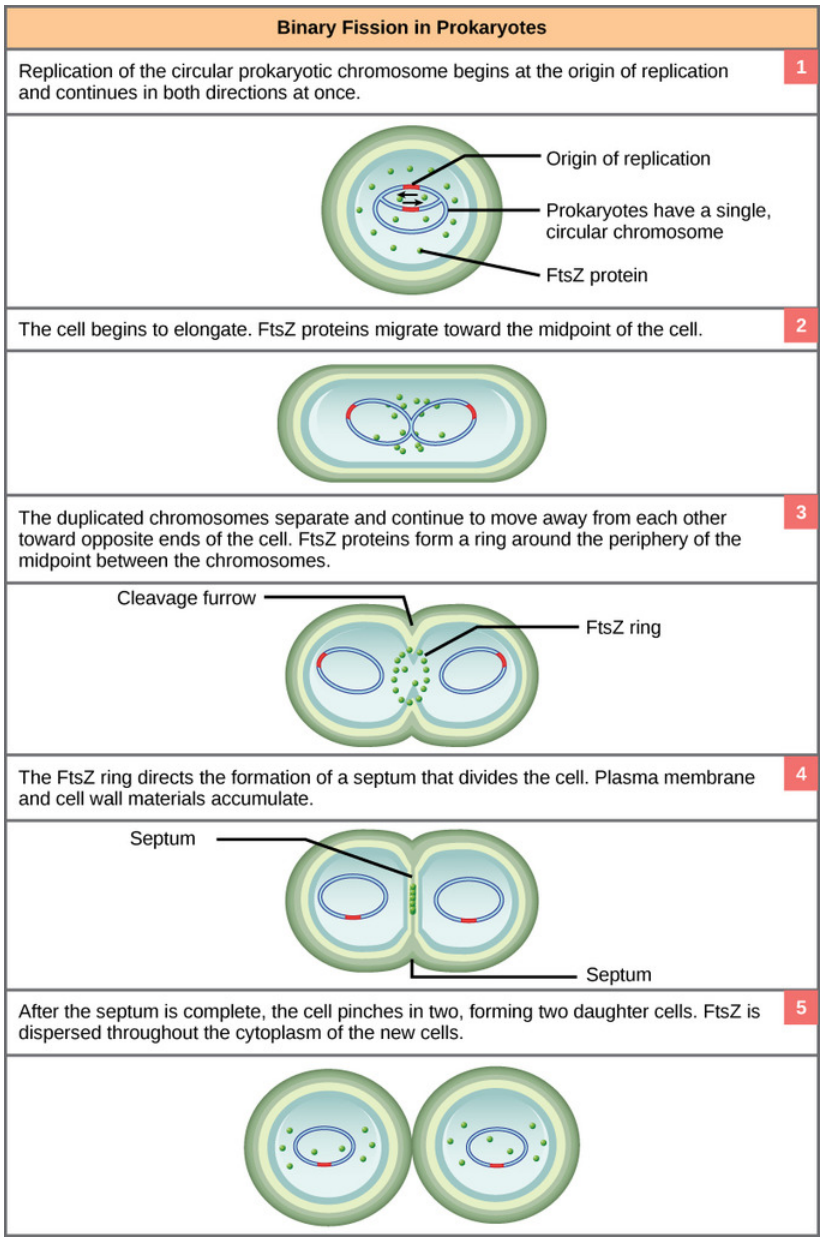


Figure: **Binary Fission**: These images show the steps of binary fission in prokaryotes.

The bacterial chromosome is attached to the plasma membrane at about the midpoint of the cell. The starting point of replication, the origin, is close to the binding site of the chromosome at the plasma membrane. Replication of the DNA is bidirectional, moving away from the origin on both strands of the loop simultaneously. As the new double strands are formed, each origin point moves away from the cell wall attachment toward the opposite ends of the cell. As the cell elongates, the growing membrane aids in the transport of the chromosomes. After the chromosomes have cleared the midpoint of the elongated cell, cytoplasmic separation begins. The formation of a ring composed of repeating units of a protein, FtsZ, directs the partition between the nucleoids. Formation of the FtsZ ring triggers the accumulation of other proteins that work together to recruit new membrane and cell wall materials to the site. A septum is formed between the nucleoids, extending gradually from the periphery toward the center of the cell. When the new cell walls are in place, the daughter cells separate.

Mitotic Spindle Apparatus

The precise timing and formation of the mitotic spindle is critical to the success of eukaryotic cell division. Prokaryotic cells, on the other hand, do not undergo karyokinesis and, therefore, have no need for a mitotic spindle. However, the FtsZ protein that plays such a vital role in prokaryotic cytokinesis is structurally and functionally very similar to tubulin, the building block of the

microtubules that make up the mitotic spindle fibers that are necessary for eukaryotes. FtsZ proteins can form filaments, rings, and other three-dimensional structures that resemble the way tubulin forms microtubules, centrioles, and various cytoskeletal components. In addition, both FtsZ and tubulin employ the same energy source, GTP (guanosine triphosphate), to rapidly assemble and disassemble complex structures.

FtsZ and tubulin are homologous structures derived from common evolutionary origins. In this example, FtsZ is the ancestor protein to tubulin (a modern protein). While both proteins are found in extant organisms, tubulin function has evolved and diversified tremendously since evolving from its FtsZ prokaryotic origin. A survey of mitotic assembly components found in present-day unicellular eukaryotes reveals crucial intermediary steps to the complex membrane-enclosed genomes of multicellular eukaryotes.

Key Points

- In bacterial replication, the DNA is attached to the plasma membrane at about the midpoint of the cell.
- The origin, or starting point of bacterial replication, is close to the binding site of the DNA to the plasma membrane.
- Replication of the bacterial DNA is bidirectional, which means it moves away from the origin on both strands simultaneously.
- The formation of the FtsZ ring, a ring composed of repeating units of protein, triggers the accumulation of other proteins that work together to acquire and bring new membrane and cell wall materials to the site.
- When new cell walls are in place, due to the formation of a septum, the daughter cells separate to form individual cells.

Key Terms

- **mitotic spindle**: the apparatus that orchestrates the movement of DNA during mitosis
- **karyokinesis**: (mitosis) the first portion of mitotic phase where division of the cell nucleus takes place
- **binary fission**: the process whereby a cell divides asexually to produce two daughter cells

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6.6.2: Fts Proteins and Cell Division

FtsZ is a protein encoded by the ftsZ gene that assembles into a ring at the future site of the septum of bacterial cell division.

LEARNING OBJECTIVES

Evaluate the role of Fts proteins in cell division

KEY TAKEAWAYS

Key Points

- FtsZ has been named after “Filamenting temperature-sensitive mutant Z”.
- During cell division, FtsZ is the first protein to move to the division site, and is essential for recruiting other proteins that produce a new cell wall between the dividing cells.
- FtsZ’s role in cell division is analogous to that of actin in eukaryotic cell division, but unlike the actin-myosin ring in eukaryotes, FtsZ has no known motor protein associated with it.

Key Terms

- **segrosomes**: multiprotein complexes that partition chromosomes/plasmids in bacteria.
- **cell division**: a process by which a cell divides into two cells.
- **septum**: a partition that separates the cells of a (septated) fungus
- **cytoskeleton**: A cellular structure like a skeleton, contained within the cytoplasm.

FtsZ is a protein encoded by the ftsZ gene that assembles into a ring at the future site of the septum of bacterial cell division. This is a prokaryotic homologue to the eukaryotic protein tubulin. FtsZ has been named after “Filamenting temperature-sensitive mutant Z”. The hypothesis was that cell division mutants of E. coli would grow as filaments due to the inability of the daughter cells to separate from one another.

FtsZ was the first protein of the prokaryotic cytoskeleton to be identified. During cell division, FtsZ is the first protein to move to the division site, and is essential for recruiting other proteins that produce a new cell wall between the dividing cells. FtsZ’s role in cell division is analogous to that of actin in eukaryotic cell division, but unlike the actin-myosin ring in eukaryotes, FtsZ has no known motor protein associated with it. The origin of the cytokinetic force thus remains unclear, but it is believed that the localized synthesis of new cell wall produces at least part of this force. It is interesting to note that L-form bacteria that lack a cell wall do not require FtsZ for division, which implies that bacteria may have retained components of an ancestral mode of cell division.

Much is known about the dynamic polymerization activities of tubulin and microtubules, but little is known about these activities in FtsZ. While it is known that single-stranded tubulin protofilaments form into 13 stranded microtubules, the multistranded structure of the FtsZ-containing Z-ring is not known. It is only speculated that the structure consists of overlapping protofilaments. Recently, proteins similar to tubulin and FtsZ have been discovered in large plasmids found in Bacillus species. They are believed to function as components of segrosomes, which are multiprotein complexes that partition chromosomes/plasmids in bacteria. The plasmid homologs of tubulin/FtsZ seem to have conserved the ability to polymerize into filaments.

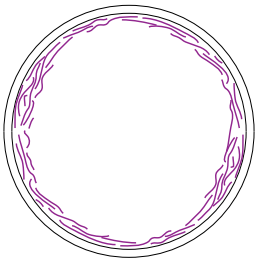


Figure: **FtsZ Filaments**: The Z-ring forms from smaller subunits of FtsZ filaments. These filaments may pull on each other and tighten to divide the cell.

FtsZ has the ability to bind to GTP, and also exhibits a GTPase domain that allows it to hydrolyze GTP to GDP and a phosphate group. In vivo, FtsZ forms filaments with a repeating arrangement of subunits, all arranged head-to-tail. These filaments form a ring around the longitudinal midpoint, or septum, of the cell. This ring is called the Z-ring. The GTP hydrolyzing activity of the protein is not essential to the formation of filaments or division. Mutants lacking the GTPase domain form twisted and disordered septa. These cells with irregular septa can still divide, although abnormally. It is unclear as to whether FtsZ actually provides the physical force that results in division or serves as a marker for other proteins to execute division.

The Z-ring forms from smaller subunits of FtsZ filaments. These filaments may pull on each other and tighten to divide the cell. If FtsZ does provide force that divides the cell, it may do so through the relative movement of subunits. In this model, FtsZ scission force comes from the relative lateral movement of subunits. Lines of FtsZ would line up together parallel and pull on each other creating a “cord” of many strings that tightens itself. In other models, FtsZ does not provide the contractile force but provides the cell a spatial scaffold for other proteins to execute the division of the cell. This is akin to the creating of a temporary structure by construction workers to access hard-to-reach places of a building. The temporary structure allows unfettered access and ensures that the workers can reach all places. If the temporary structure is not correctly built, the workers will not be able to reach certain places, and the building will be deficient.

This “scaffold theory” is supported by information that shows that the formation of the ring and localization to the membrane requires the concerted action of a number of accessory proteins. ZipA or the actin homologue FtsA permit initial FtsZ localization to the membrane. Following localization to the membrane, division proteins of the Fts family are recruited for ring assembly. Many of these proteins, such as FtsW, FtsK, and FtsQ are involved in stabilization of the Z ring and may also be active participants in the scission event. The formation of the Z-ring closely coincides with cellular processes associated with replication.

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6.6.3: MreB and Determinants of Cell Morphology

MreB is a protein found in bacteria homologous to actin.

LEARNING OBJECTIVES

Explain the role of MreB in cell morphology determination

KEY TAKEAWAYS

Key Points

- MreB proteins polymerize to form filaments that are similar to actin microfilaments.
- MreB controls the width of rod-shaped bacteria, such as Escherichia coli.
- Bacteria that are naturally spherical do not have the gene encoding MreB.

Key Terms

- **peptidoglycan**: A polymer of glycan and peptides found in bacterial cell walls.
- **cell wall**: A thick, fairly rigid layer formed around individual cells of bacteria, Archaea, fungi, plants, and algae, the cell wall is external to the cell membrane and helps the cell maintain its shape and avoid damage.
- **cytoskeleton**: A cellular structure like a skeleton, contained within the cytoplasm.



Figure: **Atomic structure of MreB, a prokaryotic structural protein**: Procaryotic MreB in cartoon representation. The fold of the protein is similar to its eukaryotic counterpart

MreB is a protein found in bacteria that has been identified as a homologue of actin, as indicated by similarities in tertiary structure and conservation of active site peptide sequence. The conservation of protein structure suggests the common ancestry of the cytoskeletal elements formed by actin and MreB, found in prokaryotes. Indeed, recent studies have found that MreB proteins polymerize to form filaments that are similar to actin microfilaments.MreB controls the width of rod-shaped bacteria, such as Escherichia coli. A mutant E. coli that creates defective MreB proteins will be spherical instead of rod-like. Also, bacteria that are naturally spherical do not have the gene encoding MreB. Prokaryotes carrying the mreB gene can also be helical in shape. MreB has long been thought to form a helical filament underneath the cytoplasmic membrane. However, this model has been brought into question by three recent publications showing that filaments cannot be seen by electron cryotomography and that GFP-MreB can be seen as patches moving around the cell circumference. It has also been shown to interact with several proteins that are proven to be involved in length growth (for instance PBP2). Therefore, MreB probably directs the synthesis and insertion of new peptidoglycan building units into the existing peptidoglycan layer to allow length growth of the bacteria.

MreB is a cytoskeleton element that assembles into filamentous structures within the bacterial cytoplasm. MreB and its homologs have been shown to interact and co-localize with cytoplasmic protein(MurB-G), membrane-imbedded proteins (MreD, MraY and RodA), as well as other molecules with large periplasmic domain in organism. Recent research shows that peptidoglycan precursors are inserted into cell wall following helical pattern which is dependent on MreB, and it's reported that MreB also promote the GT activity of PBPs. This ability of MreB is because of RodZ, an inner membrane protein containing an 80-residue,

N-terminal cytoplasmic region, and a 200-amino acid periplasmic C-terminal tail. RodZ co-localizes with MreB helices in a manner that is strictly dependent on its cytoplasmic region. MreB- RodZ complexes act as a major stabilizing factor in bacterial cell wall and ensure the insertion of new peptidoglycan in a spiral like fashion into the cell wall.

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6.6.4: Peptidoglycan Synthesis and Cell Division

LEARNING OBJECTIVES

- Examine Peptidoglycan synthesis during cell division

Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of bacteria (but not Archaea; []), forming the cell wall. The sugar component consists of alternating residues of β-(1,4) linked N-acetylglucosamine and N-acetylmuramic acid. Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids. The peptide chain can be cross-linked to the peptide chain of another strand forming the 3D mesh-like layer. Some Archaea have a similar layer of pseudopeptidoglycan or pseudomurein, where the sugar residues are β-(1,3) linked N-acetylglucosamine and N-acetyltalosaminuronic acid. That is why the cell wall of Archaea is insensitive to lysozymes, which are present in human sweat and tears as part of innate immunity.

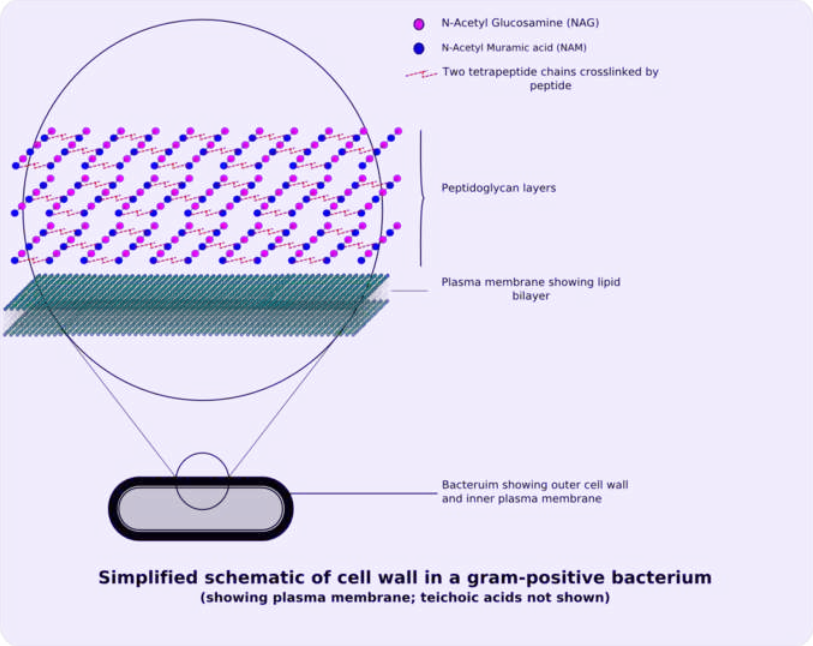


Figure: ***Simplified sc hematic of a cell wall in a Gram-positive bacteria****: Cross-linking between amino acids in different linear amino sugar chains occurs with the help of the enzyme transpeptidase and results in a 3-dimensional structure that is strong and rigid.*

Peptidoglycan serves a structural role in the bacterial cell wall giving it strength, as well as counteracting the osmotic pressure of the cytoplasm. A common misconception is that peptidoglycan gives the cell its shape. However, it is actually the MreB protein that facilitates cell shape. Peptidoglycan is also involved in binary fission during bacterial cell reproduction.

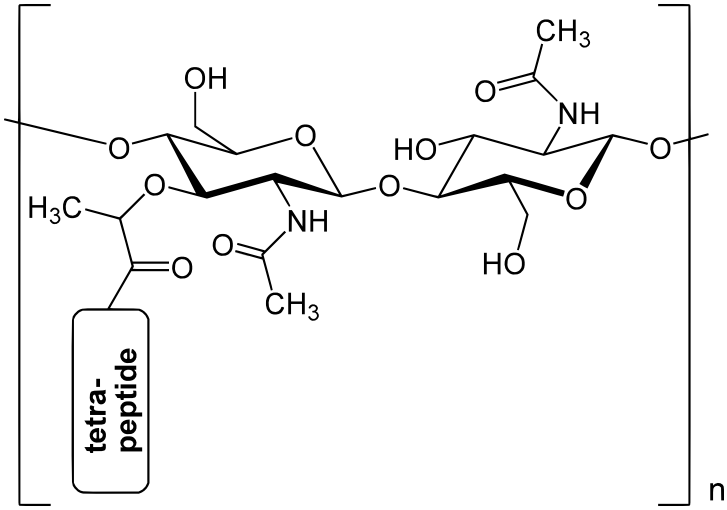


Figure: ***Peptidoglycan structure****: The peptidoglycan layer in the bacterial cell wall is a crystal lattice structure formed from linear chains of two alternating amino sugars, namely N-acetylglucosamine (GlcNAc or NAG) and N-acetylmuramic acid (MurNAc or NAM).*

The peptidoglycan layer is substantially thicker in Gram-positive bacteria (20 to 80 nanometers) than in Gram-negative bacteria (7 to 8 nanometers), with the attachment of the S-layer. Peptidoglycan forms around 90% of the dry weight of Gram-positive bacteria but only 10% of Gram-negative strains. Thus, presence of high levels of peptidoglycan is the primary determinant of the characterization of bacteria as gram-positive. In Gram-positive strains, it is important in attachment roles and stereotyping purposes. For both Gram-positive and Gram-negative bacteria, particles of approximately 2 nm can pass through the peptidoglycan. Gram-positive and Gram-negative bacteria are sensitive to different types of antiobiotics.

Key Points

- The sugar component of peptidoglycan consists of alternating residues of β-(1,4) linked N-acetylglucosamine and N-acetylmuramic acid.
- Peptidoglycan serves a structural role in the bacterial cell wall, giving structural strength but not shape, and counteracting the osmotic pressure of the cytoplasm.
- Peptidoglycan is also involved in binary fission during bacterial cell reproduction.

Key Terms

- **peptidoglycan**: A polymer of glycan and peptides found in bacterial cell walls.
- **MreB**: MreB is a protein found in bacteria that has been identified as a homologue of actin, as indicated by similarities in tertiary structure and conservation of active site peptide sequence.

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6.6.5: Generation Time

LEARNING OBJECTIVES

- Examine microbial generation times

Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. Therefore, “local doubling” of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. The doubling time is the generation time of the bacteria. If the number surviving exceeds unity on average, the bacterial population undergoes exponential growth.

The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists. The basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. In autecological studies, bacterial growth in batch culture can be modeled with four different phases: lag phase, exponential or log phase, stationary phase, and death phase.

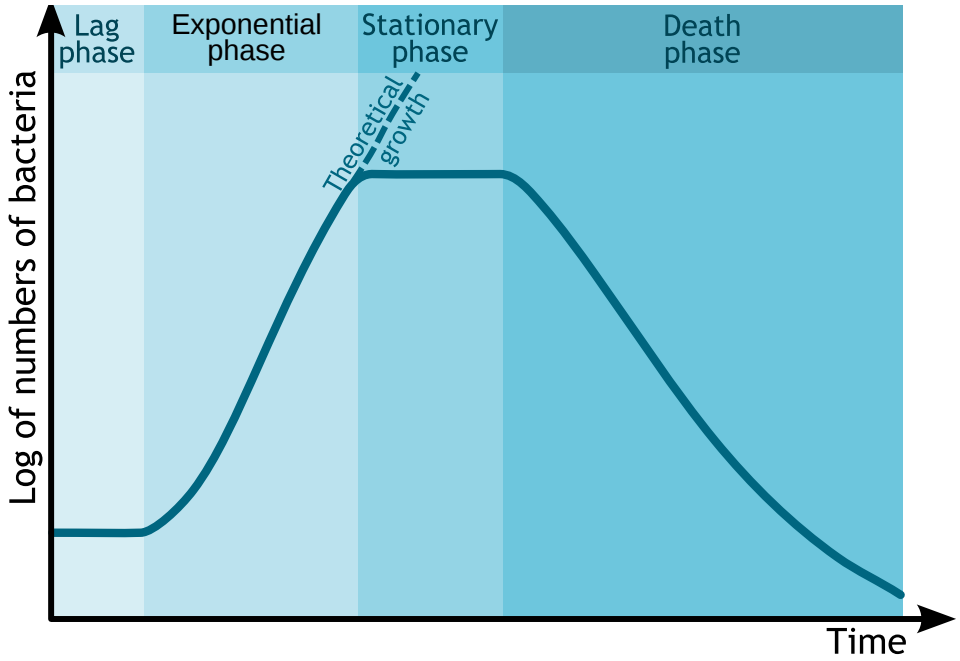


Figure: ***Bacterial Growth Curve****: This chart shows the logarithmic growth of bacteria. Note the Y-axis scale is logarithmic meaning that the number represents doubling. The phases of growth are labelled on top.*

During lag phase, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During this phase of the bacterial growth cycle, synthesis of RNA, enzymes, and other molecules occurs. The exponential phase (sometimes called the log phase or the logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time.

The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. However, exponential growth cannot continue indefinitely because the medium is soon depleted of nutrients and enriched with wastes. Finally, the stationary phase is due to a growth-limiting factor, such as depletion of a nutrient and/or the formation of inhibitory products such as organic acids. Death of cells as a function of time is rather unpredictable and very difficult to explain. At death phase, bacteria run out of nutrients and die. This basic batch culture growth model draws out and emphasizes aspects of bacterial growth which may differ from the

growth of macrofauna. It emphasizes clonality, asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients. In reality, even in batch culture, the four phases are not well defined.

Key Points

- The doubling time is the generation time of the bacteria.
- The measurement of an exponential bacterial growth curve can be done by cell counting, colony counting, or determining the turbidity of bacterial cultures.
- Bacterial growth in batch culture can be modeled with four different phases: lag phase, exponential or log phase, stationary phase, and death phase.

Key Terms

- **bacterium**: A single celled organism with no nucleus.
- **bacterial growth**: Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission.
- **doubling time**: The doubling time is the period of time required for a quantity to double in size or value. It is applied to population growth, inflation, resource extraction, consumption of goods, compound interest, the volume of malignant tumours, and many other things which tend to grow over time.
- **lag phase**: the period of bacterial growth in which bacteria adapt themselves to growth conditions; the individual bacteria are maturing and not yet able to divide
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SECTION OVERVIEW

6.7: Bacterial Population Growth

Topic hierarchy

6.7.1: Microbial Growth Cycle

6.7.2: Growth Terminology

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6.7.1: Microbial Growth Cycle

Increases in cell size are tightly linked in unicellular organisms and under optimal conditions bacteria can grow and divide rapidly.

LEARNING OBJECTIVES

Duplicate the requirements of microbial growth cycles

KEY TAKEAWAYS

Key Points

- Bacteria grow to a fixed size and then reproduce through binary fission which a form of asexual reproduction. Under optimal conditions, bacteria can grow and divide extremely rapidly.
- Different kinds of bacteria need different amounts of oxygen to survive.
- For microbial growth to process, microorganisms require certain nutrients including carbon, nitrogen, phosphorus, sulfur, and metal ions.
- Various types of bacteria thrive at different temperatures.

Key Terms

- **binary fission**: The process whereby a cell divides asexually to produce two daughter cells.
- **Anaerobe**: An anaerobic organism; one that does not require oxygen to sustain its metabolic processes.
- **Aerobe**: Any organism (but especially a bacterium) that can tolerate the presence of oxygen or that needs oxygen to survive.

Microbial Growth Cycle

All microbial metabolisms can be arranged according to three principles: 1) How the organism obtains carbon for synthesizing cell mass. 2) How the organism obtains reducing equivalents used either in energy conservation or in biosynthetic reactions. 3) How the organism obtains energy for living and growing (for more detail on this topic see atom on Growth Terminology). Unlike in multicellular organisms, increases in cell size (cell growth and reproduction by cell division) are tightly linked in unicellular organisms. Bacteria grow to a fixed size and then reproduce through binary fission which is a form of asexual reproduction. Under optimal conditions, bacteria can grow and divide extremely rapidly. These optimal conditions are discussed below.

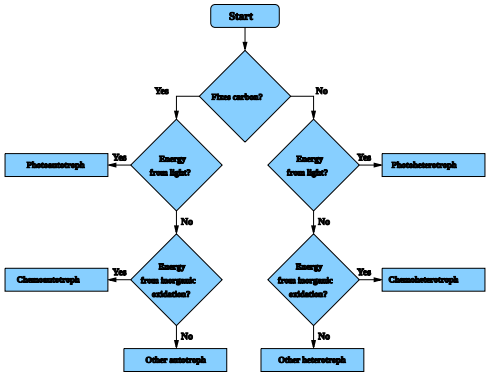


Figure: **Metabolic characteristics of microorganisms**: This is a flowchart to help determine how a microorganism undergoes growth development.

Oxygen Requirements

Different kinds of bacteria need different amounts of oxygen to survive, which determines which bacteria can infect which parts of the body. They are not able infect the skin because oxygen is present, and they can only grow in the presence of oxygen. Conversely, obligate anaerobes are killed by oxygen and carry out fermentation. Tetanus is an obligate anaerobe so it will infect areas where oxygen in limited. Aerotolerant anaerobes breath anaerobically (without oxygen), but they are able to survive when oxygen is present.

Nutrient Requirements

For microbial growth to process, microorganisms require certain nutrients including carbon, nitrogen, phosphorus, sulfur, and metal ions.

Temperature Requirements

Various types of bacteria thrive at different temperatures. Microorganisms that grow best at moderate temperatures are called mesophiles. Those surviving at high temperatures are thermophiles and microorganisms surviving at very low temperatures are called psychrophiles.

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6.7.2: Growth Terminology

The two ways that microbial organisms can be classified are as autotrophs (supply their own energy) or as heterotrophs (use the products of others).

LEARNING OBJECTIVES

Recall bacterial growth terminology

KEY TAKEAWAYS

Key Points

- An autotroph, which means self-feeding or producer, is an organism that produces complex organic compounds (such as carbohydrates and proteins) from simple substances present in its surroundings. To produce these organic compounds it either uses energy from light or inorganic chemical reactions.
- Photoautotrophs are a type of autotroph that use light (sunlight if they are green plants) as their energy source.
- Chemoautotrophs are also a type of autotroph that derive energy from chemical reactions and synthesize all necessary organic compounds from carbon dioxide.
- A heterotroph is an organism that, unlike an autotroph, cannot fix carbon and uses organic carbon for growth. Heterotrophs use the products formed by autotrophs to survive.
- Photoheterotrophs are a type of heterotroph that use light for energy, but cannot use carbon dioxide as their sole carbon source.
- Chemoheterotrophs are a type of heterotroph that are unable to fix carbon and form their own organic compounds so they must use products formed by autotrophs.

Key Terms

- **autotroph**: an organism that can synthesize its food from inorganic substances, using heat or light as a source of energy.
- **heterotroph**: An organism which requires an external supply of energy in the form of food as it cannot synthesize its own.

Growth Terminology

The two ways that microbial organisms can be classified are as autotrophs (supply their own energy) or as heterotrophs (use the products of others).

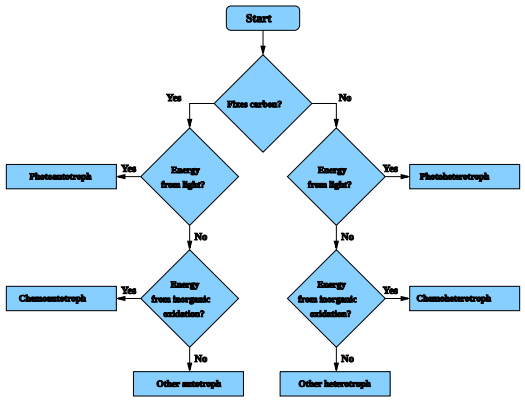


Figure: **Metabolic characteristics of microorganisms**: This is a flowchart to help determine how a microorganism undergoes growth development.

Autotrophs

An autotroph, which means self-feeding or producer, is an organism that produces complex organic compounds (such as carbohydrates, fats, and proteins) from simple substances present in its surroundings. To produce these organic compounds it either uses energy from light (by photosynthesis) or inorganic chemical reactions. Autotrophs reduce carbon dioxide (CO_2) by adding hydrogen atoms to it. This reduction process forms an organic compound that stores chemical energy. Most autotrophs use water as

their reducing agent (to gain hydrogen atoms), but some can use other hydrogen compounds like hydrogen sulfide. Autotrophs, and their formation of organic compounds, are an important component of the food chain because they produce the food necessary for larger, more complex organisms to grow.

Photoautotrophs

Photoautotrophs are a type of autotroph. Photoautotrophs use light (sunlight if they are green plants) as their energy source. They use this energy (physical) and convert it into chemical energy in the form of reduced carbon. This process produces energy that carries out various cellular metabolic processes.

Chemoautotrophs

Chemoautotrophs are also a type of autotroph. They derive their energy from chemical reactions and synthesize all necessary organic compounds from carbon dioxide. Most chemoautotrophs are bacteria and archaea that live in hostile environments (such as deep sea vents). Chemoautotrophs are thought to be the first organisms to inhabit earth.

Heterotrophs

A heterotroph is an organism that, unlike an autotroph, cannot fix carbon and uses organic carbon for growth. Heterotrophs use the products formed by autotrophs to survive.

Photoheterotrophs

Photoheterotrophs are a type of heterotroph. These organisms use light for energy, but cannot use carbon dioxide as their sole carbon source. They use compounds formed by autotrophs (such as carbohydrates, fatty acids, and alcohols) as their food.

Chemoheterotrophs

Chemoheterotrophs are a type of heterotroph. They are unable to fix carbon and form their own organic compounds so they must use products formed by autotrophs. These organisms use inorganic energy sources or organic energy sources to sustain life.

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SECTION OVERVIEW

6.8: Counting Bacteria

Topic hierarchy

6.8.1: Direct Counting

6.8.2: Viable Cell Counting

6.8.3: Measurements of Microbial Mass

6.8.4: Detecting Acid and Gas Production

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6.8.1: Direct Counting

LEARNING OBJECTIVES

- Contrast ways of directly counting bacteria

Numerous procedures in biology and medicine require that cells be counted. On almost all occasions, what gets counted is actually the concentration of the cells (for example: 5,000 cells per milliliter). By counting the cells in a known volume of a culture, the concentration can be assessed. In medicine, the concentration of various blood cells, such as red blood cells or white blood cells, can give crucial information regarding someone's health. Similarly, the concentration of bacteria, viruses, and other pathogens in blood or bodily fluids can reveal information about the progress of an infectious disease and about how a person's immune system is dealing with the infection. Knowing the cell concentration is important in molecular biology experiments in order to adjust the amount of reagents and chemicals applied to the experiment.

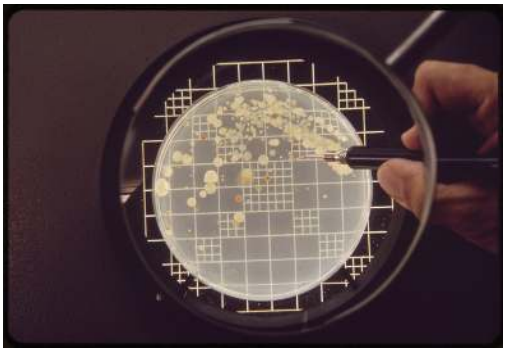


Figure: ***Counting Colonies****: An example of counting colonies on a streak plate.*

Direct counting methods include microscopic counts using a hemocytometer or a counting chamber. The hemocytometer works by creating a volumetric grid divided into differently sized cubes for accurately counting the number of particles in a cube and calculating the concentration of the entire sample. One can also quantify the number of cells in a culture by plating a known volume of the cell culture on a petri dish with a growth medium, which is also known as a streak plate. If the cells are distributed on the plate properly, it can generally be assumed that each cell will give rise to a single colony. The colonies can then be counted and, based on the known volume of the culture that was spread on the plate, the cell concentration can be calculated. Bacterial colony counts made from plating dilutions of bacteria are useful to estimate the strength of bacterial infections; for example, a urinary tract bacterial infection.

As with hemocytometers or counting chambers, cultures need to be heavily diluted prior to plating. Otherwise, instead of obtaining single colonies that can be counted, a so-called "lawn" of thousands of colonies will form, all lying atop each other. Additionally, plating is the slowest method because most microorganisms need at least 12 hours to form visible colonies. These methods of direct counting do not require sophisticated instrumentation, so they can easily be performed in most laboratories.

Key Points

- Directly counting blood cells or tissue cells by using a hemocytometer can determine the concentration of a known volume.
- Counting the number of colonies that arise on a pour plate can calculate the concentration by multiplying the count by the volume spread on the pour plate.
- Direct counting methods are easy to perform and do not require highly specialized equipment, but are often slower than other methods.

Key Terms

- **hemocytometer**: A device that counts microscopic particles. The hemocytometer works by creating a volumetric grid divided into differently sized cubes for accurately counting the number of particles in a cube and calculating the concentration of the entire sample.
- **streak plate**: A petri dish with a growth medium.

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6.8.2: Viable Cell Counting

Plate counting is used to estimate the number of viable cells that are present in a sample.

LEARNING OBJECTIVES

Explain viable cell counting

KEY TAKEAWAYS

Key Points

- The spread plate relies on bacteria growing a colony on a nutrient medium so that the colony becomes visible to the naked eye and the number of colonies on a plate can be counted.
- Selective media can be used to restrict the growth of non-target bacteria.
- The pour plate method is used when the analysis is looking for bacterial species that grow poorly in air, for example water samples.

Key Terms

- **plate count**: A means to identify the number of actively growing cells in a sample.

Viable Cell Counting

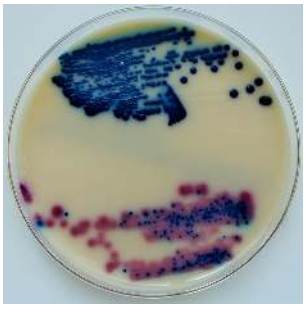


Figure: **Selective media can be used to restrict the growth of non-target bacteria.**: Urine cultured on Oxoid Brilliance UTI Agar plate. 1uL of urine spread onto the agar surface. The top sample is from patient with clinical urinary tract infection (UTI). The bottom sample is a mixed culture.

There are a variety of ways to enumerate the number of bacteria in a sample. A viable cell count allows one to identify the number of actively growing/dividing cells in a sample. The plate count method or spread plate relies on bacteria growing a colony on a nutrient medium. The colony becomes visible to the naked eye and the number of colonies on a plate can be counted. To be effective, the dilution of the original sample must be arranged so that on average between 30 and 300 colonies of the target bacterium are grown. Fewer than 30 colonies makes the interpretation statistically unsound and greater than 300 colonies often results in overlapping colonies and imprecision in the count. To ensure that an appropriate number of colonies will be generated several dilutions are normally cultured. The laboratory procedure involves making serial dilutions of the sample (1:10, 1:100, 1:1000 etc.) in sterile water and cultivating these on nutrient agar in a dish that is sealed and incubated. Typical media include Plate count agar for a general count or MacConkey agar to count gram-negative bacteria such as E. coli. Typically one set of plates is incubated at 22°C and for 24 hours and a second set at 37°C for 24 hours. The composition of the nutrient usually includes reagents that resist the growth of non-target organisms and make the target organism easily identified, often by a color change in the medium. Some recent methods include a fluorescent agent so that counting of the colonies can be automated. At the end of the incubation period the colonies are counted by eye, a procedure that takes a few moments and does not require a microscope as the colonies are typically a few millimeters across.

The pour plate method is used when the analysis is looking for bacterial species that grow poorly in air. The initial analysis is done by mixing serial dilutions of the sample in liquid nutrient agar which is then poured into bottles. The bottles are then sealed and laid on their sides to produce a sloping agar surface. Colonies that develop in the body of the medium can be counted by eye after incubation. The total number of colonies is referred to as the Total Viable Count (TVC). The unit of measurement is cfu/ml (or colony forming units per milliliter) and relates to the original sample. Calculation of this is a multiple of the counted number of

colonies multiplied by the dilution used. Examples of a viable cell count are spread plates from a serial dilution of a liquid culture and pour plates. With a spread plate one makes serial dilutions in liquid media and then spreads a known volume from the last tube in the dilution series. The colonies on the plate can then be counted and the concentration of bacteria in the original culture can be calculated. In the pour plate method a diluted bacterial sample is mixed with melted agar and then that mixture is poured into a petri dish. Again the colonies would be counted and the viable cell count calculated.

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6.8.3: Measurements of Microbial Mass

LEARNING OBJECTIVES

- Recall ways of measuring microbial mass

Bacterial growth follows three phases: the lag phase, the log phase, and the stationary phase. The measurement of an exponential bacterial growth curve in a batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements.

METHODS OF MEASUREMENT

There are several methods for measuring cell mass, including the *gravimetermethod* which uses ordinary balances to weigh a sample (dry weight/ml) after the water has been removed.



Figure: **Spectrophotometer**: This spectrophotometer can measure as little as one microliter of a sample.

An indirect method for calculating cell mass is *turbidimetry*. Cell cultures are turbid: they absorb some of the light and let the rest of it pass through. The higher the cell concentration is, the higher the turbidity. Spectrophotometers are electrical appliances that can measure turbidity very accurately. The culture is placed in a translucent cuvette; the cuvette is placed in the machine and the turbidity measured immediately. Simple mathematical formulae help convert the detected turbidity to cell concentration. Using spectrophotometry for measuring the turbidity of cultures is known as *turbidometry*. Note the difference in spelling: *turbidimetry* and *turbidometry* are not the same word.

In spectrophotometry, cultures usually do not need to be diluted, although above a certain cell density the results lose reliability. Of all the electrical appliances used for counting cells, a spectrophotometer is the cheapest and its operation the fastest and most straightforward. This has made spectrophotometry the methods of choice for quick measurements of bacterial growth and related applications. There are spectrophotometers in which several cuvettes can be inserted at one time, reducing work time even more. Additionally, there are spectrophotometers that require extremely small volumes of culture, as little as 1 microliter. This, combined with the stochastic nature of liquid cultures, enables only an estimation of cell numbers.

An additional method for the measurement of microbial mass is the quantification of cells in a culture by *plating the cells on a petri dish*. If the cells are efficiently distributed on the plate, it can be generally assumed that each cell will give rise to a single colony. The colonies can then be counted, and based on the known volume of culture that was spread on the plate the cell concentration can be calculated.

As is with counting chambers, cultures usually need to be heavily diluted prior to plating; otherwise, instead of obtaining single colonies that can be counted, a so-called “lawn” will form, resulting in thousands of colonies lying over each other. Additionally,

plating is the slowest method of all: most microorganisms need at least 12 hours to form visible colonies.

Key Points

- Calculating the dry weight of a sample enables one to calculate the cell count, but the sensitivity is limited to samples containing more than 10E8 bacteria per milliliter.
- Spectrophotometry is an indirect method for calculating cell concentrations by measuring the changes in turbidity.
- Bacteria can also be counted by using the plating method, which is based on the number of colonies formed in Petri dishes containing specific growth media.

Key Terms

- **spectrophotometry**: A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200nm – 2500nm using different controls and calibrations. [2] Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the photometric determination. [3]
- **flow cytometry**: A technique used to sort and classify cells by using fluorescent markers on their surface.
- **gravimeter**: An instrument used to measure local variations in the gravitational field

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6.8.4: Detecting Acid and Gas Production

Culture media can be used to differentiate between different kinds of bacteria by detecting acid or gas production.

LEARNING OBJECTIVES

Show how microbial acid and gas production are detected

KEY TAKEAWAYS

Key Points

- Differential media uses the biochemical characteristics of a microorganism growing in the presence of specific nutrients or indicators.
- To measure acid production one can use a pH indicator in the media.
- The Durham tube method is used to detect production of gas by microorganisms.

Key Terms

- **differential media**: Differential media or indicator media distinguish one microorganism type from another growing on the same media.

Cultures and Differential Media

A microbiological culture, or microbial culture, is created using a method for multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions. Microbial cultures are used to determine an organism's type, its abundance in the sample being tested, or both. It is one of the primary diagnostic techniques of microbiology, where it is used as a tool to determine the cause of infectious diseases by letting the agent multiply in a predetermined medium. A throat culture, for example, is taken by scraping the lining of tissue in the back of the throat and blotting the sample into a growing medium; this will allow analysis to screen for harmful microorganisms, such as *Streptococcus pyogenes*, the causative agent of strep throat. The term "culture" can be used to refer to the process of culturing organisms, to the medium they're grown in, and is more generally used informally to refer to "selectively growing" a specific kind of microorganism in the lab.

Differential media, also known as indicator media, distinguish one microorganism type from another growing on the same media. These types of media use the biochemical characteristics of a microorganism grown in the presence of specific nutrients or indicators that have been added to the medium to visibly indicate the defining characteristics of a microorganism. These indicators or nutrients include but are not limited to neutral red, phenol red, eosin y, and methylene blue. Differential media are used for the detection of microorganisms and by molecular biologists to detect recombinant strains of bacteria.

Durham Cultures

The Durham tube method is used to detect production of gas by microorganisms. They are simply smaller test tubes inserted upside down in another test tube. This small tube is initially filled with the solution in which the microorganism is to be grown. If gas is produced after inoculation and incubation, a visible gas bubble will be trapped inside the small tube. The initial air gap produced when the tube is inserted upside down is lost during sterilization, usually performed at 121°C for 15 or so minutes

Escherichia coli

Escherichia coli (*E. coli*), a rod-shaped member of the coliform group, can be distinguished from most other coliforms by its ability to ferment lactose at 44°C in the fecal coliform test, and by its growth and color reaction on certain types of culture media. When cultured on an EMB (eosin methylene blue) plate, a positive result for *E. coli* is metallic green colonies on a dark purple media. Unlike the general coliform group, *E. coli* are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination. Some strains of *E. coli* can cause serious illness in humans.

Sorbitol MacConkey Agar

Sorbitol MacConkey agar is a variant of the traditional MacConkey commonly used in the detection of *E. coli* O157:H7. Traditionally, MacConkey agar has been used to distinguish those bacteria that ferment lactose from those that do not.



Figure: **Using differential media to detect acid production**: Sorbitol fermenting non pathogenic commensal bacteria from faeces growing on Cefixime Tellurite Sorbitol MacConkey Agar.

This is an important distinction. Gut bacteria, such as *Escherichia coli*, can typically ferment lactose; important gut pathogens including *Salmonella enterica* and most *shigellas* are unable to ferment lactose. *Shigella sonnei* can ferment lactose, but only after prolonged incubation; it is referred to as a *late-lactose fermenter*.

During fermentation of sugar, acid is formed and the pH of the medium drops, changing the color of the pH indicator. Different formulations use different indicators; neutral red is often used when culturing gut bacteria because lactose fermenters turn a deep red when this pH indicator is used. Those bacteria unable to ferment lactose, often referred to as *nonlactose fermenters* (NLFs) metabolize the peptone in the medium. This releases ammonia, which raises the pH of the medium. Although some authors refer to NLFs as being colorless, in reality they turn neutral red a buffish color.

E. coli O157:H7 differs from most other strains of *E. coli* in being unable to ferment sorbitol. In sorbitol MacConkey agar, lactose is replaced by sorbitol. Most strains of *E. coli* ferment sorbitol to produce acid: *E. coli* O157:H7 can not ferment sorbitol, so this strain uses peptone to grow. This raises the pH of the medium, allowing the O157:H7 strain to be differentiated from other *E. coli* strains through the action of the pH indicator in the medium.

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SECTION OVERVIEW

6.9: Temperature and Microbial Growth

Topic hierarchy

6.9.1: Growth Rate and Temperature

6.9.2: Classification of Microorganisms by Growth Temperature

6.9.3: The Heat-Shock Response

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6.9.1: Growth Rate and Temperature

Bacteria may grow across a wide range of temperatures, from very cold to very hot.

LEARNING OBJECTIVES

Describe how the growth of bacteria is affected by temperature and how bacterial growth can be measured

KEY TAKEAWAYS

Key Points

- The basic means of measuring growth requires bacterial enumeration (cell counting).
- Methods for bacterial cell counting include: 1. direct and individual (microscopic, flow cytometry), 2. direct and bulk (biomass), 3. indirect and individual (colony counting), or 4. indirect and bulk (most probable number, turbidity, nutrient uptake).
- A mesophile is an organism that grows best in moderate temperature, neither too hot nor too cold. All human pathogens are mesophiles.
- Cold shock proteins help the cell to survive in temperatures lower than optimum growth temperature.
- Heat shock proteins help the cell to survive in temperatures greater than the optimum, possibly by condensation of the chromosome and organization of the prokaryotic nucleoid.

Key Terms

- **mesophile**: An organism, especially a microorganism, that lives and thrives at moderate temperatures.
- **psychrophile**: An organism that can live and thrive at temperatures much lower than normal; a form of extremophile.
- **thermophile**: An organism that lives and thrives at relatively high temperatures; a form of extremophile; many are members of the Archaea.

Growth Rate and Temperature

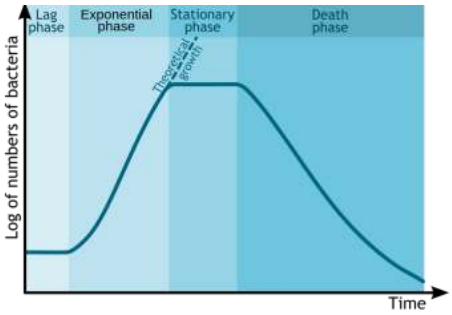


Figure: **Bacterial growth curve**: Bacterial growth in batch culture can be modeled with four different phases: (A) the lag phase, when the population stays roughly the same; (B) the exponential, or log, phase, when the population grows at an increasing rate; (C) the stationary phase, when population growth stagnates; and (D) the death phase, when bacteria begin to die off and the population decreases in size.

Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission. Providing no mutational event occurs the resulting daughter cells are genetically identical to the original cell. Hence, local doubling of the bacterial population occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists. The basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurements.



Figure: **Grand Prismatic Spring, Midway & Lower Geyser Basin, Yellowstone National Park**: This photo shows steam rising from hot and sterile deep azure blue water in the center, surrounded by huge mats of brilliant orange algae, bacteria and archaea. These colorful microorganisms are called extremophiles—these in particular are thermophiles.

Bacteria may grow across a wide range of temperatures, from very cold to very hot. A mesophile is an organism that grows best in moderate temperature, neither too hot nor too cold. All human pathogens are mesophiles. Organisms that prefer extreme environments are known as extremophiles: those that prefer cold environments are termed psychrophilic, those preferring warmer temperatures are termed thermophilic or thermotrophs and those thriving in extremely hot environments are hyperthermophilic.

For example, in molecular biology, the cold-shock domain (CSD) is a protein domain of about 70 amino acids which has been found in prokaryotic and eukaryotic DNA-binding proteins. Part of this domain is highly similar to the RNP-1 RNA-binding motif. When Escherichia coli is exposed to a temperature drop from 37 to 10 degrees Celsius, a four to five hour lag phase occurs and then growth is resumed at a reduced rate. During the lag phase, the expression of around 13 proteins, which contain cold shock domains is increased two- to ten-fold. These so-called cold shock proteins are thought to help the cell survive in temperatures lower than optimum growth temperature, by contrast with heat shock proteins, which help the cell survive in temperatures greater than the optimum, possibly by condensation of the chromosome and organization of the prokaryotic nucleoid.

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6.9.2: Classification of Microorganisms by Growth Temperature

Bacteria can be classified on the basis of cell structure, metabolism or on differences in cell components.

LEARNING OBJECTIVES

Describe how bacteria can be classified on the basis of cell structure, cellular metabolism or differences in cell components such as DNA

KEY TAKEAWAYS

Key Points

- A mesophile is an organism that grows best in moderate temperature, neither too hot nor too cold, typically between 20 and 45 °C (68 and 113 °F).The term is mainly applied to microorganisms.
- All bacteria have their own optimum environmental surroundings and temperatures in which they thrive the most.
- Thermophiles contain enzymes that can function at high temperatures. Some of these enzymes are used in molecular biology (for example, heat-stable DNA polymerases for PCR), and in washing agents.

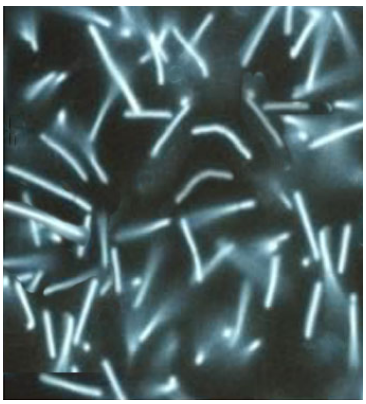
Key Terms

- **mesophile**: An organism, especially a microorganism, that lives and thrives at moderate temperatures.
- **thermophile**: An organism that lives and thrives at relatively high temperatures; a form of extremophile; many are members of the Archaea.

Classification seeks to describe the diversity of bacterial species by naming and grouping organisms based on similarities. Bacteria can be classified on the basis of cell structure, cellular metabolism, or on differences in cell components such as DNA, fatty acids, pigments, antigens and quinones.

Bacteria can be classified by their optimal growth temperature. The following are the five classifications:

- Hyperthermophile (60 degrees C and upwards)
- Thermophile (optimal growth between 45 and 122 degrees)
- Mesophile (20 and 45 degrees C)
- Psychrotrophs (will survive at 0 degrees C, but prefer mesophilic temperature
- Psychrophiles (-15 and 10 degrees C or lower)



Methanopyrus kandleri

Methanopyrus kandleri can survive and reproduce at 122 °C.

A mesophile is an organism that grows best in moderate temperature, neither too hot nor too cold, typically between 20 and 45 °C (68 and 113 °F). The term is mainly applied to microorganisms.The habitats of these organisms include especially cheese, yogurt, and mesophile organisms are often included in the process of beer and wine making. Organisms that prefer cold environments are termed psychrophilic, those preferring warmer temperatures are termed thermophilic and those thriving in extremely hot

environments are hyperthermophilic. All bacteria have their own optimum environmental surroundings and temperatures in which they thrive the most. A thermophile is an organism — a type of extremophile — that thrives at relatively high temperatures, between 45 and 122 °C (113 and 252 °F). Thermophilic eubacteria are suggested to have been among the earliest bacteria. Thermophiles are found in various geothermally heated regions of the Earth, such as hot springs like those in Yellowstone National Park. and deep sea hydrothermal vents, as well as decaying plant matter, such as peat bogs and compost.As a prerequisite for their survival, thermophiles contain enzymes that can function at high temperatures. Some of these enzymes are used in molecular biology (for example, heat-stable DNA polymerases for PCR), and in washing agents.

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6.9.3: The Heat-Shock Response

Heat shock response is a cell's response to intense heat, including up-regulation of heat shock proteins.

LEARNING OBJECTIVES

Describe how the bacterial stress response enables bacteria to survive adverse and fluctuating conditions in their immediate surroundings such as increases in temperature

KEY TAKEAWAYS

Key Points

- The bacterial stress response enables bacteria to survive adverse and fluctuating conditions in their immediate surroundings.
- A bacterial cell can react simultaneously to a wide variety of stresses and the various stress response systems interact with each other by a complex of global regulatory networks.
- The up-regulation of HSPs during heat shock is generally controlled by a single transcription factor; in eukaryotes this regulation is performed by heat shock factor (HSF), while σ32 is the heat shock sigma factor in Escherichia coli.

Key Terms

- **heat shock response**: The cellular response to heat shock.

The bacterial stress response enables bacteria to survive adverse and fluctuating conditions in their immediate surroundings. Various bacterial mechanisms recognize different environmental changes and mount an appropriate response. A bacterial cell can react simultaneously to a wide variety of stresses, and the various stress response systems interact with each other by a complex of global regulatory networks.

In biochemistry, heat shock is the "effect of subjecting a cell to a higher temperature than that of the ideal body temperature of the organism from which the cell line was derived. "

Heat shock response is the cellular response to heat shock includes the transcriptional up-regulation of genes encoding heat shock proteins (HSPs) as part of the cell's internal repair mechanism. HSPs are also called 'stress-proteins' and respond to heat, cold and oxygen deprivation by activating several cascade pathways. HSPs are also present in cells under perfectly normal conditions. Some HSPs, called 'chaperones', ensure that the cell's proteins are in the right shape and in the right place at the right time. For example, HSPs help new or misfolded proteins to fold into their correct three-dimensional conformations, which is essential for their function. They also shuttle proteins from one compartment to another inside the cell and target old or terminally misfolded proteins to proteases for degradation. Additionally, heat shock proteins are believed to play a role in the presentation of pieces of proteins (or peptides) on the cell surface to help the immune system recognize diseased cells. The up-regulation of HSPs during heat shock is generally controlled by a single transcription factor; in eukaryotes this regulation is performed by heat shock factor (HSF), while σ32 is the heat shock sigma factor in Escherichia coli.

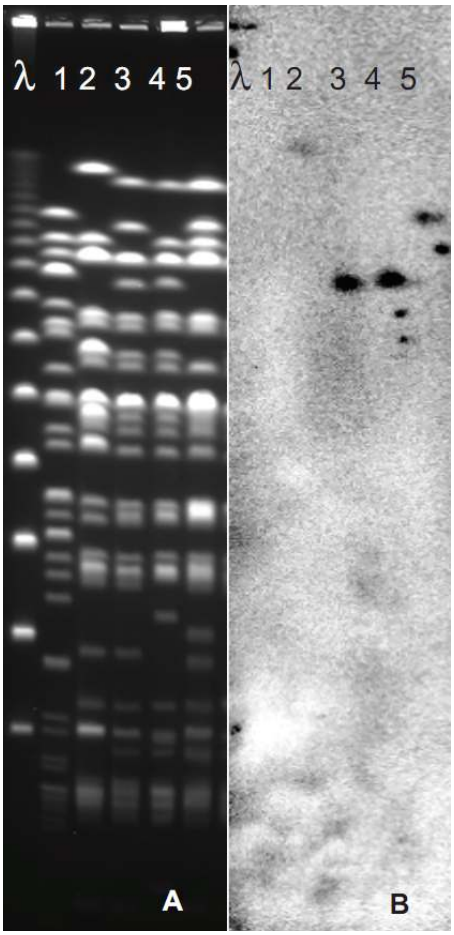


Figure: **Heat shock proteins**: Heat shock protein come in many sizes. This is an example of small heat shock proteins produced by Pseudomonas aeruginosa Clonal Variants Isolated from Diverse Niches.

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SECTION OVERVIEW

6.10: 6. 10- Other Environmental Growth Factors

Topic hierarchy

6.10.1: Gas Requirements

6.10.2: Osmotic Pressure

6.10.3: Microbial Growth at Low or High pH

6.10.4: Oxygen

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6.10.1: Gas Requirements

Cells are grown and maintained at an appropriate temperature and gas mixture of oxygen, carbon dioxide, and nitrogen in a cell incubator.

LEARNING OBJECTIVES

Compare different gas requirements of various microbes

KEY TAKEAWAYS

Key Points

- Culture conditions vary greatly for each cell type. The variation of conditions for a particular cell type can result in different phenotypes.
- Capnophiles are microorganisms that thrive in the presence of high concentrations of carbon dioxide.
- Diazotrophs are microorganisms that fix atmospheric nitrogen gas into a more usable form such as ammonia.

Key Terms

- **capnophile**: A microorganism that requires or grows best in presence of high concentrations of carbon dioxide.
- **diazotroph**: A microorganism that can fix nitrogen.

Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C and a mixture of oxygen, carbon dioxide, and nitrogen) in a cell incubator. Culture conditions vary greatly for each cell type. The variation of conditions for a particular cell type can result in different phenotypes.



Figure: **Bacteriological incubator**: Cells are grown and maintained at an appropriate temperature and gas mixture of oxygen, carbon dioxide, and nitrogen in a cell incubator.

Capnophiles are microorganisms that thrive in the presence of high concentrations of carbon dioxide. Typically, in a cell culture the CO_2 concentration is around 5%. Some capnophiles may have a metabolic requirement for carbon dioxide, while others merely compete more successfully for resources under these conditions.

Diazotrophs are microorganisms that fix atmospheric nitrogen gas into a more usable form such as ammonia. A diazotroph is an organism that is able to grow without external sources of fixed nitrogen. Some example free-living diazotrophs include:

1) obligate anaerobes that cannot tolerate oxygen even if they are not fixing nitrogen. They live in habitats low in oxygen, such as soils and decaying vegetable matter.

2) Facultative anaerobes that can grow either with or without oxygen, but they only fix nitrogen anaerobically. Often, they respire oxygen as rapidly as it is supplied, keeping the amount of free oxygen low.

3) Aerobes that require oxygen to grow, yet their nitrogenase is still debilitated if exposed to oxygen.

4) Oxygenic photosynthetic bacteria generate oxygen as a by-product of photosynthesis, yet some are able to fix nitrogen as well.

5) And finally, Anoxygenic photosynthetic bacteria that do not generate oxygen during photosynthesis as they have only a single photosystem which cannot split water. In addition, nitrogenase is expressed under nitrogen limitation.

Some higher plants, and some animals (termites), have formed associations (symbioses) with diazotrophs. Examples of those diazotrophs include: rhizobia that associate with legumes, plants of the Fabaceae family, frankias, and cyanobacteria that associate with fungi as lichens, with liverworts, with a fern, and with a cycad.

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6.10.2: Osmotic Pressure

The correct osmotic pressure in the culture medium is essential for the survival of the cells.

LEARNING OBJECTIVES

Describe osmotic effects

KEY TAKEAWAYS

Key Points

- Osmosis is the net movement of solvent molecules through a partially permeable membrane into a region of higher solute concentration in order to equalize the solute concentrations on the two sides.
- Osmosis provides the primary means by which water is transported into and out of cells.
- Osmoregulation is the homeostasis mechanism of an organism to reach balance in osmotic pressure.
- If the medium is hypotonic, the cells will gain water through osmosis.
- If the medium is hypertonic, the cells will lose water through osmosis.

Key Terms

- **osmosis**: the net movement of solvent molecules from a region of high solvent potential to a region of lower solvent potential through a partially permeable membrane
- **hypotonic**: Having a lower osmotic pressure than another.
- **isotonic**: Having the same osmotic pressure.
- **hypertonic**: Having a greater osmotic pressure than another.
- **halophile**: Organisms that thrive in high salt concentrations.

Osmotic pressure is an important factor that affects cells. Osmosis is the net movement of solvent molecules through a partially permeable membrane into a region of higher solute concentration. The intent of osmosis is to equalize the solute concentrations on the two sides. Osmosis is essential in biological systems because biological membranes are semi permeable. In general, these membranes are impermeable to large and polar molecules such as ions, proteins, and polysaccharides. However, they are permeable to non-polar and/or hydrophobic molecules like lipids as well as to small molecules like oxygen, carbon dioxide, nitrogen, nitric oxide, etc. Osmosis provides the primary means by which water is transported into and out of cells. Osmoregulation is the homeostasis mechanism of an organism to reach balance in osmotic pressure.

Having the correct osmotic pressure in the culture medium is essential. A cell can be influenced by a solution in three ways. Suppose a cell is placed in a solution of sugar or salt water. If the medium is hypotonic — a diluted solution with a higher water concentration than the cell — the cell will gain water through osmosis. If the medium is isotonic — a solution with exactly the same water concentration as the cell — there will be no net movement of water across the cell membrane. If the medium is hypertonic — a concentrated solution with a lower water concentration than the cell — the cell will lose water by osmosis.

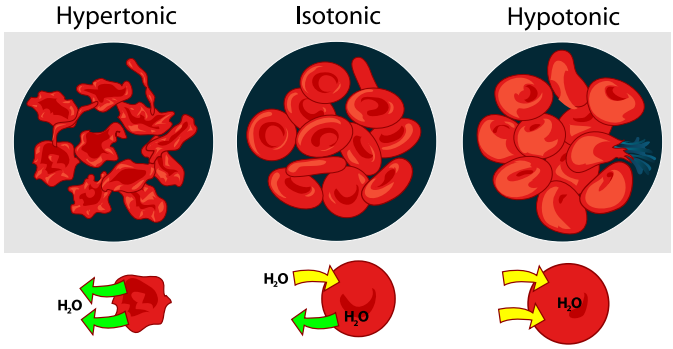


Figure: **Osmotic Pressure on Red Blood Cells**: Effect of different solutions on blood cells.

Essentially, this means that if a cell is put in a solution that has a solute concentration higher than its own, then it will shrivel up. If it is put in a solution with a lower solute concentration than its own, the cell will expand and burst.

Obligate and Facultative Halophiles

A halophile is a microorganism that can survive and replicate in a high salt concentration environment (high osmotic pressure).

Obligate halophiles are microorganisms that can only survive in high salt concentration environments. Facultative halophiles are able to survive in *both*high and normal salt concentration environments.

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6.10.3: Microbial Growth at Low or High pH

Microorganisms live and thrive within specific pH levels.

LEARNING OBJECTIVES

Differentiate microbial growth at high or low pHs

KEY TAKEAWAYS

Key Points

- Neutrophiles are organisms that thrive in neutral (pH 7) environments.
- Alkaliphiles are microbes that thrive in alkaline (pH 9-11) environments.
- Acidophilic organisms are those that thrive under highly acidic conditions (usually at pH 2.0 or below).

Key Terms

- **neutrophile**: any organism that thrives in a relatively neutral pH
- **alkaliphile**: any organism that lives and thrives in an alkaline environment, such as a soda lake; a form of extremophile
- **acidophile**: an organism that lives and thrives under acidic conditions; a form of extremophile

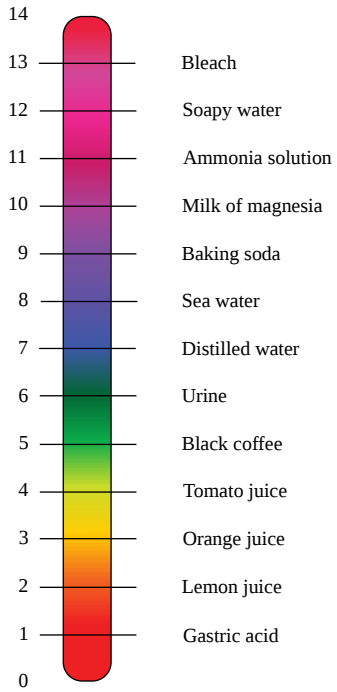


Figure: **pH scale**: A pH scale with annotated examples of chemicals at each integer pH value

In chemistry, pH is a measure of the activity of the (solvated) hydrogen ion. In other words, it is a measure of hydrogen ion concentration. Pure water has a pH very close to 7 at 25°C. Solutions with a pH less than 7 are said to be acidic, and solutions with a pH greater than 7 are said to be basic or alkaline. The pH scale is traceable to a set of standard solutions whose pH is established by international agreement. The pH of different cellular compartments, body fluids, and organs is usually tightly regulated in a process called acid-base homeostasis. Microorganisms live and thrive within specific pH levels.

Neutrophiles are organisms that thrive in neutral (pH 7) environments; extromophiles are organisms that thrive in extreme pH environments.

Alkaliphiles are microbes that thrive in alkaline environments with a pH of 9 to 11, such as playa lakes and carbonate-rich soils. To survive, alkaliphiles maintain a relatively low alkaline level of about 8 pH inside their cells by constantly pumping hydrogen ions in the form of hydronium ions (H_3O^+) across their cell membranes and into their cytoplasm.

Acidophilic organisms are those that thrive under highly acidic conditions (usually at pH 2.0 or below). Most acidophile organisms have evolved extremely efficient mechanisms to pump protons out of the intracellular space in order to keep the cytoplasm at or near neutral pH. Therefore, intracellular proteins do not need to develop acid stability through evolution. However, other acidophiles, such as Acetobacter aceti, have an acidified cytoplasm which forces nearly all proteins in the genome to evolve acid stability.

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6.10.4: Oxygen

Oxygen requirements vary among microorganisms.

LEARNING OBJECTIVES

Identify the role of oxygen in microbial growth

KEY TAKEAWAYS

Key Points

- An aerobic organism or aerobe is an organism that can survive and grow in an oxygenated environment.
- An anaerobic organism or anaerobe is any organism that does not require oxygen for growth.
- Normal microbial culturing occurs in an aerobic environment which poses a problem when culturing anaerobes; requiring one of a number of techniques to be used to keep oxygen out of the culturing setup.

Key Terms

- **anaerobic**: Without oxygen; especially of an environment or organism.
- **aerobic respiration**: metabolic reactions and processes that take place in the cells of organisms and require oxygen to convert biochemical energy from nutrients into adenosine triphosphate (ATP)
- **aerobic**: Living or occurring only in the presence of oxygen.
- **aerotolerant anaerobe**: an organism that does not require oxygen to sustain its metabolic processes, but is able to survive in the presence of oxygen

An aerobic organism or aerobe is an organism that can survive and grow in an oxygenated environment. Several varietis of aerobes exist. Obligate aerobes require oxygen for aerobic cellular respiration. In a process known as cellular respiration, these organisms use oxygen to oxidize substrates (for example sugars and fats) in order to obtain energy. Facultative anaerobes can use oxygen, but also have anaerobic (i.e. not requiring oxygen) methods of energy production. Microaerophiles are organisms that may use oxygen, but only at low concentrations. Aerotolerant organisms can survive in the presence of oxygen, but they are anaerobic because they do not use it as a terminal electron acceptor.

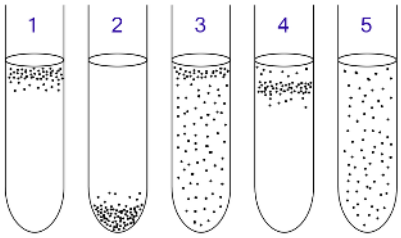


Figure: **Identity of aerobic and anaerobic bacteria**: Aerobically different bacteria behave differently when grown in liquid culture: 1) Obligate aerobic bacteria gather at the top of the test tube in order to absorb maximal amount of oxygen. 2) Obligate anaerobic bacteria gather at the bottom to avoid oxygen. 3) Facultative bacteria gather mostly at the top, since aerobic respiration is advantageous (ie, energetically favorable); but as lack of oxygen does not hurt them, they can be found all along the test tube. 4) Microaerophiles gather at the upper part of the test tube but not at the top. They require oxygen, but at a lower concentration. 5) Aerotolerant bacteria are not affected at all by oxygen, and they are evenly spread along the test tube.

An anaerobic organism or anaerobe is any organism that does not require oxygen for growth. It could possibly react negatively and may even die if oxygen is present. For practical purposes there are three categories: obligate anaerobes, which cannot use oxygen for growth and are even harmed by it. Aerotolerant organisms, which cannot use oxygen for growth, but tolerate the presence of it. And finally, facultative anaerobes, which can grow without oxygen but can utilize oxygen if it is present.

Since normal microbial culturing occurs in atmospheric air, which is an aerobic environment, the culturing of anaerobes poses a problem. Therefore, a number of techniques are employed by microbiologists when culturing anaerobic organisms, for example, handling the bacteria in a glovebox filled with nitrogen or the use of other specially-sealed containers.

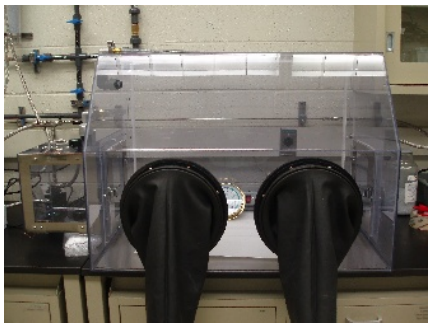


Figure: **Glovebox**: Terra Universal 100 Glovebox

The GasPak System is an isolated container that achieves an anaerobic environment by the reaction of water with sodium borohydride and sodium bicarbonate tablets to produce hydrogen gas and carbon dioxide. Hydrogen then reacts with oxygen gas on a palladium catalyst to produce more water, thereby removing oxygen gas.

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SECTION OVERVIEW

6.11: 6. 11- Microbial Growth in Communities

Topic hierarchy

6.11.1: Ecological Associations Among Microorganisms

6.11.2: Biofilms

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6.11.1: Ecological Associations Among Microorganisms

Despite an apparent simplicity, bacteria can form complex associations with other organisms.

LEARNING OBJECTIVES

Contrast the ecological associations among microorganisms

KEY TAKEAWAYS

Key Points

- Due to their small size, commensal bacteria are ubiquitous and grow on animals and plants exactly as they would grow on any other surface. Their growth can be increased by warmth and sweat; in humans, large populations of these organisms are the cause of body odor.
- Pathogenic bacteria are a major cause of human death and disease, and cause infections such as tetanus, typhoid fever, diphtheria, syphilis, cholera, foodborne illness, leprosy and tuberculosis.
- Certain bacteria form mutualistic associations, such as close spatial associations that are essential for their survival; for example, interspecies hydrogen transfer.

Key Terms

- **mutualism**: Any interaction between two species that benefits both; typically involves the exchange of substances or services.
- **parasitism**: Interaction between two organisms, in which one organism (the parasite) benefits and the other (the host) is harmed.
- **commensalism**: Describes a relationship between two living organisms where one benefits and the other is not significantly harmed or helped.

Ecological Associations Among Microorganisms

Despite an apparent simplicity, bacteria can form complex associations with other organisms. This process is known as symbiosis. These symbiotic associations can be divided into parasitism, mutualism and commensalism. Due to their small size, commensal bacteria are ubiquitous and grow on animals and plants exactly as they would grow on any other surface. However, their growth can be increased by warmth and sweat; in humans, large populations of these organisms are the cause of body odor.

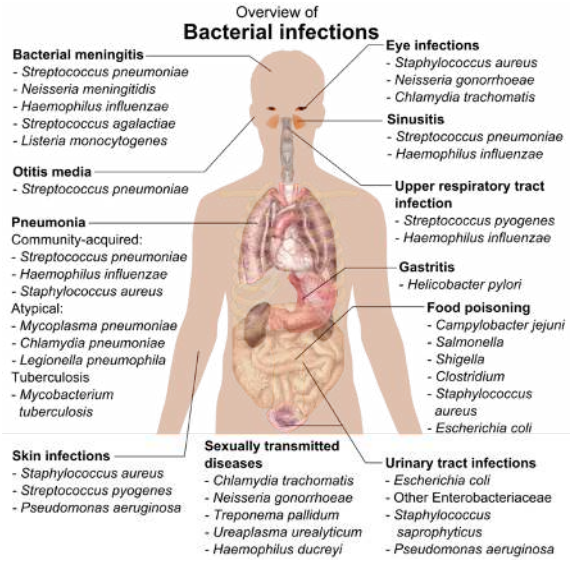


Figure: **Bacterial Infections in the Human Host**: Overview of the main bacterial infections and the most notable species involved

PREDATORS

Some species of bacteria kill and then consume other microorganisms; these species are called *predatory bacteria*. These include organisms such as*Myxococcus xanthus*, which forms swarms of cells that kill and digest any bacteria they encounter. Other bacterial predators either attach to their prey in order to digest them and absorb nutrients, such as *Vampirococcus*, or invade another cell and multiply inside the cytosol, such as *Daptobacter*. These predatory bacteria are thought to have evolved from saprophages that consumed dead microorganisms through adaptations that allowed them to entrap and kill other organisms.

MUTUALISTS

Certain bacteria form close spatial associations that are essential for their survival. One such mutualistic association, called interspecies hydrogen transfer, occurs between clusters of anaerobic bacteria that consume organic acids, such as butyric acid or propionic acid, and produce hydrogen; and methanogenic Archaea, which consume hydrogen. The bacteria in this association are unable to consume the organic acids since this reaction produces hydrogen, which accumulates in the bacteria's surroundings. Only the intimate association with the hydrogen-consuming Archaea keeps the hydrogen concentration low enough to allow the bacteria to grow.

In soil, microorganisms that reside in the rhizospehere (a zone that includes the root surface and the soil that adheres to the root after gentle shaking) carry out nitrogen fixation, converting nitrogen gas to nitrogenous compound. This serves to provide an easily absorbable form of nitrogen for many plants that cannot fix nitrogen themselves. Many other bacteria are found as symbionts in humans and other organisms. For example, the presence of over one thousand bacterial species in the normal human gut flora of the intestines can contribute to gut immunity. Synthesis vitamins such as folic acid, vitamin K, and biotin convert sugars to lactic acid (see *Lactobacillus*), as well as fermenting complex undigestible carbohydrates. The presence of this gut flora also inhibits the growth of potentially pathogenic bacteria (usually through competitive exclusion), and these beneficial bacteria are consequently sold as probioticdietary supplements.

PATHOGENS

If bacteria form a parasitic association with other organisms, they are classed as pathogens. Pathogenic bacteria are a major cause of human death and disease and cause infections such as tetanus, typhoid fever, diphtheria, syphilis, cholera, foodborne illness, leprosy, and tuberculosis. A pathogenic cause for a known medical disease may only be discovered many years after, as was the case with *Helicobacter pylori* and peptic ulcer disease. Bacterial diseases are also important in agriculture, with bacteria causing leaf spot, fire blight, and wilts in plants; as well as Johne's disease, mastitis, salmonella, and anthrax in farm animals.

Each species of pathogen has a characteristic spectrum of interactions with its human hosts. Some organisms, such as *Staphylococcus or Streptococcus*, can cause skin infections, pneumonia, meningitis and even overwhelming sepsis, a systemic inflammatory response producing shock, massive vasodilation, and death. Yet these organisms are also part of the normal human flora and usually exist on the skin or in the nose without causing any disease at all. Other organisms invariably cause disease in humans, such as the *Rickettsia*, which are obligate intracellular parasites able to grow and reproduce only within the cells of other organisms. One species of *Rickettsia* causes typhus, while another causes Rocky Mountain spotted fever. *Chlamydia*, another phylum of obligate intracellular parasites, contains species that can cause pneumonia, or urinary tract infection and may be involved in coronary heart disease. Finally, some species such as *Pseudomonas aeruginosa*, *Burkholderia cenocepacia*, and *Mycobacterium avium* are opportunistic pathogens and cause disease mainly in people suffering from immunosuppression or cystic fibrosis.

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6.11.2: Biofilms

LEARNING OBJECTIVES

- Describe biofilms

Biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface. These cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm EPS, also referred to as slime, is a polymeric conglomeration composed of extracellular DNA, proteins, and polysaccharides. Biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial, and hospital settings.

The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which, by contrast, are single cells that may float or swim in liquid. Microbes form a biofilm in response to many factors, including cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or exposure of planktonic cells to sub-inhibitory concentrations of antibiotics. When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior in which large suites of genes are differentially regulated.

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists initially form a weak, reversible adhesion to the surface via van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. Some species are not able to attach to a surface on their own but are able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able to communicate via quorum sensing using such products as AHL. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. The final stage of biofilm formation is known as development; this is the stage in which the biofilm is established and may change only in shape and size. The development of a biofilm may allow for an aggregate cell colony (or colonies) to be antibiotic-resistant.

In sum, the five stages of biofilm development are as follows:

1. Initial attachment
2. Irreversible attachment
3. Maturation I
4. Maturation II
5. Dispersion

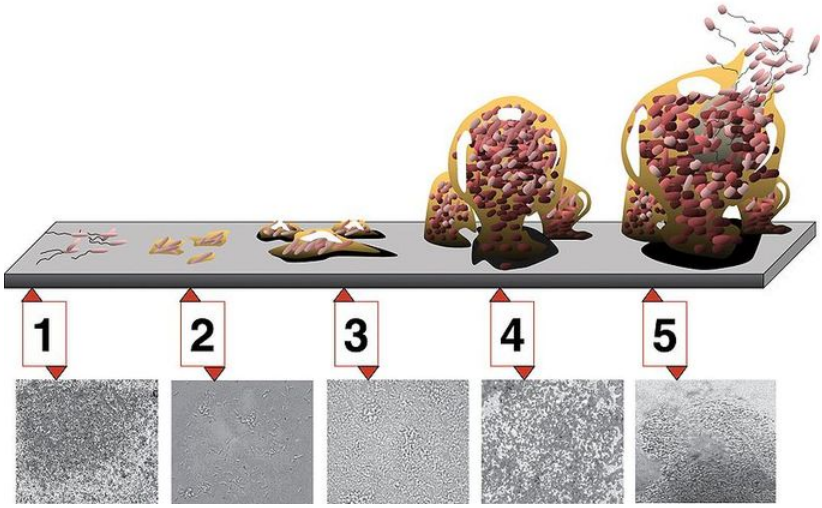


Figure: ***The Five Stages of Biofilm Development****: Stage 1: initial attachment; stage 2: irreversible attachment; stage 3: maturation I; stage 4: maturation II; stage 5: dispersion. Each stage of development in the diagram is paired with a photomicrograph of a developing Pseudomonas aeruginosa biofilm. All photomicrographs are shown at the same scale.*

Dispersal of cells from the biofilm colony is an essential stage of the biofilm life cycle. Dispersal enables biofilms to spread and colonize new surfaces. Enzymes that degrade the biofilm extracellular matrix, such as dispersin B and deoxyribonuclease, may play

a role in biofilm dispersal. Biofilm matrix-degrading enzymes may be useful as anti-biofilm agents. Recent evidence has shown that one fatty acid messenger, cis-2-decenoic acid, is capable of inducing dispersion and inhibiting growth of biofilm colonies. Secreted by Pseudomonas aeruginosa, this compound induces cyclo heteromorphic cells in several species of bacteria and the yeast Candida albicans. Nitric oxide has also been shown to trigger the dispersal of biofilms of several bacteria species at sub-toxic concentrations, so it shows potential for use in the treatment of patients that suffer from chronic infections caused by biofilms.

Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. In some cases antibiotic resistance can be increased a thousandfold. Lateral gene transfer is also greatly facilitated in biofilms and leads to a more stable structure.

However, biofilms are not always less susceptible to antibiotics. For instance, the biofilm form of *Pseudomonas aeruginosa* has no greater resistance to antimicrobials than do stationary-phase planktonic cells, although when the biofilm is compared to logarithmic-phase planktonic cells, the biofilm does show greater resistance to antimicrobials. This resistance to antibiotics in both stationary phase cells and biofilms may be due to the presence of persister cells.

Key Points

- Microbes form a biofilm in response to many factors, including cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or exposure of planktonic cells to sub-inhibitory concentrations of antibiotics.
- Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists initially adhere to the surface through weak, reversible adhesion via van der Waals forces.
- If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili.

Key Terms

- **biofilm**: an aggregate of microorganisms in which cells adhere to each other on a surface

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SECTION OVERVIEW

6.12: 6. 12- Control in Microbial Death

Topic hierarchy

6.12.1: Considerations in Microbial Control

6.12.2: Rate of Microbial Death

6.12.3: Relative Resistance of Microbes

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6.12.1: Considerations in Microbial Control

Controlling microbial growth is important in many fields but the degree of acceptable microbial levels can be quite different.

LEARNING OBJECTIVES

Discover considerations in microbial control

KEY TAKEAWAYS

Key Points

- Controlling microbial growth is important in the medical field, pharmaceutical and biotechnology industries, academic research, and food industry.
- The degree of acceptable microbial presence can differ based on the circumstances. Sterilization as a definition means that all life was terminated, whereas sanitization and disinfection terminates selectively and partially.
- Chemical agents that can eliminate or suppress microbial life are separated in different groups based on their use. The major groups are disinfectants, antiseptics, and antibiotics.
- Antibacterials are divided into two broad groups according to their biological effect on microorganisms: bactericidal agents kill bacteria, and bacteriostatic agents slow down or stall bacterial growth.

Key Terms

- **sterilization**: Any process that eliminates or kills all forms of microbial life present on a surface, solution, or solid compound.
- **microbicides**: Compounds or substances whose purpose is to reduce the infectivity of microbes, such as viruses or bacteria.
- **parenteral**: Administered by some means other than oral intake, particularly intravenously or by injection.

Considerations in Microbial Control

Ever since microbes were shown to cause diseases, people have invented different techniques to control their spread. Controlling microbial growth is important in the medical field, pharmaceutical and biotechnology industries, academic research, and food industry. Each antimicrobial substance or agent achieves a different level of microbial elimination by a certain mechanism.

TYPES OF MICROBIAL CONTROL

Sterilization (or sterilisation) is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, and spore forms) present on a surface, contained in a fluid, in medication, or in a compound. Sterilization can be achieved by applying the proper combinations of heat, chemicals, irradiation, high pressure, and filtration.

Chemical agents that can eliminate or suppress microbial life are separated in different groups based on their use.

Disinfectants are substances that are applied to non-living objects to destroy microorganisms that are living on them. Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores, so it is less effective than sterilisation. Disinfectants are different from other antimicrobial agents such as antibiotics, which destroy microorganisms within the body. Disinfectants are also different from biocides, as these are intended to destroy all forms of life, not just microorganisms. Disinfectants work by destroying the cell wall of microbes or interfering with their metabolism.

Antiseptics are antimicrobial substances that are applied to living tissue or skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are generally distinguished from antibiotics by the latter’s ability to be transported through the lymphatic system to destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non-living objects.

The term antibiotic was first used in 1942 by Selman Waksman and his collaborators in journal articles to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. This definition excluded substances that kill bacteria, but are not produced by microorganisms (such as gastric juices and hydrogen peroxide). It also excluded synthetic antibacterial compounds such as the sulfonamides. With advances in medicinal chemistry, most of today’s antibacterials chemically are semisynthetic modifications of various natural compounds.

Many antibacterial compounds are classified on the basis of chemical or biosynthetic origin into natural, semisynthetic, and synthetic. Another classification system is based on biological activity. In this classification, antibacterials are divided into two broad groups according to their biological effect on microorganisms: bactericidal agents kill bacteria, andbacteriostatic agents slow down or stall bacterial growth.

Microbicides which destroy virus particles are called viricides or antivirals.

LEVEL OF MICROBIAL PRESENCE



Figure: **Joseph Lister**: Joseph Lister was one of the first to use aseptic techniques during surgeries.

The degree of acceptable microbial presence can differ based on the circumstances. Sterilization as a definition means that all life was terminated, whereas sanitization and disinfection terminates selectively and partially. Both sanitization and disinfection reduce the number of targeted pathogenic organisms to what are considered “acceptable” levels – levels that a reasonably healthy, intact body can deal with.

In general, surgical instruments and medications that enter an already aseptic part of the body (such as the bloodstream, or penetrate the skin) must be sterilized to a high sterility assurance level (SAL). Examples of such instruments include scalpels, hypodermic needles, and artificial pacemakers. For example, medical device manufacturers design their sterilization processes for an extremely low SAL. Their “one in a million” devices should be nonsterile.

This is also essential in the manufacture of parenteral pharmaceuticals. Preparation of injectable medications and intravenous solutions for fluid replacement therapy requires not only a high sterility assurance level, but also well-designed containers to prevent entry of adventitious agents after the initial product sterilization.

Food preservation is another field where the presence of microorganisms is taken under consideration. The process usually involves preventing the growth of bacteria, fungi (such as yeasts), and other microorganisms (although some methods work by introducing benign bacteria or fungi to the food).

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6.12.2: Rate of Microbial Death

LEARNING OBJECTIVES

- Describe microbial death rates

The rate of microbial death can be determined. It is important in order to develop standard protocols for disinfection which will facilitate the sterilization routine in many industries. The goal is to find out what is the minimum time needed to achieve acceptable level of sterilization for a specific purpose. The killing agent can be different (e.g., heat, chemical with certain concentration) depending on the specific application.

When the killing factor is heat, the phrase thermal death can be used. Thermal death time is a concept used to determine how long it takes to kill a specific bacteria at a specific temperature. It was originally developed for food canning and has found applications in cosmetics, and in producing salmonella-free feeds for animals (e.g. poultry, and pharmaceuticals).

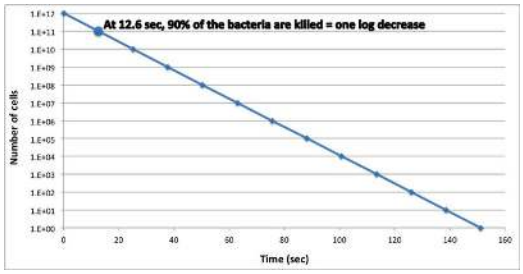


Figure: **Killing curve of C. botulinum**: This curve presents the DR value (12.6 seconds) and the 12-D reduction (151 seconds) for C. botulinum. The killing agent is heat at 121ºC.

In the food industry, it is important to reduce the amount of microbes in products to ensure proper food safety. This is usually done by thermal processing and finding ways to reduce the number of bacteria in the product. Time-temperature measurements of bacterial reduction is determined by a D-value, meaning how long it would take to reduce the bacterial population by 90% or one $\log_{10}$ at a given temperature. This D-value reference (D_R) point is 121°C.

Z or z-value is used to determine the time values with different D-values at different temperatures with its equation shown below:

$$z = T_2 - T_1 \log D_1 - \log D_2 z = T_2 - T_1 \log D_1 - \log D_2 \quad (6.12.2.1)$$

where T is temperature in °C. Such death curves can be empirically established for all bactericidal agents. This D-value is affected by pH of the product where low pH has faster D values on various foods. The D-value at an unknown temperature can be calculated knowing the D-value at a given temperature provided the Z-value is known. The target of reduction in canning is the 12-D reduction of *Clostridium botulinum*, which means that processing time will reduce the amount of this bacteria by 10^{12} bacteria per gram or milliliter. The D_R for *C. botulinum* is 12.6 seconds. A 12-D reduction will take 151 seconds.

Key Points

- When researching microbial death rate, the goal is usually to find out the minimum time needed to achieve acceptable level of sterilization for a specific purpose.
- Bacterial reduction is determined by a D-value, meaning how long it would take to reduce the bacterial population by 90% or one log10 at a given state of the killing agent.
- Microbial death curves have been developed for many agents and are used in numerous industries.

Key Terms

- **D-value**: The time needed to reduce the bacterial population by 90% or one log10 at a given temperature.
- **12-D reduction**: The time needed to reduce the amount of bacteria by 1012 bacteria per gram or milliliter.

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6.12.3: Relative Resistance of Microbes

Different microbial structures and types of microbial cells have different level of resistance to antimicrobial agents.

LEARNING OBJECTIVES

Contrast the relative resistance of microbes

KEY TAKEAWAYS

Key Points

- Endospores are considered the most resistant structure of microbes. They are resistant to most agents that would normally kill the vegetative cells they formed from.
- Mycobacterial infections are notoriously difficult to treat. Protozoa cysts are quite hard to eliminate too. Gram negative species have high levels of natural antibiotic resistance. Staphylococcus aureus is one of the major resistant human pathogens.
- Fungal cells as well as spores are more susceptible to treatments. Vegetative bacterial and yeasts cells are some of the easiest to eliminate with different treatment methods. Viruses, especially enveloped ones, are relatively easy to treat successfully with chemicals due to the presence of lipids.

Key Terms

- **horizontal gene transfer**: The transfer of genetic material from one organism to another one that is not its offspring; especially common among bacteria.
- **endospores**: An endospore is a dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum.

Different microbial structures and types of microbial cells have different level of resistance to antimicrobial agents used to eliminate them.

Endospores are considered the most resistant structure of microbes. They are resistant to most agents that would normally kill the vegetative cells from which they formed. Nearly all household cleaning products, alcohols, quaternary ammonium compounds and detergents have little effect. However, alkylating agents (e.g. ethylene oxide), and 10% bleach are effective against endospores. Endospores are able to survive boiling at 100°C for hours. Prolonged exposure to ionizing radiation, such as x-rays and gamma rays, will also kill most endospores.

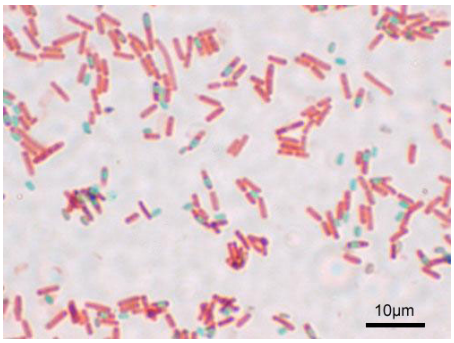


Figure: **Bacillus subtilis stained with the Schaeffer-Fulton stain.**: A stained preparation of Bacillus subtilis showing endospores as green and the vegetative cell as red.

Certain bacterial species are more resistant to treatment than others. Mycobacterial infections are notoriously difficult to treat. The organisms are hardy due to their cell wall, which is neither truly Gram negative nor positive. In addition, they are naturally resistant to a number of antibiotics that disrupt cell-wall biosynthesis, such as penicillin. Due to their unique cell wall, they can survive long exposure to acids, alkalis, detergents, oxidative bursts, lysis by complement, and many antibiotics. Most mycobacteria are susceptible to the antibiotics clarithromycin and rifamycin, but antibiotic-resistant strains have emerged.

Protozoa cysts are quite hard to eliminate too. As cysts, protozoa can survive harsh conditions, such as exposure to extreme temperatures or harmful chemicals, or long periods without access to nutrients, water, or oxygen for a period of time. Being a cyst

enables parasitic species to survive outside of a host, and allows their transmission from one host to another. Protozoa cells are also hardy to eliminate.

Gram-negative bacteria have high natural resistance to some antibiotics. Examples include *Pseudomonas* spp. which are naturally resistant to penicillin and the majority of related beta-lactam antibiotics. This ability to thrive in harsh conditions is a result of their hardy cell wall that contains porins. Their resistance to most antibiotics is attributed to efflux pumps, which pump out some antibiotics before the antibiotics are able to act.

Staphylococcus aureus is one of the major resistant pathogens. Found on the mucous membranes and the human skin of around a third of the population, it is extremely adaptable to antibiotic pressure. It was one of the earlier bacteria in which penicillin resistance was found—in 1947, just four years after the drug started being mass-produced. Methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected in Britain in 1961, and is now "quite common" in hospitals. A recent study demonstrated that the extent of horizontal gene transfer among *Staphylococcus* is much greater than previously expected—and encompasses genes with functions beyond antibiotic resistance and virulence, and beyond genes residing within the mobile genetic elements.

Fungal cells as well as spores are more susceptible to treatments. Vegetative bacterial and yeasts cells are some of the easiest to eliminate with numerous agents and methods. Viruses, especially enveloped ones, are relatively easy to treat successfully with chemicals due to the presence of lipids.

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SECTION OVERVIEW

6.13: 6. 13- Mechanisms of Microbial Control

Topic hierarchy

6.13.1: Alteration of Membrane Permeability

6.13.2: Damage to Proteins and Nucleic Acids

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6.13.1: Alteration of Membrane Permeability

LEARNING OBJECTIVES

- Illustrate the alteration of membrane permeability on microbial growth

As a phospholipid bilayer, the lipid portion of the outer membrane is impermeable to charged molecules. However, channels called porins are present in the outer membrane that allow for passive transport, across the outer membrane, of many ions, sugars, and amino acids. These molecules are present in the periplasm, the region between the cytoplasmic and outer membranes. The periplasm contains the peptidoglycan layer and also many proteins responsible for substrate binding or hydrolysis and the reception of extracellular signals. The periplasm is thought to exist as a gel-like state rather than a liquid due to the high concentration of proteins and peptidoglycan found within it. Because of its location between the cytoplasmic and outer membranes, signals received and substrates bound are available to be transported across the cytoplasmic membrane using transport and signalling proteins that are embedded there.

Antimicrobial Drugs

Examples of antimicrobial drugs that can target the microbial cell membrane to alter its functionality include polymyxin and gramicidin.

After binding to lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria, polymyxins disrupt both the outer and inner membranes. The hydrophobic tail is important in causing membrane damage, suggesting a detergent-like mode of action. Removal of the hydrophobic tail of polymyxin B yields polymyxin nonapeptide, which still binds to LPS but no longer kills the bacterial cell. However, it still increases the permeability of the bacterial cell wall to other antibiotics, indicating that it causes some degree of membrane disorganization. Gram-negative bacteria can develop resistance to polymyxins through various modifications of the LPS structure that inhibit the binding of polymyxins to LPS.

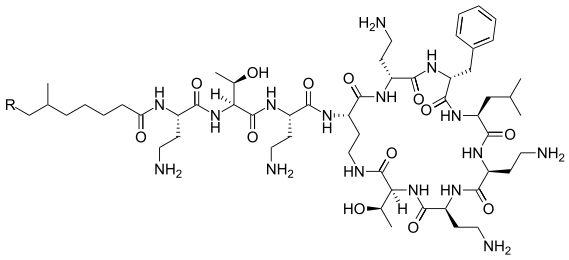


Figure: **Polymyxin B**: Polymyxin B has a hydrophobic tail that causes membrane damage.

Gramicidin is a heterogeneous mixture of six antibiotic compounds, gramicidins A, B, and C, making up 80%, 6%, and 14% respectively, all of which are obtained from the soil bacterial species *Bacillus brevis* and called collectively gramicidin D. Gramicidin D is made of linear pentadecapeptides, chains made of 15 amino acids. This is in contrast to gramicidin S, which is a cyclic peptide chain.

Gramicidin is active against gram-positive bacteria, except for gram-positive bacilli, and against selective gram-negative organisms, such as *Neisseria* bacteria. The therapeutic use of gramicidin is limited to topical application, as it induces hemolysis in lower concentrations and then bacteria cell death, so cannot be administered internally. Since the exterior epidermis is composed of dead cells, applying it to the skin's surface does not cause harm.

Gramicidin is used primarily as a topical antibiotic and is one of the three constituents of the consumer antibiotic polysporin ophthalmic solution. Gramicidin's bactericidal activity is a result of increasing the permeability of the bacterial cell membrane, allowing inorganic monovalent cations (e.g. Na+) to travel through unrestricted and thereby destroy the ion gradient between the cytoplasm and the extracellular environment.

Gramicidin D functioning as a channel was demonstrated by Hladky and Haydon, who investigated the unit conductance channel. In general, gramicidin channels are ideally selective for monovalent cations and the single-channel conductances for the alkali cations are ranked in the same order as the aqueous mobility of these ions. Divalent cations like Ca2+ block the channel by binding

near its mouth so that it is essentially impermeable to divalent cations, and also excludes anions. Cl^- in particular is excluded from the channel because its hydration shell is thermodynamically stronger than the shells of most monovalent cations. The channel is permeable to most monovalent cations, which move through the channel in single file. The channel is filled with about six water molecules, almost all of which must be displaced when an ion is transported. Thus, ions moving through the gramicidin pore carry a single file of water molecules. The flow of ion molecules and water molecules is known as flux coupling. In the presence of a second type of permeable ion, the two ions couple their flux as well. Like valinomycin and nonactin, the gramicidin channel is selective for potassium over sodium but only slightly so. It has a permeability ratio of 2.9. Though it is impermeable to anions, there are conditions under which some anion permeation may be observed. Its ability to bind and transport cations is due to the presence of cation-binding sites, one strong and the other weak, in the channel.

Key Points

- Antimicrobial drugs can target the microbial cell membrane to alter its functionality. Examples include: polymyxin and gramicidin.
- After binding to lipopolysaccharide in the outer membrane of gram-negative bacteria, polymyxins disrupt both the outer and inner membranes.
- Gramicidin's bactericidal activity is a result of increasing the permeability of the bacterial cell membrane, allowing inorganic monovalent cations (e.g. Na^+) to travel through unrestricted and thereby destroy the ion gradient between the cytoplasm and the extracellular environment.

Key Terms

- **membrane**: A flexible enclosing or separating tissue forming a plane or film and separating two environments (usually in a plant or animal).
- **antimicrobial**: An antimicrobial substance kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic).
- **permeable**: Of or relating to substance, substrate, membrane or material that absorbs or allows the passage of fluids.

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6.13.2: Damage to Proteins and Nucleic Acids

LEARNING OBJECTIVES

- Explain how microbes are controlled through DNA and protein damage

A bacteriostatic agent or bacteriostat, abbreviated Bstatic, is a biological or chemical agent that stops bacteria from reproducing, while not necessarily harming them. Depending on their application, bacteriostatic antibiotics, disinfectants, antiseptics, and preservatives can be distinguished. Upon removal of the bacteriostat, the bacteria usually start to grow again. This is in contrast to bactericides, which kill bacteria.

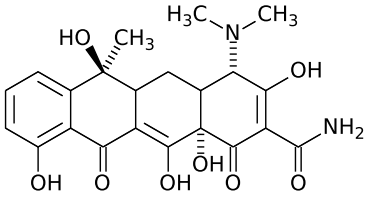


Figure: ***Tetracycline****: Chemical structure of Tetracycline ((4S,6S,12aS)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide).*

Bacteriostats are often used in plastics to prevent growth of bacteria on surfaces. Bacteriostats commonly used in laboratory work include sodium azide (which is acutely toxic) and thiomersal (which is a mutagen in mammalian cells).

Bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism. They must work together with the immune system to remove the microorganisms from the body. However, there is not always a precise distinction between them and bactericidal antibiotics. High concentrations of some bacteriostatic agents are also bactericidal, whereas low concentrations of some bacteriocidal agents are bacteriostatic.

This group of drugs include: Tetracyclines, sulfonamides, Spectinomycin, Trimethoprim, Chloramphenicol, Macrolides, and Lincosamides.

Tetracycline is a broad-spectrum polyketide antibiotic produced by the *Streptomyces* genus of Actinobacteria, indicated for use against many bacterial infections. It is a protein synthesis inhibitor. It is commonly used to treat acne today, and, more recently, rosacea, and is historically important in reducing the number of deaths from cholera. Tetracycline is marketed under the brand names Sumycin, Tetracyn, and Panmycin, among others. Actisite is a thread-like fiber formulation used in dental applications. It is also used to produce several semisynthetic derivatives, which together are known as the tetracycline antibiotics. The term “tetracycline” is also used to denote the four-ring system of this compound. “Tetracyclines” are related substances that contain the same four-ring system.

Key Points

- Bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism.
- Upon removal of the bacteriostat, the bacteria usually start to grow again.
- Bacteriostatic antibiotics must work together with the immune system to remove the microorganisms from the body.

Key Terms

- **bacteriostatic**: A drug that prevents bacterial growth and reproduction but does not necessarily kill them. When it is removed from the environment the bacteria start growing again.
- **bacteria**: A type, species, or strain of bacterium.
- **replication**: Process by which an object, person, place or idea may be copied mimicked or reproduced.

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SECTION OVERVIEW

6.14: 6. 14- Physical Antimicrobial Control

Topic hierarchy

6.14.1: Heat

6.14.2: Radiation

6.14.3: Low Temperatures

6.14.4: High Pressure

6.14.5: Desiccation

6.14.6: Osmotic Pressure

6.14.7: Filtration

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6.14.1: Heat

LEARNING OBJECTIVES

- Evaluate heat as an agent of microbrial control

Applying heat to bacterial media and utensils in research and the medical field as well as to sterilize food is one of the most common methods for control of bacterial growth. To achieve sterilization, different techniques and tools are used.

Moist Heat Sterilization

Moist heat causes destruction of micro- organisms by denaturation of macromolecules, primarily proteins. Autoclaving (pressure cooking) is a very common method for moist sterilization. It is effective in killing fungi, bacteria, spores, and viruses but does not necessarily eliminate prions. When sterilizing in this way, samples are placed into a steam chamber. The chamber is closed and heated so that steam forces air out of the vents or exhausts. Pressure is then applied so that the interior temperature reaches 121°C. This temperature is maintained for between 15 and 30 minutes. This elevated temperature and pressure is sufficient to sterilize samples of any commonly encountered microbes or spores. The chamber is then allowed to cool slowly or by passive heat dissipation.

Pressure sterilization is the prevailing method used for medical sterilization of heat-resistant tools. It is also used for sterilization of materials for microbiology and other fields calling for aseptic technique. To facilitate efficient sterilization by steam and pressure, there are several methods of verification and indication used; these include color-changing indicator tapes and biological indicators. For any method of moist heat sterilization, it is common to use biological indicators as a means of validation and confirmation. When using biological indicators, samples containing spores of heat-resistant microbes such as *Geobacillus stearothermophilis* are sterilized alongside a standard load, and are then incubated in sterile media (often contained within the sample in a glass ampoule to be broken after sterilization). A color change in the media (indicating acid production by bacteria; requires the medium to be formulated for this purpose) or the appearance of turbidity (cloudiness indicating light scattering by bacterial cells) indicates that sterilization was not achieved and the sterilization cycle may need revision or improvement. Other moist methods are boiling samples for certain period of time and Tyndallisation. Boiling is not efficient in eliminating spores. Tyndallisation inactivates spores as well, but is a more lengthy process.



Figure: ***Autoclave**: Large autoclave used for moist sterilization of media and equipment*

Dry Heat Sterilization

Dry heat destroys microorganisms by causing coagulation of proteins. The dry heat sterilization process is accomplished by conduction; that is where heat is absorbed by the exterior surface of an item and then passed inward to the next layer. Eventually, the entire item reaches the proper temperature needed to achieve sterilization. The time and temperature for dry heat sterilization is 160°C for 2 hours or 170°C for 1 hour. Instruments should be dry before sterilization since water will interfere with the process. Other heat sterilization methods include flaming and incineration. Flaming is commonly used to sterilize small equipment used to manipulate bacteria aseptically. Leaving transfer loops in the flame of a Bunsen burner or alcohol lamp until it glows red ensures that any infectious agent gets inactivated. This is commonly used for small metal or glass objects, but not for large objects (see

Incineration below). However, during the initial heating infectious material may be “sprayed” from the wire surface before it is killed, contaminating nearby surfaces and objects. Therefore, special heaters have been developed that surround the inoculating loop with a heated cage, ensuring that such sprayed material does not further contaminate the area. Another problem is that gas flames may leave residues on the object, e.g. carbon, if the object is not heated enough. A variation on flaming is to dip the object in 70% ethanol (or a higher concentration) and merely touch the object briefly to the Bunsen burner flame, but not hold it in the gas flame. The ethanol will ignite and burn off in a few seconds. 70% ethanol kills many, but not all, bacteria and viruses. It has the advantage that it leaves less residue than a gas flame. This method works well for the glass “hockey stick”-shaped bacteria spreaders. Incineration will also burn any organism to ash. It is used to sanitize medical and other bio hazardous waste before it is discarded with non-hazardous waste.

Key Points

- Different methods are used to achieve sterilization. One of the most common is applying moist heat which includes autoclaving (pressure cooking), boiling, and Tyndallisation.
- Dry heat sterilization is accomplished by conduction and is used widely for instruments.
- Other heat methods include flaming and incineration. Flaming is commonly used to sterilize small equipment used to manipulate bacteria aseptically.

Key Terms

- **sterilization**: Any process that eliminates or kills all forms of microbial life present on a surface, solution, or solid compound.
- **Tyndallisation**: Tyndallisation is the process of three successive steam treatments to achieve sterilization over the course of three days. This works by killing vegetative cells, allowing germination of surviving spores, and killing the resulting vegetative cells before they have time to form further spores.

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6.14.2: Radiation

LEARNING OBJECTIVES

- Compare non-ionizing and ionizing radiation in terms of microbe inhibition

Both non-ionizing and ionizing radiation methods are applied for sterilization.

Non-ionizing Radiation Sterilization

Ultraviolet light irradiation (UV, from a germicidal lamp) is useful only for sterilization of surfaces and some transparent objects. Many objects that are transparent to visible light such as glass, absorb UV. UV irradiation is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas. The drawback of UV radiation is that it damages some plastics, such as polystyrene foam, if they are exposed for prolonged periods of time.

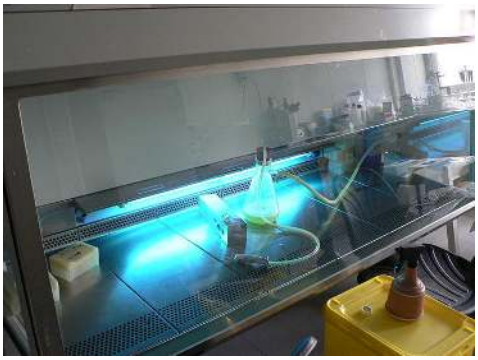


Figure: ***UV light used in the laboratory****: UV light is commonly used to irradiate and sterilize laminar flow cabinets between uses.*

Ionizing Radiation Sterilization

Ionizing radiation could be a lethal health hazard if used inappropriately. The proper use of these methods is regulated and monitored by world and national safety organizations. Any incidents that have occurred in the past are documented and thoroughly analyzed to determine root cause and improvement potential.

- **Gamma rays**: Gamma rays are very penetrating and are commonly used for sterilization of disposable medical equipment, such as syringes, needles, cannulas and IV sets, and food. The gamma radiation is emitted from a radioisotope (usually cobalt-60 or cesium-137). Cesium-137 is used in small hospital units to treat blood before transfusion to prevent **Graft-versus-host disease**. Use of a radioisotope requires shielding to ensure the safety of the operators while in use and in storage as these radioisotopes continuously emits gamma rays (cannot be turned off). An incident in Decatur, Georgia where water soluble cesium-137 leaked into the source storage pool requiring NRC intervention has led to near elimination of this radioisotope; it has been replaced by the more costly, non-water soluble cobalt-60. Sterilization by irradiation with gamma rays may, in some cases affect material properties.
- **Electron beams:** Electron beam processing is also commonly used for sterilization. Electron beams use an on-off technology and provide a much higher dosing rate than gamma or x-rays. Due to the higher dose rate, less exposure time is needed and thereby any potential degradation to polymers is reduced. A limitation is that electron beams are less penetrating than either gamma or x-rays. Facilities rely on substantial concrete shields to protect workers and the environment from radiation exposure.
- **X-rays**: High-energy X-rays are a form of ionizing energy allowing to irradiate large packages and pallet loads of medical devices. X-ray sterilization is an electricity based process that does not require chemical or radioactive material. High energy and high power X-rays are generated by an X-ray machine that can be turned off when not in use, and therefore does not require any shielding when in storage. Irradiation with X-rays or gamma rays does not make materials radioactive.
- **Subatomic particles:** Subatomic particles may be more or less penetrating, and may be generated by a radioisotope or a device, depending on the type of particle. Irradiation with particles may make materials radioactive, depending on the type of particles, their energy, and the type of target material: neutrons and very high-energy particles can make materials radioactive but have

good penetration, whereas lower energy particles (other than neutrons) cannot make materials radioactive, but have poorer penetration.

Irradiation is used by the United States Postal Service to sterilize mail in the Washington, DC area. Some foods (e.g. spices, ground meats) are irradiated for sterilization.

Key Points

- Ultraviolet light irradiation is a non-ionizing method useful only for sterilization of surfaces and some transparent objects.
- Common methods of ionizing radiation are gamma rays, electron beams, X-rays, and subatomic particles.
- However, ionizing radiation could be a lethal health hazard if used inappropriately. The proper use of these methods is regulated and monitored by world and national safety organizations.

Key Terms

- **Graft-versus-host disease**: A complication after tissue or organ transplant or blood transfusion if the blood was not irradiated. White blood cells of the transplanted tissue or organ (the graft) attack cells in the recipients body (the host).
- **NRC**: Nuclear Regulatory Commission

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6.14.3: Low Temperatures

LEARNING OBJECTIVES

- Identify how low temperatures are used for microbial control

Temperature is an important factor for microbial growth. Each species has its own optimal growth temperature at which it flourishes. Human microbial pathogens usually thrive at body temperature, 37ºC. Low temperatures usually inhibit or stop microbial growth and proliferation but often do not kill bacteria. Refrigeration (4ºC) and freezing (-20ºC or less) are commonly used in the food, pharmaceuticals and biotechnology industry.

Refrigeration preserves food by slowing down the growth and reproduction of microorganisms and the action of enzymes which cause food to rot. The introduction of commercial and domestic refrigerators drastically improved the diets of many in the 1930s by allowing foods such as fresh fruit, salads and dairy products to be stored safely for longer periods, particularly during warm weather. It also facilitated transportation of fresh food on long distances.



Figure: **The Dunedin**: This ship executed the first completely successful refrigerated shipment of food.

Refrigeration is also used to facilitate the preservation of liquid medicines or other substances used for research where microbial growth is undesirable, often combined with added preservatives. Fridge temperatures inhibit the proliferation of bacteria better than molds and fungi.

For longer periods of preservation, freezing temperatures are preferred to refrigeration. Since early times, farmers, fishermen, and trappers have preserved their game and produce in unheated buildings during the winter season. Freezing food slows down decomposition by turning residual moisture into ice, inhibiting the growth of most bacterial species.

Freezing temperatures curb the spoiling effect of microorganisms in food, but can also preserve some pathogens unharmed for long periods of time. While it kills some microorganisms by physical trauma, others are sublethally injured by freezing, and may recover to become infectious.

Frozen products do not require any added preservatives because microorganisms do not grow when the temperature of the food is below -9.5°C, which is sufficient in itself to prevent food spoilage. Long-term preservation of food may call for food storage at even lower temperatures.

Key Points

- Refrigeration (4ºC) and freezing (-20ºC or less) are commonly used in food, pharmaceutical and biotechnology industries.
- Refrigeration preserves food by slowing down the growth and reproduction of microorganisms as well as the action of enzymes which cause food to rot.
- Freezing food slows down decomposition by turning residual moisture into ice, inhibiting the growth of most bacterial species. Freezing kills some microorganisms by physical trauma, while sublethally injuring others which may recover to become infectious.

Key Terms

- **proliferation**: The process by which an organism produces others of its kind; breeding, propagation, procreation, reproduction.

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6.14.4: High Pressure

Under very high hydrostatic pressure(HHP) of up to 700 MPa, water inactivates pathogens such as *E. coli and Salmonella*.

LEARNING OBJECTIVES

Explain high pressure as a antimicrobial control

KEY TAKEAWAYS

Key Points

- High pressure processing (HPP), pascalization or bridgmanization, is a method of preserving and sterilizing food, in which a product is processed under very high pressure, leading to the inactivation of certain microorganisms and enzymes in the food.
- The frist reports showed that bacterial spores were not always inactivated by pressure, while vegetative bacteria were usually killed. Later it was discovered that using moderate pressures was more effective in eliminating spores than using higher pressures.
- Experiments were also performed with anthrax, typhoid, and tuberculosis, which was a potential health risk for the researchers.

Key Terms

- **bridgmanization**: Pascalization is also known as bridgmanization, named for physicist Percy Williams Bridgman.

Under very high hydrostatic pressure of up to 700 MPa (100,000 psi), water inactivates pathogens such as *Listeria*, *E. coli and Salmonella*.

High pressure processing (HPP), pascalization or bridgmanization, is a method of preserving and sterilizing food, in which a product is processed under very high pressure, leading to the inactivation of certain microorganisms and enzymes in the food. The technique was named after Blaise Pascal, whose work included detailing the effects of pressure on fluids. Pascalization is preferred over heat treatment in the food industry as it eliminates changes in the quality of foods due to thermal degradation, resulting in fresher taste, texture, appearance and nutrition. Processing conveniently takes place at ambient or refrigeration temperatures.

Experiments into the effects of pressure on microorganisms were first recorded in the late nineteenth century. The frist reports showed that bacterial spores were not always inactivated by pressure, while vegetative bacteria were usually killed. Around 1970, researchers renewed their efforts in studying bacterial spores after it was discovered that using moderate pressures was more effective than using higher pressures. These spores, which caused a lack of preservation in the earlier experiments, were inactivated faster by moderate pressure, but in a manner different from what occurred with vegetative microbes. When subjected to moderate pressures, bacterial spores germinate, and the resulting spores are easily killed using pressure, heat, or ionizing radiation.

Research into the effects of high pressures on microorganisms was largely focused on deep-sea organisms until the 1980s, when advancements in ceramic processing were made. This resulted in the production of machinery that allowed for processing foods at high pressures at a large scale, and generated some interest in the technique, especially in Japan. Although commercial products preserved by pascalization first emerged in 1990, the technology behind pascalization is still being perfected for widespread use.



Figure: **Japanese miso soup**: Miso soup is sterilized with high pressure.

In pascalization, food products are sealed and placed into a steel compartment containing a liquid, often water, and pumps are used to create pressure. The pumps may apply pressure constantly or intermittently. During pascalization, more than 50,000 pounds per square inch (340 MPa) may be applied for around fifteen minutes, leading to the inactivation of yeast, mold, and bacteria. In the process, the food's proteins are denatured, hydrogen bonds are fortified, and noncovalent bonds in the food are disrupted, while the product's main structure remains intact. Because pascalization is not heat-based, covalent bonds are not affected, causing no change in the food's taste.

Experiments were also performed with anthrax, typhoid, and tuberculosis, which was a potential health risk for the researchers. Indeed, before the process was improved, one employee of the Experimental Station became ill with typhoid fever.

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6.14.5: Desiccation

Desiccation is the state of extreme dryness, or the process of extreme drying and can be used to control microbial growth.

LEARNING OBJECTIVES

Show how desiccation inhibits microbes

KEY TAKEAWAYS

Key Points

- Microorganisms cannot grow and divide when desiccated, but can survive for certain periods of time, depending on their features. After the addition of water, the bacteria will start growing again, so desiccation does not provide complete sterilization.
- Pharmaceutical companies often use freeze-drying as a desiccation tool to increase the shelf life of products, such as vaccines and other injectables. Drying is also a method for food preservation.
- Freeze-drying is performed using special equipment.

Key Terms

- **desiccation**: the state of drying
- **freeze-drying**: Freeze-drying, also known as lyophilisation, lyophilization, or cryodesiccation, is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport.

Desiccation is the state of extreme dryness, or the process of extreme drying. In biology and ecology, desiccation refers to the drying out of a living organism. Microorganisms cannot grow and divide when desiccated, but can survive for certain periods of time, depending on their features. After the addition of water, the bacteria will start growing again, so desiccation does not provide complete sterilization.



Figure: **SMART Freeze Dryer**: New freeze dryer which is equipped with systems for immediate feedback on the properties of the dried product, eliminating the lengthy trial-and-error approach.

Some bacteria, such as *Deinococcus radiodurans* and *Mycobacterium* , are extremely resistant to damage from prolonged desiccation while others, such as *Neisseria gonorrhoeae*, can survive only short periods of desiccation.

Pharmaceutical companies often use freeze-drying as a desiccation tool to increase the shelf life of products, such as vaccines and other injectables. By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form. Preservation is possible because the greatly reduced water content inhibits the

action of microorganisms and enzymes that would normally spoil or degrade the substance. Another example from the pharmaceutical industry is the use of freeze-drying to produce tablets or wafers.

Drying is also a method for food preservation that works by removing water from the food, which inhibits the growth of microorganisms. Open air drying using sun and wind has been practiced since ancient times to preserve food. A solar or electric food dehydrator can greatly speed the drying process and ensure more consistent results. Water is usually removed by evaporation (air drying, sun drying, smoking, or wind drying) but, in the case of freeze-drying, food is first frozen and then the water is removed by sublimation. Bacteria, yeasts, and molds need the water in the food to grow, and drying effectively prevents them from surviving in food.

Freeze-drying is performed using special equipment. Two components are common to all types of freeze-dryers: a vacuum pump to reduce the ambient gas pressure in a vessel containing the substance to be dried, and a condenser to remove the moisture by condensation on a surface cooled to −40º to −80ºC.

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6.14.6: Osmotic Pressure

Osmotic pressure is the pressure which must be applied to a solution to prevent the inward flow of water across a semipermeable membrane.

LEARNING OBJECTIVES

Interpret osmotic pressure as a means of microbial control

KEY TAKEAWAYS

Key Points

- Osmotic pressure is of vital importance in biology as the cell's membrane is selective toward many of the solutes found in living organisms.
- When a cell is placed in a hypertonic solution, water actually flows out of the cell into the surrounding solution thereby causing the cells to shrink and lose its turgidity. Hypertonic solutions are used for antimicrobial control.
- Salt and sugar are used to create hypertonic environment for microorganisms and are commonly used as food preservatives.

Key Terms

- **turgidity**: Turgidity (turgor pressure) pushes the plasma membrane against the cell wall of plant, bacteria, and fungi cells as well as those protiat cells which have cell walls.

Osmotic pressure is the pressure which needs to be applied to a solution to prevent the inward flow of water across a semipermeable membrane. It is also defined as the minimum pressure needed to nullify osmosis.The phenomenon of osmotic pressure arises from the tendency of a pure solvent to move through a semi-permeable membrane and into a solution containing a solute to which the membrane is impermeable. This process is of vital importance in biology as the cell's membrane is selective toward many of the solutes found in living organisms.



Figure: **Salami**: The original meaning of the word is: all kind of salted (meats).

Osmosis causes water to flow from an area of low solute concentration to an area of high solute concentration until the two areas have an equal ratio of solute to water. Normally, the solute diffuses toward equilibrium as well; however, all cells are surrounded by a lipid bilayer cell membrane which permits the flow of water in and out of the cell but restricts the flow of solute under many circumstances. As a result, when a cell is placed in a hypotonic solution, water rushes into the membrane, increasing its volume. Eventually, the cell's membrane is enlarged such that it pushes against the cell's rigid wall. In an isotonic solution, water flows into the cell at the same rate it flows out. When a cell is placed in a hypertonic solution, water actually flows out of the cell into the surrounding solution causing the cells to shrink and lose its turgidity. Two of the most common substances used to create hypertonic environment for microorganisms and prevent them from growing are salt and sugar. They are widely applied in food preservation.

Table salt (sodium chloride) is the primary ingredient used in meat curing. Removal of water and addition of salt to meat creates a solute-rich environment where osmotic pressure draws water out of microorganisms, thereby retarding their growth. Doing this requires a concentration of salt of nearly 20%.

Sugar is used to preserve fruits, either in syrup with fruit such as apples, pears, peaches, apricots, plums or in crystallized form where the preserved material is cooked in sugar to the point of crystallisation and the resultant product is then stored dry. The purpose of sugaring is to create an environment hostile to microbial life and prevent food spoilage. From time to time, sugaring has also been used for non-food preservation. For example, honey was used as part of the mummification process in some ancient Egyptian rites. However, the growth of molds and fungi is not suppressed as efficiently as the growth of bacteria.

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6.14.7: Filtration

Fluids that would be damaged by heat, irradiation, or chemical sterilization can be sterilized by microfiltration using membrane filters.

LEARNING OBJECTIVES

Demonstrate microbial control using filtration

KEY TAKEAWAYS

Key Points

- A typical microfiltration membrane pore size range is 0.1-10 µm, with the most commonly used being 0.2 µm and 0.45 µm, which is sufficient to eliminate bacteria and fungi.
- Quite often, when biological samples are processed, viruses must be removed or inactivated. Nanofilters with smaller pore sizes of 20-50 nm (nanofiltration) are used.
- Filtration is commonly used for heat labile pharmaceuticals and protein solutions in processing medicines. It is also increasingly used in the treatment of drinking water.

Key Terms

- **ester**: An ester is a chemical compound consisting of a carbonyl group adjacent to an ether linkage.
- **polyethersulfone**: Thermoplastic polymers that have low protein retention. They contain the subunit aryl-SO2-aryl, the defining feature of which is the sulfone group.

Fluids that would be damaged by heat (such as fluids containing proteins like large molecule drug products, but also wine and beer), irradiation, or chemical sterilization can only be sterilized by microfiltration using membrane filters. This method is commonly used for heat labile pharmaceuticals and for protein solutions in processing medicines.

The typical microfiltration membrane pore size range is 0.1-10 µm, with the most commonly used being 0.2 µm; and 0.45 µm is sufficient to eliminate bacteria and fungi.



Figure: **Syringe Filter**: A syringe filter with a pore size of 0.22 micrometers, small enough to capture and retain bacterial and fungal cells.

Microfiltration is increasingly used in drinking water treatment. It effectively removes major pathogens and contaminants such as *Giardia lamblia* cysts, *Cryptosporidium* oocysts, and large bacteria. For this application, the filter has to be rated for 0.2 µm or smaller pore size.

Quite often when biological samples are processed, viruses must be removed or inactivated. Nanofilters with smaller pore sizes of 20-50 nm (nanofiltration) are used. The smaller the pore size, the lower the flow rate. To achieve higher total throughput or avoid premature blockage, pre-filters might be used to protect small pore membrane filters. Some studies have shown that prions can be removed or reduced by filtration.

Membrane filters used in production processes are commonly made from materials such as mixed cellulose ester or polyethersulfone. The filtration equipment and the filters may be purchased as pre-sterilized disposable units in sealed packaging, or must be sterilized by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes. To ensure proper functioning of the filter, the membrane filters are integrity tested post-use or sometimes pre-use. A non-destructive

integrity test assures the filter is undamaged, and is also a regulatory requirement enforced by agencies like the Food and Drug Administration, the European Medicines Agency, and others.

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SECTION OVERVIEW

6.15: 6. 15- Chemical Antimicrobial Control

Topic hierarchy

6.15.1: Effective Disinfection

6.15.2: Factors that Affect Germicidal Activity of Chemicals

6.15.3: Types of Disinfectants

6.15.4: Biological Control of Microbes

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6.15.1: Effective Disinfection

A perfect disinfectant would offer full microbiological sterilisation, without harming humans and would also be non-corrosive.

LEARNING OBJECTIVES

Describe the rationale for selecting a disinfectant and how their effectiveness is rated

KEY TAKEAWAYS

Key Points

- Most modern household disinfectants contain Bitrex, an exceptionally bitter substance added to discourage ingestion, as a safety measure.
- The choice of disinfectant to be used depends on the particular situation.
- One way to compare disinfectants is to compare how well they do against a known disinfectant, such as phenol, and rate them accordingly.

Key Terms

- **non-corrosive**: That does not cause corrosion.
- **disinfectant**: A substance which kills germs and/or viruses.
- **sterilisation**: Sterilization (or sterilisation) is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media.

A perfect disinfectant would also offer complete and full microbiological sterilisation, without harming humans and useful forms of life. It would also be inexpensive and non-corrosive. Most disinfectants, however, are by nature, potentially harmful (even toxic) to humans or animals. Most modern household disinfectants contain Bitrex, an exceptionally bitter substance added to discourage ingestion, as a safety measure. Those that are used indoors should never be mixed with other cleaning products, or else chemical reactions can occur.

The choice of disinfectant depends on the particular situation. Some disinfectants have a wide spectrum and kill many different types of microorganisms, while others kill a smaller range of disease-causing organisms but are preferred for other instances (they may be non-corrosive, non-toxic, or inexpensive).

There are arguments for creating or maintaining conditions that are not conducive to bacterial survival and multiplication, rather than attempting to kill them with chemicals. Bacteria can increase in number very quickly, which enables them to evolve rapidly. Should some bacteria survive a chemical attack, they give rise to new generations composed completely of bacteria that are resistant to the particular chemical used. Under a sustained chemical attack, the surviving bacteria in successive generations are increasingly resistant to the chemical used, and ultimately the chemical is rendered ineffective. For this reason, some question the wisdom of impregnating cloths, cutting boards, and worktops in the home with bactericidal chemicals.



Figure: **Disinfection**: Disinfection of a floor using disinfectant liquid applied using a mop

One way to compare disinfectants is to compare how well they do against a known disinfectant and rate them accordingly. Phenol is the standard disinfectant, and the corresponding rating system is called the “phenol coefficient. ” The disinfectant to be tested is

compared with phenol on a standard microbe (usually Salmonella typhi or Staphylococcus aureus). Disinfectants that are more effective than phenol have a coefficient > 1. Those that are less effective have a coefficient < 1.

A less specific measurement of effectiveness is the United States Environmental Protection Agency's (EPA) classification into either high, intermediate, or low level of disinfection. High-level disinfection kills all organisms, except high levels of bacterial spores, and is effected with a chemical germicide cleared for marketing as a sterilant by the U.S. Food and Drug Administration (FDA). Intermediate-level disinfection kills mycobacteria, most viruses, and bacteria with a chemical germicide registered as a "tuberculocide" by the EPA. Low-level disinfection kills some viruses and bacteria with a chemical germicide registered as a hospital disinfectant by the EPA.

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6.15.2: Factors that Affect Germicidal Activity of Chemicals

Some antiseptics are germicides, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and prevent their growth.

LEARNING OBJECTIVES

Describe how antiseptics can be bacteriocidal or bacteriostatic

KEY TAKEAWAYS

Key Points

- Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction.
- Antibacterials are antiseptics that have the proven ability to act against bacteria.
- Antiseptics are generally distinguished from antibiotics by the latter's ability to be transported through the lymphatic system to destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non-living objects.

Key Terms

- **germicides**: Some antiseptics are true germicides, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and only prevent or inhibit their growth.
- **microbes**: A microorganism or microbe is a microscopic organism that comprises either a single cell (unicellular), cell clusters, or multicellular relatively complex organisms.
- **antimicrobial**: An agent that destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action.

Antiseptics are antimicrobial substances that are applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are generally distinguished from antibiotics by the latter's ability to be transported through the lymphatic system to destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non-living objects.

Some antiseptics are true germicides, capable of destroying microbes (bacteriocidal), while others are bacteriostatic and only prevent or inhibit their growth.

Antibacterials are antiseptics that have the proven ability to act against bacteria. Microbicides that destroy virus particles are called viricides or antivirals.

Most commonly used are ethanol (60–90%), 1-propanol (60–70%), and 2-propanol/isopropanol (70–80%) or mixtures of these alcohols. They are commonly referred to as "surgical alcohol. " They are used to disinfect the skin before injections are given, often along with iodine (tincture of iodine) or some cationic surfactants (benzalkonium chloride 0.05–0.5%, chlorhexidine 0.2–4.0%, or octenidine dihydrochloride 0.1–2.0%).



Figure: **A bottle of ethanol (95%) – an antiseptic**: A bottle of ethanol (95%) – an antiseptic

Quaternary ammonium compounds include the chemicals benzalkonium chloride (BAC), cetyl trimethylammonium bromide (CTMB), cetylpyridinium chloride (Cetrim, CPC), and benzethonium chloride (BZT). Benzalkonium chloride is used in some pre-

operative skin disinfectants (conc. 0.05–0.5%) and antiseptic towels. The antimicrobial activity of Quats is inactivated by anionic surfactants, such as soaps. Related disinfectants include chlorhexidine and octenidine.

Boric acid: Used in suppositories to treat yeast infections of the vagina, in eyewashes, and as an antiviral to shorten the duration of cold sore attacks. Put into creams for burns. Also common in trace amounts in eye contact solution. A triarylmethane dye still widely used as 1% ethanol solution in Eastern Europe and ex-USSR countries for treatment of small wounds and abscesses. Efficient against gram-positive bacteria.

Chlorhexidine Gluconate: A biguanidine derivative, used in concentrations of 0.5–4.0% alone or in lower concentrations in combination with other compounds, such as alcohols. Used as a skin antiseptic and to treat inflammation of the gums (gingivitis). The microbicidal action is somewhat slow, but remanent. It is a cationic surfactant, similar to Quats.

Hydrogen peroxide: Used as a 6% (20 Vols) solution to clean and deodorize wounds and ulcers. More common 3% solutions of hydrogen peroxide have been used in household first aid for scrapes, etc. However, even this less potent form is no longer recommended for typical wound care because the strong oxidization causes scar formation and increases healing time. Gentle washing with mild soap and water or rinsing a scrape with sterile saline is a better practice.

Novel iodine antiseptics containing povidone-iodine (an iodophor, complex of povidone, a water-soluble polymer, with triiodide anions I3-, containing about 10% of active iodine) are far better tolerated, don't negatively affect wound healing, and leave a deposit of active iodine, thereby creating the so-called "remnant," or persistent, effect. The great advantage of iodine antiseptics is their wide scope of antimicrobial activity, killing all principal pathogens and, given enough time, even spores, which are considered to be the most difficult form of microorganisms to be inactivated by disinfectants and antiseptics.

Mercurochrome: Not recognized as safe and effective by the U.S. Food and Drug Administration (FDA) due to concerns about its mercury content. Other obsolete organomercury antiseptics include bis-(phenylmercuric) monohydrogenborate (Famosept).

Manuka Honey: Recognized by the U.S. Food and Drug Administration (FDA) as a medical device for use in wounds and burns. Active +15 is equal to a 15% solution of phenol.

Octenidine dihydrochloride: A cationic surfactant and bis-(dihydropyridinyl)-decane derivative, used in concentrations of 0.1–2.0%. It is similar in its action to the Quats, but is of somewhat broader spectrum of activity. Octenidine is currently increasingly used in continental Europe as a QAC's and chlorhexidine (with respect to its slow action and concerns about the carcinogenic impurity 4-chloroaniline) substitute in water- or alcohol-based skin, mucosa, and wound antiseptic. In aqueous formulations, it is often potentiated with addition of 2-phenoxyethanol.

Phenol is germicidal in strong solution, inhibitory in weaker ones. Used as a "scrub" for pre-operative hand cleansing. Used in the form of a powder as an antiseptic baby powder, where it is dusted onto the navel as it heals. Also used in mouthwashes and throat lozenges, where it has a painkilling effect as well as an antiseptic one. Example: TCP. Other phenolic antiseptics include historically important, but today rarely used (sometimes in dental surgery) thymol, today obsolete hexachlorophene, still used triclosan and sodium 3,5-dibromo-4-hydroxybenzenesulfonate (Dibromol).

Antimicrobial compound suitable for clinical use in critically colonized or infected acute and chronic wounds. The physicochemical action on the bacterial envelope prevents or impedes the development of resistant bacterial strains.

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6.15.3: Types of Disinfectants

There are multiple types of disinfectants, including but not limited to air disinfectants, alcohols, and oxidizing agents.

LEARNING OBJECTIVES

List the types of disinfectants available

KEY TAKEAWAYS

Key Points

- Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air.
- Alcohols, usually ethanol or isopropanol, are sometimes used as a disinfectant, but more often as an antiseptic.
- Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death.

Key Terms

- **disinfectants**: Disinfectants are substances that are applied to non-living objects to destroy microorganisms that are living on the objects. Disinfectants are substances that are applied to non-living objects to destroy microorganisms that are living on the objects.
- **microorganisms**: A microorganism or microbe is a microscopic organism that comprises either a single cell (unicellular), cell clusters, or multicellular relatively complex organisms.
- **antiseptic**: Any substance that inhibits the growth and reproduction of microorganisms. Generally includes only those that are used on living objects (as opposed to disinfectants) and aren't transported by the lymphatic system to destroy bacteria in the body (as opposed to antibiotics).

Types of disinfectants include: Air disinfectants, Alcohols, Aldehydes, Oxidizing agents, Phenolics, Quaternary ammonium compounds, Silver, and Copper alloy surfaces.

Air Disinfectants

Air disinfectants are typically chemical substances capable of disinfecting microorganisms suspended in the air. Disinfectants are often assumed to be limited to use on surfaces, but that is not the case. In 1928, a study found that airborne microorganisms could be killed using mists of dilute bleach. An air disinfectant must be dispersed either as an aerosol or vapor at a sufficient concentration in the air to cause the number of viable infectious microorganisms to be significantly reduced.

In the 1940s and early 1950s, further studies showed inactivation of diverse bacteria, influenza virus, and *Penicillium chrysogenum* (previously *P. notatum*) mold fungus using various glycols, principally propylene glycol and triethylene glycol. In principle, these chemical substances are ideal air disinfectants because they have both high lethality to microorganisms and low mammalian toxicity.

Although glycols are effective air disinfectants in controlled laboratory environments, it is more difficult to use them effectively in real-world environments because the disinfection of air is sensitive to continuous action. Continuous action in real-world environments with outside air exchanges at door, HVAC, and window interfaces, and in the presence of materials that adsorb and remove glycols from the air, poses engineering challenges that are not critical for surface disinfection. The engineering challenges associated with creating a sufficient concentration of the glycol vapors in the air have not to date been sufficiently addressed.

Alcohol Disinfectants

Alcohols, usually ethanol or isopropanol, are sometimes used as a disinfectant, but more often as an antiseptic, the distinction being that alcohol tends to be used on living tissue rather than nonliving surfaces. These alcohols are non-corrosive but can be a fire hazard. They also have limited residual activity due to evaporation, which results in brief contact times unless the surface is submerged. They also have a limited activity in the presence of organic material.

Alcohols are most effective when combined with purified water to facilitate diffusion through the cell membrane; 100% alcohol typically denatures only external membrane proteins. A mixture of 70% ethanol or isopropanol diluted in water is effective against a wide spectrum of bacteria, though higher concentrations are often needed to disinfect wet surfaces. Additionally, high-concentration mixtures (such as 80% ethanol + 5% isopropanol) are required to effectively inactivate lipid-enveloped viruses (such as HIV, hepatitis B, and hepatitis C). Alcohol is only partly effective against most non-enveloped viruses (such as hepatitis A), and is not at all effective against fungal and bacterial spores.

The efficacy of alcohol is enhanced when in solution with the wetting agent dodecanoic acid (coconut soap). The synergistic effect of 29.4% ethanol with dodecanoic acid is effective against a broad spectrum of bacteria, fungi, and viruses. Further testing is being performed against *Clostridium difficile* (*C. Diff*) spores using higher concentrations of ethanol and dodecanoic acid, which has been indicated to be effective with a contact time of ten minutes.

Aldehydes, such as formaldehyde and glutaraldehyde, have a wide microbiocidal activity and are sporocidal and fungicidal. They are partly inactivated by organic matter and have slight residual activity. Some bacteria have developed resistance to glutaraldehyde; it has also been found that glutaraldehyde can cause asthma and other health hazards, hence ortho-phthalaldehyde is replacing glutaraldehyde.

Oxidizing Disinfectants

Oxidizing agents act by oxidizing the cell membrane of microorganisms, which results in a loss of structure and leads to cell lysis and death. A large number of disinfectants operate in this way. Chlorine and oxygen are strong oxidizers, so their compounds figure heavily here.

Phenolics are active ingredients in some household disinfectants. They are also found in some mouthwashes and in disinfectant soap and handwashes.

Quaternary ammonium compounds (quats), such as benzalkonium chloride, are a large group of related compounds. Some concentrated formulations have been shown to be effective low-level disinfectants. Typically, quats do not exhibit effectiveness against difficult to kill non-enveloped viruses such as norovirus, rotavirus, or polio virus.

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6.15.4: Biological Control of Microbes

LEARNING OBJECTIVES

- Describe the types of antimicrobial agents available for controlling the growth of microbes

A wide variety of chemicals called antimicrobial agents are available for controlling the growth of microbes. For example:

1. **Chemotherapeutic agents**, including antibiotics, are administered into the infected body.
2. **Disinfectants** are chemical agents used on inanimate objects to lower the level of microbes present on the object. These are not capable of sterilizing, typically because they fail to kill endospores, some viruses, and organisms such as *Mycobacteriumtuberculosis*.
3. **Antiseptics** are chemicals used on living tissue to decrease the number of microbes present in that tissue.

Disinfectants and antiseptics affect bacteria in many ways. Those that result in bacterial death are called bactericidal agents. Those causing temporary inhibition of growth are bacteriostatic agents. No single antimicrobial agent is most effective for use in all situations – different situations may call for different agents. A number of factors affect selection of the best agent for any given situation – Antimicrobial agents must be selected with specific organisms and environmental conditions in mind. Additional variables to consider in the selection of an antimicrobial agent include pH, solubility, toxicity, organic material present, and cost.

Once an agent has been selected, it is important to evaluate it's effectiveness. In evaluating the effectiveness of antimicrobial agents, the concentration, length of contact, and whether it is lethal (-cidal) or inhibiting (-static) at that concentration of exposure are the important criteria.



Figure: **Maggot therapy**: a wound cleaned by maggots (fly larvae).

Prior to the advent of antibiotics, live organisms were used directly in attempts to control microbial infections. Examples of such biological control included bacteriotherapy, bacteriophage therapy, malaria therapy, probiotics, and the use of living maggots. In all cases the organisms themselves rather than a product of their metabolism were used as the potentially curative agent. The biological control of human infections was largely restricted to the treatment of surface infections of the skin and mucous membrane. Additionally, attempts were made to alter the microflora of the human intestinal tract to favor the growth of benign or beneficial bacteria or yeasts. Modern studies suggest that the use of biological control in the treatment of human infections should be re-evaluated in the light of the increasing world-wide occurrence of antibiotic-resistant bacteria, and the opportunities provided by recent developments in gene technology.

Key Points

- Most of the examples of biologic control of microbes predate the sulphonamides and penicillin.
- Maggot therapy, although repellent by modern standards, proved to be surprisingly effective.
- Today, a wide variety of chemicals called antimicrobial agents are available for controlling the growth of microbes. These include chemotherapeutic agents, disinfectants, and antiseptics.

Key Terms

- **probiotics**: live microorganisms that may confer a health benefit on the host.
- **antibiotics**: agents that inhibit bacterial growth or kill bacteria.

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CHAPTER OVERVIEW

7: Antimicrobial Drugs

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi

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SECTION OVERVIEW

7.1: Overview of Antimicrobial Therapy

Topic hierarchy

7.1.1: Origins of Antimicrobial Drugs

7.1.2: Antibiotic Discovery

7.1.3: Antibiotics and Selective Toxicity

7.1.4: Spectrum of Antimicrobial Activity

7.1.5: Antibiotic Classifications

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7.1.1: Origins of Antimicrobial Drugs

The era of antimicrobials begins when Pasteur and Joubert discover that one type of bacteria could prevent the growth of another.

Learning Objectives

- Recall the technical defintion of antibiotics

Key Points

- Antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism.
- In today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection.
- The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world.

Key Terms

- **antimicrobial**: An agent that destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action.
- **penicillin**: Any of a group of broad-spectrum antibiotics obtained from Penicillium molds or synthesized; they have a beta-lactam structure; most are active against gram-positive bacteria and used in the treatment of various infections and diseases.

The history of antimicrobials begins with the observations of Pasteur and Koch, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism.

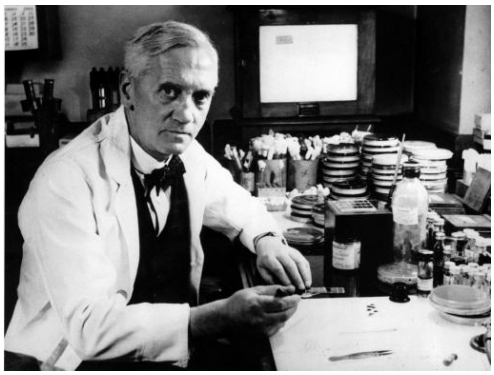


Figure: **Alexander Fleming**: In 1928 Alexander Fleming observed antibiosis against bacteria by a fungus of the genus Penicillium and postulated the effect was mediated by an antibacterial compound, penicillin, and that its antibacterial properties could be exploited for chemotherapy.

The discovery of antimicrobials like penicillin by Alexander Fleming and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

The term antibiotic was first used in 1942 by Selman Waksman and his collaborators in journal articles to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. This definition excluded substances that kill bacteria, but are not produced by microorganisms (such as gastric juices and hydrogen peroxide). It also excluded synthetic antibacterial compounds such as the sulfonamides. Many antibacterial compounds are relatively small molecules with a molecular weight of less than 2000 atomic mass units. With advances in medicinal chemistry, most of today's antibacterials chemically are semisynthetic modifications of various natural compounds.

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7.1.2: Antibiotic Discovery

Observations of antibiosis between micro-organisms led to the discovery of natural antibacterials produced by microorganisms.

Learning Objectives

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

Key Points

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

Key Terms

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

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

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



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

John Tyndall first described antagonistic activities by fungi against bacteria in England in 1875. Synthetic antibiotic chemotherapy as a science and development of antibacterials began in Germany with Paul Ehrlich in the late 1880s. Ehrlich noted certain dyes would color human, animal, or bacterial cells, while others did not. He then proposed the idea that it might be possible to create chemicals that would act as a selective drug that would bind to and kill bacteria without harming the human host. After screening hundreds of dyes against various organisms, he discovered a medicinally useful drug, the synthetic antibacterial Salvarsan now

called arsphenamine. In 1895, Vincenzo Tiberio, physician of the University of Naples discovered that a mold (Penicillium) in a water well has an antibacterial action. After this initial chemotherapeutic compound proved effective, others pursued similar lines of inquiry, but it was not until in 1928 that Alexander Fleming observed antibiosis against bacteria by a fungus of the genus Penicillium. Fleming postulated the effect was mediated by an antibacterial compound named penicillin, and that its antibacterial properties could be exploited for chemotherapy. He initially characterized some of its biological properties, but he did not pursue its further development.

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7.1.3: Antibiotics and Selective Toxicity

Antibiotics are able to selectively target specific types of bacteria without harming the infected host.

Learning Objectives

- Describe selective toxicity

Key Points

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

Key Terms

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

Selective Toxicity in Antibiotics

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

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



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

Some antibacterials have been associated with a range of adverse effects. Side-effects range from mild to very serious depending on the antibiotics used, the microbial organisms targeted, and the individual patient. Safety profiles of newer drugs are often not as

well established as for those that have a long history of use. Adverse effects range from fever and nausea to major allergic reactions, including photodermatitis and anaphylaxis. Common side-effects include diarrhea, resulting from disruption of the species composition in the intestinal flora, resulting, for example, in overgrowth of pathogenic bacteria, such as *Clostridium difficile*. Antibacterials can also affect the vaginal flora, and may lead to overgrowth of yeast species of the genus *Candida* in the vulvo-vaginal area. Additional side-effects can result from interaction with other drugs, such as elevated risk of tendon damage from administration of a quinolone antibiotic with a systemic corticosteroid.

Antibacterial Production

Despite the wide variety of known antibiotics, less than 1% of antimicrobial agents have medical or commercial value. For example, whereas penicillin has a high therapeutic index as it does not generally affect human cells, this is not so for many antibiotics. Other antibiotics simply lack advantage over those already in use, or have no other practical applications. Useful antibiotics are often discovered using a screening process. To conduct such a screen, isolates of many different microorganisms are cultured and then tested for production of diffusible products that inhibit the growth of test organisms. Most antibiotics identified in such a screen are already known and must therefore be disregarded. The remainder must be tested for their selective toxicities and therapeutic activities, and the best candidates can be examined and possibly modified. A more modern version of this approach is a rational design program. This involves screening directed towards finding new natural products that inhibit a specific target, such as an enzyme only found in the target pathogen, rather than tests to show general inhibition of a culture.

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7.1.4: Spectrum of Antimicrobial Activity

An antibiotic's spectrum can be broad or narrow.

Learning Objectives

- Compare narrow and broad spectrum antibiotics

Key Points

- Broad spectrum antibiotics act against a larger group of bacteria.
- Narrow spectrum antibiotis target specific bacteria such as Gram positive or Gram negative.
- Three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).

Key Terms

- **Gram stain**: A method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative).
- **narrow spectrum antibiotic**: A type of antibiotic that targets specific types of Gram positive or Gram negative bacteria.
- **broad spectrum antibiotic**: A type of antibiotic that can affect a wide range of bacteria.

The range of bacteria that an antibiotic affects can be divided into narrow spectrum and broad spectrum. Narrow spectrum antibiotics act against a limited group of bacteria, either gram positive or gram negative, for example sodium fusidate only acts against staphylococcal bacteria. Broad spectrum—antibiotics act against gram positive and gram negative bacteria, for example amoxicillin.

Gram staining (or Gram's method; is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram positive bacteria. A Gram positive results in a purple/blue color while a Gram negative results in a pink/red color. The Gram stain is almost always the first step in the identification of a bacterial organism, and is the default stain performed by laboratories over a sample when no specific culture is referred. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique, thus forming Gram-variable and Gram-indeterminate groups as well.

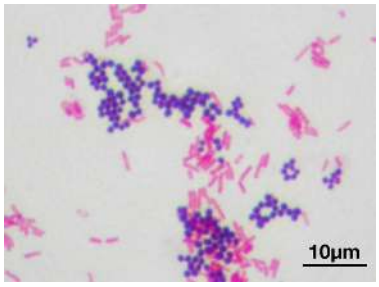


Figure: **Gram Stain**: This is a microscopic image of a Gram stain of mixed Gram-positive cocci (*Staphylococcus aureus*, purple) and Gram-negative bacilli (*Escherichia coli*, red).

A broad spectrum antibiotic acts against both Gram-positive and Gram-negative bacteria, in contrast to a narrow spectrum antibiotic, which is effective against specific families of bacteria. An example of a commonly used broad-spectrum antibiotic is ampicillin. Broad spectrum antibiotics are properly used in the following medical situations: empirically (i.e., based on the experience of the practitioner), prior to the formal identification of the causative bacteria and when there is a wide range of possible illnesses and a potentially serious illness would result if treatment is delayed. This occurs, for example, in meningitis, where the patient can become fatally ill within hours if broad-spectrum antibiotics are not initiated. Broad spectrum antibiotics are also used for drug resistant bacteria that do not respond to other, more narrow spectrum antibiotics and in the case of superinfections, where there are multiple types of bacteria causing illness, thus warranting either a broad-spectrum antibiotic or combination antibiotic therapy.

Following a 40-year hiatus in discovering new classes of antibacterial compounds, three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).

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7.1.5: Antibiotic Classifications

LEARNING OBJECTIVES

Compare the two classes of antibiotics: bactericidal and bacteriostatic antibiotic

Antibiotics can be divided into two classes based on their mechanism of action. Bactericidal antibiotics kill bacteria; bacteriostatic antibiotics inhibit their growth or reproduction.

One way that bactericidal antibodies kill bacteria is by inhibiting cell wall synthesis. Examples include the Beta-lactam antibiotics (penicillin derivatives (penams)), cephalosporins (cephems), monobactams, and carbapenems) and vancomycin. Other ways that bactericidal antibiotics kill bacteria include inhibiting bacterial enzymes or protein translation. Other batericidal agents include daptomycin, fluoroquinolones, metronidazole, nitrofurantoin, co-trimoxazole and telithromycin. Aminoglycosidic antibiotics are usually considered bactericidal, although they may be bacteriostatic with some organisms. The MBC (minimum bactericidal concentration) is the minimum concentration of drug which can kill 99.99% of the population.

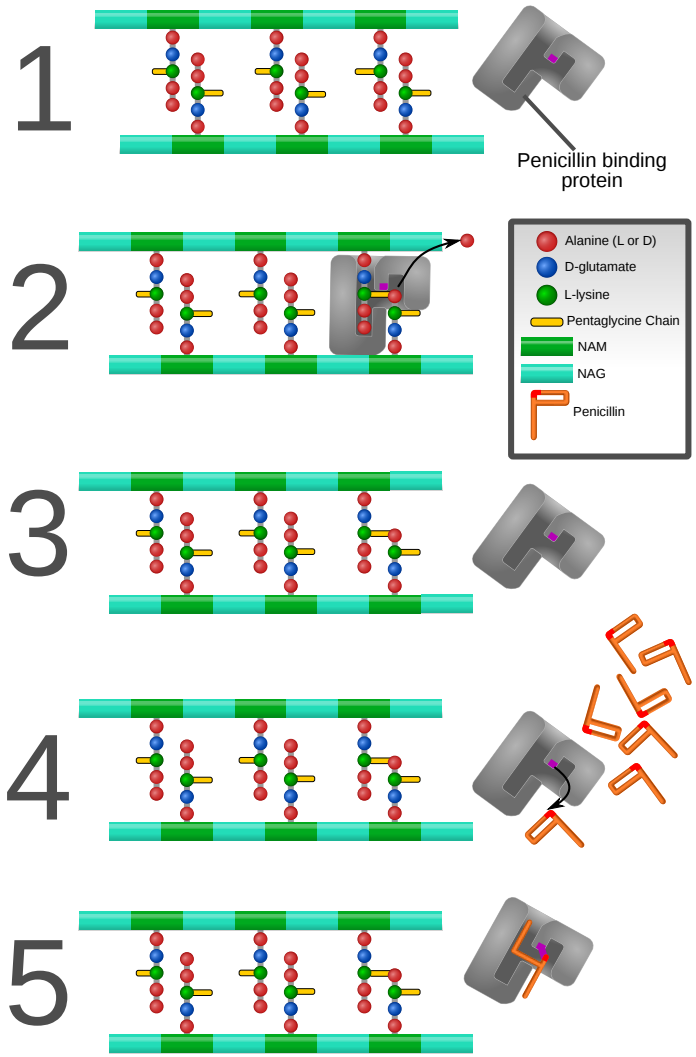


Figure: **Mechanism of penicillin inhibition**: Penicillin and most other β-lactam antibiotics act by inhibiting penicillin-binding proteins, which normally catalyze cross-linking of bacterial cell walls.

Bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism. This group includes: tetracyclines, sulfonamides, spectinomycin, trimethoprim,

chloramphenicol, macrolides and lincosamides. They must work together with the immune system to remove the microorganisms from the body. However, there is not always a precise distinction between them and bactericidal antibiotics. High concentrations of some bacteriostatic agents are also bactericidal. The MIC (minimum inhibitory concentration) is the minimum concentration of drug which can inhibit the growth of the microorganism.

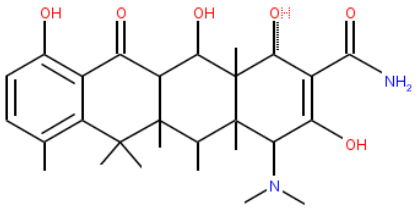


Figure: ***Structure of tetracycline****: Tetracycline antibiotics are protein synthesis inhibitors, inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex.*

Further categorization is based on their target specificity. "Narrow-spectrum" antibacterial antibiotics target specific types of bacteria, such as Gram-negative or Gram-positive bacteria, whereas broad-spectrum antibiotics affect a wide range of bacteria, usually both gram positive and gram negative cells. Following a 40-year hiatus in discovering new classes of antibacterial compounds, three new classes of antibacterial antibiotics have been brought into clinical use: cyclic lipopeptides (such as daptomycin), glycylcyclines (such as tigecycline), and oxazolidinones (such as linezolid).

Key Points

- Bactericidal antibodies inhibit cell wall synthesis.
- Bacteriostatic antibiotics limit the growth of bacteria by interfering with bacterial protein production, DNA replication, or other aspects of bacterial cellular metabolism.
- Bacteriostatic antibiotics must work together with the immune system to remove the microorganisms from the body.

Key Terms

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

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SECTION OVERVIEW

7.2: Functions of Antimicrobial Drugs

Topic hierarchy

7.2.1: Inhibiting Cell Wall Synthesis

7.2.2: Injuring the Plasma Membrane

7.2.3: Inhibiting Nucleic Acid Synthesis

7.2.4: Inhibiting Protein Synthesis

7.2.5: Inhibiting Essential Metabolite Synthesis

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7.2.1: Inhibiting Cell Wall Synthesis

β-Lactam (beta-lactam) and glycopeptide antibiotics work by inhibiting or interfering with cell wall synthesis of the target bacteria.

Learning Objectives

- Describe the two types of antimicrobial drugs that inhibit cell wall synthesis: beta-lactam and glycopeptide antibiotics

Key Points

- The peptidoglycan layer is important for cell wall structural integrity, being the outermost and primary component of the wall.
- β-Lactam antibiotics are a broad class of antibiotics that includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
- β-Lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls.
- Glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin.
- Glycopeptide antibiotics inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis.

Key Terms

- **beta-lactam antibiotic**: A broad class of antibiotics that inhibit cell wall synthesis, consisting of all antibiotic agents that contains a β-lactam nucleus in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.
- **Glycopeptide antibiotic**: Glycopeptide antibiotics are composed of glycosylated cyclic or polycyclic nonribosomal peptides. Significant glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin. This class of drugs inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis.
- **peptidoglycan**: A polymer of glycan and peptides found in bacterial cell walls.

Two types of antimicrobial drugs work by inhibiting or interfering with cell wall synthesis of the target bacteria. Antibiotics commonly target bacterial cell wall formation (of which peptidoglycan is an important component) because animal cells do not have cell walls. The peptidoglycan layer is important for cell wall structural integrity, being the outermost and primary component of the wall.

The first class of antimicrobial drugs that interfere with cell wall synthesis are the β-Lactam antibiotics (beta-lactam antibiotics), consisting of all antibiotic agents that contains a β-lactam nucleus in their molecular structures. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems. β-Lactam antibiotics are bacteriocidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The final step in the synthesis of the peptidoglycan is facilitated by penicillin-binding proteins (PBPs). PBPs vary in their affinity for binding penicillin or other β-lactam antibiotics.

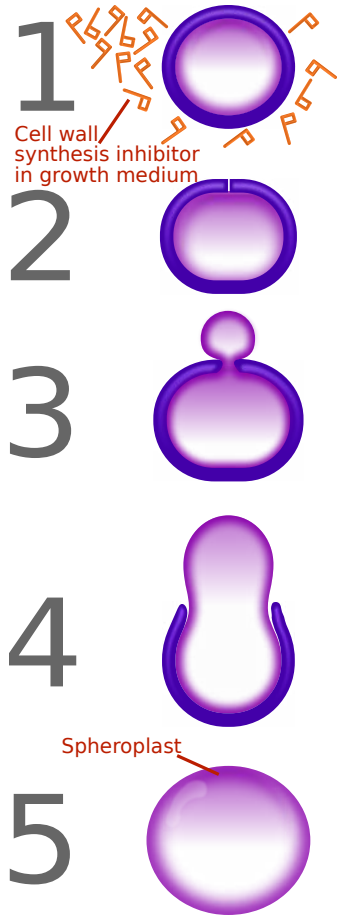


Figure: **Penicillin spheroplast generation**: Diagram depicting the failure of bacterial cell division in the presence of a cell wall synthesis inhibitor (e.g. penicillin, vancomycin).1- Penicillin (or other cell wall synthesis inhibitor) is added to the growth medium with a dividing bacterium.2- The cell begins to grow, but is unable to synthesize new cell wall to accommodate the expanding cell.3- As cellular growth continues, cytoplasm covered by plasma membrane begins to squeeze out through the gap(s) in the cell wall.4- Cell wall integrity is further violated. The cell continues to increase in size, but is unable to "pinch off" the extra cytoplasmic material into two daughter cells because the formation of a division furrow depends on the ability to synthesize new cell wall.5- The cell wall is shed entirely, forming a spheroplast, which is extremely vulnerable relative to the original cell. The loss of the cell wall also causes the cell to lose control over its shape, so even if the original bacterium were rod-shaped, the sphereoplast is generally spherical. Finally, the fact that the cell has now doubled much of its genetic and metabolic material further disrupts homeostasis, which usually leads to the cell's death.

Bacteria often develop resistance to β-lactam antibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid.

The second class of antimicrobial drugs that interfere with cell wall synthesis are the glycopeptide antibiotics, which are composed of glycosylated cyclic or polycyclic nonribosomal peptides. Significant glycopeptide antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin. This class of drugs inhibit the synthesis of cell walls in susceptible microbes by inhibiting peptidoglycan synthesis. They bind to the amino acids within the cell wall preventing the addition of new units to the peptidoglycan.

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7.2.2: Injuring the Plasma Membrane

Several types of antimicrobial drugs function by disrupting or injuring the plasma membrane.

Learning Objectives

- Discuss the function of the plasma membrane and how antimicrobial drugs target it

Key Points

- The plasma membrane or cell membrane is a biological membrane that separates the interior of all cells from the outside environment.
- Plasma membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity, and cell signaling. They serve as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton.
- Disrupting the plasma membrane causes rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis, which results in bacterial cell death.

Key Terms

- **plasma membrane**: The semipermeable membrane that surrounds the cytoplasm of a cell.
- **cell wall**: A thick, fairly rigid layer formed around individual cells of bacteria, Archaea, fungi, plants, and algae, the cell wall is external to the cell membrane and helps the cell maintain its shape and avoid damage.
- **plasma cell**: a form of lymphocyte that produces antibodies when reacted with a specific antigen; a plasmacyte

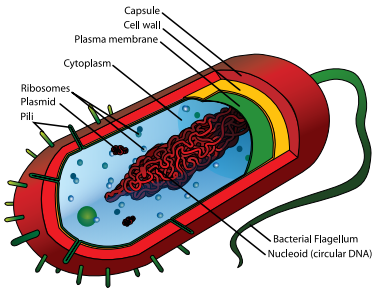


Figure: **Prokaryotic Cell**: Diagram of a typical gram-negative bacterium, with the thin cell wall sandwiched between the red outer membrane and the thin green plasma membrane.

The plasma membrane or cell membrane is a biological membrane that separates the interior of all cells from the outside environment. The plasma membrane is selectively permeable to ions and organic molecules. It controls the movement of substances in and out of cells. The membrane basically protects the cell from outside forces. It consists of the lipid bilayer with embedded proteins. Plasma membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity, and cell signaling. It serves as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton. Fungi, bacteria, and plants also have the cell wall which provides a mechanical support for the cell and precludes the passage of larger molecules. The plasma membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell and in attaching to the extracellular matrix and other cells to help group cells together to form tissues.

There are several types of antimicrobial drugs that function by disrupting or injuring the plasma membrane. One example is daptomycin, a lipopeptide which has a distinct mechanism of action, disrupting multiple aspects of bacterial cell membrane function. It appears to bind to the membrane causes rapid depolarization, resulting in a loss of membrane potential leading to inhibition of protein, DNA and RNA synthesis, which results in bacterial cell death. Another example is polymyxins antibiotics which have a general structure consisting of a cyclic peptide with a long hydrophobic tail. They disrupt the structure of the bacterial cell membrane by interacting with its phospholipids.

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7.2.3: Inhibiting Nucleic Acid Synthesis

Antimicrobial drugs inhibit nucleic acid synthesis through differences in prokaryotic and eukaryotic enzymes.

Learning Objectives

- State the steps where inhibitors of nucleic acid synthesis can exert their function

Key Points

- Some antimicrobial drugs interfere with the initiation, elongation or termination of RNA transcription.
- Some antimicrobial drugs interfere with various aspects of DNA replication.
- The antimicrobial actions of these drugs are a result of differences in the prokaryotic and eukaryotic enzymes involved in nucleic acid synthesis.

Key Terms

- **transcription**: The synthesis of RNA under the direction of DNA.
- **replication**: Process by which an object, person, place or idea may be copied mimicked or reproduced.

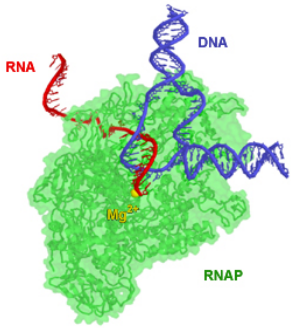


Figure: **Diagram of Transcription**: RNA Polymerase, an enzyme that produces RNA, from T. aquaticus pictured during elongation. Portions of the enzyme were made transparent so as to make the path of RNA and DNA more clear. The magnesium ion (yellow) is located at the enzyme active site.

Antimicrobial drugs can target nucleic acid (either RNA or DNA) synthesis. The antimicrobial actions of these agents are a result of differences in prokaryotic and eukaryotic enzymes involved in nucleic acid synthesis.

Prokaryotic transcription is the process in which messenger RNA transcripts of genetic material are produced for later translation into proteins. The transcription process includes the following steps: initiation, elongation and termination. Antimicrobial drugs have been developed to target each of these steps. For example, the antimicrobial rifampin binds to DNA-dependent RNA polymerase, thereby inhibiting the initiation of RNA transcription.

Other antimicrobial drugs interfere with DNA replication, the biological process that occurs in all living organisms and copies their DNA and is the basis for biological inheritance. The process starts when one double-stranded DNA molecule produces two identical copies of the molecule. In a cell, DNA replication begins at specific locations in the genome, called “origins. ” Uncoiling of DNA at the origin, and synthesis of new strands, forms a replication fork. In addition to DNA polymerase, the enzyme that synthesizes the new DNA by adding nucleotides matched to the template strand, a number of other proteins are associated with the fork and assist in the initiation and continuation of DNA synthesis. DNA replication, like all biological polymerization processes, proceeds in three enzymatically catalyzed and coordinated steps: initiation, elongation and termination. Any of the steps in the process of DNA replication can be targeted by antimicrobial drugs. For instance, quinolones inhibit DNA synthesis by interfering with the coiling of DNA strands.

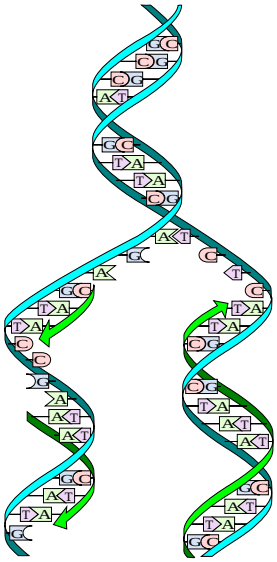


Figure: **DNA Replication**: The double helix is unwound and each strand acts as a template for the next strand. Bases are matched to synthesize the new partner strands.

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7.2.4: Inhibiting Protein Synthesis

Protein synthesis inhibitors are substances that disrupt the processes that lead directly to the generation of new proteins in cells.

Learning Objectives

- Paraphrase the general mechanism of action of protein synthesis inhibitors

Key Points

- Protein synthesis inhibitors usually act at the ribosome level, taking advantage of the major differences between prokaryotic and eukaryotic ribosome structures.
- Protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins like initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and ribosomal translocation), and termination.
- By targeting different stages of the mRNA translation, antimicrobial drugs can be changed if resistance develops.

Key Terms

- **translation**: A process occurring in the ribosome, in which a strand of messenger RNA (mRNA) guides assembly of a sequence of amino acids to make a protein.

A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins. It usually refers to substances, such as antimicrobial drugs, that act at the ribosome level. The substances take advantage of the major differences between prokaryotic and eukaryotic ribosome structures which differ in their size, sequence, structure, and the ratio of protein to RNA. The differences in structure allow some antibiotics to kill bacteria by inhibiting their ribosomes, while leaving human ribosomes unaffected.

Translation in prokaryotes involves the assembly of the components of the translation system which are: the two ribosomal subunits (the large 50S & small 30S subunits), the mRNA to be translated, the first aminoacyl tRNA, GTP (as a source of energy), and three initiation factors that help the assembly of the initiation complex. The ribosome has three sites: the A site, the P site, and the E site (not shown in). The A site is the point of entry for the aminoacyl tRNA. The P site is where the peptidyl tRNA is formed in the ribosome. The E site which is the exit site of the now uncharged tRNA after it gives its amino acid to the growing peptide chain.

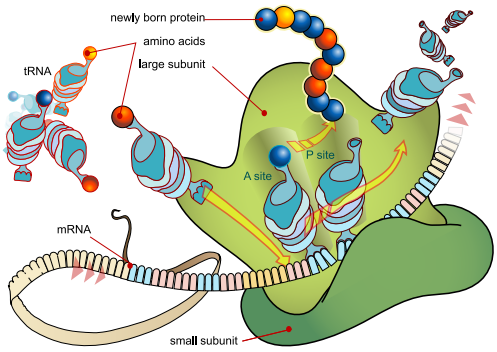


Figure: **Simplified diagram of protein synthesis**: Diagram showing how the translation of the mRNA and the synthesis of proteins is made by ribosomes.

In general, protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins like initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and ribosomal translocation), and termination. The following is a list of common antibacterial drugs and the stages which they target.

- Linezolid acts at the initiation stage, probably by preventing the formation of the initiation complex, although the mechanism is not fully understood.
- Tetracyclines and Tigecycline (a glycylcycline related to tetracyclines) block the A site on the ribosome, preventing the binding of aminoacyl tRNAs.

- Aminoglycosides, among other potential mechanisms of action, interfere with the proofreading process, causing an increased rate of error in synthesis with premature termination.
- Chloramphenicol blocks the peptidyl transfer step of elongation on the 50S ribosomal subunit in both bacteria and mitochondria.
- Macrolides, clindamycin, and aminoglycosides have evidence of inhibition of ribosomal translocation.
- Streptogramins also cause premature release of the peptide chain.

By targeting different stages of the mRNA translation, antimicrobial drugs can be changed if resistance develops to one or many of the drugs.

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7.2.5: Inhibiting Essential Metabolite Synthesis

An antimetabolite is a chemical that inhibits the use of a metabolite, a chemical that is part of normal metabolism.

Learning Objectives

- Distinguish between the three main types of antimetabolite antibiotics (antifolates, pyrimidine and purine analogues)

Key Points

- The presence of antimetabolites can have toxic effects on cells, such as halting cell growth or cell division.
- Antimetabolites are also used as antibiotics.
- The three main types of antimetabolite antibiotics are antifolates, pyrimidine analogues and purine analogues.

Key Terms

- **antimetabolite**: Any substance that competes with or inhibits the normal metabolic process, often by acting as an analogue of an essential metabolite

An antimetabolite is a chemical that inhibits the use of a metabolite, a chemical that is part of normal metabolism. Such substances are often similar in structure to the metabolite that they interfere with, such as antifolates that interfere with the use of folic acid. The presence of antimetabolites can have toxic effects on cells, such as halting cell growth or cell division.

Antimetabolites are also used as antibiotics. There are three main types of antimetabolite antibiotics. The first, antifolates impair the function of folic acid leading to disruption in the production of DNA and RNA. For example, methotrexate is a folic acid analogue, and owing to structural similarity with folic acid, methotrexate binds and inhibits the enzyme dihydrofolate reductase, and thus prevents the formation of tetrahydrofolate. Because tetrahydrofolate is essential for purine and pyrimidine synthesis, its deficiency can lead to inhibited production of DNA, RNA and proteins.

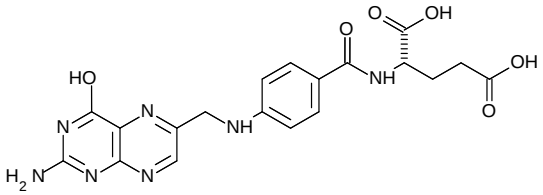


Figure: **Folic Acid Structure**: This is the chemical structure of folic acid.

The second type of antimetabolite antibiotics consist of pyrimidine analogues which mimic the structure of metabolic pyrimidines. Three nucleobases found in nucleic acids, cytosine (C), thymine (T), and uracil (U), are pyrimidine derivatives and the pyrimidine analogues disrupt their formation and consequently disrupt DNA and RNA synthesis.

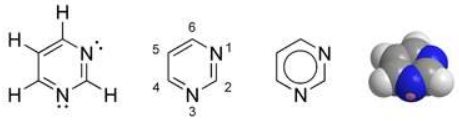


Figure: **Pyrimidine Structure**: This is the chemical structure of pyrimidine.

The purine analogues are the third type of antimetabolite antibiotics and they mimic the structure of metabolic purines. Two of the four bases in nucleic acids, adenine and guanine, are purines. Purine analogues disrupt nucleic acid production. For example, azathioprine is the main immunosuppressive cytotoxic substance that is widely used in transplants to control rejection reactions by inhibiting DNA synthesis in lymphocytes.

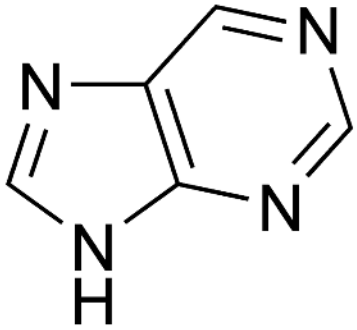


Figure: **Purine Structure**: This is the chemical structure of purine.

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SECTION OVERVIEW

7.3: Commonly Used Antimicrobial Drugs

Topic hierarchy

7.3.1: Synthetic Antimicrobial Drugs

7.3.2: Naturally Occurring Antimicrobial Drugs- Antibiotics

7.3.3: Beta-Lactam Antibiotics- Penicillins and Cephalosporins

7.3.4: Antibiotics from Prokaryotes

7.3.5: Antimycobacterial Antibiotics

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7.3.1: Synthetic Antimicrobial Drugs

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans.

Learning Objectives

- Recall the synthetic antimicrobial drugs that are sulfonamide and sulphonamide based

Key Points

- The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world.
- With the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents.
- Synthetic agents include: sulphonamides, cotrimoxazole, quinolones, anti-virals, anti-fungals, anti-cancer drugs, anti-malarials, anti-tuberculosis drugs, anti-leprotics, and anti-protozoals.

Key Terms

- **antimicrobial**: An agent that destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action.
- **microorganism**: An organism that is too small to be seen by the unaided eye, especially a single-celled organism, such as a bacterium.
- **bacteria**: A type, species, or strain of bacterium.

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.

The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become resistant to the poisons and/or heavy metals.

Antimicrobial nanotechnology is a recent addition to the fight against disease-causing organisms, replacing heavy metals and toxins, and may some day be used as a viable alternative.

Infections that are acquired during a hospital visit are called "hospital acquired infections" or nosocomial infections. Similarly, when the infectious disease is picked up in the non-hospital setting, it is considered "community acquired".

Synthetic agents include: sulphonamides, cotrimoxazole, quinolones, anti-virals, anti-fungals, anti-cancer drugs, anti-malarials, anti-tuberculosis drugs, anti-leprotics, and anti-protozoals.

Sulfonamide or sulphonamide is the basis of several groups of drugs. The original antibacterial sulfonamides (sometimes called sulfa drugs or sulpha drugs) are synthetic antimicrobial agents that contain the sulfonamide group. Some sulfonamides are also devoid of antibacterial activity, e.g., the anticonvulsant sultiame. The sulfonylureas and thiazide diuretics are newer drug groups based on the antibacterial sulfonamides.

Sulfa allergies are common, and medications containing sulfonamides are prescribed carefully. It is important to make a distinction between sulfa drugs and other sulfur-containing drugs and additives, such as sulfates and sulfites, which are chemically unrelated to

the sulfonamide group and do not cause the same hypersensitivity reactions seen in the sulfonamides.

In bacteria, antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthetase (DHPS), an enzyme involved in folate synthesis. As such, the microorganism will be “starved” of folate and die.

The sulfonamide chemical moiety is also present in other medications that are not antimicrobials, including thiazide diuretics (including hydrochlorothiazide, metolazone, and indapamide, among others), loop diuretics (including furosemide, bumetanide, and torsemide), sulfonylureas (including glipizide, glyburide, among others), and some COX-2 inhibitors (e.g., celecoxib), and acetazolamide.

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7.3.2: Naturally Occurring Antimicrobial Drugs- Antibiotics

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans.

Learning Objectives

- Discuss the mechanism of action for protein synthesis inhibitors used as antimicrobial drugs, and recognize various naturally occuring antimicrobial drugs

Key Points

- There are mainly two classes of antimicrobial drugs: those obtained from natural sources (i.e. beta-lactam antibiotic (such as penicillins, cephalosporins) or protein synthesis inhibitors (such as aminoglycosides, macrolides, tetracyclines, chloramphenicol, polypeptides); and synthetic agents.
- A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such because the nitrogen atom is attached to the β-carbon relative to the carbonyl.
- A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins.

Key Terms

- **β-lactam**: A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.
- **antimicrobial**: An agent that destroys microbes, inhibits their growth, or prevents or counteracts their pathogenic action.
- **microorganism**: An organism that is too small to be seen by the unaided eye, especially a single-celled organism, such as a bacterium.

An antimicrobial is a substance that kills or inhibits the growth of microorganisms bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

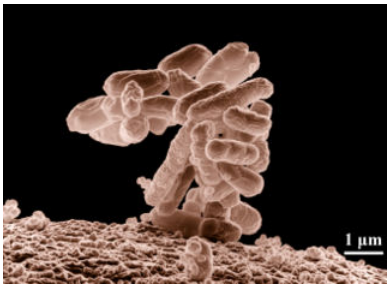


Figure: **A cluster of Escherichia coli Bacteria magnified 10,000 times.**: A cluster of Escherichia coli Bacteria magnified 10,000 times.

The discovery of antimicrobials, like penicillin and tetracycline, paved the way for better health for millions of people around the world. Before penicillin became a viable medical treatment in the early 1940's, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.

However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely. This allowed the microbe to survive, change, and become resistant to the poisons and/or heavy metals.

Antimicrobial nanotechnology is a recent addition to the fight against disease causing organisms. It replaces heavy metals and toxins and may someday be a viable alternative.

Infections that are acquired during a hospital visit are called "hospital acquired infections" or nosocomial infections. Similarly, when the infectious disease is picked up in the non-hospital setting it is considered "community acquired. "

There are mainly two classes of antimicrobial drugs: those obtained from natural sources (i.e. beta-lactam) antibiotic (such as penicillins, cephalosporins) or protein synthesis inhibitors (such as aminoglycosides, macrolides, tetracyclines, chloramphenicol, polypeptides); and synthetic agents.

A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.

A protein synthesis inhibitor is a substance that stops or slows the growth or proliferation of cells by disrupting the processes that lead directly to the generation of new proteins. While a broad interpretation of this definition could be used to describe nearly any antibiotic, in practice, it usually refers to substances that act at the ribosome level (either the ribosome itself or the translation factor), taking advantage of the major differences between prokaryotic and eukaryotic ribosome structures. Toxins such as ricin also function via protein synthesis inhibition. Ricin acts at the eukaryotic 60S.

In general, protein synthesis inhibitors work at different stages of prokaryotic mRNA translation into proteins, like initiation, elongation (including aminoacyl tRNA entry, proofreading, peptidyl transfer, and ribosomal translocation), and termination. Rifamycin inhibits prokaryotic DNA transcription into mRNA by inhibiting DNA-dependent RNA polymerase by binding its beta-subunit. Linezolid acts at the initiation stage probably by preventing the formation of the initiation complex, although the mechanism is not fully understood.

Tetracyclines and Tigecycline (a glycylcycline related to tetracyclines) block the A site on the ribosome, preventing the binding of aminoacyl tRNAs. Aminoglycosides, among other potential mechanisms of action, interfere with the proofreading process, causing increased rate of error in synthesis with premature termination. Chloramphenicol blocks the peptidyl transfer step of elongation on the 50S ribosomal subunit in both bacteria and mitochondria. Macrolides (as well as inhibiting ribosomal translocation and other potential mechanisms) bind to the 50s ribosomal subunits, inhibiting peptidyl transfer. Quinupristin/dalfopristin act synergistically, with dalfopristin, enhancing the binding of quinupristin as well as inhibiting peptidyl transfer. Quinupristin binds to a nearby site on the 50S ribosomal subunit and prevents elongation of the polypeptide. It also causes incomplete chains to be released. Macrolides, clindamycin, and aminoglycosides (with all these three having other potential mechanisms of action as well) have evidence of inhibition of ribosomal translocation. Fusidic acid prevents the turnover of elongation factor G (EF-G) from the ribosome. Macrolides and clindamycin (both also having other potential mechanisms) cause premature dissociation of the peptidyl-tRNA from the ribosome. Puromycin has a structure similar to that of the tyrosinyl aminoacyl-tRNA. Therefore, it binds to the ribosomal A site and participates in peptide bond formation, producing peptidyl-puromycin. However, it does not engage in translocation and quickly dissociates from the ribosome, causing a premature termination of polypeptide synthesis.

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7.3.3: Beta-Lactam Antibiotics- Penicillins and Cephalosporins

The β-lactam ring is part of the core structure of several antibiotic families.

Learning Objectives

- Recognize the classes of beta-lactams and their mechanisms of action

Key Points

- The principal antibiotic families of which the β-lactam ring is part of the core structure are the penicillins, cephalosporins, carbapenems, and monobactams, which are also called β-lactam antibiotics.
- A β-lactam (beta-lactam) ring is a four-membered lactam (cyclic amide). -Lactams are classified according to their core ring structures.
- The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was previously known as “Cephalosporium”.

Key Terms

- **cephalosporins**: The cephalosporins are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was previously known as “Cephalosporium”.
- **antibiotic**: Any substance that can destroy or inhibit the growth of bacteria and similar microorganisms.
- **β-lactam**: A β-lactam (beta-lactam) ring is a four-membered lactam. A lactam is a cyclic amide. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.
- **β-lactam**: Any of a class of cyclic amides, that are the nitrogen analogs of lactones, formed by heating amino acids; the tautomeric enol forms are known as lactims.

A β-lactam (beta-lactam) ring, is a four-membered lactam. It is named as such, because the nitrogen atom is attached to the β-carbon relative to the carbonyl. The simplest β-lactam possible is 2-azetidinone.

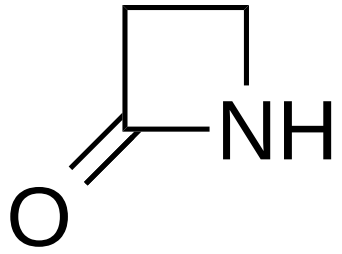


Figure: **β-Lactam**: β-Lactam ring is a four-membered lactam.

The β-lactam ring is part of the core structure of several antibiotic families, the principal ones being the penicillins, cephalosporins, carbapenems, and monobactams, which are, therefore, also called β-lactam antibiotics. Nearly all of these antibiotics work by inhibiting bacterial cell wall biosynthesis. This has a lethal effect on bacteria. Bacteria do, however, contain within their populations, in smaller quantities, bacteria that are resistant against β-lactam antibiotics. They do this by expressing the β-lactamase gene. When bacterial populations have these resistant subgroups, treatment with β-lactam can result in the resistant strain becoming more prevalent and so, more virulent.

β-Lactams are classified according to their core ring structures:

- β-Lactams fused to saturated five-membered rings;
- β-Lactams containing thiazolidine rings are named *penams*;
- β-Lactams containing pyrrolidine rings are named *carbapenams*;
- β-Lactams fused to oxazolidine rings are named *oxapenams* or *clavams*;
- β-Lactams fused to unsaturated five-membered rings;
- β-Lactams containing 2,3-dihydrothiazole rings are named *penems*;
- β-Lactams containing 2,3-dihydro-1H-pyrrole rings are named *carbapenems*;
- β-Lactams fused to unsaturated, six-membered rings;

- β-Lactams containing 3,6-dihydro-2H-1,3-thiazine rings are named *cephems*;
- β-Lactams containing 1,2,3,4-tetrahydropyridine rings are named *carbacephems*;
- β-Lactams containing 3,6-dihydro-2H-1,3-oxazine rings are named *oxacephems*; and
- β-Lactams not fused to any other ring are named *monobactams*.

Penicillin (sometimes abbreviated PCN or pen) is a group of antibiotics derived from *Penicillium fungi.* They include penicillin G, procaine penicillin, benzathine penicillin, and penicillin V. Penicillin antibiotics are historically significant because they are the first drugs that were effective against many previously serious diseases, such as syphilis, and infections caused by staphylococci and streptococci. Penicillins are still widely used today, though many types of bacteria are now resistant. All penicillins are β-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms.

The cephalosporins (sg. /ˌsɛfələʊspɔːrɨn/) are a class of β-lactam antibiotics originally derived from the fungus Acremonium, which was previously known as “Cephalosporium”. Together with cephamycins, they constitute a subgroup of β-lactam antibiotics called *cephems*. Cephalosporins are indicated for the prophylaxis and treatment of infections caused by bacteria susceptible to this particular form of antibiotic. First-generation cephalosporins are active predominantly against Gram-positive bacteria, and successive generations have increased activity against Gram-negative bacteria (albeit often with reduced activity against Gram-positive organisms).

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7.3.4: Antibiotics from Prokaryotes

Most of the currently available antibiotics are produced by prokaryotes mainly by bacteria from the genus *Streptomyces*.

Learning Objectives

- Explain the role of Streptomyces and other prokaryotes in antibiotic production

Key Points

- Gramicidin is one of the first antibiotics to be manufactured commercially. It is a heterogeneous mixture of six antibiotic compounds, all of which are obtained from the soil bacterial species Bacillus brevis.
- Streptomyces is the largest antibiotic-producing genus, producing antibacterial, antifungal, and antiparasitic drugs, and also a wide range of other bioactive compounds, such as immunosuppressants. They produce over two-thirds of the clinically useful antibiotics of natural origin.
- Members of the Streptomyces genus are the source for numerous antibacterial pharmaceutical agents; among the most important of these are: Chloramphenicol (from S. venezuelae), Lincomycin (from S. lincolnensis), Neomycin (from S. fradiae), Tetracycline (from S. rimosus and S. aureofaciens).
- Some Pseudomonas spp. might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide.

Key Terms

- **antibiotic**: Any substance that can destroy or inhibit the growth of bacteria and similar microorganisms.
- **beta-lactamase**: An enzyme produced by certain bacteria, responsible for their resistance to beta-lactam antibiotics such as penicillin.

Even though penicillin drugs, antibiotics produced by molds, were the first antibiotics successfully used to treat many serious infections, most of the naturally produced antibiotics are synthesized by bacteria. In 1939 the French microbiologist René Dubos isolated the substance tyrothricin and later showed that it was composed of two substances, gramicidin (20%) and tyrocidine (80%). These were the first antibiotics to be manufactured commercially. Gramicidin is a heterogeneous mixture of six antibiotic compounds, all of which are obtained from the soil bacterial species *Bacillus brevis* and called collectively gramicidin D.

Streptomyces is the largest antibiotic-producing genus, producing antibacterial, antifungal, and antiparasitic drugs, and also a wide range of other bioactive compounds, such as immunosuppressants. They produce over two-thirds of the clinically useful antibiotics of natural origin. The now uncommonly-used streptomycin takes its name directly from *Streptomyces*. Aminoglycosides, class of antibiotics, that are derived from bacteria of the *Streptomyces* genus are named with the suffix -mycin, whereas those that are derived from *Micromonospora* are named with the suffix -micin. However, this nomenclature system is not specific for aminoglycosides.



Figure: **Supernatant of a Streptomyces davawensis culture**: The picture shows the typical red color of the antibiotic Roseoflavin secreted by the Streptomyces cells in the culture.

Streptomycetes are characterised by a complex secondary metabolism. Almost all of the bioactive compounds produced by *Streptomyces* are initiated during the time coinciding with the aerial hyphal formation from the substrate mycelium.

Streptomycetes produce numerous antifungal compounds of medicinal importance, including nystatin (from *S. noursei*), amphotericin B (from *S. nodosus*), and natamycin (from *S. natalensis*).

Members of the *Streptomyces* genus are the source for numerous antibacterial pharmaceutical agents; among the most important of these are: Chloramphenicol (from *S. venezuelae*), Daptomycin (from *S. roseosporus*), Fosfomycin (from *S. fradiae*), Lincomycin (from *S. lincolnensis*), Neomycin (from *S. fradiae*), Puromycin (from *S. alboniger*), Streptomycin (from *S. griseus*), Tetracycline (from *S. rimosus* and *S. aureofaciens*).

Clavulanic acid (from *S. clavuligerus*) is a drug used in combination with some antibiotics (like amoxicillin) to block and/or weaken some bacterial-resistance mechanisms by irreversible beta-lactamase inhibition.

Other bacterial species produce antibiotics as well. Such an example are some *Pseudomonas* species which produce antimicrobial compounds. *P. aurantiaca* produces di-2,4-diacetylfluoroglucylmethane, a compound antibiotically active against Gram-positive organisms. Other *Pseudomonas* spp. might produce compounds antagonistic to other soil microbes, such as phenazine-type antibiotics or hydrogen cyanide.

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7.3.5: Antimycobacterial Antibiotics

Antimycobacterial antibiotics target microbes classified as mycobacterium.

Learning Objectives

- Compare and contrast the drugs used for treatment of Mycobacterium tuberculosis and Mycobacterium leprae

Key Points

- The standard “short” course treatment for TB is isoniazid, rifampicin, pyrazinamide, and ethambutol for two months, then isoniazid and rifampicin alone for another four months.
- The standard treatment for leprosy is a multidrug therapy that includes dapsone, clofazimine and rifampicin.
- Mycobacterium are defined by their ability to grow in a mold-like fashion on the surface of liquids when cultured.

Key Terms

- **tuberculosis**: Tuberculosis, MTB, or TB (short for tubercle bacillus) is a common, and in many cases lethal, infectious disease caused by various strains of mycobacteria, usually Mycobacterium tuberculosis.
- **infectious disease**: Infectious diseases, also known as transmissible diseases or communicable diseases comprise of clinically evident illness (i.e., characteristic medical signs and/or symptoms of disease) resulting from the infection, presence and growth of pathogenic biological agents in an individual host organism. In certain cases, infectious diseases may be asymptomatic for much or even all of their course in a given host. In the latter case, the disease may only be defined as a “disease” (which by definition means an illness) in hosts who secondarily become ill after contact with an asymptomatic carrier. An infection is not synonymous with an infectious disease, as some infections do not cause illness in a host.
- **leprosy**: Leprosy, also known as Hansen’s disease (HD), is a chronic disease caused by the bacteria Mycobacterium leprae and Mycobacterium lepromatosis.
- **isoniazid**: a medication used in the prevention and treatment of tuberculosis, having the chemical formula C6H7N3O

Antimycobacterial antibiotics are a class of antimicrobial drugs that target mycobacterium. Mycobacterium is a genus of Actinobacteria that includes pathogens known to cause serious and infectious disease. The types of pathogens considered to be mycobacterium include Mycobacterium tuberculosis (tuberculosis) and Mycobacterium leprae (leprosy). Mycobacterium grow in a mold-like manner on the surface of liquids when cultured. Antiomycobacterial antibiotics specifically target these types of microbes.

A type of antimycobacterial antibiotic includes the class of drugs used for tuberculosis (TB) treatment. The standard “short” course treatment for TB is isoniazid, rifampicin (also known as rifampin in the United States), pyrazinamide and ethambutol for two months, then isoniazid and rifampicin alone for another four months. The patient is considered cured at six months (although there is still a relapse rate of 2 to 3%). For latent tuberculosis, the standard treatment is six to nine months of isoniazid alone.

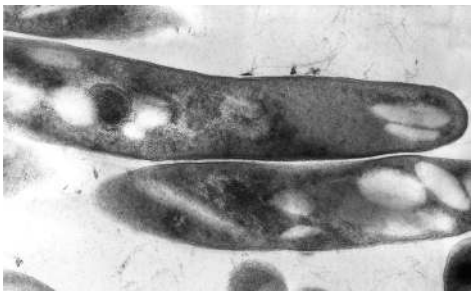


Figure: **Mycobacterium**: Mycobacterium are a class of bacteria defined by their ability to grow in a mold-like manner. Here, a TEM of Mycobacterium tuberculosis, the causative agent of tuberculosis. Antimycobacterial antibiotics target mycobacterium.

If the organism is known to be fully sensitive, then it is treated with isoniazid, rifampicin and pyrazinamide for two months, followed by isoniazid and rifampicin for four months. Ethambutol need not be used. Most regimens have an initial high-intensity phase, followed by a continuation phase (also called a consolidation phase or eradication phase) – the high-intensity phase is given first, then the continuation phase.

There are six classes of second-line drugs (SLDs) used for the treatment of TB. A drug may be classed as second-line instead of first-line for one of three possible reasons: it may be less effective than the first-line drugs (e.g., p-aminosalicylic acid); or, it may have toxic side-effects (e.g., cycloserine); or it may be unavailable in many developing countries (e.g., fluoroquinolones): aminoglycosides: e.g., amikacin (AMK), kanamycin (KM); polypeptides: e.g., capreomycin, viomycin, enviomycin; Fluoroquinolones: e.g., ciprofloxacin (CIP), levofloxacin, moxifloxacin (MXF); thioamides: e.g. ethionamide, prothionamide.

For treatment of leprosy, caused by Mycobacterium leprae, the traditional antimycobacterial drugs include promin (the first treatment introduced to fight leprosy) and dapsone (which eventually become obsolete as Mycobacterium leprae quickly evolved resistance). Modern drugs which were developed in response to the resistance was clofazimine and rifampicin. The use of multidrug therapies including dapsone, clofazimine and rifampicin were advantageous due to the low risk of antibiotic resistance. However, the use of these multidrug treatments was costly and only adopted in endemic countries when the World Health Assembly passed a resolution to eliminate leprosy in 1991.

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SECTION OVERVIEW

7.4: Interactions Between Drug and Host

Topic hierarchy

7.4.1: Organ Toxicity

7.4.2: Allergic Responses to Drugs

7.4.3: Suppression and Alteration of Microbiota by Antimicrobials

7.4.4: Effects of Drug Combinations

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7.4.1: Organ Toxicity

The accumulation of antimicrobial drugs and their metabolic byproducts in organs can be toxic, leading to organ damage.

Learning Objectives

- Outline the two major types of organ toxicity and their effects, recognizing additional types of toxicity

Key Points

- Antimicrobial drugs can have unintended side effects, including being toxic to organs.
- The liver and kidney are particularly susceptible to organ toxicity as they are the sites of toxin filtration and toxin metabolic breakdown.
- Almost any organ or tissue in the human body can be affected by antimicrobial toxicity.
- The toxic effects of antimicrobial drugs, while potentially harmful are very rare.

Key Terms

- **antimicrobrial drugs**: A drug administered to a patient, with the purpose of killing or slowing the growth of a microorganism, including protozoans, fungi and bacteria.
- **antibiotics**: A chemical that slows the growth of, or kills a bacteria.

The use of antimicrobial drugs can have many unintended side-effects. The use, particularly when repeated, of many drugs can lead to an accumulation of a drug, or harmful byproducts from the metabolism of a drug, in tissues or organs. This accumulation of toxic chemicals can lead to organ damage, and in extreme cases, even organ failure and death. Two severe types of organ toxicity associated with antimicrobial drugs are nephrotoxicity and hepatotoxicity, toxicity of the kidneys and liver respectively.

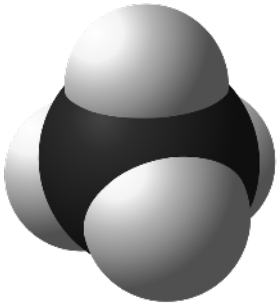


Figure: **Methane**: This space-filling model of methane shows the approximately spherical nature of this molecule.

The liver and kidneys are common organs affected by chemical toxicity. The kidneys are responsible for the filtration of the blood, so it is not surprising that deleterious agents in the blood may accumulate there. The liver is an important site for the breakdown of most metabolites in the body, and is referred to as the “metabolic clearing house” of the body. As drugs are quite often broken down in the liver, they can accumulate there and cause damage, or the byproducts of a drug’s metabolism can be toxic. In addition to direct damage to the liver by an antimicrobial drug, antimicrobial drugs can lead to the formation of dangerous toxins through the breakdown of microbes or due to interaction with other tissues in the body. These secondary toxins from drug metabolism then accumulate in the liver, potentially causing damage. Drug damage to the liver or kidneys can be particularly catastrophic as these organs are needed for the proper “cleaning” of the body. If they are compromised, this leads to further accumulation of potentially toxic metabolites further damaging organs in the body.



Figure: **Sun Poisoning**: This rash seen on a forearm is a typical reaction observed when an antibiotic causes phototoxicity.

Some of the toxic effects can be more benign, as is the case with ototoxicity, or damage to the ear. Use of some antibiotics, such as gentamicin, can cause a lose of hearing, or tinnitus (“ringing in the ears”). Ototoxicity is usually temporary with antibiotics, but permanent hearing damage, while rare, has been reported. Some antibiotics such as Tetracycline can cause phototoxicity, also known as sun poisoning; the result of which is that very short exposures to direct sunlight can cause severe skin irritation, with the appearance being quite similar to a rash or sunburn. The Tetracycline in a patient’s skin becomes toxic when exposed to sunlight, which causes an allergic reaction and leads to rash on the affected area. Broad-spectrum antibiotics in the family of fluoroquinolones can cause neurotoxicity by directly damaging neuronal receptors. This can lead to any number of psychological effects from mild cognitive dysfunction (brain fog) to more severe effects, such as hallucinations and suicidal thoughts.

While a few specific examples have been outlined here, toxic effects are not limited to the organs mentioned. In fact, almost any tissue or organ can be affected by antimicrobial drugs. However, it should be noted that the side-effects due to broad-spectrum antibiotics are actually quite rare, with organ damage being even more rare.The potential side-effects of antibiotics or other antimicrobial drugs are offset by the benefits of combating the microbial infection.

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7.4.2: Allergic Responses to Drugs

Antimicrobrial drugs can cause immune responses which can be fatal.

Learning Objectives

- Explain the physiology of an immune response responsible for an allergic reaction to drugs

Key Points

- Antimicrobial drugs, can like almost any other substance, act as an allergen.
- Using a drug may not induce an allergic response the first time it is taken, however, subsequent use of the antimicrobial drugs may lead to allergic reactions.
- The most common antimicrobial drug allergy is penicillin. 1-5% of people who have taken it have suspected penicillin allergies.

Key Terms

- **allergen**: A substance, known as an antigen, which stimulates an immune response from a sensitive individual.
- **anaphylaxis**: A rapid and severe allergic reaction which can lead to death
- **Beta-lactam antibiotics**: A broad class of antibiotics of which penicillin is a member.

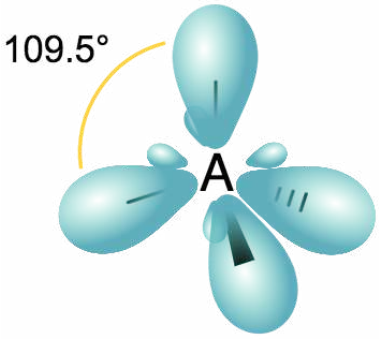


Figure: **sp3 hybridization**: In the process of sp^3 hybridization in methane, the single 2s and three 2p orbitals of carbon mix into four sp^3 hybrid orbitals, which are chemically and geometrically identical. Bond angles are 109.5°.

Virtually any substance, when exposed internally or externally to the body, can act as an allergen and illicit an immune system response, such is the case with antimicrobial drugs. While the drug acts as an allergen, the drug itself is not causing direct damage to the individual, but rather it is the response of an individual's immune system which is deleterious. An allergic reaction is the body's response to clear a foreign substance. Once the body recognizes a substance as foreign (in this case an antimicrobial drug), it starts producing antibodies, specifically immunoglobulin E (IgE) against the drug. The IgE binds directly to the drug and sets off a cascade of events, including the activation of receptors on immune system cells. This results in the production of histamines. The worst allergic reactions can be very severe and result in anaphylaxis. Anaphylaxis is an extremely severe allergic reaction caused when histamines are overproduced leading to severe contraction of bronchial muscles. In the most extreme cases, the airways close, which can lead to death. Other less severe symptoms of an allergic reaction can include, hives, angioedema (tissue swelling under the skin, often on the face), tight throat, coughing, wheezing, or watery eyes.

The exposure to a drug may not elicit an allergic reaction during the first exposure, but after the first exposure, the body creates antibodies and memory lymphocyte cells against the drug, therefore later exposures to the drug will illicit an immune response. There are many factors that can determine if an individual is sensitive to an antimicrobial drug, as with other allergens. Some factors include genetics and past exposures to other allergens, typically a person who has allergies to other things, such as various foods, is more prone to have or develop drug allergies. The most reported drug allergy is to Beta-lactam antibiotics, of which penicillin is the most well-known type, affecting 1-5% of people who take penicillin. While the most severe cases can result in anaphylaxis, most reactions are not severe. Additionally the allergic reaction may not even be due to the penicillin, as dyes and other chemicals added to antimicrobial drugs may in fact cause the allergic response instead. Taken together, recent studies show that perhaps only 1/5 people who suspect they have an allergy to penicillin do indeed have such an allergy.

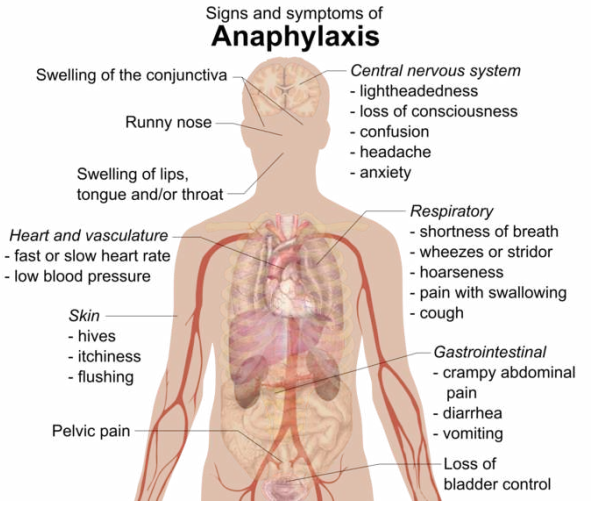


Figure: **Anaphylaxis**: A representation of the signs and symptoms of anaphylaxis that result from an allergic reaction. Signs include CNS symptoms such as confusion or lightheadedness, respiratory symptoms such as shortness of breath, gastrointestinal issues such as pain or vomiting, skin issues such as hives or itchiness, vascular symptoms such as change in heart rate or low blood pressure, and swelling of the mouth or eyes.

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7.4.3: Suppression and Alteration of Microbiota by Antimicrobials

Our bodies depend upon, and host, a vast number of complex microbial flora that can be affected negatively by antimicrobial treatments.

Learning Objectives

- Describe the role and function of the microbiota

Key Points

- The intestinal system has many different species of microbes and huge numbers of individual microbes; we rely on these microbes for proper metabolism of food.
- The use of antimicrobial agents to slow down or kill pathogenic microbes can often kill beneficial bacteria, causing deleterious health effects.
- Our body hosts some microbes that inhibit the growth of pathogenic microbes; using antimicrobial agents can alter the flora allowing pathogenic microbes to overgrow and cause diseases.

Key Terms

- **Candidal vulvovaginitis**: Candidal vulvovaginitis or vaginal thrush, or yeast infection, is an infection of the vagina's mucous membranes by Candida albicans.
- **microbiota**: The microbial flora harbored by normal, healthy individuals.
- **pathogenic bacteria**: Bacteria which infect and cause deleterious health effects.

The human body hosts thousands of different species of microbial organisms, known as the microbial flora or microbiota. Microbiota serve many functions in our body; most notable is the gut flora, crucial for the proper digestion of food, carbohydrate fermentation, and nutrient absorption. The gut flora in the human intestinal system has hundreds of species of microbes and over 100 trillion individual microbes; in comparison, the human body has around 10 trillion cells. Most of these microbes are bacterial and fungal. This is especially a problem when broad-spectrum antimicrobial agents are used, as antimicrobial treatments while helping to clear up pathogenic microbes from the body will often kill symbiotic bacteria. In addition, some microbial infections are due to translocation, the movement of advantageous bacteria to parts of the body where they might be harmful. An example is gut flora getting into the body's blood stream. The treatment of translocated or pathogenic bacteria may necessitate the use of antibiotics that will kill symbiotic bacteria. Antimicrobial agents which can kill beneficial gut flora can reduce the numbers of individual microbes or reduce the species of beneficial bacteria. In the case of the gut flora, this may impair the ability of a patient to properly metabolize food. If advantageous bacteria do not repopulate the intestine, this can lead to serious malnutrition problems.

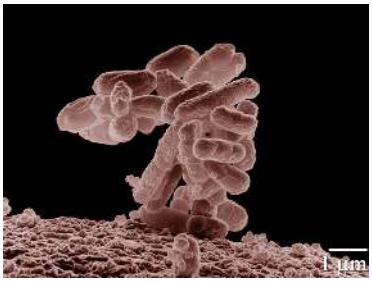


Figure: **Gut bacteria**: This is an electron micrograph, at 10,000X magnification. The oblong structures are Escherichia coli (E. coli), a symbiotic bacteria found in the human intestinal system.

In addition to serving a necessary function as gut flora due in metabolism of food, some microbiota in our bodies serve the function of keeping pathogenic microbes from inhabiting or dominating other flora at locations in our body. This is exemplified by Candida albicans, a yeast which is often found on humans. C. albicans is normally harmless, but when women take some antibiotics this can kill beneficial bacteria, specifically lactobacilli, in the vulvo-vaginal area. Without lactobacilli, C. albicans growth is not suppressed and can thus overgrow. This causes candidal vulvovaginitis, or yeast infections, a potentially painful infection of the vaginal

mucous membranes by overgrown C. albicans. Yeast infections can be caused by antibiotics, as well as using aggressive topical cleaning agents such as detergents which again kill off beneficial lactobacilli allowing C. albicans to overgrow.

Fortunately there are antimicrobial agents that specifically target pathogenic bacterial species, which opposed to broad-spectrum treatments can reduce harmful effects on beneficial microbes. Sometimes the use of broad-spectrum antimicrobial agents is unavoidable; in these situations, consuming foods such as yogurt which contains beneficial bacteria can replenish the body's symbiotic microbes. In extreme cases microbes can be transplanted from a healthy individual to someone with whose symbiotic microbes have been compromised.

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7.4.4: Effects of Drug Combinations

Antimicrobial drugs can interact with other drugs in deleterious ways or can be used in combination to combat microbial infections.

Learning Objectives

- Give examples of interactions that can render an antimicrobial ineffective

Key Points

- The interaction between one antimicrobial agent and another is very complex, along with the way they target microbes and the organism.
- While it is not certain that a drug may interact with antibiotics, it is considered wise to err on the side that there are potentially unknown and harmful interactions from mixing drugs.
- The use of more than one antimicrobial agent is an effective and widely used practice to reduce the chance that microbes will resist a treatment.

Key Terms

- **contraindication**: In medicine, a contraindication is a condition or factor that serves as a reason to withhold a certain medical treatment.
- **tuberculosis**: An infectious disease of humans and animals caused by a species of mycobacterium mainly infecting the lungs where it causes tubercles characterized by the expectoration of mucus and sputum, fever, weight loss, and chest pain, and transmitted through inhalation or ingestion of bacteria.
- **combination therapy**: Combination therapy is the use of more than one medication or other therapy. Most often, these terms refer to the simultaneous administration of two or more medications to treat a single disease.

Pharmacodynamics is the field that attempts to understand the unintended effects of the use of two or more drugs. Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body or on microorganisms or parasites within or on the body. It also looks at the mechanisms of drug action and the relationship between drug concentration and effect. These changes are extraordinarily difficult to classify given the wide variety of modes of action that exist and the fact that many drugs can cause their effect through a number of different mechanisms. This wide diversity also means that, in all but the most obvious cases, it is important to investigate and understand these mechanisms. The well-founded suspicion exists that there are more unknown interactions than known ones.

Two well described interactions between antimicrobial drugs and other drugs are between antibiotics and alcohol and antibiotics and the birth control pill. Interactions between alcohol and certain antibacterials may occur, cause side-effects, and decrease effectiveness of antibacterial therapy. Potential risks of side-effects and effectiveness depend on the type of antibacterial administered. Despite the lack of a categorical contraindication, the belief that alcohol and antibacterials should never be mixed is widespread. Some antibacterials may inhibit the breakdown of alcohol, which may result in alcohol-induced vomiting, nausea, and shortness of breath. Other effects of alcohol on antibacterial activity include altered activity of the liver enzymes that break down the antibacterial compound. In addition, serum levels bacteriostatic antibacterials may be reduced by alcohol consumption, resulting in reduced efficacy and diminished pharmacotherapeutic effect.

Another well studied interaction is between antibiotics and the contraceptive pill. The majority of studies indicate that antibiotics do not interfere with contraceptive pills. In cases where antibacterials have been suggested to affect the efficiency of birth control pills may be due to an increase in the activities of hepatic liver enzymes causing increased breakdown of the pill's active ingredients. Effects on the intestinal flora, which might result in reduced absorption of estrogens in the colon, have also been suggested, but such suggestions have been inconclusive and controversial. Clinicians have recommended that extra contraceptive measures be applied during therapies using antibacterials that are suspected to interact with oral contraceptives.

Additionally, when dealing with a microbial infection, sometimes the use of two or more antibiotics can effectively combat the infection while each drug individually has little or no effect. This method is called combination therapy and is used when the nature of a microbial infection is unknown, as typified by the combination of the antibiotics ampicillin and sulbactam. The use of two antibiotics with different modes of microbial inhibition increases the chance that the treatment will combat the microbial infection.

Further, tuberculosis has been treated with combination therapy for over fifty years. This is due to the phenomenon of resistance, whereby a micro-organism gains the ability to resist an antimicrobial drug, while initially the drug effectively slowed the growth of or even killed the target micro-organism. Treating tuberculosis, or other pathogenic microbes with more than one antibiotic reduces the chance that the microbe will adapt and survive the treatment, especially if the two drugs have different methods of reducing the microbe's normal functions.



Figure: **Tuberculosis**: This x-ray of a tuberculosis patient shows the lung on the left side completely infected and the right lung partially infected (the dark areas), with tuberculosis.

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SECTION OVERVIEW

7.5: Measuring Drug Susceptibility

Topic hierarchy

7.5.1: Minimal Inhibitory Concentration (MIC)

7.5.2: Kirby-Bauer Disk Susceptibility Test

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7.5.1: Minimal Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration is the lowest drug concentration that prevents visible microorganism growth after overnight incubation.

Learning Objectives

- Analyze data to interpret minimal inhibitory concentration values

Key Points

- Minimum inhibitory concentration (MIC) can be determined by culturing microorganisms in liquid media or on plates of solid growth medium.
- A lower MIC value indicates that less drug is required for inhibiting growth of the organism; therefore, drugs with lower MIC scores are more effective antimicrobial agents.
- By identifying appropriate drugs and their effective concentrations, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains.

Key Terms

- **culture**: The process of growing a bacterial or other biological entity in an artificial medium.
- **minimum inhibitory concentration**: This is the lowest concentration of an antimicrobial drug that prevents visible growth of a microorganism after overnight incubation with media.
- **bacteriostatic**: A drug that prevents bacterial growth and reproduction but does not necessarily kill them. When it is removed from the environment the bacteria start growing again.

Definition and Measurement

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation. MICs can be determined on plates of solid growth medium (called agar, shown in the “Kirby-Bauer Disk Susceptibility Test” atom) or broth dilution methods (in liquid growth media, shown in) after a pure culture is isolated. For example, to identify the MIC via broth dilution, identical doses of bacteria are cultured in wells of liquid media containing progressively lower concentrations of the drug. The minimum inhibitory concentration of the antibiotic is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth. There are also several commercial methods available to experimentally measure MIC values.



Figure: **Microbroth Dilution Method**: To identify the lowest concentration required for a given antibiotic to inhibit bacterial growth, an identical amount of bacteria is introduced into wells of liquid media containing progressively lower concentrations of the drug. (Here, the dilution series of the drug is set up from left to right: for example, well E1 might contain 100 units of drug; E2, 50 units; E3, 25 units; E4, 12.5 units; etc.). Because bacterial growth made the media in well E5 cloudy and the media in well E4 is indistinguishable from clear media, this indicates that the minimum inhibitory concentration is between the drug concentrations in wells E4 and E5. (Image courtesy of Microrao, Dept. of Microbiology, JJMMC, Davangere).

Significance and Applications

An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Because a lower MIC value indicates that less of the drug is required in order to inhibit growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents. Currently, there are a few web-based, freely accessible MIC databases. MIC scores are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. Clinicians use MIC scores to choose which antibiotics to administer to patients with specific infections and to identify an effective dose of antibiotic. This is important because populations of bacteria exposed to an insufficient concentration of a particular drug or to a broad-spectrum antibiotic (one designed to inhibit many strains of bacteria) can evolve resistance to these drugs. Therefore, MIC scores aid in improving outcomes for patients and preventing evolution of drug-resistant microbial strains.

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7.5.2: Kirby-Bauer Disk Susceptibility Test

Learning Objectives

- Review the procedure for the Kirby-Bauer antibiotic tes

Kirby-Bauer antibiotic testing (also called KB testing or disk diffusion antibiotic sensitivity testing) uses antibiotic-containing wafers or disks to test whether particular bacteria are susceptible to specific antibiotics. First, a pure culture of bacteria is isolated from the patient. Then, a known quantity of bacteria are grown overnight on agar (solid growth media) plates in the presence of a thin wafer that contains a known amount of a relevant antibiotic. If the bacteria are susceptible to the particular antibiotic from a wafer, an area of clear media where bacteria are not able to grow surrounds the wafer, which is known as the zone of inhibition. A larger zone of inhibition around an antibiotic-containing disk indicates that the bacteria are more sensitive to the antibiotic in the disk.

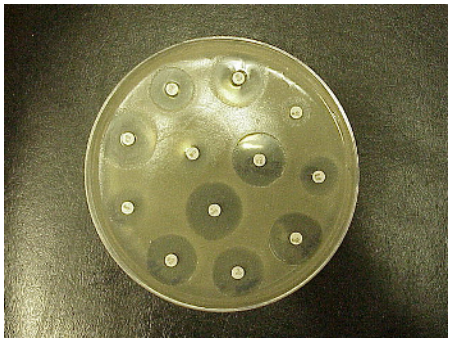


Figure: ***Kirby-Bauer test****: In Kirby–Bauer testing, discs containing antibiotics are placed on agar where bacteria are growing, and the antibiotics diffuse out into the agar. If an antibiotic stops the bacteria from growing, one can see circular areas around the wafers where bacteria have not grown.*

KB tests are performed under standardized conditions and standard-sized zones of inhibition have been established for each antibiotic. KB test results are usually reported as sensitive, intermediate, or resistant, based on the size of the zone of inhibition. If the observed zone of inhibition is greater than or equal to the size of the standard zone, the microorganism is considered to be sensitive to the antibiotic. Conversely, if the observed zone of inhibition is smaller than the standard size, the microorganism is considered to be resistant. The size of a zone of inhibition in a KB test is inversely related to the minimum inhibitory concentration (MIC), which is the amount of antibiotic required to prevent bacterial growth in an overnight culture. The MIC (in µg/ml) can be calculated from known standard-curve (linear regression) graphs based on the diameter of the observed inhibition zone diameter (in millimeters).

Clinicians can use KB test results to choose appropriate antibiotics to combat a particular infection in a patient. Administering antibiotics that specifically target the particular bacteria that are causing the infection can avoid using broad-spectrum antibiotics, which target many types of bacteria. Thus, clinical application of KB testing results can decrease the frequency with which antibiotic-resistant bacteria evolve.

Key Points

- KB tests are performed under standard conditions, so the minimum inhibitory concentration for a given antibiotic can be calculated by comparing the observed zone of inhibition ‘s size to known values.
- Clinicians use KB test results to choose antibiotics effective against the specific bacteria causing a patient’s infection. Using specifically-targeted antibiotics helps decrease the frequency of drug-resistant bacteria evolving.

Key Terms

- **Kirby-Bauer antibiotic testing**: This is a method to determine the sensitivity of microorganisms to specific antimicrobial drugs; greater drug efficacy yields larger microbe-free zones surrounding drug-containing disks after overnight growth on solid media.

- **zone of inhibition**: This is an area of media where bacteria are unable to grow, due to presence of a drug that impedes their growth.
- **minimum inhibitory concentration**: This is the lowest concentration of an antimicrobial drug that prevents visible growth of a microorganism after overnight incubation with media.

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SECTION OVERVIEW

7.6: Drug Resistance

Topic hierarchy

7.6.1: Mechanisms of Resistance

7.6.2: Antibiotic Misuse

7.6.3: Cost and Prevention of Resistance

7.6.4: Biofilms, Persisters, and Antibiotic Tolerance

7.6.5: Finding New Antimicrobial Drugs

7.6.6: Antimicrobial Peptides

7.6.7: Antisense Agents

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7.6.1: Mechanisms of Resistance

Development of microbial resistance to antimicrobial agents requires alterations in the microbe's cell physiology and structure.

Learning Objectives

- Describe the mechanisms bacteria use to develop antimicrobial resistance and the factors that can lead to it

Key Points

- Antimicrobial resistance can be mediated by the environment or the microorganism itself.
- Environmentally-mediated antimicrobial resistance results from physical or chemical characteristics of the environment that can affect the antimicrobial agent or the microorganism.
- Microorganism-mediated antimicrobial resistance can be intrinsic or acquired.

Key Terms

- **intrinsic**: innate, inherent, inseparable from the thing itself, essential.

Development of microbial resistance to antimicrobial agents requires alterations in the microbe 's cell physiology and structure. Antimicrobial resistance is defined as the loss of susceptibility to an extent that the drug is no longer effective for clinical use against an organism. Resistance can be mediated by the environment or the microorganism itself.

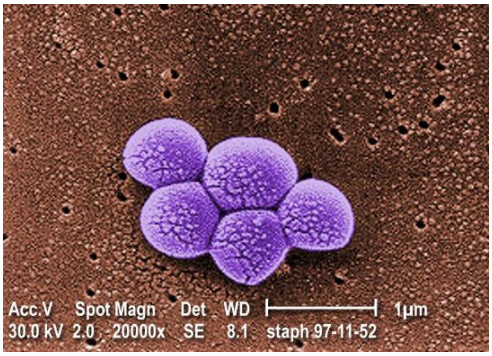


Figure: **Resistant bacterial strain**: Methicillin-resistant Staphylococcus aureus.

Environmentally-mediated antimicrobial resistance is affected by the environment's chemical and physical properties such as pH, anaerobic conditions, cation concentrations (calcium, magnesium), and thymine-thymidine content (available metabolites and nutrients).

Microorganism-mediated antimicrobial resistance is due to genetically-encoded traits of the microorganism and can be divided into intrinsic or acquired. Intrinsic resistance is considered to be a natural and inherited property with high predictability. Once the identity of the organism is known, the aspects of its anti-microbial resistance are also recognized. On the other hand, acquired resistance results from a change in the organism's genetic makeup. This trait is associated with only some strains of an organism's group but not the others. It is also an unpredictable trait and necessitates the development of laboratory methods to detect it. Microorganism-mediated antimicrobial resistance is acquired by gene change or exchange such as genetic mutations, acquisition of genes from other organisms via gene transfer mechanisms, or a combination of mutational and gene transfer events. Some common pathways bacteria use to effect antimicrobial resistance include: enzymatic degradation or modification of the antimicrobial agent, decreased uptake or accumulation of the antimicrobial agent, altered antimicrobial target, circumvention of consequences of antimicrobial actions, uncoupling of antimicrobial agent-target interaction, or any combination of these mechanisms.

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7.6.2: Antibiotic Misuse

LEARNING OBJECTIVES

- Explain the effects of antibiotic misuse

With the introduction of antibiotics into medical practice, clinically-relevant bacteria have had to adopt resistance mechanisms as part of their survival strategy. Antibiotic resistance occurs when antibiotics no longer work against disease-causing bacteria. These infections are difficult to treat and can mean longer-lasting illnesses, more doctor visits or extended hospital stays, and the need for more expensive and toxic medications. Some resistant infections can even cause death. Developing new antibiotics and other treatments to keep pace with antibiotic-resistant strains of bacteria is necessary. However, using antibiotics wisely is equally important for preventing the spread of resistant strains.

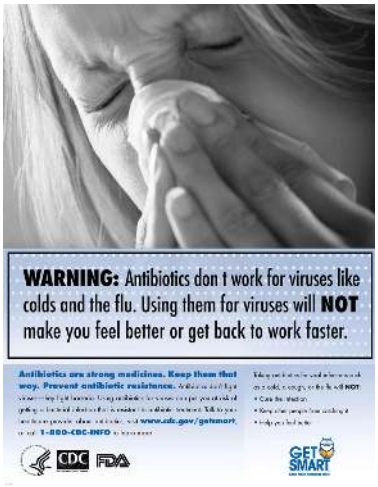


Figure: ***Figure* 7.6.2.1***: Antibiotic misuse. Antibiotics are not effective against viral infections. Misusing them leads to resistant bacterial strains.*

Antibiotic misuse has contributed largely to the emergence of new resistant strains. It is caused by taking an antibiotic too often for a condition it cannot treat such as viral infections and the common cold or in the wrong doses. It can also be manifested by not finishing a course of antibiotics as prescribed (stopping the antibiotic before the infection is fully cleared from the body). Overuse of antibiotics affects the bodsy's normal flora and disrupts the balance between beneficial bacteria that help digestion for example, and harmful bacteria. Excessive use of antibiotics in intensive farming units, particularly pig and poultry farms, is also seen as a growing threat. Scientists say antimicrobial resistance may be passing between animals and humans through food consumption, making the need to cut unnecessary use of antibiotics in farming even more urgent. Responsible antibiotic use in industry, and good practice for patients and physicians, are essential to keep resistant bacterial strains curable, and antibiotic treatment affordable to patients.

Key Points

- Antimicrobial resistance is a major public health concern.
- Antimicrobial resistance is brought about by antibiotic misuse, such as overuse, misuse, or interrupted treatment.
- Food industries, physicians, and patients play a role in minimizing the spread of resistance by adhering to good antibiotic practice.

Key Terms

- **course of antibiotics**: a period of continuous treatment with a drug.

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7.6.3: Cost and Prevention of Resistance

LEARNING OBJECTIVES

- Examine the causes and effects of multidrug-resistant organisms on healthcare

Prevention and control of microbial-resistant organisms is one of the most complex management issues that health care professionals face. The clinical and financial burden to patients and health care providers is staggering. Patients who are infected with bacterial strains resistant to more than one type or class of drugs (multidrug-resistant organisms, MDRO) often have an increased risk of prolonged illness, extended hospital stay, and mortality.

The cost of care for these patients can be more than double compared to those without an MDRO infection. The alternative medication they are prescribed to overcome the infection is often substantially more costly. Multidrug resistance forces healthcare providers to use antibiotics that are more expensive or more toxic to the patient.



Figure: ***Antibiotics****: Antibiotic misuse is a major cause of the staggering healthcare costs for the treatment of resistant bacterial strains.*

When no antibiotic is effective, healthcare providers may be limited to providing supportive care rather than directly treating an infection. In a 2008 study of attributable medical costs for antibiotic resistant infections, it was estimated that infections in 188 patients from a single healthcare institution cost between $13.35 and $18.75 million dollars.

Research and development of new drugs effective against resistant bacterial strains also comes at a cost. To prevent antimicrobial resistance, the patient and the healthcare provider should discuss the appropriate medicine for the illness. Patients should follow prescription directions and should not share or take medicine that was prescribed for someone else; these virtues should be strictly practiced. Healthy lifestyle habits, including proper diet, exercise, and sleeping patterns, as well as good hygiene such as frequent hand washing, can help prevent illness. These practices, therefore, also help prevent the overuse or misuse of antibiotics and the emergence of problematic resistant strains.

Key Points

- Antimicrobial resistance to available drugs requires the development of new drugs to effectively treat resistant strains and reduce mortality from bacterial infections.
- Antimicrobial resistance can be prevented by practicing good hygiene, and being responsible with antibiotic use.
- Treating antibiotic-resistant bacterial strains is expensive for both the patient and the healthcare provider. The treatment requires extended hospital stay and costly medications.

Key Terms

- **multidrug resistance**: A condition enabling a disease-causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism.

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7.6.4: Biofilms, Persisters, and Antibiotic Tolerance

Biofilms and persisters are bacterial communities responsible for chronic diseases and antibiotic tolerance.

Learning Objectives

- Explain the role of biofilms and persisters in multidrug tolerance, distinguishing this from multidrug resistance

Key Points

- Biofilms are aggregates of microbial cells that form to avoid antimicrobial agents or attack by the immune system.
- Persisters are slow-growing, dormant microbial cells that can tolerate antibiotic treatment.
- Biofilms and persisters are responsible for chronic bacterial infections and recurrent disease.
- Antibiotic tolerance is different from antibiotic resistance but equally important as a public health burden for eradication of serious bacterial diseases.

Key Terms

- **gingivitis**: inflammation of the gums or gingivae
- **extracellular matrix**: All the connective tissues and fibers that are not part of a cell, but rather provide support.

Biofilms are bacteria that have formed a gated community. Biofilms are composed of an aggregate of bacterial cells and are essentially considered a multi-cellular organism. They are characterized by structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances. They live on solid surfaces (e.g., catheters,) and the extracellular material they produce protects them from external threats, such as attacks by the body's immune cells. The property of biofilms constitute a penetration barrier for most antibiotics therefore preventing the drug from reaching the microbes. It is being widely recognized that bacterial biofilms are responsible for several chronic diseases that are difficult to treat, hence hard to eradicate (e.g., cystitis, endocarditis, urinary tract infections, gingivitis, dental plaque, and other yet to be identified conditions). They differ from free-floating or planktonic bacteria that cause acute infections and are managed by antimicrobial drugs.

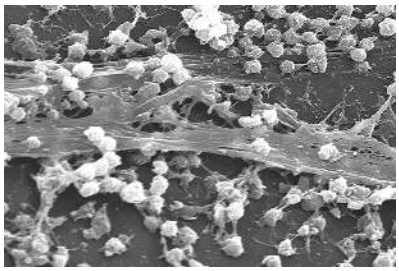


Figure: **Staphylococcus aureus biofilm**: Staphylococcus aureus forming a biofilm on a catheter.

Persisters are multidrug tolerant cells present in all bacterial populations. Bacterial populations that produce persister cells that neither grow nor die in the presence of microbicidal antibiotics are largely responsible for high levels of biofilm tolerance to antimicrobials. Persisters are not mutants, but rather phenotypic variants of the wild-type that upon inoculation produce a culture with similar levels of tolerance. Elimination of persisters remains an obstacle for the eradication of some tenacious and highly recurrent bacterial infections. Biofilms and persisters are the cause of multidrug tolerance. Multidrug tolerance differs from multidrug resistance in that it is not caused by mutant microbes but rather by microbial cells that exist in a transient, dormant state. These non-dividing cells often survive antibiotic exposure targeted to kill highly proliferating bacteria.

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7.6.5: Finding New Antimicrobial Drugs

Antimicrobial resistance has created a public health crisis in the treatment of infectious diseases and necessitates the discovery of new drugs.

Learning Objectives

- Explain the reasons for low production of new antibiotics and discuss the proposed mechanisms to evade antimicrobial resistance

Key Points

- Finding new antimicrobial drugs requires researchers, pharmaceutical, and biotech companies to invest in new technology and discover new sources for antibiotic development.
- Proposed mechanisms to circumvent antimicrobial resistance range from exploring the list of resistance genes to antibody-based therapy and vaccines.
- Finding new candidates to target is essential but it needs to be accompanied by awareness on antibiotic misuse with the prospect to eliminate the root of the problem.

Key Terms

- **mimetic**: A substance with similar pharmacological effects to another substance.

Antimicrobial resistance: the problem

Antibiotics, more than any other medicines, have improved the life expectancy of mankind, however, multi-drug resistance has become common in pathogenic bacteria and multiple drugs are losing efficacy. Recent reports on the occurrence of panresistant gram-negative strains, i.e. strains resistant to every registered antibacterial drug, indicate that we are on the verge to lose the battle, taking us back to the pre-antibiotic era. There is world-wide consensus that the medical need for novel antiinfective drugs is enormous and that we are running out of time. Many achievements of modern medicine, not only treatment of infectious diseases, depend on the availability of efficacious antibiotics, still, the antibacterial development pipeline is slow and the number of new drugs reaching the market is alarmingly low. There are many reasons for this at all levels of the discovery and development process. Investments into antibiotic research and technologies is minimal; socioeconomic considerations together with regulatory hurdles have prompted pharmaceutical companies to exit the field and innovative biotech companies were confronted with problems beyond their control. Answers are needed as to where and how we can find new lead compounds with unprecedented activities?

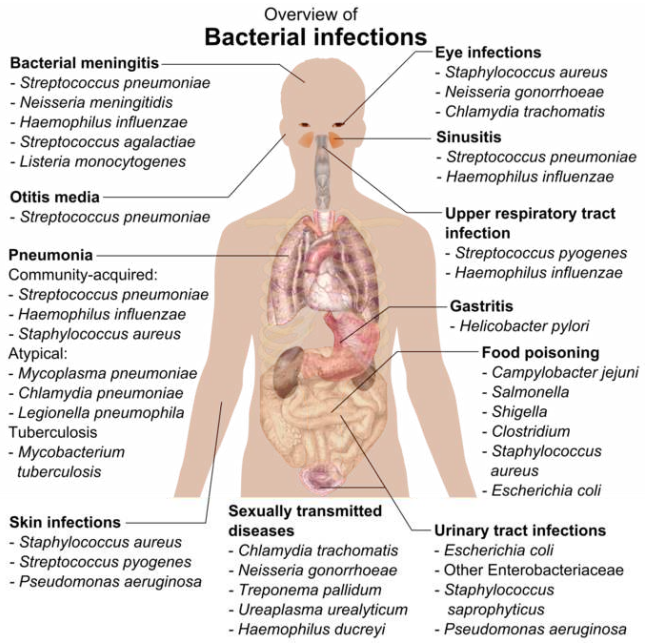


Figure: **Bacterial infections of the human body**: most bacterial species listed in this figure have developed resistance to available antibiotics necessitating new drug discovery.

Finding new antimicrobial drugs: the solution

Research on new antimicrobial compounds is geared towards innovative targets to circumvent resistance. Some of the proposed areas to investigate include: collecting and examining the list of antimicrobial resistance genes (e.g. exploring the resistome), targeting teichoic acid biosynthesis as a new method to compromise the bacterial wall integrity, producing ribosomal inhibitors to target protein synthesis, targeting outer-membrane transporters with protein epitope mimetics (e.g. mimetics of the cationic antimicrobial peptides that form part of the immune response to microbes), and developing antibody-based strategies and vaccines. The initiative to develop new antimicrobial agents is urgently needed but is a long process from invention, to development, to actual clinical application. It is also necessary to initiate a worldwide awareness on antibiotic misuse and overuse as a mean to address the root of the problem for antimicrobial resistance.

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7.6.6: Antimicrobial Peptides

Antimicrobial peptides exhibit cytotoxic activity against all microbes.

Learning Objectives

- Discuss the structure, mechanism, and targets of antimicrobial peptides

Key Points

- Antimicrobial peptides (AMPs) are a unique and assorted group of molecules produced by living organisms of all types, considered to be part of the innate immunity of a host.
- These peptides demonstrate potent antimicrobial activity and are rapidly mobilized to neutralize a broad range of microbes, such as viruses, bacteria, protozoa, and fungi.
- The ability of these natural molecules to kill multidrug-resistant microorganisms has gained them considerable attention and clinical interest.

Key Terms

- **neutropenia**: A hematological disorder characterized by an abnormally low neutrophil count.
- **atopic dermatitis**: An atopic, hereditary, and non-contagious skin disease characterized by chronic inflammation of the skin.

A first line of defense against pathogenic insult is called the innate immune system, which is followed by acquired immune responses associated with the activation of T and B cells aimed against specific antigens. In contrast to the clonal, acquired adaptive immunity, endogenous peptide antibiotics or antimicrobial peptides provide a fast and energy-effective mechanism as front-line defense.

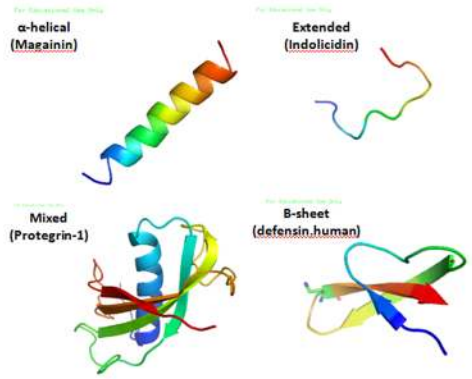


Figure: **Various AMPs**: These are various antimicrobial peptide structures.

Antimicrobial peptides (AMPs) are small molecular weight proteins with broad spectrum antimicrobial activity against bacteria, viruses, and fungi. They are classified on the basis of their structure and amino acid motifs. Peptides of the defensin, cathelicidin, and histatin classes are found in humans. These evolutionarily conserved peptides are usually positively charged and have both a hydrophobic and hydrophilic side that enables the molecule to be soluble in aqueous environments yet also enter lipid-rich membranes. Once in a target microbial membrane, the peptide kills target cells through diverse mechanisms. AMPs secrete lytic enzymes, nutrient-binding proteins or contain sites that target specific microbial macromolecules.

Cathelicidins and defensins are major groups of epidermal AMPs. Decreased levels of these peptides have been noted for patients with atopic dermatitis and Kostmann's syndrome, a congenital neutropenia. AMPs have proven effective against multidrug-resistant microbes. In addition to important antimicrobial properties, growing evidence indicates that AMPs alter the host immune response through receptor-dependent interactions. AMPs have been shown to be important in such diverse functions as angiogenesis, wound healing, cytokine release, chemotaxis, and regulation of the adaptive immune system. These peptides qualify as innovative drugs that might be used as antibiotics, anti-lipopolysaccharide drugs, or modifiers of inflammation reactions.

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7.6.7: Antisense Agents

LEARNING OBJECTIVES

- Discuss the mechanism of antisense agents and the advantages and disadvantages of antisense therapy.

Antisense agents are synthetic, single-stranded short sequences of DNA bases designed to hybridize to specific sequences of messenger RNA (mRNA) forming a duplex. This DNA-RNA coupling attracts an endogenous nuclease, RNase H that destroys the bound RNA and frees the DNA antisense to rehybridize with another copy of mRNA. In this way, the effect is not only highly specific but prolonged because of the recycling of the antisense DNA sequence. When this agent binds to the pathogen DNA or messenger RNA, the biosynthesis of target proteins is disrupted. Therefore, there are at least two ways in which antisense agents act to effectively reduce the amount of pathogenic protein being synthesized – RNase H based degradation of RNA and prevention of ribosomal assembly and translation. This approach has a great advantage. It prevents a pathogenic protein from being produced, rather than trying to selectively neutralize it once it is made.

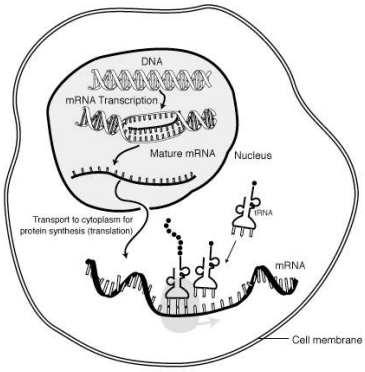


Figure: **DNA to Protein**: Role of messenger RNA in protein synthesis.

Antisense agents can be specifically targeted to genes that control expression of antibiotic resistance mechanisms, thereby potentially restoring an antibiotic-sensitive phenotype to the cell. A limiting factor in their potential application as therapeutic agents for bacterial infections is their poor uptake by bacterial cells. These agents have been successfully developed for the treatment of viral infections such as cytomegalovirus, hepatitis C, and HIV infections. The advantage of antisense therapy is that they can be manufactured fairly fast, they produce a lasting clinical effect, and they are highly specific to the target. Antisense agents also exhibit efficacy in broader clinical applications such as cancer therapy.

Key Points

- Antisense agents have broad applications in several diseases. Their use for treating microbial infections is promising.
- They are synthetic oligonucleotides that can be manufactured quickly and their biological effect is long-lasting.
- Their use for the treatment of antibiotic resistant bacterial infections is possible but limited by their poor uptake by the bacterial cell. Studies are being developed to improve their penetration into the cell.

Key Terms

- **messenger RNA**: RNA that encodes and carries information from DNA during transcription to sites of protein synthesis to undergo translation in order to yield a protein
- **nuclease**: Any of several enzymes capable of cleaving the phosphodiester bonds between the nucleotide subunits of nucleic acids.
- **hybridize**: To combine complementary subunits of multiple biological macromolecules.

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SECTION OVERVIEW

7.7: Antiviral Drugs

Topic hierarchy

7.7.1: Antiviral Agents that Prevent Virus Uncoating or Release

7.7.2: Antiviral DNA Synthesis Inhibitors

7.7.3: Nucleotide and Nonnucleotide Reverse Transcriptase Inhibitors

7.7.4: Protease Inhibitors

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7.7.1: Antiviral Agents that Prevent Virus Uncoating or Release

Different approches are used to target the initial and final steps of a virus life cycle.

Learning Objectives

- Compare the mechanisms of the discussed antiviral drugs

Key Points

- The attachment step is targeted by molecules that will block the receptor on the host cell surface, or on the viral capsid region responsible for binding to the host receptor.
- Drugs that target the uncoating step bind to, and inactivate, proteins on the capsid surface responsible for the uncoating.
- The release step is targeted by drugs that inhibit the activity of neuraminidase, an enzyme on the viral surface.

Key Terms

- **sialic acid**: A derivative of neuraminic acid (a nine-carbon monosaccharide) that is often the sugar part of glycoproteins.

A viral infection starts with entry of the virus into the cell. The entry mechanism is complex, consists of multiple steps and involves host cell structures.

Targeting the Attachment Step

Virus infection starts with a virus attaching to the host cell by binding to a receptor molecule. There are two main strategies used to design antiviral drugs at this step:

- Using molecules that will bind to the cell receptor and inactivate it; thus preventing the virus from attachment. Examples include anti-receptor antibodies or natural ligands that can bind to the receptor.
- Using receptor-like molecules to bind to the virus and inactivate it before it meets the cell. These include anti-virus antibodies (with specificity against the viral structure that binds to the receptor) or synthetic molecules that mimic the receptor.

The search for such drugs, however, is very expensive and time-consuming.

Targeting the Uncoating Step

Another drug target is the uncoating step during viral infection. Uncoating is the process of capsid disintegration, which leads to the release of the genomic material. This step is performed by viral or host enzymes, or by capsid dissociation alone. Drugs that can perform such functions are used against the influenza virus, rhinoviruses (the cause of the common cold), and enteroviruses (gastrointestinal infections, meningitis, etc.). It is believed that such drugs prevent the virus from uncoating by blocking the proteins on the capsid responsible for uncoating, such as ion channel proteins. An example of such a drug is Rimantadine, which blocks the ion channel in the influenza virus. The ion channel has an important role in disintegrating the viral capsid.

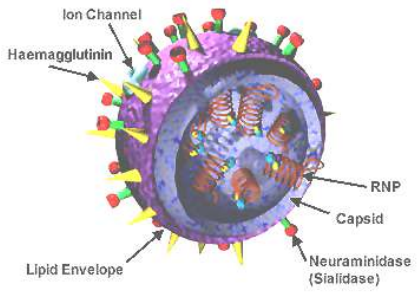


Figure: **Structure (3D) of the Influenza Virus**: The image depicts the major components of the virus structure, including the neuraminidase.

Targeting the Release of the Newly Formed Viral Particles

The last step in the virus life cycle—release from the cell—has been targeted by drugs as well. Neuraminidase is an enzyme on the capsid of influenza virus. It cleaves sialic acid from glycoproteins on the surface of the host cell and allows the viral particles to leave the cell. Tamiflu and Relenza are trend names of two drugs used to treat influenza infections by targeting neuraminidase.

Since viruses use many structures in the host cells to replicate, designing or discovering good antiviral drugs that will not affect the eukaryotic cells is a challenging task. Serious side effects are often observed with the use of antiviral drugs, as is resistance against the drugs. Developing drugs that inhibit different steps in the virus life cycle is of critical importance.

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7.7.2: Antiviral DNA Synthesis Inhibitors

Inhibiting DNA synthesis during viral replication is another key approach in battling viral infections.

Learning Objectives

- Review the mechanism of action for antiviral DNA synthesis inhibitors and recognize the types of these inhibitors

Key Points

- Drugs such as acyclovir, are nucleoside analogues that lack a free 3′ group that is needed for the addition of the next nucleotide. When added into a growing DNA chain they stop its synthesis.
- Another drug, foscarnet, mimics pyrophosphates and inactivates the activity of the viral DNA polymerase.
- Resistance can develop against both of these groups of drugs.

Key Terms

- **CMV retinitis**: An inflammation of the eye's retina caused by CMV. It can lead to blindness.

Inhibiting DNA synthesis during viral replication is another approach to battle viral infections.

The most common strategy used for this approach is to use molecules that mimic the structure of a nucleoside. The similarity is good enough to ensure its incorporation into the newly synthesized DNA chain. However, the nucleoside analogue lacks free 3′ end needed for the addition of the next nucleotide. This prevents the incorporation of the next nucleotide and terminates the elongation of the DNA chain.

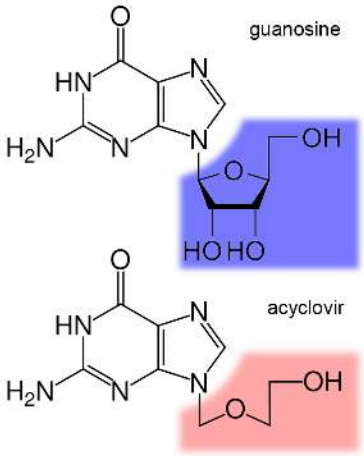


Figure: **Comparison of acyclovir and guanosine**: Acyclovir does not contain a sugar molecule with a 3′-OH group and will interrupt the synthesis of a newly synthesized nucleotide chain if added to it. The guanosine depicted in this specific image is used for RNA synthesis but acyclovir inhibits the synthesis of DNA synthesis.

One of the most often used antiviral drugs that works with the described mechanism is acyclovir (aciclovir), a guanosine analogue. It is used to treat herpes simplex virus infections (type 1 and type 2) as well as chicken pox and shingles. It was designed based on nucleosides isolated from a Caribbean sponge. After administration, the molecule gets activated by phosphorylation both by viral and host cell kinases and the resulting nucleotide incorporated into the newly synthesized DNA resulting in premature chain termination. The drug has very low cytotoxicity and there is low resistance to it.

Other drugs that are also nucleoside analogues and have the same mode of actions are ganciclovir (a synthetic analogue of 2′-deoxy-guanosine) and vidarabine(an adenosine analog). However, both drugs are more toxic and have more serious side effects than acyclovir.

Another type of drug that is a DNA synthesis inhibitor is foscarnet. It mimics pyrophosphate and inactivates the activity of the DNA polymerase. This inhibitor is active against the viral DNA polymerases at doses much lower than the ones needed to inhibit

the human polymerases. This drug is used in cases of resistance against acyclovir and ganciclovir nucleoside analogue chemicals. It is also used to treat cytomegalovirus infection (CMV) and specifically CMV retinitis.

Another antiviral drug that targets DNA synthesis is hydroxycarbamide, commonly referred to as a hydroxyurea. Hydroxycarbamide can be used an antiretroviral drug against HIV/AIDS. The mechanism of hydroxycarbamide is thought to be based on the reduction of production of deoxyribonucleotides; therefore, inhibiting DNA synthesis. Hydroxycarbamide is thought to inhibit the enzyme ribonucleotide reductase.

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7.7.3: Nucleotide and Nonnucleotide Reverse Transcriptase Inhibitors

Reverse transcriptase in viruses is inhibited by nucleoside (nucleotide) analogues or drugs that change the conformation of the enzyme.

Learning Objectives

- Summarize the mechanism of action for reverse transcriptase inhibitors

Key Points

- Nucleoside and nucleotide inhibitors are competitive substrate inhibitors that mimic the structure of a normal nucleotide but lack the 3′ hydroxyl group needed for the addition of the next nucleotide for DNA elongation.
- Non-nucleotide inhibitors bind to a site different than the active one and cause rearrangements of the protein domains needed for DNA polymerization.
- Mutations in the reverse transcriptase gene can cause resistance to both types of drugs.

Key Terms

- **competitive substrate inhibitors**: Molecules that bind to the active site of an enzyme and prevent the real substrate from binding to it.
- **non-competitive inhibitors**: Molecules that bind to sites other than the active site of an enzyme while still being able to indirectly inhibit its function.
- **nucleotide**: the monomer comprising DNA or RNA biopolymer molecules, consisting of a nitrogenous heterocyclic base; a five-carbon pentose sugar; and a phosphate group

Reverse transcriptase is an enzyme that has the ability to transcribe single-stranded DNA from a single-stranded RNA chain. This is the reverse of the usual flow of information when RNA is synthesized from DNA. Viruses that use reverse transcriptase to convert their genetic material (RNA) into DNA are called retroviruses. One of the most prominent representative of a retrovirus is HIV. Due to the high prevalence of HIV/AIDS in the world, it is important to have drugs that will prevent or cure the infection. This enzyme is also found in tumors and cancer cells.

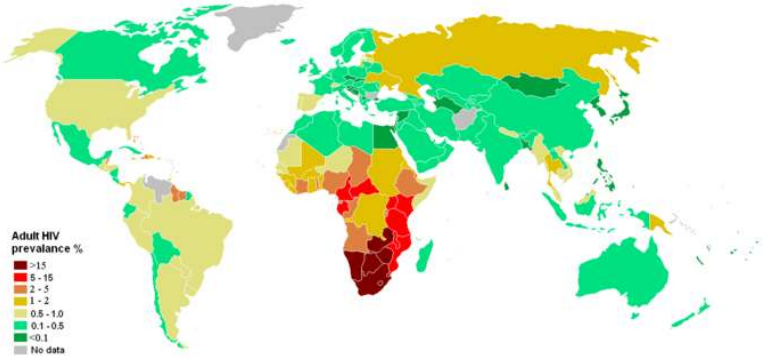


Figure: **Estimation of adults with HIV/AIDS by country**: The data shows the people, between 18-49 years, by country who are affected by HIV/AIDS. The information is provided by UNAIDS based on a report from July 2008

Drugs that inhibit the function of this enzyme are divided into three groups:

- nucleoside analog reverse transcriptase inhibitors
- nucleotide analog reverse transcriptase inhibitors
- non-nucleoside reverse transcriptase inhibitors

The first two inhibitors act on the same principle. They mimic, respectively, nucleosides or nucleotides but lack a free hydroxyl group at the 3′ end. The major difference between them is that the nucleosides need to be phosphorylated by cellular kinases. The enzyme reverse transcriptase recognizes them as regular nucleotides and inserts them into the newly synthesized DNA chain. But once inserted the elongation stops at them because no more nucleotides can be added due to the lack of the 3′ hydroxyl group and the inability of the formation of 5′-3′ phosphodiester bond. This process is called chain termination. Nucleoside and nucleotide

inhibitors are also called competitive substrate inhibitors. Examples of such drugs are Zidovudine (AZT) and Lamivudine. AZT was the first FDA approved drug for the treatment of HIV. Lamivudine is used for the treatment of both HIV and hepatitis B. Since some viruses, such as hepatitis B, carry RNA-dependent DNA polymerases reverse transcriptase inhibitors can be used to treat these infections as well.

Non-nucleotide reverse transcriptase inhibitors bind to a different site, not the active one, of the reverse transcriptase enzyme. That leads to conformational changes that distort the position of the DNA binding sites in the enzyme and lead to halt in DNA polymerization. Non-nucleotide inhibitors are non-competitive inhibitorsof reverse transcriptase. Such drugs are Efavirenz and Nevirapine.

Resistance occurs to all drug groups. The mechanisms for resistance against the nucleoside (nucleotide) inhibitors are two. The first one is due to mutations in the N-terminal polymerase domain of the reverse transcriptase that makes it less likely to incorporate the analogues. The second mechanism is caused by mutations in the transcriptase that allow the removal of the incorporated inhibitor and hence restart of DNA replication.

Resistance to the non-nucleotide inhibitors is caused by mutations in the inhibitor binding site of the enzyme. Such mutations prevent the binding of the inhibitor to the enzyme.

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7.7.4: Protease Inhibitors

Protease inhibitors target viral proteases which are key enzymes for the completion of viral maturation.

Learning Objectives

- Describe the mechanism of action for protease inhibitors

Key Points

- Protease inhibitors mimic peptides or are chemicals that can be inserted in the active site of a protease. They prevent it from binding the viral polyproteins.
- Such drugs were one of the first to be used against HIV. They are an inseparable part of the HIV/AIDS therapy.
- Mutations in the enzyme active site and other sites, which cause conformational changes, can cause resistance.

Key Terms

- **cross-resistance**: Bacterial or viral resistance to a chemical which causes resistance to other chemicals of the same group.

Proteases are enzymes that have the ability to cut proteins into peptides. They are used by some viruses (e.g., HIV) to cleave precursor long protein chains into individual proteins. This allows the completion of the assembly step in the viral life cycle where the proteins and the viral RNA come together to form virion particles ready to exit the cell.

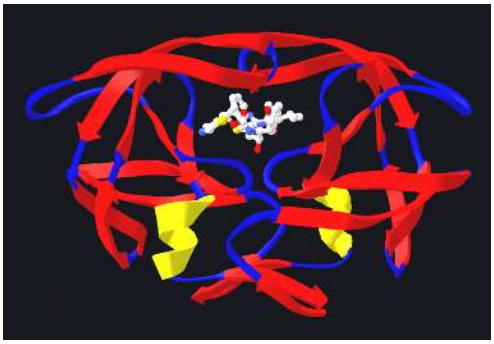


Figure: **HIV protease with bound protease inhibitor**: The drug is ritonavir depicted here with a white molecule in the middle of the enzyme structure.

The design of protease inhibitors, that could be used to battle HIV, started soon after the discovery of the virus. The first approved protease inhibitor drug was released on the market in 1995, only 10 years after the discovery of HIV. These drugs are an inseparable part of an HIV therapy. Natural protease inhibitors are found in Shiitake mushrooms. The experimental protease inhibitor drugs Zmapp and Brincidofovir are currently being tested to treat the ebola virus disease.

Protease inhibitors are short peptide-like molecules that are competitive inhibitors of the enzyme. Instead of -NH-CO- peptide link, they contain -(CH_2-CH(OH)-). When such a peptide gets into the enzyme active site, the protease is unable to cut the linkage and gets inactivated. This leads to a lack of cleavage of the polypeptide chains of two crucial viral proteins, Gag and Pol, which are essential structural and enzymatic proteins of HIV. Their absence blocks the formation of mature virion particles.

Saquinavir is the first clinically used peptide-like inhibitor. Some protease inhibitors do not mimic peptides in their structure. One such drug is *Nelfinavir*. In general, protease inhibitors exhibit the unusual side effect of fat storage in non-typical organs and tissues. The reasons for this are still unclear.

Mutations in the enzyme active site and other sites, which cause conformational changes, can cause resistance. Quite often one mutation can lead to resistance to many different drugs simultaneously since they all share the same mode of action. This is called cross-resistance. It is one of the major drawbacks of protease inhibitors therapy.

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SECTION OVERVIEW

7.8: Other Antimicrobial Drugs

Topic hierarchy

7.8.1: Antifungal Drugs

7.8.2: Antiprotozoan and Antihelminthic Drugs

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7.8.1: Antifungal Drugs

LEARNING OBJECTIVES

- Compare and contrast the mechanisms of action for: polyene, azole,allylamine and echinocandin antifungals

The field of mycology deals with the study of fungi and has resulted in the identification of over 60,000 species of fungi. Fungi are classified as eukaryotic organisms which are primarily classified based on spore production. The classification and identification of a species of fungi by spore class, in combination with the basic biological mechanism(s) it uses to sustain life, is key in developing anti-fungal drugs.

The development of antifungal drugs focuses on the classes of mycotic diseases for which fungi are responsible. These classes include hypersensitivity—allergic reactions based on the presence of mold and spores; mycotoxicoses—diseases based on the presence of fungi that produce toxins in animal feed and human food products; mycetismus—mushroom poisoning; and lastly, mycoses—characterized by infection.

Disease-causing fungi are targeted and then drug classes are classified based on drug structure or mechanism. Some of the major classes of antifungal medication include polyene antifungals, azole antifungals, allylamines, and echinocandins. It is important to note that antifungal drugs are not limited to these classes, as there are additional drugs capable of targeting fungi that do not fall into these categories.

Polyene Antifungals

Polyene antifungals are characterized by the presence of multiple conjugated double bonds in the drug structure. This specific antifungal drug class targets the fungal cell membrane. The target sterol is ergosterol, which is specific to fungi cell membranes; in animal cell membranes cholesterol is the key sterol. The fungal cell membrane becomes leaky, resulting in movement of essential cellular contents, such as organic molecules and ions, out of the cell. Amphotericin B is an example of a polyene antifungal; it is selective for ergosterol and can be used as a broad spectrum drug administered intravenously.

Azole Antifungals

Azole antifungals are characterized by the ability to inhibit ergosterol synthesis in fungal membranes. The biosynthesis of ergosterol requires the enzyme lanosterol 14 α-demethylase. This enzyme is needed to convert lanosterol to ergosterol. Targeting this enzyme prevents ergosterol production. Thus, the fungal membrane structure is depleted of ergosterol and the fungus dies. The various types of azole classified drugs include imidazole, triazole and thiazole antifungals. Azole drugs are broad-spectrum drugs and treat fungal infections of the skin or mouth. An example of an azole drug is Clotrimazole, commonly used to treat athlete's foot, ringworm, vaginal yeast infections, and oral thrush.



Figure: **Ringworm**: Ringworm on a human leg.

Allylamine Antifungals

Allylamine antifungals are characterized by the ability to inhibit fungal squalene metabolism. The biosynthesis of ergosterol requires an enzyme called squalene peroxidase. Squalene peroxidase is responsible for catalyzing the first step in ergosterol biosynthesis; inhibition of this enzyme results in disruption of ergosterol synthesis. The inhibition of squalene metabolism is toxic to the fungi because of the buildup of squalene and the inhibition of ergosterol synthesis. An example of an allylamine drug is Terbinafine, which is commonly used to treat fungal skin infections.

Echinocandin Antifungals

Echinocandins are characterized by their ability to inhibit synthesis of a key component of the *fungal cell wall*, while previously discussed drugs target the *fungal cell membrane*. Cell wall synthesis requires the production of glucan by the enzyme 1,3-β glucan synthase. Echinocandins specifically inhibit glucan synthesis by targeting that enzyme. This class of drug is most effective when administered by injection, as it is poorly absorbed when administered orally. Echinocandin injection allows the drug to treat a systemic infection of the sort typically seen in immunocompromised patients. An example of an echinocandin based drug is Caspofungin. Caspofungin blocks cell-wall synthesis by disrupting glucan synthesis; it can target invasive candidiasis and aspergillus.

Additional Antifungal Drugs

The major classes of antifungal drugs are discussed above are not the only drugs capable of effectively targeting fungi. The emergence of alternative medicine as a hot field of research has also increased the list of available antifungal compounds. For example, numerous compounds and essential oils found in nature have been found to have antifungal properties that could be utilized for treatment. Examples of these include coconut oil, orange oil, olive leaf, and zinc.

The identification of additional antifungal compounds is key to the development of drugs that can replace existing drugs to which fungi have developed resistance. The discovery process for effective and fungi-specific drugs is enduring and laborious, as the drugs must be specific for fungi cells. However, with the expansion of molecular studies in fungal organisms, the opportunity to identify novel and fungal specific mechanisms will allow for the development of new drugs.

Key Points

- The various classes of antifungal drugs exploit the unique fungal structure.
- The synthesis of both cell membrane and cell wall components are key targets for antifungal drugs.
- Classes of antifungal medications include: polyene antifungals, azole drugs, allylamines and echinocandins.

Key Terms

- **ergosterol**: major component of fungal cell membranes.
- **mycology**: the study of fungi

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7.8.2: Antiprotozoan and Antihelminthic Drugs

Antiprotozoan and antihelminthic drugs are characterized based on structure and the mechanism of action by which they target the organism.

Learning Objectives

- Describe the objective of drugs against helminths anf the disadvantages to developing drugs against protozoa

Key Points

- Antiprotozoan drugs are typically species specific.
- It is essential that antiprotozoan drugs target pathways and mechanisms specific to protozoa as overlap in the basic biology can result in inaccurate targeting of the drug to both the host and protozoa.
- Antihelminthic drugs typically focus on the disruption of structural mechanisms that maintain body structure resulting in paralysis of the helminth which promotes expulsion.

Key Terms

- **helminth**: A parasitic roundworm or flatworm.
- **protozoa**: Protozoa are a diverse group of unicellular eukaryotic organisms, many of which are motile. Originally, protozoa had been defined as unicellular protists with animal-like behavior, e.g., movement. Protozoa were regarded as the partner group of protists to protophyta, which have plant-like behavior, e.g., photosynthesis.
- **Parasite**: Parasitism is a non-mutual relationship between organisms of different species where one organism, the parasite, benefits at the expense of the other, the host.

Parasites are organisms that live on or in a host organism to obtain food. Two major classes of parasitic organisms include protozoa and helminths.

Protozoa are unicellular eukaryotic organisms that are classified as either free-living or parasitic organisms. Protozoa are further classified based on their mode of locomotion and include: Sarcodina (amoeba); Mastigophora (flagellates); Ciliophora (ciliates); and Sporozoa (non motile in adult form). Some examples of diseases caused by protozoa include: Malaria, Giardia, Trichomoniasis, and Leishmaniasis.

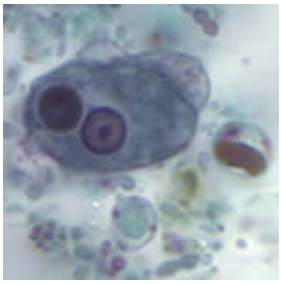


Figure: **Entamoeba histolytica**: Entamoeba histolytica is classified as a protozoa, specifically a Sarcodina.

Helminths are multicellular eukaryotic organisms that are also classified as either free-living or parasitic chemoheterotrophic organisms. Helminths are parasitic worms and are divided into three major groups including: flatworms (platyhelminths); thorny-headed worms (acanthocephalins); and roundworms (nematodes and hookworms).



Figure: **Hookworms**: Hookworms attached to the intestinal mucosa.

The variation that exists between protozoa contributes to complications associated with developing effective drugs. The lack of similarities between protozoans demands the need for highly specific drugs and medications against individual pathogens. In addition, protozoa are eukaryotic and exhibit similar properties and metabolic pathways as human cells. Therefore, the drugs that are developed to target protozoans are classified by either their mechanism of action or the organism for which they target. In terms of mechanism of actions, most antiprotozoan drugs specifically target the organism to prevent its growth and reproduction.

Protozoal diseases can also be prevented by targeting the route of transmission and/or targeting vector organisms. For example, Malaria is caused by the protozoan Plasmodia. This parasite is injected into humans via mosquitoes. The development of antimalarial drugs are based on the life cycle of Plasmodiain both the mosquito and human host.

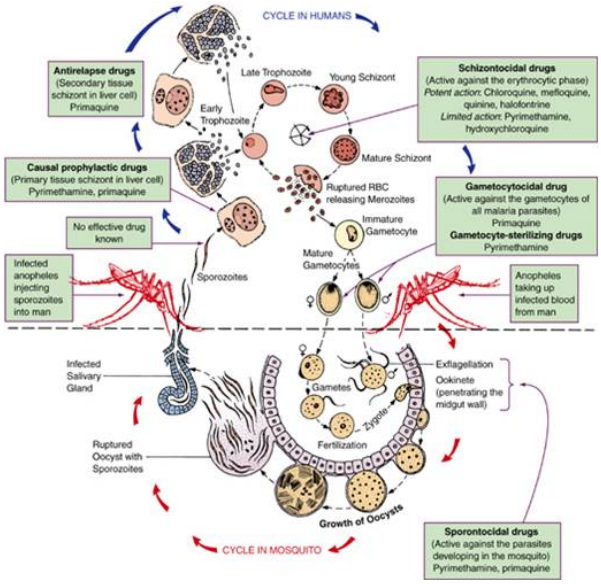


Figure: **Life Cycle of Plasmodium**: Malaria, a protzoan based disease, is transmitted via an arthropod vector. The development of antimalarial drugs focuses on the life cycle in both the mosquito and human hosts.

Other types of antiprotozan drugs specifically target metabolic mechanisms utilized by the parasite. For example, African sleeping sickness is caused by trypanosomes. The drug Eflornithine attacks this parasite by targeting an enzyme responsible for regulating cell division.

Helminths are characterized as various types of parasitic worms, which are effectively targeted by promoting expulsion from the body. Parasitic helminths worms include: tapeworms, flukes, leeches and hookworms. The drugs utilized to target helminths are characterized based on chemical structure and mechanism of action. A few examples of the major drug classes include: piperazine, benzimidazole, levamisole, pyrantel, morantel and emodepside.

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