ESC4103 - ENVIRONMENTAL MICROBIOLOGY



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Detailed Licensing

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CHAPTER OVERVIEW

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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.

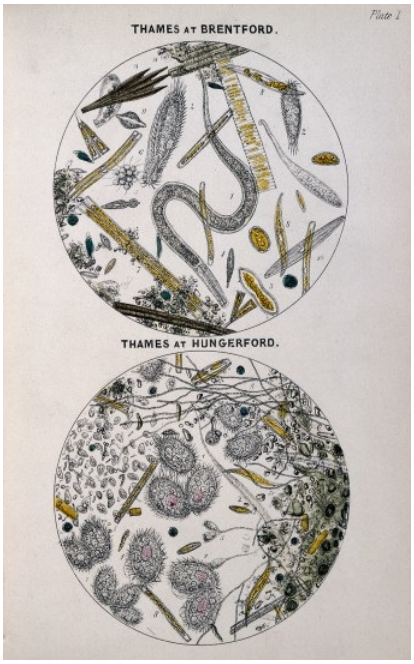


Figure: ***A Drawing of Microbes****: This is a drawing of what Arthur Hill Hassall saw under a microscope in a sample of water taken from the River Thames at two locations. Hassall was able to identify many microscopic organisms not perceptible to the unaided eye,.*

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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1.2: 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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1.3: 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.

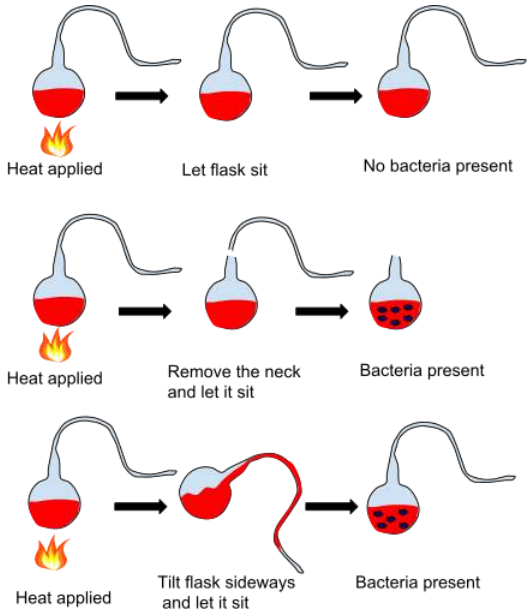


Figure: ***Pasteur's test of spontaneous generation***: *By sterilizing a food source and keeping it isolated from the outside, Pasteur observed no putrefaction of the food source (top panel). Upon exposure to the outside environment, Pasteur observed the putrefaction of the food source (bottom panel). 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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1.4: Koch and Pure Culture

Learning Objectives

- Explain Robert Koch's postulates

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



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

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

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

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

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Key Points

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

Key Terms

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

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CHAPTER OVERVIEW

2: Types and habitats

2.1: 1.2A Types of Microorganisms
2.2: Classification of Microorganisms
2.3: Microbes and the Origin of Life on Earth
2.4: Environmental Diversity of Microbes

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2.1: 1.2A Types of Microorganisms

Learning Objectives

- Define the differences between microbial organisms.

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.

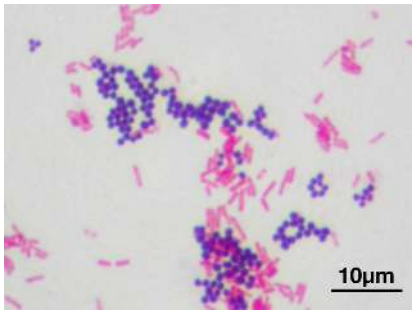


Figure: **Gram Stain**: This is a microscopic image of a Gram stain of mixed Gram-positive cocci (Staphylococcus aureus, purple) and Gram-negative bacilli (Escherichia coli, red).

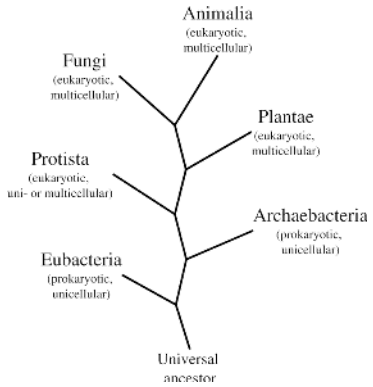


Figure: **Types of microorganisms**: This tree of life shows the different types of microorganisms.

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.

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2.2: Classification of Microorganisms

Learning Objectives

- Assess how early life changed the earth

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.

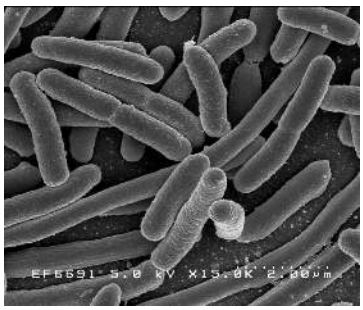


Figure: **Classification of E. coli**: Domain: Bacteria, Kingdom: Eubacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Enterobacteriales, Family: Enterobacteriaceae, Genus: Escherichia, Species: E. coli.

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.

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.

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2.3: Microbes and the Origin of Life on Earth

Learning Objectives

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

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.

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.

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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.

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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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CHAPTER OVERVIEW

3: Microbial Ecology

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  - 3.1A: Microbes and Ecosystem Niches
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SECTION OVERVIEW

3.1: Microbial Ecology

Topic hierarchy

3.1A: Microbes and Ecosystem Niches

3.1B: Organization of Ecosystems

3.1C: Role of Microbes in Biogeochemical Cycling

3.1D: Microbial Environments and Microenvironments

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3.1A: Microbes and Ecosystem Niches

Learning Objectives

- Evaluate microbes and the niches they occupy

Microbes and Ecosystem Niches

Microbial life is amazingly diverse and microorganisms quite literally cover the planet. In fact, it has been estimated that there are 100,000,000 times more microbial cells on the planet than there are stars in the observable universe! Microbes live in all parts of the biosphere where there is liquid water, including soil, hot springs, the ocean floor, acid lakes, deserts, geysers, rocks, and even the mammalian gut.

By virtue of their omnipresence, microbes impact the entire biosphere; indeed, microbial metabolic processes (including nitrogen fixation, methane metabolism, and sulfur metabolism) collectively control global biogeochemical cycling. The ability of microbes to contribute substantially to the function of every ecosystem is a reflection their tremendous biological diversity.



Figure: **A Biofilm of Thermophilic Bacteria**: Thermophiles, which thrive at relatively high temperatures, occupy a unique ecological niche. This image shows a colony of thermophilic bacteria at Mickey Hot Springs in Oregon, USA.

Microbes are vital to every ecosystem on Earth and are particularly important in zones where light cannot approach (that is, where photosynthesis cannot be the basic means to collect energy). Microorganisms participate in a host of fundamental ecological processes including production, decomposition, and fixation. They can also have additional indirect effects on the ecosystem through symbiotic relationships with other organisms. In addition, microbial processes can be co-opted for biodegradation or bioremediation of domestic, agricultural, and industrial wastes, making the study of microbial ecology particularly important for biotechnological and environmental applications.

Each species in an ecosystem is thought to occupy a separate, unique niche. The ecological niche of a microorganism describes how it responds to the distribution of resources and competing species, as well as the ways in which it alters those same factors in turn. In essence, the niche is a complex description of the ways in which a microbial species uses its environment.

The precise ecological niche of a microbe is primarily determined by the specific metabolic properties of that organism. For example, microbial organisms that can obtain energy from the oxidation of inorganic compounds (such as iron-reducing bacteria) will likely occupy a different niche from those that obtain energy from light (such as cyanobacteria). Even among photosynthetic bacteria, there are various species that contain different photosynthetic pigments (such as chlorophylls and carotenoids) that allow them to take advantage of different portions of the electromagnetic spectrum; therefore, even microbes with similar metabolic properties may inhabit unique niches.

Key Points

- Microbes live in all parts of the biosphere where there is liquid water.
- By virtue of their omnipresence, microbes impact the entire biosphere.
- Each microbial species in an ecosystem is thought to occupy a unique niche, which is a complex description of the ways in which an organism uses its environment.
- The precise ecological niche of a microbe is primarily determined by the specific metabolic properties of that organism.

Key Terms

- **bioremediation**: The use of biological organisms, usually microorganisms, to remove contaminants, especially from polluted water.

- **niche**: A function within an ecological system to which an organism is especially suited.
- **biosphere**: The part of the Earth and its atmosphere capable of supporting life.

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3.1B: Organization of Ecosystems

Learning Objectives

- Illustrate the organization of ecosystems

Although ecologists tend to regard ecosystems as basic structural units, it can be difficult (if not impossible) to formally define the boundaries of a given ecosystem. As such, ecosystems are better thought of as *conceptual* rather than *actual* geographical locations. Rarely are ecosystems isolated from one another; rather, they should be considered parts of a larger functioning whole that together comprise the biosphere ("the place on Earth's surface where life dwells").

Despite the fact that clear boundaries between ecosystems may be difficult to identify, the myriad interactions that take place *within* an ecological community can often be observed and defined. These interactions may be best described by detailing feeding connections (what eats what) among biota in an ecosystem, thereby linking the ecosystem into a unified system of exchange.

All life forms in an ecosystem can be broadly grouped into one of two categories (called trophic levels):

- Autotrophs, which produce organic matter (food) from inorganic substances; and
- Heterotrophs, which must feed on other organisms in order to obtain organic matter.

In general, trophic levels are used to describe the way in which a particular organism within an ecosystem gets its food. Using this description, we can restate and reorganize the categories above to define the three basic ways organisms acquire their food:

- Producers (autotrophs) do not usually eat other organisms but pull nutrients from the soil or the ocean and manufacture their own food using photosynthesis. In this way, it is the energy from the sun that usually powers the base of the food chain.
- Consumers (heterotrophs) cannot manufacture their own food and need to consume other organisms.
- Decomposers break down dead plant and animal material and wastes and release them into the ecosystem as energy and nutrients for recycling.

Within ecosystems, the biotic factors that comprise the categories above can be organized into a food chain in which autotrophic producers use materials and nutrients recycled by decomposers to make their own food; the producers are in turn eaten by heterotrophic consumers. In real world ecosystems, there are multiple food chains for most organisms (since most organisms eat more than one kind of food or are eaten by more than one type of predator). Additionally, the movement of mineral nutrients in the food chain is cyclic rather than linear. As a consequence, the intricate network of intersecting and overlapping food chains for an ecosystem is more commonly represented as a food web. A food web depicts a collection of heterotrophic consumers that network and cycle the flow of energy and nutrients from a productive base of self-feeding autotrophs.

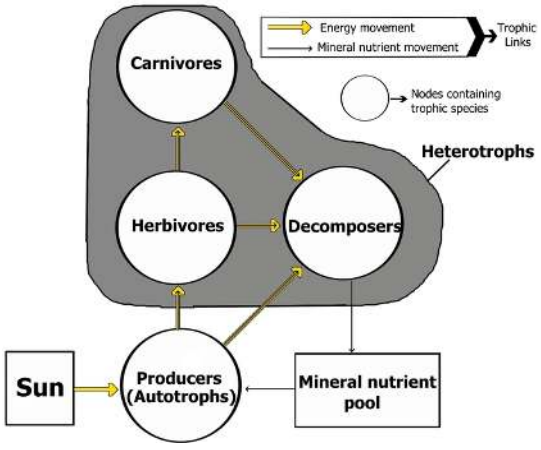


Figure: **A simplified food web**: This image shows a simplified food web model of energy and mineral nutrient movement in an ecosystem. Energy flow is unidirectional (noncyclic) and mineral nutrient movement is cyclic.

Microorganisms play a vital role in every ecological community by serving both as producers and as decomposers. Although plants are the most common primary producers, autotrophic photosynthetic microbes (such as cyanobacteria and algae) can harness light energy to generate organic matter. Additionally, in zones where light cannot penetrate (and thus photosynthesis cannot be the basic

means to produce energy), chemosynthetic microbes provide energy and carbon to the other organisms in the ecosystem. Other microbes are decomposers, with the ability to recycle nutrients from dead organic matter and other organisms' waste products. Decomposition is critical as most of the carbon and energy incorporated into plant tissues during photosynthesis remains uneaten when the plant tissue dies (and therefore must be broken down before it can be made available for recycling).

Key Points

- An ecosystem is a unified system of exchange made up of autotrophic producers, heterotrophic consumers, and decomposers.
- A food web depicts a collection of heterotrophic consumers that network and cycle the flow of energy and nutrients from a productive base of self-feeding autotrophs.
- Microorganisms play a vital role in every ecological community by serving as both producers and decomposers.

Key Terms

- **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.

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3.1C: Role of Microbes in Biogeochemical Cycling

Learning Objectives

- Explain the role microbes play in biogeochemical cycling

Microbial Role in Biogeochemical Cycling

Nutrients move through the ecosystem in biogeochemical cycles. A biogeochemical cycle is a pathway by which a chemical element (such as carbon or nitrogen) circulates through the biotic (living) and the abiotic (non-living) factors of an ecosystem. The elements that move through the factors of an ecosystem are not lost but are instead recycled or accumulated in places called reservoirs (or “sinks”) where they can be held for a long period of time. Elements, chemical compounds, and other forms of matter are passed from one organism to another and from one part of the biosphere to another through these biogeochemical cycles.

Ecosystems have many biogeochemical cycles operating as a part of the system. A good example of a molecule that is cycled within an ecosystem is water, which is always recycled through the water cycle. Water undergoes evaporation, condensation, and then falls back to Earth as rain (or other forms of precipitation). This typifies the cycling that is observed for all of the principal elements of life.

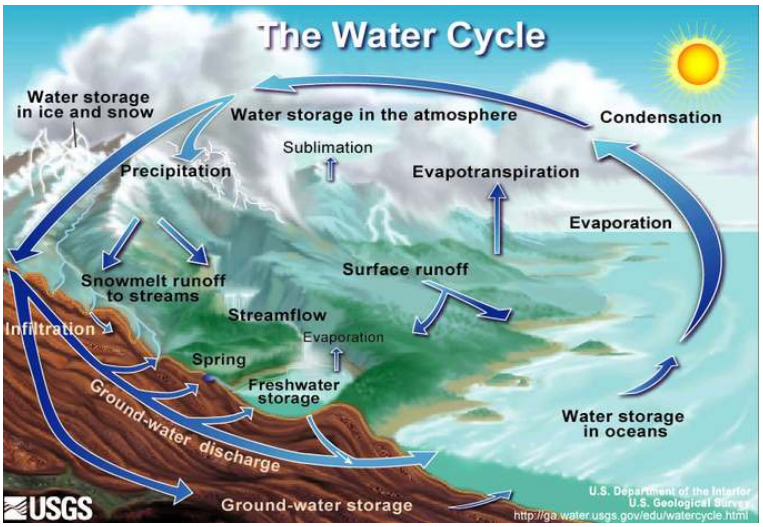


Figure: **The Water Cycle**: Water is recycled in an ecosystem through the water cycle.

Although biogeochemical cycles in a given ecosystem are coordinated by the full complement of living organisms and abiotic factors that make up that system, microorganisms play a primary role in regulating biogeochemical systems in virtually all of our planet’s environments. This includes extreme environments such as acid lakes and hydrothermal vents, and even includes living systems such as the human gut. The key collective metabolic processes of microbes (including nitrogen fixation, carbon fixation, methane metabolism, and sulfur metabolism) effectively control global biogeochemical cycling. Incredibly, production by microbes is so immense that global biogeochemistry would likely not change even if eukaryotic life were totally absent!

Microbes comprise the backbone of every ecological system, particularly those in which there is no light (i.e. systems in which energy cannot be collected through photosynthesis). Two key examples of critical biogeochemical processes carried out by microorganisms are discussed below.

The Carbon Cycle

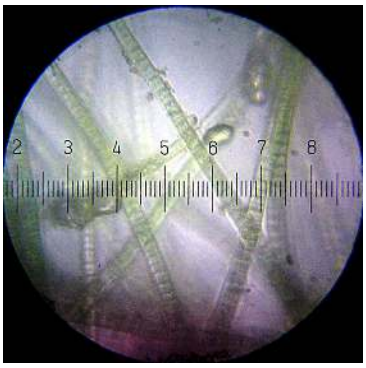


Figure: **Cyanobacteria**: Cyanobacteria, also known as blue-green bacteria, blue-green algae, and Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis

Carbon is critical for life because it is the essential building block of all organic compounds. Plants and animals utilize carbon to produce carbohydrates, fats, and proteins, which can then be used to build their internal structures or to obtain energy.

Carbon in the form of carbon dioxide (CO_2) is readily obtained from the atmosphere, but before it can be incorporated into living organisms it must be transformed into a usable organic form. The transformative process by which carbon dioxide is taken up from the atmospheric reservoir and "fixed" into organic substances is called carbon fixation. Perhaps the best known example of carbon fixation is photosynthesis, a process by which energy derived from sunlight is harnessed to form organic compounds. Photosynthesis depends on the activity of microorganisms such as cyanobacteria; indeed, the fact that there is oxygen in the Earth's atmosphere at all is a consequence of the photosynthetic activity of ancient microbes.

The Nitrogen Cycle

Nitrogen is essential for all forms of life because it is required for synthesis of the basic building blocks of life (e.g., DNA, RNA, and amino acids). The Earth's atmosphere is primarily composed of nitrogen, but atmospheric nitrogen (N_2) is relatively unusable for biological organisms. Consequently, chemical processing of nitrogen (or nitrogen fixation) is necessary to convert gaseous nitrogen into forms that living organisms can use. Almost all of the nitrogen fixation that occurs on the planet is carried out by bacteria that have the enzyme nitrogenase, which combines N_2 with hydrogen to produce a useful form of nitrogen (such as ammonia). Thus, microorganisms are absolutely essential for plant and animal life forms, which cannot fix nitrogen on their own.

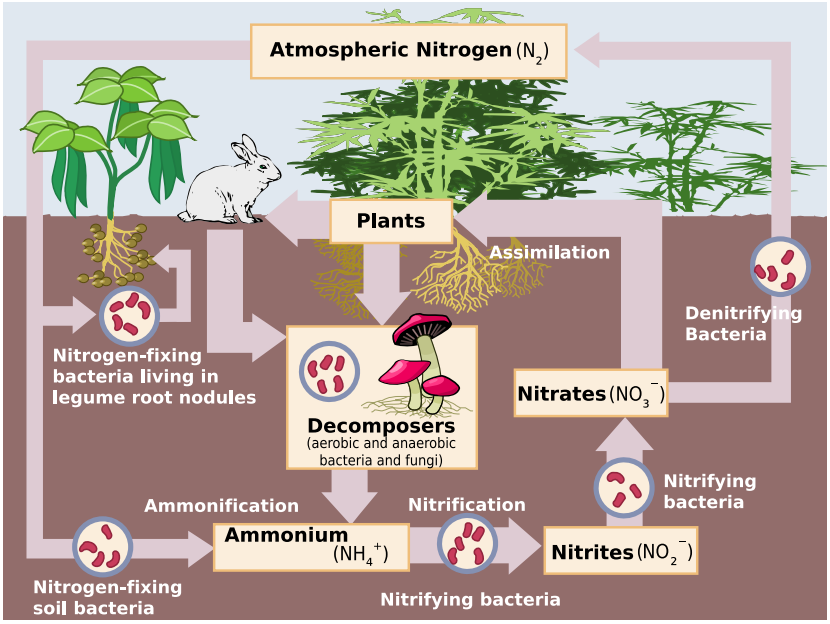


Figure: **The Role of Microbes in the Nitrogen Cycle**: The processing of nitrogen into a biologically useful form requires the activity of microorganisms.

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Key Points

- A biogeochemical cycle is a pathway by which a chemical element (such as carbon or nitrogen) circulates through and is recycled by an ecosystem.
- Microorganisms play a primary role in regulating biogeochemical systems in virtually all of our planet ‘s environments.
- Microbes participate in essential biogeochemical cycling events such as carbon and nitrogen fixation.

Key Terms

- **photosynthesis**: The process by which plants and other photoautotrophs generate carbohydrates and oxygen from carbon dioxide, water, and light energy in chloroplasts.
- **biogeochemistry**: The scientific study of biological, geological, and chemical processes in the natural environment and especially of their mutual relationships.
- **nitrogenase**: The enzyme, in nitrogen-fixing bacteria, that catalyzes the conversion of atmospheric nitrogen into ammonia.

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3.1D: Microbial Environments and Microenvironments

Learning Objectives

- Define microenvironments

Microorganisms are found on practically every habitable square inch of the planet. They live and thrive in all parts of the biosphere where there is liquid water, including hostile environments such as the poles, deserts, geysers, rocks, and the deep sea. Additionally, while microbes are often free-living, many have intimate symbiotic relationships with other larger organisms. Clearly, microbes have adapted to extreme and intolerant conditions, and it is this adaptation that has yielded tremendous biological diversity among microorganisms.

Like all extant organisms, microbes have evolved to thrive within a given environmental context. Microorganisms are ubiquitous despite the fact that the planet is host to extraordinarily diverse environments. Therefore, microbes have adapted to fill every ecological niche on the planet. For example, extremophilic species have been found that can tolerate the following environmental extremes:

- Temperatures as high as 130 °C (266 °F) and as low as −17 °C (1 °F)
- Highly alkaline (pH 0) and highly acidic (pH 11.5) environments
- Extremely saline environments (including those in which the salt concentration is saturating)
- Extremely high (1,000-2,000 atm) and low (0 atm) pressures (some bacteria can survive for prolonged periods in a pressure-less vacuum, meaning they might even survive in space)
- High ionizing radiation (up to 15,000 Gy; as a reference, a mere 5 Gy would kill a human!)

These evolutionary adaptations have allowed microbial life to extend into much of the Earth's atmosphere, crust, and hydrosphere (the water found over, under, and on the surface of a planet).

In addition to occupying a unique niche within an ecosystem, microbes are potentially sensitive to subtle environmental differences between adjacent areas. These differences define so-called microenvironments (or microhabitats) that can be distinguished from the immediate surroundings by such factors as the amount of incident light, the degree of moisture, and the range of temperatures. For example, the side of a tree that is shaded from sunlight is a microenvironment that typically supports a somewhat different community of microorganisms than would be found on the side that receives regular light. Microbes, therefore, are not only adapted to their habitat, but also to the immediate environment, thus promoting increased diversity among microbial species within an ecosystem.

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Key Points

- Microorganisms are found in practically every habitat present on the planet.
- Microorganisms have evolved to survive in extraordinarily diverse environments, including extreme, hostile, or otherwise intolerant ecological systems.
- In addition to occupying a unique niche within an ecosystem, microbes adapt to microenvironments (or microhabitats) that can be distinguished from the immediate surroundings by such factors as the amount of incident light, the degree of moisture, and the range of temperatures.

Key Terms

- **microenvironment**: The very small environment in the immediate vicinity of an organism.
- **extremophilic**: Of or pertaining to the extremophiles, a class of organism that thrives under extreme conditions of temperature, salinity, and so on; commercially important as a source of enzymes that operate under similar conditions.
- **ubiquitous**: Being everywhere at once: omnipresent.

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SECTION OVERVIEW

3.2: Soil and Plant Microbiology

Topic hierarchy

3.2A: Soil Composition

3.2B: Physical Properties of Soil

3.2C: Mycorrhiza

3.2D: Wetland Soils

3.2E: Endophytes and Plants

3.2F: Mycorrhizae- The Symbiotic Relationship between Fungi and Roots

3.2F: Plant Pathogens

3.2G: Nitrogen Fixation- Root and Bacteria Interactions

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3.2A: Soil Composition

Learning Objectives

- Explain soil composition

Plants obtain inorganic elements from the soil, which serves as a natural medium for land plants. Soil is the outer, loose layer that covers the surface of Earth. Soil quality, a major determinant, along with climate, of plant distribution and growth, depends not only on the chemical composition of the soil, but also the topography (regional surface features) and the presence of living organisms.

Soil consists of these major components:

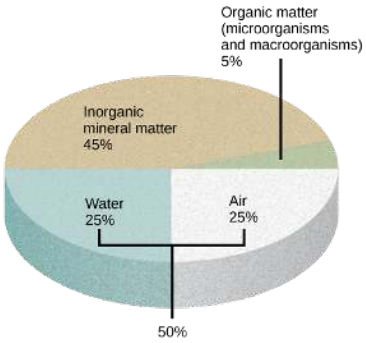


Figure: **Components of soil**: The four major components of soil are shown: inorganic minerals, organic matter, water, and air.

- inorganic mineral matter, about 40 to 45 percent of the soil volume
- organic matter, about 5 percent of the soil volume
- water, about 25 percent of the soil volume
- air, about 25 percent of the soil volume

The amount of each of the four major components of soil depends on the quantity of vegetation, soil compaction, and water present in the soil. A good, healthy soil has sufficient air, water, minerals, and organic material to promote and sustain plant life.

The organic material of soil, called humus, is made up of microorganisms (dead and alive), and dead animals and plants in varying stages of decay. Humus improves soil structure, providing plants with water and minerals. The inorganic material of soil is composed of rock, slowly broken down into smaller particles that vary in size. Soil particles that are 0.1 to 2 mm in diameter are sand. Soil particles between 0.002 and 0.1 mm are called silt, and even smaller particles, less than 0.002 mm in diameter, are called clay. Some soils have no dominant particle size, containing a mixture of sand, silt, and humus; these soils are called **loams**.

Key Points

- The chemical composition of the soil, the topography, and the presence of living organisms determines the quality of soil.
- In general, soil contains 40-45% inorganic matter, 5% organic matter, 25% water, and 25% air.
- In order to sustain plant life, the proper mix of air, water, minerals, and organic material is required.
- Humus, the organic material in soil, is composed of microorganisms (dead and alive) and decaying plants.
- The inorganic material of soil is composed of rock, which is broken down into small particles of sand (0.1 to 2 mm), silt (0.002 to 0.1 mm), and clay (less than 0.002 mm).
- Loam is a soil that is a mix sand, silt, and humus.

Key Terms

- **loam**: soil with no dominant particle size that contains a mixture of sand, silt, and humus
- **humus**: a large group of natural organic compounds found in the soil composed of decaying plants and dead and living microorganisms

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3.2B: Physical Properties of Soil

Learning Objectives

- Describe the physical properties or profile of soil

Soils are named and classified based on their horizons. The soil profile has four distinct layers:

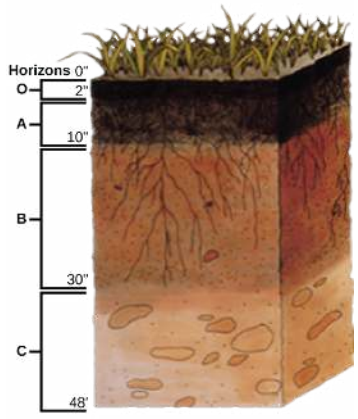


Figure: **Soil profile**: This soil profile shows the different soil layers (O horizon, A horizon, B horizon, and C horizon) found in typical soils.

1. The O horizon has freshly-decomposing organic matter, humus, at its surface, with decomposed vegetation at its base. Humus enriches the soil with nutrients, enhancing soil moisture retention. Topsoil, the top layer of soil, is usually two to three inches deep, but this depth can vary considerably. For instance, river deltas, such as the Mississippi River delta, have deep layers of topsoil. Topsoil is rich in organic material. Microbial processes occur there; it is responsible for plant production.
2. The A horizon consists of a mixture of organic material with inorganic products of weathering; it is the beginning of true mineral soil. This horizon is typically darkly colored because of the presence of organic matter. In this area, rainwater percolates through the soil and carries materials from the surface.
3. The B horizon, or subsoil, is an accumulation of mostly fine material that has moved downward, resulting in a dense layer in the soil. In some soils, the B horizon contains nodules or a layer of calcium carbonate.
4. The C horizon, or soil base, includes the parent material, plus the organic and inorganic material that is broken down to form soil. The parent material may be either created in its natural place or transported from elsewhere to its present location. Beneath the C horizon lies bedrock.

Some soils may have additional layers, or lack one of these layers. The thickness of the layers is also variable, depending on the factors that influence soil formation. In general, immature soils may have O, A, and C horizons, whereas mature soils may display all of these, plus additional layers.

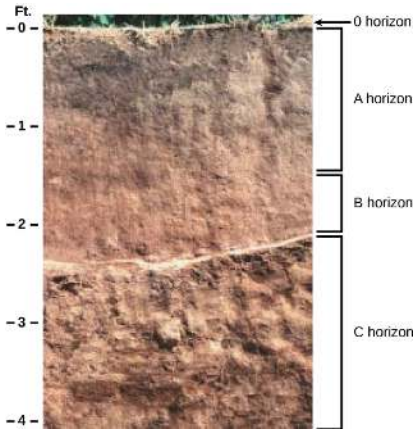


Figure: **Mature soil**: The San Joaquin soil is a mature soil that has an O horizon, A horizon, B horizon, and C horizon.

Key Points

- The O horizon, or topsoil, is made of decaying organisms and plant life; it is responsible for plant production.
- The A horizon is of a mixture of organic material and inorganic products of weathering; it is the beginning of true mineral soil.
- The B horizon, or subsoil, is a dense layer of mostly fine material that has been pushed down from the topsoil.
- The C horizon, or soil base, is located just above bedrock and is made of parent, organic, and inorganic material.

Key Terms

- **topsoil**: top layer of soil containing humus at its surface and decomposing vegetation at its base; the most fertile soil
- **subsoil**: dense layer of soil containing fine material that has moved downward; the layer of earth that is below the topsoil

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3.2C: Mycorrhiza

Learning Objectives

- Evaluate mycorrhiza as a plant symbiote

A mycorrhiza is a symbiotic (generally mutualistic, but occasionally weakly pathogenic) association between a fungus and the roots of a vascular plant.

In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in arbuscular mycorrhizal fungi (AMF or AM) or extracellularly as in ectomycorrhizal fungi. They are an important component of soil life and soil chemistry. Mycorrhizas form a mutualistic relationship with the roots of most plant species. While only a small proportion of all species has been examined, 95% of those plant families are predominantly mycorrhizal.

They are named after their presence in the plant's rhizosphere (root system). This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates, such as glucose and sucrose. The carbohydrates are translocated from their source (usually leaves) to root tissue and on to the plant's fungal partners. In return, the plant gains the benefits of the mycelium's higher absorptive capacity for water and mineral nutrients due to the comparatively large surface area of mycelium: root ratio, thus improving the plant's mineral absorption capabilities. Plant roots alone may be incapable of taking up phosphate ions that are demineralized in soils with a basic pH. The mycelium of the mycorrhizal fungus can, however, access these phosphorus sources and make them available to the plants they colonize.

Suillus tomentosus, a fungus, produces specialized structures, known as tuberculate ectomycorrhizae, with its plant host lodgepole pine (Pinus contorta var. latifolia). These structures have in turn been shown to host nitrogen fixing bacteria which contribute a significant amount of nitrogen and allow the pines to colonize nutrient-poor sites.

Plants grown in sterile soils and growth media often perform poorly without the addition of spores or hyphae of mycorrhizal fungi to colonize the plant roots and aid in the uptake of soil mineral nutrients.

Fungi have been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils. Pine trees inoculated with Pisolithus tinctorius planted in several contaminated sites displayed high tolerance to the prevailing contaminant, survivorship, and growth.

Mycorrhizas are present in 92% of plant families studied (80% of species), with arbuscular mycorrhizas being the ancestral and predominant form and the most prevalent symbiotic association found in the plant kingdom. The structure of arbuscular mycorrhizas has been highly conserved since their first appearance in the fossil record.

Key Points

- In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellularly as in arbuscular mycorrhizal fungi (AMF or AM) or extracellularly as in ectomycorrhizal fungi.
- Mycorrhiza are named after their presence in the plant's rhizosphere (root system). This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates, such as glucose and sucrose.
- Plants grown in sterile soils and growth media often perform poorly without the addition of spores or hyphae of mycorrhizal fungi to colonize the plant roots and aid in the uptake of soil mineral nutrients.

Key Terms

- **mycorrhiza**: A symbiotic relationship between the mycelium of a fungus and the roots of a plant.

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3.2D: Wetland Soils

Learning Objectives

- Assess the composition of wetland soils

A wetland is a land area that is saturated with water, either permanently or seasonally, such that it takes on the characteristics of a distinct ecosystem. Primarily, the factor that distinguishes wetlands from other land forms or water bodies is the characteristic vegetation that is adapted to its unique soil conditions: Wetlands consist primarily of hydric soil, which supports aquatic plants. The water found in wetlands can be saltwater, freshwater, or brackish. Main wetland types include swamps, marshes, bogs and fens. Sub-types include mangrove, carr, pocosin, and varzea.

Wetlands play a number of roles in the environment, principally water purification, flood control, and shoreline stability. Wetlands are also considered the most biologically diverse of all ecosystems, serving as home to a wide range of plant and animal life.

In balanced soil, plants grow in an active and steady environment. The mineral content of the soil and its heartiful structure are important for their well-being, but it is the life in the earth that powers its cycles and provides its fertility. Without the activities of soil organisms, organic materials would accumulate and litter the soil surface, and there would be no food for plants.

The soil biota includes:

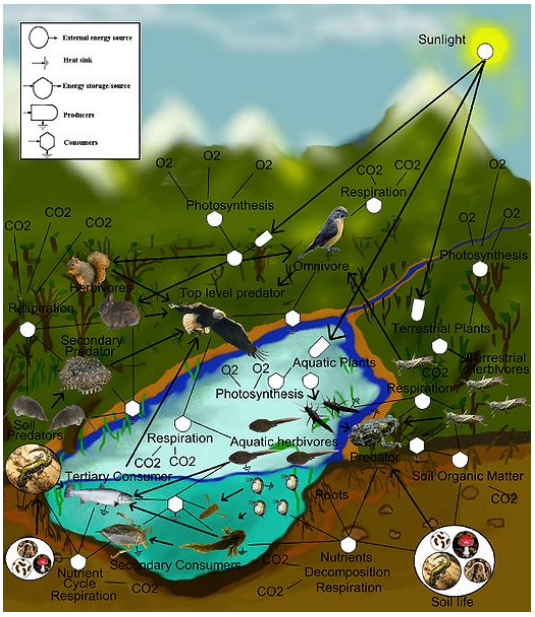


Figure: **A freshwater aquatic and terrestrial food-web.**: The food web is a complex and interconnected.

Megafauna: size range – 20 mm upward, e.g. moles, rabbits, and rodents.

Mesofauna: size range – 100 micrometres to 2 mm, e.g. tardigrades, mites, and springtails.

Microfauna and Microflora: size range – 1 to 100 micrometres, e.g. yeasts, bacteria (commonly actinobacteria), fungi, protozoa, roundworms, and rotifers.

Of these, bacteria and fungi play key roles in maintaining a healthy soil. They act as decomposers that break down organic materials to produce detritus and other breakdown products. Soil detritivores, like earthworms, ingest detritus and decompose it. Saprotrophs, well represented by fungi and bacteria, extract soluble nutrients from delitro. The ants (macrofaunas) help by breaking down in the same way but they also provide the motion part as they move in their armies. Also the rodents, wood-eaters help the soil to be more absorbent.

Nutrient cycling in lakes and freshwater wetlands depends heavily on redox conditions. Under a few millimeters of water heterotrophic bacteria metabolize and consume oxygen. They therefore deplete the soil of oxygen and create the need for anaerobic

respiration. Some anaerobic microbial processes include denitrification, sulfate reduction and methanogenesis and are responsible for the release of N_2 (nitrogen), H_2S (hydrogen sulfide) and CH_4 (methane). Other anaerobic microbial processes are linked to changes in the oxidation state of iron and manganese. As a result of anaerobic decomposition, the soil stores large amounts of organic carbon because decomposition is incomplete.

The redox potential describes which way chemical reactions will proceed in oxygen deficient soils and controls the nutrient cycling in flooded systems. Redox potential, or reduction potential, is used to express the likelihood of an environment to receive electrons and therefore become reduced. For example, if a system already has plenty of electrons (anoxic, organic-rich shale) it is reduced and will likely donate electrons to a part of the system that has a low concentration of electrons, or an oxidized environment, to equilibrate to the chemical gradient. The oxidized environment has high redox potential, whereas the reduced environment has a low redox potential.

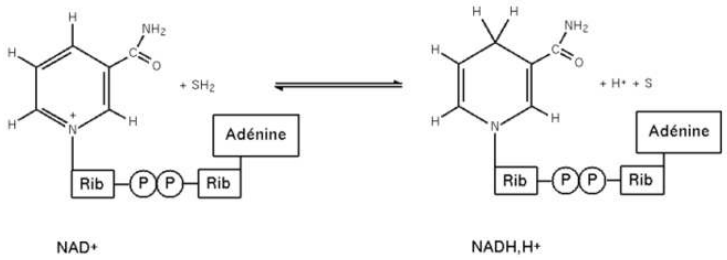


Figure: **Redox Potential**: The redox potential describes which way chemical reactions will proceed in oxygen deficient soils and controls the nutrient cycling in flooded systems.

The redox potential is controlled by the oxidation state of the chemical species, pH and the amount of oxygen (O_2) there is in the system. The oxidizing environment accepts electrons because of the presence of O_2, which acts as electron acceptors:

$$O_2 + 4e^- + 4H^+ \rightarrow H_2O$$

This equation will tend to move to the right in acidic conditions which causes higher redox potentials to be found at lower pH levels. Bacteria, heterotrophic organisms, consume oxygen while decomposing organic material which depletes the soils of oxygen, thus increasing the redox potential. In low redox conditions the deposition of ferrous iron (Fe^{2+}) will increase with decreasing decomposition rates, thus preserving organic remains and depositing humus.

Key Points

- Nutrient cycling in lakes and freshwater wetlands depends heavily on redox conditions.
- Some anaerobic microbial processes include denitrification, sulfate reduction and methanogenesis and are responsible for the release of N2 (nitrogen), H2S (hydrogen sulfide) and CH4 (methane).
- Other anaerobic microbial processes are linked to changes in the oxidation state of iron and manganese and as a result of anaerobic decomposition, the soil stores large amounts of organic carbon because decomposition is incomplete.

Key Terms

- **denitrification**: The process by which a nitrate becomes molecular nitrogen, especially by the action of bacteria.
- **methanogenesis**: The generation of methane by anaerobic bacteria.
- **heterotroph**: An organism that requires an external supply of energy in the form of food as it cannot synthesize its own.

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3.2E: Endophytes and Plants

Learning Objectives

- Evaluate endophytes as plant pathogens

An endophyte is an endosymbiont, often a bacterium or fungus, that lives within a plant for at least part of its life without causing apparent disease. Endophytes are ubiquitous and have been found in all the species of plants studied to date. However, most of these endophyte/plant relationships are not well understood. Many economically important forage and turfgrasses (e.g., Festuca spp., Lolium spp.) carry fungal endophytes (Neotyphodium spp.) which may improve the ability of these grasses to tolerate abiotic stresses such as drought, as well as improve their resistance to insect and mammalian herbivores.

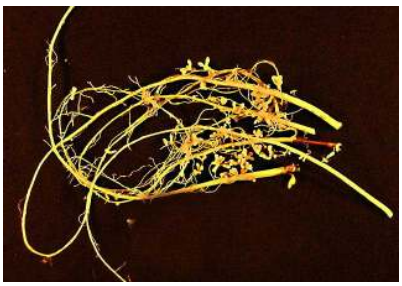


Figure: **Root nodules on a legume**: Soybean root nodules, each containing billions of Bradyrhizobium bacteria.

Endophytes may be transmitted either vertically (directly from parent to offspring) or horizontally (from individual to unrelated individual). Vertically transmitted fungal endophytes are asexual and transmit via fungal hyphae penetrating the host's seeds (e.g., Neotyphodium). Since their reproductive fitness is intimately tied to that of their host plant, these fungi are often mutualistic. Conversely, horizontally transmitted fungal endophytes are sexual and transmit via spores that can be spread by wind and/or insect vectors. Since they spread in a similar way to pathogens, horizontally transmitted endophytes are often closely related to pathogenic fungi, although they are not pathogenic themselves.

Endophytes may benefit host plants by preventing pathogenic organisms from colonizing them. Extensive colonization of the plant tissue by endophytes creates a "barrier effect," where the local endophytes outcompete and prevent pathogenic organisms from taking hold. Endophytes may also produce chemicals which inhibit the growth of competitors, including pathogenic organisms. Some bacterial endophytes have proven to increase plant growth. The presence of fungal endophytes can cause higher rates of water loss in leaves. However, certain fungal endophytes help plants survive drought and heat. Plant use of endophytic fungi in defense is a very common phenomenon, primarily involving the arbuscular mycorrhizal fungi.

The wide range of compounds produced by endophytes have been shown to combat pathogens and even cancers in animals including humans. One notable endophyte with medicinal benefits to humans was discovered by Gary Strobel: Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana (Himalayan Yew) was found to produce taxol. Endophytes are also being investigated for roles in agriculture and biofuels production. Inoculating crop plants with certain endophytes may provide increased disease or parasite resistance while others may possess metabolic processes that convert cellulose and other carbon sources into "myco-diesel" hydrocarbons and hydrocarbon derivatives. Piriformospora indica is an interesting endophytic fungus of the order Sebacinales, the fungus is capable of colonizing roots and forming symbiotic relationship with every possible plant on earth. P. indica has also been shown to increase both crop yield and plant defence of a variety of crops (barley, tomato, maize, etc.) against root-pathogens.

It is speculated that there may be many thousands of endophytes useful to mankind. However, since there are few scientists working in this field, and since forests and areas of biodiversity are rapidly being destroyed, many useful endophytes for curing disease might be permanently lost for medicinal use before they are discovered. The effects of climate change on endophytes are being investigated. Studies of plants grown at different climates or at increased carbon dioxide levels have different distributions of endophytic species.

Key Points

- Endophytes are ubiquitous and have been found in all the species of plants studied to date.
- Vertically transmitted fungal endophytes are asexual and transmit via fungal hyphae penetrating the host ‘s seeds (e.g., Neotyphodium).
- The wide range of compounds produced by endophytes have been shown to combat pathogens and even cancers in animals including humans.

Key Terms

- **endophyte**: Any organism that lives inside another plant.

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3.2F: Mycorrhizae- The Symbiotic Relationship between Fungi and Roots

Learning Objectives

- Describe the symbiotic relationship of mycorrhizae and plant roots

Mycorrhizae: The Symbiotic Relationship between Fungi and Roots



Mycorrhizae: Hyphae proliferate within the mycorrhizae, which appears as off-white fuzz in this image. These hyphae greatly increase the surface area of the plant root, allowing it to reach areas that are not depleted of nutrients.

A nutrient depletion zone can develop when there is rapid soil solution uptake, low nutrient concentration, low diffusion rate, or low soil moisture. These conditions are very common; therefore, most plants rely on fungi to facilitate the uptake of minerals from the soil. Mycorrhizae, known as root fungi, form symbiotic associations with plant roots. In these associations, the fungi are actually integrated into the physical structure of the root. The fungi colonize the living root tissue during active plant growth.



Figure: **Ectomycorrhizae**: Ectomycorrhizae form sheaths, called a mantle, around the roots of plants, as shown in this image.

Through mycorrhization, the plant obtains phosphate and other minerals, such as zinc and copper, from the soil. The fungus obtains nutrients, such as sugars, from the plant root. Mycorrhizae help increase the surface area of the plant root system because hyphae, which are narrow, can spread beyond the nutrient depletion zone. Hyphae are long extensions of the fungus, which can grow into small soil pores that allow access to phosphorus otherwise unavailable to the plant. The beneficial effect on the plant is best observed in poor soils. The benefit to fungi is that they can obtain up to 20 percent of the total carbon accessed by plants. Mycorrhizae function as a physical barrier to pathogens. They also provides an induction of generalized host defense mechanisms, which sometimes involves the production of antibiotic compounds by the fungi. Fungi have also been found to have a protective role for plants rooted in soils with high metal concentrations, such as acidic and contaminated soils.

There are two types of mycorrhizae: ectomycorrhizae and endomycorrhizae. Ectomycorrhizae form an extensive dense sheath around the roots, called a mantle. Hyphae from the fungi extend from the mantle into the soil, which increases the surface area for water and mineral absorption. This type of mycorrhizae is found in forest trees, especially conifers, birches, and oaks. Endomycorrhizae, also called arbuscular mycorrhizae, do not form a dense sheath over the root. Instead, the fungal mycelium is embedded within the root tissue. Endomycorrhizae are found in the roots of more than 80 percent of terrestrial plants.

Key Points

- Because nutrients are often depleted in the soil, most plants form symbiotic relationships called mycorrhizae with fungi that integrate into the plant's root.

- The relationship between plants and fungi is symbiotic because the plant obtains phosphate and other minerals through the fungus, while the fungus obtains sugars from the plant root.
- The long extensions of the fungus, called hyphae, help increase the surface area of the plant root system so that it can extend beyond the area of nutrient depletion.
- Ectomycorrhizae are a type of mycorrhizae that form a dense sheath around the plant roots, called a mantle, from which the hyphae grow; in endomycorrhizae, mycelium is embedded within the root tissue, as opposed to forming a sheath around it.
- In endomycorrhizae, mycelium is embedded within the root tissue, as opposed to forming a sheath around it; these are found in the roots of most terrestrial plants.

Key Terms

- **mycorrhiza**: a symbiotic association between a fungus and the roots of a vascular plant
- **hypha**: a long, branching, filamentous structure of a fungus that is the main mode of vegetative growth
- **mycelium**: the vegetative part of any fungus, consisting of a mass of branching, threadlike hyphae, often underground

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3.2F: Plant Pathogens

Learning Objectives

- Break down the types and modes of plant pathogenicity

Most bacteria that are associated with plants are actually saprophytic, and do no harm to the plant itself. However, a small number, around 100 species, are able to cause disease. Bacterial diseases are much more prevalent in sub-tropical and tropical regions of the world. Most plant pathogenic bacteria are rod shaped (bacilli). In order to be able to colonise the plant they have specific pathogenicity factors. There are 4 main bacterial pathogenicity factors:



Figure: **Tobacco Mosaic Disease**: Photo of a tobacco leaf with symptoms of tobacco mosaic virus.

- Cell wall -degrading enzymes: These are used to break down the plant cell wall in order to release the nutrients inside.
- Toxins: These can be non- host -specific, which damage all plants, or host-specific, which cause damage only on a host plant.
- Phytohormones: example Agrobacterium changes the level of Auxin to cause tumours.
- Effector proteins: These can be secreted into the extracellular environment or directly into the host cell, often via the Type three secretion system. Some effectors are known to suppress host defense processes. This can include: reducing the plants internal signaling mechanisms or reduction of phytochemicals production. Bacteria, fungus and oomycetes are known for this function.

Significant bacterial plant pathogens:

- Burkholderia
- Proteobacteria
- *Xanthomonas* spp.
- *Pseudomonas* spp.
- Pseudomonas syringae pv. tomato causes tomato plants to produce less fruit, and it “continues to adapt to the tomato by minimizing its recognition by the tomato immune system. “

The majority of phytopathogenic fungi belong to the Ascomycetes and the Basidiomycetes. The fungi reproduce both sexually and asexually via the production of spores and other structures. Spores may be spread long distances by air or water, or they may be soil borne. Many soil inhabiting fungi are capable of living saprotrophically, carrying out the part of their lifecycle in the soil. These are known as facultative saprotrophs. Fungal diseases may be controlled through the use of fungicides and other agriculture practices, however new races of fungi often evolve that are resistant to various fungicides. · Biotrophic fungal pathogens colonize living plant tissue and obtain nutrients from living host cells. Necrotrophic fungal pathogens infect and kill host tissue and extract nutrients from the dead host cells.

Significant fungal plant pathogens include:

- *Fusarium* spp. (causal agents of Fusarium wilt disease)
- *Thielaviopsis* spp. (causal agents of: canker rot, black root rot, *Thielaviopsis* root rot)
- *Verticillium* spp.
- *Magnaporthe grisea* (causal agent of blast of rice and gray leaf spot in turfgrasse

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Key Points

- The majority of phytopathogenic fungi belong to the Ascomycetes and the Basidiomycetes.
- Many soil inhabiting fungi are capable of living saprotrophically, carrying out the part of their lifecycle in the soil.
- Bacterial plant pathogens are much more prevalent in sub-tropical and tropical regions of the world.

Key Terms

- **Type three secretion system**: Type three secretion system (often written Type III secretion system and abbreviated TTSS or T3SS, also called Injectisome or Injectosome) is a protein appendage found in several Gram-negative bacteria. In pathogenic bacteria, the needle-like structure is used as a sensory probe to detect the presence of eukaryotic organisms and secrete proteins that help the bacteria infect them. The proteins are secreted directly from the bacterial cell into the eukaryotic cell, also known as “the host” cell.

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3.2G: Nitrogen Fixation- Root and Bacteria Interactions

Learning Objectives

- Explain the process and importance of nitrogen fixation

Nitrogen Fixation: Root and Bacteria Interactions

Nitrogen is an important macronutrient because it is part of nucleic acids and proteins. Atmospheric nitrogen, which is the diatomic molecule N_2, or dinitrogen, is the largest pool of nitrogen in terrestrial ecosystems. However, plants cannot take advantage of this nitrogen because they do not have the necessary enzymes to convert it into biologically useful forms. However, nitrogen can be "fixed." It can be converted to ammonia (NH_3) through biological, physical, or chemical processes. Biological nitrogen fixation (BNF), the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), is exclusively carried out by prokaryotes, such as soil bacteria or cyanobacteria. Biological processes contribute 65 percent of the nitrogen used in agriculture.

The most important source of BNF is the symbiotic interaction between soil bacteria and legume plants, including many crops important to humans. The NH_3 resulting from fixation can be transported into plant tissue and incorporated into amino acids, which are then made into plant proteins. Some legume seeds, such as soybeans and peanuts, contain high levels of protein and are among the most important agricultural sources of protein in the world.

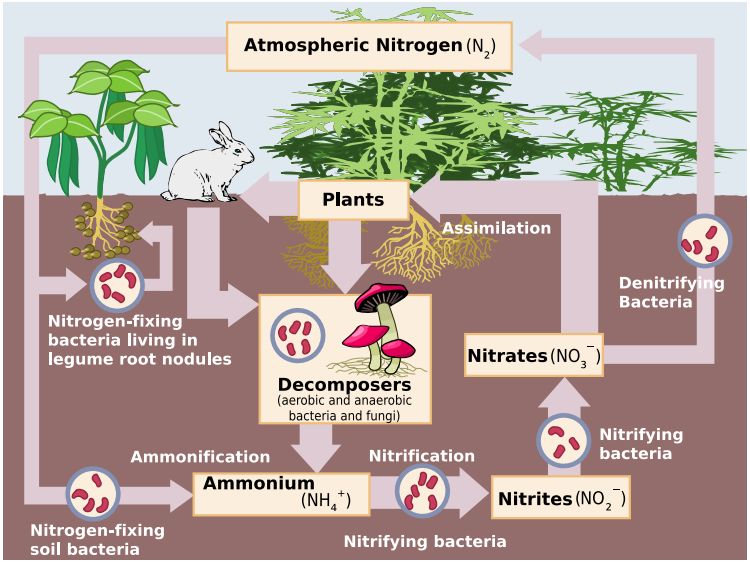


Figure: **Diagram of the Nitrogen Cycle**: Schematic representation of the nitrogen cycle. Abiotic nitrogen fixation has been omitted.



Figure: **Nitrogen fixation in crops**: Some common edible legumes, such as (a) peanuts, (b) beans, and (c) chickpeas, are able to interact symbiotically with soil bacteria that fix nitrogen.

Soil bacteria, collectively called rhizobia, symbiotically interact with legume roots to form specialized structures called nodules in which nitrogen fixation takes place. This process entails the reduction of atmospheric nitrogen to ammonia by means of the enzyme nitrogenase. Therefore, using rhizobia is a natural and environmentally-friendly way to fertilize plants as opposed to chemical fertilization that uses a non-renewable resource, such as natural gas. Through symbiotic nitrogen fixation, the plant benefits from using an endless source of nitrogen from the atmosphere. The process simultaneously contributes to soil fertility because the plant root system leaves behind some of the biologically-available nitrogen. As in any symbiosis, both organisms benefit from the

interaction: the plant obtains ammonia and bacteria obtain carbon compounds generated through photosynthesis, as well as a protected niche in which to grow.

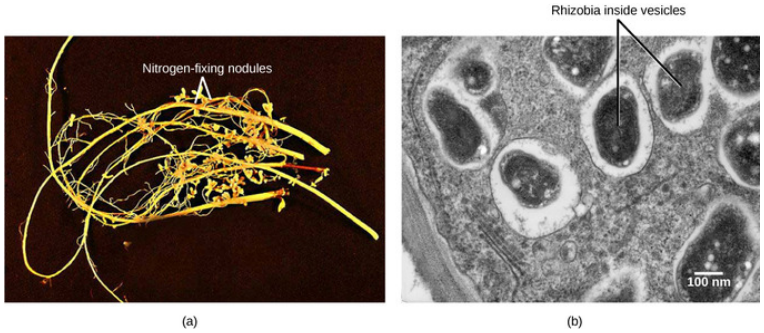


Figure: **Rhizobia**: Soybean roots contain (a) nitrogen-fixing nodules. Cells within the nodules are infected with Bradyrhyzobium japonicum, a rhizobia or "root-loving" bacterium. The bacteria are encased in (b) vesicles inside the cell, as can be seen in this transmission electron micrograph.

Key Points

- Diatomic nitrogen is abundant in the atmosphere and soil, but plants are unable to use it because they do not have the necessary enzyme, nitrogenase, to convert it into a form that they can use to make proteins.
- Soil bacteria, or rhizobia, are able to perform biological nitrogen fixation in which atmospheric nitrogen gas (N_2) is converted into the ammonia (NH_3) that plants are able to use to synthesize proteins.
- Both the plants and the bacteria benefit from the process of nitrogen fixation; the plant obtains the nitrogen it needs to synthesize proteins, while the bacteria obtain carbon from the plant and a secure environment to inhabit within the plant roots.

Key Terms

- **rhizobia**: any of various bacteria, of the genus Rhizobium, that form nodules on the roots of legumes and fix nitrogen
- **nitrogen fixation**: the conversion of atmospheric nitrogen into ammonia and organic derivatives, by natural means, especially by microorganisms in the soil, into a form that can be assimilated by plants
- **nodule**: structures that occur on the roots of plants that associate with symbiotic nitrogen-fixing bacteria

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SECTION OVERVIEW

3.3: Aquatic Microbiology

Topic hierarchy

3.3A: Marine Habitats

3.3B: Planktonic Communities

3.3C: Planktonic Food Webs

3.3D: Ocean Floor

3.3E: Cold-Seep Ecosystems

3.3F: The Deep Sea and Barophilism

3.3G: Sea Coral and Sea Anemone Zooxanthellae

3.3H: Sponge Communities

3.3I: Freshwater Environments

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3.3A: Marine Habitats

Learning Objectives

- Describe marine habitats

The marine environment supplies many kinds of habitats that support life. Marine life partially depends on the saltwater that is in the sea (“marine” comes from the Latin “mare,” meaning sea or ocean). A habitat is an ecological or environmental area inhabited by one or more living species.



Figure: **Marine Habitats**: Coral reefs provide marine habitats for tube sponges, which in turn become marine habitats for fishes.

Marine habitats can be divided into coastal and open ocean habitats. Coastal habitats are found in the area that extends from as far as the tide comes in on the shoreline, out to the edge of the continental shelf. Most marine life is found in coastal habitats, even though the shelf area occupies only seven percent of the total ocean area. Open ocean habitats are found in the deep ocean beyond the edge of the continental shelf.

Alternatively, marine habitats can be divided into pelagic and demersal habitats. Pelagic habitats are found near the surface or in the open water column, away from the bottom of the ocean. Demersal habitats are near or on the bottom of the ocean. An organism living in a pelagic habitat is said to be a pelagic organism, as in pelagic fish. Similarly, an organism living in a demersal habitat is said to be a demersal organism, as in demersal fish. Pelagic habitats are intrinsically shifting and ephemeral, depending on what ocean currents are doing.

Marine habitats can be modified by their inhabitants. Some marine organisms, like corals, kelp, mangroves and seagrasses, are ecosystem engineers, which reshape the marine environment to the point where they create habitats for other organisms.

Marine habitats include coastal zones, intertidal zones, sandy shores, rocky shores, mudflats, swamps and salt marshes, estuaries, kelp forests, seagrasses, and coral reefs. In addition, in the open ocean there are surface waters, deep sea and sea floor.

Intertidal zones (those areas close to shore) are constantly being exposed and covered by the ocean’s tides. A huge array of life lives within this zone.

Sandy shores, also called beaches, are coastal shorelines where sand accumulates. Waves and currents shift the sand, continually building and eroding the shoreline. Longshore currents flow parallel to the beaches, making waves break obliquely on the sand. These currents transport large amounts of sand along coasts, forming spits, barrier islands and tombolos. Longshore currents also commonly create offshore bars, which give beaches some stability by reducing erosion.

The relative solidity of rocky shores seems to give them a permanence compared to the shifting nature of sandy shores. This apparent stability is not real over even quite short geological time scales, but it is real enough over the short life of an organism. In contrast to sandy shores, plants and animals can anchor themselves to the rocks.

Mudflats are coastal wetlands that form when mud is deposited by tides or rivers. They are found in sheltered areas such as bays, bayous, lagoons, and estuaries. Mudflats may be viewed geologically as exposed layers of bay mud, resulting from deposition of

estuarine silts, clays and marine animal detritus. Most of the sediment within a mudflat is within the intertidal zone, and thus the flat is submerged and exposed approximately twice daily.

Mangrove swamps and salt marshes form important coastal habitats in topical and temperate areas respectively. An estuary is a partly enclosed coastal body of water with one or more rivers or streams flowing into it, and with a free connection to the open sea.

Kelp forests are underwater areas with a high density of kelp. They are recognized as one of the most productive and dynamic ecosystems on Earth. Smaller areas of anchored kelp are called kelp beds. Kelp forests occur worldwide throughout temperate and polar coastal oceans.

Seagrasses are flowering plants from one of four plant families which grow in marine environments. They are called seagrasses because the leaves are long and narrow and are very often green, and because the plants often grow in large meadows, which look like grassland.

Reefs comprise some of the densest and most diverse habitats in the world. The best-known types of reefs are tropical coral reefs, which exist in most tropical waters; however, reefs can also exist in cold water. Reefs are built up by corals and other calcium-depositing animals, usually on top of a rocky outcrop on the ocean floor. Reefs can also grow on other surfaces; this has made it possible to create artificial reefs. Coral reefs also support a huge community of life, including the corals themselves, their symbiotic zooxanthellae, tropical fish, and many other organisms.

Key Points

- Marine habitats can be divided into coastal and open ocean habitats.
- Coastal habitats are found in the area that extends from as far as the tide comes in on the shoreline out to the edge of the continental shelf.
- Open ocean habitats are found in the deep ocean beyond the edge of the continental shelf.
- Most marine life is found in coastal habitats, even though the shelf area occupies only seven percent of the total ocean area.

Key Terms

- **coastal**: Relating to the coast; on or near the coast, as a coastal town, a coastal breeze.
- **habitat**: A specific place or natural conditions in which a plant or animal lives.
- **marine**: Of, or pertaining to, the sea (marine biology, marine insurance).

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3.3B: Planktonic Communities

Learning Objectives

- Recall Planktonic communities

Plankton (singular plankter) are any organisms that live in the water column and are incapable of swimming against a current. They provide a crucial source of food to many large aquatic organisms, such as fish and whales.

These organisms include drifting animals, plants, archaea, algae, or bacteria that inhabit the pelagic zone of oceans, seas, or bodies of fresh water. That is, plankton are defined by their ecological niche rather than phylogenetic or taxonomic classification.

Although many planktic (or planktonic) species are microscopic in size, plankton consists organisms covering a wide range of sizes, including large organisms such as jellyfish.

Plankton are primarily divided into broad functional (or trophic level) groups: Phytoplankton, Zooplankton, and Bacterioplankton.

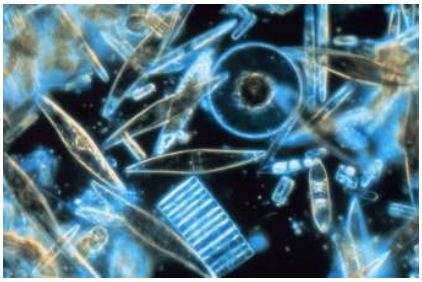


Figure: **Diatoms**: Assorted diatoms as seen through a microscope. These specimens were living between crystals of annual sea ice in McMurdo Sound, Antarctica. Image digitized from original 35mm Ektachrome slide. These tiny phytoplankton are encased within a silicate cell wall.

Phytoplankton (from Greek phyton, or plant), autotrophic, prokaryotic, or eukaryotic algae live near the water surface where there is sufficient light to support photosynthesis. Among the more important groups are the diatoms, cyanobacteria, dinoflagellates, and coccolithophores.

Zooplankton (from Greek zoon, or animal), small protozoans or metazoans (e.g. crustaceans and other animals) that feed on other plankton and telonemia. Some of the eggs and larvae of larger animals, such as fish, crustaceans, and annelids, are included here.

Bacterioplankton, bacteria and archaea, which play an important role in remineralising organic material down the water column (note that the prokaryotic phytoplankton are also bacterioplankton).

This scheme divides the plankton community into broad producer, consumer, and recycler groups. However, determining the trophic level of some plankton is not straightforward. For example, although most dinoflagellates are either photosynthetic producers or heterotrophic consumers, many species are mixotrophic depending upon circumstances.

Aside from representing the bottom few levels of a food chain that supports commercially important fisheries, plankton ecosystems play a role in the biogeochemical cycles of many important chemical elements, including the ocean's carbon cycle.

Primarily by grazing on phytoplankton, zooplankton provides carbon to the planktic foodweb, either respiring it to provide metabolic energy, or upon death as biomass or detritus. Typically more dense than seawater, organic material tends to sink. In open ocean ecosystems away from the coasts this transports carbon from surface waters to the deep. This process is known as the biological pump, and is one reason that oceans constitute the largest carbon sink on earth.

It might be possible to increase the ocean's uptake of carbon dioxide generated through human activities by increasing plankton production through "seeding," primarily with the micronutrient iron. However, this technique may not be practical at a large scale. Ocean oxygen depletion and resultant methane production (caused by the excess production remineralizing at depth) is one potential drawback.

The growth of phytoplankton populations is dependent on light levels and nutrient availability. The main factor limiting growth varies from region to region in the world's oceans. On a broad scale, growth of phytoplankton in the oligotrophic tropical and

subtropical gyres is generally limited by nutrient supply, while light often limits phytoplankton growth in subarctic gyres. Environmental variability at multiple scales influences the nutrient and light available for phytoplankton. As these organisms form the base of the marine food web, this variability in phytoplankton growth influences higher trophic levels. For example, at interannual scales phytoplankton levels temporarily plummet during El Nino periods, influencing populations of zooplankton, fishes, sea birds, and marine mammals.

The effects of anthropogenic warming on the global population of phytoplankton are an area of active research. Changes in the vertical stratification of the water column, the rate of temperature-dependent biological reactions, and the atmospheric supply of nutrients are expected to have important impacts on future phytoplankton productivity. Additionally, changes in the mortality of phytoplankton due to rates of zooplankton grazing may be significant.

Freshly hatched fish larvae are also plankton for a few days as long as they cannot swim against currents. Zooplankton are the initial prey item for almost all fish larvae as they switch from their yolk sacs to external feeding. Fish rely on the density and distribution of zooplankton to match that of new larvae, which can otherwise starve. Natural factors (e.g., current variations) and man-made factors (e.g. river dams) can strongly affect zooplankton, which can in turn strongly affect larval survival and therefore breeding success.

Key Points

- Plankton are primarily divided into broad functional (or trophic level) groups: Phytoplankton, Zooplankton, and Bacterioplankton.
- Plankton cover a wide range of sizes, including microscopic to large organisms such as jellyfish.
- Plankton community into broad producer, consumer, and recycler groups.

Key Terms

- **trophic**: Describing the relationships between the feeding habits of organisms in a food chain.
- **plankton**: Plankton (singular plankter) are any organisms that live in the water column and are incapable of swimming against a current. They provide a crucial source of food to many large aquatic organisms, such as fish and whales.
- **organisms**: An organism is any contiguous living system (such as animal, fungus, micro-organism, or plant). In at least some form, all types of organisms are capable of response to stimuli, reproduction, growth and development, and maintenance of homeostasis as a stable whole.

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3.3C: Planktonic Food Webs

Learning Objectives

- Differentiate planktonic food webs

Plankton communities are divided into broad categories of producer, consumer and recycler groups. However, determining the trophic level of some plankton is not straightforward. For example, although most dinoflagellates are either photosynthetic producers or heterotrophic consumers, many species are mixotrophic, depending upon circumstances.



Figure: **Photomontage of plankton organisms**: Plankton are any water-column organisms that are incapable of swimming against a current.

Aside from representing the bottom few levels of a food chain that supports commercially important fisheries, plankton ecosystems play a role in the biogeochemical cycles of many important chemical elements, including the ocean's carbon cycle.

Primarily by grazing on phytoplankton, zooplankton provide carbon to the planktic foodweb, either respiring it to provide metabolic energy, or upon death as biomass or detritus. Typically more dense than seawater, organic material tends to sink. In open-ocean ecosystems away from the coasts this transports carbon from surface waters to the deep. This process is known as the biological pump, and is one reason that oceans constitute the largest carbon sink on Earth.

It might be possible to increase the ocean's uptake of carbon dioxide generated through human activities by increasing plankton production through "seeding", primarily with the micronutrient iron. However, this technique may not be practical on a large scale. Ocean oxygen depletion and resultant methane production (caused by the excess production of remineralising at depth) is one potential drawback.

The growth of phytoplankton populations is dependent on light levels and nutrient availability. The chief factor limiting growth varies from region to region in the world's oceans. On a broad scale, growth of phytoplankton in the oligotrophic tropical and subtropical gyres is generally limited by nutrient supply, while light often limits phytoplankton growth in subarctic gyres. Environmental variability at multiple scales influences the nutrient and light available for phytoplankton. As these organisms form the base of the marine food web, this variability in phytoplankton growth influences higher trophic levels. For example, at interannual scales phytoplankton levels temporarily plummet during El Nino periods, influencing populations of zooplankton, fish, sea birds, and marine mammals.

The effects of anthropogenic warming on the global population of phytoplankton is an area of active research. Changes in the vertical stratification of the water column, the rate of temperature-dependent biological reactions, and the atmospheric supply of nutrients are expected to have important impacts on future phytoplankton productivity. Additionally, changes in the mortality of phytoplankton due to rates of zooplankton grazing may be significant.

Freshly-hatched fish larvae are also plankton for a few days as long as they cannot swim against currents. Zooplankton are the initial prey item for almost all fish larvae as they switch from their yolk sacs to external feeding. Fish rely on the density and distribution of zooplankton to match that of new larvae, which can otherwise starve. Natural factors (e.g., current variations) and man-made factors (e.g. river dams) can strongly affect zooplankton, which can in turn strongly affect larval survival, and therefore breeding success.

Key Points

- Plankton communities represent the bottom few levels of a food chain that supports commercially important fisheries.
- Plankton ecosystems also play a crucial role in the biogeochemical cycles of many important chemical elements, including the ocean's carbon cycle.
- The growth of phytoplankton populations is dependent on light levels and nutrient availability.

Key Terms

- **plankton**: Plankton (singular plankter) are any organisms that live in the water column and are incapable of swimming against a current. They provide a crucial source of food to many large aquatic organisms, such as fish and whales.
- **ecosystems**: Communities of living organisms (plants, animals and microbes) in conjunction with the nonliving components of their environment (things like air, water, and mineral soil), interacting as a system; linked together through nutrient cycles and energy flows.
- **biogeochemical cycles**: A biogeochemical cycle or substance turnover or cycling of substances is a pathway by which a chemical element or molecule moves through both biotic (biosphere) and abiotic (lithosphere, atmosphere, and hydrosphere) compartments of Earth. A cycle is a series of change which comes back to the starting point and which can be repeated.

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3.3D: Ocean Floor

Learning Objectives

- Explain the importance of microbes and hydrothermal vents to underwater ecosystems

Microorganisms, by their omnipresence, impact the entire biosphere. Microbial life plays a primary role in regulating biogeochemical systems in virtually all of our planet ‘s environments, including some of the most extreme, from frozen environments and acidic lakes, to hydrothermal vents at the bottom of deepest oceans, and some of the most familiar, such as the human small intestine.

Microbes, especially bacteria, often engage in symbiotic relationships (either positive or negative) with other organisms, and these relationships affect the ecosystem. One example of these fundamental symbioses are chloroplasts, which allow eukaryotes to conduct photosynthesis. Chloroplasts are considered to be endosymbiotic cyanobacteria, a group of bacteria that are thought to be the origins of aerobic photosynthesis.

They are the backbone of all ecosystems, but even more so in the zones where light cannot approach and therefore photosynthesis cannot be the basic means to collect energy. In such zones, chemosynthetic microbes provide energy and carbon to the other organisms. Other microbes are decomposers, with the ability to recycle nutrients from other organisms’ waste poducts. These microbes play a vital role in biogeochemical cycles. The nitrogen cycle, the phosphorus cycle and the carbon cycle all depend on microorganisms in one way or another. For example, nitrogen which makes up 78% of the planet’s atmosphere is “indigestible” for most organisms, and the flow of nitrogen into the biosphere depends on a microbial process called fixation.

Recently there has been the discovery of abundant marine life in the deep sea, especially around hydrothermal vents. Large deep sea communities of marine life have been discovered around black and white smokers – hydrothermal vents emitting typical chemicals toxic to humans and most of the vertebrates. This marine life receives its energy from both the extreme temperature difference (typically a drop of 150 degrees) and from chemosynthesis by bacteria.

Brine pools are another seabed feature, usually connected to cold seeps. Hydrothermal vents along the mid-ocean ridge spreading centers act as oases, as do their opposites, cold seeps. Such places support unique biomes and many new microbes and other lifeforms have been discovered at these locations. The deepest recorded oceanic trench measured to date is the Mariana Trench, near the Philippines, in the Pacific Ocean at 10,924 m (35,838 ft). At such depths, water pressure is extreme. There is no sunlight, but some life still exists. A white flatfish, a shrimp, and a jellyfish were seen by the American crew of the bathyscaphe Trieste when it dove to the bottom in 1960. Marine life also flourishes around seamounts that rise from the depths, where fish and other sea life congregate to spawn and feed.

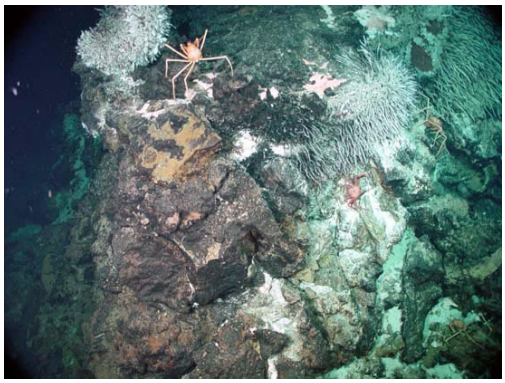


Figure: Zooarium chimney provides a habitat for vent biota.

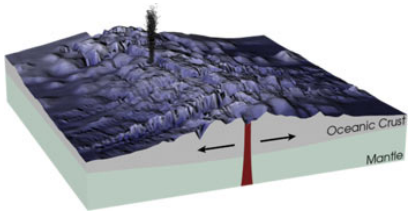


Figure: **Oceanic ridge with deep sea vent**: Oceanic ridge with deep sea vent.

Key Points

- Recently, there has been the discovery of abundant marine life in the deep sea, especially around hydrothermal vents.
- Hydrothermal vents along the mid-ocean ridge spreading centers act as oases and support unique biomes and many new microbes.
- Each area of the seabed has typical features such as common soil composition, typical topography, salinity of water layers above it, marine life, magnetic direction of rocks, and sedimenting.

Key Terms

- **plankton**: Plankton (singular plankter) are any organisms that live in the water column and are incapable of swimming against a current. They provide a crucial source of food to many large aquatic organisms, such as fish and whales.
- **ecosystems**: Communities of living organisms (plants, animals and microbes) in conjunction with the nonliving components of their environment (things like air, water, and mineral soil), interacting as a system; linked together through nutrient cycles and energy flows.
- **biogeochemical cycles**: A biogeochemical cycle or substance turnover or cycling of substances is a pathway by which a chemical element or molecule moves through both biotic (biosphere) and abiotic (lithosphere, atmosphere, and hydrosphere) compartments of Earth. A cycle is a series of change which comes back to the starting point and which can be repeated.

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3.3E: Cold-Seep Ecosystems

Learning Objectives

- Outline the organisms that live in cold-seep ecosystems

A cold seep (sometimes called a cold vent) is an area of the ocean floor where hydrogen sulfide, methane, and other hydrocarbon-rich fluid seepage occurs, often in the form of a brine pool. “Cold” does not mean temperature of seepage is lower than surrounding sea water. Actually, its temperature is often slightly higher. Cold seeps constitute a biome supporting several endemic species.

Cold seeps develop unique topography over time, where reactions between methane and seawater create carbonate rock formations and reefs. These reactions may also be dependent on bacterial activity. Ikaite, a hydrous calcium carbonate, can be associated with oxidizing methane at cold seeps.

Types of cold seeps can be distinguished according to the depth, as shallow cold seeps and deep cold seeps. Cold seeps can also be distinguished in detail, as follows: oil/gas seeps, gas seeps, methane seeps, gas hydrate seeps, brine seeps, are forming brine pools, pockmarks and mud volcanos.

Organisms living in cold seeps are known as extremophiles. Biological research in cold seeps and hydrothermal vents has been mostly focused on the microbiology and the prominent chemosynthetic macro-invertebrates. Much less research has been done on the smaller benthic fraction at the size of the meiofauna (<1 mm).

Community composition’s orderly shift from one set of species to another is called ecological succession. The first type of organism to take advantage of this deep-sea energy source is bacteria. Aggregating into bacterial mats at cold seeps, these bacteria metabolize methane and hydrogen sulfide (another gas that emerges from seeps) for energy. This process of obtaining energy from chemicals is known as chemosynthesis.



Figure: **A Beggiatoa bacterial mat at the Blake Ridge**: Beggiatoa spp. bacterial mat at a seep on Blake Ridge, off the coast of South Carolina. The red dots are range-finding laser beams. Beggiatoa are able to detoxify hydrogen sulfide in soil.

During this initial stage, when methane is relatively abundant, dense mussel beds also form near the cold seep. Mostly composed of species in the genus Bathymodiolus, these mussels do not directly consume food. Instead, they are nourished by symbiotic bacteria that also produce energy from methane, similar to their relatives that form mats. Chemosynthetic bivalves are prominent constituents of the fauna of cold seeps and are represented in that setting by five families: Solemyidae, Lucinidae, Vesicomyidae, Thyasiridae, and Mytilidae.

This microbial activity produces calcium carbonate ($CaCO3$), which is deposited on the seafloor and forms a layer of rock. During a period lasting up to several decades, these rock formations attract siboglinid tubeworms, which settle and grow along with the mussels. Like the mussels, tubeworms rely on chemosynthetic bacteria (in this case, a type that needs hydrogen sulfide instead of methane) for survival. True to any symbiotic relationship, a tubeworm also provides for their bacteria by appropriating hydrogen sulfide from the environment. The sulfide not only comes from the water, but is also mined from the sediment through an extensive “root” system a tubeworm “bush” establishes in the hard, carbonate substrate. A tubeworm bush can contain hundreds of individual worms, which can grow a meter or more above the sediment.

Cold seeps do not last indefinitely. As the rate of gas seepage slowly decrease, the shorter-lived, methane-hungry mussels (or more precisely, their methane-hungry bacterial symbionts) start to die off. At this stage, tubeworms become the dominant organism in a seep community. As long as there is some sulfide in the sediment, the sulfide-mining tubeworms can persist. Individuals of one tubeworm species Lamellibrachia luymesi have been estimated to live for over 250 years in such conditions.

Key Points

- Cold seeps develop unique topography over time, where reactions between methane and seawater create carbonate rock formations and reefs.
- Types of cold seeps can be distinguished according to the depth, as shallow cold seeps and deep cold seeps.
- Organisms living in cold seeps are known as extremophiles.

Key Terms

- **cold seep**: A cold seep (sometimes called a cold vent) is an area of the ocean floor where hydrogen sulfide, methane, and other hydrocarbon-rich fluid seepage occurs, often in the form of a brine pool. “Cold” does not mean temperature of seepage is lower than surrounding sea water. Actually, its temperature is often slightly higher.
- **topography**: A detailed graphic representation of the surface features of a place or object.
- **extremophiles**: An extremophile (from Latin extremus, meaning “extreme,” and Greek philiā (φ), meaning “love”) is an organism that thrives in physically or geochemically extreme conditions that are detrimental to most life on earth.

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3.3F: The Deep Sea and Barophilism

Learning Objectives

- Indicate how barophiles survive in the deep sea

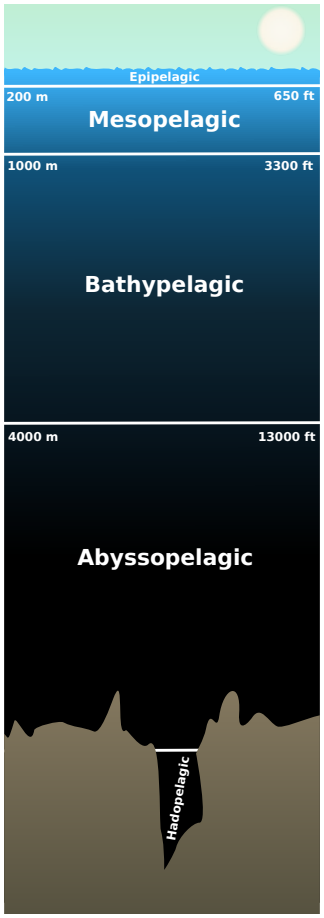


Figure: **Deep Sea Pelagic Zones**: Mesopelagic, bathyl, abyssal, and hadal zones.

Deep sea communities currently remain largely unexplored, due the technological and logististical challenges, and the expense involved in visiting these remote biomes. Because of the unique challenges (particularly the high barometric pressure, extremes of temperature, and absence of light), it was long believed that little life existed in this hostile environment. Since the 19th century however, research has demonstrated that significant biodiversity exists in the deep sea.

The three main sources of energy and nutrients for deep sea communities are marine snow, whale falls, and chemosynthesis at hydrothermal vents and cold seeps.

Zones of the deep sea include the mesopelagic zone, the bathyal zone, the abyssal zone, and the hadal zone.

A piezophile, also called a barophile, is an organism which thrives at high pressures, such as deep sea bacteria or archaea. They are generally found on ocean floors, where pressure often exceeds 380 atm (38 MPa). Some have been found at the bottom of the Pacific Ocean where the maximum pressure is roughly 117 MPa. The high pressures experienced by these organisms can cause the normally fluid cell membrane to become waxy and relatively impermeable to nutrients. These organisms have adapted in novel ways to become tolerant of these pressures in order to colonize deep sea habitats. One example, xenophyophores, have been found in the deepest ocean trench, 6.6 miles (10,541 meters) below the surface.

Barotolerant bacteria are able to survive at high pressures, but can exist in less extreme environments as well. Obligate barophiles cannot survive outside of such environments. For example, the Halomonas species Halomonas salaria requires a pressure of 1000

atm (100 MPa) and a temperature of three degrees Celsius. Most piezophiles grow in darkness and are usually very UV-sensitive; they lack many mechanisms of DNA repair.

Key Points

- The three main sources of energy and nutrients for deep sea communities are marine snow, whale falls, and chemosynthesis.
- Zones of the deep sea include the mesopelagic zone, the bathyal zone, the abyssal zone, and the hadal zone.
- Organisms have adapted in novel ways to become tolerant of the high pressures and cool temperatures in order to colonize deep sea habitats.

Key Terms

- **deep sea**: The deeper part of the sea or ocean in which no light penetrates.
- **piezophile**: A piezophile (also called a barophile) is an organism which thrives at high pressures, such as deep sea bacteria or archaea.
- **chemosynthesis**: The production of carbohydrates and other compounds from simple compounds such as carbon dioxide, using the oxidation of chemical nutrients as a source of energy rather than sunlight; it is limited to certain bacteria and fungi.

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3.3G: Sea Coral and Sea Anemone Zooxanthellae

Learning Objectives

- Outline the role Zooxanthellae play in animal sybiosis

Symbiodinium are colloquially called “zooxanthellae” (or “zoox”), and animals symbiotic with algae in this genus are said to be “zooxanthellate”. The term was loosely used to refer to any golden-brown endosymbionts, including diatoms and other dinoflagellates. The genus Symbiodinium encompasses the largest and most prevalent group of endosymbiotic dinoflagellates known to science. These unicellular algae commonly reside in the endoderm of tropical cnidarians such as corals, sea anemones, and jellyfish, where they translocate products of photosynthesis to the host and in turn receive inorganic nutrients (e.g. CO2, NH4+). They are also harbored by various species of sponges, flatworms, mollusks (e.g. giant clams), foraminifera (soritids), and some ciliates. Generally, these dinoflagellates enter the host cell through phagocytosis, persist as intracellular symbionts, reproduce, and disperse to the environment (note that in most mollusks, Symbiodinium are inter- rather than intra-cellular). Cnidarians that are associated with Symbiodinium occur mostly in warm oligotrophic (nutrient-poor) marine environments where they are often the dominant constituents of benthic communities. These dinoflagellates are therefore among the most abundant eukaryotic microbes found in coral reef ecosystems.

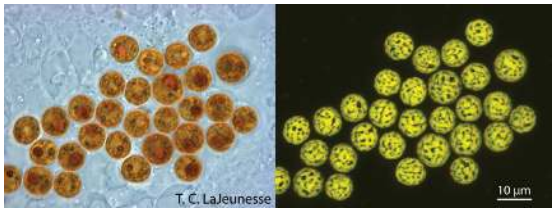


Figure: **Symbiodinium cell**: Symbiodinium cell living inside a jellyfish.

Symbiodinium are known primarily for their role as mutualistic endosymbionts. In hosts, they usually occur in high densities, ranging from hundreds of thousands to millions per square centimeter. The successful culturing of swimming gymnodinioid cells from coral led to the discovery that “zooxanthellae” were actually dinoflagellates. Each Symbiodinium cell is coccoid in hospite (living in a host cell) and surrounded by a membrane that originates from the host cell plasmalemma during phagocytosis. This membrane probably undergoes some modification to its protein content, which functions to limit or prevent phago-lysosome fusion. The vacuole structure containing the symbiont is therefore termed the symbiosome, and only a single symbiont cell is found within each symbiosome. It is unclear how this membrane expands to accommodate a dividing symbiont cell. Under normal conditions, symbiont and host cells exchange organic and inorganic molecules that enable the growth and proliferation of both partners.

Key Points

- Zooxanthellae species are members of the phylum Dinoflagellata. The most common genus is Symbiodinium.
- Each Symbiodinium cell is coccoid in hospite (living in a host cell) and surrounded by a membrane that originates from the host cell plasmalemma during phagocytosis.
- Zooxanthellates mutualistic relationships with reef-building corals form the basis of a highly diverse and productive ecosystem.

Key Terms

- **endosymbiont**: An organism that lives within the body or cells of another organism.
- **phagocytosis**: the process by which a cell incorporates foreign particles intracellularly.
- **benthic**: Pertaining to the benthos; living on the seafloor, as opposed to floating in the ocean.

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3.3H: Sponge Communities

Learning Objectives

- Compare the types of sponge communities

Sponge reefs serve an important ecological function as habitat, breeding, and nursery areas for fish and invertebrates. The reefs are currently threatened by the fishery, offshore oil, and gas industries. Attempts are being made to protect these unique ecosystems through fishery closures, and potentially the establishment of Marine Protected Areas (MAPs) around the sponge reefs.



Figure: **Aphrocallistes vastus**: Aphrocallistes vastus (Cloud sponge), is a major reef-building species.

Hexactinellids

Hexactinellid sponge reefs were common in the late Jurassic period, and were believed to have gone extinct during or shortly after the Cretaceous period. Living sponge reefs were discovered in the Queen Charlotte Basin (QCB) in 1987-1988, and were reported in the Georgia Basin (GB) in 2005. These sponge reefs are considered to be “living fossils. ”

Hexactinellids, or “glassy” sponges, are characterized by a rigid framework of spicules made of silica. Unlike other poriferans, hexactinellids do not possess the ability to contract. Another unique feature of glassy sponges is that their tissues are made up almost entirely of syncytia. In a syncytium there are many nuclei in a continuous cytoplasm; nuclei are not packaged in discrete cells.

As a result, the sponge has a distinctive electrical conduction system across its body. This allows the sponge to rapidly respond to disturbances, such as a physical impact or excessive sediment in the water. The sponge’s response is to stop feeding. It will try to resume feeding after 20-30 minutes, but will stop again if the irritation is still present.

Hexactinellids are exclusively marine and are found throughout the world in deep (>1000 m) oceans. Individual sponges grow at a rate of 0-7 cm/year, and can live to be at least 220 years old. Little is known about hexactinellid sponge reproduction. Like all poriferans, the hexactinellids are filter feeders. They obtain nutrition from direct absorption of dissolved substances, and to a lesser extent from particulate materials. There are no known predators of healthy reef sponges. This is likely because the sponges possess very little organic tissue; the siliceous skeleton accounts for 90% of the sponge body weight.

Hexasterophorans

Hexasterophoran sponges have spicules called hexactines that have six rays set at right angles. Orders within hexasterophora are classified by how tightly the spicules interlock with Lyssanctinosan spicules less tightly interlocked than those of Hexactinosan sponges.

The primary frame-building sponges are all members of the order Hexactinosa, and include the species Chonelasma/Heterochone calyx (chalice sponge), Aphrocallistes vastus (cloud sponge), and Farrea occa. Hexactinosan sponges have a rigid scaffolding of “fused” spicules that persists after the death of the sponge.

Lyssactinosa

Other sponge species abundant on sponge reefs are members of the order Lyssactinosa (Rosselid sponges) and include Rhabdocalyptus dawsoni (boot sponge), Acanthascus platei, Acanthascus cactus and Staurocalyptus dowlingi. Rosselid sponges have a “woven” or “loose” siliceous skeleton that does not persist after the death of the sponge, and are capable of forming mats, but not reefs.

Sponge Reefs

Each living sponge on the surface of the reef can be over 1.5 m tall. The reefs are composed of mounds called “bioherms” that are up to 21 m high, and sheets called “biostromes” that are 2-10 m thick, and may be many km wide. Each sponge in the order Hexactinosa has a rigid skeleton that persists after the death of the animal. This provides an excellent substrate for sponge larvae to settle upon, and new sponges grow on the framework of past generations. The growth of sponge reefs is thus analogous to that of coral reefs. The tendrils of new sponges wrap around spicules of older, deceased sponges. The tendrils will later form the basal plate of the adult sponge that firmly anchors the animal to the reef.

Deep ocean currents carry fine sediments that are captured by the scaffolding of sponge reefs. A sediment matrix of silt, clay, and some sand forms around the base of the sponge bioherms. The sediment matrix is soft near the surface, and firm below one metre deep. Dead sponges become covered in sediment, but do not lose their supportive siliceous skeleton. The sponge sediments have high levels of silica and organic carbon. The reefs grow parallel to the glacial troughs, and the morphology of reefs is due to deep currents.

Hexactinellids first appeared in the fossil record during the late Proterozoic, and the first Hexactinosans were found in the late Devonian. Hexactinellid sponge reefs were first identified in the middle Triassic (245-208 million years ago). The sponges reached their full extent in the late Jurassic (208-146 million years ago), when a discontinuous reef system 7,000 km long stretched across the northern Tethys and North Atlantic basins. This chain of sponge reefs is the largest known biostructure to have ever existed on Earth.

Key Points

- Sponge reefs are considered to be “living fossils”.
- Hexactinellids, or “glassy” sponges are characterized by a rigid framework of spicules made of silica.
- A unique feature of glassy sponges is that their tissues are made up almost entirely of syncytia.

Key Terms

- **silica**: Any of the silica group of the silicate minerals.
- **Sponge reefs**: Sponge reefs serve an important ecological function as habitat, breeding, and nursery areas for fish and invertebrates. The reefs are currently threatened by the fishery, offshore oil, and gas industries.
- **syncytia**: A syncytia (plural syncytia) is a multinucleate cell which can result from multiple cell fusions of uninuclear cells (i.e., cells with a single nucleus), in contrast to a coenocyte, which can result from multiple nuclear divisions without accompanying cytokinesis.

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3.3I: Freshwater Environments

Learning Objectives

- Generalize the characteristics of freshwater environments

Fresh water is naturally occurring water on the Earth's surface in ice sheets, ice caps, glaciers, bogs, ponds, lakes, rivers and streams, and underground as groundwater in aquifers and underground streams. Fresh water is generally characterized by having low concentrations of dissolved salts and other total dissolved solids. The term specifically excludes seawater and brackish water but it does include mineral rich waters such as chalybeate springs. The term "sweet water" has been used to describe fresh water in contrast to salt water.

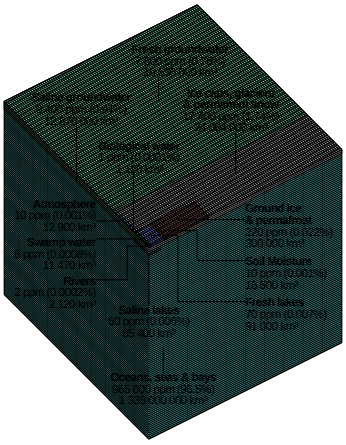


Figure: **Distribution (by volume) of water on Earth**: Visualization of the distribution (by volume) of water on Earth. Each tiny cube (such as the one representing biological water) corresponds to approximately 1000 km³ of water, with a mass of about 1 trillion tonnes (200000 times that of the Great Pyramid of Giza). The entire block comprises 1 million tiny cubes.

Scientifically, freshwater habitats are divided into lentic systems, which are the stillwaters including ponds, lakes, swamps and mires; lotic systems, which are running water; and groundwater which flows in rocks and aquifers. There is, in addition, a zone which bridges between groundwater and lotic systems – the hyporheic zone – which underlies many larger rivers and can contain substantially more water than is seen in the open channel. It may also be in direct contact with the underlying underground water.

Fresh water creates a hypotonic environment for aquatic organisms. This is problematic for some organisms with pervious skins or with gill membranes, whose cell membranes may burst if excess water is not excreted. Some protists accomplish this using contractile vacuoles, while freshwater fish excrete excess water via the kidney. Although most aquatic organisms have a limited ability to regulate their osmotic balance and therefore can only live within a narrow range of salinity, diadromous fish have the ability to migrate between fresh water and saline water bodies. During these migrations they undergo changes to adapt to the surroundings of the changed salinities; these processes are hormonally controlled. The eel (Anguilla anguilla) uses the hormone prolactin, while in salmon (Salmo salar) the hormone cortisol plays a key role during this process.

Many sea birds have special glands at the base of the bill through which excess salt is excreted. Similarly the marine iguanas on the Galápagos Islands excrete excess salt through a nasal gland and they sneeze out a very salty excretion.

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Key Points

- Freshwater habitats are divided into lentic systems (which are the stillwaters including ponds, lakes, swamps and mires) and lotic systems, which are running water; and groundwater which flows in rocks and aquifers.
- Fresh water creates a hypotonic environment for aquatic organisms.
- Most aquatic organisms have a limited ability to regulate their osmotic balance and therefore can only live within a narrow range of salinity.

Key Terms

- **hypotonic**: Having a lower osmotic pressure than another.
- **Freshwater**: Fresh water is naturally occurring water on the Earth's surface in ice sheets, ice caps, glaciers, bogs, ponds, lakes, rivers and streams, and underground as groundwater in aquifers and underground streams. Fresh water is generally characterized by having low concentrations of dissolved salts and other total dissolved solids.
- **osmotic balance**: Osmoregulation is the active regulation of the osmotic pressure of an organism's fluids to maintain the homeostasis of the organism's water content; that is, it keeps the organism's fluids from becoming too diluted or too concentrated.

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SECTION OVERVIEW

3.4: Nutrient Cycles

Topic hierarchy

3.4A: Sources and Sinks of Essential Elements

3.4B: The Carbon Cycle

3.4C: Syntrophy and Methanogenesis

3.4D: The Phosphorus Cycle

3.4E: The Nitrogen Cycle

3.4F: The Sulfur Cycle

3.4G: The Iron Cycle

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3.4A: Sources and Sinks of Essential Elements

Learning Objectives

- Identify sources and sinks of essential elements

Most important substances on Earth, such as oxygen, nitrogen, and water undergo turnover or cycling through both the biotic (living) and abiotic (geological, atmospheric, and hydrologic) compartments of the Earth. Flows of nutrients from living to non-living components of the Earth are called biogeochemical cycles.

Nutrient Cycles and the Biosphere

Ecosystems hinge on biogeochemical cycles. The nitrogen cycle, the phosphorous cycle, the sulfur cycle, and the carbon cycle all involve assimilation of these nutrients into living things. These elements are transferred among living things through food webs, until organisms ultimately die and release them back into the geosphere.

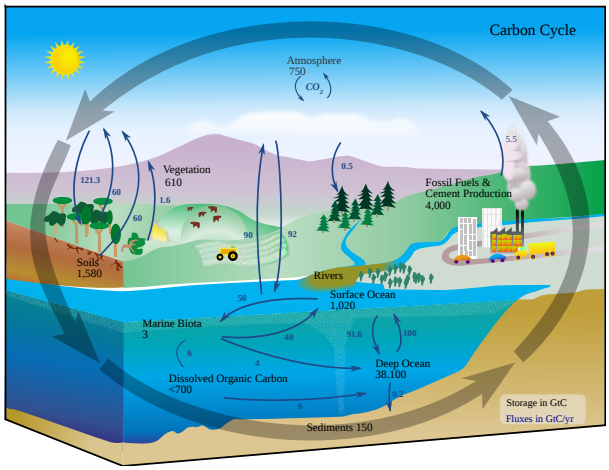


Figure: **The Carbon Cycle**: The element carbon moves from the biosphere to the geosphere and the hydrosphere. This flow from abiotic to biotic compartments of the Earth is typical of biogeochemical cycles.

Reservoirs of Essential Elements

Chemicals are sometimes sequestered for long periods of time and taken out of circulation. Locations where elements are stored for long periods of time are called reservoirs. Coal is a reservoir for carbon, and coal deposits can house carbon for thousands of years. The atmosphere is considered a reservoir for nitrogen.

Humans and Biogeochemical Cycles

Although the Earth receives energy from the Sun, the chemical composition of the planet is more or less fixed. Matter is occasionally added by meteorites, but supplies of essential elements generally do not change. However, human activity can change the proportion of nutrients that are in reservoirs and in circulation. For example, coal is a resevoir of carbon, but the human use of fossil fuels has released carbon into the atmosphere, increasing the amount of carbon in circulation. Likewise, phosphorous and nitrogen are extracted from geological reservoirs and used in phosphorous, and excesses of these elements have caused the overgrowth of plant matter and the disruption of many ecosystems.

Key Points

- Biogeochemical cycles are pathways by which nutrients flow between the abiotic and abiotic compartments of the Earth. The abiotic portion of the Earth includes the lithosphere (the geological component of the Earth) and the hydrosphere (the Earth's water).
- Ecosystems rely on biogeochemical cycles. Many of the nutrients that living things depend on, such as carbon, nitrogen, and phosphorous are in constant circulation.

- Essential elements are often stored in reservoirs, where they can be taken out of circulation for years. For example, coal is a reservoir for carbon.
- Humans can affect biogeochemical cycles. Humans extract carbon and nitrogen from the geosphere and use them for energy and fertilizer. This has increased the amount of these elements in circulation, which has detrimental effects on ecosystems.

Key Terms

- **Reservoir**: Reservoirs are places where essential elements are sequestered for long periods of time.
- **biogeochemical cycle**: A pathway by which a chemical element or molecule moves through both biotic (biosphere) and abiotic (lithosphere, atmosphere, and hydropshere) compartments of the planet.

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3.4B: The Carbon Cycle

Learning Objectives

- Outline the flow of carbon through the biosphere and abiotic matter on earth

The carbon cycle describes the flow of carbon between the biosphere, the geosphere, and the atmosphere, and is essential to maintaining life on earth.

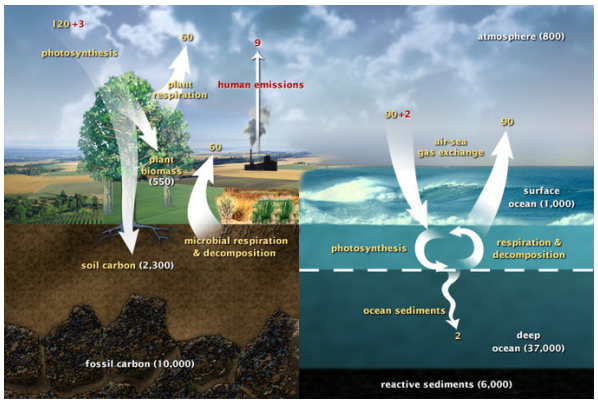


Figure: **The Carbon Cycle**: The carbon cycle describes the flow of carbon between the atmosphere, the biosphere, and the geosphere.

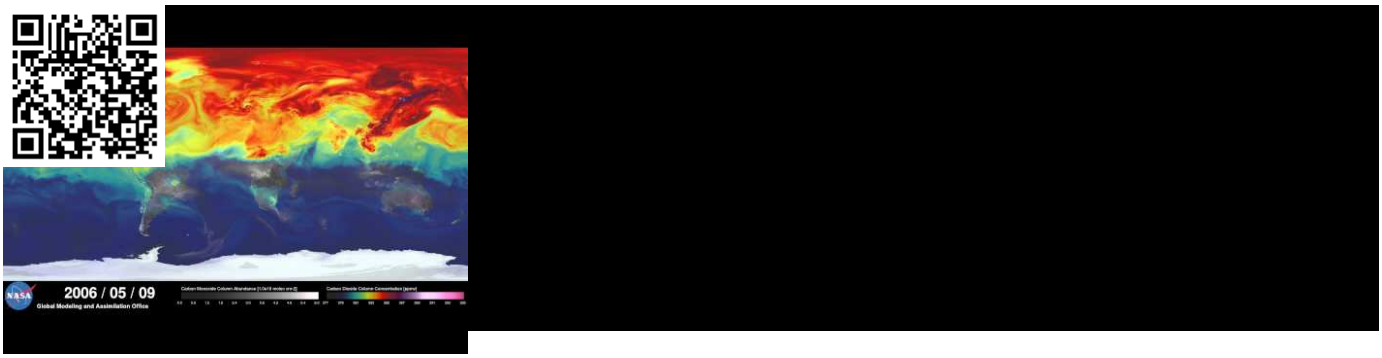
Atmospheric Carbon Dioxide: Carbon in the earth's atmosphere exists in two main forms: carbon dioxide and methane. Carbon dioxide leaves the atmosphere through photosynthesis, thus entering the terrestrial and marine biospheres. Carbon dioxide also dissolves directly from the atmosphere into bodies of water (oceans, lakes, etc.), as well as dissolving in precipitation as raindrops fall through the atmosphere. When dissolved in water, carbon dioxide reacts with water molecules and forms carbonic acid, which contributes to ocean acidity. Human activity over the past two centuries has significantly increased the amount of carbon in the atmosphere, mainly in the form of carbon dioxide, both by modifying ecosystems ' ability to extract carbon dioxide from the atmosphere and by emitting it directly, e.g. by burning fossil fuels and manufacturing concrete.

Terrestrial Biosphere: The terrestrial biosphere includes the organic carbon in all land-living organisms, both alive and dead, as well as carbon stored in soils. Although people often imagine plants as the most important part of the terrestrial carbon cycle, microorganisms such as single celled algae and chemoautotrophic bacteria are also important in converting atmospheric CO_2 into terrestrial carbon. Carbon is incorporated into living things as part of organic molecules, either through photosynthesis or by animals that consume plants and algae. Some of the carbon in living things is released through respiration, while the rest remains in the tissue. Once organisms die, bacteria break down their tissues, releasing CO_2 back into the atmosphere or into the soil.

Marine Biosphere: The carbon cycle in the marine biosphere is very similar to that in the terrestrial ecosystem. CO_2 dissolves in the water and algae, plants and bacteria convert it into organic carbon. Carbon may transfer between organisms (from producers to consumers). Their tissues are ultimately broken down by bacteria and CO_2 is released back into the ocean or atmosphere.

NASA | A Year in the Life of Earth's CO2: An ultra-high-resolution NASA computer model has given scientists a stunning new look at how carbon dioxide in the atmosphere travels around the globe. Plumes of carbon dioxide in the simulation swirl and shift as winds disperse the greenhouse gas away from its sources. The simulation also illustrates differences in carbon dioxide levels in the northern and southern hemispheres and distinct swings in global carbon dioxide concentrations as the growth cycle of plants and trees changes with the seasons. The carbon dioxide visualization was produced by a computer model called GEOS-5, created by scientists at NASA Goddard Space Flight Center's Global Modeling and Assimilation Office. The visualization is a product of a simulation called a "Nature Run." The Nature Run ingests real data on atmospheric conditions and the emission of greenhouse gases and both natural and man-made particulates. The model is then left to run on its own and simulate the natural behavior of the Earth's atmosphere. This Nature Run simulates January 2006 through December 2006. While Goddard scientists worked with a "beta" version of the Nature Run internally for several years, they released this updated, improved version to the scientific community for the first time in the fall of 2014.





Geologic Carbon: The earth's crust also contains carbon. Much of the earth's carbon is stored in the mantle, and has been there since the earth formed. Much of the carbon on the earth's lithosphere (about 80%) is stored in limestone, which was formed from the calcium carbonate from the shells of marine animals. The rest of the carbon on the earth's surface is stored in Kerogens, which were formed through the sedimentation and burial of terrestrial organisms under high heat and pressure.

Key Points

- Atmospheric carbon is usually in the form of CO_2. Carbon dioxide is converted to organic carbon through photosynthesis by primary producers such as plants, bacteria, and algae.
- Some organic carbon is returned to the atmosphere as CO_2 during respiration. The rest of the organic carbon may cycle from organism to organism through the food chain. When an organism dies, it is decomposed by bacteria and its carbon is released into the atmosphere or the soil.
- Carbon is also found in the earth's crust, primarily as limestone and kerogens.

Key Terms

- **lithosphere**: The rigid, mechanically strong, outer layer of the earth; divided into twelve major tectonic plates.
- **chemoautotrophic**: An organism obtaining its nutrition through the oxidation of non-organic compounds (or other chemical processes); as opposed to the process of photosynthesis.
- **carbon cycle**: The physical cycle of carbon through the Earth's biosphere, geosphere, hydrosphere and atmosphere that includes such processes as photosynthesis, decomposition, respiration and carbonification.

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3.4C: Syntrophy and Methanogenesis

Learning Objectives

- Assess syntrophy methanogenesis

Syntrophy or cross feeding is when one species lives off the products of another species. A frequently cited example of syntrophy are methanogenic archaea bacteria and their partner bacteria that perform anaerobic fermentation.



Figure: **Methanogenic Bacteria in Termites**: Methanogenic bacteria have a syntrophic relationship with protozoans living in the guts of termites. The protozoans break down cellulose, releasing H2 which is then used in methanogenesis.

Methanogenesis in microbes is a form of anaerobic respiration, performed by bacteria in the domain Archaea. Unlike other microorganisms, methanogens do not use oxygen to respire; but rather oxygen inhibits the growth of methanogens. In methanogenesis, carbon is used as the terminal electron receptor instead of oxygen. Although there are a variety of potential carbon based compounds that are used as electron receptors, the two best described pathways involve the use of carbon dioxide and acetic acid as terminal electron acceptors.

Acetic Acid: $CO2+4H2 \rightarrow CH4+2H2OCO2+4H2 \rightarrow CH4+2H2O$

Carbon Dioxide: $CH3COOH \rightarrow CH4+CO2CH3COOH \rightarrow CH4+CO2$

Many methanogenic bacteria that live in close association with bacteria produce fermentation products such as fatty acids longer than two carbon atoms, alcohols longer than one carbon atom, and branched chain and aromatic fatty acids. These products cannot be used in methanogenesis. Partner bacteria of the methanogenic archea therefore process these products. By oxydizing them to acetate, they allow them to be used in methanogenesis.

Methanogenic bacteria are important in the decomposition of biomass in most ecosystems. Only methanogenesis and fermentation can occur in the absence of electron acceptors other than carbon. Fermentation only allows the breakdown of larger organic compounds, and produces small organic compounds that can be used in methanogenesis. The semi-final products of decay (hydrogen, small organics, and carbon dioxide) are then removed by methanogenesis. Without methanogenesis, a great deal of carbon (in the form of fermentation products) would accumulate in anaerobic environments.

Methanogenic archea bacteria can also form associations with other organisms. For example, they may also associate with protozoans living in the guts of termites. The protozoans break down the cellulose consumed by termites, and release hydrogen, which is then used in methanogenesis.

Key Points

- Methanogenic bacteria are only found in the domain Archea, which are bacteria with no nucleus or other organelles.
- Methanogenesis is a form of respiration in which carbon rather than oxygen is used as an electron acceptor.
- Bacteria that perform anaerobic fermentation often partner with methanogenic bacteria. During anaerobic fermentation, large organic molecules are broken down into hydrogen and acetic acid, which can be used in methanogenic respiration.
- There are other examples of syntrophic relationships between methanogenic bacteria and mircoorganisms: protozoans in the guts of termites break down cellulose and produce hydrogen which can be used in methanogenesis.

Key Terms

- **Archea**: A domain of single-celled microorganisms. These microbes have no cell nucleus or any other membrane-bound organelles within their cells.
- **syntrophy**: A phenomenon where one species lives off the products of another species.
- **methanogenesis**: The generation of methane by anaerobic bacteria.

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3.4D: The Phosphorus Cycle

Learning Objectives

- Explain the phosphorous cycle

Phosphorus is an important element for living things because it is neccesary for nucleotides and ATP. Plants assimilate phosphorous from the environment and then convert it from inorganic phosphorous to organic phosphorous. Phosphorous can be transfered to other organisms when they consume the plants and algae. Animals either release phosphorous through urination or defecation, when they die and are broken down by bacteria. The organic phosphorous is released and converted back into inorganic phosphorous through decomposition. The phosphorous cycle differs from other nutrient cycles, because it never passes through a gaseous phase like the nitrogen or carbon cycles.

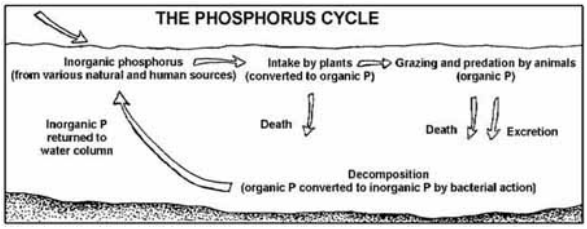


Figure: **The aquatic phosphorous cycle**: Phosphorous is converted between its organic and inorganic forms. Plants convert phosphorous to its organic form, and bacteria convert it back to the inorganic form through decomposition

Phosphorous levels follow a seasonal pattern in aquatic ecosystems. In the spring, inorganic phosphorous is released from the sediment by convection currents in the warming water. When phosphorous levels are high, algae and plants reproduce rapidly. Much of the phosphorous is then converted to organic phosphorous, and primary productivity then declines. Later in the summer, the plants and algae begin to die off, and bacteria decompose them, and inorganic phosphorus is released back into the ecosystem. As phosphorous levels begin to increase at the end of the summer, primary plants and algae begin to rapidly grow again.

The phosphorous cycle is affected by human activities. Although phosphorous is normally a limiting nutrient, most agricultural fertilizers contain phosphorous. Run-off and drainage from farms can flood aquatic ecosystems with excess phosphorus. Artificial phosphorous can cause over growth of algae and plants in aquatic ecosytems. When the excess plant material is broken down, the decomposing bacteria can use up all the oxygen in the water causing dead zones. Most bodies of water gradually become more productive over time through the slow, natural accumulation of nutrients in a process called eutrophication. However, overgrowth of algae due to phosphorous fertilizer is called “cultural eutrophication” or “hypertrophication,” and is generally negative for ecosystems.



Figure: **Hypertrophication on the Potomac River**: The bright green color of the water is the result of algae blooms in response to the addition of phosphorous based fertilizers.

Key Points

- Phosphorous is important for the production of ATP and nucleotides.
- Inorganic phosphorous is found in the soil or water. Plants and algae assimilate inorganic phosphorus into their cells, and transfer it to other animals that consume them.

- When organisms die, their phosphorous is released by decomposer bacteria.
- Aquatic phosphorous follows a seasonal cycle, inorganic phosphorous peaks in the spring causing rapid algae and plant growth, and then declines. As plants die, it is re-released into the water.
- Phosphorous based fertilizers can cause excessive algae growtin in aquatic systems, which can have negative impacts on the environment.

Key Terms

- **hypertrophication**: the ecosystem response to the addition of artificial or natural substances, such as nitrates and phosphates, through fertilizers or sewage, to an aquatic system. This response is usually an increase in primary production.

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3.4E: The Nitrogen Cycle

Learning Objectives

- Describe the nitrogen cycle and how it is affected by human activity

The nitrogen cycle describes the conversion of nitrogen between different chemical forms. The majority of the earth's atmosphere (about 78%) is composed of atmospheric nitrogen, but it is not in a form that is usable to living things. Complex species interactions allow organisms to convert nitrogen to usable forms and exchange it between themselves. Nitrogen is essential for the formation of amino acids and nucleotides. It is essential for all living things.

Fixation: In order for organisms to use atmospheric nitrogen (N_2), it must be "fixed" or converted into ammonia (NH_3). This can happen occasionally through a lightning strike, but the bulk of nitrogen fixation is done by free living or symbiotic bacteria. These bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia. It is then further converted by the bacteria to make their own organic compounds. Some nitrogen fixing bacteria live in the root nodules of legumes where they produce ammonia in exchange for sugars. Today, about 30% of the total fixed nitrogen is manufactured in chemical plants for fertilizer.

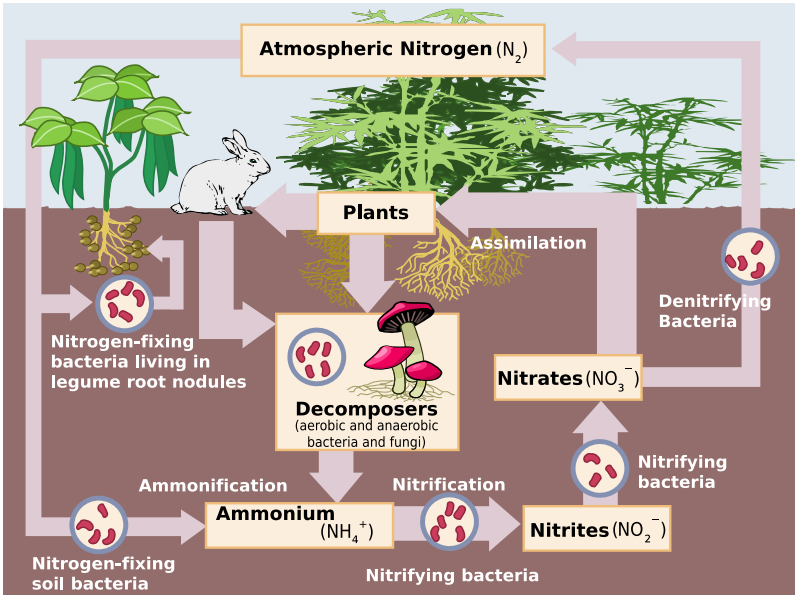


Figure: **The role of soil bacteria in the Nitrogen cycle**: Nitrogen transitions between various biologically useful forms.

Nitrificaton: Nitrification is the conversion of ammonia (NH_3) to nitrate (NO_3^-). It is usually performed by soil living bacteria, such as nitrobacter. This is important because plants can assimilate nitrate into their tissues, and they rely on bacteria to convert it from ammonia to a usable form. Nitrification is performed mainly by the genus of bacteria, *Nitrobacter*.

Ammonification /Mineralization: In ammonification, bacteria or fungi convert the organic nitrogen from dead organisms back into ammonium (NH_4^+). Nitrification can also work on ammonium. It can either be cycled back into a plant usable form through nitrification or returned to the atmosphere through de-nitrification.

De-Nitrification: Nitrogen in its nitrate form (NO_3^-) is converted back into atmospheric nitrogen gas (N_2) by bacterial species such as Pseudomonas and Clostridium, usually in anaerobic conditions. These bacteria use nitrate as an electron acceptor instead of oxygen during respiration.

Key Points

- Nitrogen is converted from atmospheric nitrogen (N2) into usable forms, such as NO2-, in a process known as fixation. The majority of nitrogen is fixed by bacteria, most of which are symbiotic with plants.

- Recently fixed ammonia is then converted to biologically useful forms by specialized bacteria. This occurs in two steps: first, bacteria convert ammonia in to (nitrites) NO_2^-, and then other bacteria species convert it to NO_3^- (nitrate).
- Nitriates are a form of nitrogen that is usable by plants. It is assimilated into plant tissue as protein. The nitrogen is passed through the food chain by animals that consume the plants, and then released into the soil by decomposer bacteria when they die.
- De-nitrifying bacteria convert NO_2^- back into atmospheric nitrogen (N_2), completing the cycle.

Key Terms

- **de-nitrification**: A microbially facilitated process of nitrate reduction that may ultimately produce molecular nitrogen (N_2) through a series of intermediate gaseous nitrogen oxide products.
- **nitrification**: The biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of these nitrites into nitrates.
- **ammonification**: The formation of ammonia or its compounds from nitrogenous compounds, especially as a result of bacterial decomposition.

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3.4F: The Sulfur Cycle

Learning Objectives

- Describe the sulfur cycle

The Sulfur Cycle

The sulfur cycle describes the movement of sulfur through the atmosphere, mineral forms, and through living things. Although sulfur is primarily found in sedimentary rocks or sea water, it is particularly important to living things because it is a component of many proteins.

Sulfur is released from geologic sources through the weathering of rocks. Once sulfur is exposed to the air, it combines with oxygen, and becomes sulfate SO_4. Plants and microbes assimilate sulfate and convert it into organic forms. As animals consume plants, the sulfur is moved through the food chain and released when organisms die and decompose.

Some bacteria – for example Proteus, Campylobacter, Pseudomonas and Salmonella – have the ability to reduce sulfur, but can also use oxygen and other terminal electron acceptors. Others, such as Desulfuromonas, use only sulfur. These bacteria get their energy by reducing elemental sulfur to hydrogen sulfide. They may combine this reaction with the oxidation of acetate, succinate, or other organic compounds.

The most well known sulfur reducing bacteria are those in the domain Archea, which are some of the oldest forms of life on Earth. They are often extremophiles, living in hot springs and thermal vents where other organisms cannot live. Lots of bacteria reduce small amounts of sulfates to synthesize sulfur-containing cell components; this is known as assimilatory sulfate reduction. By contrast, the sulfate-reducing bacteria considered here reduce sulfate in large amounts to obtain energy and expel the resulting sulfide as waste. This process is known as dissimilatory sulfate reduction. In a sense, they breathe sulfate.

Sulfur metabolic pathways for bacteria have important medical implications. For example, Mycobacterium tuberculosis (the bacteria causing tuberculosis) and Mycobacterium leprae (which causes leoprosy) both utilize sulfur, so the sulfur pathway is a target of drug development to control these bacteria.

Key Points

- The sulfur cycle describes the movement of sulfur through the geosphere and biosphere. Sulfur is released from rocks through weathering, and then assimilated by microbes and plants. It is then passed up the food chain and assimilated by plants and animals, and released when they decompose.
- Many bacteria can reduce sulfur in small amounts, but some specialized bacteria can perform respiration entirely using sulfur. They use sulfur or sulfate as an electron receptor in their respiration, and release sulfide as waste. This is a common form of anaerobic respiration in microbes.
- Sulfur reducing pathways are found in many pathogenic bacteria species. Tuberculosis and leprosy are both caused by bacterial species that reduce sulfur, so the sulfur reduction pathway is an important target of drug development.

Key Terms

- **extremophile**: An organism that lives under extreme conditions of temperature, salinity, and so on. They are commercially important as a source of enzymes that operate under similar conditions.
- **assimilatory sulfate reduction**: The reduction of 3′-Phosphoadenosine-5′-phosphosulfate, a more elaborated sulfateester, leads also to hydrogen sulfide, the product used in biosynthesis (e.g., for the production of cysteine because the sulfate sulfur is assimilated).

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3.4G: The Iron Cycle

Learning Objectives

- Compare the terrestrial and marine iron cycles

Iron (Fe) follows a geochemical cycle like many other nutrients. Iron is typically released into the soil or into the ocean through the weathering of rocks or through volcanic eruptions.

The Terrestrial Iron Cycle: In terrestrial ecosystems, plants first absorb iron through their roots from the soil. Iron is required to produce chlorophyl, and plants require sufficient iron to perform photosynthesis. Animals acquire iron when they consume plants, and iron is utilized by vertebrates in hemoglobin, the oxygen-binding protein found in red blood cells. Animals lacking in iron often become anemic and cannot transmit adequate oxygen. Bacteria then release iron back into the soil when they decompose animal tissue.

The Marine Iron Cycle: The oceanic iron cycle is similar to the terrestrial iron cycle, except that the primary producers that absorb iron are typically phytoplankton or cyanobacteria. Iron is then assimilated by consumers when they eat the bacteria or plankton. The role of iron in ocean ecosystems was first discovered when English biologist Joseph Hart noticed “desolate zones,” which are regions that lacked plankton but were rich in nutrients. He hypothesized that iron was the limiting nutrient in these areas. In the past three decades there has been research into using iron fertilization to promote alagal growth in the world’s oceans. Scientists hoped that by adding iron to ocean ecosystems, plants might grown and sequester atmospheric CO2. Iron fertilization was thought to be a possible method for removing the excess CO2 responsible for climate change. Thus far, the results of iron fertilization experiments have been mixed, and there is concern among scientists about the possible consequences of tampering nutrient cycles.

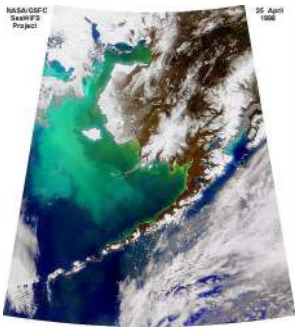


Figure: **Algal bloom**: Algae bloom in the Bering Sea after a natural iron fertilization event.

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Key Points

- Iron is an important limiting nutrient for plants, which use it to produce chlorophyll. Photosynthesis depends on adequate iron supply. Plants assimilate iron from the soil into their roots.
- Animals consume plants and use the iron to produce hemoglobin, the oxygen transports protein found in red blood cells. When animals die, decomposing bacteria return iron to the soil.
- The marine iron cycle is very similar to the terrestrial iron cycle, except that phytoplankton and cyanobacteria assimilate iron.
- Iron fertilization has been studied as a method for sequestering carbon. Scientists have hoped that by adding iron to the ocean, plankton might be able to sequester the excess CO2 responsible for climate change. However, there is concern about the long term effects of this strategy.

Key Terms

- **hemoglobin**: the iron-containing oxygen transport metalloprotein in the red blood cells of all vertebrates

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SECTION OVERVIEW

3.5: Microbial Symbioses

Topic hierarchy

3.5A: Mutualism vs. Symbiosis

3.5B: The Rumen and Ruminant Animals

3.5C: Hydrothermal Vent Microbial Ecosystems

3.5D: Squid-Aliivibrio Symbiosis

3.5E: Mutualistic Relationships with Fungi and Fungivores

3.5F: Agrobacterium and Crown Gall Disease

3.5G: The Legume-Root Nodule Symbiosis

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3.5A: Mutualism vs. Symbiosis

Learning Objectives

- Compare Mutualism and Symbiosis

Symbiosis is any relationship between two or more biological species. Such relationships are usually long term and have a strong impact on the fitness of one or both organisms. Symbiotic relationships are categorized by the benefits and physical relationships experienced by each species.

Common types of symbiosis are categorized by the degree to which each species benefits from the interaction:

- Mutualism: In mutualistic interactions, both species benefit from the interaction. A classic example of mutualism is the relationship between insects that pollinate plants and the plants that provide those insects with nectar or pollen. Another classic example is the behavior of mutualistic bacteria in ecology and human health. Gut bacteria in particular are very important for digestion in humans and other species. In humans, gut bacteria assist in breaking down additional carbohydrates, out-competing harmful bacteria, and producing hormones to direct fat storage. Humans lacking healthy mutualistic gut flora can suffer a variety of diseases, such as irritable bowel syndrome. Some ruminant animals, like cows or deer, rely on special mutualistic bacteria to help them break down the tough cellulose in the plants they eat. In return, the bacteria get a steady supply of food.
- Commensalism: In commensalism, one organism benefits while the other organism neither benefits nor suffers from the interaction. For example, a spider may build a web on a plant and benefit substantially, while the plant remains unaffected. Similarly, a clown fish might live inside a sea anemone and receive protection from predators, while the anemone neither benefits nor suffers.
- Parasitism: Parasites are organisms that harm their symbiotic partners. Parasitism is incredibly common in nature: depending on the definition, more than half of all species may go through at least one parasitic stage in their life cycle. There are many well-documented examples of parasitic bacteria and microorganisms throughout this text.

Symbiosis can also be characterized by an organism's physical relationship with its partner.

- Endosymbiosis: a relationship in which one of the symbiotic species lives inside the tissue the other. For example, Coral polyps have special algae called zooxanthelle that live inside their cells. Zooxanthelle provide sugars to the coral through photosynthesis. Similarly, nitrogen-fixing fungi often live inside the cells of plants, providing nitrogen in exchange for the sugars of photosynthesis.
- Ectosymbiosis: a relationship in which one species lives on the outside surface of the other. Barnacles that live on whales and bromeliads that live on tropical trees are examples of endosymbionts.

These categories can be paired with the above terms to better describe the species' interactions. For example, you might say that a gut bacteria is an "endosymbiotic mutualist," or that a flea is an "ectosymbiotic parasite. "

Key Points

- Mutualism, a relationship in which both species benefit, is common in nature. In microbiology, there are many examples of mutualistic bacteria in the gut that aid digestion in both humans and animals.
- Commensalism is a relationship between species in which one benefits and the other is unaffected. Humans are host to a variety of commensal bacteria in their bodies that do not harm them but rely on them for survival (e.g. bacteria that consume dead skin).
- Parasitic relationships, in which one species benefits and the other suffers, are very common in nature. Most of the microorganisms studied in medical microbiology are parasitic and feed on human tissue. For example, cholera, leshmaniasis, and Giardia are all parasitic microbes.
- Symbiotic relationships can also be classified by the physical relationship between the two species. Endosymbionts live inside the tissues of the host, while ectosymbionts live outside of their partner species.

Key Terms

- **commensalism**: A class of relationship between two organisms in which one organism benefits without affecting the other
- **symbiosis**: A close and often long-term interaction between two or more different biological species
- **mutualism**: A relationship between individuals of different species in which both individuals benefit

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3.5B: The Rumen and Ruminant Animals

Learning Objectives

- Identify how ruminant animals host symbiotic bacteria

A Ruminant's Multi-chambered Stomach

Ruminants are mammals that digest plant based food by processing it in a series of chambers in their stomachs. There are about 150 species of ruminants, including both domestic and wild species. Ruminating mammals include cattle, goats, sheep, giraffes, bison, moose, elk, yaks, water buffalo, deer, camels, alpacas, llamas, and antelope.

Ruminants differ from non-ruminants (called monogastrics) because they have a four-chambered stomach. The four compartments are called the rumen, the reticulum, the omasum, and the abomasum. The rumen and the reticulum are connected and work in concert and are therefore sometimes called the "reticulorumen".

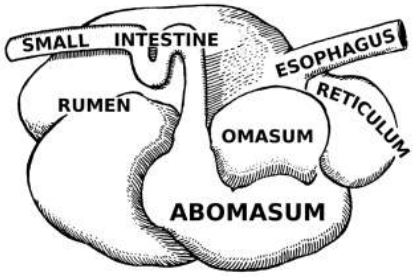


Figure: **The digestive tract of a ruminant**: The ruminant digestive tract has four compartments, the rumen, the reticulum, the omasum, and abomasum.

The Ruminant Digestive Process

- Ruminants chew plant matter to mix it with saliva and swallow. The food then enters the first two stomach chambers, the reticulum and rumen (or reticulorumen).
- The reticulum and rumen work together to separate solids and liquids. Contractions push solid food particles back up into the rumen, while liquids are drained into the reticulum. Specialized microbe species live in the rumen and help ruminants break down cellulose.
- Solids are formed into a bolus, called "cud," in the rumen and the solid cud is regurgitated back up to the mouth where it is chewed a second time, and returned to the reticulorumen to repeat the process.
- Liquid digesta in the reticulum is passed into the omasum where nutrients and water are absorbed into the blood stream.
- After this, digesta is passed into the abomasum, which is similar to the stomach of other animals. After the abomasum, digesta moves through the large and small intestines.

Ruminants are of interest to microbiologists because they have unique species of bacteria, yeasts, protozoa, and fungi in their rumens. The plant matter consumed by ruminants is high in cellulose, but vertebrates cannot produce cellulase which is the enzyme required to break down cellulose. Thus ruminants depend on the symbiotic microbes in their guts to break down cellulose for digestion. There is no oxygen in the rumen, so bacteria in the rumen are typically anaerobes or facultative anaerobes.

Key Points

- Ruminant animals use a special four-chambered stomach with a unique microbial flora to digest tough cellulose found in the plants in their diets. Most vertebrates cannot make cellulase, the enzyme that breaks down cellulose, but microbes in the rumen produce it for them.
- Ruminants chew and ingest plant matter and then swallow it. The plant matter is separated into liquids and solids in the rumen, and liquids drain into the reticulum. Solids in the rumen are then regurgitated into the mouth to be chewed and further broken down.

- Liquids pass from the reticulum into the omasum, where sugars, fatty acids, and other nutrients are absorbed into the blood stream.
- After the omasum, food passes into the abomasum, which is much like the stomach in non-ruminant (monogastric) animals, and from there moves into the small intestine, where it is digested.

Key Terms

- **Rumen**: The first chamber in the alimentary canal of ruminant animals. It serves as the primary site for microbial fermentation of ingested feed.
- **Abomasum**: The fourth and final stomach compartment in ruminants. It secretes rennin – the artificial form of which is called rennet, and is used in cheese creation.
- **Omasum**: The third compartment of the stomach in ruminants. Though its functions have not been well-studied, it appears to primarily aid in the absorption of water, magnesium, and the volatile fatty acids produced.

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3.5C: Hydrothermal Vent Microbial Ecosystems

Learning Objectives

- Describe hydrothermal vent microbial ecosystems

Hydrothermal Vents and Their Microbial Communities

A hydrothermal vent is a fissure in the earth's surface from which geothermally heated water issues. They are typically found deep below the surface of the ocean. Hydrothermal vents are of interest to microbiologists because they have unique microbial communities found nowhere else on earth.

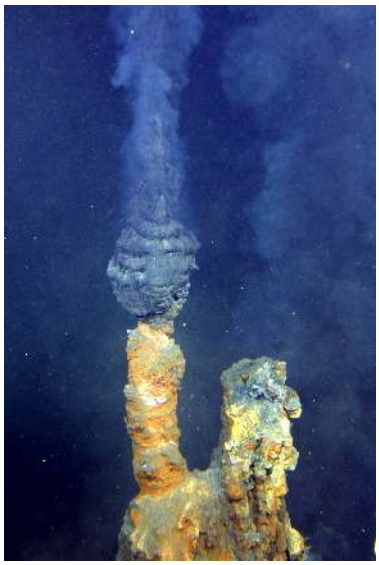


Figure: **Hydrothermal Vents**: Hydrothermal vents are cracks in the earth's crust where geothermally heated water leaks out.

In most shallow water and terrestrial ecosystems, energy comes from sunlight, but in the deep ocean there is total darkness. However, hydrothermal vents often expel nutrient rich water, containing methane and sulfur compounds. Vent bacteria can synthesize all the compounds they need to live from these nutrients, a process called chemosynthesis. These bacteria form the basis of the entire hydrothermal vent ecosystem.

The chemosynthetic bacteria grow into a thick mat, covering the hydrothermal vent, and this is the first trophic level of the ecosystem. Snails, shrimp crabs, tube worms, and fish feed on the bacterial mat and attract larger organisms such as squid and octopuses. Many of these species are specially adapted to live in the dark and lack eyes. Hydrothermal vents are biodiversity hot spots because they have many species that are uniquely adapted to live in this harsh environment. For example, the Pompeii tube worm *Alvinella pompejana* can resist temperatures up to 176°F. These ecosystems are almost entirely independent of sunlight (although the dissolved oxygen used by some animals does ultimately come from plants at the surface).

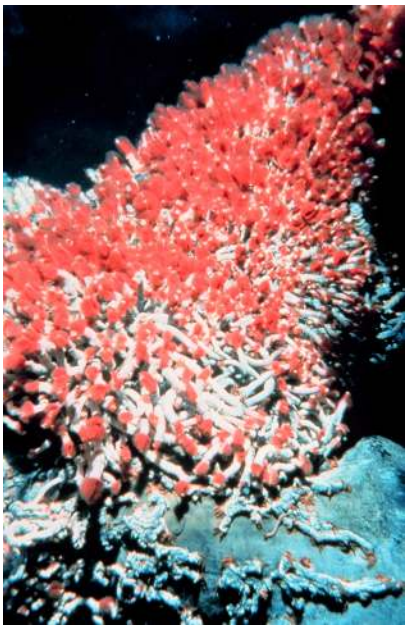


Figure: **Tubeworms Living Near A Hydrothermal Vent**: Some species of tube worms are specially adapted to withstand the high temperatures found at hydrothermal vents.



Figure: **Crabs near a hydrothermal vent**: The ecosystems around hydrothermal vents rely on mats chemosynthetic bacteria, and many species feed on the bacteria. Hydrothermal vents are some of the most unique ecosystems in the world

Despite being some of the most remote ecosystems in the world, hydrothermal vents are under threat from mining companies. As mineral resources on land have become depleted, mining companies have turned to deep sea geothermal vents to extract metals and sulfur. Although the technology for deep sea mining is new, conservation biologists are concerned that mining hydrothermal vents will destroy these fragile and unique ecosystems.

Key Points

- Hydrothermal vents emit nutrient rich, geothermally heated water. Mats of chemosynthetic bacteria grow around the vents and synthesize carbohydrates from the carbon dioxide ejected by the vent.
- Many species of crabs, worms, snails, and tube worms depend on these bacterial mats for food. These species are often specially adapted to life in the lightless, high pressure, and hot environment of the vent.
- Vents are the target of exploitation of the mining industry, which is a cause for concern among marine biologists. Mining could damage these very unique and diverse ecosystems.

Key Terms

- **chemosynthesis**: The production of carbohydrates and other compounds from simple compounds such as carbon dioxide, using the oxidation of chemical nutrients as a source of energy rather than sunlight; it is limited to certain bacteria and fungi.
- **geothermal**: Pertaining to heat energy extracted from reservoirs in the earth's interior.

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3.5D: Squid-Aliivibrio Symbiosis

Learning Objectives

- Explain the symbiotic relationship of squid and aliivibrio

Bioluminescence

A special category of symbiotic relationships involve bioluminescence, where light producing bacteria are hosted by another organism. One of the best studied examples of bioluminescence is the Hawaiian bobtail squid (*Euprymna scolopes*) and its mutualistic bacteria, *Aliivibrio fischeri*. Aliivibrio fischeri inhabits a special light organ in the squid's mantle. The bacteria are fed a sugar and amino acid solution by the squid. In return, they produce light to hide the squid's silhouette when viewed from below, allowing the squid to match ambient light conditions.



Figure: **Bobtail Squid**: Bobtail squid rely on their mutualist bacteria Allivibrio fischerii to generate light. The bacteria inhabits a special light organ in the squid's mantle and receives sugars and amino acids in exchange for light.

Bobtail squid hatchlings do not have *Aliivibrio fischeri* naturally in their bodies. They are born with a special light organ structure, with cilliated cells at the opening designed to trap passing *A. fischeri*, but must obtain the bacteria from sea water. To do this, the squid secretes a special mucus whenever its cells detect peptidoglycan (which is found in the cell walls of bacteria). The mucus collects near the opening of the light organ which traps passing bacteria. The squid weeds out unwanted bacteria in several ways. For instance, *A. fischeri* is able to survive in the mucus better than other species. It is also a very mobile bacteria, and is able to swim against the current created by the cilia at the mouth of the light organ.

The squid also creates a hostile environment at the entrance to the light organ by secreting an enzyme that splits hydrogen peroxide, creating a toxic environment for most bacteria. *Aliivibrio fischeri* can capture hydrogen peroxide before the squid can use it as a toxin, and thus can survive in the hostile chemical environment. Once *A. fischeri* has passed these hurdles at the opening of the light organ, it can colonize chambers of the light organ and begin enjoying the benefits of symbiosis.

Despite all the effort that goes into obtaining *Aliivibrio fischeri*, the squid ejects 95% of its bacteria every day. It not fully understood why the squid cleans out its light organ, but the bacteria require a great deal of sugar and amino acids, so it may be most useful to the squid to host bacteria only when they are needed. It may also provide a supply of bacteria for squid hatchlings.

Key Points

- Squid rely on Allivibrio bacteria to generate light that allows them to blend in with the light coming from above. Animals below them cannot see their shadow when they view the squid from below.
- Squid use mucus to attract many species of bacteria into their light organ, but they sort out Aliivibiro in several ways. Ciliated cells in the light organ create a current that expels most bacteria, and the squid uses hydrogen peroxide to create a hostile environment that Aliivibrio can resist.
- Once inside the light organ, the Aliivibrio bacteria receive sugars and amino acids from the squid. However, this is costly to the squid, and the squid clears out its light organ during the day so that it does not have to constantly maintain a colony of Aliivibrio bacteria.

Key Terms

- **cilia**: Organelles found in eukaryotic cells. Cilia are slender protuberances that project from the much larger cell body.
- **BIoluminescence**: The emission of light by a living organism.

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3.5E: Mutualistic Relationships with Fungi and Fungivores

Learning Objectives

- Describe mutualistic relationships with fungi

Mutualistic Relationships

Symbiosis is the ecological interaction between two organisms that live together. However, the definition does not describe the quality of the interaction. When both members of the association benefit, the symbiotic relationship is called mutualistic. Fungi form mutualistic associations with many types of organisms, including cyanobacteria, plants, and animals.

Fungi & Plant Mutualism

Mycorrhiza, which comes from the Greek words “myco” meaning fungus and “rhizo” meaning root, refers to the association between vascular plant roots and their symbiotic fungi. About 90 percent of all plant species have mycorrhizal partners. In a mycorrhizal association, the fungal mycelia use their extensive network of hyphae and large surface area in contact with the soil to channel water and minerals from the soil into the plant, thereby increasing a plant’s nutrient uptake. In exchange, the plant supplies the products of photosynthesis to fuel the metabolism of the fungus.

Mycorrhizae display many characteristics of primitive fungi: they produce simple spores, show little diversification, do not have a sexual reproductive cycle, and cannot live outside of a mycorrhizal association. There are a number of types of mycorrhizae. Ectomycorrhizae (“outside” mycorrhiza) depend on fungi enveloping the roots in a sheath (called a mantle) and a Hartig net of hyphae that extends into the roots between cells. The fungal partner can belong to the Ascomycota, Basidiomycota, or Zygomycota. In a second type, the Glomeromycete fungi form vesicular–arbuscular interactions with arbuscular mycorrhiza (sometimes called endomycorrhizae). In these mycorrhiza, the fungi form arbuscules that penetrate root cells and are the site of the metabolic exchanges between the fungus and the host plant. The arbuscules (from the Latin for “little trees”) have a shrub-like appearance. Orchids rely on a third type of mycorrhiza. Orchids are epiphytes that form small seeds without much storage to sustain germination and growth. Their seeds will not germinate without a mycorrhizal partner (usually a Basidiomycete). After nutrients in the seed are depleted, fungal symbionts support the growth of the orchid by providing necessary carbohydrates and minerals. Some orchids continue to be mycorrhizal throughout their lifecycle.

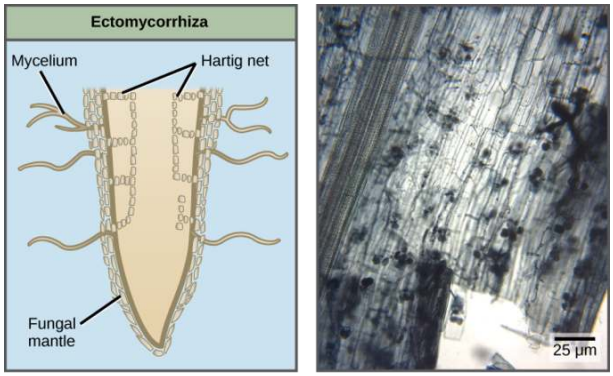


Figure: **Mycorrhizal fungi**: (a) Ectomycorrhiza and (b) arbuscular mycorrhiza have different mechanisms for interacting with the roots of plants.

Lichens

Lichens display a range of colors and textures. They can survive in the most unusual and hostile habitats. They cover rocks, gravestones, tree bark, and the ground in the tundra where plant roots cannot penetrate. Lichens can survive extended periods of drought: they become completely desiccated and then rapidly become active once water is available again. Lichens fulfill many ecological roles, including acting as indicator species, which allow scientists to track the health of a habitat because of their sensitivity to air pollution.



Figure: **Lichen: fungi and cyanobateria**: Lichens have many forms. They may be (a) crust-like, (b) hair-like, or (c) leaf-like.

Lichens are not a single organism, but, rather, an example of a mutualism in which a fungus (usually a member of the Ascomycota or Basidiomycota phyla) lives in close contact with a photosynthetic organism (a eukaryotic alga or a prokaryotic cyanobacterium). Generally, neither the fungus nor the photosynthetic organism can survive alone outside of the symbiotic relationship. The body of a lichen, referred to as a thallus, is formed of hyphae wrapped around the photosynthetic partner. The photosynthetic organism provides carbon and energy in the form of carbohydrates. Some cyanobacteria fix nitrogen from the atmosphere, contributing nitrogenous compounds to the association. In return, the fungus supplies minerals and protection from dryness and excessive light by encasing the algae in its mycelium. The fungus also attaches the symbiotic organism to the substrate.

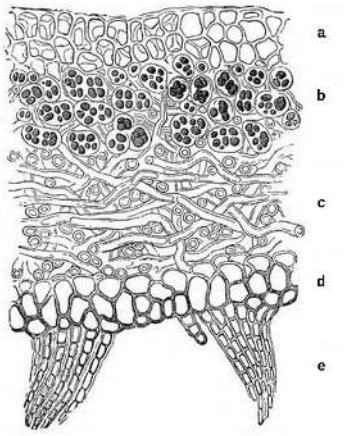


Figure: **Thallus of lichen**: This cross-section of a lichen thallus shows the (a) upper cortex of fungal hyphae, which provides protection; the (b) algal zone where photosynthesis occurs, the (c) medulla of fungal hyphae, and the (d) lower cortex, which also provides protection and may have (e) rhizines to anchor the thallus to the substrate.

The thallus of lichens grows very slowly, expanding its diameter a few millimeters per year. Both the fungus and the alga participate in the formation of dispersal units for reproduction. Lichens produce soredia, clusters of algal cells surrounded by mycelia. Soredia are dispersed by wind and water and form new lichens.

Fungi & Animal Mutualism

Fungi have evolved mutualisms with numerous insects. Arthropods (jointed, legged invertebrates, such as insects) depend on the fungus for protection from predators and pathogens, while the fungus obtains nutrients and a way to disseminate spores into new environments. The association between species of Basidiomycota and scale insects is one example. The fungal mycelium covers and protects the insect colonies. The scale insects foster a flow of nutrients from the parasitized plant to the fungus. In a second example, leaf-cutting ants of Central and South America literally farm fungi. They cut disks of leaves from plants and pile them up in gardens. Fungi are cultivated in these disk gardens, digesting the cellulose in the leaves that the ants cannot break down. Once smaller sugar molecules are produced and consumed by the fungi, the fungi in turn become a meal for the ants. The insects also patrol their garden, preying on competing fungi. Both ants and fungi benefit from the association. The fungus receives a steady supply of leaves and freedom from competition, while the ants feed on the fungi they cultivate.

Key Points

- Mutualistic relationships are those where both members of an association benefit; Fungi form these types of relationships with various other Kingdoms of life.
- Mycorrhiza, formed from an association between plant roots and primitive fungi, help increase a plant’s nutrient uptake; in return, the plant supplies the fungi with photosynthesis products for their metabolic use.

- In lichen, fungi live in close proximity with photosynthetic cyanobateria; the algae provide fungi with carbon and energy while the fungi supplies minerals and protection to the algae.
- Mutualistic relationships between fungi and animals involves numerous insects; Arthropods depend on fungi for protection, while fungi receive nutrients in return and ensure a way to disseminate the spores into new environments.

Key Terms

- **mycorrhiza**: a symbiotic association between a fungus and the roots of a vascular plant
- **lichen**: any of many symbiotic organisms, being associations of fungi and algae; often found as white or yellow patches on old walls, etc.
- **thallus**: vegetative body of a fungus

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3.5F: Agrobacterium and Crown Gall Disease

Learning Objectives

- Summarize the symbiotic relationship between plants and agrobacterium

Crown Gall Disease is caused by a bacteria called *Agrobacterium tumefaciens*. The disease manifests as a tumor-like growth usually at the junction of the root and shoot. *A. tumefaciens* can transfer part of its DNA to the host plant, through a plasmid – a bacterial DNA molecule that is independent of a chromosome. The new DNA segment causes the plant to produce unusual amino acids and plant hormones which provide the bacteria with carbon and nitrogen.

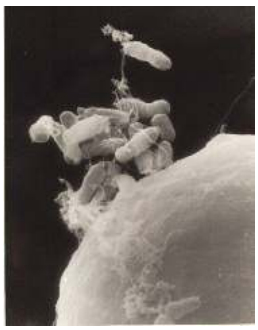


Figure: **Smart plants cue farmers to nutrient deficiencies**: A. tumefaciens attaching itself to a plant cell

Bacteria normally use plasmids for horizontal gene transfer, so they can share genes with related bacteria to help them cope with stressful environments. For example, plasmids can confer on bacteria the ability to fix nitrogen, or to resist antibiotic compounds. Typically bacteria transfer plasmids through conjugation: a donor bacteria creates a tube called a pilus that penetrates the cell wall of the recipient bacteria and the plasmid DNA passes through the tube. The other bacteria either integrates the plasmid into its chromosomes, or it remains free-floating in the cytoplasm. In either case, the recipient bacteria receives new genetic material.

In the case of Crown Gall Disease, *A. tumefaciens* transfers a plasmid containing T-DNA into the cells of its host plant through conjugation, as it would with another bacteria. However, once inside the plant cell, the DNA integrates semi-randomly into the genome of the plant and changes the behavior of the celll.

The new plasmid genes are expressed by the plant cells, and cause them to secrete enzymes that produce the amino acids octopine or nopaline. It also carries genes for the biosynthesis of the plant hormones, auxin and cytokinins, and for the biosynthesis of opines, providing a carbon and nitrogen source for the bacteria.

These opines can be used by very few other bacteria and give *A. tumefaciens* a competitive advantage.

Key Points

- Crown Gall Disease is caused by *Agrobacterium tumefacien*s, a bacteria that infects plants. The bacteria causes tumors on the stem of its host.
- *Agrobacterium tumefaciens* manipulates its hosts by transferring a DNA plasmid to the cells of its host. Plasmids are normally used to transfer DNA from bacteria to bacteria.
- Once in the host cell, the plasmid integrates itself into the host plant cell's genome and forces the host to produce unique amino acids and other substances which nourish the bacteria. These compounds are unusable by most bacteria, so Argobacteria can out-compete other species.

Key Terms

- **plasmid**: A circle of double-stranded DNA that is separate from the chromosomes, which is found in bacteria and protozoa.
- **pilus**: A hair-like appendage found on the cell surface of many bacteria.

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3.5G: The Legume-Root Nodule Symbiosis

Learning Objectives

- Evaluate legume and nitrogen-fixing bacteria symbiosis

Legumes and their Nitrogen-Fixing Bacteria

Many legumes have root nodules that provide a home for symbiotic nitrogen-fixing bacteria called *rhizobia*. This relationship is particularly common in nitrogen-limited conditions. The Rhizobia convert nitrogen gas from the atmosphere into ammonia, which is then used in the formation of amino acids and nucleotides.

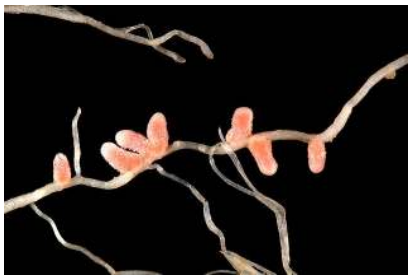


Figure: **Root Nodules**: Root nodules are formed when nitrogen fixing bacteria called rhizobia enter the cells of a host plant.

Rhizobia normally live in the soil and can exist without a host plant. However, when legume plants encounter low nitrogen conditions and want to form a symbiotic relationship with rhizobia they release flavinoids into the soil. Rhizobia respond by releasing nodulation factor (sometimes just called *nod factor*), which stimulates nodule formation in plant roots. Exposure to nod factor triggers the formation of deformed root hairs, which permit rhizobia to enter the plant. Rhizobia then form an infection thread, which is an intercellular tube that penetrates the cells of the host plant, and the bacteria then enter the host plants cells through the deformed root hair. Rhizobia can also enter the root by inserting themselves between cracks between root cells; this method of infection is called *crack entry*. Bacteria enter the root cells from the intercellular spaces, also using an infection thread to penetrate cell walls. Infection triggers rapid cell division in the root cells, forming a nodule of tissue.

The relationship between a host legume and the rhizobia is symbiotic, providing benefits to both participants. Once the rhizobia have established themselves in the root nodule, the plant provides carbohydrates in the form of malate and succinate, and the rhizobia provide ammonia for the formation of amino acids. Many legumes are popular agricultural crops specifically because they require very little fertilizer: their rhiziobia fix nitrogen for them. Used properly some legumes can even serve as fertilizer for later crops, binding nitrogen in the plant remains in the soil.



Figure: **Soy Beans**: Soy beans are a type of legume crop that rely on rhizobia

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Key Points

- Rhizobia normally live in the soil, but when there is limited soil nitrogen, legumes release flavonoids which signal to rhizobia that the plant is seeking symbiotic bacteria.
- When exposed to flavonoids, the Rhizobia release *nodulation factor*, which stimulates the plant to create deformed root hairs. Rhizobia then form an ” infection thread” which allows them to enter the root cells through the root hairs.
- Once the rhizobia are inside the root cells, the root cells divide rapidly, forming a nodule.
- The rhizobia create ammonia from nitrogen in the air, which is used by the plant to create amino acids and nucleotides. The plant provides the bacteria with sugars.

Key Terms

- **Nodulation Factor**: Signaling molecules produced by bacteria known as rhizobia during the initiation of nodules on the root of legumes. A symbiosis is formed when legumes take up the bacteria.

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SECTION OVERVIEW

4.1: Types of Metabolism

Topic hierarchy

4.1A: Photoautotrophs and Photohetrotrophs

4.1B: Chemoautotrophs and Chemohetrotrophs

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4.1A: Photoautotrophs and Photohetrotrophs

Learning Objectives

- Differentiate photoautotrophs from photoheterotrophs

Phototrophs are organisms that use light as their source of energy to produce ATP and carry out various cellular processes. Not all phototrophs are photosynthetic but they all constitute a food source for heterotrophic organisms. All phototrophs either use electron transport chain or direct proton pumping to establish an electro-chemical gradient utilized by ATP synthase to provide molecular energy for the cell. Phototrophs can be of two types based on their metabolism.

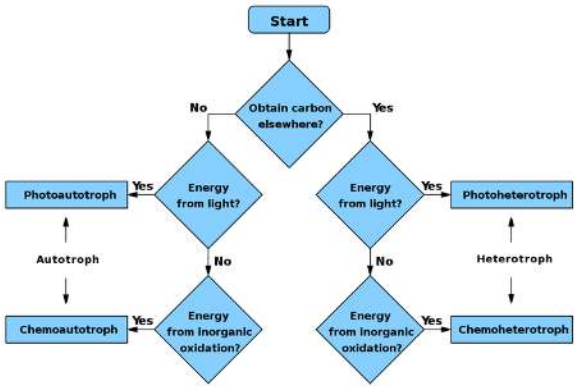


Figure: **Types of microbial metabolism**: Flowchart summarizing the types of microbial metabolism.

Photoautotrophs

An autotroph is an organism able to make its own food. Photoautotrophs are organisms that carry out photosynthesis. Using energy from sunlight, carbon dioxide and water are converted into organic materials to be used in cellular functions such as biosynthesis and respiration. In an ecological context, they provide nutrition for all other forms of life (besides other autotrophs such as chemotrophs). In terrestrial environments plants are the predominant variety, while aquatic environments include a range of phototrophic organisms such as algae, protists, and bacteria. In photosynthetic bacteria and cyanobacteria that build up carbon dioxide and water into organic cell materials using energy from sunlight, starch is produced as final product. This process is an essential storage form of carbon, which can be used when light conditions are too poor to satisfy the immediate needs of the organism.

Photoheterotrophs

A heterotroph is an organism that depends on organic matter already produced by other organisms for its nourishment. Photoheterotrophs obtain their energy from sunlight and carbon from organic material and not carbon dioxide. Most of the well-recognized phototrophs are autotrophs, also known as photoautotrophs, and can fix carbon. They can be contrasted with chemotrophs that obtain their energy by the oxidation of electron donors in their environments. Photoheterotrophs produce ATP through photophosphorylation but use environmentally obtained organic compounds to build structures and other bio-molecules. Photoautotrophic organisms are sometimes referred to as holophytic.

Key Points

- Phototrophs are organisms that carry out photon capture to acquire energy.
- Photoautotrophs convert inorganic materials into organic materials for use in cellular functions such as biosynthesis and respiration and provide nutrition for many other forms of life.
- Photoheterotrophs depend on light for their source of energy and mostly organic compounds from the environment for their source of carbon.

Key Terms

- **ATP synthase**: an important enzyme that catalyzes the conversion of adenosine diphosphate into adenosine triphosphate.
- **photosynthesis**: The process by which plants and other photoautotrophs generate carbohydrates and oxygen from carbon dioxide, water, and light energy in chloroplasts.

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4.1B: Chemoautotrophs and Chemohetrotrophs

Learning Objectives

- Compare chemoautotrophs and chemoheterotrophs

Chemotrophs are a class of organisms that obtain their energy through the oxidation of inorganic molecules, such as iron and magnesium. The most common type of chemotrophic organisms are prokaryotic and include both bacteria and fungi. All of these organisms require carbon to survive and reproduce. The ability of chemotrophs to produce their own organic or carbon-containing molecules differentiates these organisms into two different classifications–chemoautotrophs and chemoheterotrophs.

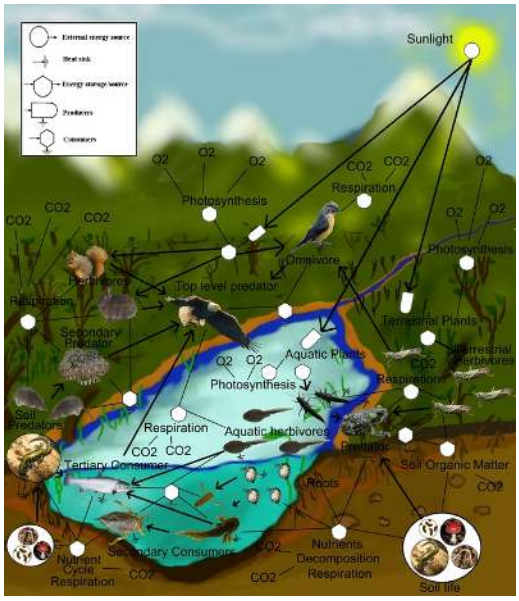


Figure: **Organismal and environmental interactions in a wetland**: sources of energy and carbon for each trophic level.

Chemoautotrophs

Chemoautotrophs are able to synthesize their own organic molecules from the fixation of carbon dioxide. These organisms are able to produce their own source of food, or energy. The energy required for this process comes from the oxidation of inorganic molecules such as iron, sulfur or magnesium. Chemoautotrophs are able to thrive in very harsh environments, such as deep sea vents, due to their lack of dependence on outside sources of carbon other than carbon dioxide. Chemoautotrophs include nitrogen fixing bacteria located in the soil, iron oxidizing bacteria located in the lava beds, and sulfur oxidizing bacteria located in deep sea thermal vents.

Chemoheterotrophs

Chemoheterotrophs, unlike chemoautotrophs, are unable to synthesize their own organic molecules. Instead, these organisms must ingest preformed carbon molecules, such as carbohydrates and lipids, synthesized by other organisms. They do, however, still obtain energy from the oxidation of inorganic molecules like the chemoautotrophs. Chemoheterotrophs are only able to thrive in environments that are capable of sustaining other forms of life due to their dependence on these organisms for carbon sources. Chemoheterotrophs are the most abundant type of chemotrophic organisms and include most bacteria, fungi and protozoa.

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Key Points

- Chemotrophs are organisms that obtain energy by the oxidation of electron donors in their environment.
- Chemoautotrophs use inorganic energy sources to synthesize organic compounds from carbon dioxide.
- Chemoheterotrophs are unable to utilize carbon dioxide to form their own organic compounds. Their carbon source is rather derived from sulfur, carbohydrates, lipids, and proteins.

Key Terms

- **inorganic molecule**: lacks carbon and hydrogen atoms.

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SECTION OVERVIEW

4.2: Energy Production

Topic hierarchy

4.2A: Control of Catabolic Pathways

4.2B: Transforming Chemical Energy

4.2C: Connecting Other Sugars to Glucose Metabolism

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4.2A: Control of Catabolic Pathways

Learning Objectives

- Explain how catabolic pathways are controlled

Enzymes, proteins, electron carriers, and pumps that play roles in glycolysis, the citric acid cycle, and the electron transport chain tend to catalyze non-reversible reactions. In other words, if the initial reaction takes place, the pathway is committed to proceeding with the remaining reactions. Whether a particular enzyme activity is released depends upon the energy needs of the cell (as reflected by the levels of ATP, ADP, and AMP).

Glycolysis

The control of glycolysis begins with the first enzyme in the pathway, hexokinase. This enzyme catalyzes the phosphorylation of glucose, which helps to prepare the compound for cleavage in a later step. The presence of the negatively-charged phosphate in the molecule also prevents the sugar from leaving the cell. When hexokinase is inhibited, glucose diffuses out of the cell and does not become a substrate for the respiration pathways in that tissue. The product of the hexokinase reaction is glucose-6-phosphate, which accumulates when a later enzyme, phosphofructokinase, is inhibited.

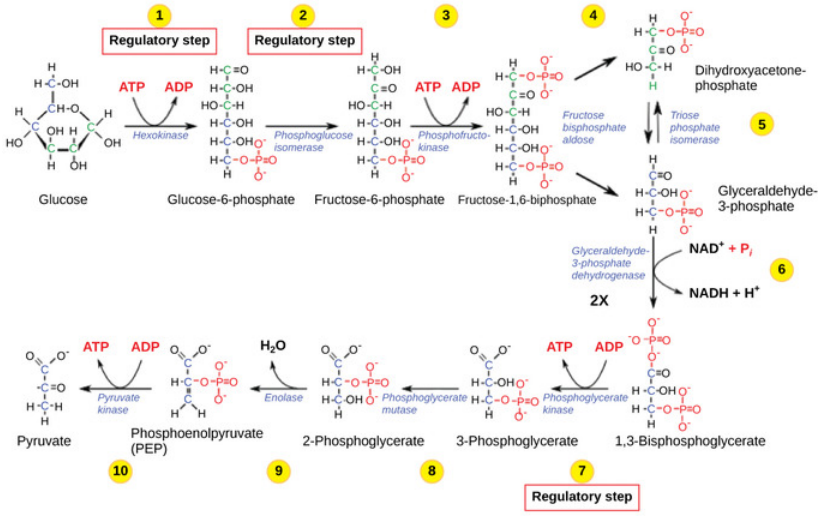


Figure: **Glycolysis**: The glycolysis pathway is primarily regulated at the three key enzymatic steps (1, 2, and 7) as indicated. Note that the first two steps that are regulated occur early in the pathway and involve hydrolysis of ATP.

Phosphofructokinase is the main enzyme controlled in glycolysis. High levels of ATP, citrate, or a lower, more acidic pH decrease the enzyme's activity. An increase in citrate concentration can occur because of a blockage in the citric acid cycle. Fermentation, with its production of organic acids like lactic acid, frequently accounts for the increased acidity in a cell; however, the products of fermentation do not typically accumulate in cells.

The last step in glycolysis is catalyzed by pyruvate kinase. The pyruvate produced can proceed to be catabolized or converted into the amino acid alanine. If no more energy is needed and alanine is in adequate supply, the enzyme is inhibited. The enzyme's activity is increased when fructose-1,6-bisphosphate levels increase. (Recall that fructose-1,6-bisphosphate is an intermediate in the first half of glycolysis.) The regulation of pyruvate kinase involves phosphorylation, resulting in a less-active enzyme. Dephosphorylation by a phosphatase reactivates it. Pyruvate kinase is also regulated by ATP (a negative allosteric effect).

If more energy is needed, more pyruvate will be converted into acetyl CoA through the action of pyruvate dehydrogenase. If either acetyl groups or NADH accumulate, there is less need for the reaction and the rate decreases. Pyruvate dehydrogenase is also regulated by phosphorylation: a kinase phosphorylates it to form an inactive enzyme, and a phosphatase reactivates it. The kinase and the phosphatase are also regulated.

Citric Acid Cycle

The citric acid cycle is controlled through the enzymes that catalyze the reactions that make the first two molecules of NADH. These enzymes are isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. When adequate ATP and NADH levels are available, the rates of these reactions decrease. When more ATP is needed, as reflected in rising ADP levels, the rate increases. α-Ketoglutarate dehydrogenase will also be affected by the levels of succinyl CoA, a subsequent intermediate in the cycle, causing a decrease in activity. A decrease in the rate of operation of the pathway at this point is not necessarily negative as the increased levels of the α-ketoglutarate not used by the citric acid cycle can be used by the cell for amino acid (glutamate) synthesis.

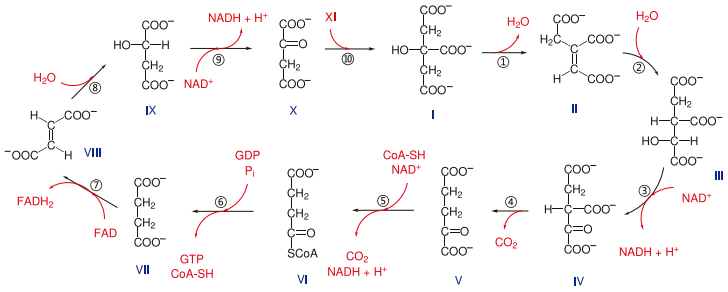


Figure: **Citric Acid Cycle**: Enzymes, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, catalyze the reactions that make the first two molecules of NADH in the citric acid cycle. Rates of the reaction decrease when sufficient ATP and NADH levels are reached.

Electron Transport Chain

Specific enzymes of the electron transport chain are unaffected by feedback inhibition, but the rate of electron transport through the pathway is affected by the levels of ADP and ATP. Greater ATP consumption by a cell is indicated by a buildup of ADP. As ATP usage decreases, the concentration of ADP decreases: ATP begins to build up in the cell. This change in the relative concentration of ADP to ATP triggers the cell to slow down the electron transport chain.

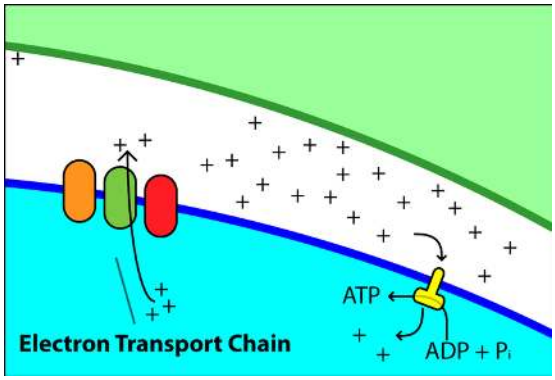


Figure: **Electron Chain Transport**: Levels of ADP and ATP affect the rate of electron transport through this type of chain transport.

Key Points

- Glycolysis, the citric acid cycle, and the electron transport chain are catabolic pathways that bring forth non-reversible reactions.
- Glycolysis control begins with hexokinase, which catalyzes the phosphorylation of glucose; its product is glucose-6- phosphate, which accumulates when phosphofructokinase is inhibited.
- The citric acid cycle is controlled through the enzymes that break down the reactions that make the first two molecules of NADH.
- The rate of electron transport through the electron transport chain is affected by the levels of ADP and ATP, whereas specific enzymes of the electron transport chain are unaffected by feedback inhibition.

Key Terms

- **phosphofructokinase**: any of a group of kinase enzymes that convert fructose phosphates to biphosphate
- **glycolysis**: the cellular metabolic pathway of the simple sugar glucose to yield pyruvic acid and ATP as an energy source
- **kinase**: any of a group of enzymes that transfers phosphate groups from high-energy donor molecules, such as ATP, to specific target molecules (substrates); the process is termed phosphorylation

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4.2B: Transforming Chemical Energy

Learning Objectives

- Discuss the importance of cellular respiration

Introduction: Cellular Respiration

An electrical energy plant converts energy from one form to another form that can be more easily used. For example, geothermal energy plants start with underground thermal energy (heat) and transform it into electrical energy that will be transported to homes and factories.



Figure: **Energy Plant**: This geothermal energy plant transforms thermal energy from deep in the ground into electrical energy, which can be easily used.

Like a generating plant, living organisms must take in energy from their environment and convert it into to a form their cells can use. Organisms ingest large molecules, like carbohydrates, proteins, and fats, and convert them into smaller molecules like carbon dioxide and water. This process is called cellular respiration, a form of catabolism, and makes energy available for the cell to use. The energy released by cellular respiration is temporarily captured by the formation of adenosine triphosphate (ATP) within the cell. ATP is the principle form of stored energy used for cellular functions and is frequently referred to as the energy currency of the cell.

The nutrients broken down through cellular respiration lose electrons throughout the process and are said to be oxidized. When oxygen is used to help drive the oxidation of nutrients the process is called aerobic respiration. Aerobic respiration is common among the eukaryotes, including humans, and takes place mostly within the mitochondria. Respiration occurs within the cytoplasm of prokaryotes. Several prokaryotes and a few eukaryotes use an inorganic molecule other than oxygen to drive the oxidation of their nutrients in a process called anaerobic respiration. Electron acceptors for anaerobic respiration include nitrate, sulfate, carbon dioxide, and several metal ions.

The energy released during cellular respiration is then used in other biological processes. These processes build larger molecules that are essential to an organism's survival, such as amino acids, DNA, and proteins. Because they synthesize new molecules, these processes are examples of anabolism.

Key Points

- Organisms ingest organic molecules like the carbohydrate glucose to obtain the energy needed for cellular functions.
- The energy in glucose can be extracted in a series of chemical reactions known as cellular respiration.
- Cellular respiration produces energy in the form of ATP, which is the universal energy currency for cells.

Key Terms

- **aerobic respiration**: the process of converting the biochemical energy in nutrients to ATP in the presence of oxygen
- **adenosine triphosphate**: a multifunctional nucleoside triphosphate used in cells as a coenzyme, often called the "molecular unit of energy currency" in intracellular energy transfer
- **cellular respiration**: the set of the metabolic reactions and processes that take place in the cells of organisms to convert biochemical energy from nutrients into adenosine triphosphate (ATP)
- **catabolism**: the breakdown of large molecules into smaller ones usually accompanied by the release of energy

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4.2C: Connecting Other Sugars to Glucose Metabolism

Learning Objectives

- Identify the types of sugars involved in glucose metabolism

You have learned about the catabolism of glucose, which provides energy to living cells. But living things consume more than glucose for food. How does a turkey sandwich end up as ATP in your cells? This happens because all of the catabolic pathways for carbohydrates, proteins, and lipids eventually connect into glycolysis and the citric acid cycle pathways.

Metabolic pathways should be thought of as porous; that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways. Like sugars and amino acids, the catabolic pathways of lipids are also connected to the glucose catabolism pathways.

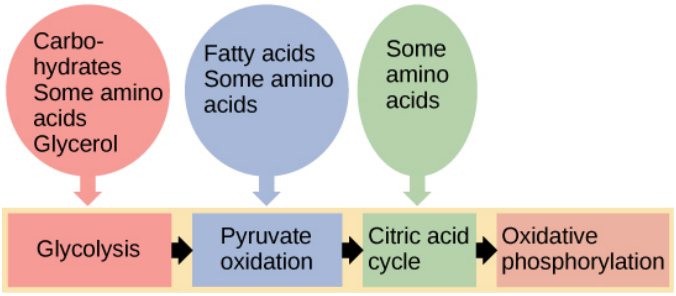


Figure: **Glycogen Pathway**: Glycogen from the liver and muscles, hydrolyzed into glucose-1-phosphate, together with fats and proteins, can feed into the catabolic pathways for carbohydrates.

Glycogen, a polymer of glucose, is an energy-storage molecule in animals. When there is adequate ATP present, excess glucose is shunted into glycogen for storage. Glycogen is made and stored in both the liver and muscles. The glycogen is hydrolyzed into the glucose monomer, glucose-1-phosphate (G-1-P), if blood sugar levels drop. The presence of glycogen as a source of glucose allows ATP to be produced for a longer period of time during exercise. Glycogen is broken down into G-1-P and converted into glucose-6-phosphate (G-6-P) in both muscle and liver cells; this product enters the glycolytic pathway.

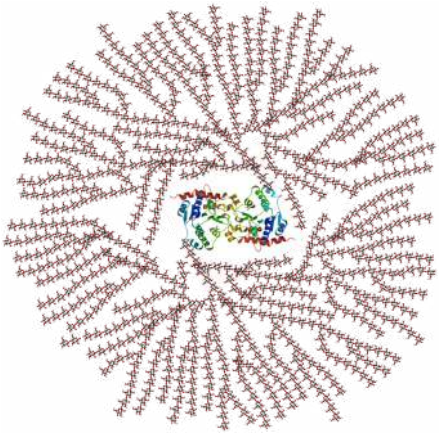


Figure: **Glycogen Structure**: Schematic two-dimensional cross-sectional view of glycogen: A core protein of glycogenin is surrounded by branches of glucose units. The entire globular granule may contain around 30,000 glucose units.

Galactose is the sugar in milk. Infants have an enzyme in the small intestine that metabolizes lactose to galactose and glucose. In areas where milk products are regularly consumed, adults have also evolved this enzyme. Galactose is converted in the liver to G-6-P and can thus enter the glycolytic pathway.

Fructose is one of the three dietary monosaccharides (along with glucose and galactose) which are absorbed directly into the bloodstream during digestion. Fructose is absorbed from the small intestine and then passes to the liver to be metabolized, primarily to glycogen. The catabolism of both fructose and galactose produces the same number of ATP molecules as glucose.

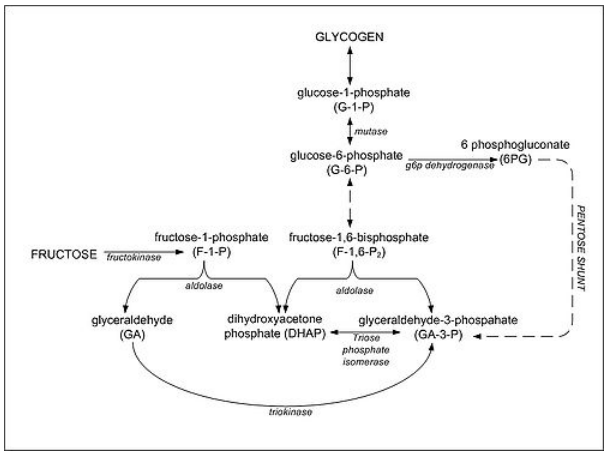


Figure: **Fructose Metabolism**: Although the metabolism of fructose and glucose share many of the same intermediate structures, they have very different metabolic fates in human metabolism.

Sucrose is a disaccharide with a molecule of glucose and a molecule of fructose bonded together with a glycosidic linkage. The catabolism of sucrose breaks it down to monomers of glucose and fructose. The glucose can directly enter the glycolytic pathway while fructose must first be converted to glycogen, which can be broken down to G-1-P and enter the glycolytic pathway as described above.

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Key Points

- When blood sugar levels drop, glycogen is broken down into glucose -1-phosphate, which is then converted to glucose-6-phosphate and enters glycolysis for ATP production.
- In the liver, galactose is converted to glucose-6-phosphate in order to enter the glycolytic pathway.
- Fructose is converted into glycogen in the liver and then follows the same pathway as glycogen to enter glycolysis.
- Sucrose is broken down into glucose and fructose; glucose enters the pathway directly while fructose is converted to glycogen.

Key Terms

- **disaccharide**: A sugar, such as sucrose, maltose, or lactose, consisting of two monosaccharides combined together.
- **glycogen**: A polysaccharide that is the main form of carbohydrate storage in animals; converted to glucose as needed.
- **monosaccharide**: A simple sugar such as glucose, fructose, or deoxyribose that has a single ring.

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SECTION OVERVIEW

4.3: Catabolism

Topic hierarchy

4.3A: Types of Catabolism

4.3B: Pyruvic Acid and Metabolism

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4.3A: Types of Catabolism

Learning Objectives

- Summarize various types of catabolism included in metabolism (catabolism of carbohydrates, proteins and fats)

Catabolism is the set of metabolic processes that break down large molecules. These include breaking down and oxidizing food molecules. The purpose of catabolic reactions is to provide the energy and components needed by anabolic reactions. The exact nature of these catabolic reactions differ from organism to organism; organisms can be classified based on their sources of energy and carbon, their primary nutritional groups. Organic molecules are used as a source of energy by organotrophs, while lithotrophs use inorganic substrates and phototrophs capture sunlight as chemical energy.

All these different forms of metabolism depend on redox reactions that involve the transfer of electrons from reduced donor molecules such as organic molecules, water, ammonia, hydrogen sulfide or ferrous ions to acceptor molecules such as oxygen, nitrate or sulfate. In animals these reactions involve complex organic molecules being broken down to simpler molecules, such as carbon dioxide and water. In photosynthetic organisms such as plants and cyanobacteria, these electron-transfer reactions do not release energy, but are used as a way of storing energy absorbed from sunlight.

The most common set of catabolic reactions in animals can be separated into three main stages. In the first, large organic molecules such as proteins, polysaccharides, or lipids are digested into their smaller components outside cells. Next, these smaller molecules are taken up by cells and converted to yet smaller molecules, usually the acetyl coenzyme *A (acetyl-CoA)*, which releases some energy. Finally, the acetyl group on the CoA is oxidized to water and carbon dioxide in the citric acid cycle and electron transport chain, releasing the energy that is stored by reducing the coenzyme nicotinamide adenine dinucleotide (NAD+) into NADH.

Macromolecules such as starch, cellulose or proteins cannot be rapidly taken up by cells and must be broken into their smaller units before they can be used in cell metabolism. Several common classes of enzymes digest these polymers. These digestive enzymes include proteases that digest proteins into amino acids, as well as glycoside hydrolases that digest polysaccharides into monosaccharides. Microbes secrete digestive enzymes into their surroundings, while animals only secrete these enzymes from specialized cells in their guts. The amino acids or sugars released by these extracellular enzymes are then pumped into cells by specific active transport proteins. A simplified schematic of the catabolism of carbohydrates, proteins and fats is shown in.

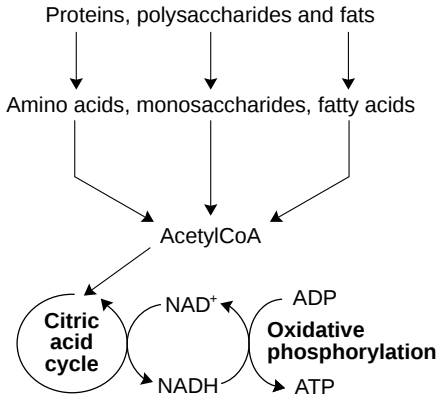


Figure: **Catabolism**: A simplified outline of the catabolism of proteins, carbohydrates and fats

Carbohydrate Catabolism

Carbohydrate catabolism is the breakdown of carbohydrates into smaller units. Carbohydrates are usually taken into cells once they have been digested into monosaccharides. Once inside, the major route of breakdown is glycolysis, where sugars such as glucose and fructose are converted into pyruvate and some ATP is generated. Pyruvate is an intermediate in several metabolic pathways, but the majority is converted to acetyl-CoA and fed into the citric acid cycle. Although some more ATP is generated in the citric acid cycle, the most important product is NADH, which is made from NAD+ as the acetyl-CoA is oxidized. This oxidation releases

carbon dioxide as a waste product. In anaerobic conditions, glycolysis produces lactate, through the enzyme lactate dehydrogenase re-oxidizing NADH to NAD+ for re-use in glycolysis.

The Pentose Phosphate Pathway

An alternative route for glucose breakdown is the pentose phosphate pathway, which reduces the coenzyme NADPH and produces pentose sugars such as ribose, the sugar component of nucleic acids. Fats are catabolised by hydrolysis to free fatty acids and glycerol. The glycerol initiates glycolysis and the fatty acids are broken down by beta oxidation to release acetyl-CoA, which then is fed into the citric acid cycle. Fatty acids release more energy upon oxidation than carbohydrates because carbohydrates contain more oxygen in their structures.

Amino acids are either used to synthesize proteins and other biomolecules, or oxidized to urea and carbon dioxide as a source of energy. The oxidation pathway starts with the removal of the amino group by a transaminase. The amino group is fed into the urea cycle, leaving a deaminated carbon skeleton in the form of a keto acid. Several of these keto acids are intermediates in the citric acid cycle, for example the deamination of glutamate forms α-ketoglutarate. The glucogenic amino acids can also be converted into glucose, through gluconeogenesis.

Key Points

- The purpose of the catabolic reactions is to provide the energy and components needed by anabolic reactions.
- Microbes simply secrete digestive enzymes into their surroundings, while animals only secrete these enzymes from specialized cells in their guts.
- Fats are catabolised by hydrolysis to free fatty acids and glycerol.
- Amino acids are either used to synthesize proteins and other biomolecules, or oxidized to urea and carbon dioxide as a source of energy.
- Carbohydrates are usually taken into cells once they have been digested into monosaccharides and then processed inside the cell via glycolysis.

Key Terms

- **polymer**: A long or larger molecule consisting of a chain or network of many repeating units, formed by chemically bonding together many identical or similar small molecules called monomers. A polymer is formed by polymerization, the joining of many monomer molecules.
- **acetyl CoA**: Acetyl coenzyme A or acetyl-CoA is an important molecule in metabolism, used in many biochemical reactions. Its main function is to convey the carbon atoms within the acetyl group to the citric acid cycle (Krebs cycle) to be oxidized for energy production.
- **catabolism**: Destructive metabolism, usually includes the release of energy and breakdown of materials.

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4.3B: Pyruvic Acid and Metabolism

Learning Objectives

- Outline the metabolic processes that involve pyruvate

Pyruvic acid ($CH_3COCOOH$; is an organic acid, a ketone, and the simplest of the alpha-keto acids. The carboxylate (COO^-) anion of pyruvic acid. The Brønsted–Lowry conjugate base, CH_3COCOO^-, is known as pyruvate, and is a key intersection in several metabolic pathways.

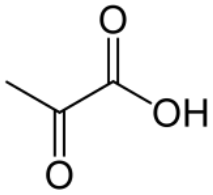


Figure: ***Pyruvic acid****: Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through acetyl-CoA. It can also be used to construct the amino acid alanine and be converted into ethanol. Pyruvic acid supplies energy to living cells through the citric acid cycle (also known as the Krebs cycle) when oxygen is present (aerobic respiration), and alternatively ferments to produce lactic acid when oxygen is lacking (fermentation).*

Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through acetyl-CoA. It can also be used to construct the amino acid alanine, and it can be converted into ethanol.

Pyruvic acid supplies energy to living cells through the citric acid cycle (also known as the Krebs cycle) when oxygen is present (aerobic respiration); when oxygen is lacking, it ferments to produce lactic acid. Pyruvate is an important chemical compound in biochemistry. It is the output of the anaerobic metabolism of glucose known as glycolysis. One molecule of glucose breaks down into two molecules of pyruvate, which are then used to provide further energy in one of two ways. Pyruvate is converted into acetyl- coenzyme A, which is the main input for a series of reactions known as the Krebs cycle. Pyruvate is also converted to oxaloacetate by an anaplerotic reaction, which replenishes Krebs cycle intermediates; also, oxaloacetate is used for gluconeogenesis. These reactions are named after Hans Adolf Krebs, the biochemist awarded the 1953 Nobel Prize for physiology, jointly with Fritz Lipmann, for research into metabolic processes. The cycle is also known as the citric acid cycle or tri-carboxylic acid cycle, because citric acid is one of the intermediate compounds formed during the reactions.

If insufficient oxygen is available, the acid is broken down anaerobically, creating lactate in animals and ethanol in plants and microorganisms. Pyruvate from glycolysis is converted by fermentation to lactate using the enzyme lactate dehydrogenase and the coenzyme NADH in lactate fermentation. Alternatively it is converted to acetaldehyde and then to ethanol in alcoholic fermentation.

Pyruvate is a key intersection in the network of metabolic pathways. Pyruvate can be converted into carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine, and to ethanol. Therefore, it unites several key metabolic processes.

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Key Points

- Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through acetyl-CoA.
- Pyruvic acid supplies energy to living cells through the citric acid cycle (also known as the Krebs cycle) when oxygen is present (aerobic respiration); it ferments to produce lactic acid when oxygen is lacking (fermentation).
- Pyruvate is the output of the anaerobic metabolism of glucose known as glycolysis.
- Pyruvate can be converted into carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine, and to ethanol.

Key Terms

- **pyruvic acid**: A colourless liquid; an important intermediate in the metabolism of proteins and carbohydrates, and in fermentation.
- **conjugate base**: Any compound, of general formula Xn+, which can be transformed into a conjugate acid HX(n+1)+ by the gain of a proton.
- **Krebs cycle**: A series of enzymatic reactions that occurs in all aerobic organisms; it involves the oxidative metabolism of acetyl units and serves as the main source of cellular energy.

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SECTION OVERVIEW

4.4: Glycolysis

Topic hierarchy

4.4A: Importance of Glycolysis

4.4B: Electron Donors and Acceptors

4.4C: ATP Yield

4.4D: Respiration and Proton Motive Force

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4.4A: Importance of Glycolysis

Learning Objectives

- Explain the importance of glycolysis to cells

Nearly all of the energy used by living cells comes to them from the energy in the bonds of the sugar glucose. Glucose enters heterotrophic cells in two ways. One method is through secondary active transport in which the transport takes place against the glucose concentration gradient. The other mechanism uses a group of integral proteins called GLUT proteins, also known as glucose transporter proteins. These transporters assist in the facilitated diffusion of glucose. Glycolysis is the first pathway used in the breakdown of glucose to extract energy. It takes place in the cytoplasm of both prokaryotic and eukaryotic cells. It was probably one of the earliest metabolic pathways to evolve since it is used by nearly all of the organisms on earth. The process does not use oxygen and is, therefore, anaerobic.

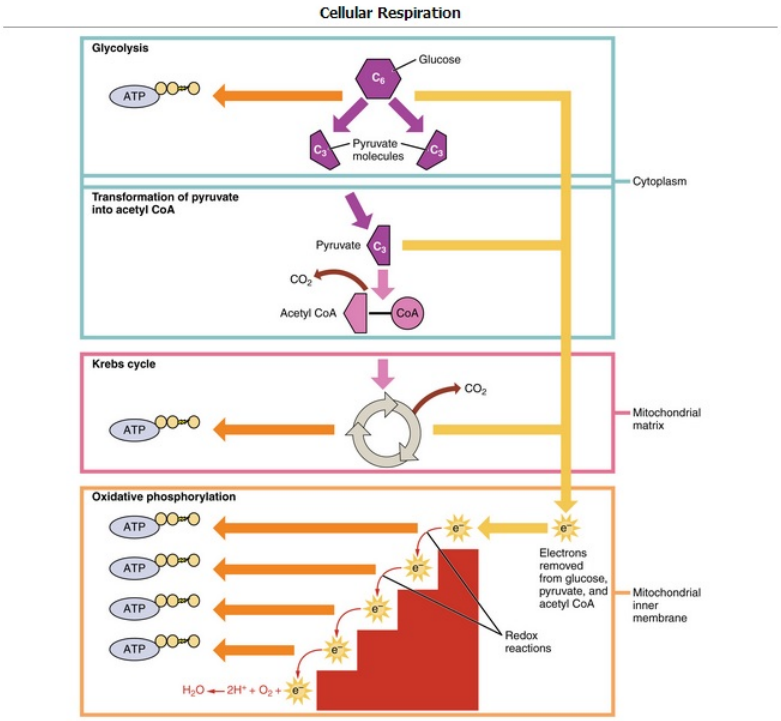


Figure: ***Cellular Respiration****: Glycolysis is the first pathway of cellular respiration that oxidizes glucose molecules. It is followed by the Krebs cycle and oxidative phosphorylation to produce ATP.*

Glycolysis is the first of the main metabolic pathways of cellular respiration to produce energy in the form of ATP. Through two distinct phases, the six-carbon ring of glucose is cleaved into two three-carbon sugars of pyruvate through a series of enzymatic reactions. The first phase of glycolysis requires energy, while the second phase completes the conversion to pyruvate and produces ATP and NADH for the cell to use for energy. Overall, the process of glycolysis produces a net gain of two pyruvate molecules, two ATP molecules, and two NADH molecules for the cell to use for energy. Following the conversion of glucose to pyruvate, the glycolytic pathway is linked to the Krebs Cycle, where further ATP will be produced for the cell's energy needs.

Key Points

- Glycolysis is present in nearly all living organisms.
- Glucose is the source of almost all energy used by cells.
- Overall, glycolysis produces two pyruvate molecules, a net gain of two ATP molecules, and two NADH molecules.

Key Terms

- **glycolysis**: the cellular metabolic pathway of the simple sugar glucose to yield pyruvic acid and ATP as an energy source
- **heterotroph**: an organism that requires an external supply of energy in the form of food, as it cannot synthesize its own

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4.4B: Electron Donors and Acceptors

Learning Objectives

- Recognize the various types of electron donors and acceptors

In prokaryotes (bacteria and archaea) there are several different electron donors and several different electron acceptors. Note that electrons can enter the chain at three levels: at the level of a dehydrogenase, at the level of the quinone pool, or at the level of a mobile cytochrome electron carrier. These levels correspond to successively more positive redox potentials, or to successively decreased potential differences relative to the terminal electron acceptor. In other words, they correspond to successively smaller Gibbs free energy changes for the overall redox reaction *Donor* → *Acceptor*.

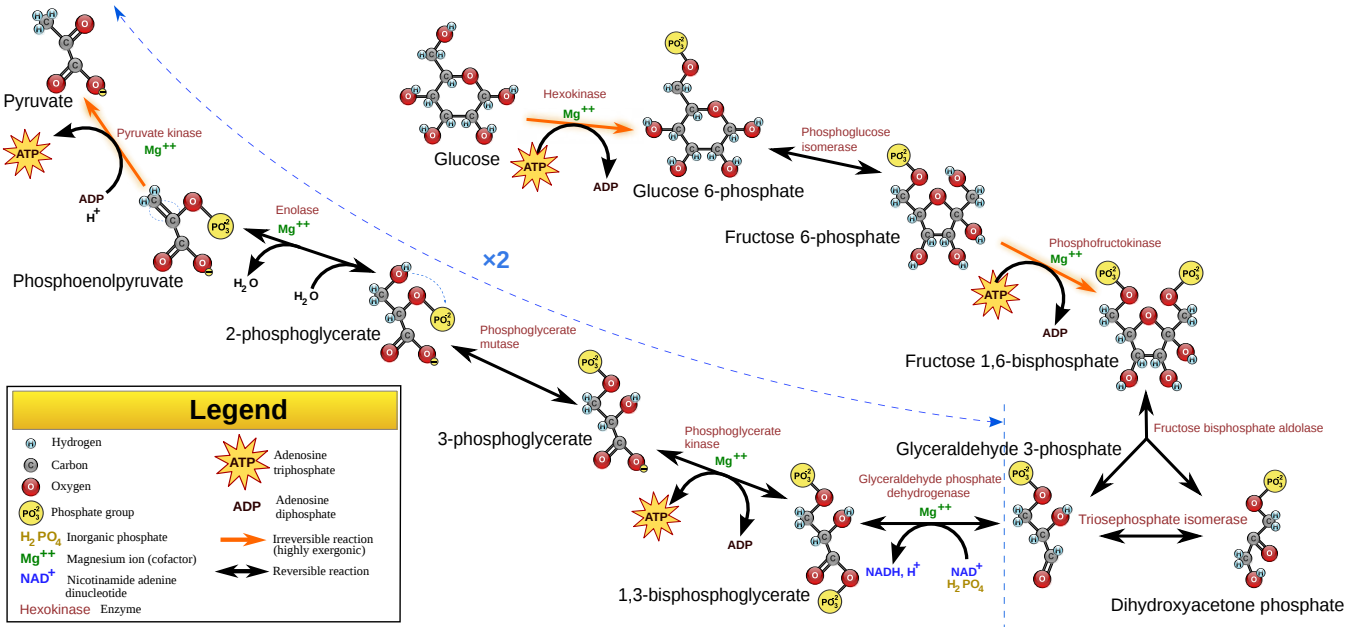


Figure: ***Glycolysis Pathway Overview****: An overview of the glycolytic pathway. This pathway, comprised of a series of reactions, produces many intermediates and molecules utilized as substrates for biosynthesis in additional pathways.*

Individual bacteria use multiple electron transport chains, often simultaneously. Bacteria can use a number of different electron donors, a number of different dehydrogenases, a number of different oxidases and reductases, and a number of different electron acceptors. For example, *E. coli* (when growing aerobically using glucose as an energy source) uses two different NADH dehydrogenases and two different quinol oxidases, for a total of four different electron transport chains operating simultaneously.

A common feature of all electron transport chains is the presence of a proton pump to create a transmembrane proton gradient. Bacterial electron transport chains may contain as many as three proton pumps, like mitochondria, or they may contain only one or two. They always contain at least one proton pump.

In the present day biosphere, the most common electron donors are organic molecules. Organisms that use organic molecules as an energy source are called *organotrophs*. Organotrophs (animals, fungi, protists) and *phototrophs* (plants and algae) constitute the vast majority of all familiar life forms.

Some prokaryotes can use inorganic matter as an energy source. Such organisms are called *lithotrophs* (“rock-eaters”). Inorganic electron donors include hydrogen, carbon monoxide, ammonia, nitrite, sulfur, sulfide, and ferrous iron. Lithotrophs have been found growing in rock formations thousands of meters below the surface of Earth. Because of their volume of distribution, lithotrophs may actually out number organotrophs and phototrophs in our biosphere.

The use of inorganic electron donors as an energy source is of particular interest in the study of evolution. This type of metabolism must logically have preceded the use of organic molecules as an energy source.

Just as there are a number of different electron donors (organic matter in organotrophs, inorganic matter in lithotrophs), there are a number of different electron acceptors, both organic and inorganic. If oxygen is available, it is invariably used as the terminal electron acceptor, because it generates the greatest Gibbs free energy change and produces the most energy.

In anaerobic environments, different electron acceptors are used, including nitrate, nitrite, ferric iron, sulfate, carbon dioxide, and small organic molecules such as fumarate.

Since electron transport chains are redox processes, they can be described as the sum of two redox pairs. For example, the mitochondrial electron transport chain can be described as the sum of the NAD^+/NADH redox pair and the O_2/H_2O redox pair. NADH is the electron donor and O_2 is the electron acceptor.

Not every donor-acceptor combination is thermodynamically possible. The redox potential of the acceptor must be more positive than the redox potential of the donor. Furthermore, actual environmental conditions may be far different from *standard* conditions (1 molar concentrations, 1 atm partial pressures, pH = 7), which apply to *standard* redox potentials. For example, hydrogen-evolving bacteria grow at an ambient partial pressure of hydrogen gas of 10^{-4} atm. The associated redox reaction, which is thermodynamically favorable in nature, is thermodynamically impossible under “standard” conditions.

Bacterial electron transport pathways are, in general, inducible. Depending on their environment, bacteria can synthesize different transmembrane complexes and produce different electron transport chains in their cell membranes. Bacteria select their electron transport chains from a DNA library containing multiple possible dehydrogenases, terminal oxidases and terminal reductases. The situation is often summarized by saying that electron transport chains in bacteria are *branched*, *modular*, and *inducible*.

Key Points

- Bacterial electron transport chains may contain as many as three proton pumps.
- The most common electron donors are organic molecules.
- There are a number of different electron acceptors, both organic and inorganic. If oxygen is available, it is invariably used as the terminal electron acceptor.

Key Terms

- **organotroph**: An organism that obtains its energy from organic compounds.
- **lithotroph**: An organism that obtains its energy from inorganic compounds (such as ammonia) via electron transfer.

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4.4C: ATP Yield

Learning Objectives

- Describe the origins of variability in the amount of ATP that is produced per molecule of glucose consumed

ATP Yield

In a eukaryotic cell, the process of cellular respiration can metabolize one molecule of glucose into 30 to 32 ATP. The process of glycolysis only produces two ATP, while all the rest are produced during the electron transport chain. Clearly, the electron transport chain is vastly more efficient, but it can only be carried out in the presence of oxygen.

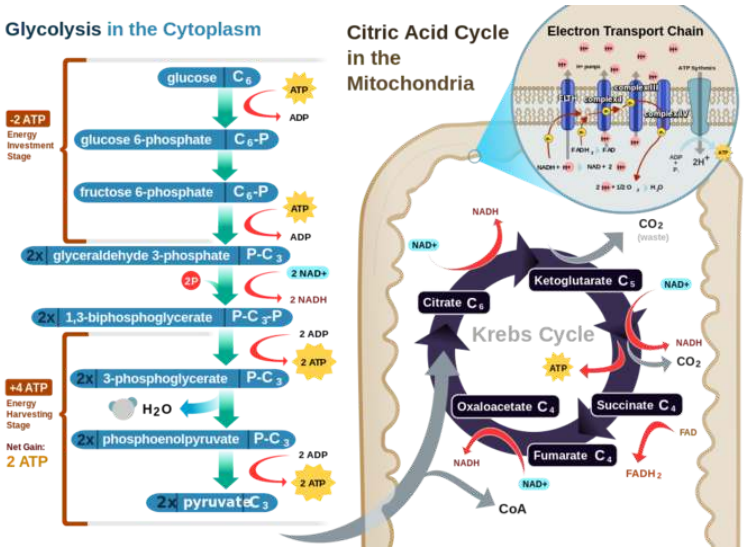


Figure: **Cellular respiration in a eukaryotic cell**: Glycolysis on the left portion of this illustration can be seen to yield 2 ATP molecules, while the Electron Transport Chain portion at the upper right will yield the remaining 30-32 ATP molecules under the presence of oxygen.

The number of ATP molecules generated via the catabolism of glucose can vary substantially. For example, the number of hydrogen ions the electron transport chain complexes can pump through the membrane varies between species. Another source of variance occurs during the shuttle of electrons across the membranes of the mitochondria. The NADH generated from glycolysis cannot easily enter mitochondria. Thus, electrons are picked up on the inside of mitochondria by either NAD^+ or FAD^+. These FAD^+ molecules can transport fewer ions; consequently, fewer ATP molecules are generated when FAD^+ acts as a carrier. NAD^+ is used as the electron transporter in the liver, and FAD^+ acts in the brain.

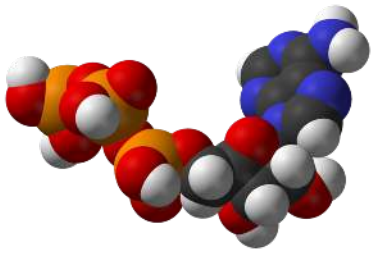


Figure: **Adenosine triphosphate**: ATP is the main source of energy in many living organisms.

Another factor that affects the yield of ATP molecules generated from glucose is the fact that intermediate compounds in these pathways are used for other purposes. Glucose catabolism connects with the pathways that build or break down all other biochemical compounds in cells, but the result is not always ideal. For example, sugars other than glucose are fed into the glycolytic pathway for energy extraction. Moreover, the five-carbon sugars that form nucleic acids are made from intermediates in glycolysis. Certain nonessential amino acids can be made from intermediates of both glycolysis and the citric acid cycle. Lipids,

such as cholesterol and triglycerides, are also made from intermediates in these pathways, and both amino acids and triglycerides are broken down for energy through these pathways. Overall, in living systems, these pathways of glucose catabolism extract about 34 percent of the energy contained in glucose.

Key Points

- While glucose catabolism always produces energy, the amount of energy (in terms of ATP equivalents) produced can vary, especially across different species.
- The number of hydrogen ions the electron transport chain complexes can pump through the membrane varies between species.
- NAD^+ provides more ATP than FAD^+ in the electron transport chain and can lead to variance in ATP production.
- The use of intermediates from glucose catabolism in other biosynthetic pathways, such as amino acid synthesis, can lower the yield of ATP.

Key Terms

- **catabolism**: Destructive metabolism, usually including the release of energy and breakdown of materials.

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4.4D: Respiration and Proton Motive Force

Learning Objectives

- Describe the role of the proton motive force in respiration

Cellular Respiration

Cellular respiration is a set of metabolic reactions and processes that take place within the cells of organisms to convert biochemical energy from nutrients into adenosine triphosphate (ATP). The reactions involved in this respiration are considered to be catabolic reactions that release energy as larger molecules are broken down into smaller ones and high-energy bonds are broken. Respiration is one of the key ways a cell gains useful energy to fuel cellular activity.

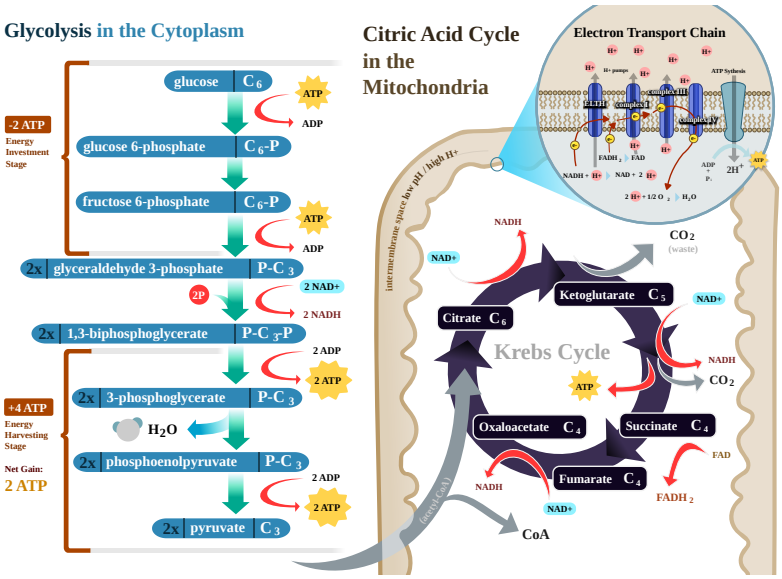


Figure: **Overview of Cellular Respiration**: A diagram of cellular respiration including glycolysis, Krebs cycle (AKA citric acid cycle), and the electron transport chain.

Chemically, cellular respiration is considered an exothermic redox reaction. The overall reaction is broken into many smaller ones when it occurs in the body. Most of these smaller reactions are redox reactions themselves. Although technically, cellular respiration is a combustion reaction, it does not resemble one when it occurs in a living cell. This is because it occurs in many separate steps. While the overall reaction is a combustion reaction, no single reaction that comprises it is a combustion reaction.

Aerobic and Anaerobic Reactions

Aerobic reactions require oxygen for ATP generation. Although carbohydrates, fats and proteins can be used as reactants, the preferred method is the process of glycolysis. During glycolysis, pyruvate is formed from glucose metabolism. During aerobic conditions, the pyruvate enters the mitochondrion to be fully oxidized by the Krebs cycle. The products of the Krebs cycle include energy in the form of ATP (via substrate level phosphorylation), NADH, and FADH2.

The simplified reaction is as follows:

$C_6H_{12}O_6$ (s) + 6 O_2 (g) → 6 CO_2 (g) + 6 H_2O (l) + heat

ΔG = -2880 kJ per mole of $C_6H_{12}O_6$

A negative ΔG indicates that the reaction can occur spontaneously.

Aerobic metabolism is up to 15 times more efficient than anaerobic metabolism, which yields two molecules ATP per one molecule glucose. Both types of metabolism share the initial pathway of glycolysis, but aerobic metabolism continues with the Krebs cycle and oxidative phosphorylation. In eukaryotic cells, the post-glycolytic reactions take place in the mitochondria, while in prokaryotic cells, these reactions take place in the cytoplasm.

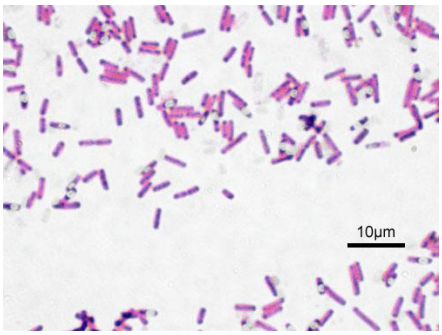


Figure: **Humans use of prokaryotes**: This is a microscopic image of Bacillus subtilis (ATCC 6633) with a gram staining of magnification: 1,000. The oval, unstained structures are spores.

Glycolysis

Glycolysis takes place in the cytosol, does not require oxygen, and can therefore function under anaerobic conditions. The process converts one molecule of glucose into two molecules of pyruvate, generating energy in the form of two net molecules of ATP. Four molecules of ATP per glucose are actually produced, but two of these are consumed as part of the preparatory phase. The initial phosphorylation of glucose is required to destabilize the molecule for cleavage into two pyruvate. During the pay-off phase of glycolysis, four phosphate groups are transferred to ADP by substrate-level phosphorylation to make four ATP, and two NADH are produced when the pyruvate are oxidized. The overall reaction can be expressed this way:

$$\text{Glucose} + 2\ NAD^+ + 2\ P_i + 2\ ADP \rightarrow 2\ \text{pyruvate} + 2\ NADH + 2\ ATP + 2\ H^+ + 2\ H_2O + \text{heat}$$

Starting with glucose, one ATP is used to donate a phosphate to glucose to produce glucose 6-phosphate. With the help of glycogen phosphorylase, glycogen can change into glucose 6-phosphate as well. During energy metabolism, glucose 6-phosphate turns into fructose 6-phosphate. With the help of phosphofructokinase, an additional ATP can be used to turn phosphorylate fructose 6-phosphate into fructose 1, 6-diphosphate. Fructose 1, 6-diphosphate then splits into two phosphorylated molecules with three carbon chains that later degrades into pyruvate.

Making Proton Gradients

Some archaea, the most notable ones being halobacteria, make proton gradients by pumping in protons from the environment. They are able to do this with the help of the solar-driven enzyme bacteriorhodopsin, which is used to drive the molecular motor enzyme ATP synthase to make the necessary conformational changes required to synthesize ATP. By running ATP synthase in reverse, proton gradients are also made by bacteria and are used to drive flagella. The F1FO ATP synthase is a reversible enzyme. Large enough quantities of ATP cause it to create a transmembrane proton gradient. This is used by fermenting bacteria, which lack an electron transport chain, and which hydrolyze ATP to make a proton gradient. Bacteria use these gradients for flagella and for the transportation of nutrients into the cell. In respiring bacteria under physiological conditions, ATP synthase, in general, runs in the opposite direction. This creates ATP while using the proton motive force created by the electron transport chain as a source of energy. The overall process of creating energy in this fashion is termed oxidative phosphorylation.

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Key Points

- The reactions involved in respiration are catabolic reactions, which break large molecules into smaller ones, releasing energy in the process as they break high-energy bonds.
- Aerobic respiration requires oxygen in order to generate energy (ATP).

- Aerobic metabolism is up to 15 times more efficient than anaerobic metabolism (which yields two molecules ATP per one molecule glucose).
- With the help of the solar-driven enzyme bacteriorhodopsin, some bacteria make proton gradients by pumping in protons from the environment.

Key Terms

- **exothermic**: releasing energy in the form of heat
- **redox**: a reversible process in which one reaction is an oxidation and the reverse is a reduction

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SECTION OVERVIEW

4.5: Respiratory ETS and ATP Synthase

Topic hierarchy

4.5A: Cofactors and Energy Transitions

4.5B: Oxidoreductase Protein Complexes

4.5C: F10 ATP Synthase

4.5D: Sodium Pumps as an Alternative to Proton Pump

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4.5A: Cofactors and Energy Transitions

Learning Objectives

- Recognize the various types of cofactors involved in biochemical reactions

A cofactor is a non- protein chemical compound that is bound to a protein and is required for the protein's biological activity. These proteins are commonly enzymes. Cofactors can be considered "helper molecules" that assist in biochemical transformations.

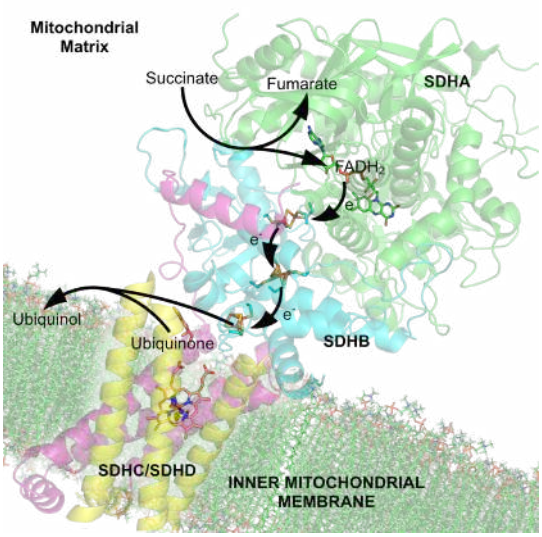


Figure: **Cofactor**: The succinate dehydrogenase complex showing several cofactors, including flavin, iron-sulfur centers, and heme.

Cofactors are either organic or inorganic. They can also be classified depending on how tightly they bind to an enzyme, with loosely-bound cofactors termed coenzymes and tightly-bound cofactors termed prosthetic groups. Some sources also limit the use of the term "cofactor" to inorganic substances. An inactive enzyme without the cofactor is called an apoenzyme, while the complete enzyme with cofactor is the holoenzyme.

Some enzymes or enzyme complexes require several cofactors. For example, the multienzyme complex pyruvate dehydrogenase at the junction of glycolysis and the citric acid cycle requires five organic cofactors and one metal ion: loosely bound thiamine pyrophosphate (TPP), covalently bound lipoamide and flavin adenine dinucleotide (FAD), and the cosubstrates nicotinamide adenine dinucleotide (NAD+) and coenzyme A (CoA), and a metal ion (Mg2+).

Organic cofactors are often vitamins or are made from vitamins. Many contain the nucleotide adenosine monophosphate (AMP) as part of their structures, such as ATP, coenzyme A, FAD, and NAD+. This common structure may reflect a common evolutionary origin as part of ribozymes in an ancient RNA world. It has been suggested that the AMP part of the molecule can be considered a kind of "handle" by which the enzyme can "grasp" the coenzyme to switch it between different catalytic centers.

Cofactors can be divided into two broad groups: organic cofactors, such as flavin or heme, and inorganic cofactors, such as the metal ions Mg2+, Cu+, Mn2+, or iron-sulfur clusters.

Vitamins can serve as precursors to many organic cofactors (e.g., vitamins B1, B2, B6, B12, niacin, folic acid) or as coenzymes themselves (e.g., vitamin C). However, vitamins do have other functions in the body. Many organic cofactors also contain a nucleotide, such as the electron carriers NAD and FAD, and coenzyme A, which carries acyl groups. Most of these cofactors are found in a huge variety of species, and some are universal to all forms of life. An exception to this wide distribution is a group of unique cofactors that evolved in methanogens, which are restricted to this group of archaea.

Metabolism involves a vast array of chemical reactions, but most fall under a few basic types of reactions that involve the transfer of functional groups. This common chemistry allows cells to use a small set of metabolic intermediates to carry chemical groups between different reactions. These group-transfer intermediates are the loosely-bound organic cofactors, often called coenzymes.

Each class of group-transfer reaction is carried out by a particular cofactor, which is the substrate for a set of enzymes that produce it and a set of enzymes that consume it. An example of this is the dehydrogenases that use nicotinamide adenine dinucleotide (NAD+) as a cofactor. Here, hundreds of separate types of enzymes remove electrons from their substrates and reduce NAD+ to NADH. This reduced cofactor is then a substrate for any of the reductases in the cell that require electrons to reduce their substrates.

Therefore, these cofactors are continuously recycled as part of metabolism. As an example, the total quantity of ATP in the human body is about 0.1 mole. This ATP is constantly being broken down into ADP, and then converted back into ATP. Therefore, at any given time, the total amount of ATP + ADP remains fairly constant. The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily, which is around 50 to 75 kg. In typical situations, humans use up their body weight of ATP over the course of the day. This means that each ATP molecule is recycled 1,000 to 1,500 times daily.

The term is used in other areas of biology to refer more broadly to non-protein (or even protein) molecules that either activate, inhibit, or are required for the protein to function. For example, ligands such as hormones that bind to and activate receptor proteins are termed cofactors or coactivators, whereas molecules that inhibit receptor proteins are termed corepressors.

Key Points

- Cofactors are commonly enzymes, and cofactors can be considered “helper molecules ” that assist in biochemical transformations.
- Some enzymes or enzyme complexes require several cofactors.
- Each class of group-transfer reaction is carried out by a particular cofactor, which is the substrate for a set of enzymes that produce it, and a set of enzymes that consume it.

Key Terms

- **cofactor**: A substance, especially a coenzyme or a metal, that must be present for an enzyme to function.
- **enzymes**: Enzymes are large biological molecules responsible for the thousands of chemical interconversions that sustain life. They are highly selective catalysts, greatly accelerating both the rate and specificity of metabolic reactions, from the digestion of food to the synthesis of DNA.
- **reaction**: A chemical reaction is a process that leads to the transformation of one set of chemical substances to another. Classically, chemical reactions encompass changes that strictly involve the motion of electrons in the forming and breaking of chemical bonds between atoms, and can often be described by a chemical equation.
- **apoenzyme**: an inactive haloenzyme lacking a cofactor

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4.5B: Oxidoreductase Protein Complexes

Learning Objectives

- Recognize the function of oxidoreductase protein complexes

In biochemistry, an oxidoreductase is an enzyme that catalyzes the transfer of electrons from one molecule, the reductant, also called the electron donor, to another the oxidant, also called the electron acceptor. This group of enzymes usually utilizes NADP or NAD+ as cofactors.

For example, an enzyme that catalyzed this reaction would be an oxidoreductase: A– + B → A + B–. In this example, A is the reductant (electron donor) and B is the oxidant (electron acceptor).

In biochemical reactions, the redox reactions are sometimes more difficult to see, such as this reaction from glycolysis: Pi + glyceraldehyde-3-phosphate + NAD+ → NADH + H+ + 1,3-bisphosphoglycerate. In this reaction, NAD+ is the oxidant (electron acceptor) and glyceraldehyde-3-phosphate is the reductant (electron donor).

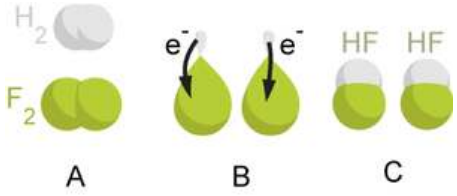


Figure: **Illustration of a redox reaction**: Illustration of a redox reaction

Oxidoreductases are classified as EC 1 in the EC number classification of enzymes. Oxidoreductases can be further classified into 22 subclasses:

- EC 1.1 includes oxidoreductases that act on the CH-OH group of donors (alcohol oxidoreductases);
- EC 1.2 includes oxidoreductases that act on the aldehyde or oxo group of donors;
- EC 1.3 includes oxidoreductases that act on the CH-CH group of donors (CH-CH oxidoreductases);
- EC 1.4 includes oxidoreductases that act on the CH-NH_2 group of donors (Amino acid oxidoreductases, Monoamine oxidase);
- EC 1.5 includes oxidoreductases that act on CH-NH group of donors;
- EC 1.6 includes oxidoreductases that act on NADH or NADPH;
- EC 1.7 includes oxidoreductases that act on other nitrogenous compounds as donors;
- EC 1.8 includes oxidoreductases that act on a sulfur group of donors;
- EC 1.9 includes oxidoreductases that act on a heme group of donors;
- EC 1.10 includes oxidoreductases that act on diphenols and related substances as donors;
- EC 1.11 includes oxidoreductases that act on peroxide as an acceptor (peroxidases);
- EC 1.12 includes oxidoreductases that act on hydrogen as donors;
- EC 1.13 includes oxidoreductases that act on single donors with incorporation of molecular oxygen (oxygenases);
- EC 1.14 includes oxidoreductases that act on paired donors with incorporation of molecular oxygen;
- EC 1.15 includes oxidoreductases that act on superoxide radicals as acceptors;
- EC 1.16 includes oxidoreductases that oxidize metal ions; EC 1.17 includes oxidoreductases that act on CH or CH2 groups;
- EC 1.18 includes oxidoreductases that act on iron-sulfur proteins as donors;
- EC 1.19 includes oxidoreductases that act on reduced flavodoxin as a donor;
- EC 1.20 includes oxidoreductases that act on phosphorus or arsenic in donors;
- EC 1.21 includes oxidoreductases that act on X-H and Y-H to form an X-Y bond; and EC 1.97 includes other oxidoreductases.

Key Points

- The reductant is the electron donor.
- The oxidant is the electron acceptor.
- This group of enzymes usually utilizes NADP or NAD+ as cofactors.

Key Terms

- **oxidoreductase**: Any enzyme that catalyzes an oxidation-reduction (redox) reaction.
- **enzyme**: A globular protein that catalyses a biological chemical reaction.
- **catalyzes**: Catalysis is the change in rate of a chemical reaction due to the participation of a substance called a catalyst. Unlike other reagents that participate in the chemical reaction, a catalyst is not consumed by the reaction itself.

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4.5C: F10 ATP Synthase

Learning Objectives

- Discuss the structure and function of ATP synthase, including the F1 and FO components

ATP synthase is an important enzyme that provides energy for the cell to use through the synthesis of adenosine triphosphate (ATP). ATP is the most commonly used “energy currency” of cells from most organisms. It is formed from adenosine diphosphate (ADP) and inorganic phosphate (Pi), and needs energy.

The overall reaction sequence is: ATP synthase + ADP + Pi → ATP Synthase + ATP

Energy is often released in the form of protium or H+, moving down an electrochemical gradient, such as from the lumen into the stroma of chloroplasts or from the inter-membrane space into the matrix in mitochondria.

Located within the mitochondria, ATP synthase consists of 2 regions: the FO portion is within the membrane and the F1 portion of the ATP synthase is above the membrane, inside the matrix of the mitochondria.

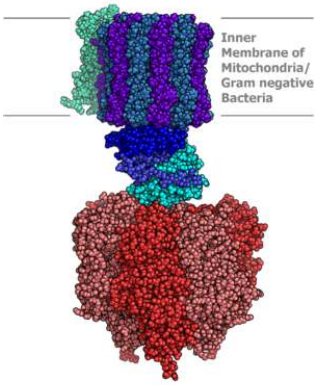


Figure: **ATP synthase**: Molecular model of ATP synthase by X-ray diffraction method

The nomenclature of the enzyme suffers from a long history. The F1 fraction derives its name from the term “Fraction 1” and FO (written as a subscript letter “o”, not “zero”) derives its name from being the oligomycin binding fraction. Oligomycin, an antibiotic, is able to inhibit the FO unit of ATP synthase.

F1- ATP Synthase structure: The F1 particle is large and can be seen in the transmission electron microscope by negative staining. These are particles of 9 nm diameter that pepper the inner mitochondrial membrane. They were originally called elementary particles and were thought to contain the entire respiratory apparatus of the mitochondrion, but, through a long series of experiments, Ephraim Racker and his colleagues (who first isolated the F1 particle in 1961) were able to show that this particle is correlated with ATPase activity in uncoupled mitochondria and with the ATPase activity in submitochondrial particles created by exposing mitochondria to ultrasound. This ATPase activity was further associated with the creation of ATP by a long series of experiments in many laboratories.

The FO region of ATP synthase is a proton pore that is embedded in the mitochondrial membrane. It consists of three main subunits A, B, and C, and (in humans) six additional subunits, d, e, f, g, F6, and 8 (or A6L).

E. coli ATP synthase is the simplest known form of ATP synthase, with 8 different subunit types.

Key Points

- Energy is often released in the form of protium or H+, moving down an electrochemical gradient.
- ATP synthase consists of 2 regions: the FO portion is within the membrane and the F1 portion of the ATP synthase is above the membrane, inside the matrix of the mitochondria.
- E. coli ATP synthase is the simplest known form of ATP synthase, with 8 different subunit types.

Key Terms

- **synthase**: Any enzyme that catalyzes the synthesis of a biological compound but, unlike synthetases, does not make use of ATP as a source of energy
- **adenosine triphosphate**: Adenosine-5′-triphosphate (ATP) is a multifunctional nucleoside triphosphate used in cells as a coenzyme. It is often called the “molecular unit of currency” of intracellular energy transfer.
- **enzyme**: A globular protein that catalyses a biological chemical reaction.

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4.5D: Sodium Pumps as an Alternative to Proton Pump

Learning Objectives

- Describe the mechanisms of sodium pumps and its role as an alternative proton pump

What Are Sodium Pumps?

Na+/K+-ATPase (Sodium-potassium adenosine triphosphatase, also known as Na+/K+ pump, sodium-potassium pump, or sodium pump) is an antiporter enzyme (EC 3.6.3.9) (an electrogenic transmembrane ATPase) located in the plasma membrane of all animal cells.

Active transport is responsible for cells containing relatively high concentrations of potassium ions but low concentrations of sodium ions. The mechanism responsible for this is the sodium-potassium pump, which moves these two ions in opposite directions across the plasma membrane. This was investigated by following the passage of radioactively labeled ions across the plasma membrane of certain cells. It was found that the concentrations of sodium and potassium ions on the two sides of the membrane are interdependent, suggesting that the same carrier transports both ions. It is now known that the carrier is an ATP-ase and that it pumps three sodium ions out of the cell for every two potassium ions pumped in.

Discovery and Significance

The sodium-potassium pump was discovered in the 1950s by Danish scientist Jens Christian Skou. It marked an important step in our understanding of how ions get into and out of cells, and has a particular significance for excitable cells like nervous cells, which depend on this pump for responding to stimuli and transmitting impulses.

The Na+/K+-ATPase helps maintain resting potential, avail transport and regulate cellular volume. It also functions as signal transducer/integrator to regulate MAPK pathway, ROS, as well as intracellular calcium. In most animal cells, the Na+/K+-ATPase is responsible for about 1/5 of the cell's energy expenditure. For neurons, the Na+/K+-ATPase can be responsible for up to 2/3 of the cell's energy expenditure.

Functions

Functions include resting potential, transport, controlling cell volume and acting as a signal transducer. The pump, while binding ATP, binds 3 intracellular Na+ ions. ATP is hydrolyzed, leading to phosphorylation of the pump at a highly conserved aspartate residue and subsequent release of ADP. A conformational change in the pump exposes the Na+ ions to the outside. The phosphorylated form of the pump has a low affinity for Na+ ions, so they are released.The pump binds 2 extracellular K+ ions. This causes the dephosphorylation of the pump, reverting it to its previous conformational state, transporting the K+ ions into the cell.The unphosphorylated form of the pump has a higher affinity for Na+ ions than K+ ions, so the two bound K+ ions are released. ATP binds, and the process starts again.

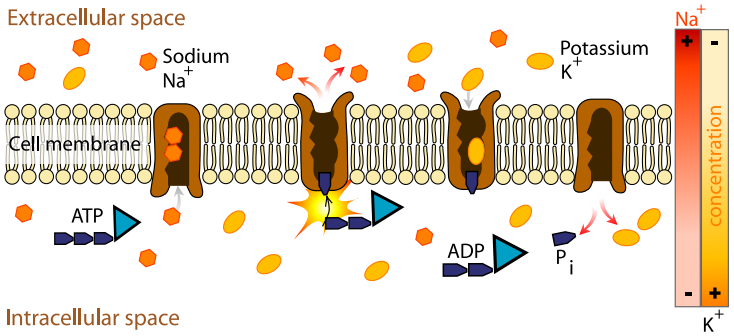


Figure: **Na+/K+-ATPase**: Na+/K+-ATPase Mechanism of Action

Proton Motive Force

Most bacteria rely on proton motive force as a source of energy for a variety of cellular processes. Usually, an H+ cycle includes generation of the transmembrane electrochemical gradient of H+ (proton motive force) by primary transport systems (H+ pumps)

and its use for ATP synthesis, solute transport, motility and reverse electron transport. A substantial body of evidence indicates, however, that certain extremophilic bacteria can use Na+ as a coupling ion in an Na+ cycle instead of, or in addition to, the H+ cycle. As in the H+ cycle, a fully operational Na+ cycle would include a primary Na+ pump that directly couples Na+ translocation to a chemical reaction, an Na+-transporting membrane ATP synthetase, a number of Na+-dependent membrane transporters, and an Na+-dependent flagellar motor. While certain Na+-dependent functions, like Na+-dependent uptake of melibiose, proline, and glutamate, have been observed in many bacteria, the ion gradients that served as energy sources for these transports have been generated by primary H+ pumps and converted to Na+ gradients by Na+/H+ antiporters.

One could think of several possible explanations for the widespread distribution of the elements of the Na+ cycle among pathogenic bacteria. First, Na+-based membrane energetics could improve the versatility of a pathogen by providing it with additional means of ATP synthesis, motility and solute uptake. This would improve its chances for colonization of the host cells and survival in the host organisms where defense mechanisms, including generation of superoxide radicals, impair the integrity of the bacterial membrane and decrease the levels of the proton motive force. Second, because Na+ concentrations in most natural environments are almost 10^6-fold higher than H+ concentrations, sodium motive force levels are unlikely to change as rapidly as proton motive force levels, making sodium motive force a much more reliable source of energy.

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Key Points

- The Na+/K+-ATPase helps maintain resting potential, avail transport and regulate cellular volume.
- The pump, while binding ATP, binds 3 intracellular Na+ ions. ATP is hydrolyzed, leading to phosphorylation of the pump at a highly conserved aspartate residue and subsequent release of ADP.
- Some extremophilic bacteria can use Na+ as a coupling ion in an Na+ cycle instead of, or in addition to, the H+ cycle.
- Na+-based membrane energetics provide an additional means of ATP synthesis, motility and solute uptake for pathogenic microbes.
- Because Na+ concentrations in most natural environments are almost 10^6-fold higher than H+ concentrations, sodium motive force levels are unlikely to change as rapidly as proton motive force levels, making sodium motive force a much more reliable source of energy.

Key Terms

- **antiporter**: A cell protein that acts within an antiport to transport different molecules or ions across the membrane in opposite directions
- **resting potential**: The nearly latent membrane potential of inactive cells.
- **hydrolyzed**: Hydrolysis usually means the cleavage of chemical bonds by the addition of water.

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SECTION OVERVIEW

4.6: The Citric Acid (Krebs) Cycle

Topic hierarchy

4.6A: Citric Acid Cycle

4.6B: Breakdown of Pyruvate

4.6C: Acetyl CoA and the Citric Acid Cycle

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4.6A: Citric Acid Cycle

Learning Objectives

- List the steps of the Krebs (or citric acid) cycle

Like the conversion of pyruvate to acetyl CoA, the citric acid cycle takes place in the matrix of the mitochondria. Almost all of the enzymes of the citric acid cycle are soluble, with the single exception of the enzyme succinate dehydrogenase, which is embedded in the inner membrane of the mitochondrion. Unlike glycolysis, the citric acid cycle is a closed loop: the last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one GTP/ATP, and reduced forms of NADH and FADH2. This is considered an aerobic pathway because the NADH and FADH2 produced must transfer their electrons to the next pathway in the system, which will use oxygen. If this transfer does not occur, the oxidation steps of the citric acid cycle also do not occur. Note that the citric acid cycle produces very little ATP directly and does not directly consume oxygen.

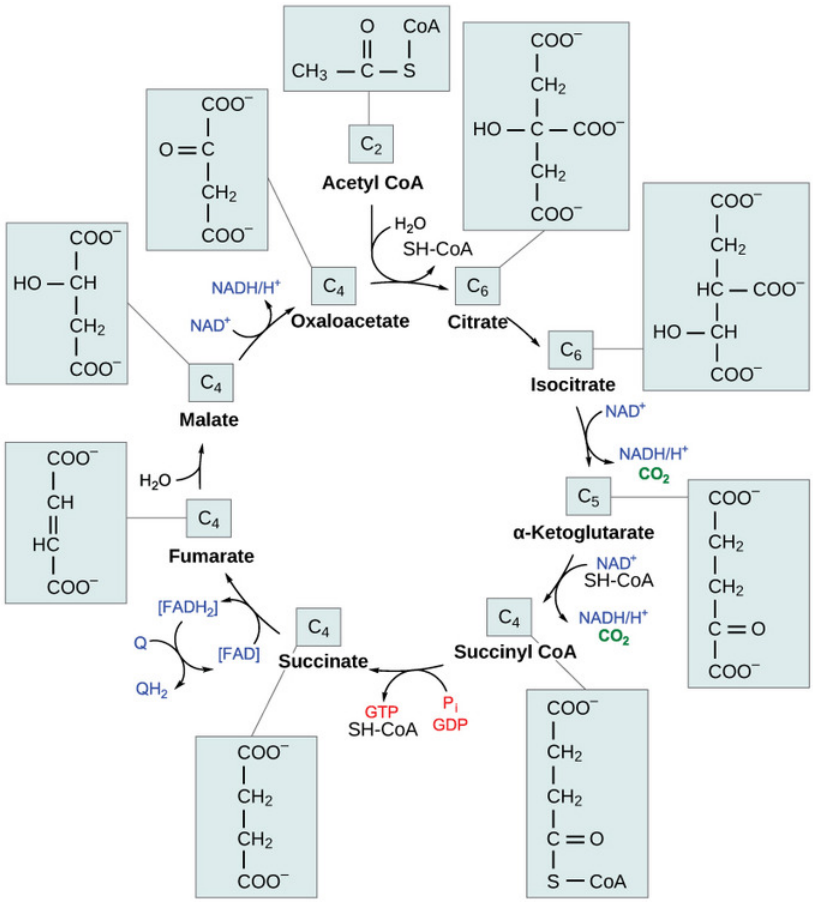


Figure: ***The citric acid cycle****: In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NAD+ molecules are reduced to NADH, one FAD molecule is reduced to* $FADH_2$*, and one ATP or GTP (depending on the cell type) is produced (by substrate-level phosphorylation). Because the final product of the citric acid cycle is also the first reactant, the cycle runs continuously in the presence of sufficient reactants.*

Steps in the Citric Acid Cycle

Step 1. The first step is a condensation step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. CoA is bound to a sulfhydryl group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible because it is highly exergonic. The rate of this reaction is

controlled by negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

Step 2. Citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

Steps 3 and 4. In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD+ to NADH. This step is also regulated by negative feedback from ATP and NADH and by a positive effect of ADP. Steps three and four are both oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. α-Ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

Step 5. A phosphate group is substituted for coenzyme A, and a high- energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6. Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. The energy contained in the electrons of these atoms is insufficient to reduce NAD^+ but adequate to reduce FAD. Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

Step 7. Water is added to fumarate during step seven, and malate is produced. The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced.

Products of the Citric Acid Cycle

Two carbon atoms come into the citric acid cycle from each acetyl group, representing four out of the six carbons of one glucose molecule. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not necessarily contain the most recently-added carbon atoms. The two acetyl carbon atoms will eventually be released on later turns of the cycle; thus, all six carbon atoms from the original glucose molecule are eventually incorporated into carbon dioxide. Each turn of the cycle forms three NADH molecules and one $FADH_2$ molecule. These carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One GTP or ATP is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is amphibolic (both catabolic and anabolic).

Key Points

- The four-carbon molecule, oxaloacetate, that began the cycle is regenerated after the eight steps of the citric acid cycle.
- The eight steps of the citric acid cycle are a series of redox, dehydration, hydration, and decarboxylation reactions.
- Each turn of the cycle forms one GTP or ATP as well as three NADH molecules and one FADH2 molecule, which will be used in further steps of cellular respiration to produce ATP for the cell.

Key Terms

- **citric acid cycle**: a series of chemical reactions used by all aerobic organisms to generate energy through the oxidization of acetate derived from carbohydrates, fats, and proteins into carbon dioxide
- **Krebs cycle**: a series of enzymatic reactions that occurs in all aerobic organisms; it involves the oxidative metabolism of acetyl units and serves as the main source of cellular energy
- **mitochondria**: in cell biology, a mitochondrion (plural mitochondria) is a membrane-enclosed organelle, often described as “cellular power plants” because they generate most of the ATP

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4.6B: Breakdown of Pyruvate

Learning Objectives

- Explain why cells break down pyruvate

In order for pyruvate, the product of glycolysis, to enter the next pathway, it must undergo several changes to become acetyl Coenzyme A (acetyl CoA). Acetyl CoA is a molecule that is further converted to oxaloacetate, which enters the citric acid cycle (Krebs cycle). The conversion of pyruvate to acetyl CoA is a three-step process.

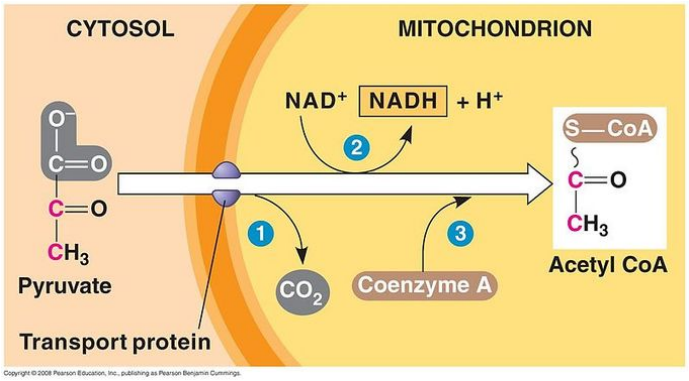


Figure: **Breakdown of Pyruvate**: Each pyruvate molecule loses a carboxylic group in the form of carbon dioxide. The remaining two carbons are then transferred to the enzyme CoA to produce Acetyl CoA.

Step 1. A carboxyl group is removed from pyruvate, releasing a molecule of carbon dioxide into the surrounding medium. (Note: carbon dioxide is one carbon attached to two oxygen atoms and is one of the major end products of cellular respiration.) The result of this step is a two-carbon hydroxyethyl group bound to the enzyme pyruvate dehydrogenase; the lost carbon dioxide is the first of the six carbons from the original glucose molecule to be removed. This step proceeds twice for every molecule of glucose metabolized (remember: there are two pyruvate molecules produced at the end of glycolysis); thus, two of the six carbons will have been removed at the end of both of these steps.

Step 2. The hydroxyethyl group is oxidized to an acetyl group, and the electrons are picked up by NAD^+, forming NADH (the reduced form of NAD+). The high- energy electrons from NADH will be used later by the cell to generate ATP for energy.

Step 3. The enzyme-bound acetyl group is transferred to CoA, producing a molecule of acetyl CoA. This molecule of acetyl CoA is then further converted to be used in the next pathway of metabolism, the citric acid cycle.

Key Points

- In the conversion of pyruvate to acetyl CoA, each pyruvate molecule loses one carbon atom with the release of carbon dioxide.
- During the breakdown of pyruvate, electrons are transferred to NAD+ to produce NADH, which will be used by the cell to produce ATP.
- In the final step of the breakdown of pyruvate, an acetyl group is transferred to Coenzyme A to produce acetyl CoA.

Key Terms

- **acetyl CoA**: a molecule that conveys the carbon atoms from glycolysis (pyruvate) to the citric acid cycle to be oxidized for energy production

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4.6C: Acetyl CoA and the Citric Acid Cycle

Learning Objectives

- Recall the citric acid cycle

The citric acid cycle, shown in —also known as the tricarboxylic acid cycle (TCA cycle) or the Krebs cycle—is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetate—derived from carbohydrates, fats, and proteins—into carbon dioxide. The cycle provides precursors including certain amino acids as well as the reducing agent NADH that is used in numerous biochemical reactions. Its central importance to many biochemical pathways suggests that it was one of the earliest established components of cellular metabolism; it may have originated abiogenically.

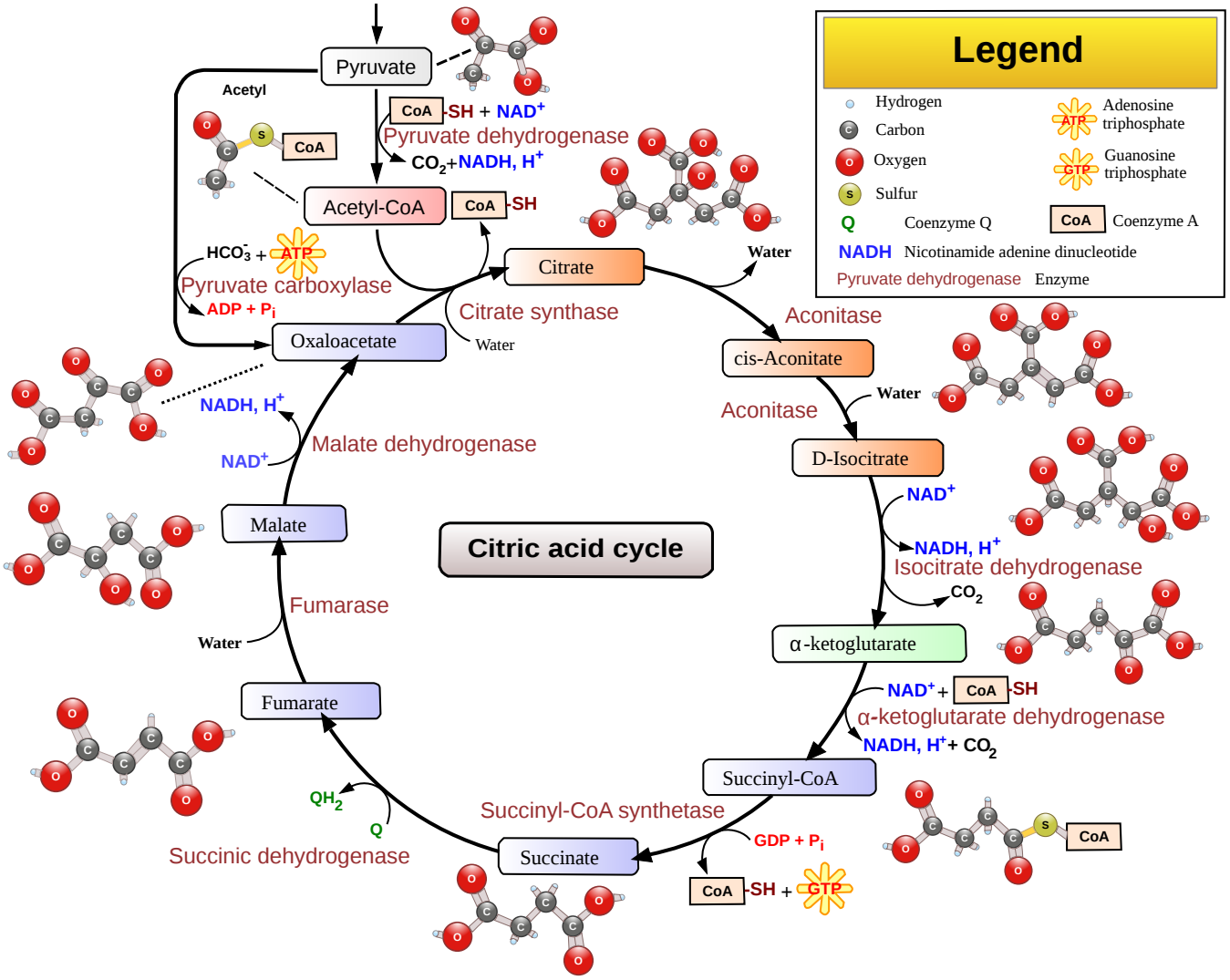


Figure: **The Citric Acid Cycle**: The citric acid cycle, or Krebs cycle, is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidization of acetate—derived from carbohydrates, fats, and proteins—into carbon dioxide. In addition, the cycle provides precursors including certain amino acids as well as the reducing agent NADH that is used in numerous biochemical reactions.

The name of this metabolic pathway is derived from citric acid, a type of tricarboxylic acid that is first consumed and then regenerated by this sequence of reactions to complete the cycle. The cycle consumes acetate (in the form of acetyl-CoA) and water, reduces NAD^+ to NADH, and produces carbon dioxide. The NADH generated by the TCA cycle is fed into the oxidative phosphorylation pathway. The net result of these two closely linked pathways is the oxidation of nutrients to produce usable energy in the form of ATP.

Components of the TCA cycle were derived from anaerobic bacteria, and the TCA cycle itself may have evolved more than once. Theoretically there are several alternatives to the TCA cycle, however the TCA cycle appears to be the most efficient. If several alternatives independently evolved, they all rapidly converged to the TCA cycle.

The citric acid cycle is a key component of the metabolic pathway by which all aerobic organisms generate energy. Through the catabolism of sugars, fats, and proteins, a two carbon organic product acetate in the form of acetyl-CoA is produced. Acetyl-CoA along with two equivalents of water (H_2O) are consumed by the citric acid cycle, producing two equivalents of carbon dioxide (CO_2) and one equivalent of HS-CoA. In addition, one complete turn of the cycle converts three equivalents of nicotinamide adenine dinucleotide (NAD^+) into three equivalents of reduced NAD^+ (NADH), one equivalent of ubiquinone (Q) into one equivalent of reduced ubiquinone (QH_2), and one equivalent each of guanosine diphosphate (GDP) and inorganic phosphate (P_i) into one equivalent of guanosine triphosphate (GTP). The NADH and QH_2 that is generated by the citric acid cycle is used by the oxidative phosphorylation pathway to generate energy-rich adenosine triphosphate (ATP).

One of the primary sources of acetyl-CoA is sugars that are broken down by glycolysis to produce pyruvate that, in turn, is decarboxylated by the enzyme pyruvate dehydrogenase. This generates acetyl-CoA according to the following reaction scheme:

$CH_3C(=O)C(=O)O^-$ (pyruvate) + HSCoA + NAD^+ → CH3C(=O)SCoA (acetyl-CoA) + NADH + H^+ + CO_2

The product of this reaction, acetyl-CoA, is the starting point for the citric acid cycle.

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Key Points

- The Krebs cycle is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidization of acetate—derived from carbohydrates, fats, and proteins —into carbon dioxide.
- Theoretically there are several alternatives to the TCA cycle, but the TCA cycle appears to be the most efficient.
- The citric acid cycle consumes acetate (in the form of acetyl-CoA) and water, reduces NAD+ to NADH, and produces carbon dioxide.

Key Terms

- **acetyl CoA**: Acetyl coenzyme A or acetyl-CoA is an important molecule in metabolism, used in many biochemical reactions. Its main function is to convey the carbon atoms within the acetyl group to the citric acid cycle (Krebs cycle) to be oxidized for energy production.
- **citric acid cycle**: An alternative name for the Krebs cycle.
- **glycolysis**: The cellular degradation of the simple sugar glucose to yield pyruvic acid and ATP as an energy source.

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SECTION OVERVIEW

4.7: Alternatives to Glycolysis

Topic hierarchy

4.7A: The Entner-Doudoroff Pathway

4.7B: Aerobic Hydrocarbon Oxidation

4.7C: The Pentose Phosphate Shunt

4.7D: Organic Acid Metabolism

4.7E: Lipid Metabolism

4.7F: Connecting Proteins to Glucose Metabolism

4.7G: Methylotrophy and Methanotrophy

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4.7A: The Entner-Doudoroff Pathway

Learning Objectives

- Distinguish between the Entner-Doudoroff pathway and glycolysis

The Entner–Doudoroff pathway describes an alternate series of reactions that catabolize glucose to pyruvate using a set of enzymes different from those used in either glycolysis or the pentose phosphate pathway. Glycolysis (from glycose, an older term for glucose + -lysis degradation) is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, CH_3COCOO^-. The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide). Most bacteria use glycolysis and the pentose phosphate pathway. This pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.

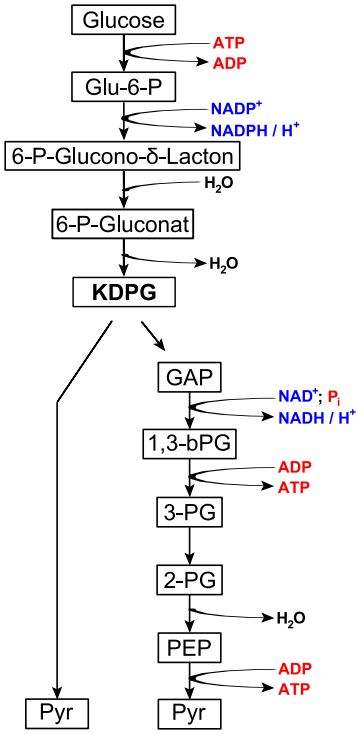


Figure: ***The Entner–Doudoroff Pathway****: This is a diagram of the Entner-Doudoroff pathway (KDPG: 2-keto-3-deoxy-6-phosphogluconate).*

Distinct features of the Entner–Doudoroff pathway are that it occurs only in prokaryotes and it uses 6-phosphogluconate dehydratase and 2-keto-3-deoxyphosphogluconate aldolase to create pyruvate from glucose. The Entner–Doudoroff pathway has a net yield of 1 ATP for every glucose molecule processed, as well as 1 NADH and 1 NADPH. By comparison, glycolysis has a net yield of 2 ATP and 2 NADH for every one glucose molecule processed.

There are a few bacteria that substitute classic glycolysis with the Entner-Doudoroff pathway. They may lack enzymes essential for glycolysis, such as phosphofructokinase-1. This pathway is generally found in *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Agrobacterium*, and a few other Gram-negative genera. Very few Gram-positive bacteria have this pathway, with *Enterococcus faecalis* being a rare exception. Most organisms that use the pathway are aerobes due to the low ATP yield per glucose such as Pseudomonas, a genus of Gram-negative bacteria, and Azotobacter, a genus of Gram-negative bacteria.

Key Points

- Glycolysis is the metabolic pathway that converts glucose into pyruvate and hydrogen ions.
- The Entner-Doudoroff pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.
- There are a few bacteria that substitute classic glycolysis with the Entner-Doudoroff pathway.

Key Terms

- **glycolysis**: The metabolic pathway that converts glucose into pyruvate and hydrogen ions.
- **ATP**: Adenosine-5′-triphosphate (ATP) is a multifunctional nucleoside triphosphate used in cells as a coenzyme. It is often called the “molecular unit of currency” of intracellular energy transfer. ATP transports chemical energy within cells for metabolism.

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4.7B: Aerobic Hydrocarbon Oxidation

Learning Objectives

- Discuss the advantages of organisms that can undergo aerobic hydrocarbon oxidation

Microbes can utilize hydrocarbons via oxidation as an energy source.

Microbes can use many different carbon sources for energy. The best known and perhaps most common example is glucose. Microbes can utilize hydrocarbons via a stepwise oxidation of a hydrocarbon by oxygen produces water and, successively, an alcohol, an aldehyde or a ketone, a carboxylic acid, and then a peroxide. Note the presence of oxygen, thus defining this as aerobic hydrocarbon oxidation. There are examples of anaerobic hydrocarbon oxidation, which will not be discussed here. This is of special interest as many of the environment pollutants released by human industry are often hydrocarbon based. One of the best examples is oil spills. Understanding how microbes digest hydrocarbons has started the field of microbial biodegradation, a type of bioremediation. The goal of this is to find ways of using microbes to degrade hydrocarbon spills or waste into less dangerous byproducts such as alcohol.

Hydrocarbon utilizing microorganisms, mostly Cladosporium resinae and Pseudomonas aeruginosa, colloquially known as “HUM bugs,” are commonly present in jet fuel. They live in the water-fuel interface of the water droplets, form dark black/brown/green, gel-like mats, and cause microbial corrosion to plastic and rubber parts of the aircraft fuel system by consuming them, and to the metal parts by the means of their acidic metabolic products. They are also incorrectly called algae due to their appearance. FSII, which is added to the fuel, acts as a growth retardant for them. There are about 250 kinds of bacteria that can live in jet fuel, but fewer than a dozen are meaningfully harmful.

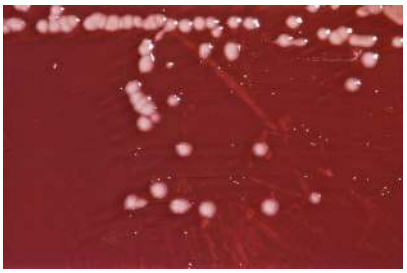


Figure: **Pseudomonas aeruginosa**: P. aeruginosa is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon-using microorganism (or “HUM bug”), causing microbial corrosion. [3] It creates dark, gellish mats sometimes improperly called “algae” because of their appearance.

Biosurfactants are surface-active substances synthesized by living cells. Interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, possibility of large-scale production, selectivity, performance under extreme conditions, and potential applications in environmental protection. Biosurfactants enhance the emulsification of hydrocarbons, have the potential to solubilize hydrocarbon contaminants, and increase their availability for microbial degradation. The use of chemicals for the treatment of a hydrocarbon polluted site may contaminate the environment with their by-products, whereas biological treatment may efficiently destroy pollutants, while being biodegradable themselves. Therefore, biosurfactant-producing microorganisms may play an important role in the accelerated bioremediation of hydrocarbon-contaminated sites. These compounds can also be used in enhanced oil recovery and may be considered for other potential applications in environmental protection. Other applications include herbicides and pesticides formulations, detergents, healthcare and cosmetics, pulp and paper, coal, textiles, ceramic processing and food industries, uranium ore-processing, and mechanical dewatering of peat. Several microorganisms are known to synthesize surface-active agents; most of them are bacteria and yeasts. When grown on hydrocarbon substrate as the carbon source, these microorganisms synthesize a wide range of chemicals with surface activity, such as glycolipid, phospholipid, and others. These chemicals are synthesized to emulsify the hydrocarbon substrate and facilitate its transport into the cells. In some bacterial species such as Pseudomonas aeruginosa, biosurfactants are also involved in a group motility behavior called swarming motility.

Key Points

- Microbes in aerobic conditions can use hydrocarbons via oxidation of the hydrocarbon. This leads to byproducts such as water, alcohol, and peroxide.
- Many hydrocarbons are environmentally damaging, thus the break down of hydrocarbons by microbes is of special interest.
- HUM bugs can function as biosurfactants to facilitate the emulsification of hydrocarbons.

Key Terms

- **hydrocarbon**: A compound consisting only of carbon and hydrogen atoms.
- **biosurfactant**: Surface-active substances synthesized by living cells.
- **bioremediation**: The use of biological organisms, usually microorganisms, to remove contaminants, especially from polluted water.

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4.7C: The Pentose Phosphate Shunt

Learning Objectives

- Outline the two major phases of the pentose phosphate shunt: oxidative and non-oxidative phases

The pentose phosphate pathway (PPP; also called the phosphogluconate pathway and the hexose monophosphate shunt) is a process that breaks down glucose-6-phosphate into NADPH and pentoses (5-carbon sugars) for use in downstream biological processes. There are two distinct phases in the pathway: the oxidative phase and the non-oxidative phase. The first is the oxidative phase in which glucose-6-phosphate is converted to ribulose-5-phosphate. During this process two molecules of $NADP^+$are reduced to NADPH. The overall reaction for this process is:

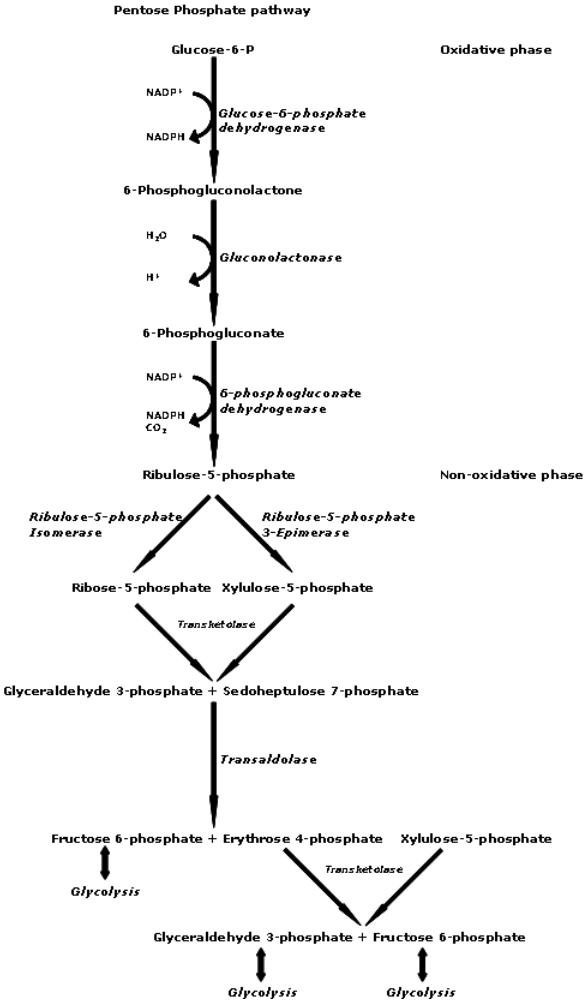


Figure: ***Figure 1 The Pentose Phosphate Pathway****: The pentose phosphate pathway generates reducing equivalents in the form of NADPH. It is used in reductive biosynthesis reactions within cells (e.g. fatty acid synthesis). It produces ribulose-5-phosphate, used in the synthesis of nucleotides. It also produces nucleic acids and erythrose-4-phosphate, used in the synthesis of aromatic amino acids.*

$$\text{Glucose 6-phosphate} + 2\ NADP^+ + H_2O \rightarrow \text{ribulose-5-phosphate} + 2\ NADPH + 2\ H^+ + CO_2$$

The second phase of this pathway is the non-oxidative synthesis of 5-carbon sugars. Depending on the body's state, ribulose-5-phosphate can reversibly isomerize to ribose-5-phosphate. Ribulose-5-phosphate can alternatively undergo a series of isomerizations as well as transaldolations and transketolations that result in the production of other pentose phosphates including fructose-6-phosphate, erythrose-4-phosphate, and glyceraldehyde-3-phosphate (both intermediates in glycolysis). These

compounds are used in a variety of different biological processes including production of nucleotides and nucleic acids (ribose-5-phosphate), as well as synthesis of aromatic amino acids (erythrose-4-phosphate).

Glucose-6-phosphate dehydrogenase is the rate-controlling enzyme in this pathway. It is allosterically stimulated by $NADP^+$. NADPH-utilizing pathways, such as fatty acid synthesis, generate $NADP^+$, which stimulates glucose-6-phosphate dehydrogenase to produce more NADPH. In mammals, the PPP occurs exclusively in the cytoplasm; it is found to be most active in the liver, mammary gland, and adrenal cortex. The ratio of NADPH:$NADP^+$ is normally about 100:1 in liver cytosol, making the cytosol a highly-reducing environment.

The PPP is one of the three main ways the body creates molecules with reducing power, accounting for approximately 60% of NADPH production in humans. While the PPP does involve oxidation of glucose, its primary role is anabolic rather than catabolic, using the energy stored in NADPH to synthesize large, complex molecules from small precursors.

Additionally, NADPH can be used by cells to prevent oxidative stress. NADPH reduces glutathione via glutathione reductase, which converts reactive H_2O_2 into H_2O by glutathione peroxidase. For example, erythrocytes generate a large amount of NADPH through the pentose phosphate pathway to use in the reduction of glutathione.

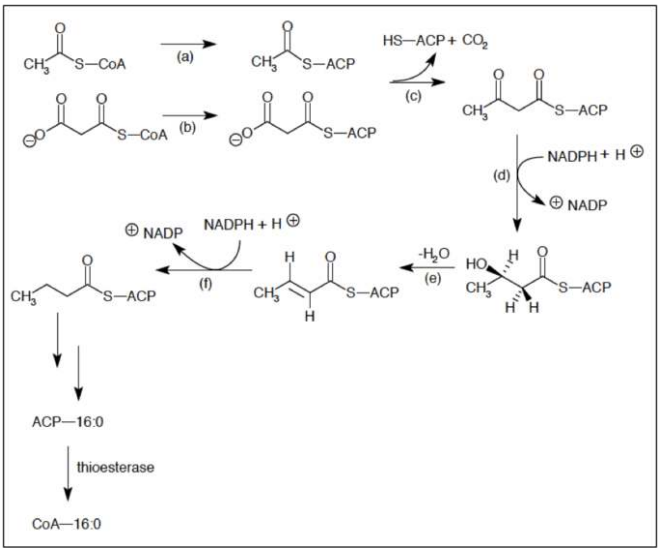


Figure: **Fatty Acid Synthesis**: Synthesis of Straight-Chain Saturated Fatty Acids

Key Points

- There are two distinct phases in the pathway: the oxidative phase and the non-oxidative phase.
- In the oxidative phase, two molecules of $NADP^+$ are reduced to NADPH, utilizing the energy from the conversion of glucose-6-phosphate into ribulose-5-phosphate. These NADPH molecules can then be used as an energy source in elsewhere in the cell.
- The non-oxidative phase generates 5-carbon sugars, which can be used in the synthesis of nucleotides, nucleic acids, and amino acids.
- The pentose phosphate pathway is an alternative to glycolysis.

Key Terms

- **glycolysis**: The cellular degradation of the simple sugar glucose to yield pyruvic acid and ATP as an energy source.
- **NADPH**: Nicotinamide adenine dinucleotide phosphate (NADP) carrying electrons and bonded with a hydrogen (H) ion; the reduced form of NADP+.
- **oxidative stress**: Damage caused to cells or tissue by reactive oxygen species.

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4.7D: Organic Acid Metabolism

Microbes can harness energy and carbon derived from organic acids by using a variety of dedicated metabolic enzymes.

LEARNING OBJECTIVES

Give examples of types of organic acid metabolism that are used by microorganisms for a sole source of energy

KEY TAKEAWAYS

Key Points

- Some microbes are capable of utilizing organic acids such as fatty acids, amino acids, or straight-chain unsaturated acids (e.g., lactate) as a sole source of energy.
- Metabolism of the organic acid formate is important in methylotrophic organisms. It is vital in the catabolism of C1 compounds (such as methanol).
- Many bacteria are capable of utilizing fatty acids as sole energy and carbon sources through the cyclic β-oxidation pathway, which ultimately yields acetyl-CoA.

Key Terms

- **fatty acid**: Any of a class of aliphatic carboxylic acids, of general formula CnH2n+1COOH, that occur combined with glycerol as animal or vegetable oils and fats. Only those with an even number of carbon atoms are normally found in natural fats.
- **acyl**: Any of class of organic radicals, RCO-, formed by the removal of a hydroxyl group from a carboxylic acid.
- **acetyl CoA**: Acetyl coenzyme A or acetyl-CoA is an important molecule in metabolism, used in many biochemical reactions. Its main function is to convey the carbon atoms within the acetyl group to the citric acid cycle (Krebs cycle) to be oxidized for energy production.

Organic Acid Metabolism

A great many organisms generate organic acids (such as lactate) as a byproduct of fermentation. Some microbes are capable of utilizing such compounds as a sole source of energy.

The most commonly metabolized organic acids are the carboxylic acids, which are organic acids containing at least one carboxyl (-COOH) group. The general formula of a carboxylic acid is R-COOH, where R is a monovalent functional group. Many types of carboxylic acids can be metabolized by microbes, including:

- Fatty acids (carboxylic acids with long acyl tails)
- Amino acids (the building blocks of proteins)
- Straight-chained, saturated acids (e.g., formate, acetate, and palmitate)

FORMATE METABOLISM

Formate metabolism is important in methylotrophic organisms. It is vital in the catabolism of C_1 compounds such as methanol (see the “Methylotrophy and Methanotrophy” atom for more information on C_1 compound utilization). Methylotrophic microbes convert single-carbon compounds to formaldehyde, which is oxidized to formate by formaldehyde dehydrogenase. Degradation of formate is then catalyzed by formate dehydrogenase (FDH), which oxidizes formate to ultimately yield CO_2. It permits the donation of electrons to a second substrate (such as NAD+) in the process. This is a critical late step in the hydrocarbon utilization pathway. The ability to metabolize formate is also critical in bacterial anaerobic metabolism, in which case formate is also oxidized by an FDH enzyme but the electrons are donated to cytochromes (proteins involved in electron transport).

FATTY ACID METABOLISM

Many bacteria are capable of utilizing fatty acids of various tail lengths as sole energy and carbon sources. This process requires the β-oxidation pathway, a cyclic process that catalyzes the sequential shortening of fatty acid acyl chains to the final product, acetyl-CoA. The step-by-step process occurs as follows:

1. Fatty acid chains are converted to enoyl-CoA (catalyzed by acyl-CoA dehydrogenase).
2. Enoyl-CoA is converted to 3-hydroxyacyl-CoA (catalyzed by enoyl-CoA hydratase).

3. 3-hydroxyacyl-CoA is converted to 3-ketoacyl-CoA (catalyzed by 3-hydroxyacyl-CoA dehydrogenase).
4. 3-ketoacyl-CoA is thiolated (by 3-ketoacyl-CoA thiolase) to yield one molecule of acetyl-CoA and a derivative of the original input fatty acid that is now shorter by two carbons.

The fatty acid chain that is left over after the thiolation step can then reenter the β-oxidation pathway, which can cycle until the fatty acid has been completely reduced to acetyl-CoA. Acertyl-CoA is the entry molecule for the TCA cycle. The TCA cycle is the process used by all aerobic organisms to generate energy.

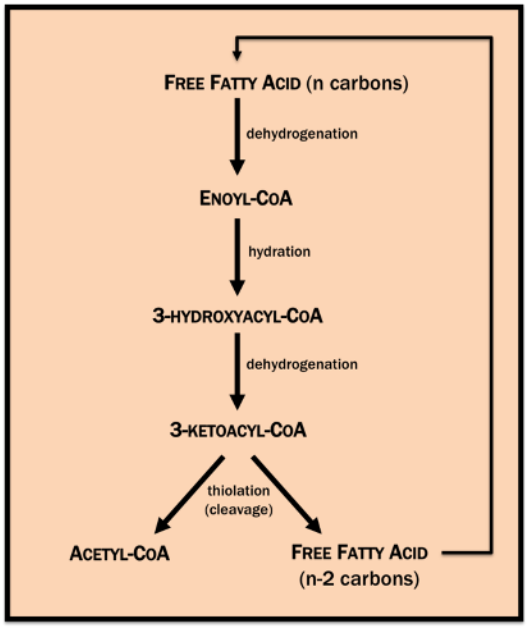


Figure: **β-oxidation of fatty acids**: Free fatty acids are broken down to acetyl-CoA by dedicated enzymes in the β-oxidation pathway.

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4.7E: Lipid Metabolism

Learning Objectives

- Outline the process of lipid metabolism, specifically beta-oxidation

Lipid Metabolism

Lipids are universal biological molecules. Not only does this broad class of compounds represent the primary structural component of biological membranes in all organisms, they also serve a number of vital roles in microorganisms. Among these, lipids can be metabolized by microbes for use as a primary energy source. Although not stated explicitly, the “Organic Acid Metabolism” atom in this module introduces the concept of lipid metabolism by describing the process of fatty acid metabolism through β-oxidation. This atom will expand on the metabolic pathway that enables degradation and utilization of lipids. Fatty acids are the building blocks of lipids. They are made of a hydrocarbon chain of variable length that terminates with a carboxylic acid group (-COOH). The fatty acid structure (see below) is one of the most fundamental categories of biological lipids. It is commonly used as a building block of more structurally complex lipids (such as phospholipids and triglycerides). When metabolized, fatty acids yield large quantities of ATP, which is why these molecules are important energy sources. Lipids are an energy and carbon source. Before complex lipids can be used to produce energy, they must first be hydrolyzed. This requires the activity of hydrolytic enzymes called lipases, which release fatty acids from derivatives such as phospholipids. These fatty acids can then enter a dedicated pathway that promotes step-wise lipid processing that ultimately yields acetyl-CoA, a critical metabolite that conveys carbon atoms to the TCA cycle (aka Krebs cycle or citric acid cycle) to be oxidized for energy production.

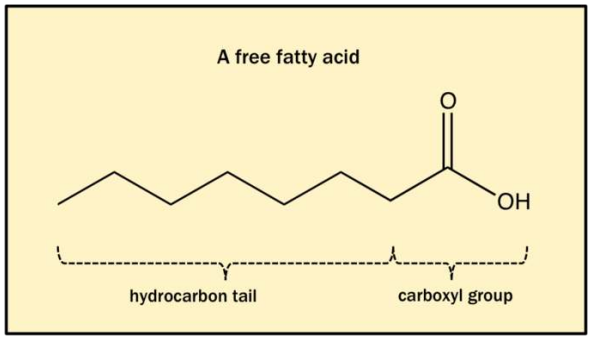


Figure: **An example of a fatty acid**: A fatty acid is a carboxylic acid with a long aliphatic tail that may be either saturated or unsaturated. The molecule shown here is the eight-carbon saturated fatty acid known as octanoic acid (or caprylic acid).

β-oxidation

The metabolic process by which fatty acids and their lipidic derivatives are broken down is called β-oxidation. This process bears significant similarity to the mechanism by which fatty acids are synthesized, except in reverse. In brief, the oxidation of lipids proceeds as follows: two-carbon fragments are removed sequentially from the carboxyl end of the fatty acid after dehydrogenation, hydration, and oxidation to form a keto acid, which is then cleaved by thiolysis. The acetyl-CoA molecule liberated by this process is eventually converted into ATP through the TCA cycle.

β-oxidation can be broken down into a series of discrete steps:

1. Activation: Before fatty acids can be metabolized, they must be “activated. ” This activation step involves the addition of a coenzyme A (CoA) molecule to the end of a long-chain fatty acid, after which the activated fatty acid (fatty acyl -CoA) enters the β-oxidation pathway.
2. Oxidation: The initial step of β-oxidation is catalyzed by acyl-CoA dehydrogenase, which oxidizes the fatty acyl-CoA molecule to yield enoyl-CoA. As a result of this process, a trans double bond is introduced into the acyl chain.
3. Hydration: In the second step, enoyl-CoA hydratase hydrates the double bond introduced in the previous step, yielding an alcohol (-C-OH).
4. Oxidation: Hydroxyacyl-CoA dehydrogenase oxidizes the alcohol formed in the previous step to a carbonyl (-C=O).

5. Cleavage: A thiolase then cleaves off acetyl-CoA from the oxidized molecule, which also yields an acyl-CoA that is two carbons shorter than the original molecule that entered the β-oxidation pathway.

This cycle repeats until the fatty acid has been completely reduced to acetyl-CoA, which is fed through the TCA cycle to ultimately yield cellular energy in the form of ATP.

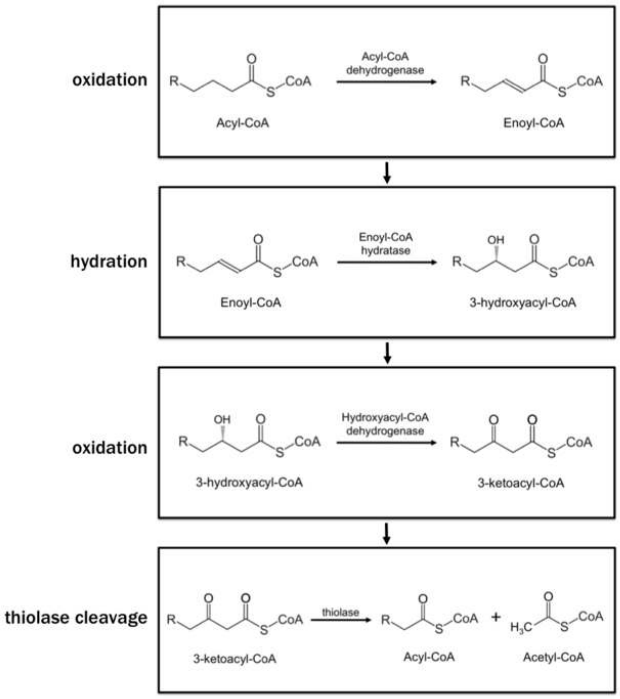


Figure: **β-oxidation**: The sequential steps of the β-oxidation pathway.

Key Points

- In addition to their role as the primary component of cell membranes, lipids can be metabolized for use as a primary energy source.
- Lipid metabolism involves the degradation of fatty acids, which are fundamental biological molecules and the building blocks of more structurally complex lipids.
- In order to be metabolized by the cell, lipids are hydrolyzed to yield free fatty acids that then converted to acetyl-CoA through the β- oxidation pathway.
- One major feature of anaerobic digestion is the production of biogas (with the most useful component being methane), which can be used in generators for electricity production and/or in boilers for heating purposes.

Key Terms

- **carboxylic acid**: Any of a class of organic compounds containing a carboxyl functional group.
- **coenzyme A**: A coenzyme, formed from pantothenic acid and adenosine triphosphate, that is necessary for fatty acid synthesis and metabolism.

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4.7F: Connecting Proteins to Glucose Metabolism

Learning Objectives

- Describe the role played by proteins in glucose metabolism

Metabolic pathways should be thought of as porous; that is, substances enter from other pathways and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways. Proteins are a good example of this phenomenon. They can be broken down into their constituent amino acids and used at various steps of the pathway of glucose catabolism.

Proteins are hydrolyzed by a variety of enzymes in cells. Most of the time, the amino acids are recycled into the synthesis of new proteins or are used as precursors in the synthesis of other important biological molecules, such as hormones, nucleotides, or neurotransmitters. However, if there are excess amino acids, or if the body is in a state of starvation, some amino acids will be shunted into the pathways of glucose catabolism.

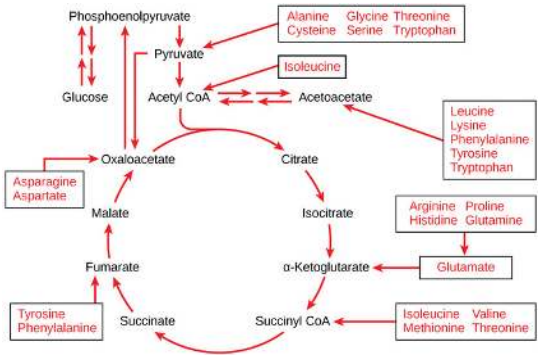


Figure: **Connection of Amino Acids to Glucose Metabolism Pathways**: The carbon skeletons of certain amino acids (indicated in boxes) are derived from proteins and can feed into pyruvate, acetyl CoA, and the citric acid cycle.

Each amino acid must have its amino group removed (deamination) prior to the carbon chain's entry into these pathways. When the amino group is removed from an amino acid, it is converted into ammonia through the urea cycle. The remaining atoms of the amino acid result in a keto acid: a carbon chain with one ketone and one carboxylic acid group. In mammals, the liver synthesizes urea from two ammonia molecules and a carbon dioxide molecule. Thus, urea is the principal waste product in mammals produced from the nitrogen originating in amino acids; it leaves the body in urine. The keto acid can then enter the citric acid cycle.

When deaminated, amino acids can enter the pathways of glucose metabolism as pyruvate, acetyl CoA, or several components of the citric acid cycle. For example, deaminated asparagine and aspartate are converted into oxaloacetate and enter glucose catabolism in the citric acid cycle. Deaminated amino acids can also be converted into another intermediate molecule before entering the pathways. Several amino acids can enter glucose catabolism at multiple locations.

Key Points

- Amino acids must be deaminated before entering any of the pathways of glucose catabolism: the amino group is converted to ammonia, which is used by the liver in the synthesis of urea.
- Deaminated amino acids can be converted into pyruvate, acetyl CoA, or some components of the citric acid cycle to enter the pathways of glucose catabolism.
- Several amino acids can enter the glucose catabolism pathways at multiple locations.

Key Terms

- **catabolism**: Destructive metabolism, usually including the release of energy and breakdown of materials.
- **keto acid**: Any carboxylic acid that also contains a ketone group.
- **deamination**: The removal of an amino group from a compound.

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4.7G: Methylotrophy and Methanotrophy

Learning Objectives

- Distinguish between methylotrophs and methanotrophs and their energy sources

Methylotrophs

Multiple diverse microorganisms have evolved the intriguing ability to utilize single-carbon (C_1) compounds (e.g. methanol or methane) or multi-carbon compounds lacking carbon bonds (e.g. dimethyl ether and dimethylamine) as the sole energy source for their growth. Microbes with this capability are known as methylotrophs.

Methylotrophs, in general, aerobically utilize C_1 compounds by oxidizing them to yield formaldehyde. Formaldehyde, in turn, can either be "burned" for energy (by dissimilation to CO_2) or assimilated into biomass, allowing the cell to grow using molecules like methanol as a sole carbon source. Because methanol is more abundant, more easily purified, and cheaper than sugar carbon sources (e.g. glucose), methylotrophs are particularly useful in biotechnology for the production of amino acids, vitamins, recombinant proteins, single-cell proteins, co- enzymes, and cytochromes.

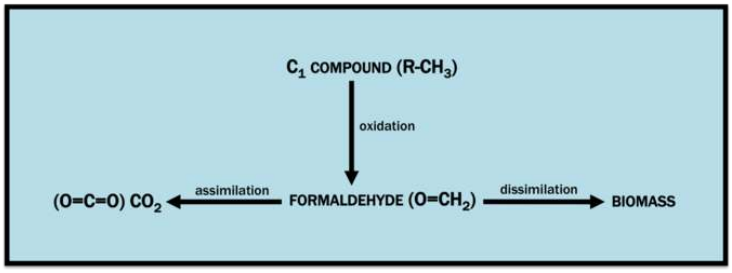


Figure: **Methylotrophy**: This is the general utilization pathway for C1 compounds. Molecules of this type are converted to formaldehyde by oxidation and ultimately used as a source of energy and carbon.

Here are examples of methylotrophs:

- Methanosarcina, which can both utilize and produce methane;
- Methylococcus capsulatus, which requires methane to survive; and
- Pichia pastoris, a biotechnologically important model organism that can use methanol as a carbon and energy source.

Methanotrophs

Some methylotrophs can degrade the greenhouse gas methane. Organisms of this type are referred to as methanotrophs. Methanotrophs are able to metabolize methane as their only source of carbon and energy. Most known methanotrophs are bacteria that strictly require methane for growth ("obligate methanotrophs"). The fact that some methylotrophs can also make use of multi-carbon compounds distinguishes them from methanotrophs, which are usually fastidious methane and methanol oxidizers.

Methanotrophs occur mostly in soils. They are especially common near environments where methane is produced, such as:

- oceans
- mud
- marshes
- underground environments
- soils
- rice paddies
- landfills

Methanotrophs are of special interest to researchers studying global warming because they prevent a potential greenhouse gas (methane), far more potent than carbon dioxide, from being released into the atmosphere. Methanophilic ("methane-loving") bacteria, therefore, are significant in the global methane budget.

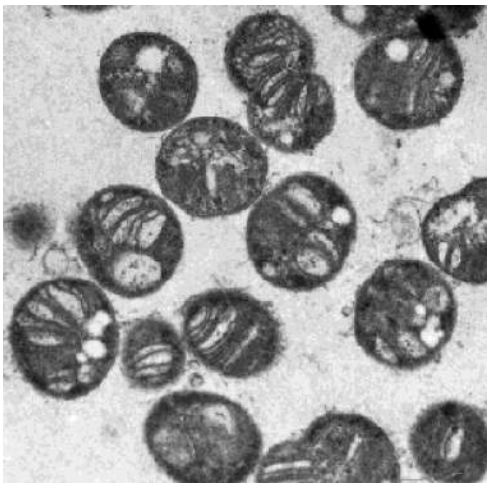


Figure: **Methylococcus capsulatus**: The Methylococcaceae have internal membranes (in the form of flattened discs) that contain pMMOs, which catalyze methane oxidation.

Methane Utilization

Methanotrophs oxidize methane by first initiating reduction of oxygen (O_2) to water (H_2O) and oxidation of methane (CH_4) to a more active species, methanol (CH_3OH), using oxidoreductase enzymes called methane monooxygenases (MMOs). Two types of MMO have been isolated from methanotrophs:

- soluble methane monooxygenase (sMMO), which is found in the cell cytoplasm.
- particulate methane monooxygenase (pMMO), which is found in the cell membrane.

Cells containing pMMO demonstrate higher growth capabilities and higher affinity for methane than cells that contain sMMO. Because pMMO is a membrane protein, cells that use it for methane metabolism characteristically have a system of internal membranes within which methane oxidation occurs.

As in the general case described above for methylotrophs, methanotrophs ultimately oxidize the methanol produced by MMOs to yield formaldehyde. The method of formaldehyde fixation differs between various methanotrophic organisms. This difference (along with variability in membrane structure) divides methanotrophs into several subgroups, such as the Methylococcaceae, Methylocystaceae, and Verrucomicrobiae. Although the mechanism by which it occurs is not entirely clear, it is also apparent that certain bacteria can utilize methane anaerobically by essentially running the methanogenesis pathway (normally used by methanogenic bacteria to produce methane) in reverse. This typically occurs in microbes dwelling in marine sediments where oxygen is scarce or altogether absent.

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Key Points

- Microbes with the ability to utilize single-carbon (C1) compounds (or multi-carbon compounds lacking carbon bonds) as the sole energy source for their growth are known as methylotrophs.
- Methanotrophs, a specific type of methylotroph, are able to metabolize methane as their only source of carbon and energy.
- Methylotrophs aerobically utilize C1 compounds by oxidizing them to yield formaldehyde, which in turn can either be used for energy or assimilated into biomass.

Key Terms

- **methylotroph**: Any organism that utilizes simple methyl compounds (such as methane or methanol) as a source of carbon and of energy.

- **monooxygenase**: Any oxygenase enzyme that catalyzes the incorporation of a single atom of molecular oxygen into a substrate, the other atom being reduced to water; active in the metabolism of many foreign compounds.
- **methanogenesis**: The generation of methane by anaerobic bacteria.

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SECTION OVERVIEW

4.8: Fermentation

Topic hierarchy

4.8A: Anaerobic Cellular Respiration

4.8B: Clostridial and Propionic Acid Fermentation

4.8C: Fermentation Without Substrate-Level Phosphorylation

4.8C: Syntrophy

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4.8A: Anaerobic Cellular Respiration

Learning Objectives

- Describe the process of anaerobic cellular respiration.

Anaerobic Cellular Respiration

The production of energy requires oxygen. The electron transport chain, where the majority of ATP is formed, requires a large input of oxygen. However, many organisms have developed strategies to carry out metabolism without oxygen, or can switch from aerobic to anaerobic cell respiration when oxygen is scarce.

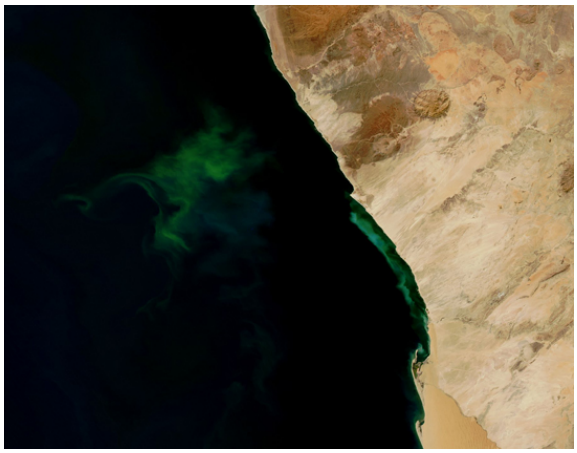


Figure: ***Anaerobic bacteria****: The green color seen in these coastal waters is from an eruption of hydrogen sulfide-producing bacteria. These anaerobic, sulfate-reducing bacteria release hydrogen sulfide gas as they decompose algae in the water.*

During cellular respiration, some living systems use an organic molecule as the final electron acceptor. Processes that use an organic molecule to regenerate NAD^+ from NADH are collectively referred to as fermentation. In contrast, some living systems use an inorganic molecule as a final electron acceptor. Both methods are called anaerobic cellular respiration, where organisms convert energy for their use in the absence of oxygen.

Certain prokaryotes, including some species of bacteria and archaea, use anaerobic respiration. For example, the group of archaea called methanogens reduces carbon dioxide to methane to oxidize NADH. These microorganisms are found in soil and in the digestive tracts of ruminants, such as cows and sheep. Similarly, sulfate-reducing bacteria and archaea, most of which are anaerobic, reduce sulfate to hydrogen sulfide to regenerate NAD^+ from NADH.

Eukaryotes can also undergo anaerobic respiration. Some examples include alcohol fermentation in yeast and lactic acid fermentation in mammals.

Lactic Acid Fermentation

The fermentation method used by animals and certain bacteria (like those in yogurt) is called lactic acid fermentation. This type of fermentation is used routinely in mammalian red blood cells and in skeletal muscle that has an insufficient oxygen supply to allow aerobic respiration to continue (that is, in muscles used to the point of fatigue). The excess amount of lactate in those muscles is what causes the burning sensation in your legs while running. This pain is a signal to rest the overworked muscles so they can recover. In these muscles, lactic acid accumulation must be removed by the blood circulation and the lactate brought to the liver for further metabolism. The chemical reactions of lactic acid fermentation are the following:

$$\text{Pyruvic acid} + NADH \leftrightarrow \text{lactic acid} + NAD^+$$

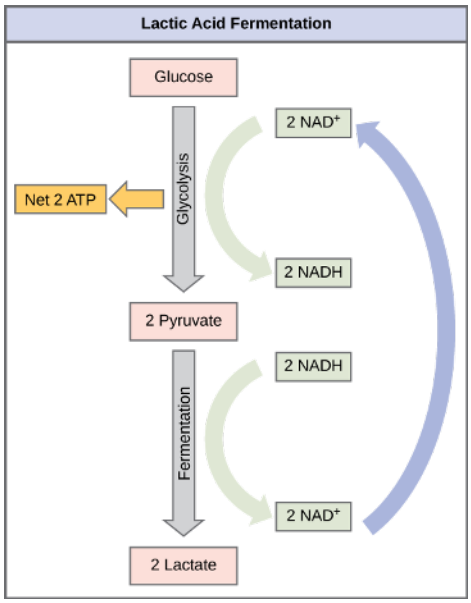


Figure: ***Lactic acid fermentation****: Lactic acid fermentation is common in muscle cells that have run out of oxygen.*

The enzyme used in this reaction is lactate dehydrogenase (LDH). The reaction can proceed in either direction, but the reaction from left to right is inhibited by acidic conditions. Such lactic acid accumulation was once believed to cause muscle stiffness, fatigue, and soreness, although more recent research disputes this hypothesis. Once the lactic acid has been removed from the muscle and circulated to the liver, it can be reconverted into pyruvic acid and further catabolized for energy.

Alcohol Fermentation

Another familiar fermentation process is alcohol fermentation, which produces ethanol, an alcohol. The use of alcohol fermentation can be traced back in history for thousands of years. The chemical reactions of alcoholic fermentation are the following (Note: CO_2 does not participate in the second reaction):

$$\text{Pyruvic acid} \rightarrow \text{CO2} + \text{acetaldehyde} + \text{NADH} \rightarrow \text{ethanol} + \text{NAD}^+$$



Figure: ***Alcohol Fermentation****: Fermentation of grape juice into wine produces CO2 as a byproduct. Fermentation tanks have valves so that the pressure inside the tanks created by the carbon dioxide produced can be released.*

The first reaction is catalyzed by pyruvate decarboxylase, a cytoplasmic enzyme, with a coenzyme of thiamine pyrophosphate (TPP, derived from vitamin B_1 and also called thiamine). A carboxyl group is removed from pyruvic acid, releasing carbon dioxide as a gas. The loss of carbon dioxide reduces the size of the molecule by one carbon, making acetaldehyde. The second reaction is catalyzed by alcohol dehydrogenase to oxidize NADH to NAD^+ and reduce acetaldehyde to ethanol.

The fermentation of pyruvic acid by yeast produces the ethanol found in alcoholic beverages. Ethanol tolerance of yeast is variable, ranging from about 5 percent to 21 percent, depending on the yeast strain and environmental conditions.

Other Types of Fermentation

Various methods of fermentation are used by assorted organisms to ensure an adequate supply of NAD^+ for the sixth step in glycolysis. Without these pathways, that step would not occur and no ATP would be harvested from the breakdown of glucose.Other fermentation methods also occur in bacteria. Many prokaryotes are facultatively anaerobic. This means that they can switch between aerobic respiration and fermentation, depending on the availability of oxygen. Certain prokaryotes, like *Clostridia*, are obligate anaerobes. Obligate anaerobes live and grow in the absence of molecular oxygen. Oxygen is a poison to these microorganisms, killing them on exposure.

It should be noted that all forms of fermentation, except lactic acid fermentation, produce gas. The production of particular types of gas is used as an indicator of the fermentation of specific carbohydrates, which plays a role in the laboratory identification of the bacteria.

Key Points

- Anaerobic respiration is a type of respiration where oxygen is not used; instead, organic or inorganic molecules are used as final electron acceptors.
- Fermentation includes processes that use an organic molecule to regenerate NAD^+ from NADH.
- Types of fermentation include lactic acid fermentation and alcohol fermentation, in which ethanol is produced.
- All forms of fermentation except lactic acid fermentation produce gas, which plays a role in the laboratory identification of bacteria.
- Some types of prokaryotes are facultatively anaerobic, which means that they can switch between aerobic respiration and fermentation, depending on the availability of oxygen.

Key Terms

- **archaea**: A group of single-celled microorganisms. They have no cell nucleus or any other membrane-bound organelles within their cells.
- **anaerobic respiration**: A form of respiration using electron acceptors other than oxygen.
- **fermentation**: An anaerobic biochemical reaction. When this reaction occurs in yeast, enzymes catalyze the conversion of sugars to alcohol or acetic acid with the evolution of carbon dioxide.

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4.8B: Clostridial and Propionic Acid Fermentation

Learning Objectives

- Discuss the process of acidogenesis and the production of propionate

Four Stages of Anaerobic Digestion

Acidogenesis is the second stage in the four stages of anaerobic digestion: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is a chemical reaction wherein particulates are solubilized and large polymers are converted into simpler monomers. Acidogenesis is a biological reaction wherein simple monomers are converted into volatile fatty acids. Acetogenes is a biological reaction wherein volatile fatty acids are converted into acetic acid, carbon dioxide, and hydrogen. Finally, methanogenesis is a biological reaction wherein acetates are converted into methane and carbon dioxide, and hydrogen is consumed.



Figure: **Biofuel production can come from plants, algae, and bacteria.**: Biohydrogen is defined as hydrogen produced biologically, most commonly by algae, bacteria, and archaea. Species of the Clostridium genus allow hydrogen production, a potential biofuel, in mixed cultures.

Anaerobic digestion is a complex biochemical process of mediated reactions undertaken by a consortium of microorganisms to convert organic compounds into methane and carbon dioxide. It is a stabilization process, reducing odor, pathogens, and mass reduction. Hydrolytic bacteria form a variety of reduced end-products from the fermentation of a given substrate.

One fundamental question in anaerobic digestion concerns the metabolic features that control carbon and electron flow. This flow is directed toward a reduced end-product during pure culture and mixed methanogenic cultures of hydrolytic bacteria. *Thermoanaerobium brockii* is a representative thermophilic, hydrolytic bacterium, which ferments glucose, via the Embden–Meyerhof Parnas Pathway.

Acidogenisis

Acidogenic activity was found in the early 20th century, but it was not until mid-1960s that the engineering of phases separation was assumed in order to improve the stability and waste digester treatment. In this phase, complex molecules (carbohydrates, lipids, and proteins) are depolymerized into soluble compounds by hydrolytic enzymes (cellulases, hemicellulases, amylases, lipases and proteases). The hydrolyzed compounds are fermented into volatile fatty acids (acetate, propionate, butyrate, and lactate), neutral compounds (ethanol, methanol), ammonia, hydrogen and carbon dioxide. Acetogenesis is one of the main reactions of this stage. In this reaction, the intermediary metabolites produced are metabolized to acetate, hydrogen, and carbonic gas by the three main groups of bacteria—homoacetogens, syntrophes, and sulphoreductors. For the acetic acid production are considered three kind of bacteria: Clostridium aceticum, *Acetobacter woodii*, and *Clostridium termoautotrophicum*.

In 1979, Winter and Wolfe demonstrated that *A. wodii* in syntrophic association with *Methanosarcina* produce methane and carbon dioxide from fructose, instead of three molecules of acetate. *C. thermoaceticum* and *C. formiaceticum* are able to reduce the carbonic gas to acetate, but they do not have hydrogenases to inhabilite the hydrogen use, so they can produce three molecules of acetate from fructose. Acetic acid is equally a co-metabolite of the organic substrates' fermentation (sugars, glycerol, lactic acid, etc.) by diverse groups of microorganisms, which produce different acids:

- propionic bacteria (propionate + acetate)
- *Clostridium* (butyrate + acetate)
- Enterobacteria (acetate + lactate)
- Hetero-fermentative bacteria (acetate, propionate, butyrate, valerate, etc.)

Key Points

- Acetogenesis is the third stage in the four stages of anaerobic digestion.
- Acetogenesis end products are acetate, hydrogen, and carbonic gas.
- Acetogenesis occurs in three main groups of bacteria: homoacetogens, syntrophes, and sulphoreductors.

Key Terms

- **acetogenesis**: The anaerobic production of acetic acid or acetate by bacteria.
- **metabolite**: Any substance produced by, or taking part in, a metabolic reaction.

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4.8C: Fermentation Without Substrate-Level Phosphorylation

Learning Objectives

- Give examples of various types of fermentation: homolactic, heterolactic and alcoholic

Fermentation is the process of extracting energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound. In contrast, respiration is where electrons are donated to an exogenous electron acceptor, such as oxygen, via an electron transport chain. Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (adenosine triphosphate) by glycolysis.

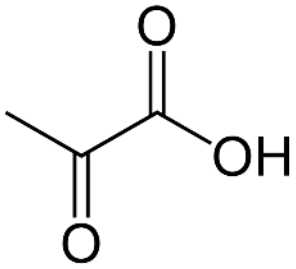


Figure: ***Pyruvic acid****: Pyruvic acid can be made from glucose through glycolysis, converted back to carbohydrates (such as glucose) via gluconeogenesis, or to fatty acids through acetyl-CoA. It can also be used to construct the amino acid alanine and be converted into ethanol. Pyruvic acid supplies energy to living cells through the citric acid cycle (also known as the Krebs cycle) when oxygen is present (aerobic respiration), and alternatively ferments to produce lactic acid when oxygen is lacking (fermentation).*

During fermentation, pyruvate is metabolised to various compounds. Homolactic fermentation is the production of lactic acid from pyruvate; alcoholic fermentation is the conversion of pyruvate into ethanol and carbon dioxide; and heterolactic fermentation is the production of lactic acid as well as other acids and alcohols. Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption (a phenomenon known as the Crabtree effect). The antibiotic activity of Hops also inhibits aerobic metabolism in Yeast.

Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, lactose, and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone. Yeast carries out fermentation in the production of ethanol in beers, wines, and other alcoholic drinks, along with the production of large quantities of carbon dioxide. Fermentation occurs in mammalian muscle during periods of intense exercise where oxygen supply becomes limited, resulting in the creation of lactic acid.

Key Points

- Fermentation without substrate level phosphorylation uses an endogenous electron acceptor, which is usually an organic compound.
- Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (adenosine triphosphate) by glycolysis.
- During fermentation, pyruvate is metabolised to various compounds such as lactic acid, ethanol and carbon dioxide or other acids.

Key Terms

- **fermentation**: Any of many anaerobic biochemical reactions in which an enzyme (or several enzymes produced by a microorganism) catalyses the conversion of one substance into another; especially the conversion (using yeast) of sugars to alcohol or acetic acid with the evolution of carbon dioxide.
- **substrate**: a surface on which an organism grows or to which it is attached
- **oxidative phosphorylation**: Oxidative phosphorylation (or OXPHOS in short) is a metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP).

- **electron acceptor**: An electron acceptor is a chemical entity that accepts electrons transferred to it from another compound. It is an oxidizing agent that, by virtue of its accepting electrons, is itself reduced in the process.

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4.8C: Syntrophy

Learning Objectives

- Give examples of syntrophy in microbial metabolism

Syntrophy, or symbiosis, is the phenomenon involving one species living off the products of another species. For example, house dust mites live off human skin flakes. A healthy human being produces about 1 gram of skin flakes per day. These mites can also produce chemicals that stimulate the production of skin flakes. People can become allergic to these compounds. Another example are the many organisms that feast on feces or dung. A cow eats a lot of grass, the cellulose of which is transformed into lipids by micro-organisms in the cow's large intestine.



Figure: ***House dust mite****: The house dust mite (sometimes referred to by allergists as HDM) is a cosmopolitan guest in human habitation. Dust mites feed on organic detritus such as flakes of shed human skin and flourish in the stable environment of dwellings.*

These microorganisms cannot use the lipids because of a lack of dioxygen in the intestine, so the cow does not take up all the lipids produced. When the processed grass leaves the intestine as dung and comes into open air, many organisms, such as the dung beetle, feast on it. Yet another example is the community of micro-organisms in soil that live off leaf litter. Leaves typically last one year and are then replaced by new ones. These microorganisms mineralize the discarded leaves and release nutrients that are taken up by the plant. Such relationships are called reciprocal syntrophy because the plant lives off the products of micro-organisms. Many symbiotic relationships are based on syntrophy. Finally, anaerobic fermentation/methanogenesis is an example of a syntrophic relationship between different groups of microorganisms. Although fermentative bacteria are not strictly dependent on syntrophyic relationships, they still gain profit from the activities of the hydrogen-scavenging organisms. The fermentative bacteria gain maximum energy yield when protons are used as electron acceptor with concurrent H2 production.

Fermentation is a specific type of heterotrophic metabolism that uses organic carbon instead of oxygen as a terminal electron acceptor. This means that these organisms do not use an electron transport chain to oxidize NADH to NAD+ and therefore must have an alternative method of using this reducing power and maintaining a supply of NAD+ for the proper functioning of normal metabolic pathways (e.g. glycolysis). As oxygen is not required, fermentative organisms are anaerobic. Many organisms can use fermentation under anaerobic conditions and aerobic respiration when oxygen is present. These organisms are facultative anaerobes. To avoid the overproduction of NADH, obligately fermentative organisms usually do not have a complete citric acid cycle. Instead of using an ATP synthase as in respiration, ATP in fermentative organisms is produced by substrate-level phosphorylation where a phosphate group is transferred from a high-energy organic compound to ADP to form ATP. As a result of the need to produce high energy phosphate-containing organic compounds (generally in the form of CoA-esters) fermentative organisms use NADH and other cofactors to produce many different reduced metabolic by-products, often including hydrogen gas (H2). These reduced organic compounds are generally small organic acids and alcohols derived from pyruvate, the end product of glycolysis. Examples include ethanol, acetate, lactate, and butyrate. Fermentative organisms are very important industrially and are used to make many different types of food products. The different metabolic end products produced by each specific bacterial species are responsible for the different tastes and properties of each food.

The best studied example of syntrophy in microbial metabolism is the oxidation of fermentative end products (such as acetate, ethanol and butyrate) by organisms such as Syntrophomonas. Alone, the oxidation of butyrate to acetate and hydrogen gas is energetically unfavorable. However, when a hydrogenotrophic (hydrogen-using) methanogen is present the use of the hydrogen gas

will significantly lower the concentration of hydrogen (down to 10−5 atm) and thereby shift the equilibrium of the butyrate oxidation reaction under standard conditions (ΔG°) to non-standard conditions (ΔG'). Because the concentration of one product is lowered, the reaction is "pulled" towards the products and shifted towards net energetically favorable conditions (for butyrate oxidation: ΔG°= +48.2 kJ/mol, but ΔG' = -8.9 kJ/mol at 10−5 atm hydrogen and even lower if also the initially produced acetate is further metabolized by methanogens). Conversely, the available free energy from methanogenesis is lowered from ΔG°= -131 kJ/mol under standard conditions to ΔG' = -17 kJ/mol at 10−5 atm hydrogen. This is an example of intraspecies hydrogen transfer. In this way, low energy-yielding carbon sources can be used by a consortium of organisms to achieve further degradation and eventual mineralization of these compounds. These reactions help prevent the excess sequestration of carbon over geologic time scales, releasing it back to the biosphere in usable forms such as methane and CO2.

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Key Points

- Anaerobic fermentation / methanogenesis is an example of a syntrophic relationship between different groups of microorganisms.
- Fermentation is a specific type of heterotrophic metabolism that uses organic carbon instead of oxygen as a terminal electron acceptor.
- The best studied example of syntrophy in microbial metabolism is the oxidation of fermentative end products (such as acetate, ethanol and butyrate) by organisms such as Syntrophomonas.

Key Terms

- **syntrophy**: The relationship between the individuals of different species (especially of bacteria) in which one or both benefit nutritionally from the presence of the other.
- **symbiosis**: A close, prolonged association between two or more organisms of different species, regardless of benefit to the members.
- **fermentation**: Any of many anaerobic biochemical reactions in which an enzyme (or several enzymes produced by a microorganism) catalyses the conversion of one substance into another; especially the conversion (using yeast) of sugars to alcohol or acetic acid with the evolution of carbon dioxide.

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SECTION OVERVIEW

4.9: Anaerobic Respiration

Topic hierarchy

4.9A: Electron Donors and Acceptors in Anaerobic Respiration

4.9B: Nitrate Reduction and Denitrification

4.9C: Sulfate and Sulfur Reduction

4.9D: Methanogenesis

4.9E: Proton Reduction

4.9F: Anoxic Hydrocarbon Oxidation

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4.9A: Electron Donors and Acceptors in Anaerobic Respiration

Learning Objectives

- Describe various types of electron acceptors and donors including: nitrate, sulfate, hydrgoen, carbon dioxide and ferric iron

Anaerobic respiration is the formation of ATP without oxygen. This method still incorporates the respiratory electron transport chain, but without using oxygen as the terminal electron acceptor. Instead, molecules such as sulfate (SO_4^{2-}), nitrate (NO_3^-), or sulfur (S) are used as electron acceptors. These molecules have a lower reduction potential than oxygen; thus, less energy is formed per molecule of glucose in anaerobic versus aerobic conditions.

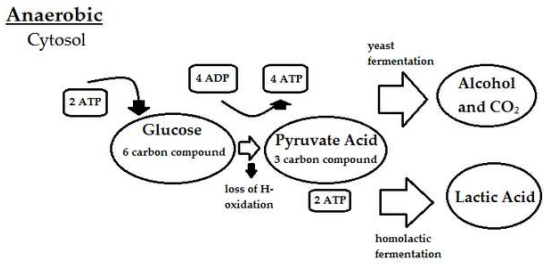


Figure: **Anaerobic Respiration**: A molecule other than oxygen is used as the terminal electron acceptor in anaerobic respiration.

Many different types of electron acceptors may be used for anaerobic respiration. Denitrification is the utilization of nitrate (NO_3^-) as the terminal electron acceptor. Nitrate, like oxygen, has a high reduction potential. This process is widespread, and used by many members of Proteobacteria. Many denitrifying bacteria can also use ferric iron (Fe^{3+}) and different organic electron acceptors.

Sulfate reduction uses sulfate (SO_2^{-4}) as the electron acceptor, producing hydrogen sulfide (H_2S) as a metabolic end product. Sulfate reduction is a relatively energetically poor process, and is used by many Gram negative bacteria found within the δ-Proteobacteria. It is also used in Gram-positive organisms related to Desulfotomaculum or the archaeon Archaeoglobus.

Sulfate reduction requires the use of electron donors, such as the carbon compounds lactate and pyruvate (organotrophic reducers), or hydrogen gas (lithotrophic reducers). Some unusual autotrophic sulfate-reducing bacteria, such as *Desulfotignum phosphitoxidans*, can use phosphite (HPO_3^-) as an electron donor. Others, such as certain *Desulfovibrio* species, are capable of sulfur disproportionation (splitting one compound into an electron donor and an electron acceptor) using elemental sulfur (S0), sulfite (SO_3^{-2}), and thiosulfate ($S_2O_3^{2-}$) to produce both hydrogen sulfide (H_2S) and sulfate (SO^{2-}).

Acetogenesis is a type of microbial metabolism that uses hydrogen (H_2) as an electron donor and carbon dioxide (CO_2) as an electron acceptor to produce acetate, the same electron donors and acceptors used in methanogenesis.

Ferric iron (Fe^{3+}) is a widespread anaerobic terminal electron acceptor used by both autotrophic and heterotrophic organisms. Electron flow in these organisms is similar to those in electron transport, ending in oxygen or nitrate, except that in ferric iron-reducing organisms the final enzyme in this system is a ferric iron reductase. Since some ferric iron-reducing bacteria (*e.g.G. metallireducens*) can use toxic hydrocarbons (*e.g.* toluene) as a carbon source, there is significant interest in using these organisms as bioremediation agents in ferric iron contaminated aquifers.

Other inorganic electron acceptors include the reduction of Manganic ion (Mn^{4+}) to manganous (Mn^{2+}), Selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}) to selenium (Se), Arsenate (AsO_4^{3-}) to arsenite (AsO_3^{3-}), and Uranyl (UO_2^{2+}) to uranium dioxide (UO_2)

Organic compounds may also be used as electron acceptors in anaerobic respiration. These include the reduction of fumarate to succinate, Trimethylamine N-oxide (TMAO) to trimethylamine (TMA), and Dimethyl sulfoxide (DMSO) to Dimethyl sulfide (DMS).

Key Points

- Both inorganic and organic compounds may be used as electron acceptors in anaerobic respiration. Inorganic compounds include sulfate (SO_4^{2-}), nitrate (NO_3^-), and ferric iron (Fe^{3+}). Organic compounds include DMSO.
- These molecules have a lower reduction potential than oxygen. Therefore, less energy is formed per molecule of glucose in anaerobic versus aerobic conditions.
- The reduction of certain inorganic compounds by anaerobic microbes is often ecologically significant.

Key Terms

- **anaerobic**: Without oxygen; especially of an environment or organism.
- **reduction**: A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- **anaerobic respiration**: metabolic reactions and processes that take place in the cells of organisms that use electron acceptors other than oxygen

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4.9B: Nitrate Reduction and Denitrification

Learning Objectives

- Outline the processes of nitrate reduction and denitrification and the organisms that utilize it

In anaerobic respiration, denitrification utilizes nitrate (NO_3^-) as a terminal electron acceptor in the respiratory electron transport chain. Denitrification is a widely used process; many facultative anaerobes use denitrification because nitrate, like oxygen, has a high reduction potential

Denitrification is a microbially facilitated process involving the stepwise reduction of nitrate to nitrite (NO_2^-) nitric oxide (NO), nitrous oxide (N_2O), and, eventually, to dinitrogen (N_2) by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. The complete denitrification process can be expressed as a redox reaction: $2\ NO^{3-} + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$.

Protons are transported across the membrane by the initial NADH reductase, quinones and nitrous oxide reductase to produce the electrochemical gradient critical for respiration. Some organisms (e.g. *E. coli*) only produce nitrate reductase and therefore can accomplish only the first reduction leading to the accumulation of nitrite. Others (e.g. *Paracoccus denitrificans* or *Pseudomonas stutzeri*) reduce nitrate completely. Complete denitrification is an environmentally significant process because some intermediates of denitrification (nitric oxide and nitrous oxide) are significant greenhouse gases that react with sunlight and ozone to produce nitric acid, a component of acid rain. Denitrification is also important in biological wastewater treatment, where it can be used to reduce the amount of nitrogen released into the environment, thereby reducing eutrophication.

Denitrification takes place under special conditions in both terrestrial and marine ecosystems. In general, it occurs where oxygen is depleted and bacteria respire nitrate as a substitute terminal electron acceptor. Due to the high concentration of oxygen in our atmosphere, denitrification only takes place in anaerobic environments where oxygen consumption exceeds the oxygen supply and where sufficient quantities of nitrate are present. These environments may include certain soils and groundwater, wetlands, oil reservoirs, poorly ventilated corners of the ocean, and in sea floor sediments.

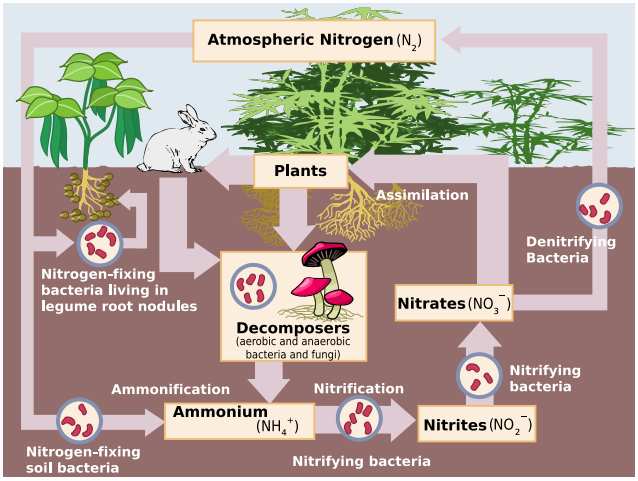


Figure: **The role of soil bacteria in the Nitrogen cycle**: Denitrification is an important process in maintaining ecosystems. Generally, denitrification takes place in environments depleted of oxygen.

Denitrification is performed primarily by heterotrophic bacteria (e.g. *Paracoccus denitrificans*), although autotrophic denitrifiers have also been identified (e.g., *Thiobacillus denitrificans*). Generally, several species of bacteria are involved in the complete reduction of nitrate to molecular nitrogen, and more than one enzymatic pathway have been identified in the reduction process.

Rhizobia are soil bacteria with the unique ability to establish a N_2-fixing symbiosis on legume roots. When faced with a shortage of oxygen, some *rhizobia* species are able to switch from O_2-respiration to using nitrates to support respiration.

The direct reduction of nitrate to ammonium (dissimilatory nitrate reduction) can be performed by organisms with the nrf- gene. This is a less common method of nitrate reduction than denitrification in most ecosystems. Other genes involved in denitrification

include nir (nitrite reductase) and nos (nitrous oxide reductase), which are possessed by such organisms as *Alcaligenes faecalis*, *Alcaligenes xylosoxidans*, *Pseudomonas spp*, *Bradyrhizobium japonicum*, and *Blastobacter denitrificans*.

Key Points

- Denitrification generally proceeds through a stepwise reduction of some combination of the following intermediate forms: $NO_3^- \rightarrow NO_2^- \rightarrow NO + N_2O \rightarrow N_2$.
- Generally, several species of bacteria are involved in the complete reduction of nitrate to molecular nitrogen, and more than one enzymatic pathway has been identified in the reduction process.
- Complete denitrification is an environmentally significant process as some intermediates of denitrification (nitric oxide and nitrous oxide) are significant greenhouse gases that react with sunlight and ozone to produce nitric acid, a component of acid rain.

Key Terms

- **electron acceptor**: An electron acceptor is a chemical entity that accepts electrons transferred to it from another compound. It is an oxidizing agent that, by virtue of its accepting electrons, is itself reduced in the process.
- **eutrophication**: The process of becoming eutrophic.
- **facultative**: Not obligate; optional, discretionary or elective

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4.9C: Sulfate and Sulfur Reduction

Learning Objectives

- Outline the process of sulfate and sulfur reduction including its various purposes

Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain. Compared to aerobic respiration, sulfate reduction is a relatively energetically poor process, though it is a vital mechanism for bacteria and archaea living in oxygen-depleted, sulfate-rich environments.

Many sulfate reducers are organotrophic, using carbon compounds, such as lactate and pyruvate (among many others) as electron donors, while others are lithotrophic, and use hydrogen gas (H_2) as an electron donor. Some unusual autotrophic sulfate-reducing bacteria (e.g., *Desulfotignum phosphitoxidans*) can use phosphite (HPO^{3-}) as an electron donor, whereas others (e.g., *Desulfovibrio sulfodismutans*, *Desulfocapsa thiozymogenes*, and *Desulfocapsa sulfoexigens*) are capable of sulfur disproportionation (splitting one compound into two different compounds, in this case an electron donor and an electron acceptor) using elemental sulfur (S0), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$) to produce both hydrogen sulfide (H_2S) and sulfate (SO_4^{2-}).

Before sulfate can be used as an electron acceptor, it must be activated. This is done by the enzyme ATP-sulfurylase, which uses ATP and sulfate to create adenosine 5′-phosphosulfate (APS). APS is subsequently reduced to sulfite and AMP. Sulfite is then further reduced to sulfide, while AMP is turned into ADP using another molecule of ATP. The overall process, thus, involves an investment of two molecules of the energy carrier ATP, which must to be regained from the reduction.

All sulfate-reducing organisms are strict anaerobes. Because sulfate is energetically stable, it must be activated by adenylation to form APS (adenosine 5′-phosphosulfate) to form APS before it can be metabolized, thereby consuming ATP. The APS is then reduced by the enzyme APS reductase to form sulfite (SO_3^{2-}) and AMP. In organisms that use carbon compounds as electron donors, the ATP consumed is accounted for by fermentation of the carbon substrate. The hydrogen produced during fermentation is actually what drives respiration during sulfate reduction.

Sulfate-reducing bacteria can be traced back to 3.5 billion years ago and are considered to be among the oldest forms of microorganisms, having contributed to the sulfur cycle soon after life emerged on Earth. Sulfate-reducing bacteria are common in anaerobic environments (such as seawater, sediment, and water rich in decaying organic material) where they aid in the degradation of organic materials. In these anaerobic environments, fermenting bacteria extract energy from large organic molecules; the resulting smaller compounds (such as organic acids and alcohols) are further oxidized by acetogens, methanogens, and the competing sulfate-reducing bacteria.

Many bacteria reduce small amounts of sulfates in order to synthesize sulfur-containing cell components; this is known as assimilatory sulfate reduction. By contrast, sulfate-reducing bacteria reduce sulfate in large amounts to obtain energy and expel the resulting sulfide as waste; this is known as “dissimilatory sulfate reduction. ” Most sulfate-reducing bacteria can also reduce other oxidized inorganic sulfur compounds, such as sulfite, thiosulfate, or elemental sulfur (which is reduced to sulfide as hydrogen sulfide).

Toxic hydrogen sulfide is one waste product of sulfate-reducing bacteria; its rotten egg odor is often a marker for the presence of sulfate-reducing bacteria in nature. Sulfate-reducing bacteria are responsible for the sulfurous odors of salt marshes and mud flats. Much of the hydrogen sulfide will react with metal ions in the water to produce metal sulfides. These metal sulfides, such as ferrous sulfide (FeS), are insoluble and often black or brown, leading to the dark color of sludge. Thus, the black color of sludge on a pond is due to metal sulfides that result from the action of sulfate-reducing bacteria.



Figure: **Black sludge**: The black color of this pond is due to metal sulfides that result from the action of sulfate-reducing bacteria.

Some sulfate-reducing bacteria play a role in the anaerobic oxidation of methane (CH_4+ $SO4^{2-}$ → HCO_3– + HS– + H_2O). An important fraction of the methane formed by methanogens below the seabed is oxidized by sulfate-reducing bacteria in the transition zone separating the methanogenesis from the sulfate reduction activity in the sediments.This process is also considered a major sink for sulfate in marine sediments. In hydrofracturing fluids used to frack shale formations to recover methane (shale gas), biocide compounds are often added to water to inhibit the microbial activity of sulfate-reducing bacteria in order to avoid anaerobic methane oxidation and to minimize potential production loss.

Sulfate-reducing bacteria often create problems when metal structures are exposed to sulfate-containing water. The interaction of water and metal creates a layer of molecular hydrogen on the metal surface. Sulfate-reducing bacteria oxidize this hydrogen, creating hydrogen sulfide, which contributes to corrosion. Hydrogen sulfide from sulfate-reducing bacteria also plays a role in the biogenic sulfide corrosion of concrete, and sours crude oil.

Sulfate-reducing bacteria may be utilized for cleaning up contaminated soils; some species are able to reduce hydrocarbons, such as benzene, toluene, ethylbenzene, and xylene. Sulfate-reducing bacteria may also be a way to deal with acid mine waters.

Key Points

- Sulfate reduction is a vital mechanism for bacteria and archaea living in oxygen-depleted, sulfate-rich environments.
- Sulfate reducers may be organotrophic, using carbon compounds, such as lactate and pyruvate as electron donors, or lithotrophic, and use hydrogen gas (H_2) as an electron donor.
- Before sulfate can be used as an electron acceptor, it must be activated by ATP -sulfurylase, which uses ATP and sulfate to create adenosine 5′-phosphosulfate (APS).
- Sulfate-reducing bacteria can be traced back to 3.5 billion years ago and are considered to be among the oldest forms of microorganisms, having contributed to the sulfur cycle soon after life emerged on Earth.
- Toxic hydrogen sulfide is one waste product of sulfate-reducing bactera, and is the source of the rotten egg odor.
- Sulfate-reducing bacteria may be utilized for cleaning up contaminated soils.

Key Terms

- **lithotrophic**: Obtains electrons for respiration from inorganic substrates.
- **organotrophic**: Obtains electrons for respiration from organic substrates.

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4.9D: Methanogenesis

Learning Objectives

- Recognize the characteristics associated with methanogenesis

Methanogenesis, or biomethanation, is a form of anaerobic respiration that uses carbon as the terminal electron acceptor, resulting in the production of methane. The carbon is sourced from a small number of low molecular weight organic compounds, such as carbon dioxide, acetic acid, formic acid (formate), methanol, methylamines, dimethyl sulfide, and methanethiol. The two best described pathways of methanogenesis use carbon dioxide or acetic acid as the terminal electron acceptor:

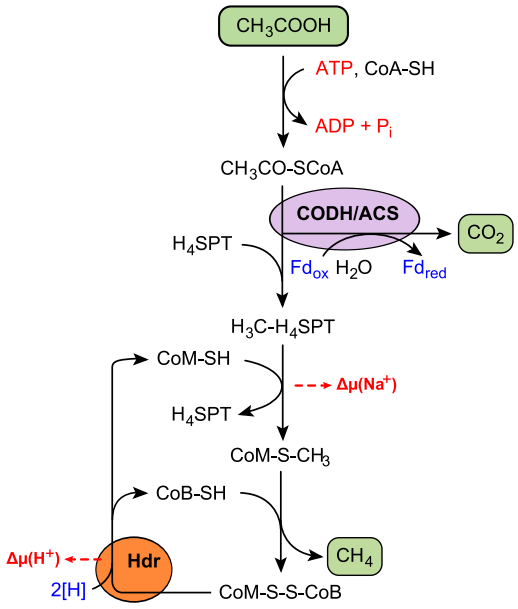


Figure: **Methanogenesis of acetate**: Acetate is broken down to methane by methanogenesis, a type of anaerobic respiration.

$CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O$

$CH_3COOH \rightarrow CH_4 + CO_2$

The biochemistry of methanogenesis is relatively complex. It involves the coenzymes and cofactors F420, coenzyme B, coenzyme M, methanofuran, and methanopterin.

Microbes capable of producing methane are called methanogens. They have been identified only from the domain Archaea – a group that is phylogenetically distinct from eukaryotes and bacteria – though many live in close association with anaerobic bacteria. The production of methane is an important and widespread form of microbial metabolism, and in most environments, it is the final step in the decomposition of biomass.

During the decay process, electron acceptors (such as oxygen, ferric iron, sulfate, and nitrate) become depleted, while hydrogen (H2), carbon dioxide, and light organics produced by fermentation accumulate. During advanced stages of organic decay, all electron acceptors become depleted except carbon dioxide, which is a product of most catabolic processes. It is not depleted like other potential electron acceptors.

Only methanogenesis and fermentation can occur in the absence of electron acceptors other than carbon. Fermentation only allows the breakdown of larger organic compounds, and produces small organic compounds. Methanogenesis effectively removes the semi-final products of decay: hydrogen, small organics, and carbon dioxide. Without methanogenesis, a great deal of carbon (in the form of fermentation products) would accumulate in anaerobic environments.

Methanogenesis also occurs in the guts of humans and other animals, especially ruminants. In the rumen, anaerobic organisms, including methanogens, digest cellulose into forms usable by the animal. Without these microorganisms, animals such as cattle would not be able to consume grass. The useful products of methanogenesis are absorbed by the gut. Methane is released from the

animal mainly by belching (eructation). The average cow emits around 250 liters of methane per day. Some, but not all, humans emit methane in their flatus!

Some experiments even suggest that leaf tissues of living plants emit methane, although other research indicates that the plants themselves do not actually generate methane; they are just absorbing methane from the soil and then emitting it through their leaf tissues. There may still be some unknown mechanism by which plants produce methane, but that is by no means certain.

Methane is one of the earth's most important greenhouse gases, with a global warming potential 25 times greater than carbon dioxide (averaged over 100 years). Therefore, the methane produced by methanogenesis in livestock is a considerable contributor to global warming.

Methanogenesis can also be beneficially exploited. It is the primary pathway that breaks down organic matter in landfills (which can release large volumes of methane into the atmosphere if left uncontrolled), and can be used to treat organic waste and to produce useful compounds. Biogenic methane can be collected and used as a sustainable alternative to fossil fuels.

Key Points

- Carbon dioxide or acetic acid are the most commonly used electron acceptor in methanogenesis.
- Microbes capable of producing methane are called methanogens. They have been identified only from the domain Archaea – a group that is phylogenetically distinct from eukaryotes and bacteria.
- The production of methane is an important and widespread form of microbial metabolism. In most environments, it is the final step in the decomposition of biomass.
- Methane is a major greenhouse gas. The average cow emits around 250 liters of methane a day as a result of the breakdown of cellulose by methanogens. Therefore, the large scale raising of cattle for meat is a considerable contributor to global warming.

Key Terms

- **methanethiol**: A colourless gas, a thiol with a smell like rotten cabbage, found naturally in plants and animals.
- **cofactor**: A substance, especially a coenzyme or a metal, that must be present for an enzyme to function.
- **fermentation**: Any of many anaerobic biochemical reactions in which an enzyme (or several enzymes produced by a microorganism) catalyses the conversion of one substance into another; especially the conversion (using yeast) of sugars to alcohol or acetic acid with the evolution of carbon dioxide.

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4.9E: Proton Reduction

Learning Objectives

- Outline the role of the proton motive force in metabolism

Proton Gradients in Reductive Metabolism

Biological energy is frequently stored and released by means of redox reactions, or the transfer of electrons. Reduction occurs when an oxidant gains an electron. Photosynthesis involves the reduction of carbon dioxide into sugars and the oxidation of water into molecular oxygen. The reverse reaction, respiration, oxidizes sugars (loses an electron) to produce carbon dioxide and water. As intermediate steps, the reduced carbon compounds are used to reduce nicotinamide adenine dinucleotide (NAD+), which then contributes to the creation of a proton gradient. This then drives the synthesis of adenosine triphosphate (ATP) and is maintained by the reduction of oxygen, or alternative receptors for anaerobic respiration. In animal cells, the mitochondria performs similar functions.

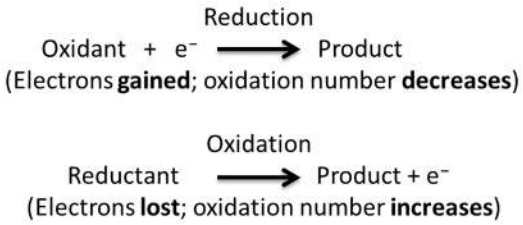


Figure: **The Basics of Redox**: In every redox reaction you have two halves: reduction and oxidation.

An electrochemical gradient represents one of the many interchangeable forms of potential energy through which energy may be conserved. In biological processes, the direction an ion moves by diffusion or active transport across a membrane is determined by the electrochemical gradient. In the mitochondria and chloroplasts, proton gradients are used to generate a chemiosmotic potential that is also known as a proton motive force. This potential energy is used for the synthesis of ATP by phosphorylation. An electrochemical gradient has two components. First, the electrical component is caused by a charge difference across the lipid membrane. Second, a chemical component is caused by a differential concentration of ions across the membrane. The combination of these two factors determines the thermodynamically favorable direction for an ion's movement across a membrane. The electrochemical potential difference between the two sides of the membrane in mitochondria, chloroplasts, bacteria, and other membranous compartments that engage in active transport involving proton pumps, is at times called a chemiosmotic potential or proton motive force.

In respiring bacteria under physiological conditions, ATP synthase, in general, runs in the opposite direction, creating ATP while using the proton motive force created by the electron transport chain as a source of energy. The overall process of creating energy in this fashion is termed oxidative phosphorylation. The same process takes place in the mitochondria, where ATP synthase is located in the inner mitochondrial membrane, so that F1 part sticks into the mitochondrial matrix where ATP synthesis takes place.

Cellular respiration (both aerobic and anaerobic) utilizes highly reduced species such as NADH and FADH2 to establish an electrochemical gradient (often a proton gradient) across a membrane, resulting in an electrical potential or ion concentration difference across the membrane. The reduced species are oxidized by a series of respiratory integral membrane proteins with sequentially increasing reduction potentials, the final electron acceptor being oxygen (in aerobic respiration) or another species (in anaerobic respiration). The membrane in question is the inner mitochondrial membrane in eukaryotes and the cell membrane in prokaryotes. A proton motive force or pmf drives protons down the gradient (across the membrane) through the proton channel of ATP synthase. The resulting current drives ATP synthesis from ADP and inorganic phosphate.

Proton reduction is important for setting up electrochemical gradients for anaerobic respiration. For example, in denitrification, protons are transported across the membrane by the initial NADH reductase, quinones, and nitrous oxide reductase to produce the electrochemical gradient critical for respiration. In organisms that use hydrogen as an energy source, hydrogen is oxidized by a membrane-bound hydrogenase causing proton pumping via electron transfer to various quinones and cytochromes. Sulfur oxidation

is a two step process that occurs because energetically sulfide is a better electron donor than inorganic sulfur or thiosulfate, allowing for a greater number of protons to be translocated across the membrane.

In contrast, fermentation does not utilize an electrochemical gradient. Instead, it only uses substrate-level phosphorylation to produce ATP. The electron acceptor NAD+ is regenerated from NADH formed in oxidative steps of the fermentation pathway by the reduction of oxidized compounds. These oxidized compounds are often formed during the fermentation pathway itself, but may also be external. For example, in homofermentative lactic acid bacteria, NADH formed during the oxidation of glyceraldehyde-3-phosphate is oxidized back to NAD+ by the reduction of pyruvate to lactic acid at a later stage in the pathway. In yeast, acetaldehyde is reduced to ethanol.

Key Points

- In denitrification, protons are transported across the membrane by the initial NADH reductase, quinones, and nitrous oxide reductase to produce the electrochemical gradient critical for respiration.
- An electrochemical gradient represents one of the many interchangeable forms of potential energy through which energy may be conserved. In biological processes, the direction an ion moves by diffusion or active transport across a membrane is determined by the electrochemical gradient.
- In mitochondria and chloroplasts, proton gradients are used to generate a chemiosmotic potential that is also known as a proton motive force.

Key Terms

- **phosphorylation**: The process of transferring a phosphate group from a donor to an acceptor; often catalysed by enzymes

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4.9F: Anoxic Hydrocarbon Oxidation

Learning Objectives

- Describe the process of anoxic hydrocarbon oxidation in regards to marine environments

Hydrocarbons are organic compounds consisting entirely of hydrogen and carbon. The majority of hydrocarbons occur naturally in crude oil, where decomposed organic matter provides an abundance of carbon and hydrogen. The combustion of hydrocarbons is the primary energy source for current civilizations.

Crude oil contains aromatic compounds that are toxic to most forms of life. Their release into the environment by human spills and natural seepages can have detrimental effects. Marine environments are especially vulnerable. Despite its toxicity, a considerable fraction of crude oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities. Although it was once thought that hydrocarbon compounds could only be degraded in the presence of oxygen, the discovery of anaerobic hydrocarbon-degrading bacteria and pathways show that the anaerobic degradation of hydrocarbons occurs naturally.



Figure: **Contaminated soil**: Microbes may be used to degrade toxic hydrocarbons in anaerobic environments.

The facultative denitrifying proteobacteria *Aromatoleum aromaticum* strain EbN1 was the first to be determined as an anaerobic hydrocarbon degrader, using toluene or ethylbenzene as substrates. Some sulfate-reducing bacteria can reduce hydrocarbons such as benzene, toluene, ethylbenzene, and xylene, and have been used to clean up contaminated soils. The genome of the iron-reducing and hydrocarbon degrading species *Geobacter metallireducens* was recently determined.

Anaerobic oxidation of methane (AOM) is a microbial process that occurs in anoxic marine sediments. During this process, the hydrocarbon methane is oxidized with sulfate as the terminal electron acceptor: $CH_4 + SO_4^{2-} \rightarrow HCO^{3-} + HS^- + H_2O$. It is believed that AOM is mediated by a syntrophic aggregation of methanotrophic archaea and sulfate-reducing bacteria, although the exact mechanisms of this syntrophic relationship are still poorly understood. AOM is considered to be a very important process in reducing the emission of methane (a greenhouse gas) from the ocean into the atmosphere. It is estimated that almost 90% of all the methane that arises from marine sediments is oxidized anaerobically by this process. Recent investigations have shown that some syntrophic pairings are able to oxidize methane with nitrate instead of sulfate.

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Key Points

- Hydrocarbons are organic compounds consisting entirely of hydrogen and carbon.
- The majority of hydrocarbons occur naturally in crude oil, where decomposed organic matter provides an abundance of carbon and hydrogen. The combustion of hydrocarbons is the primary energy source for current civilizations.
- Anaerobic oxidation of methane (AOM) is a microbial process that occurs in anoxic marine sediments. AOM is considered to be a very important process, reducing the emission of methane (a greenhouse gas) from the ocean into the atmosphere by up to 90%.

Key Terms

- **methanotrophic**: The ability to metabolize methane as an only source of carbon and energy.
- **syntrophic**: When one species lives off the products of another species.
- **anoxic**: Lacking oxygen.

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SECTION OVERVIEW

4.10: Chemolithotrophy

Topic hierarchy

4.10A: The Energetics of Chemolithotrophy

4.10B: Hydrogen Oxidation

4.10C: Oxidation of Reduced Sulfur Compounds

4.10D: Iron Oxidation

4.10E: Nitrification

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4.10G: Benzoate Catabolism

4.10H: Polycyclic Aromatic Hydrocarbons

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4.10A: The Energetics of Chemolithotrophy

Learning Objectives

- Outline the characteristics associated with chemolithotrophs

A lithotroph is an organism that uses an inorganic substrate (usually of mineral origin) to obtain reducing equivalents for use in biosynthesis (e.g., carbon dioxide fixation) or energy conservation via aerobic or anaerobic respiration. Known chemolithotrophs are exclusively microbes; no known macrofauna possesses the ability to utilize inorganic compounds as energy sources. Macrofauna and lithotrophs can form symbiotic relationships, in which case the lithotrophs are called “prokaryotic symbionts”. An example of this is chemolithotrophic bacteria in deep sea worms or plastids, which are organelles within plant cells that may have evolved from photolithotrophic cyanobacteria-like organisms.



Figure: ***Gollner Riftia pachyptila****: Giant tube worms (Riftia pachyptila have an organ containing chemosynthetic bacteria instead of a gut.*

Chemotrophs are organisms that obtain energy through the oxidation of electron donors in their environments. These molecules can be organic (chemoorganotrophs) or inorganic (chemolithotrophs). The chemotroph designation is in contrast to phototrophs, which utilize solar energy. Chemotrophs can be either autotrophic or heterotrophic. Chemoautotrophs generally fall into several groups: methanogens, halophiles, sulfur oxidizers and reducers, nitrifiers, anammox bacteria, and thermoacidophiles. Chemolithotrophic growth could be dramatically fast, such as Thiomicrospira crunogena with a doubling time around one hour.

In chemolithotrophs, the compounds – the electron donors – are oxidized in the cell, and the electrons are channeled into respiratory chains, ultimately producing ATP. The electron acceptor can be oxygen (in aerobic bacteria), but a variety of other electron acceptors, organic and inorganic, are also used by various species. Unlike water, the hydrogen compounds used in chemosynthesis are high in energy. Other lithotrophs are able to directly utilize inorganic substances, e.g., iron, hydrogen sulfide, elemental sulfur, or thiosulfate, for some or all of their energy needs.

Key Points

- Chemotrophs are organisms that obtain energy by the oxidation of electron donors in their environments. These molecules can be organic (chemoorganotrophs) or inorganic (chemolithotrophs).
- In chemolithotrophs, the compounds – the electron donors – are oxidized in the cell, and the electrons are channeled into respiratory chains, ultimately producing ATP.
- The electron acceptor can be oxygen (in aerobic bacteria), but a variety of other electron acceptors, organic and inorganic, are also used by various species.

Key Terms

- **chemolithotroph**: chemoautotroph
- **symbiont**: An organism that lives in a symbiotic relationship; a symbiote.
- **chemotroph**: an organism that obtains energy by the oxidation of electron-donating molecules in the environment
- **lithotroph**: An organism that obtains its energy from inorganic compounds (such as ammonia) via electron transfer.

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4.10B: Hydrogen Oxidation

Learning Objectives

- Discuss the process of hydrogen oxidation in organisms that use hydrogen aerobically

Chemolithotrophy is a type of metabolism where energy is obtained from the oxidation of inorganic compounds. Most chemolithotrophic organisms are also autotrophic. There are two major objectives to chemolithotrophy: the generation of energy (via ATP) and the generation of reducing power (via NADH). Hydrogen oxidizing bacteria (sometimes called **Knallgas-bacteria**) are bacteria that oxidize hydrogen. These bacteria include *Hydrogenobacter thermophilus*, *Hydrogenovibrio marinus*, and *Helicobacter pylori*. There are both Gram positive and Gram negative knallgas bacteria.

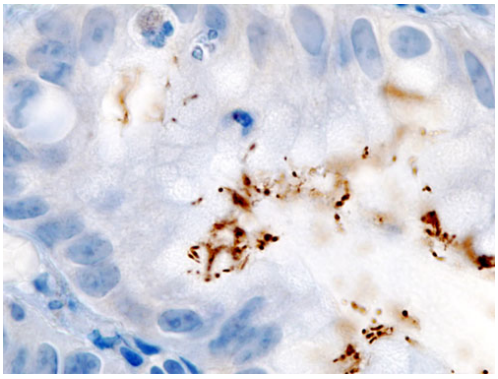


Figure: ***Immunohistochemical staining of H. pylori from a gastric biopsy****: Colonization with H. pylori is not a disease in and of itself, but a condition associated with a number of disorders of the upper gastrointestinal tract. Testing for H. pylori is recommended if there is peptic ulcer disease, low grade gastric MALT lymphoma, after endoscopic resection of early gastric cancer, if there are first degree relatives with gastric cancer, and in certain cases of dyspepsia, not routinely.*

Most grow best under microaerophilic conditions. They do this because the hydrogenase enzyme used in hydrogen oxidation is inhibited by the presence of oxygen, but oxygen is still needed as a terminal electron acceptor.

Many organisms are capable of using hydrogen (H_2) as a source of energy. While there are several mechanisms of anaerobic hydrogen oxidation (e.g. sulfate reducing- and acetogenic bacteria), hydrogen can also be used as an energy source aerobically. In these organisms, hydrogen is oxidized by a membrane-bound hydrogenase causing proton pumping via electron transfer to various quinones and cytochromes. In many organisms, a second cytoplasmic hydrogenase is used to generate reducing power in the form of NADH, which is subsequently used to fix carbon dioxide via the Calvin cycle. Hydrogen-oxidizing organisms, such as *Cupriavidus necator* (formerly *Ralstonia eutropha*), often inhabit oxic-anoxic interfaces in nature to take advantage of the hydrogen produced by anaerobic fermentative organisms while still maintaining a supply of oxygen.

Helicobacter pylori (H. pylori), previously named *Campylobacter pyloridis*, is a Gram-negative, microaerophilic bacterium found in the stomach. It was identified in 1982 by Barry Marshall and Robin Warren. They found that it was present in patients with chronic gastritis and gastric ulcers, conditions that were not previously believed to have a microbial cause. It is also linked to the development of duodenal ulcers and stomach cancer. However, over 80 percent of individuals infected with the bacterium are asymptomatic. It has been postulated that it may play an important role in the natural stomach ecology. More than 50% of the world's population harbor H. pylori in their upper gastrointestinal tract. Infection is more prevalent in developing countries and incidence is decreasing in Western countries. H. pylori's helix shape (from which the generic name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach.

Key Points

- In some organisms, hydrogen is oxidized by a membrane-bound hydrogenase causing proton pumping via electron transfer to various quinones and cytochromes.
- Hydrogen-oxidizing organisms, such as Cupriavidus necator (formerly Ralstonia eutropha), often inhabit oxic-anoxic interfaces in nature to take advantage of the hydrogen produced by anaerobic fermentative organisms while still maintaining a supply of oxygen.

- Helicobacter pylori is a Gram-negative, microaerophilic bacterium found in the stomach. It has been postulated that it may play an important role in the natural stomach ecology.

Key Terms

- **Knallgas-bacteria**: Bacteria which oxidize hydrogen.
- **calvin cycle**: A series of biochemical reactions that take place in the stroma of chloroplasts in photosynthetic organisms.

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4.10C: Oxidation of Reduced Sulfur Compounds

Learning Objectives

- Describe the process of sulfur oxidation

Sulfur is an essential element for all life, and it is widely used in biochemical processes. In metabolic reactions, sulfur compounds serve as both fuels and respiratory (oxygen-alternative) materials for simple organisms. Sulfur is an important part of many enzymes and antioxidant molecules such as glutathione and thioredoxin.

Sulfur Oxidation

Sulfur oxidation involves the oxidation of reduced sulfur compounds such as sulfide (H_2S), inorganic sulfur (S_0), and thiosulfate ($S_2O_2^{-3}$) to form sulfuric acid (H_2SO_4). An example of a sulfur-oxidizing bacterium is Paracoccus.

Generally, the oxidation of sulfide occurs in stages, with inorganic sulfur being stored either inside or outside of the cell until needed. The two step process occurs because sulfide is a better electron donor than inorganic sulfur or thiosulfate; this allows a greater number of protons to be translocated across the membrane. Sulfur-oxidizing organisms generate reducing power for carbon dioxide fixation via the Calvin cycle using reverse electron flow—an energy-requiring process that pushes the electrons against their thermodynamic gradient to produce NADH. Biochemically, reduced sulfur compounds are converted to sulfite (SO_2^{-3}) and, subsequently, sulfate (SO_2^{-4}) by the enzyme sulfite oxidase. Some organisms, however, accomplish the same oxidation using a reversal of the APS reductase system used by sulfate-reducing bacteria (see above). In all cases the energy liberated is transferred to the electron transport chain for ATP and NADH production. In addition to aerobic sulfur oxidation, some organisms (e.g. Thiobacillus denitrificans) use nitrate (NO^{-3}) as a terminal electron acceptor and therefore grow anaerobically.

Beggiatoa

A classic example of a sulfur-oxidizing bacterium is Beggiatoa, a microbe originally described by Sergei Winogradsky, one of the founders of environmental microbiology. Beggiatoa can be found in marine or freshwater environments. They can usually be found in habitats that have high levels of hydrogen sulfide. These environments include cold seeps, sulfur springs, sewage contaminated water, mud layers of lakes, and near deep hydrothermal vents. Beggiatoa can also be found in the rhizosphere of swamp plants. During his research in Anton de Bary's laboratory of botany in 1887, Russian botanist Winogradsky found that Beggiatoa oxidized hydrogen sulfide (H_2S) as an energy source, forming intracellular sulfur droplets. Winogradsky referred to this form of metabolism as inorgoxidation (oxidation of inorganic compounds). The finding represented the first discovery of lithotrophy.



Figure: **A Beggiatoa bacterial mat at the Blake Ridge**: Beggiatoa spp. bacterial mat at a seep on Blake Ridge, off the coast of South Carolina. The red dots are range-finding laser beams. Beggiatoa are able to detoxify hydrogen sulfide in soil.

Beggiatoa can grow chemoorgano-heterotrophically by oxidizing organic compounds to carbon dioxide in the presence of oxygen, though high concentrations of oxygen can be a limiting factor. Organic compounds are also the carbon source for biosynthesis. Some species may oxidize hydrogen sulfide to elemental sulfur as a supplemental source of energy (facultatively litho-heterotroph). This sulfur is stored intracellularly. Some species have the ability of chemolithoautotrophic growth, using sulfide

oxidation for energy and carbon dioxide as a source of carbon for biosynthesis. In this metabolic process, internal stored nitrate is the electron acceptor and reduced to ammonia.

Sulfide oxidation: $2H_2S + O_2 \rightarrow 2S + 2H_2O$

Marine autotrophic Beggiatoa species are able to oxidize intracellular sulfur to sulfate. The reduction of elemental sulfur frequently occurs when oxygen is lacking. Sulfur is reduced to sulfide at the cost of stored carbon or by added hydrogen gas. This may be a survival strategy to bridge periods without oxygen

Key Points

- The oxidation of sulfide occurs in stages, with inorganic sulfur being stored either inside or outside of the cell until needed.
- This two step process occurs because sulfide is a better electron donor than inorganic sulfur or thiosulfate; this allows a greater number of protons to be translocated across the membrane.
- Sulfur-oxidizing organisms generate reducing power for carbon dioxide fixation via the Calvin cycle using reverse electron flow—an energy-requiring process that pushes the electrons against their thermodynamic gradient to produce NADH.

Key Terms

- **calvin cycle**: A series of biochemical reactions that take place in the stroma of chloroplasts in photosynthetic organisms.
- **thiosulfate**: Any salt or ester of thiosulfuric acid.
- **chemolithoautotrophic**: The characteristic of a microorganism that obtains energy from the oxidation of inorganic compounds and carbon from the fixation of carbon dioxide.

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4.10D: Iron Oxidation

Learning Objectives

- Outline the purpose of iron oxidation and the three types of ferrous iron-oxidizing microbes (acidophiles, microaerophiles and anaerobic photosynthetic bacteria)

Ferric iron (Fe^{3+}) is a widespread anaerobic terminal electron acceptor both for autotrophic and heterotrophic organisms. Electron flow in these organisms is similar to those in electron transport, ending in oxygen or nitrate, except that in ferric iron-reducing organisms the final enzyme in this system is a ferric iron reductase. Model organisms include *Shewanella putrefaciens* and *Geobacter metallireducens*. Since some ferric iron-reducing bacteria (e.g. G. metallireducens) can use toxic hydrocarbons such as toluene as a carbon source, there is significant interest in using these organisms as bioremediation agents in ferric iron-rich contaminated aquifers.



Figure: ***Iron Bacteria****: Common effects of excess iron in water are a reddish-brown color and stained laundry. Iron bacteria are a natural part of the environment in most parts of the world. These microorganisms combine dissolved iron or manganese with oxygen and use it to form rust-colored deposits. In the process, the bacteria produce a brown slime that builds up on well screens, pipes, and plumbing fixtures. Bacteria known to feed on iron are Thiobacillus ferrooxidans and Leptospirillum ferrooxidans.*

Ferrous iron is a soluble form of iron that is stable at extremely low pHs or under anaerobic conditions. Under aerobic, moderate pH conditions ferrous iron is oxidized spontaneously to the ferric (Fe^{3+}) form and is hydrolyzed abiotically to insoluble ferric hydroxide ($Fe(OH)_3$). There are three distinct types of ferrous iron-oxidizing microbes. The first are acidophiles, such as the bacteria Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans, as well as the archaeon Ferroplasma. These microbes oxidize iron in environments that have a very low pH and are important in acid mine drainage. The second type of microbes oxidizes ferrous iron at cirum-neutral pH. These micro-organisms (for example Gallionella ferruginea or Leptothrix ochracea) live at the oxic-anoxic interfaces and are microaerophiles. The third type of iron-oxidizing microbes is anaerobic photosynthetic bacteria such as Rhodopseudomonas, which use ferrous iron to produce NADH for autotrophic carbon dioxide fixation. Biochemically, aerobic iron oxidation is a very energetically poor process which therefore requires large amounts of iron to be oxidized by the enzyme rusticyanin to facilitate the formation of proton motive force. Like sulfur oxidation, reverse electron flow must be used to form the NADH used for carbon dioxide fixation via the Calvin cycle.

Although ferric iron is the most prevalent inorganic electron acceptor, a number of organisms (including the iron-reducing bacteria mentioned above) can use other inorganic ions in anaerobic respiration. While these processes may often be less significant ecologically, they are of considerable interest for bioremediation, especially when heavy metals or radionuclides are used as electron acceptors. Examples include:

- Manganic ion (Mn^{4+}) reduction to manganous ion]] (Mn^{2+})
- Selenate (SeO_2^{-4}) reduction to selenite (SeO_2^{-3}) and selenite reduction to inorganic selenium (Se_0)
- Arsenate (AsO_3^{-4}) reduction to arsenite (AsO_3^{-3})
- Uranyl ion ion (UO_2^{+2}) reduction to uranium dioxide (UO_2)

Key Points

- Ferrous iron is a soluble form of iron that is stable at extremely low pHs or under anaerobic conditions. Under aerobic, moderate pH conditions ferrous iron is oxidized spontaneously to the ferric (Fe^{3+}) form and is hydrolyzed abiotically to insoluble ferric hydroxide ($Fe(OH)_3$).
- Three distinct types of ferrous iron-oxidizing microbes: acidophiles, microaerophiles that oxidize ferrous iron at cirum-neutral pH, anaerobic photosynthetic bacteria which use ferrous iron to produce NADH for autotrophic carbon dioxide fixation.
- Although ferric iron is the most prevalent inorganic electron acceptor, a number of organisms can use other inorganic ions in anaerobic respiration.

Key Terms

- **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.

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4.10E: Nitrification

Learning Objectives

- Describe the process of nitrification and its importance

Nitrification is the process by which ammonia (NH_3) or ammonium ($NH4^+$) is converted to nitrate (NO_3^-). Nitrification is the net result of two distinct processes: oxidation of ammonium to nitrite (NO_2^-) by nitrosifying or ammonia-oxidizing bacteria and oxidation of nitrite (NO_2^-) to nitrate (NO_3^-) by the nitrite-oxidizing bacteria. Nitrification is an important step in the nitrogen cycle in soil. Nitrification is an aerobic process performed by small groups of autotrophic bacteria and archaea.

Chemistry of Nitrogen Compound Oxidation

Nitrification is a process of nitrogen compound oxidation (effectively, loss of electrons from the nitrogen atom to the oxygen atoms):

1. $2\ NH_4^+ + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 4\ H^+$ (Nitrosomonas)
2. $2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$ (Nitrobacter, Nitrospina)
3. $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$
4. $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

Both of these processes are extremely energetically poor, which leads to very slow growth rates for both types of organisms.

Ammonium Oxidation

The transformation of ammonia to nitrite is usually the rate limiting step of nitrification. Biochemically, ammonium oxidation occurs by the stepwise oxidation of ammonium to hydroxylamine (NH_2OH) by the enzyme ammonium monooxygenase in the cytoplasm, followed by the oxidation of hydroxylamine to nitrite by the enzyme hydroxylamine oxidoreductase in the periplasm. Electron and proton cycling are very complex, but as a net result only one proton is translocated across the membrane per molecule of ammonium oxidized.

Nitrite Reduction

Nitrite reduction is much simpler, with nitrite being oxidized by the enzyme nitrite oxidoreductase coupled to proton translocation by a very short electron transport chain, again leading to very low growth rates for these organisms. Oxygen is required in ammonium and nitrite oxidation, meaning that both nitrosifying and nitrite-oxidizing bacteria are aerobes. As in sulfur and iron oxidation, NADH for carbon dioxide fixation using the Calvin cycle is generated by reverse electron flow, thereby placing a further metabolic burden on an already energy-poor process.

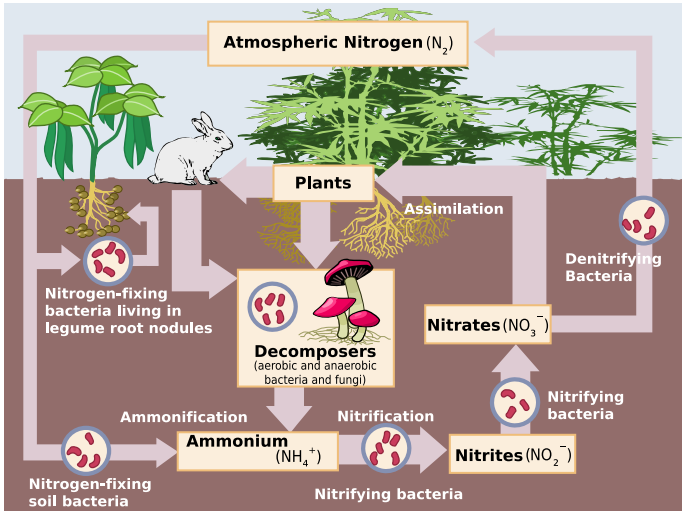


Figure: ***The nitrogen cycle****: Schematic representation of the flow of nitrogen through the environment. The importance of bacteria in the cycle is immediately recognized as being a key element in the cycle, providing different forms of nitrogen compounds assimilable by higher organisms.*

Human Applications of Nitrification

Nitrification is important in agricultural systems, where fertilizer is often applied as ammonia. Nitrification also plays an important role in the removal of nitrogen from municipal wastewater. The conventional removal is nitrification, followed by denitrification.

Key Points

- Nitrification is actually the net result of two distinct processes: the oxidation of ammonia (NH_3) or ammonium (NH_4^+) to nitrite (NO_2^-) by ammonia-oxidizing bacteria (e.g. Nitrosomonas) and the oxidation of nitrite (NO_2^-) to nitrate (NO_3^-) by the nitrite-oxidizing bacteria (e.g. Nitrobacter).
- Nitrification is extremely energetically poor leading to very slow growth rates for both types of organisms.
- Oxygen is required in ammonium and nitrite oxidation; ammonia-oxidizing and nitrite-oxidizing bacteria are aerobes.

Key Terms

- **chemolithotrophy**: A type of metabolism where energy is obtained from the oxidation of inorganic compounds.
- **nitrification**: The biological oxidation of ammonia or ammonium with oxygen into nitrite followed by the oxidation of these nitrites into nitrates.

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4.10F: Anammox

Learning Objectives

- Describe the overall process of ANaerobic AMMonium OXidation (Anammox) and its purpose

Anammox, an abbreviation for ANaerobic AMMonium OXidation, is a globally significant microbial process of the nitrogen cycle. The bacteria mediating this process were identified in 1999, and at the time were a great surprise to the scientific community. Anammox takes place in many natural environments, contributing up to 50% of the dinitrogen gas produced in the oceans. In this biological process, nitrite and ammonium are converted directly into dinitrogen gas. The overall catabolic reaction is:



Figure: **Enrichment Culture of Anammox Bacterium (Radboud University, Nijmegen)**: Enrichment culture of the anammox bacterium, Kuenenia stuttgartiensis.

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$.

This form of metabolism involves the coupling of ammonia oxidation to nitrite reduction. Since oxygen is not required for the process, these organisms are strict anaerobes. Amazingly, hydrazine (N_2H_4 — rocket fuel) is produced as an intermediate during anammox metabolism. To deal with the high toxicity of hydrazine, anammox bacteria have a hydrazine-containing intracellular organelle called the anammoxasome (a compartment inside the cytoplasm which is the locus of anammox catabolism), which is surrounded by an unusual and highly compact ladderane lipid membrane. Further, the membranes of these bacteria mainly consist of ladderane lipids so far unique in biology. Of special interest is the conversion to hydrazine (normally used as a high-energy rocket fuel, and poisonous to most living organisms) as an intermediate. A final striking feature of the organism is the extremely slow growth rate. The doubling time is nearly two weeks. The anammox process was originally found to occur only from 20°C to 43°C but more recently, anammox has been observed at temperatures from 36°C to 52°C in hot springs and 60°C to 85°C at hydrothermal vents located along the Mid-Atlantic Ridge.

Anammox organisms are autotrophs although the mechanism for carbon dioxide fixation is still unclear. Because of this property, these organisms could be used industrially to remove nitrogen in wastewater treatment processes. The bacteria that perform the anammox process belong to the bacterial phylum Planctomycetes (e.g. Candidatus Brocadia anammoxidans), of which Planctomyces and Pirellula are the best known genera. Currently five genera of anammox bacteria have been (provisionally) defined: Brocadia, Kuenenia, Anammoxoglobus, Jettenia (all fresh water species), and Scalindua (marine species).

Key Points

- The bacteria mediating this process were identified in 1999, and at the time were a great surprise for the scientific community.
- This form of metabolism occurs in members of the Planctomycetes (e.g. Candidatus Brocadia anammoxidans) and involves the coupling of ammonia oxidation to nitrite reduction.
- To deal with the high toxicity of hydrazine, anammox bacteria contain a hydrazine-containing intracellular organelle called the anammoxasome, surrounded by highly compact ladderane lipid membrane. These lipids are unique in nature, as is the use of hydrazine as a metabolic intermediate.

Key Terms

- **Anammox**: An abbreviation for ANaerobic AMMonium OXidation, a globally significant microbial process of the nitrogen cycle.
- **anaerobes**: Organisms that do not require oxygen for growth.
- **ladderane**: Any of a class of polycyclic hydrocarbons, consisting of repeating cyclobutane moieties, that resemble ladders

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4.10G: Benzoate Catabolism

Learning Objectives

- List a function of Rhodococcus in the scientific community

Key Points

- In the absence of water-splitting, photosynthesis is anoxygenic. Therefore, hydrogen production is sustained without inhibition from generated oxygen.
- Strains of Rhodococcus are applicably important owing to their ability to catabolize a wide range of compounds and produce bioactive steroids, acrylamide, and acrylic acid, and their involvement in fossil fuel biodesulfurization.
- The use of Rhodococcus is borne out of its ability to metabolize harmful environmental pollutants, such as toluene, naphthalene, herbicides, and PCBs.

Key Terms

- **catabolism**: Destructive metabolism, usually includes the release of energy and breakdown of materials.
- **Rhodococcus**: a genus of aerobic, nonsporulating, nonmotile Gram-positive bacteria closely related to Mycobacteria and Corynebacteria.
- **benzoate**: Any salt or ester of benzoic acid.

Benzoate catabolism is a series of chemical reactions resulting in the breakdown of benzoate. The purple non-sulphur (PNS) bacteria Rhodobacter sphaeroides is able to produce hydrogen from a wide range of organic compounds (chiefly organic acids) and light. The photo-system required for hydrogen production in Rhodobacter (PS-I) differ from its oxygenic photosystem (PS-II) due to the requirement of organic acids and the inability to oxidize water. In the absence of water-splitting, photosynthesis is anoxygenic. Therefore, hydrogen production is sustained without inhibition from generated oxygen. In PNS bacteria, hydrogen production is due to catalysis by nitrogenase. Hydrogenases are also present but the production of hydrogen by [FeFe]-hydrogenase is less than ten times the hydrogen uptake by [NiFe]-hydrogenase. Only under nitrogen-deficient conditions is nitrogenase activity sufficient to overcome uptake hydrogenase activity, resulting in net generation of hydrogen.

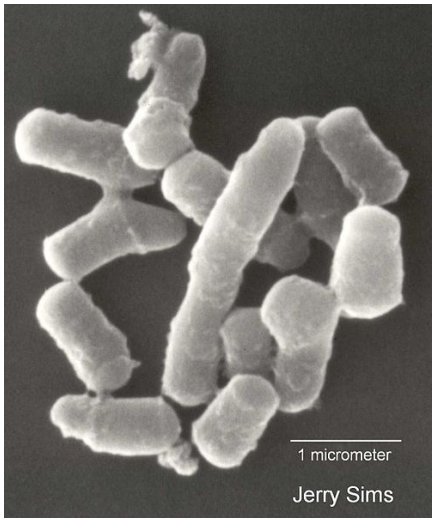


Figure: **Scanning electron micrograph of Rhodococcus sp. strain Q1**: Rhodococcus sp. strain Q1 grown on quinoline – the organism can use quinoline as a sole source of carbon, nitrogen and energy, tolerating concentrations up to 3.88 millimoles per liter.

Rhodococcus is a genus of aerobic, nonsporulating, nonmotile Gram-positive bacteria closely related to Mycobacteria and Corynebacteria. While a few species are pathogenic, most are benign and have been found to thrive in a broad range of environments, including soil, water, and eukaryotic cells. Fully sequenced in October 2006, the genome is known to be 9.7 megabasepairs long and 67% G/C. Strains of Rhodococcus are applicably important owing to their ability to catabolize a wide range of compounds and produce bioactive steroids, acrylamide, and acrylic acid, and their involvement in fossil fuel

biodesulfurization. This genetic and catabolic diversity is not only due to the large bacterial chromosome, but also to the presence of three large linear plasmids. Rhodococcus is also an experimentally advantageous system owing to a relatively fast growth rate and simple developmental cycle. However, as it stands now, Rhodococcus is not well characterized. Another important application of Rhodococcus comes from bioconversion, using biological systems to convert cheap starting material into more valuable compounds. This use of Rhodococcus is borne out of its ability to metabolize harmful environmental pollutants, such as toluene, naphthalene, herbicides, and PCBs. Rhodococci typically metabolize aromatic substrates by first oxygenating the aromatic ring to form a diol (two alcohol groups). Then, the ring is cleaved with intra/extradiol mechanisms, opening the ring and exposing the substrate to further metabolism. Since the chemistry here is very stereospecific, the diols are created with predictable chirality. While controlling the chirality of chemical reaction presents a significant challenge for synthetic chemists, biological processes can be used instead to faithfully produce chiral molecules in cases where direct chemical synthesis is infeasible or inefficient. An example of this is the use of Rhodococcus to produce indene, a precursor to the AIDS drug Crixivan^{TM}, a protease inhibitor, and containing two of the five chiral centers needed in the complex.

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4.10H: Polycyclic Aromatic Hydrocarbons

Learning Objectives

- Recognize various sources of polycyclic aromatic hydrocarbons and means of removal (bio-, phy

PAHs

Polycyclic aromatic hydrocarbons (PAHs), also known as poly-aromatic hydrocarbons or polynuclear aromatic hydrocarbons, are seen in. PAHs are potent atmospheric pollutants that consist of fused aromatic rings and do not contain heteroatoms or carry substituents. Naphthalene is the simplest example of a PAH. PAHs occur in oil, coal, and tar deposits, and are produced as byproducts of fuel burning (whether fossil fuel or biomass). As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic. PAHs are also found in cooked foods—studies have found PAHs in meat cooked at high temperatures such as grilling or barbecuing, and in smoked fish. They are also found in the interstellar medium, comets, and meteorites. PAHs are a candidate for the molecule acted as a basis for the earliest forms of life. In graphene the PAH motif is extended to large 2D sheets.

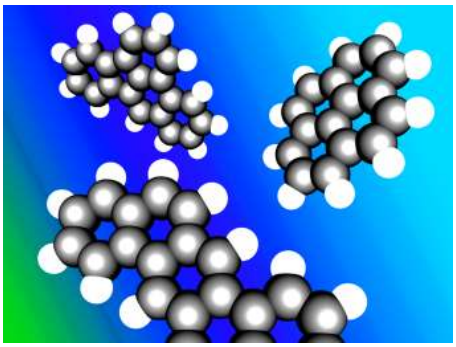


Figure: **Polycyclic Aromatic Hydrocarbons**: An image showing three examples of polycyclic aromatic hydrocarbons. Clockwise from top left, the molecules are: benz[e]acephenanthrylene, pyrene and dibenz[a,h]anthracene.

Natural crude oil and coal deposits contain significant amounts of PAHs from chemical conversion of natural product molecules, such as steroids, to aromatic hydrocarbons. They are also found in processed fossil fuels, tar, and various edible oils.

PLFA Analysis

Phospholipid -derived fatty acids (PLFA) are widely used in microbial ecology as chemotaxonomic markers of bacteria and other organisms. Phospholipids are the primary lipids composing cellular membranes. They can be esterified to many types of fatty acids. Once the phospholipids of an unknown sample are esterfied, the composition of the resulting PLFA can be compared to the PLFA of known organisms to determine the identity of the sample organism. PLFA analysis may be combined with stable isotope probing to determine which microbes are metabolically active in a sample.

The basic premise for PLFA analysis is that as individual organisms (especially bacteria and fungi) die, phospholipids are rapidly degraded and the remaining phospholipid content of the sample is assumed to be from living organisms. As the phospholipids of different groups of bacteria and fungi contain a variety of somewhat unique fatty acids, they can serve as useful biomarkers for such groups. PLFA profiles and composition can be determined by purifying the phospholipids and then cleaving the fatty acids for further analysis.

Bioremediation

Bioremediation is the use of micro-organism metabolism to remove pollutants. Technologies can be generally classified as *in situ* or *ex situ*. *In situ*bioremediation involves treating the contaminated material at the site, while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation related technologies are phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have found success by adding

matched microbe strains to the medium to enhance the resident microbe population ‘s ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators. Not all contaminants, however, are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. The assimilation of metals such as mercury into the food chain may worsen matters.

Phytoremediation

Phytoremediation is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or recycled for industrial use. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

Mycoremediation

Mycoremediation, is a form of bioremediation, the process of using fungi to degrade or sequester contaminants in the environment. Stimulating microbial and enzyme activity, mycelium reduces toxins *in situ*. Some fungi are hyperaccumulators, capable of absorbing and concentrating heavy metals in the mushroom fruit bodies. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These organic compounds are composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants. The key to mycoremediation is determining the right fungal species to target a specific pollutant.

Gallaecimonas is a recently described genus of bacteria. It is a Gram-negative, rod-shaped, halotolerant bacterium in the class Gammaproteobacteria. It can degrade high molecular mass polycyclic aromatic hydrocarbons of 4 and 5 rings. The 16S rRNA gene sequences of the type strain CEE_131(T) proved to be distantly related to those of Rheinheimera and Serratia.

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Key Points

- Polycyclic aromatic hydrocarbons (PAHs) occur in oil, coal, and tar deposits, and they are produced as byproducts of fuel burning (whether fossil fuel or biomass).
- Bioremediation is the use of microorganism metabolism to remove pollutants. These technologies can be generally classified as *in situ* or *ex situ*.
- Mycoremediation is a form of bioremediation that uses fungi to degrade or sequester contaminants in the environment. Stimulating microbial and enzyme activity, mycelium reduces toxins *in situ*.

Key Terms

- **Polycyclic aromatic hydrocarbons**: also known as poly-aromatic hydrocarbons or polynuclear aromatic hydrocarbons, are potent atmospheric pollutants that consist of fused aromatic rings and do not contain heteroatoms or carry substituents.
- **carcinogenic**: Causing or tending to cause cancer.
- **mutagenic**: Capable of causing mutation.

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SECTION OVERVIEW

4.11: Phototrophy

Topic hierarchy

4.11A: The Purpose and Process of Photosynthesis

4.11B: Main Structures and Summary of Photosynthesis

4.11C: The Two Parts of Photosynthesis

4.11D: Bacteriorhodopsin

4.11E: Carotenoids and Phycobilins

4.11F: Facultative Phototrophy

4.11G: Oxygenic Photosynthesis

4.11H: Anoxygenic Photosynthesis

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4.11A: The Purpose and Process of Photosynthesis

Learning Objectives

- Describe the process of photosynthesis

The Importance of Photosynthesis

The processes of all organisms—from bacteria to humans—require energy. To get this energy, many organisms access stored energy by eating food. Carnivores eat other animals and herbivores eat plants. But where does the stored energy in food originate? All of this energy can be traced back to the process of photosynthesis and light energy from the sun.

Photosynthesis is essential to all life on earth. It is the only biological process that captures energy from outer space (sunlight) and converts it into chemical energy in the form of G3P (
Glyceraldehyde 3-phosphate) which in turn can be made into sugars and other molecular compounds. Plants use these compounds in all of their metabolic processes; plants do not need to consume other organisms for food because they build all the molecules they need. Unlike plants, animals need to consume other organisms to consume the molecules they need for their metabolic processes.

The Process of Photosynthesis

During photosynthesis, molecules in leaves capture sunlight and energize electrons, which are then stored in the covalent bonds of carbohydrate molecules. That energy within those covalent bonds will be released when they are broken during cell respiration. How long lasting and stable are those covalent bonds? The energy extracted today by the burning of coal and petroleum products represents sunlight energy captured and stored by photosynthesis almost 200 million years ago.

Plants, algae, and a group of bacteria called cyanobacteria are the only organisms capable of performing photosynthesis. Because they use light to manufacture their own food, they are called photoautotrophs (“self-feeders using light”). Other organisms, such as animals, fungi, and most other bacteria, are termed heterotrophs (“other feeders”) because they must rely on the sugars produced by photosynthetic organisms for their energy needs. A third very interesting group of bacteria synthesize sugars, not by using sunlight’s energy, but by extracting energy from inorganic chemical compounds; hence, they are referred to as chemoautotrophs.

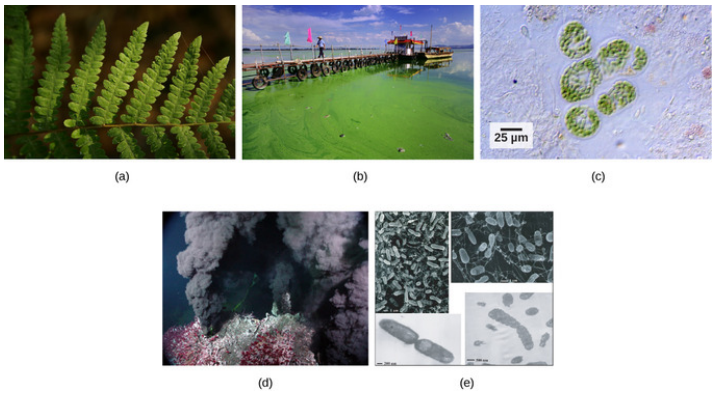


Figure: **Photosynthetic and Chemosynthetic Organisms**: Photoautotrophs, including (a) plants, (b) algae, and (c) cyanobacteria, synthesize their organic compounds via photosynthesis using sunlight as an energy source. Cyanobacteria and planktonic algae can grow over enormous areas in water, at times completely covering the surface. In a (d) deep sea vent, chemoautotrophs, such as these (e) thermophilic bacteria, capture energy from inorganic compounds to produce organic compounds. The ecosystem surrounding the vents has a diverse array of animals, such as tubeworms, crustaceans, and octopi that derive energy from the bacteria.

The importance of photosynthesis is not just that it can capture sunlight’s energy. A lizard sunning itself on a cold day can use the sun’s energy to warm up. Photosynthesis is vital because it evolved as a way to store the energy in solar radiation (the “photo-” part) as high-energy electrons in the carbon-carbon bonds of carbohydrate molecules (the “-synthesis” part). Those carbohydrates are the energy source that heterotrophs use to power the synthesis of ATP via respiration. Therefore, photosynthesis powers 99

percent of Earth's ecosystems. When a top predator, such as a wolf, preys on a deer, the wolf is at the end of an energy path that went from nuclear reactions on the surface of the sun, to light, to photosynthesis, to vegetation, to deer, and finally to wolf.

Key Points

- Photosynthesis evolved as a way to store the energy in solar radiation as high-energy electrons in carbohydrate molecules.
- Plants, algae, and cyanobacteria, known as photoautotrophs, are the only organisms capable of performing photosynthesis.
- Heterotrophs, unable to produce their own food, rely on the carbohydrates produced by photosynthetic organisms for their energy needs.

Key Terms

- **photosynthesis**: the process by which plants and other photoautotrophs generate carbohydrates and oxygen from carbon dioxide, water, and light energy in chloroplasts
- **photoautotroph**: an organism that can synthesize its own food by using light as a source of energy
- **chemoautotroph**: a simple organism, such as a protozoan, that derives its energy from chemical processes rather than photosynthesis

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4.11B: Main Structures and Summary of Photosynthesis

Learning Objectives

- Describe the main structures involved in photosynthesis and recall the chemical equation that summarizes the process of photosynthesis

Overview of Photosynthesis

Photosynthesis is a multi-step process that requires sunlight, carbon dioxide, and water as substrates. It produces oxygen and glyceraldehyde-3-phosphate (G3P or GA3P), simple carbohydrate molecules that are high in energy and can subsequently be converted into glucose, sucrose, or other sugar molecules. These sugar molecules contain covalent bonds that store energy. Organisms break down these molecules to release energy for use in cellular work.

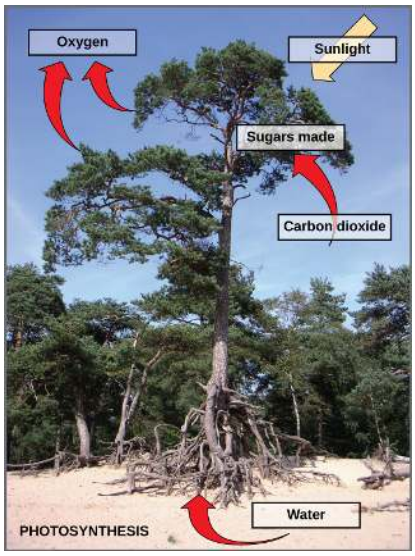


Figure: **Photosynthesis**: Photosynthesis uses solar energy, carbon dioxide, and water to produce energy-storing carbohydrates. Oxygen is generated as a waste product of photosynthesis.

The energy from sunlight drives the reaction of carbon dioxide and water molecules to produce sugar and oxygen, as seen in the chemical equation for photosynthesis. Though the equation looks simple, it is carried out through many complex steps. Before learning the details of how photoautotrophs convert light energy into chemical energy, it is important to become familiar with the structures involved.

Photosynthesis Equation

Carbon dioxide	+	Water	SUNLIGHT →	Sugar	+	Oxygen
$6CO_2$		$6H_2O$		$C_6H_{12}O_6$		$6O_2$

Figure: **Chemical equation for photosynthesis**: The basic equation for photosynthesis is deceptively simple. In reality, the process includes many steps involving intermediate reactants and products. Glucose, the primary energy source in cells, is made from two three-carbon GA3P molecules.

Photosynthesis and the Leaf

In plants, photosynthesis generally takes place in leaves, which consist of several layers of cells. The process of photosynthesis occurs in a middle layer called the mesophyll. The gas exchange of carbon dioxide and oxygen occurs through small, regulated openings called stomata (singular: stoma), which also play a role in the plant's regulation of water balance. The stomata are typically located on the underside of the leaf, which minimizes water loss. Each stoma is flanked by guard cells that regulate the opening and closing of the stomata by swelling or shrinking in response to osmotic changes.

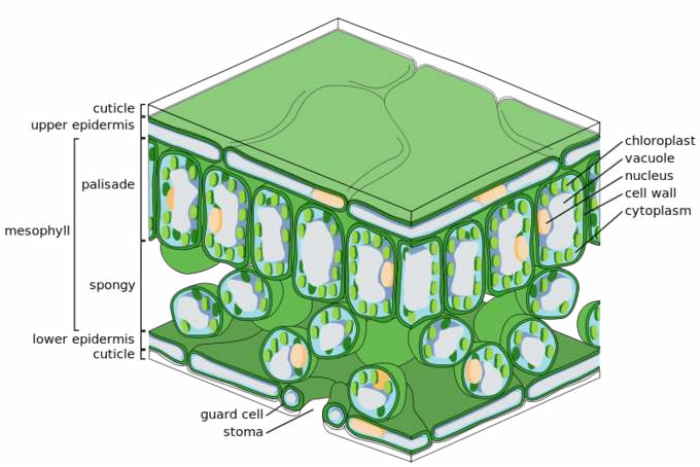


Figure: **Structure of a leaf (cross-section)**: Photosynthesis takes place in the mesophyll. The palisade layer contains most of the chloroplast and principal region in which photosynthesis is carried out. The airy spongy layer is the region of storage and gas exchange. The stomata regulate carbon dioxide and water balance.

Photosynthesis within the Chloroplast

In all autotrophic eukaryotes, photosynthesis takes place inside an organelle called a chloroplast. For plants, chloroplast-containing cells exist in the mesophyll. Chloroplasts have a double membrane envelope composed of an outer membrane and an inner membrane. Within the double membrane are stacked, disc-shaped structures called thylakoids.

Embedded in the thylakoid membrane is chlorophyll, a pigment that absorbs certain portions of the visible spectrum and captures energy from sunlight. Chlorophyll gives plants their green color and is responsible for the initial interaction between light and plant material, as well as numerous proteins that make up the electron transport chain. The thylakoid membrane encloses an internal space called the thylakoid lumen. A stack of thylakoids is called a granum, and the liquid-filled space surrounding the granum is the stroma or “bed.”

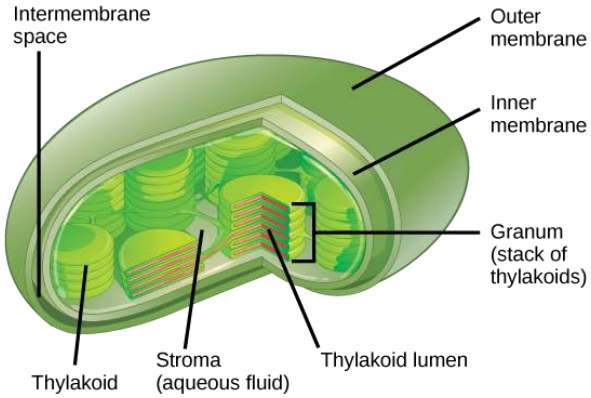


Figure: **Structure of the Chloroplast**: Photosynthesis takes place in chloroplasts, which have an outer membrane and an inner membrane. Stacks of thylakoids called grana form a third membrane layer.

Key Points

- The chemical equation for photosynthesis is $6CO2+6H2O \rightarrow C6H12O6+6O2.6CO2+6H2O \rightarrow C6H12O6+6O2$.
- In plants, the process of photosynthesis takes place in the mesophyll of the leaves, inside the chloroplasts.
- Chloroplasts contain disc-shaped structures called thylakoids, which contain the pigment chlorophyll.
- Chlorophyll absorbs certain portions of the visible spectrum and captures energy from sunlight.

Key Terms

- **chloroplast**: An organelle found in the cells of green plants and photosynthetic algae where photosynthesis takes place.
- **mesophyll**: A layer of cells that comprises most of the interior of the leaf between the upper and lower layers of epidermis.
- **stoma**: A pore in the leaf and stem epidermis that is used for gaseous exchange.

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4.11C: The Two Parts of Photosynthesis

Learning Objectives

- Distinguish between the two parts of photosynthesis

Photosynthesis takes place in two sequential stages:

1. The light-dependent reactions;
2. The light-independent reactions, or Calvin Cycle.

Light-Dependent Reactions

Just as the name implies, light-dependent reactions require sunlight. In the light-dependent reactions, energy from sunlight is absorbed by chlorophyll and converted into stored chemical energy, in the form of the electron carrier molecule NADPH (nicotinamide adenine dinucleotide phosphate) and the energy currency molecule ATP (adenosine triphosphate). The light-dependent reactions take place in the thylakoid membranes in the granum (stack of thylakoids), within the chloroplast.

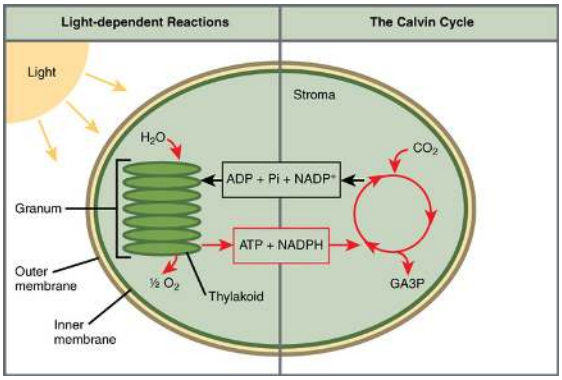


Figure: **The two stages of photosynthesis**: Photosynthesis takes place in two stages: light-dependent reactions and the Calvin cycle (light-independent reactions). Light-dependent reactions, which take place in the thylakoid membrane, use light energy to make ATP and NADPH. The Calvin cycle, which takes place in the stroma, uses energy derived from these compounds to make GA3P from CO2.

Photosystems

The process that converts light energy into chemical energy takes place in a multi-protein complex called a photosystem. Two types of photosystems are embedded in the thylakoid membrane: photosystem II (PSII) and photosystem I (PSI). Each photosystem plays a key role in capturing the energy from sunlight by exciting electrons. These energized electrons are transported by “energy carrier” molecules, which power the light-independent reactions.

Photosystems consist of a light-harvesting complex and a reaction center. Pigments in the light-harvesting complex pass light energy to two special chlorophyll *a* molecules in the reaction center. The light excites an electron from the chlorophyll *a* pair, which passes to the primary electron acceptor. The excited electron must then be replaced. In photosystem II, the electron comes from the splitting of water, which releases oxygen as a waste product. In photosystem I, the electron comes from the chloroplast electron transport chain.

The two photosystems oxidize different sources of the low-energy electron supply, deliver their energized electrons to different places, and respond to different wavelengths of light.

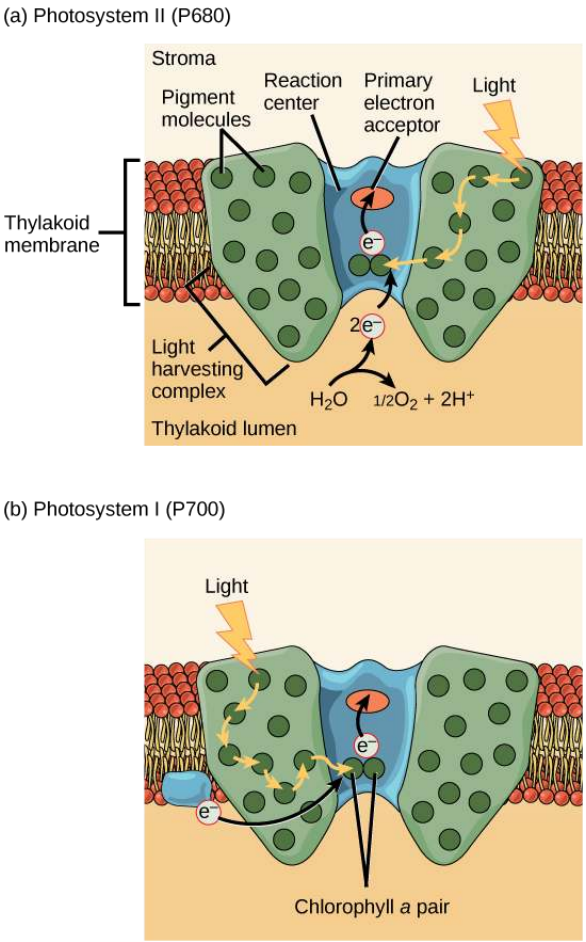


Figure: **Photosystems I & II**: As explained above, the photosystems manipulate electrons with energy harvested from light.

Light-Independent Reactions

In the light-independent reactions or Calvin cycle, the energized electrons from the light-dependent reactions provide the energy to form carbohydrates from carbon dioxide molecules. The light-independent reactions are sometimes called the Calvin cycle because of the cyclical nature of the process.

Although the light-independent reactions do not use light as a reactant (and as a result can take place at day or night), they require the products of the light-dependent reactions to function. The light-independent molecules depend on the energy carrier molecules, ATP and NADPH, to drive the construction of new carbohydrate molecules. After the energy is transferred, the energy carrier molecules return to the light-dependent reactions to obtain more energized electrons. In addition, several enzymes of the light-independent reactions are activated by light.

Key Points

- In light-dependent reactions, the energy from sunlight is absorbed by chlorophyll and converted into chemical energy in the form of electron carrier molecules like ATP and NADPH.
- Light energy is harnessed in Photosystems I and II, both of which are present in the thylakoid membranes of chloroplasts.
- In light-independent reactions (the Calvin cycle), carbohydrate molecules are assembled from carbon dioxide using the chemical energy harvested during the light-dependent reactions.

Key Terms

- **photosystem**: Either of two biochemical systems active in chloroplasts that are part of photosynthesis.

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4.11D: Bacteriorhodopsin

Learning Objectives

- Discuss the function of bacteriorhodopsin

Bacteriorhodopsin is a protein used by Archaea, the most notable one being Halobacteria. It acts as a proton pump; that is, it captures light energy and uses it to move protons across the membrane out of the cell. The resulting proton gradient is subsequently converted into chemical energy. The resulting proton gradient is subsequently converted into chemical energy.

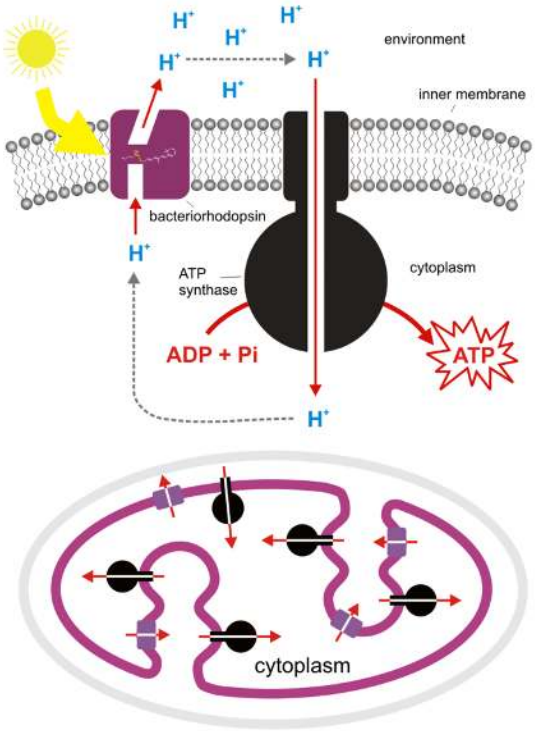


Figure: **ATP generation via bacteriorhodopsin**: Chemiosmotic coupling between sun energy, bacteriorhodopsin and phosphorylation by ATP synthase (chemical energy) during photosynthesis in Halobacterium salinarum (syn. H. halobium).

Bacteriorhodopsin is an integral membrane protein usually found in two-dimensional crystalline patches known as “purple membrane”, which can occupy up to nearly 50% of the surface area of the archaeal cell. The bacteriorhodopsin forms repeating elements that are arranged in chains. Each chain has seven transmembrane alpha helices and contains one molecule of retinal buried deep within, the typical structure for retinylidene proteins. It is the retinal molecule that changes its conformation when absorbing a photon, resulting in a conformational change of the surrounding protein and the proton pumping action. This releases a proton from a “holding site” into the extracellular side (EC) of the membrane. Reprotonation of the retinal molecule by restores its original isomerized form. This results in a second proton being released to the EC side. The releases the proton from its “holding site,” where a new cycle may begin.

The bacteriorhodopsin molecule is purple and is most efficient at absorbing green light (wavelength 500-650 nm, with the absorption maximum at 568 nm). Bacteriorhodopsin belongs to a family of bacterial proteins related to vertebrate rhodopsins, the pigments that sense light in the retina. Many molecules have homology to bacteriorhodopsin, including the light-driven chloride pump halorhodopsin, and some directly light-activated channels like channelrhodopsin. All other phototrophic systems in bacteria, algae, and plants use chlorophylls or bacteriochlorophylls rather than bacteriorhodopsin. These also produce a proton gradient, but in a quite different and more indirect way involving an electron transfer chain consisting of several other proteins. Furthermore, chlorophylls are aided in capturing light energy by other pigments known as “antennas”; these are not present in bacteriorhodopsin-based systems. Last, chlorophyll-based phototrophy is coupled to carbon fixation (the incorporation of carbon dioxide into larger

organic molecules) and for that reason is photosynthesis, which is not true for bacteriorhodopsin-based system. Thus, it is likely that phototrophy independently evolved at least twice, once in bacteria and once in archaea.

Key Points

- Bacteriorhodopsin is a proton pump found in Archaea, it takes light energy and coverts it into chemical energy, ATP, that can be used by the cell for cellular functions.
- Bacteriorhodopsin forms chains, which contain retinal molecule within, it is the retinal molecule that absorbs a photon from light, it then changes the confirmation of the nearby Bacteriorhodopsin protein, allowing it to act as a proton pump.
- While chlorophyll based ATP generation depends on a protein gradient, like bacteriorhodopsin, but with striking differences, suggesting that phototrophy evolved in bacteria and archaea independently of each other.

Key Terms

- **isomerized**: converted from one isomer to another
- **retinal**: One of several yellow or red carotenoid pigments formed from rhodopsin by the action of light; retinene
- **phototrophy**: The synthesis of an organism's food from inorganic material using light as a source of energy

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4.11E: Carotenoids and Phycobilins

Learning Objectives

- Distinguish between carotenoids and phycobilins



Figure: **Microbial Mats Around the Grand Prismatic Spring**: Thermophiles produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park. The color of the mats of algae and bacteria is due to the ratio of chlorophyll to carotenoid molecules produced by the organisms. During summertime the chlorophyll content of the organisms is low and thus the mats appear orange, red, or yellow. However during the winter, the mats are usually dark green, because sunlight is more scarce and the microbes produce more chlorophyll to compensate, thereby masking the carotenoid colors.

Photosynthesis in many plants and algae depend on chlorophylls which absorb light closer to the ultraviolet side of the spectrum, and emit light in the green end of the spectrum. However during certain times of the year or in various location most of the light may be shifted to other wavelengths away from the ultraviolet spectrum. To deal with these problems, organisms dependent on photosynthesis express various compounds that allow them to absorb different spectrum of light. Notably are carotenoids and phycobilins.

Chromoplasts of plants and some other photosynthetic organisms like algae, some bacteria, and some fungi. Carotenoids can be produced from fats and other basic organic metabolic building blocks by all these organisms. Carotenoids generally cannot be manufactured by species in the animal kingdom so animals obtain carotenoids in their diets, and may employ them in various ways in metabolism.There are over 600 known carotenoids; they are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons, and contain no oxygen). All carotenoids are tetraterpenoids, meaning that they are produced from 8 isoprene molecules and contain 40 carbon atoms. Carotenoids in general absorb blue light. They serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they protect chlorophyll from photodamage.

Phycobilins (from Greek: φ (phykos) meaning “alga”, and from Latin: bilis meaning “bile”) are chromophores (light-capturing molecules) found in cyanobacteria and in the chloroplasts of red algae, glaucophytes and some cryptomonads (though not in green algae and higher plants). They are unique among the photosynthetic pigments in that they are bonded to certain water-soluble proteins, known as phycobiliproteins. Phycobiliproteins then pass the light energy to chlorophylls for photosynthesis.The phycobilins are especially efficient at absorbing red, orange, yellow, and green light, wavelengths that are not well absorbed by chlorophyll a. Organisms growing in shallow waters tend to contain phycobilins that can capture yellow/red light, while those at greater depth often contain more of the phycobilins that can capture green light, which is relatively more abundant there.

Key Points

- Chlorophylls absorb light most efficiently at the ultraviolet end of the spectrum, however not all light that an organism gets is at that wavelength. Thus many photosynthetic organisms rely on accessory compounds to get light from different spectrums.
- Caretenoids aid in the absorption of light in the blue-range spectrum, while at the same time help with the oxidative stress due to the photosynthetic process.
- Phycobilins aid in the absorption of light in the red, orange, yellow, and green light, wavelengths.

Key Terms

- **isoprene**: An unsaturated hydrocarbon, C5H8, that is readily polymerized; natural rubber (caoutchouc) is cis-1,4-polyisoprene, and trans-1,4-polyisoprene is present in gutta-percha and balata; it is the structural basis for the terpenes.

- **photosynthesis**: The process by which plants and other photoautotrophs generate carbohydrates and oxygen from carbon dioxide, water, and light energy in chloroplasts.

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4.11F: Facultative Phototrophy

Learning Objectives

- Recognize the traits associated with the classification of facultative phototrophy

An autotroph or “producer”, is an organism that produces complex organic compounds (such as carbohydrates, fats, and proteins) from simple substances present in its surroundings, generally using energy from light (photosynthesis) or inorganic chemical reactions (chemosynthesis). They are the producers in a food chain, such as plants on land or algae in water. They are able to make their own food, and do not need a living energy or carbon source. Autotrophs can reduce carbon dioxide to make organic compounds, creating a store of chemical energy. Phototrophs, a type of autotroph, convert physical energy from sunlight (in case of green plants) into chemical energy in the form of reduced carbon.

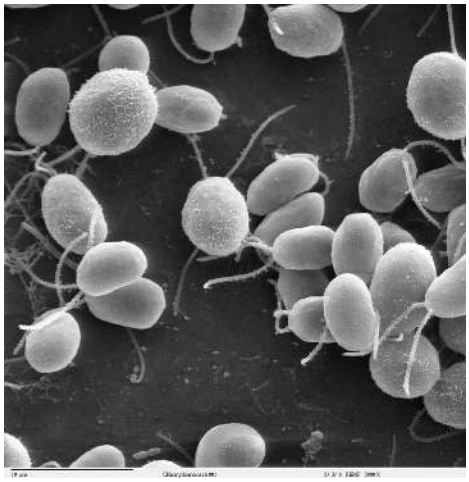


Figure: **Chlamydomanas reinhardtii**: Scanning electron microscope image, showing an example of green algae (Chlorophyta).

In terms of biology facultative means “optional” or “discretionary” the antonym of which is obligate meaning “by necessity”. Thus facultative phototrophy means an organism that can switch between phototrophy to make organix compounds and other means of getting cellular energy. Probably the best studied example of a facultative phototrophy is Chlamydomonas reinhardtii.

Chlamydomonas reinhardtii is a single celled green alga about 10 micrometres in diameter that swims with two flagella. It has a cell wall made of hydroxyproline-rich glycoproteins, a large cup-shaped chloroplast, a large pyrenoid, and an “eyespot” that senses light. Although widely distributed worldwide in soil and fresh water, C. reinhardtii is primarily used as a model organism in biology in a wide range of subfields. When illuminated, C. reinhardtii can grow in media lacking organic carbon and chemical energy sources, and can also grow in the dark when supplied with these. C. reinhardtii is also of interest in the biofuel field, as a source of hydrogen. As one can imagine switching energy sources under varying conditions allows facultative microbes to live in different conditions, in the case of a facultative phototroph it can rely of light other energy sources.

Key Points

- Phototrophs can obtain cellular energy from light as well as using light to fix carbon to make complex macromolecules on which to survive.
- Chlamydomonas reinhardtii is an organism that can rely on photosynthetic and chemical energy sources, depending on conditions.
- Facultative means optional, in terms of biology it refers to an organism that can switch energy sources for survival.

Key Terms

- **autotroph**: Any organism that can synthesize its food from inorganic substances, using heat or light as a source of energy.
- **pyrenoid**: any of several transparent structures found in the chloroplast of certain algae etc.; they are responsible for the fixation of carbon dioxide and the formation of starch

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4.11G: Oxygenic Photosynthesis

Learning Objectives

- Describe oxygenic photosynthesis

In plants, algae and cyanobacteria, photosynthesis releases oxygen. This is called oxygenic photosynthesis. Although there are some differences between oxygenic photosynthesis in plants, algae, and cyanobacteria, the overall process is quite similar in these organisms. Photosynthesis is not only needed by photosynthetic organism for energy but also for carbon fixation.

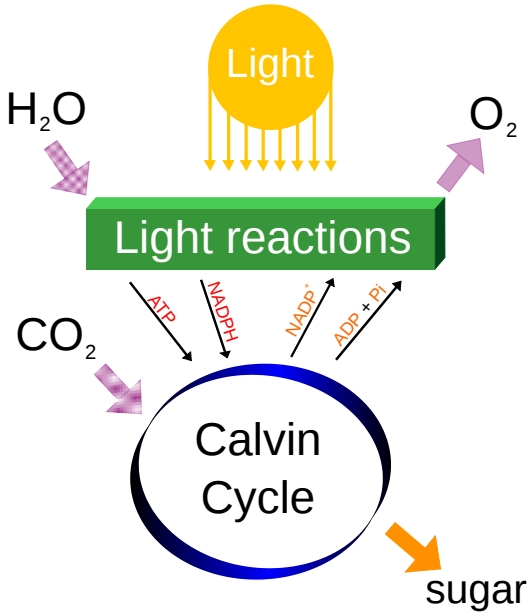


Figure: **Photosynthesis overview**: Photosynthesis changes sunlight into chemical energy, splits water to liberate O2, and fixes CO2 into sugar.

Carbon dioxide is converted into sugars in a process called carbon fixation. Carbon fixation is a redox reaction, so photosynthesis needs to supply both a source of energy to drive this process, and the electrons needed to convert carbon dioxide into a carbohydrate, which is a reduction reaction. In general outline, photosynthesis is the opposite of cellular respiration, where glucose and other compounds are oxidized to produce carbon dioxide, water, and release chemical energy. However, the two processes take place through a different sequence of chemical reactions and in different cellular compartments. The general equation for photosynthesis is therefore:

2n CO2 + 2n DH2 + photons → 2(CH_2O)n + 2n DO

Carbon dioxide + electron donor + light energy → carbohydrate + oxidized electron donor.

In oxygenic photosynthesis water is the electron donor and, since its hydrolysis releases oxygen, the equation for this process is:

2n CO_2 + 4n H_2O + photons → 2(CH_2O)n + 2n O_2 + 2n H_2O

carbon dioxide + water + light energy → carbohydrate + oxygen + water

Often 2n water molecules are cancelled on both sides, yielding:

2n CO_2 + 2n H_2O + photons → 2(CH_2O)n + 2n O_2

carbon dioxide + water + light energy → carbohydrate + oxygen

In plants, algae and cyanobacteria, photosynthesis releases oxygen. This is called oxygenic photosynthesis. Although there are some differences between oxygenic photosynthesis in plants, algae, and cyanobacteria, the overall process is quite similar in these organisms.

Key Points

- Plants, algae and cyanobacteria release oxygen during photosynthesis.
- Photosynthesis is also needed for carbon fixation.
- While different organisms may have differences during oxygenic photosynthesis, they all follow the general equation of, carbon dioxide + water + light energy → carbohydrate + oxygen.

Key Terms

- **cyanobacteria**: Cyanobacteria, also known as blue-green bacteria, blue-green algae, and Cyanophyta, is a phylum of bacteria that obtain their energy through photosynthesis.
- **oxygenic**: of, relating to, containing or producing oxygen

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4.11H: Anoxygenic Photosynthesis

Learning Objectives

- Discuss the characteristics that classify a specific type of chlorophototrophy as anoxygenic photosynthesis

Phototrophy is the process by which organisms trap light energy (photons) and store it as chemical energy in the form of ATP and/or reducing power in NADPH. There are two major types of phototrophy: chlorophyll-based chlorophototrophy and rhodopsin-based retinalophototrophy. Chlorophototrophy can further be divided into oxygenic photosynthesis and anoxygenic phototrophy. Oxygenic and anoxygenic photosynthesizing organisms undergo different reactions either in the presence of light or with no direct contribution of light to the chemical reaction (colloquially called “light reactions” and “dark reactions”, respectively).



Figure: **Green d winogradsky**: A column containing green sulfur bacteria which uses anoxygenic photosynthesis.

Anoxygenic photosynthesis is the phototrophic process where light energy is captured and converted to ATP, without the production of oxygen. Water is therefore not used as an electron donor. There are several groups of bacteria that undergo anoxygenic photosynthesis: Green sulfur bacteria, green and red filamentous anoxygenic phototrophs (FAPs), phototrophic purple bacteria, phototrophic Acidobacteria, and phototrophic heliobacteria. Anoxygenic phototrophs have photosynthetic pigments called bacteriochlorophylls (similar to chlorophyll found in eukaryotes). Bacteriochlorophyll a and b have wavelengths of maximum absorption at 775 nm and 790 nm, respectively in ether. In vivo however, due to shared extended resonance structures, these pigments were found to maximally absorb wavelengths out further into the near-infrared. Bacteriochlorophylls c-g have the corresponding “peak” absorbance at more blue wavelengths when dissolved in an organic solvent, but are similarly red-shifted within their natural environment (with the exception of bacteriochlorophyll f, which has not been naturally observed).Unlike oxygenic phototrophs, anoxygenic photosynthesis only functions using (by phylum) either one of two possible types of photosystem. This restricts them to cyclic electron flow and are therefore unable to produce O_2 from the oxidization of H_2O.

The cyclic nature of the electron flow is typified in purple non-sulfur bacteria. The electron transport chain of purple non-sulfur bacteria begins when the reaction centre bacteriochlorophyll pair, P870, becomes excited from the absorption of light. Excited P870 will then donate an electron to Bacteriopheophytin, which then passes it on to a series of electron carriers down the electron chain. In the process, it will generate a proton motor force (PMF) which can then be used to synthesize ATP by oxidative phosphorylation. The electron returns to P870 at the end of the chain so it can be used again once light excites the reaction-center. Therefore electrons are not left over to oxidize H_2O into O_2.

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Key Points

- Anoxygenic photosynthesis produces cellular energy (ATP), without oxygen as a by-product.
- As opposed to eukaryotic organisms, which rely on chlorophylls for photosynthesis, anoxygenic organisms rely on bacteriochlorophylls.

- The electron transport chain of anoxygenic phototrophs is cyclic, meaning the electrons used during photosynthesis are fed back into the system, therefore no electrons are left over to oxidize water into oxygen.

Key Terms

- **electron transport chain**: An electron transport chain (ETC) couples electron transfer between an electron donor (such as NADH) and an electron acceptor (such as O2) with the transfer of H+ ions (protons) across a membrane. The resulting electrochemical proton gradient is used to generate chemical energy in the form of adenosine triphosphate (ATP). Electron transport chains are the cellular mechanisms used for extracting energy from sunlight in photosynthesis and also from redox reactions, such as the oxidation of sugars (respiration).
- **electron donor**: An electron donor is a chemical entity that donates electrons to another compound. It is a reducing agent that, by virtue of its donating electrons, is itself oxidized in the process.
- **anoxygenic**: That does not involve the production of oxygen

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SECTION OVERVIEW

4.12: Biosynthesis

Topic hierarchy

4.12A: Substrates for Biosysnthesis

4.12B: Biosynthesis and Energy

4.12C: The Calvin Cycle

4.12D: Intermediates Produced During the Calvin Cycle

4.12E: Regulation of the Calvin Cycle

4.12F: The Reverse TCA Cycle

4.12G: The Acetyl-CoA Pathway

4.12H: The 3-Hydroxypropionate Cycle

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4.12A: Substrates for Biosysnthesis

Learning Objectives

- Describe the importance of substrates for biosynthesis

Microorganisms have numerous pathways and processes in place to ensure both energy and nutrient production. These pathways are necessary for survival and cellular function. The major metabolic pathways require substrates to be acted upon for the formation of larger, more complex products. Biosynthetic processes are defined by the production of more complex products that are required for growth and maintenance of life. These processes require pathways that are often multi-step. There are various components deemed necessary for biosynthetic processes to occur, including: precursor compounds, chemical energy, and carious catalytic enzymes.

TCA Cycle

The citric acid cycle, commonly referred to as the Krebs cycle, is characterized by the production of energy through the oxidation of acetate derived from carbohydrates, fats, and proteins into carbon dioxide. The cycle is one of the major metabolic processes utilized to generate energy. The citric acid cycle, comprised of a series of chemical reactions, provides precursors for additional biochemical pathways. These precursors are used as substrates for the biogenesis of large complex products. The precursors include amino acids and reducing agents such as NADH. Additional pathways that require precursors formed by the TCA include amino acid and nucleotide synthesis.

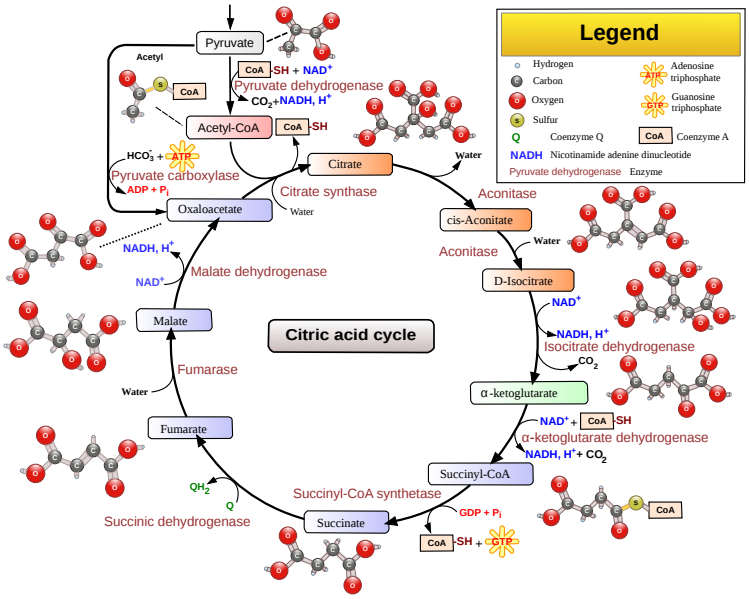


Figure: **The Citric Acid Cycle**: An overview of the Citric Acid Cycle.

Glycolysis

An additional central metabolic pathway includes glycolysis. Glycolysis is characterized by a series of reactions that results in the conversion of glucose into pyruvate. This process is characterized by the production of various intermediates and molecules that function as substrates in additional pathways. Additional pathways that require substrates or metabolites produced by the glycolytic pathway include: gluconeogenesis, lipid metabolism, the pentose phosphate pathway, and the TCA.

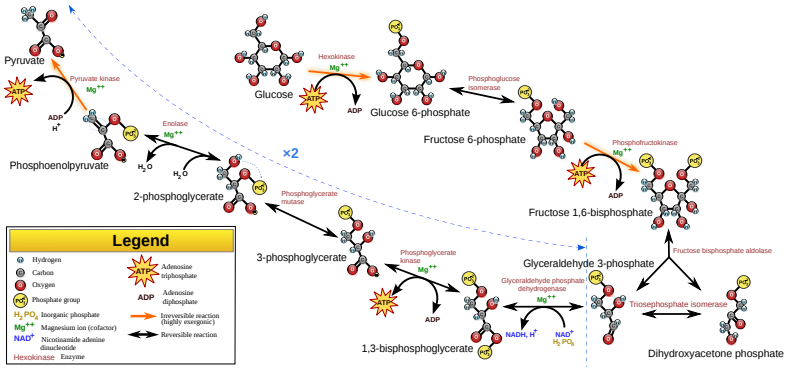


Figure: **Glycolysis Pathway Overview**: An overview of the glycolytic pathway. This pathway, comprised of a series of reactions, produces many intermediates and molecules utilized as substrates for biosynthesis in additional pathways.

Key Points

- Biogenesis or anabolism, requires substrates to be acted upon that result in the formation of larger more complex molecules.
- A central metabolic pathway that produces precursors and substrates used in biosynthetic processes is the TCA cycle.
- A central metabolic pathway that produces precursors and substrates used in biosynthetic processes is glycolysis.

Key Terms

- **reducing agent**: A substance that functions in reducing or donating electrons to another substance until that specific substance becomes oxidized.
- **oxidation**: A reaction in which the atoms of an element lose electrons and the valence of the element increases.

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4.12B: Biosynthesis and Energy

Learning Objectives

- Discuss the principles of biosynthesis and provide examples

Biosynthesis and Energy

Biosynthesis in living organisms is a process in which substrates are converted to more complex products. The products which are produced as a result of biosynthesis are necessary for cellular and metabolic processes deemed essential for survival. Biosynthesis is often referred to as the anabolism branch of metabolism that results in complex proteins such as vitamins.

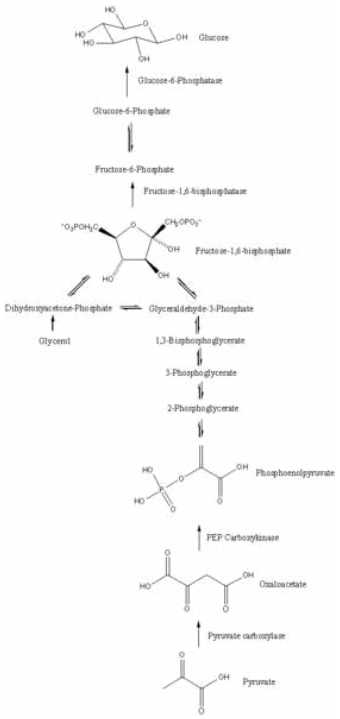


Figure: **Overview of Gluconeogenesis**: A biosynthetic pathway is utilized in microorganisms to produce glucose.

A majority of the organic compounds required by microorganisms are produced via biosynthetic pathways. The components which are utilized by biosynthetic pathways to promote the production of large molecules include chemical energy and catalytic enzymes. Biosynthetic building blocks utilized by organisms include amino acids, purines, pyrimidines, lipids, sugars, and enzyme cofactors. There are numerous mechanisms in place to ensure biosynthetic pathways are properly controlled so a cell will produce a specific amount of a compound. Biosynthetic metabolism (also known as anabolism) involves the synthesis of macromolecules from specific building blocks. A majority of these processes are considered to be multi-step or multi-enzymatic processes.

Carbon Dioxide Fixation

Carbon dioxide fixation is necessary to ensure carbon dioxide can be converted into organic carbon. The major pathways utilized to ensure fixation of carbon dioxide include: the Calvin cycle, the reductive TCA cycle, and the acetyl-CoA pathway. The Calvin cycle involves utilizing carbon dioxide and water to form organic compounds. The reductive TCA cycle, commonly referred to as the reverse Krebs cycle, also produces carbon compounds from carbon dioxide and water. In the acetyl-CoA pathway, carbon dioxide is reduced to carbon monoxide and then acetyl-CoA.

Glucose and Fructose Synthesis

An additional biosynthetic pathway utilized by microorganisms includes the synthesis of sugars and polysaccharides. The ability to synthesize sugars and polysaccharides from noncarbohydrate precursors is key to survival in numerous microorganisms. The

process of gluconeogenesis, characterized by the production of glucose or fructose from noncarbohydrate precursors, is an ubiquitous process. This process utilizes precursors such as pyruvate, lactate, or glycerol.

Key Points

- Anabolism is the form of metabolism responsible for building large complexes from precursors.
- The three categories of carbon fixation pathways are the Calvin cycle, the reverse TCA, and acetyl-CoA pathways.
- One example of a biosynthetic process is gluconeogenesis, which is responsible for the production of glucose from noncarbohydrate precursors.

Key Terms

- **anabolism**: Anabolism is the set of metabolic pathways that construct molecules from smaller units.
- **biosynthesis**: Biosynthesis is an enzyme-catalyzed process in cells of living organisms by which substrates are converted to more complex products.
- **metabolic**: Of or pertaining to metabolism; as, metabolic activity; metabolic force.

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4.12C: The Calvin Cycle

Learning Objectives

- Describe the Calvin Cycle

The Calvin Cycle

In plants, carbon dioxide (CO_2) enters the leaves through stomata, where it diffuses over short distances through intercellular spaces until it reaches the mesophyll cells. Once in the mesophyll cells, CO_2 diffuses into the stroma of the chloroplast, the site of light-independent reactions of photosynthesis. These reactions actually have several names associated with them. Other names for light-independent reactions include the Calvin cycle, the Calvin-Benson cycle, and dark reactions. The most outdated name is dark reactions, which can be misleading because it implies incorrectly that the reaction only occurs at night or is independent of light, which is why most scientists and instructors no longer use it.

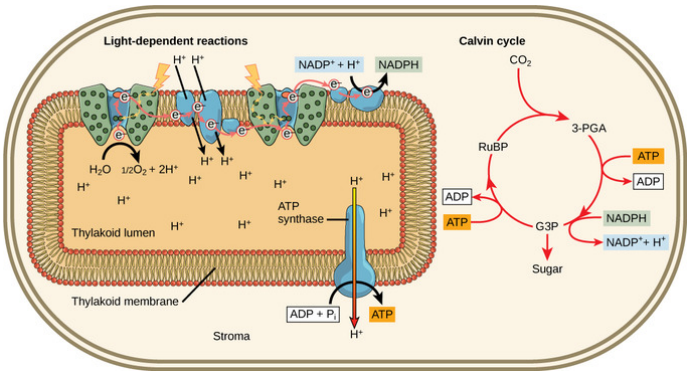


Figure: **Light Reactions**: Light-dependent reactions harness energy from the sun to produce chemical bonds, ATP, and NADPH. These energy-carrying molecules are made in the stroma where the Calvin cycle takes place. The Calvin cycle is not totally independent of light since it relies on ATP and NADH, which are products of the light-dependent reactions.

The light-independent reactions of the Calvin cycle can be organized into three basic stages: fixation, reduction, and regeneration.

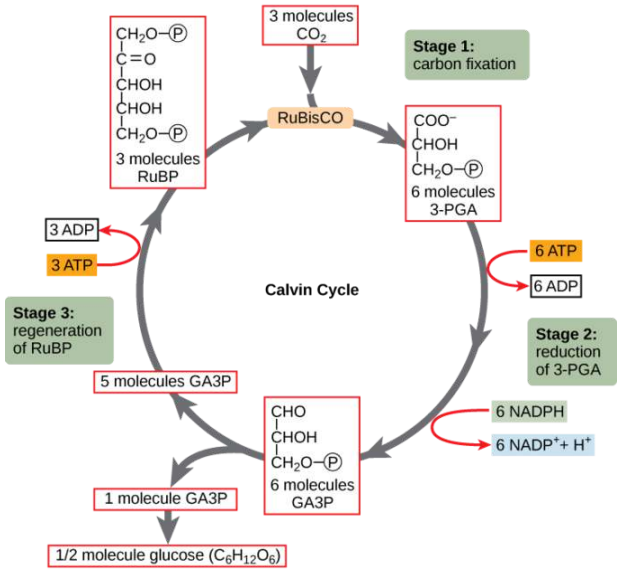


Figure: **The Calvin Cycle**: The Calvin cycle has three stages. In stage 1, the enzyme RuBisCO incorporates carbon dioxide into an organic molecule, 3-PGA. In stage 2, the organic molecule is reduced using electrons supplied by NADPH. In stage 3, RuBP, the molecule that starts the cycle, is regenerated so that the cycle can continue. Only one carbon dioxide molecule is incorporated at a time, so the cycle must be completed three times to produce a single three-carbon GA3P molecule, and six times to produce a six-carbon glucose molecule.

Stage 1: Fixation

In the stroma, in addition to CO_2,two other components are present to initiate the light-independent reactions: an enzyme called ribulose bisphosphate carboxylase (RuBisCO) and three molecules of ribulose bisphosphate (RuBP). RuBP has five atoms of carbon, flanked by two phosphates. RuBisCO catalyzes a reaction between CO_2 and RuBP. For each CO_2 molecule that reacts with one RuBP, two molecules of 3-phosphoglyceric acid (3-PGA) form. 3-PGA has three carbons and one phosphate. Each turn of the cycle involves only one RuBP and one carbon dioxide and forms two molecules of 3-PGA. The number of carbon atoms remains the same, as the atoms move to form new bonds during the reactions (3 atoms from $3CO_2$ + 15 atoms from 3RuBP = 18 atoms in 3 atoms of 3-PGA). This process is called carbon fixation because CO_2 is "fixed" from an inorganic form into organic molecules.

Stage 2: Reduction

ATP and NADPH are used to convert the six molecules of 3-PGA into six molecules of a chemical called glyceraldehyde 3-phosphate (G3P). This is a reduction reaction because it involves the gain of electrons by 3-PGA. Recall that a reduction is the gain of an electron by an atom or molecule. Six molecules of both ATP and NADPH are used. For ATP, energy is released with the loss of the terminal phosphate atom, converting it to ADP; for NADPH, both energy and a hydrogen atom are lost, converting it into $NADP^+$. Both of these molecules return to the nearby light-dependent reactions to be reused and reenergized.

Stage 3: Regeneration

At this point, only one of the G3P molecules leaves the Calvin cycle and is sent to the cytoplasm to contribute to the formation of other compounds needed by the plant. Because the G3P exported from the chloroplast has three carbon atoms, it takes three "turns" of the Calvin cycle to fix enough net carbon to export one G3P. But each turn makes two G3Ps, thus three turns make six G3Ps. One is exported while the remaining five G3P molecules remain in the cycle and are used to regenerate RuBP, which enables the system to prepare for more CO_2 to be fixed. Three more molecules of ATP are used in these regeneration reactions.

Key Points

- The Calvin cycle refers to the light-independent reactions in photosynthesis that take place in three key steps.
- Although the Calvin Cycle is not directly dependent on light, it is indirectly dependent on light since the necessary energy carriers (ATP and NADPH) are products of light-dependent reactions.
- In fixation, the first stage of the Calvin cycle, light-independent reactions are initiated; CO_2 is fixed from an inorganic to an organic molecule.
- In the second stage, ATP and NADPH are used to reduce 3-PGA into G3P; then ATP and NADPH are converted to ADP and $NADP^+$, respectively.
- In the last stage of the Calvin Cycle, RuBP is regenerated, which enables the system to prepare for more CO_2 to be fixed.

Key Terms

- **light-independent reaction**: chemical reactions during photosynthesis that convert carbon dioxide and other compounds into glucose, taking place in the stroma
- **rubisco**: (ribulose bisphosphate carboxylase) a plant enzyme which catalyzes the fixing of atmospheric carbon dioxide during photosynthesis by catalyzing the reaction between carbon dioxide and RuBP
- **ribulose bisphosphate**: an organic substance that is involved in photosynthesis, reacts with carbon dioxide to form 3-PGA

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4.12D: Intermediates Produced During the Calvin Cycle

Learning Objectives

- Outline the function of the intermediates produced in the major phases of the Calvin Cycle

The Calvin Cycle is characterized as a carbon fixation pathway. The Calvin Cycle is also referred to as the reductive pentose phosphate cycle or the Calvin-Benson-Bassham cycle. The process of carbon fixation involves the reduction of carbon dioxide to organic compounds by living organisms. The Calvin cycle is most often associated with carbon fixation in autotrophic organisms, such as plants, and is recognized as a dark reaction. In organisms that require carbon fixation, the Calvin cycle is a means to obtain energy and necessary components for growth. Some examples of microorganisms that utilize the Calvin cycle include cyanobacteria, purple bacteria, and nitrifying bacteria. Specifically, the Calvin cycle involves reducing carbon dioxide to the sugar triose phosphate, most commonly known as glyceraldehyde 3-phosphate (GAP). Throughout the Calvin Cycle, there are numerous intermediate molecules made which are consistently withdrawn and utilized to create cellular material and participate in cellular processes. The Calvin cycle can be divided into three major phases which include: Phase 1: carbon fixation; Phase 2: reduction; and Phase 3: regeneration of ribulose. The following is a brief overview of the intermediates created during the Calvin cycle.

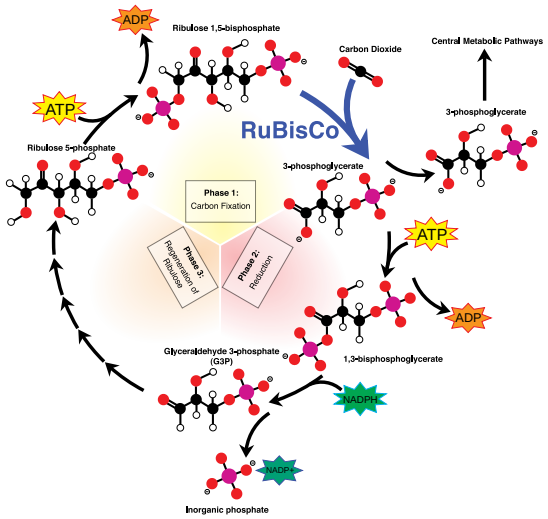


Figure: **The Calvin Cycle**: An overview of the Calvin Cycle.

Phase 1: Carbon Fixation Intermediates

During phase 1 of this cycle, the CO2 molecule is incorporated into one of two 3-phosphoglycerate molecules (3-PGA). This process requires the enzyme RuBisCO and both ATP and NADPH. Once 3-PGA is formed, one of two molecules formed continues into the reduction phase (phase 2). The additional 3-PGA is utilized in additional metabolic pathways such as glycolysis and gluconeogenesis. The structure of 3-PGA allows it to be combined and rearranged to form sugars which can be transported to additional cells or stored for energy.

Phase 2: Reduction

During phase 2 of this cycle, the newly formed 3-PGA undergoes phosphorylation by the enzyme phosphoglycerate kinase which utilizes ATP. The result of this phosphorylation is the production of 1,3-bisphosphoglycerates and ADP products. The ADP product that is produced via the breakdown of ATP will be utilized in additional pathways and be converted back into ATP. The inter conversions of ATP to ADP and ADP to ATP is a key process in supplying energy in numerous processes. This energy is necessary for cellular growth and metabolic processes.

Once the bisphosphoglycerate molecules are formed, they must be converted and further reduced to GAP by NADPH. The intermediate of this product is the conversion of NADPH to NADP+ and an inorganic phosphate ion. NADP+ is a coenzyme which is necessary for the function of NADPH. The functions that require NADP+ include anabolic reactions such as lipid and nucleic

acid synthesis. The inorganic phosphate ion is often a result of regulatory metabolic processes. The phosphate ions are used in processes such as buffering cells, conversions of AMP/ADP to ATP and production of materials involved in structure such as bone and teeth. It is important to note that these intermediates or products (inorganic phosphate, NADP+ and ADP) processed by phase 2 are often regenerated back into the cycle.

Phase 3: Regeneration of Ribulose

The GAP molecules at this point are the end product of the Calvin cycle, which is responsible for reducing carbon to a sugar form. However, additional GAP molecules that are formed will be converted to ribulose-1,5-bisphosphate (RuBP), which is responsible for the conversion of CO2 to 3-PGA in phase 1, via numerous steps. The G3P, which is destined to exit the cycle, will be used for carbohydrate synthesis and additional pathways.

Key Points

- The Calvin Cycle can be divided into three major phases: Phase 1: carbon fixation; Phase 2: reduction; Phase 3: regeneration.
- The intermediates of the Calvin Cycle include ADP, NADP+, inorganic phosphate, and 3-phosphoglycerate.
- Many of the intermediates or products of the Calvin Cycle are regenerated back into earlier stages of the process.

Key Terms

- **autotroph**: Any organism that can synthesize its food from inorganic substances, using heat or light as a source of energy.
- **coenzyme**: Any small molecule that is necessary for the functioning of an enzyme.
- **phosphorylation**: The process of transferring a phosphate group from a donor to an acceptor; often catalysed by enzymes

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4.12E: Regulation of the Calvin Cycle

Learning Objectives

- Outline the three major phases of the Calvin cycle: carbon fixation, reduction, and regeneration of ribulose

The Calvin cycle is a process utilized to ensure carbon dioxide fixation. In this process, carbon dioxide and water are converted into organic compounds that are necessary for metabolic and cellular processes. There are various organisms that utilize the Calvin cycle for production of organic compounds including cyanobacteria and purple and green bacteria. The Calvin cycle requires various enzymes to ensure proper regulation occurs and can be divided into three major phases:

1. carbon fixation,
2. reduction, and
3. regeneration of ribulose.

Each of these phases are tightly regulated and require unique and specific enzymes.

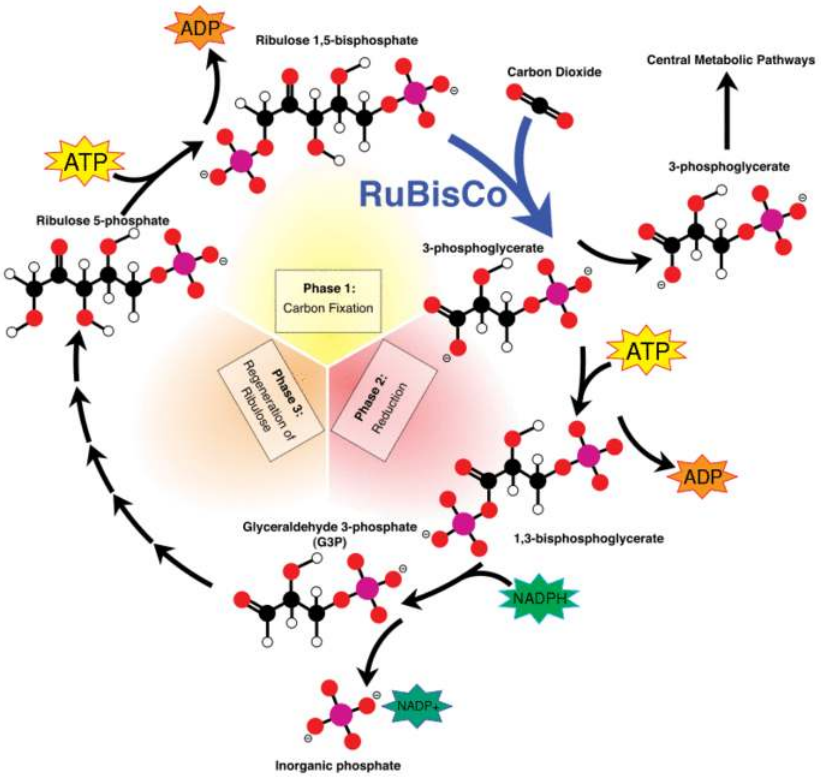


Figure: ***Figure 4.12E.1:*** *Overview of the Calvin cycle. An overview of the Calvin cycle and the three major phases. (CC SA-BY 3.0; Mike Jones).*

During the first phase of the Calvin cycle, carbon fixation occurs. The carbon dioxide is combined with ribulose 1,5-bisphosphate to form two 3-phosphoglycerate molecules (3-PG). The enzyme that catalyzes this specific reaction is ribulose bisphosphate carboxylase (RuBisCO). RuBisCO is identified as the most abundant enzyme on earth, to date. RuBisCO is the first enzyme utilized in the process of carbon fixation and its enzymatic activity is highly regulated. RuBisCO is only active during the day as its substrate, ribulose 1,5-bisphosphate, is not generated in the dark. RuBisCO enzymatic activity is regulated by numerous factors including: ions, RuBisCO activase, ATP /ADP and reduction/oxidation states, phosphate and carbon dioxide. The various factors influencing RuBisCO activity directly affect phase 1 of the Calvin cycle.

During the second phase of the Calvin cycle, reduction occurs. The 3-PG molecules synthesized in phase 1 are reduced to glyceraldehyde-3-phosphate (G3P). This reducing process is mediated by both ATP and NADPH. One of the two G3P molecules formed are further converted to dihydroxyacetone phosphate (DHAP) and the enzyme aldolase is used to combine G3P and DHAP

to form fructose-1,6-bisphosphate. The enzyme aldolase is typically characterized as a glycolytic enzyme with the ability to split fructose 1,6-bisphosphate into DHAP and G3P. However, in this specific phase of the Calvin cycle, it is used in reverse. Therefore, aldolase is said to regulate a reverse reaction in the Calvin cycle. Additionally, aldolase can be utilized to promote a reverse reaction in gluconeogenesis as well. The fructose-1,6-bisphosphate formed in phase 2 is then converted into fructose-6-phosphate.

During the third phase of the Calvin cycle, regeneration of RuBisCO occurs. This specific phase involves a series of reactions in which there are a variety of enzymes required to ensure proper regulation. This phase is characterized by the conversion of G3P, which was produced in earlier phase, back to ribulose 1,5-bisphosphate. This process requires ATP and specific enzymes. The enzymes involved in this process include: triose phosphate isomerase, aldolase, fructose-1,6-bisphosphatase, transketolase, sedoheptulase-1,7-bisphosphatase, phosphopentose isomerase, phosphopentose epimerase, and phosphoribulokinase. The following is a brief summary of each enzyme and its role in the regeneration of ribulose 1,5-bisphosphate in the order it appears in this specific phase.

1. Triose phosphate isomerase: converts all G3P molecules into DHAP
2. Aldolase and fructose-1,6-bisphosphatase: converts G3P and DHAP into fructose 6-phosphate
3. Transketolase: removes two carbon molecules in fructose 6-phosphate to produce erythrose 4-phosphate (E4P); the two removed carbons are added to G3P to produce xylulose-5-phosphate (Xu5P)
4. Aldolase: converts E4P and a DHAP to sedoheptulose-1,7-bisphosphate
5. Sedoheptulase-1,7-bisphosphatase: cleaves the sedohetpulose-1,7-bisphosphate into sedoheptulase-7-phosphate (S7P)
6. Transketolase: removes two carbons from S7P and two carbons are transferred to one of the G3P molecules producing ribose-5-phosphate (R5P)and another Xu5P
7. Phosphopentose isomerase: converts the R5P into ribulose-5-phosphate (Ru5P)
8. Phosphopentose epimerase: converts the Xu5P into Ru5P
9. Phosphoribulokinase: phosphorylates Ru5P into ribulose-1,5-bisphosphate

After this final enzyme performs this conversion, the Calvin cycle is considered complete. The regulation of the Calvin cycle requires many key enzymes to ensure proper carbon fixation.

Key Points

- In this process, carbon dioxide and water are converted into organic compounds that are necessary for metabolic and cellular processes.
- The three phases of the Calvin cycle, fixation, reduction, and regeneration require specific enzymes to ensure proper regulation.
- The last phase of the Calvin cycle, regeneration, is considered the most complex and regulated phase of the cycle.

Key Terms

- **calvin cycle**: A series of biochemical reactions that take place in the stroma of chloroplasts in photosynthetic organisms.
- **gluconeogenesis**: A metabolic process which glucose is formed from non-carbohydrate precursors.
- **ribulose**: A ketopentose whose phosphate derivatives participate in photosynthesis.

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4.12F: The Reverse TCA Cycle

Learning Objectives

- List the enzymes and function that are unique to the reverse TCA cycle (ATP citrate lyase; 2-oxoglutarate:ferredoxin oxidoreductase; pyruvate:ferredoxin oxidoreductase)

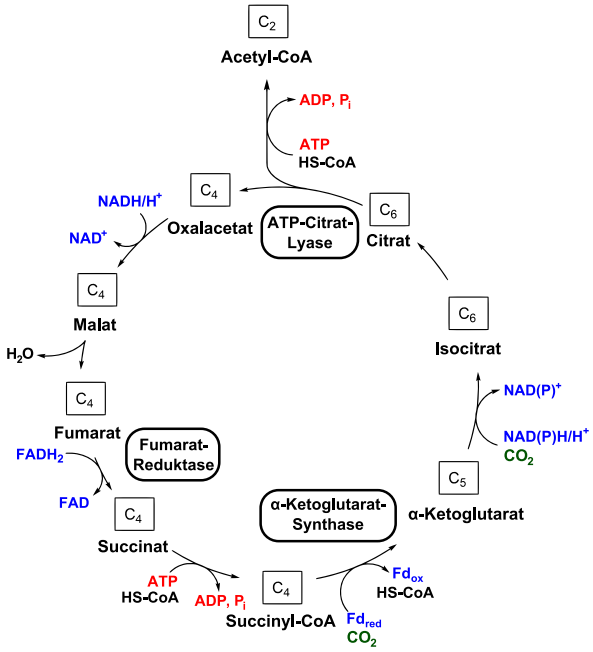


Figure: **The Reverse Citric Acid Cycle**: An overview of the reverse citric acid cycle.

The citric acid cycle (TCA) or Krebs cycle, is a process utilized by numerous organisms to generate energy via the oxidation of acetate derived from carbohydrates, fats, and proteins into carbon dioxide. The cycle plays a critical role in the maintenance of numerous central metabolic processes. However, there are numerous organisms that undergo reverse TCA or reverse Krebs cycles. This process is characterized by the production of carbon compounds from carbon dioxide and water. The chemical reactions that occur are the reverse of what is seen in the TCA cycle. There are numerous anaerobic organisms that utilize a cyclic reverse TCA cycle and an example includes organisms classified as Thermoproteus. The following is a brief overview of the reverse TCA cycle.

Reverse TCA Summary

The reverse TCA cycle is a series of chemical reactions by which organisms produce carbon compounds from carbon dioxide and water. The reverse TCA cycle requires electron donors and often times, bacteria will use hydrogen, sulfide or thiosulfate for this purpose. The reverse TCA is considered to be an alternative to photosynthesis which produces organic molecules as well. Reverse TCA, a form of carbon fixation, utilizes numerous ATP molecules, hydrogen and carbon dioxide to generate an acetyl CoA. This process requires a number of reduction reactions using various carbon compounds. The enzymes, unique to reverse TCA, that function in catalyzing these reactions include: ATP citrate lyase, 2-oxoglutarate:ferredoxin oxidoreductase, and pyruvate:ferredoxin oxidoreductase. ATP citrate lyase is one of the key enzymes that function in reverse TCA. ATP citrate lyase is the enzyme responsible for cleaving citrate into oxaloacetate and acetyl CoA. These enzymes are unique to reverse TCA and are necessary for the reductive carboxylation to occur.

In reverse TCA, the following occurs in a cyclic manner:

1) oxaloacetate is converted to malate (NADH/H+ is utilized and NAD+ is produced)

2) malate is converted to fumarate (H20 molecule is produced)

3) fumarate is converted to succinate via a fumarate-reductase enzyme ($FADH_2$ is converted to FAD)

4) succinate is converted to succinyl-CoA (ATP is hydrolyzed to ADP+Pi)

5) succincyl CoA is converted to alpha-ketoglutarate via an alpha-ketoglutarate synthase (reduction of carbon dioxide occurs and oxidation of coenzyme A)

6) alpha-ketoglutarate is converted to isocitrate (NAD(P)H/H+ and CO_2 is broken down to NAD(P+)

7) isocitrate is converted to citrate

8) ATP citrate lyase is then used to convert citrate to oxaloacetate and acetyl CoA (ATP is hydrolyzed to ADP and Pi).

9) Pathway is cyclic and continues cycle from step 1

An example of a microorganism that utilizes reverse TCA includes Thermoproteus. Thermoproteusis type of prokaryotic that is characterized as a hydrogen-sulfur autotroph. The organisms classified as Thermoproteus utilizes sulfur reduction for metabolic processes. As previously mentioned, organisms that use reverse TCA may use sulfur as an electron donor to carry out this metabolic process.

Key Points

- The TCA cycle utilizes complex carbon molecules and oxidizes them to carbon dioxide and water.
- The reverse TCA utilizes carbon dioxide and water to produce carbon molecules.
- There are three major enzymes that are unique to reverse TCA including ATP citrate lyase which converts citrate into oxaloacetate and acetyl CoA.

Key Terms

- **Krebs cycle**: A series of enzymatic reactions that occurs in all aerobic organisms; it involves the oxidative metabolism of acetyl units and serves as the main source of cellular energy.
- **ATP citrate lyase**: ATP citrate lyase is an enzyme that represents an important step in fatty acid biosynthesis. This step in fatty acid biosynthesis occurs because ATP citrate lyase is the link between the metabolism of carbohydrates (which causes energy) and the production of fatty acids.
- **carboxylation**: A reaction that introduces a carboxylic acid into a molecule.

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4.12G: The Acetyl-CoA Pathway

Learning Objectives

- Describe the role of the carbon monoxide dehydrogenase and acetyl-CoA synthetase in the acetyl-CoA pathway

The acetyl coenzyme A (CoA) pathway, commonly referred to as the Wood-Ljungdahl pathway or the reductive acetyl-CoA pathway, is one of the major metabolic pathways utilized by bacteria. This specific pathway is characterized by the use of hydrogen as an electron donor and carbon dioxide as an electron acceptor to produce acetyl-CoA as the final product. Acetyl-CoA is a major component in numerous metabolic processes as it plays a key role in the citric acid cycle. The main function of acetyl-CoA in the citric cycle is to transport carbon atoms. In regards to molecular structure, acetyl-CoA functions as the thioester between conezyme A and acetic acid. Specific types of organisms that utilize this pathway include archaea classified as methanogens and acetate-producing bacteria as well. The following is a brief overview of the acetyl-CoA pathway..

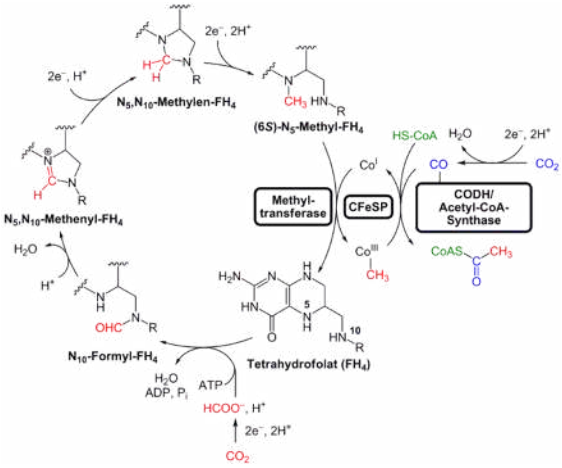


Figure: **Acetyl-CoA Pathway**: An overview of the acetyl-CoA pathway

The acetyl-CoA pathway begins with the reduction of a carbon dioxide to carbon monoxide. The other carbon dioxide is reduced to a carbonyl group. The two major enzymes involved in these processes are carbon monoxide dehydrogenase and acetyl CoA synthase complex. The carbon dioxide that is reduced to a carbonyl group, via the carbon monoxide dehydrogenase, is combined with the methyl group to form acetyl-CoA. The acetyl-CoA synthase complex is responsible for this reaction.

Carbon Monoxide Dehydrogenase

Carbon monoxide dehydrogenase, the enzyme responsible for the reduction of a carbon dioxide to a carbonyl group, functions in numerous biochemical processes. These processes include metabolism of methanogens, acetogenic and sulfate-reducing bacteria. Specifically, the acetyl-CoA pathway is utilized by bacteria that are classified as methanogens and acetate-producing organisms. The carbon monoxide dehydrogenase allows organisms to use carbon dioxide as a source of carbon and carbon monoxide as a source of energy.The carbon monoxide dehydrogenase can also form a complex with the acetyl-CoA synthase complex which is key in the acetyl-CoA pathway.

Acetyl-CoA Synthetase

Acetyl-CoA synthetase is a class of enzymes that is key to the acetyl-CoA pathway. The acetyl-CoA synthetase functions in combining the carbon monoxide and a methyl group to produce acetyl-CoA..

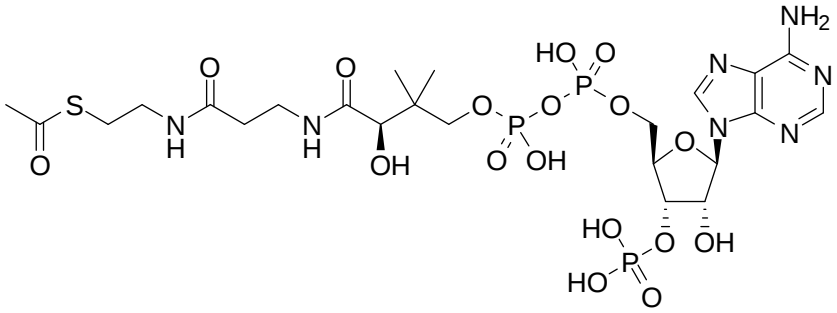


Figure: **Acetyl-CoA Structure**: Schematic of the structure of acetyl-CoA

Microorganisms and the Acetyl-CoA Pathway

The ability to utilize the acetyl-CoA pathway is advantageous due to the ability to utilize both hydrogen and carbon dioxide to produce acetyl-CoA. Specific types of bacteria which utilize the acetyl-CoA pathway include methanogens and acetate-producing bacteria.

Methanogens

Methanogens are types of organisms, classified as archaea, that exhibit the ability to produce methane as a metabolic byproduct. Methanogens, which are found in numerous environments including wetlands, marine sediments, hot springs and hydrothermal vents, are able to use carbon dioxide as a source of carbon for growth. In addition, the carbon dioxide is used as an electron acceptor in the production of methane. Methanogens are able to utilize the acetyl-CoA pathway to fix carbon dioxide.

Acetogens

Acetate producing bacteria, or acetogens, are a class of microorganisms that are able to generate acetate as a product of anaerobic respiration. This process, known as acetogenesis, will occur in organisms that are typically found in anaerobic environments. Acetogens are able to use carbon dioxide as a source of carbon and hydrogen as a source of energy.

Key Points

- The acetyl-CoA pathway utilizes two major enzymes in the production of acetyl-CoA: carbon monoxide dehydrogenase and acetyl-CoA synthase.
- Carbon monoxide dehydrogenase functions in the reduction of carbon dioxide to a methyl group.
- Acetyl-CoA synthase functions in combining carbon monoxide and a methyl group to produce acetyl-CoA.

Key Terms

- **acetogenesis**: The anaerobic production of acetic acid or acetate by bacteria.

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4.12H: The 3-Hydroxypropionate Cycle

Learning Objectives

- Recall the two major phases and known steps in the 3-hydroxypropionate cycle

Carbon fixation is a key pathway in numerous microorganisms, resulting in the formation of organic compounds deemed necessary for cellular processes. One of the pathways that is utilized for carbon fixation is the 3-hydroxypropionate cycle. Specifically, in this cycle, the carbon dioxide is fixed by acetyl-CoA and propionyl-CoA carboxylases. This process results in the formation of malyl-CoA which is further split into acetyl-CoA and glyoxylate. Propionyl-CoA carboxylase is an enzyme that functions in the carboxylation of propionyl CoA. This enzyme functions in the mitochondrial matrix and is biotin dependent. The acetyl-CoA carboxylase utilized in this cycle is biotin-dependent as well and catalyzes the carboxylation of acetyl-CoA to malonyl-CoA.

This pathway produces pyruvate via conversion of bicarbonate and also results in the production of intermediates such as acetyl-CoA, gloxylate and succinyl-CoA. To date, this pathway has been identified in organisms classified as green non sulfur bacteria, specifically *Chloroflexus aurantiacus* *()* and in chemotrophic archaea. The green non sulfur bacteria uses reduced sulfur compounds, such as hydrogen sulfide or thiosulfate as an electron donor for metabolism. The ability of *Chloroflexus aurantiacus* to utilize this pathway is unique. The 3-hydroxypropionate cycle is a newly discovered pathway, thus, the exact details involving this process in regards to enzymes and intracellular components are still currently under investigation. However, the cycle can be broken down into two major phases, carbon dioxide fixation and glyoxylate assimilation. Glyoxylate, the conjugate base of glyoxylic acid, is the form that exists at a neutral pH. The importance of glyoxylate within microorganisms is in its ability to convert fatty acids into carbohydrates. Numerous types of organisms including bacteria, fungi and plants can utilize glyoxylate for these processes.

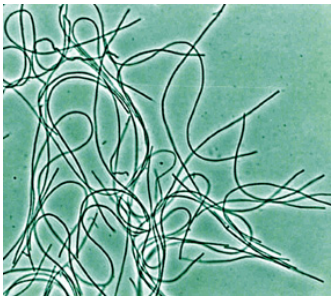


Figure: ***Chloroflexus aurantiacus****: An image of Chloroflexus aurantiacus, a green nonsulfur bacteria that utilizes the 3-hydroxypropionate pathway.*

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Key Points

- The 3-hydroxypropionate cycle is a newly identified pathway and many of the exact details which occur are currently under investigation.
- This pathway is utilized in green non sulfur bacteria such as Chloroflexus aurantiacus.
- The pathway can be divided into major phases which includes carbon dioxide fixation and gloxylate assimilation.

Key Terms

- **glyoxylate**: a salt or ester of glyoxylic acid
- **carboxylation**: A reaction that introduces a carboxylic acid into a molecule.

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SECTION OVERVIEW

4.13: Anabolism

Topic hierarchy

4.13A: Polysaccharide Biosynthesis

4.13B: Lipid Biosynthesis

4.13C: Regulation by Biosynthetic Enzymes

4.13D: Bacterial Polyesters

4.13E: Polyketide Antibiotics

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4.13A: Polysaccharide Biosynthesis

Learning Objectives

- Describe the mechanism of polysaccharide biosynthesis and its importance in bacteria

Polysaccharides are long carbohydrate molecules of repeated monomer units joined together by glycosidic bonds. They range in structure from linear to highly branched. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water.

One of the most common building block of polysaccharides is glucose. However, glucose has to be in its activated forms. There are two forms of activated glucose: UDP-glucose and ADP-glucose.

Uridine diphosphate glucose (uracil-diphosphate glucose, UDP-glucose) is a nucleotide sugar. Components UDP-glucose consists of the pyrophosphate group, the pentose sugar ribose, glucose, and the nucleobase uracil. It is used in nucleotide sugars metabolism as an activated form of glucose as a substrate for enzymes called glucosyltransferases. UDP-glucose can also be used as a precursor of lipopolysaccharides, and peptidoglycan. ADP-glucose is usually the precursor for glycogen production in bacteria.

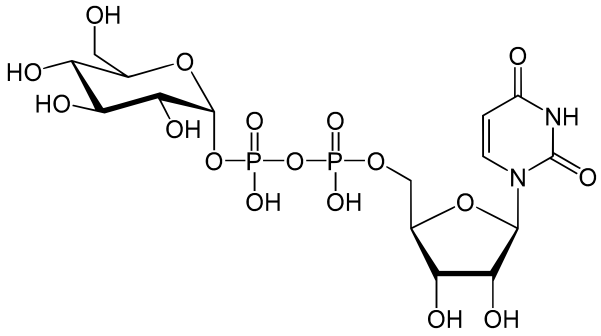


Figure: **Structure of UDP-glucose**: UDP-Glucose consists of the pyrophosphate group, the pentose sugar ribose, glucose, and the nucleobase uracil.

When the cells are grown on a carbon source different than glucose, then polysaccharides are synthesized using a different pathway. Gluconeogenesis (abbreviated GNG) is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as phosphoenolpyruvate (PEP). PEP is formed from the decarboxylation of oxaloacetate and hydrolysis of one guanosine triphosphate molecule. This reaction is a rate-limiting step in gluconeogenesis.

Pathogenic bacteria commonly produce a thick, mucous-like, layer of polysaccharide. This “capsule” cloaks antigenic proteins on the bacterial surface that would otherwise provoke an immune response and thereby lead to the destruction of the bacteria. Bacteria and many other microbes, including fungi and algae, often secrete polysaccharides as an evolutionary adaptation to help them adhere to surfaces and to prevent them from drying out. Humans have developed some of these polysaccharides into useful products including xanthan gum, dextran, welan gum, gellan gum, diutan gum, and pullulan.

Key Points

- Uridine diphosphate glucose (UDP-glucose) is a nucleotide sugar. UDP-glucose consists of the pyrophosphate group, the pentose sugar ribose, glucose, and the nucleobase uracil. It is used as a substrate for enzymes called glucosyltransferases.
- UDP-glucose can also be used as a precursor of lipopolysaccharides, and peptidoglycan. ADP-glucose is usually the precursor for glycogen production in bacteria.
- When the cells are grown on a carbon source different than glucose, gluconeogenesis (abbreviated GNG) is the metabolic pathway used to produce glucose from non- carbohydrate carbon substrates such as phosphoenolpyruvate (PEP).
- Pathogenic bacteria can produce a thick, mucous-like, layer of polysaccharide. This “capsule” cloaks antigenic proteins on the bacterial surface. Bacteria and other microbes, secrete polysaccharides as an evolutionary adaptation to help them adhere to surfaces and to prevent them from drying out.

Key Terms

- **Polysaccharides**: Polysaccharides are long, carbohydrate molecules of repeated monomer units joined together by glycosidic bonds. They range in structure from linear to highly branched. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit.
- **gluconeogenesis**: Gluconeogenesis (abbreviated GNG) is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol, and glucogenic amino acids.
- **glucosyltransferases**: Glucosyltransferases are a type of glycosyltransferase that enable the transfer of glucose such as glycogen synthesis.

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4.13B: Lipid Biosynthesis

Learning Objectives

- Outline the characteristics and processes of lipid biosynthesis, including:; lipogenesis and fatty acid biosynthesis

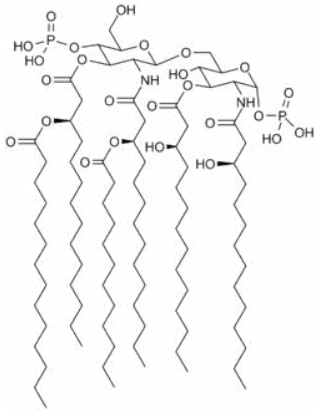


Figure: **Chemical structure of lipid A as found in E. coli**: Chemical structure of lipid A as found in E. coli

Lipids constitute a broad group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules.

Lipids may be broadly defined as hydrophobic or amphiphilic small molecules. The amphiphilic nature of some lipids allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment. Biological lipids originate entirely or in part from two distinct types of biochemical subunits or “building-blocks”: ketoacyl and isoprene groups. Using this approach, lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides (derived from condensation of ketoacyl subunits); and sterol lipids and prenol lipids (derived from condensation of isoprene subunits).

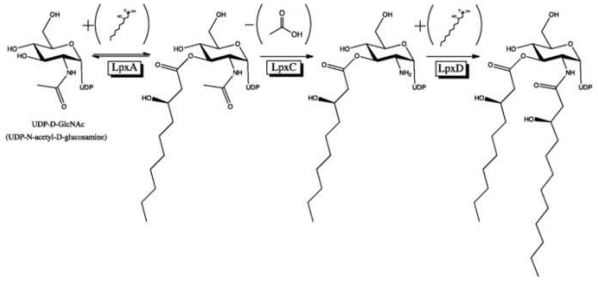


Figure: **Synthesis of the UDP-diacylglucosamine precursor of Lipid A**: Synthesis of the UDP-diacylglucosamine precursor of Lipid AThe synthesis of unsaturated fatty acids involves a desaturation reaction, whereby a double bond is introduced into the fatty acyl chain. For example, in humans, the desaturation of stearic acid by stearoyl-CoA desaturase-1 produces oleic acid. The doubly unsaturated fatty acid linoleic acid as well as the triply unsaturated α-linolenic acid cannot be synthesized in mammalian tissues, and are therefore essential fatty acids and must be obtained from the diet. Triglyceride synthesis takes place in the endoplasmic reticulum by metabolic pathways in which acyl groups in fatty acyl-CoAs are transferred to the hydroxyl groups of glycerol-3-phosphate and diacylglycerol. Terpenes and isoprenoids, including the carotenoids, are made by the assembly and modification of isoprene units donated from the reactive precursors isopentenyl pyrophosphate and dimethylallyl pyrophosphate. These precursors can be made in different ways. In archaea, the mevalonate pathway produces these compounds from acetyl-CoA, while in plants and bacteria the non-mevalonate pathway uses pyruvate and glyceraldehyde 3-phosphate as substrates.

Although humans and other mammals use various biosynthetic pathways to both break down and synthesize lipids, some essential lipids cannot be made this way and must be obtained from the diet.

In animals, when there is an oversupply of dietary carbohydrate, the excess carbohydrate is converted to triglycerides. This involves the synthesis of fatty acids from acetyl-CoA and the esterification of fatty acids in the production of triglycerides, a process called lipogenesis. Fatty acids are made by fatty acid synthases that polymerize and then reduce acetyl-CoA units. The acyl

chains in the fatty acids are extended by a cycle of reactions that add the acetyl group, reduce it to an alcohol, dehydrate it to an alkene group and then reduce it again to an alkane group.

The enzymes of fatty acid biosynthesis are divided into two groups, in animals and fungi all these fatty acid synthase reactions are carried out by a single multifunctional protein, while in plant plastids and bacteria separate enzymes perform each step in the pathway. The fatty acids may be subsequently converted to triglycerides that are packaged in lipoproteins and secreted from the liver.

One important reaction that uses these activated isoprene donors is steroid biosynthesis. Here, the isoprene units are joined together to make squalene and then folded up and formed into a set of rings to make lanosterol. Lanosterol can then be converted into other steroids such as cholesterol and ergosterol.

Key Points

- Lipid A is a lipid component of an endotoxin held responsible for toxicity of Gram-negative bacteria.
- The synthesis of unsaturated fatty acids involves a desaturation reaction, whereby a double bond is introduced into the fatty acyl chain.
- In archaea, the mevalonate pathway produces reactive precursors isopentenyl pyrophosphate and dimethylallyl pyrophosphate from acetyl-CoA, while in plants and bacteria the non-mevalonate pathway uses pyruvate and glyceraldehyde 3-phosphate as substrates.

Key Terms

- **lipid A**: Lipid A is a lipid component of an endotoxin held responsible for toxicity of Gram-negative bacteria. It is the innermost of the three regions of the lipopolysaccharide (LPS, also called endotoxin) molecule, and its hydrophobic nature allows it to anchor the LPS to the outer membrane.
- **endotoxin**: Any toxin secreted by a microorganism and released into the surrounding environment only when it dies.
- **lipogenesis**: The biochemical production of fat, especially the conversion of carbohydrate into fat so that it may be stored as a long-term source of energy when food is scarce.

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4.13C: Regulation by Biosynthetic Enzymes

Learning Objectives

- Discuss how attenuation can regulate expression of biosynthetic enzymes

Attenuation (in genetics) is a proposed mechanism of control in some bacterial operons that results in premature termination of transcription. It is based on the fact that, in bacteria, transcription and translation can and do proceed simultaneously. Attenuation involves a provisional stop signal (attenuator), located in the DNA segment that corresponds to the leader sequence of mRNA. During attenuation, the ribosome becomes stalled (delayed) in the attenuator region in the mRNA leader. Depending on the metabolic conditions, the attenuator either stops transcription at that point or allows read-through to the structural gene part of the mRNA and synthesis of the appropriate protein.

Attenuation is a regulatory feature found throughout Archaea and Bacteria causing premature termination of transcription. Attenuators are 5′-cis acting regulatory regions that fold into one of two alternative RNA structures that determine the success of transcription. The folding is modulated by a sensing mechanism producing either a Rho-independent terminator, resulting in interrupted transcription and a non-functional RNA product; or an anti-terminator structure, resulting in a functional RNA transcript. There are now many equivalent examples where the translation, not transcription, is terminated by sequestering the Shine-Dalgarno sequence (ribosomal binding site) in a hairpin-loop structure. While not meeting the previous definition of (transcriptional) attenuation, these are now considered to be variants of the same phenomena and are included in this article. Attenuation is an ancient regulatory system, prevalent in many bacterial species providing fast and sensitive regulation of gene operons and is commonly used to repress genes in the presence of their own product (or a downstream metabolite). What is an attenuator? Attenuator is a nucleotide sequence in DNA that can lead to premature termination of transcription.

Attenuators may be classified according to the type of molecule which induces the change in RNA structure. It is likely that transcription-attenuation mechanisms developed early, perhaps prior to the archaea/bacteria separation and have since evolved to use a number of different sensing molecules (the tryptophan biosynthetic operon has been found to use three different mechanisms in different organisms.)

An example is the trp gene in bacteria. When there is a high level of tryptophan in the region, it is inefficient for the bacterium to synthesize more. When the RNA polymerase binds and transcribes the trp gene, the ribosome will start translating. (This differs from eukaryotic cells, where RNA must exit the nucleus before translation starts.) The attenuator sequence, which is located between the mRNA leader sequence (5′ UTR) and trp operon gene sequence, contains four domains, where domain 3 can pair with domain 2 or domain 4.

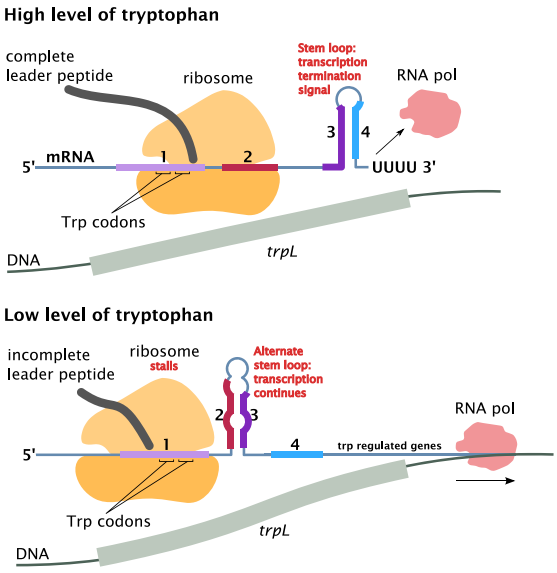


Figure: **Mechanism of transcriptional attenuation of the trp operon.**: Mechanism of transcriptional attenuation of the trp operon.

The attenuator sequence at domain 1 contains instruction for peptide synthesis that requires tryptophans. A high level of tryptophan will permit ribosomes to translate the attenuator sequence domains 1 and 2, allowing domains 3 and 4 to form a hairpin structure, which results in termination of transcription of the trp operon. Since the protein coding genes are not transcribed due to rho independent termination, no tryptophan is synthesized.

In contrast, a low level of tryptophan means that the ribosome will stall at domain 1, causing the domains 2 and 3 to form a different hairpin structure that does not signal termination of transcription. Therefore, the rest of the operon will be transcribed and translated, so that tryptophan can be produced. Thus, domain 4 is an attenuator. Without domain 4, translation can continue regardless of the level of tryptophan. The attenuator sequence has its codons translated into a leader peptide, but is not part of the trp operon gene sequence. The attenuator allows more time for the attenuator sequence domains to form loop structures, but does not produce a protein that is used in later tryptophan synthesis.

Key Points

- Attenuators may be classified according to the type of molecule that induces the change in RNA structure.
- Attenuator is a nucleotide sequence in DNA that can lead to premature termination of transcription.
- An example of attenuation is the trp gene in bacteria.

Key Terms

- **transcription**: The synthesis of RNA under the direction of DNA.
- **termination**: The process of terminating or the state of being terminated.
- **Attenuation**: Attenuation (in genetics) is a proposed mechanism of control in some bacterial operons that results in premature termination of transcription. It is based on the fact that, in bacteria, transcription and translation can and do proceed simultaneously.

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4.13D: Bacterial Polyesters

Learning Objectives

- Summarize the process of PHA production and its applications

Polyhydroxyalkanoates, or PHAs, are linear polyesters produced in nature by bacterial fermentation of sugar or lipids. They are produced by the bacteria to store carbon and energy. More than 150 different monomers can be combined within this family to give materials extremely diverse properties. These plastics are biodegradeable and are used in the production of bioplastics. They can be either thermoplastic or elastomeric materials, with melting points ranging from 40 to 180°C.

The mechanical qualities and biocompatibility of PHA can also be changed by blending, modifying the surface or combining PHA with other polymers, enzymes and inorganic materials, making it possible for a wider range of applications.

PROCESS OF PHA PRODUCTION

To produce PHA, a culture of a micro-organism such as Alcaligenes eutrophus is placed in a suitable medium and fed appropriate nutrients so that it multiplies rapidly. The biosynthesis of PHA is usually caused by certain deficiency conditions (e.g. lack of macro elements such as phosphorus, nitrogen, trace elements, or lack of oxygen) and the excess supply of carbon sources. Recombinants Bacillus subtilis str. pBE2C1 and Bacillus subtilis str. pBE2C1AB were used in production of polyhydroxyalkanoates (PHA) and it was shown that they could use malt waste as carbon source for lower cost of PHA production. As raw material for the fermentation, carbohydrates such as glucose and sucrose can be used, but also vegetable oil or glycerine from biodiesel production. Researchers in the industry are working on methods with which transgenic crops will be developed that express PHA synthesis routes from bacteria and so produce PHA as energy storage in their tissues. Another group of researchers at Micromidas is working to develop methods of producing PHA from municipal waste water. Another even larger scale synthesis can be done with the help of soil organisms. For lack of nitrogen and phosphorus they produce a kilogram of PHA from three kilograms of sugar.

Polyesters are deposited in the form of highly refractive granules in the cells. Depending upon the microorganism and the cultivation conditions, homo- or copolyesters with different hydroxyalkanic acids are generated. PHAs granules are then recovered by disrupting the cells. In the industrial production of PHA, the polyester is extracted and purified from the bacteria by optimizing the conditions of microbial fermentation of sugar or glucose. Once the population has reached a substantial level, the nutrient composition is changed to force the micro-organism to synthesize PHA. The yield of PHA obtained from the intracellular inclusions can be as high as 80% of the organism's dry weight.

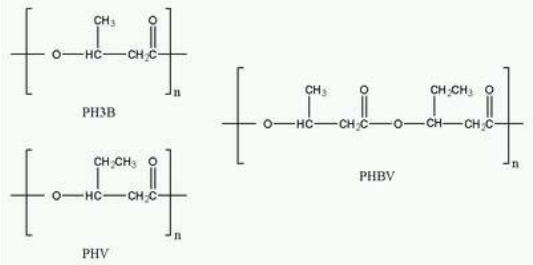


Figure: **Chemical structures of P3HB, PHV and their copolymer PHBV**: Chemical structures of P3HB, PHV and their copolymer PHBV

PHA SYNTHASES

PHA synthases are the key enzymes of PHA biosynthesis. They use the coenzyme A – thioester of (r)-hydroxy fatty acids as substrates. The two classes of PHA synthases differ in the specific use of hydroxyfattyacids of short or medium chain length. The resulting PHA is of the two types: Poly (HA SCL) from hydroxy fatty acids with short chain lengths including three to five carbon atoms are synthesized by numerous bacteria, including Ralstonia eutropha and Alcaligenes latus (PHB). Poly (HA MCL) from hydroxy fatty acids with middle chain lengths including six to 14 carbon atoms, can be made for example, by Pseudomonas putida. A few bacteria, including Aeromonas hydrophila and Thiococcus pfennigii, synthesize copolyester from the above two types of

hydroxy fatty acids. The simplest and most commonly occurring form of PHA is the fermentative production of poly-beta-hydroxybutyrate) (poly-3-hydroxybutyrate, P3HB), which consists of 1000 to 30000 hydroxy fatty acid monomers.

PHA APPLICATIONS

PHAs are processed mainly via injection molding, extrusion and extrusion bubbles into films and hollow bodies. A PHA copolymer called PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) is less stiff and tougher, and it may be used as packaging material. There are also applications for PHA produced by micro-organisms within the medical and pharmaceutical industries, primarily due to their biodegradability. Some of the fixation and orthopaedic applications that have been devised for these polymers include:

- sutures and suture fasteners
- meniscus repair and regeneration devices
- rivets, tacks, staples, and screws
- bone plates and bone plating systems
- surgical mesh, repair patches, and cardiovascular patches
- vein valves, bone marrow scaffolds
- ligament and tendon grafts
- ocular cell implants
- skin substitutes, bone graft substitutes, and wound dressings

Key Points

- To produce PHA, a culture of a micro- organism such as Alcaligenes eutrophus is placed in a suitable medium and fed appropriate nutrients so that it multiplies rapidly.
- PHA synthases are the key enzymes of PHA biosynthesis.
- There are potential applications for PHA produced by micro-organisms within the medical and pharmaceutical industries, primarily due to their biodegradability.

Key Terms

- **Polyhydroxyalkanoates**: Polyhydroxyalkanoates or PHAs are linear polyesters produced in nature by bacterial fermentation of sugar or lipids.
- **fermentation**: Any of many anaerobic biochemical reactions in which an enzyme (or several enzymes produced by a microorganism) catalyses the conversion of one substance into another; especially the conversion (using yeast) of sugars to alcohol or acetic acid with the evolution of carbon dioxide.
- **biodegradability**: The capacity of a material to decompose over time as a result of biological activity, especially to be broken down by microorganisms

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4.13E: Polyketide Antibiotics

Learning Objectives

- Describe the characteristics associated with polyketides, including: type I, II and III polyketides

Polyketides are secondary metabolites produced from bacteria, fungi, plants, and animals.

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Unlike primary metabolites, the absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, and recreational drugs.

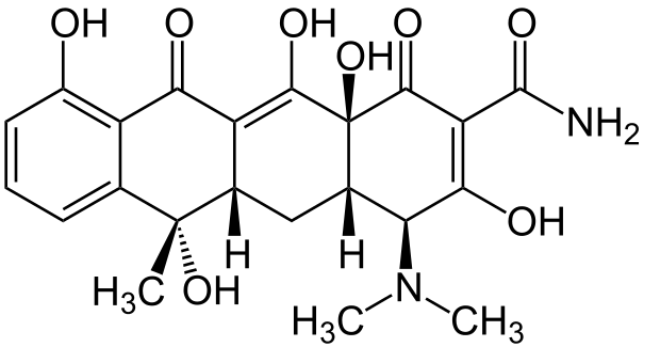


Figure: **Tetracycline structural formula**

Polyketides are usually biosynthesized through the decarboxylative condensation of malonyl-CoA derived extender units in a similar process to fatty acid biosynthesis (a Claisen condensation). The polyketide chains produced by a minimal polyketide synthase are often further derivitized and modified into bioactive natural products.

Polyketides are structurally a very diverse family of natural products with diverse biological activities and pharmacological properties. They are broadly divided into three classes: type I polyketides (often macrolides produced by multimodular megasynthases), type II polyketides (often aromatic molecules produced by the iterative action of dissociated enzymes), and type III polyketides (often small aromatic molecules produced by fungal species). Polyketide antibiotics, antifungals, cytostatics, anticholesteremic, antiparasitics, coccidiostats, animal growth promoters, and natural insecticides are in commercial use.

Examples of polyketides include: Macrolides; Pikromycin, the first isolated macrolide; the antibiotics erythromycin A; clarithromycin, and azithromycin; the immunosuppressant tacrolimus; Radicicol and Pochonin family (HSP90 inhibitor); Polyene antibiotics; Amphotericin; Tetracyclines and the tetracycline family of antibiotics.

Polyketides are synthesized by one or more specialized and highly complex polyketide synthase (PKS) enzymes.

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Key Points

- Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism.
- Polyketides are usually biosynthesized through the decarboxylative condensation of malonyl-CoA derived extender units in a similar process to fatty acid biosynthesis.
- Polyketides are structurally a very diverse family of natural products with diverse biological activities and pharmacological properties.

Key Terms

- **Polyketides**: Polyketides are secondary metabolites from bacteria, fungi, plants, and animals. Polyketides are usually biosynthesized through the decarboxylative condensation of malonyl-CoA derived extender units in a similar process to fatty acid synthesis (a Claisen condensation).
- **metabolites**: Metabolites are the intermediates and products of metabolism. The term metabolite is usually restricted to small molecules. Metabolites have various functions, including fuel, structure, signaling, stimulatory and inhibitory effects on enzymes, catalytic activity of their own (usually as a cofactor to an enzyme), defense, and interactions with other organisms (e.g. pigments, odorants, and pheromones).
- **biosynthesized**: Biosynthesis (also called biogenesis or “anabolism”) is an enzyme-catalyzed process in cells of living organisms by which substrates are converted to more complex products. The biosynthesis process often consists of several enzymatic steps in which the product of one step is used as substrate in the following step.

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SECTION OVERVIEW

4.14: Amino Acids and Nucleotide Biosynthesis

Topic hierarchy

4.14A: Amino Acid Synthesis

4.14B: Purine and Pyrimidine Synthesis

4.14C: Nonribosomal Peptide Antibiotics

4.14D: Biosynthesis of Tetrapyrroles

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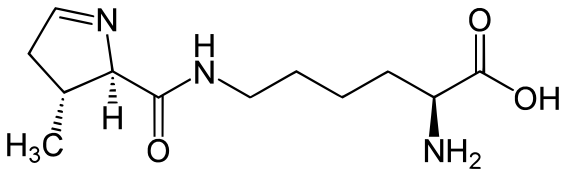
4.14A: Amino Acid Synthesis

Learning Objectives

- Recognize the factors involved in amino acid synthesis

Amino acids are the structural units that make up proteins. They join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins. These polymers are linear and unbranched, with each amino acid within the chain attached to two neighboring amino acids. The process of making proteins is called translation and involves the step-by-step addition of amino acids to a growing protein chain by a ribozyme that is called a ribosome. The order in which the amino acids are added is read through the genetic code from an mRNA template, which is a RNA copy of one of the organism ‘s genes.

Twenty-two amino acids are naturally incorporated into polypeptides and are called proteinogenic or natural amino acids. Of these, 20 are encoded by the universal genetic code. The remaining two, selenocysteine and pyrrolysine, are incorporated into proteins by unique synthetic mechanisms. Selenocysteine is incorporated when the mRNA being translated includes a SECIS element, which causes the UGA codon to encode selenocysteine instead of a stop codon. Pyrrolysine is used by some methanogenic archaea in enzymes that they use to produce methane. It is coded with the codon UAG, which is normally a stop codon in other organisms. Pyrrolysine (abbreviated as Pyl or O) is a naturally occurring amino acid similar to lysine, but with an added pyrroline ring linked to the end of the lysine side chain. Produced by a specific tRNA and aminoacyl tRNA synthetase, it forms part of an unusual genetic code in these organisms. It is considered the 22nd proteinogenic amino acid. This UAG codon is followed by a PYLIS downstream sequence.



Structure of Pyrrolysine: Pyrrolysine (abbreviated as Pyl or O) is a naturally occurring, genetically coded amino acid used by some methanogenic archaea and one known bacterium in enzymes that are part of their methane-producing metabolism.

Organisms vary in their ability to synthesize the 20 common amino acids. Most bacteria and plants can synthesize all 20. Some simple parasites, such as the bacteria *Mycoplasma pneumoniae*, lack all amino acid synthesis and take their amino acids directly from their hosts. All amino acids are synthesized from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway. Nitrogen is provided by glutamate and glutamine. Amino acid synthesis depends on the formation of the appropriate alpha-keto acid, which is then transaminated to form an amino acid. Amino acids are made into proteins by being joined together in a chain by peptide bonds. Each different protein has a unique sequence of amino acid residues: this is its primary structure. Just as the letters of the alphabet can be combined to form an almost endless variety of words, amino acids can be linked in varying sequences to form a huge variety of proteins. Proteins are made from amino acids that have been activated by attachment to a transfer RNA molecule through an ester bond.

Aside from the 22 standard amino acids, there are many other amino acids that are called non-proteinogenic or non-standard. Those either are not found in proteins (for example carnitine, GABA) or are not produced directly and in isolation by standard cellular machinery (for example, hydroxyproline and selenomethionine). Non-standard amino acids that are found in proteins are formed by post-translational modification, which is modification after translation during protein synthesis. These modifications are often essential for the function or regulation of a protein. For example, the carboxylation of glutamate allows for better binding of calcium cations. The hydroxylation of proline is critical for maintaining connective tissues. Another example is the formation of hypusine in the translation initiation factor EIF5A, through modification of a lysine residue. Such modifications can also determine the localization of the protein, e.g., the addition of long hydrophobic groups can cause a protein to bind to a phospholipid membrane. Some nonstandard amino acids are not found in proteins. Examples include lanthionine, 2-aminoisobutyric acid, dehydroalanine, and the neurotransmitter gamma-aminobutyric acid. Nonstandard amino acids often occur as intermediates in the metabolic pathways for standard amino acids — for example, ornithine and citrulline occur in the urea cycle, part of amino acid catabolism.

Key Points

- All amino acids are synthesized from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway.
- Amino acid synthesis depends on the formation of the appropriate alpha-keto acid, which is then transaminated to form an amino acid.
- Of the 22 amino acids naturally incorporated into proteins, 20 are encoded by the universal genetic code and the remaining two, selenocysteine and pyrrolysine, are incorporated into proteins by unique synthetic mechanisms.

Key Terms

- **pyrrolysine**: An amino acid found in methanogenic bacteria.
- **selenocysteine**: A naturally-occurring amino acid, present in several enzymes, whose structure is that of cysteine but with the sulfur atom replaced by one of selenium.
- **genetic code**: The set of rules by which the sequence of bases in DNA are translated into the amino acid sequence of proteins

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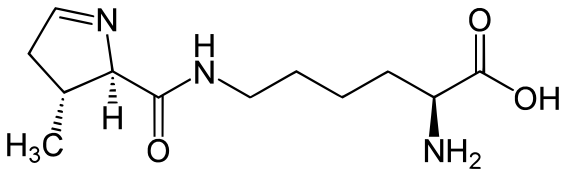
4.14B: Purine and Pyrimidine Synthesis

Learning Objectives

- Recognize the factors involved in amino acid synthesis

Amino acids are the structural units that make up proteins. They join together to form short polymer chains called peptides or longer chains called either polypeptides or proteins. These polymers are linear and unbranched, with each amino acid within the chain attached to two neighboring amino acids. The process of making proteins is called translation and involves the step-by-step addition of amino acids to a growing protein chain by a ribozyme that is called a ribosome. The order in which the amino acids are added is read through the genetic code from an mRNA template, which is a RNA copy of one of the organism ‘s genes.

Twenty-two amino acids are naturally incorporated into polypeptides and are called proteinogenic or natural amino acids. Of these, 20 are encoded by the universal genetic code. The remaining two, selenocysteine and pyrrolysine, are incorporated into proteins by unique synthetic mechanisms. Selenocysteine is incorporated when the mRNA being translated includes a SECIS element, which causes the UGA codon to encode selenocysteine instead of a stop codon. Pyrrolysine is used by some methanogenic archaea in enzymes that they use to produce methane. It is coded with the codon UAG, which is normally a stop codon in other organisms. Pyrrolysine (abbreviated as Pyl or O) is a naturally occurring amino acid similar to lysine, but with an added pyrroline ring linked to the end of the lysine side chain. Produced by a specific tRNA and aminoacyl tRNA synthetase, it forms part of an unusual genetic code in these organisms. It is considered the 22nd proteinogenic amino acid. This UAG codon is followed by a PYLIS downstream sequence.



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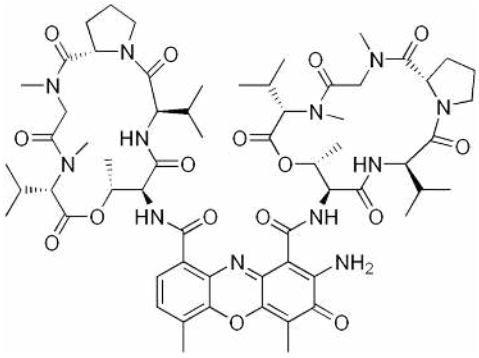
4.14C: Nonribosomal Peptide Antibiotics

Learning Objectives

- Outline the characteristics associated with nonribosomal peptides and the production of antibiotics

Nonribosomal peptides (NRP) are a class of peptide secondary metabolites, usually produced by microorganisms like bacteria and fungi. Nonribosomal peptides are also found in higher organisms (such as nudibranchs) but are thought to be made by bacteria inside these organisms. While there exists a wide range of peptides that are not synthesized by ribosomes, the term nonribosomal peptide typically refers to a very specific set of these as discussed in this article.

Nonribosomal peptides are synthesized by nonribosomal peptide synthetases, which, unlike the ribosomes, are independent of messenger RNA. Each nonribosomal peptide synthetase can synthesize only one type of peptide. Nonribosomal peptides often have a cyclic and/or branched structures, can contain non-proteinogenic amino acids including D-amino acids, carry modifications like N-methyl and N-formyl groups, or are glycosylated, acylated, halogenated, or hydroxylated. Cyclization of amino acids against the peptide "backbone" is often performed, resulting in oxazolines and thiazolines; these can be further oxidized or reduced. On occasion, dehydration is performed on serines, resulting in dehydroalanine. This is just a sampling of the various manipulations and variations that nonribosomal peptides can perform. Nonribosomal peptides are often dimers or trimers of identical sequences chained together or cyclized, or even branched.



Actinomycin D: Actinomycin D (also known generically as Actinomycin or Dactinomycin), is the most significant member of actinomycines, which are a class of polypeptide antibiotics isolated from soil bacteria of the genus Streptomyces. As one of the older chemotherapy drugs, it has been used for many years.

Nonribosomal peptides are a very diverse family of natural products with an extremely broad range of biological activities and pharmacological properties. They are often toxins, siderophores, or pigments. Nonribosomal peptide antibiotics (for example, actinomycin D), cytostatics, and immunosuppressants are used commercially.

Nonribosomal peptides are synthesized by one or more specialized nonribosomal peptide-synthetase (NRPS) enzymes. The NRPS genes for a certain peptide are usually organized in one operon in bacteria and in gene clusters in eukaryotes. However the first fungal NRP to be found was ciclosporin. It is synthesized by a single 1.6MDa NRPS. The enzymes are organized in modules that are responsible for the introduction of one additional amino acid. Each module consists of several domains with defined functions, separated by short spacer regions of about 15 amino acids.

The biosynthesis of nonribosomal peptides shares characteristics with the polyketide and fatty acid biosynthesis. Due to these structural and mechanistic similarities, some nonribosomal peptide synthetases contain polyketide synthase modules for the insertion of acetate or propionate-derived subunits into the peptide chain.

Key Points

- Nonribosomal peptides are produced by microorganisms like bacteria and fungi but are also found in higher organisms such as nudibranchs where they are thought to be made by bacteria inside these organisms.

- Nonribosomal peptides are synthesized by nonribosomal peptide synthetases, which, unlike the ribosomes, are independent of messenger RNA.
- The biosynthesis of nonribosomal peptides shares characteristics with the polyketide and fatty acid biosynthesis.

Key Terms

- **peptide**: A class of organic compounds consisting of various numbers of amino acids, in which the amine of one is reacted with the carboxylic acid of the next to form an amide bond.
- **metabolite**: Any substance produced by, or taking part in, a metabolic reaction.
- **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 Fe3+ binding agents known.

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4.14D: Biosynthesis of Tetrapyrroles

Learning Objectives

- Recall the process of tetrapyrrole biosynthesis

Porphyrins are a group of organic compounds, many naturally occurring. One of the best-known porphyrins is heme, the pigment in red blood cells. Heme is a cofactor of the protein hemoglobin. The main "application" of porphyrins is their role in supporting aerobic life. For example, complexes of meso-tetraphenylporphyrin, e.g., the iron(III) chloride complex (TPPFeCl), catalyze a variety of reactions of potential interest in organic synthesis.

Porphyrins are heterocyclic macrocycles composed of four modified pyrrole subunits interconnected at their α carbon atoms via methine bridges (=CH-). Porphyrins are aromatic, obeying Hückel's rule for aromaticity, possessing 4n+2 π electrons (n=4 for the shortest cyclic path) delocalized over the macrocycle. Thus, porphyrin macrocycles are highly conjugated systems. As a consequence, they typically have very intense absorption bands in the visible region and may be deeply colored. (The name porphyrin comes from a Greek word for purple.) The macrocycle has 26 pi electrons in total. The parent porphyrin is porphine, and substituted porphines are called porphyrins.

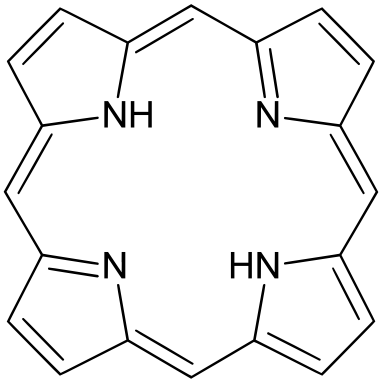


Figure: **Structure of Porphine**: Porphine is the simplest porphyrin, an aromatic organic compound.

Porphyrins are the conjugate acids of ligands that bind metals to form complexes. The metal ion usually has a charge of 2+ or 3+. A schematic equation for these syntheses is:

H_2porphyrin + [MLn]2+ → M(porphyrinate)Ln-4 + 4 L + 2 H+

where M=metal ion and L=a ligand

A porphyrin without a metal ion in its cavity is a free base. Some iron-containing porphyrins are called hemes. Heme-containing proteins, or hemoproteins, are found extensively in nature. Hemoglobin and myoglobin are two O2-binding proteins that contain iron porphyrins. Various cytochromes are also hemoproteins. Several other heterocycles are related to porphyrins. These include corrins, chlorins, bacteriochlorophylls and corphins. Chlorins (2,3-dihydroporphyrin) are more reduced, contain more hydrogen than porphyrins, and feature a pyrroline subunit. This structure occurs in a chlorophyll molecule. Replacement of two of the four pyrrolic subunits with pyrrolinic subunits results in either a bacteriochlorin (as found in some photosynthetic bacteria) or an isobacteriochlorin, depending on the relative positions of the reduced rings. Some porphyrin derivatives follow Hückel's rule, but most do not.

The "committed step" for porphyrin biosynthesis is the formation of δ-aminolevulinic acid (δ-ALA, 5-ALA or dALA) by the reaction of the amino acid glycine with succinyl-CoA from the citric acid cycle. Two molecules of dALA combine to give porphobilinogen (PBG), which contains a pyrrole ring. Four PBGs are then combined through deamination into hydroxymethyl bilane (HMB), which is hydrolysed to form the circular tetrapyrrole uroporphyrinogen III. This molecule undergoes a number of further modifications. Intermediates are used in different species to form particular substances, but, in humans, the main end-product protoporphyrin IX is combined with iron to form heme. Bile pigments are the breakdown products of heme.

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Key Points

- Porphyrins are a group of organic compounds, many naturally occurring, such as heme.
- Porphyrins are aromatic, obeying Hückel's rule for aromaticity, possessing $4n+2$ π electrons ($n=4$ for the shortest cyclic path) delocalized over the macrocycle.
- Porphyrin macrocycles are highly conjugated systems and typically have very intense absorption bands in the visible region, and may be deeply colored.

Key Terms

- **heterocyclic**: Having one or more atoms other than carbon in at least one of its rings.
- **porphyrin**: Any of a class of heterocyclic compounds containing four pyrrole rings arranged in a square; they are important in biochemistry in a form with a metal atom in the central cavity (hemoglobin with iron, chlorophyll with magnesium, etc.).
- **aromatic**: Having a closed ring of alternate single and double bonds with delocalized electrons.

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SECTION OVERVIEW

4.15: Nitrogen Fixation

Topic hierarchy

4.15A: Nitrogenase and Nitrogen Fixation

4.15B: Early Discoveries in Nitrogen Fixation

4.15C: Nitrogen Fixation Mechanism

4.15D: Anaerobiosis and N_2 Fixation

4.15E: Genetics and Regulation of N_2 Fixation

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4.15A: Nitrogenase and Nitrogen Fixation

Learning Objectives

- Describe the importance of nitrogen fixation

Nitrogen fixation also refers to other biological conversions of nitrogen, such as its conversion to nitrogen dioxide. Nitrogen fixation is a process by which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_3). Atmospheric nitrogen or elemental nitrogen (N_2) is relatively inert: it does not easily react with other chemicals to form new compounds. Dinitrogen is quite inert because of the strength of its N≡N triple bond. To break one nitrogen atom away from another requires breaking all three of these chemical bonds. Fixation processes free up the nitrogen atoms from their diatomic form (N_2) to be used in other ways. Nitrogen fixation, natural and synthetic, is essential for all forms of life because nitrogen is required to biosynthesize basic building blocks of plants, animals, and other life forms, e.g., nucleotides for DNA and RNA and amino acids for proteins. Therefore, nitrogen fixation is essential for agriculture and the manufacture of fertilizer. Microorganisms that fix nitrogen are bacteria called diazotrophs.

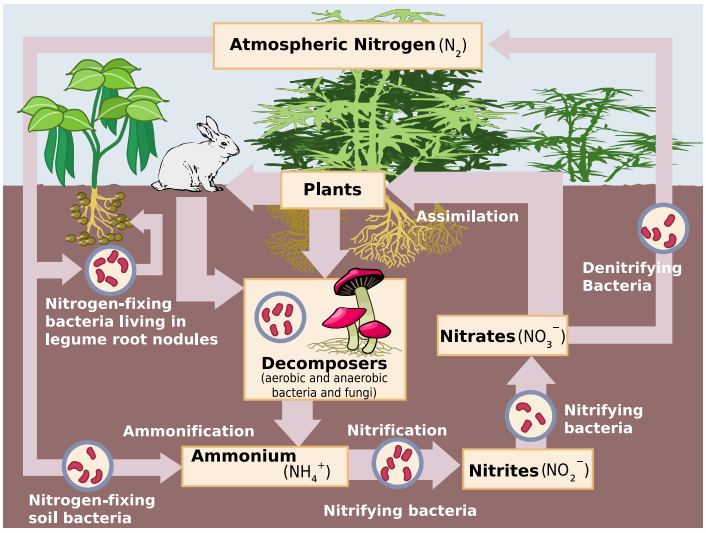


Figure: **The role of soil bacteria in the Nitrogen cycle**: Nitrogen transitions between various biologically useful forms.

Some higher plants, and some animals (termites), have formed associations (symbioses) with diazotrophs. Diazotrophs are microbes. They are intensively studied by microbiologists. Biological nitrogen fixation was discovered by the German agronomist Hermann Hellriegel and Dutch microbiologist Martinus Beijerinck. Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. Nitrogenases are enzymes used by some organisms to fix atmospheric nitrogen gas (N_2). There is only one known family of enzymes that accomplishes this process. All nitrogenases have an iron – and sulfur-containing cofactor that includes a heterometal complex in the active site (e.g., FeMoCo). In most species, this heterometal complex has a central molybdenum atom. However, in some species it is replaced by a vanadium or iron atom. Enzymes responsible for nitrogenase action are very susceptible to destruction by oxygen. Many bacteria cease production of the enzyme in the presence of oxygen. Many nitrogen-fixing organisms exist only in anaerobic conditions, respiring to draw down oxygen levels, or binding the oxygen with proteins.

Key Points

- Nitrogen fixation takes elemental nitrogen (N_2) and converts it into a ammonia, a format usable by biological organism.
- The fixed form of nitrogen (NH_3) is needed as an essential component of DNA and proteins. Therefore, it is needed for all life on earth.
- Nitrogen fixation is carried out by the enzyme nitrogenase, which are found in microbes.

Key Terms

- **fixation**: The act of uniting chemically with a solid substance or in a solid form; reduction to a non-volatile condition; — said of gaseous elements.
- **cofactor**: A substance, especially a coenzyme or a metal, that must be present for an enzyme to function.
- **heterometal**: Describing a complex containing two (or more) different metals
- **nitrogen fixation**: the conversion of atmospheric nitrogen into ammonia and organic derivatives, by natural means, especially such conversion, by microorganisms in the soil, into a form that can be assimilated by plants

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4.15B: Early Discoveries in Nitrogen Fixation

Learning Objectives

- Outline the early discoveries of nitrogen fixation

For thousands of years farmers were aware that plants belonging to the legume family, such as peas and soy beans, promoted crop growth when planted with other non-legumes such as wheat. Growing a legume crop in a field could also result in the next year's crop of non-legume plants giving a far greater yield. This led to the practice of crop rotation, a practice which can be traced back to techniques recorded in Roman literature.



Figure: ***Martinus Beijerinck****: Work done by Martinus Beijerinck was key to the discovery of rhizobia, symbiotic bacteria found on the roots of legumes and responsible for nitrogen fixation.*

While the ancient Romans were aware of the improved results gained through crop rotation, they did not know that these benefits were brought about through the replenishment of nitrogen in the soil. Later people knew legumes did replenish nitrogen in the soil, but did not know how atmospheric (N_2) was converted into ammonium (NH_3) by legumes until research done in the 19thcentury. Hermann Hellriegel (1831-1895), a noted German agricultural chemist, discovered that leguminous plants took atmospheric nitrogen and replenished the ammonium in the soil through the process now known as nitrogen fixation. He found that the nodules on the roots of legumes are the location where nitrogen fixation takes place.

HELLRIEGEL'S AND BEIJERINCK'S DISCOVERIES

Hellriegel did not determine what factors in the root nodules carried out nitrogen fixation. Martinus Willem Beijerinck (March 16, 1851 – January 1, 1931), a Dutch microbiologist and botanist, explored the mechanism responsible, discovering that the root nodules contained microbes. He further demonstrated that these microbes were bacteria, which he named rhizobia. These rhizobia perform the chemical processes of nitrogen fixation. In addition to having discovered this biochemical reaction vital to soil fertility and agriculture, Beijerinck is responsible for the discovery of this classic example of symbiosis between plants and bacteria. The bacteria in the root nodules are needed to provide nitrogen for legume growth, while the rhizobia are dependent on the root nodules as a environment to grow.and a source of nutrition.

Key Points

- A key benefit of crop rotation is the introduction of nitrogen into the soil. Although farmers have used this technique for millennia, it wasn't until the 19th century scientists determined how this fertilization occurred.
- Nitrogen fixation occurs in root nodules of plants belonging to the legume family.
- The root nodules of legumes contain symbiotic bacteria which contain the enzymes needed for nitrogen fixation.

Key Terms

- **legume**: Any of a large family (Leguminosae syn. Fabaceae) of dicotyledonous herbs, shrubs, and trees having fruits that are legumes or loments, bearing nodules on the roots that contain nitrogen-fixing bacteria, and including important food and forage plants (as peas, beans, or clovers).
- **symbiosis**: A close, prolonged association between two or more organisms of different species, regardless of benefit to the members.

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4.15C: Nitrogen Fixation Mechanism

Learning Objectives

- Distinguish between component I and II of the nitrogenase enzyme and its role in biological nitrogen fixation

Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase. The reaction for BNF is:

$$\mathbf{N_2 + 8\,H^+ + 8\,e^- \longrightarrow 2\,NH_3 + H_2.} \qquad \textbf{(4.15C.1)}$$

This type of reaction results in N_2 gaining electrons (see above equation) and is thus termed a reduction reaction. The exact mechanism of catalysis is unknown due to the technical difficulties biochemists have in actually visualizing this reaction in vitro, so the exact sequence of the steps of this reaction are not completely understood. Despite this, a great deal is known of the process. While the equilibrium formation of ammonia from molecular hydrogen and nitrogen has an overall negative enthalpy of reaction (i.e. it gives off energy), the energy barrier to activation is very high without the assistance of catalysis, which is done by nitrogenases. The enzymatic reduction of N_2 to ammonia therefore requires an input of chemical energy, released from ATP hydrolysis, to overcome the activation energy barrier.

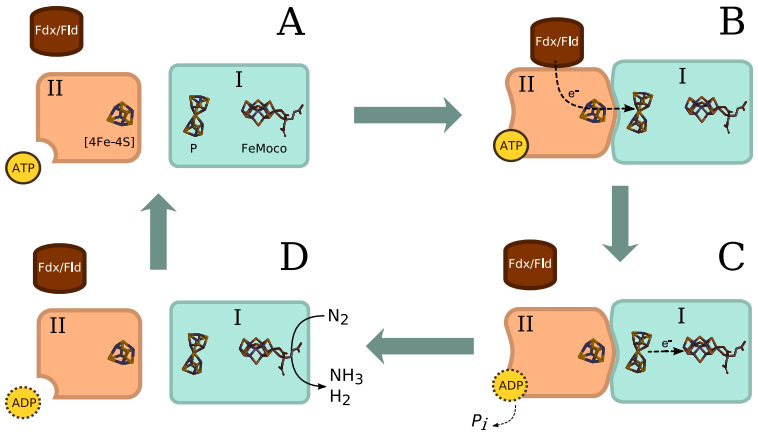


Figure: ***A General Catalytic Mechanism Scheme for Nitrogenase****: A) Components I and II are dissociated; II is ready for reduction. B) ATP binds to component II, which receives electrons from an electron donor (ferredoxin or flavodoxin); binding of ATP induces an allosteric conformational change which allows association of the two proteins. Electrons flow from the [4Fe-4S] cluster on II to the P cluster on I. C) Electrons are further shuttled to the iron-molybdenum cofactor (FeMoco), and ATP is hydrolised to ADP. This step is repeated several times before a molecule of N2 can bind to FeMoco. D) The protein complex dissociates, and nitrogenase reduces dinitrogen to ammonia and dihydrogen. Legend:I: component I (dinitrogenase; MoFe protein); II: component II (dinitrogen reductase; Fe protein); ATP: adenosine triphosphate; ADP: adenosine diphosphate; Fdx: ferredoxin; Fld: flavodoxin.*

Nitrogenase is made up of two soluble proteins: component I and II. Component I known as MoFe protein or nitrogenase contains 2 Mo atoms, 28 to 34 Fe atoms, and 26 to 28 acid-labile sulfides, also known as a iron-molybdenum cofactor (FeMoco). Component I is composed of two copies each of two subunits (α and β); each subunit's stability depends on the other in vivo. Component II known as Fe protein or nitrogenase reductase is composed of two copies of a single subunit. This protein has four non-heme Fe atoms and four acid-labile sulfides (4Fe-4S). Substrate binding and reduction takes place on component I, which binds to ATP and ferredoxin or flavodoxin proteins (Fdx or Fld) (see step B).

The hydrolysis of ATP supplies the energy for the reaction while the Fdx/Fld proteins supply the electrons. Note this is a reduction reaction which means that electrons must be added to the N_2 to reduce it to NH_4. Thus, the role of component II is to supply electrons, one at a time to component I. ATP is not hydrolyzed to ADP until component II transfers an electron to component I (see step C and D). 21-25 ATPs are required for each N_2 fixed. The association of nitrogenase component I and II and later dissociation occurs several times to allow the fixation of one N_2 molecule (see step B and D).

Nitrogenase ultimately bonds each atom of nitrogen to three hydrogen atoms to form ammonia (NH_3). The nitrogenase reaction additionally produces molecular hydrogen as a side product, which is of special interest for people trying to produce H_2 as an

alternative energy source to fossil fuels.

Key Points

- Nitrogen fixation does result in the release of energy, but the activation of this reaction takes energy in the form of ATP hydrolysis.
- Nitrogenases are metalloenzymes, which are proteins that have metalic molecules as subunits.
- While a great deal is known about how nitrogenases reduce nitrogen, some steps are unknown.

Key Terms

- **sulfide**: Any compound of sulfur and a metal or other electropositive element or group.
- **reduction**: A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- **enthalpy**: In thermodynamics, a measure of the heat content of a chemical or physical system.

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4.15D: Anaerobiosis and N_2 Fixation

Learning Objectives

- Outline the various mechanisms utilized by nitrogen-fixing bacteria to protect nitrogenases from oxygen

Central to nitrogen fixation (N_2 to NH_3) are the enzymes that do the actual fixation, these are known as nitrogenases. Due to the oxidation carried out by oxygen, most nitrogenases, which are essential large reduction complexes are irreversibly inhibited by O_2, which degradatively oxidizes the Fe-S cofactors. In essence, O_2binds to the iron (Fe) found in nitrogenases and blocks their ability to bind to N_2. To protect nitrogenases, there are mechanisms for nitrogen fixers to protect nitrogenase from oxygen in vivo. One known exception is the nitrogenase of Streptomyces thermoautotrophicus, which is unaffected by the presence of oxygen. This is complicated by the fact the bacteria still need the presence of oxygen for proper respiration.

Some microbes have a proteoglycan rich extra cellular matrix which traps a layer of water, often referred to as a slime layer. This slime layer acts as a barrier for oxygen. The ability of some nitrogen fixers such as azotobacteraceae to employ an oxygen-amendable nitrogenase under aerobic conditions has been attributed to a high metabolic rate, allowing oxygen reduction at the cell membrane; however, the effectiveness of this mechanism is in question.

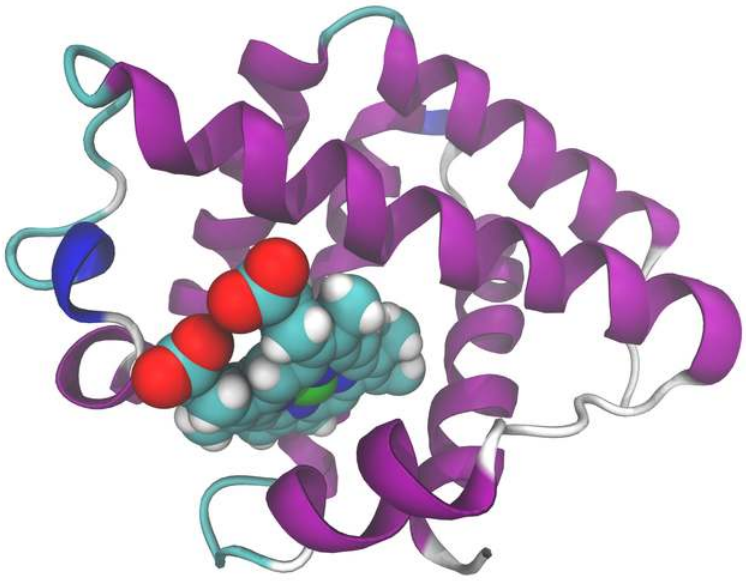


Figure: ***Leghemoglobin****: Leghemoglobin, the protein which binds to oxygen, allowing nitrogenases to function in an oxygen free environment. The ribbons represent protein folds, while the conglomerate of spheres are the postion of the iron contain heme group which binds the oxygen.*

Many rhizobia, nitrogen fixing bacteria, live in a symbiotic relationship with plants known as legumes. They have an interesting strategy to deal with O_2. In plants infected with Rhizobium, (legumes such as alfalfa or soybeans), the presence of oxygen in the root nodules would reduce the activity of the oxygen-sensitive nitrogenase. In these situations, the roots of such plants produce a protein known as leghemoglobin (also leghaemoglobin or legoglobin). Leghemoglobin buffers the concentration of free oxygen in the cytoplasm of infected plant cells to ensure the proper function of root nodules. Leghemoglobin is a nitrogen or oxygen carrier; naturally occurring oxygen and nitrogen interact similarly with this protein. Leghemoglobin buffers the concentration of free oxygen in the cytoplasm of infected plant cells to ensure the proper function of root nodules. It has close chemical and structural similarities to hemoglobin, and, like hemoglobin, is red in colour. Leghemoglobin has a high affinity for oxygen, about ten times higher than of human hemoglobin. This allows an oxygen concentration that is low enough to allow nitrogenase to function but not so high as to bind all the O_2 in the bacteria, providing the bacteria with oxygen for respiration.

Leghemoglobin is produced by legumes in response to the roots being infected by rhizobia, as part of the symbiotic interaction between the plant and these nitrogen-fixing bacterium. Interestingly, it is widely believed that leghemoglobin is the product of both

the plant and the bacterium in which a protein precursor is produced by the plant and the heme (an iron atom bound in a porphyrin ring, which binds O_2) is produced by the bacterium. The protein and heme come together to function, allowing the bacteria to fix-nitrogen, giving the plant usable nitrogen and thus the plant provides the rhizobia a home.

Key Points

- The iron (Fe) found in nitrogenases is very sensitive to oxygen, if there is too much oxygen this will in the end disrupt nitrogenase function.
- Some bacteria produce barriers which protect themselves from oxygen, while others use proteins such as leghemoglobin to bind up oxygen which may interfere with nitrogenases.
- Portions of leghemoglobin are thought to be produced by rhizobia residing in plant nodules, while other parts are produced by the plant, an elegant example of symbiosis.

Key Terms

- **oxidation**: A reaction in which the atoms of an element lose electrons and the valence of the element increases.
- **reduction**: A reaction in which electrons are gained and valence is reduced; often by the removal of oxygen or the addition of hydrogen.
- **proteoglycan**: Any of many glycoproteins that have heteropolysaccharide side chains

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4.15E: Genetics and Regulation of N_2 Fixation

Learning Objectives

- Discuss the role of the nif genes in controlling nitrogen fixation

The fixation of atmospheric nitrogen (N_2) is a very energy intensive endeavor. If there is no need for N_2 fixation, the production of proteins needed for fixation are tightly controlled. The nif genes are responsible for the coding of proteins related and associated with the fixation of atmospheric nitrogen into a form of nitrogen available to plants. These genes are found in nitrogen fixing bacteria and cyanobacteria. The nif genes are found in both free living nitrogen fixing bacteria and in symbiotic bacteria in various plants.

The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen. The primary enzyme encoded by the nif genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen to other nitrogen forms such as ammonia, which plants can use for various purposes. Besides the nitrogenase enzyme, the nif genes also encode a number of regulatory proteins involved in nitrogen fixation. The expression of the nif genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment). Nitrogen fixation is regulated by nif regulon, which is a set of seven operons which includes 17 nif genes. Nif genes have both positive and negative regulators. Some of nif genes are: Nif A, D, L,K, F,H S,U,Y,W,Z.

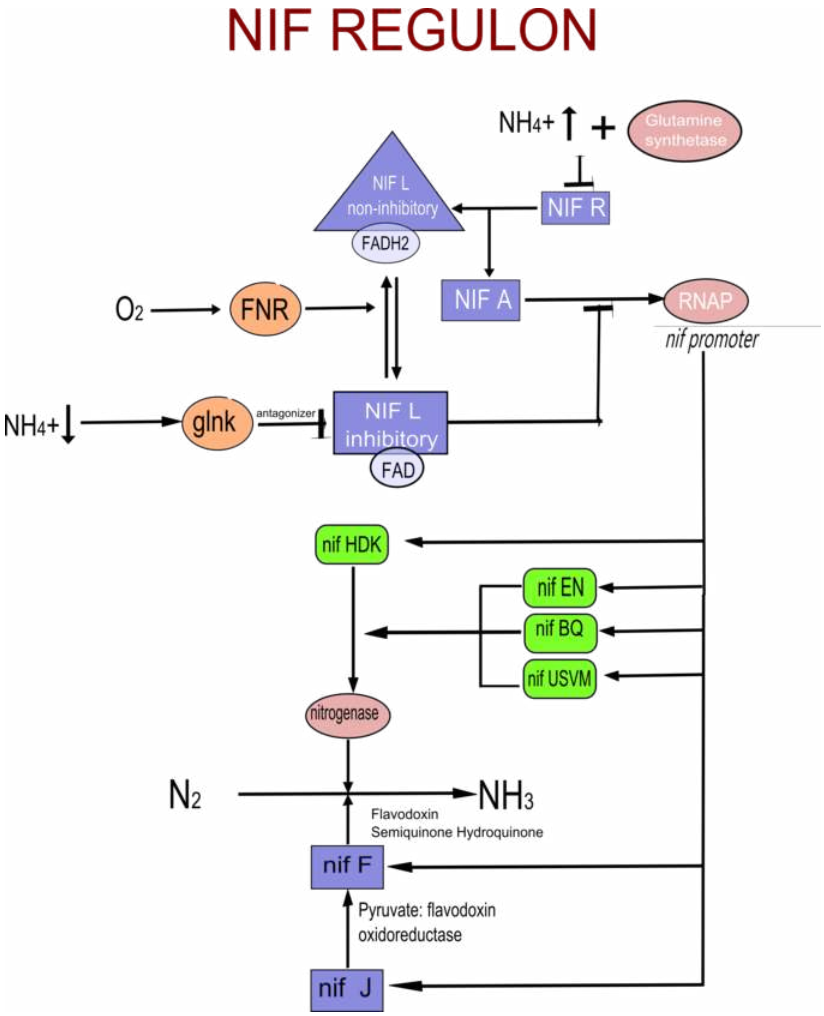


Figure: ***Nif Regulon**: This is a schematic representing many of the proteins in the nif regulon and where they act in the pathway needed for nitrogen fixation.*

Activation of nif genes transcription is done by the nitrogen sensitive NifA protein. When there isn't enough fixed nitrogen factor available for the plant's use, NtrC, which is a RNA polymerase, triggers NifA's expression. NifA then activates the rest of the transcription for the nif genes. If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated, NifL. In turn, NifL inhibits NifA activity, which results in the inhibition of nitrogenase formation. NifL is then regulated by other proteins that are sensors for the levels of O_2 and ammonium in the surrounding environment.

The nif genes can be found on bacteria's chromosomes, but many times they are found on bacteria's plasmids with other genes related to nitrogen fixation, such as the genes needed for the bacteria to communicate with the plant host.

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Key Points

- Nitrogen fixation takes a great deal of energy. If the conditions are not favorable for nitrogen fixation or there is enough ammonia around, nitrogen-fixing bacteria turn off the production of proteins needed for nitrogen fixation.
- Nitrogen fixing protein production is regulated by the nif regulon.
- The nif regulon contains factors which both turn on and off the production of proteins needed for nitrogen-fixation.

Key Terms

- **regulon**: A group of genes that is regulated by the same regulatory molecule. The genes of a regulon share a common regulatory element binding site or promoter. The genes comprising a regulon may be located non-contiguously in the genome.
- **operon**: A unit of genetic material that functions in a coordinated manner by means of an operator, a promoter, and structural genes that are transcribed together.

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SECTION OVERVIEW

5.1: Genes

5.1A: Bacterial Genomes

5.1B: DNA Replication in Prokaryotes

5.1C: Gene Inversion

5.1D: Slipped-Strand Mispairing

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5.1A: Bacterial Genomes

Learning Objectives

- Explain the basic features of bacterial genomes

Bacterial genomes are generally smaller and less variant in size between species when compared with genomes of animals and single cell eukaryotes. Bacterial genomes can range in size anywhere from 139 kbp to 13,000 kbp. Recent advances in sequencing technology led to the discovery of a high correlation between the number of genes and the genome size of bacteria, suggesting that bacteria have relatively small amounts of junk DNA.

Studies have since shown that a large number of bacterial species have undergone genomic degradation resulting in a decrease in genome size from their ancestral state. Over the years, researchers have proposed several theories to explain the general trend of bacterial genome decay and the relatively small size of bacterial genomes. Compelling evidence indicates that the apparent degradation of bacterial genomes is owed to a deletional bias.

In prokaryotes, most of the genome (85-90%) is non-repetitive DNA, which means coding DNA mainly forms it, while non-coding regions only take a small part. Most biological entities that are more complex than a virus sometimes or always carry additional genetic material besides that which resides in their chromosomes. In some contexts, such as sequencing the genome of a pathogenic microbe, “genome” is meant to include information stored on this auxiliary material, which is carried in plasmids. In such circumstances then, “genome” describes all of the genes and information on non-coding DNA that have the potential to be present.

Amongst species of bacteria, there is relatively little variation in genome size when compared with the genome sizes of other major groups of life. Genome size is of little relevance when considering the number of functional genes in eukaryotic species. In bacteria however, the strong correlation between the number of genes and the genome size makes the size of bacterial genomes an interesting topic for research and discussion. The general trends of bacterial evolution indicate that bacteria started as free-living organisms. Evolutionary paths led some bacteria to become pathogens and symbionts.

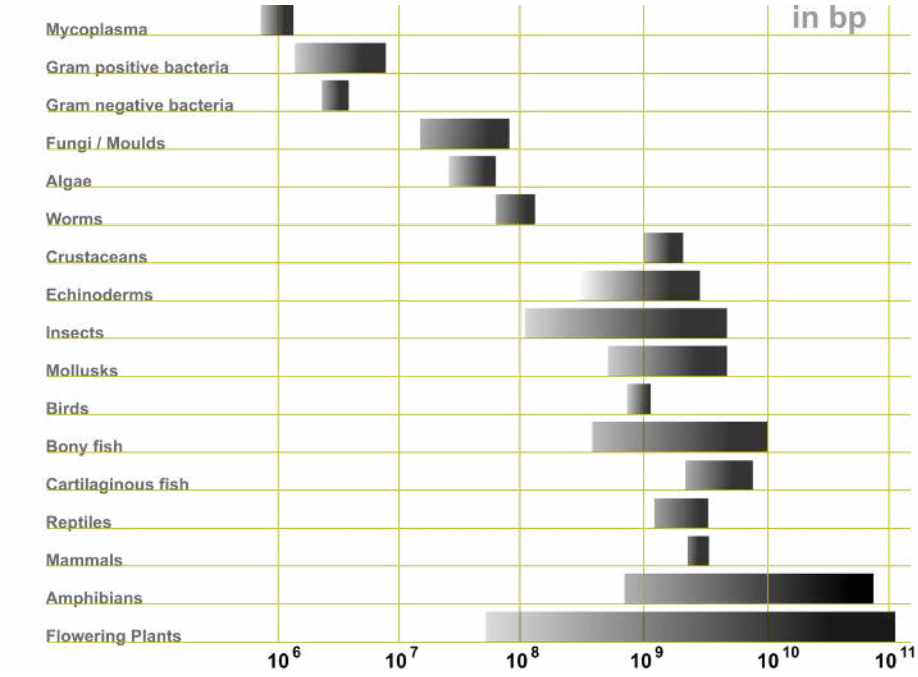


***Figure 7.1A: Graph of variation in estimated genome sizes in base pairs.**: Unlike eukaryotes, bacteria show a strong correlation between genome size and number of functional genes in a genome. Genome size ranges (in base pairs) of various life forms. (CC BY-SA 4.0; Abizar).*

The lifestyles of bacteria play an integral role in their respective genome sizes. Free-living bacteria have the largest genomes out of the three types of bacteria; however, they have fewer pseudogenes than bacteria that have recently acquired pathogenicity.

Facultative and recently evolved pathogenic bacteria exhibit a smaller genome size than free-living bacteria, yet they have more pseudogenes than any other form of bacteria. Obligate bacterial symbionts or pathogens have the smallest genomes and the fewest number of pseudogenes of the three groups. The relationship between life-styles of bacteria and genome size raises questions as to the mechanisms of bacterial genome evolution.

Researchers have developed several theories to explain the patterns of genome size evolution amongst bacteria. One theory predicts that bacteria have smaller genomes due to a selective pressure on genome size to ensure faster replication. The theory is based upon the logical premise that smaller bacterial genomes will take less time to replicate. Subsequently, smaller genomes will be selected preferentially due to enhanced fitness.

Deletional bias selection is but one process involved in evolution. Two other major processes (mutation and genetic drift) can be used to explain the genome sizes of various types of bacteria.

Evidence of a deletional bias is present in the respective genome sizes of free-living bacteria, facultative and recently derived parasites and obligate parasites and symbionts. Free-living bacteria tend to have large population sizes and are subject to more opportunity for gene transfer. As such, selection can effectively operate on free-living bacteria to remove deleterious sequences resulting in a relatively small number of pseudogenes. Continually, further selective pressure is evident as free-living bacteria must produce all gene-products independent of a host. Given that there is sufficient opportunity for gene transfer to occur and there are selective pressures against even slightly deleterious deletions, it is intuitive that free-living bacteria should have the largest bacterial genomes of all bacteria types. Recently formed parasites undergo severe bottlenecks and can rely on host environments to provide gene products. As such, in recently formed and facultative parasites, there is an accumulation of pseudogenes and transposable elements due to a lack of selective pressure against deletions. The population bottlenecks reduce gene transfer and as such, deletional bias ensures the reduction of genome size in parasitic bacteria.

Key Points

- In prokaryotes, most of the genome (85-90%) is non-repetitive, coding DNA, while the remaining DNA is non-coding.
- The genome of a pathogenic microbe, “genome” is meant to include information stored on this auxiliary material, which is carried in plasmids.
- The lifestyles of bacteria play an integral role in their respective genome sizes. Free-living bacteria have the largest genomes out of the three types of bacteria; however, they have fewer pseudogenes than bacteria that have recently acquired pathogenicity.

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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5.1B: DNA Replication in Prokaryotes

Prokaryotic DNA is replicated by DNA polymerase III in the 5′ to 3′ direction at a rate of 1000 nucleotides per second.

LEARNING OBJECTIVES

Explain the functions of the enzymes involved in prokaryotic DNA replication

Key Takeaways

DNA Replication in Prokaryotes

DNA replication employs a large number of proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme DNA polymerase, which adds nucleotides one by one to the growing DNA chain that are complementary to the template strand. The addition of nucleotides requires energy; this energy is obtained from the nucleotides that have three phosphates attached to them, similar to ATP which has three phosphate groups attached. When the bond between the phosphates is broken, the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain. In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. DNA pol III is the enzyme required for DNA synthesis; DNA pol I and DNA pol II are primarily required for repair.

There are specific nucleotide sequences called origins of replication where replication begins. In *E. coli*, which has a single origin of replication on its one chromosome (as do most prokaryotes), it is approximately 245 base pairs long and is rich in AT sequences. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called helicase unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called replication forks are formed. Two replication forks at the origin of replication are extended bi-directionally as replication proceeds. Single-strand binding proteins coat the strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix. DNA polymerase is able to add nucleotides only in the 5′ to 3′ direction (a new DNA strand can be extended only in this direction). It also requires a free 3′-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide. This means that it cannot add nucleotides if a free 3′-OH group is not available. Another enzyme, RNA primase, synthesizes an RNA primer that is about five to ten nucleotides long and complementary to the DNA, priming DNA synthesis. A primer provides the free 3′-OH end to start replication. DNA polymerase then extends this RNA primer, adding nucleotides one by one that are complementary to the template strand.

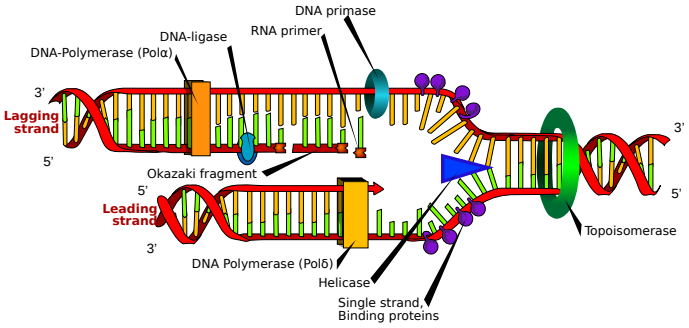


Figure: **DNA Replication in Prokaryotes**: A replication fork is formed when helicase separates the DNA strands at the origin of replication. The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this supercoiling. Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming. Primase synthesizes an RNA primer. DNA polymerase III uses this primer to synthesize the daughter DNA strand. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. DNA polymerase I replaces the RNA primer with DNA. DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule.

The replication fork moves at the rate of 1000 nucleotides per second. DNA polymerase can only extend in the 5′ to 3′ direction, which poses a slight problem at the replication fork. As we know, the DNA double helix is anti-parallel; that is, one strand is in the 5′ to 3′ direction and the other is oriented in the 3′ to 5′ direction. One strand (the leading strand), complementary to the 3′ to 5′

parental DNA strand, is synthesized continuously towards the replication fork because the polymerase can add nucleotides in this direction. The other strand (the lagging strand), complementary to the 5′ to 3′ parental DNA, is extended away from the replication fork in small fragments known as Okazaki fragments, each requiring a primer to start the synthesis. Okazaki fragments are named after the Japanese scientist who first discovered them.

The leading strand can be extended by one primer alone, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3′ to 5′, while that of the leading strand will be 5′ to 3′. The sliding clamp (a ring-shaped protein that binds to the DNA) holds the DNA polymerase in place as it continues to add nucleotides. Topoisomerase prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA pol I, while the gaps are filled in by deoxyribonucleotides. The nicks that remain between the newly-synthesized DNA (that replaced the RNA primer) and the previously-synthesized DNA are sealed by the enzyme DNA ligase that catalyzes the formation of phosphodiester linkage between the 3′-OH end of one nucleotide and the 5′ phosphate end of the other fragment.

The table summarizes the enzymes involved in prokaryotic DNA replication and the functions of each.

Prokaryotic DNA Replication: Enzymes and Their Function	
Enzyme/protein	Specific Function
DNA pol I	Exonuclease activity removes RNA primer and replaces with newly synthesized DNA
DNA pol II	Repair function
DNA pol III	Main enzyme that adds nucletides in the 5′ – 3′ direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Topoisomerase	Helps relieve the stress on DNA when unwinding by causing breaks and then resealing the DNA
Single-strand binding proteins (SSB)	Binds to single-stranded DNA to avoid DNA rewinding back.

Key Points

- Helicase separates the DNA to form a replication fork at the origin of replication where DNA replication begins.
- Replication forks extend bi-directionally as replication continues.
- Okazaki fragments are formed on the lagging strand, while the leading strand is replicated continuously.
- DNA ligase seals the gaps between the Okazaki fragments.
- Primase synthesizes an RNA primer with a free 3′-OH, which DNA polymerase III uses to synthesize the daughter strands.

Key Terms

- **DNA replication**: a biological process occuring in all living organisms that is the basis for biological inheritance
- **helicase**: an enzyme that unwinds the DNA helix ahead of the replication machinery
- **origin of replication**: a particular sequence in a genome at which replication is initiated

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5.1C: Gene Inversion

Learning Objectives

- Explain how gene sequence inversions can have a regulatory effect

Recombining sequences in site-specific reactions are usually short and occur at a single target site within the recombining sequence. For this to occur, there is typically one or more cofactors (to name a few: DNA-binding proteins and the presence or absence of DNA binding sites) and a site specific recombinase. There is also a change in orientation of the DNA that will affect gene expression or the structure of the gene product. This is done by changing the spatial arrangement of the promoter or the regulatory elements.

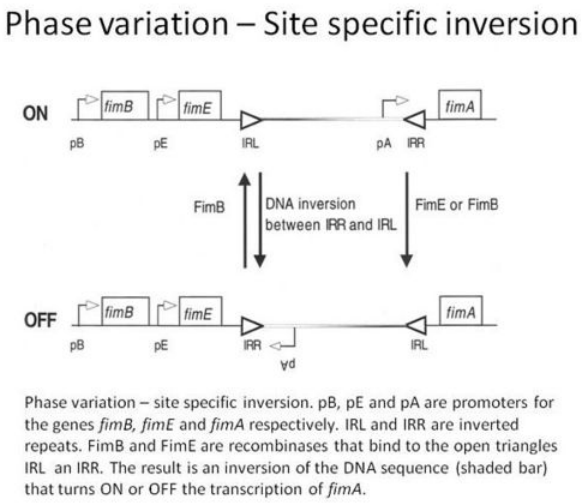


Figure: ***Phase variation Site specific Inversion****: Phase and Antigenic Variation in Bacteria. pA is the promoter for FimA, pB is the promoter for FimB and pE is the promoter for FimE. IRR is inverted repeat right and IRL is inverted repeat left. FimB and FimE are recombinases that can change the orientation of the FimA promoter by inverting the IRR and IRL.*

Through the utilization of specific recombinases, a particular DNA sequence is inverted, resulting in an ON to OFF switch, and vice versa, of the gene located within or next to this switch. Many bacterial species can utilize inversion to change the expression of certain genes for the benefit of the bacterium during infection. The inversion event can be simple by involving the toggle in expression of one gene, like *E. coli* pilin expression; or more complicated by involving multiple genes in the expression of multiple types of flagellin by *S. typhimurium*. Fimbrial adhesion by the type I fimbriae in *E. coli* undergoes site specific inversion to regulate the expression of *fimA*, the major subunit of the pili, depending on the stage of infection. The invertible element has a promoter within it that depending on the orientation will turn on or off the transcription of *fimA*. The inversion is mediated by two recombinases, FimB and FimE, and regulatory proteins H-NS, Integration Host Factor (IHF) and Leucine responsive protein (LRP). The FimE recombinase has the capability to only invert the element and turn expression from on to off, while FimB can mediate the inversion in both directions.

Key Points

- Recombining sequences in site-specific reactions are usually short and occur at a single target site. For this to occur, there is typically one or more cofactors (to name a few: DNA -binding proteins and the presence or absence of DNA binding sites) and a site specific recombinase.
- Many bacterial species can utilize inversion to change the expression of certain genes for the benefit of the bacterium during infection.

- The inversion event can be simple by involving the toggle in expression of one gene, like *E. coli* pilin expression; or more complicated by involving multiple genes in the expression of multiple types of flagellin by *S. typhimurium*.

Key Terms

- **recombinase**: Any of several enzymes that mediate recombination of DNA fragments between maternal and paternal chromosomes in prokaryotes.

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5.1D: Slipped-Strand Mispairing

Slipped strand mispairing (SSM) is a process that produces mispairing of short repeat sequences during DNA synthesis.

LEARNING OBJECTIVES

Explain how slipped-strand mispairing can be used as a mechanism to regulate gene expression

Key Takeaways

Slipped strand mispairing (SSM) is a process that produces mispairing of short repeat sequences between the mother and daughter strand during DNA synthesis. This RecA-independent mechanism can transpire during either DNA replication or DNA repair and can be on the leading or lagging strand and can result in an increase or decrease in the number of short repeat sequences. The short repeat sequences are 1 to 7 nucleotides and can be homogeneous or heterogeneous repetitive DNA sequences.

Altered gene expression is a result of SSM and depending where the increase or decrease of the short repeat sequences occurs in relation to the promoter will either regulate at the level of transcription or translation. The outcome is an ON or OFF phase of a gene or genes.

Transcriptional regulation occurs in several ways. One possibility is if the repeats are located in the promoter region at the RNA polymerase binding site, -10 and -35, upstream of the gene(s). The opportunistic pathogen *H. influenzae* has two divergently oriented promoters in fimbriae geneshifA and hifB. The overlapping promoter regions have repeats of the dinucleotide TA in the -10 and -35 sequences. Through SSM the TA repeat region can undergo addition or subtraction of TA dinucleotides which results in the reversible ON phase or OFF phase of transcription of the hifA and hifB. The second way that SSM induces transcriptional regulation is by changing the short repeat sequences located outside the promoter. If there is a change in the short repeat sequence, it can affect the binding of a regulatory protein, such as an activator or repressor. It can also lead to differences in post-transcriptional stability of mRNA.

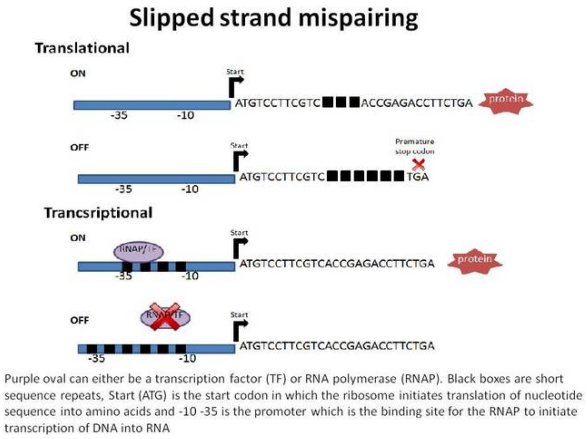


Figure: **Slip strand mispairing**: Purple ovals can either be a transcription factor (TF) or RNA polymerase (RNAP). Black boxes are short sequence repeats. Start (ATG) is the start codon in which the ribosome initiates translation of nucleotide sequence into amino acids, and (-10 -35) is the promoter which is the binding site for the RNAP to initiate transcription of DNA into RNA.

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Key Points

- Altered gene expression is a result of SSM and depending where the increase or decrease of the short repeat sequences occurs in relation to the promoter will either regulate at the level of transcription or translation. The outcome is an ON or OFF phase of a gene or genes.

- SSM can result in an increase or decrease in the number of short repeat sequences. The short repeat sequences are 1 to 7 nucleotides and can be homogeneous or heterogeneous repetitive DNA sequences.
- Transcriptional regulation can occur if the repeats are located in the promoter region at the RNA polymerase binding site, -10 and -35 upstream of the gene(s).
- SSM induces transcriptional regulation is by changing the short repeat sequences located outside the promoter. If there is a change in the short repeat sequence it can affect the binding of a regulatory protein, such as an activator or repressor.

Key Terms

- **Slipped strand mispairing**: a process that produces mispairing off short repeat sequences between the mother and daughter strand during DNA synthesis.

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SECTION OVERVIEW

5.2: Prokaryotic Genomes

Topic hierarchy

5.2A: Bacterial Chromosomes in the Nucleoid

5.2B: Supercoiling

5.2C: Size Variation and ORF Contents in Genomes

5.2D: Bioinformatic Analyses and Gene Distributions

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5.2A: Bacterial Chromosomes in the Nucleoid

Learning Objectives

- Evaluate the nucleoid in prokaryotes

The Nucleoid

The *nucleoid* (meaning nucleus-like) is an irregularly-shaped region within the cell of a prokaryote that contains all or most of the genetic material. In contrast to the nucleus of a eukaryotic cell, it is not surrounded by a nuclear membrane. The genome of prokaryotic organisms generally is a circular, double-stranded piece of DNA, of which multiple copies may exist at any time. The length of a genome varies widely, but is generally at least a few million base pairs.

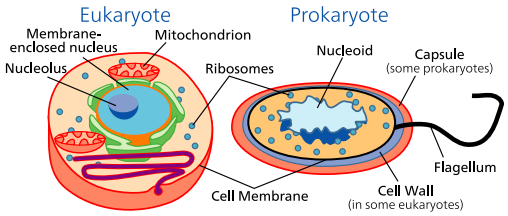


Figure: **Prokaryote cell nucleoid**: Prokaryote cell (right) showing the nucleoid in comparison to a eukaryotic cell (left) showing the nucleus.

The nucleoid can be clearly visualized on an electron micrograph at high magnification, where it is clearly visible against the cytosol. Sometimes even strands of what is thought to be DNA are visible. The nucleoid can also be seen under a light microscope.by staining it with the Feulgen stain, which specifically stains DNA. The DNA-intercalating stains DAPI and ethidium bromide are widely used for fluorescence microscopy of nucleoids.

Experimental evidence suggests that the nucleoid is largely composed of about 60% DNA, plus a small amount of RNA and protein. The latter two constituents are likely to be mainly messenger RNA and the transcription factor proteins found regulating the bacterial genome. Proteins helping to maintain the supercoiled structure of the nucleic acid are known as *nucleoid proteins* or *nucleoid-associated proteins*, and are distinct from histones of eukaryotic nuclei. In contrast to histones, the DNA-binding proteins of the nucleoid do not form nucleosomes, in which DNA is wrapped around a protein core. Instead, these proteins often use other mechanisms, such as DNA looping, to promote compaction.

The Genophore

A *genophore* is the DNA of a prokaryote. It is commonly referred to as a prokaryotic chromosome. The term “chromosome” is misleading, because the genophore lacks chromatin. The genophore is compacted through a mechanism known as supercoiling, but a chromosome is additionally compacted through the use of chromatin. The genophore is circular in most prokaryotes, and linear in very few. The circular nature of the genophore allows replication to occur without telomeres. Genophores are generally of a much smaller size than Eukaryotic chromosomes. A genophore can be as small as 580,073 base pairs (Mycoplasma genitalium). Many eukaryotes (such as plants and animals) carry genophores in organelles such as mitochondria and chloroplasts. These organelles are very similar to true prokaryotes.

Key Points

- The genome of prokaryotic organisms generally is a circular, double-stranded piece of DNA, multiple copies of which may exist at any time.
- The length of a genome varies widely, but is generally at least a few million base pairs.
- A genophore is the DNA of a prokaryote. It is commonly referred to as a prokaryotic chromosome.

Key Terms

- **nucleoid**: The irregularly-shaped region within a prokaryote cell where the genetic material is localized.
- **prokaryote**: An organism characterized by the absence of a nucleus or any other membrane-bound organelles.

- **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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5.2B: Supercoiling

Learning Objectives

- Assess the role of supercoiling in prokaryotic genomes

DNA supercoiling refers to the over- or under-winding of a DNA strand, and is an expression of the strain on that strand. Supercoiling is important in a number of biological processes, such as compacting DNA. Additionally, certain enzymes such as topoisomerases are able to change DNA topology to facilitate functions such as DNA replication or transcription. Mathematical expressions are used to describe supercoiling by comparing different coiled states to relaxed B-form DNA.

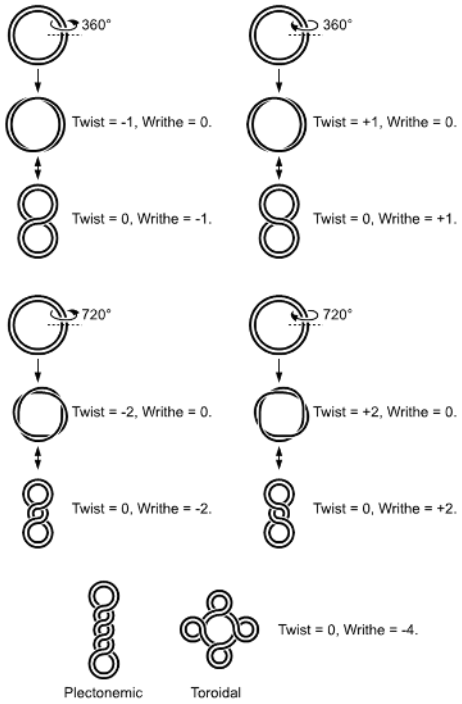


Figure: ***Supercoiled Structure of Circular DNA****: This is a supercoiled structure of circular DNA molecules with low writhe. Note that the helical nature of the DNA duplex is omitted for clarity.*

As a general rule, the DNA of most organisms is negatively supercoiled. In a “relaxed” double-helical segment of B-DNA, the two strands twist around the helical axis once every 10.4 to 10.5 base pairs of sequence. Adding or subtracting twists, as some enzymes can do, imposes strain. If a DNA segment under twist strain were closed into a circle by joining its two ends and then allowed to move freely, the circular DNA would contort into a new shape, such as a simple figure-eight. Such a contortion is a supercoil.

The simple figure eight is the simplest supercoil, and is the shape a circular DNA assumes to accommodate one too many or one too few helical twists. The two lobes of the figure eight will appear rotated either clockwise or counterclockwise with respect to one another, depending on whether the helix is over or underwound. For each additional helical twist being accommodated, the lobes will show one more rotation about their axis.

The noun form “supercoil” is rarely used in the context of DNA topology. Instead, global contortions of a circular DNA, such as the rotation of the figure-eight lobes above, are referred to as writhe. The above example illustrates that twist and writhe are interconvertible. “Supercoiling” is an abstract mathematical property representing the sum of twist and writhe. The twist is the number of helical turns in the DNA and the writhe is the number of times the double helix crosses over on itself (these are the supercoils).

Extra helical twists are positive and lead to positive supercoiling, while subtractive twisting causes negative supercoiling. Many topoisomerase enzymes sense supercoiling and either generate or dissipate it as they change DNA topology. In part because chromosomes may be very large, segments in the middle may act as if their ends are anchored. As a result, they may be unable to

distribute excess twist to the rest of the chromosome or to absorb twist to recover from underwinding—the segments may become supercoiled, in other words. In response to supercoiling, they will assume an amount of writhe, just as if their ends were joined.

Supercoiled DNA forms two structures; a *plectoneme* or a *toroid*, or a combination of both. A negatively supercoiled DNA molecule will produce either a one-start left-handed helix, the toroid, or a two-start right-handed helix with terminal loops, the plectoneme. Plectonemes are typically more common in nature, and this is the shape most bacterial plasmids will take. For larger molecules, it is common for hybrid structures to form – a loop on a toroid can extend into a plectoneme. If all the loops on a toroid extend, it becomes a branch point in the plectonemic structure.

The Importance of DNA Supercoiling

DNA supercoiling is important for DNA packaging within all cells. Because the length of DNA can be thousands of times that of a cell, packaging this genetic material into the cell or nucleus (in eukaryotes) is a difficult feat. Supercoiling of DNA reduces the space and allows for much more DNA to be packaged. In prokaryotes, plectonemic supercoils are predominant, because of the circular chromosome and relatively small amount of genetic material. In eukaryotes, DNA supercoiling exists on many levels of both plectonemic and solenoidal supercoils, with the solenoidal supercoiling proving the most effective in compacting the DNA. Solenoidal supercoiling is achieved with histones to form a 10 nm fiber. This fiber is further coiled into a 30 nm fiber, and further coiled upon itself numerous times more.

DNA packaging is greatly increased during nuclear division events such as mitosis or meiosis, where DNA must be compacted and segregated to daughter cells. Condensins and cohesins are structural maintenance of chromosome (SMC) proteins that aid in the condensation of sister chromatids and the linkage of the centromere in sister chromatids. These SMC proteins induce positive supercoils.

Supercoiling is also required for DNA and RNA synthesis. Because DNA must be unwound for DNA and RNA polymerase action, supercoils will result. The region ahead of the polymerase complex will be unwound; this stress is compensated with positive supercoils ahead of the complex. Behind the complex, DNA is rewound and there will be compensatory negative supercoils. It is important to note that topoisomerases such as DNA gyrase (Type II Topoisomerase) play a role in relieving some of the stress during DNA and RNA synthesis.

Key Points

- As a general rule, the DNA of most organisms is negatively supercoiled.
- The simple figure eight is the simplest supercoil, and is the shape a circular DNA assumes to accommodate one too many or one too few helical twists.
- DNA supercoiling is important for DNA packaging within all cells.

Key Terms

- **supercoiling**: The coiling of the DNA helix upon itself; can cause disruption to transcription and lead to cell death.
- **DNA**: A biopolymer of deoxyribonucleic acids (a type of nucleic acid) that has four different chemical groups, called bases: adenine, guanine, cytosine, and thymine.
- **chromosome**: A structure in the cell nucleus that contains DNA, histone protein, and other structural proteins.

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5.2C: Size Variation and ORF Contents in Genomes

Learning Objectives

- Explain prokaryotic genome size variation and ORFs

In molecular genetics, an open reading frame (ORF) is the part of a reading frame that contains no stop codons. The transcription termination pause site is located after the ORF, beyond the translation stop codon, because if transcription were to cease before the stop codon, an incomplete protein would be made during translation.

Normally, inserts which interrupt the reading frame of a subsequent region after the start codon cause frameshift mutation of the sequence and dislocate the sequences for stop codons.

Open reading frames are used as one piece of evidence to assist in gene prediction. Long ORFs are often used, along with other evidence, to initially identify candidate protein coding regions in a DNA sequence. The presence of an ORF does not necessarily mean that the region is ever translated. For example, in a randomly generated DNA sequence with an equal percentage of each nucleotide, a stop-codon would be expected once every 21 codons. A simple gene prediction algorithm for prokaryotes might look for a start codon followed by an open reading frame that is long enough to encode a typical protein, where the codon usage of that region matches the frequency characteristic for the given organism ‘s coding regions. Even a long open reading frame by itself is not conclusive evidence for the presence of a gene.

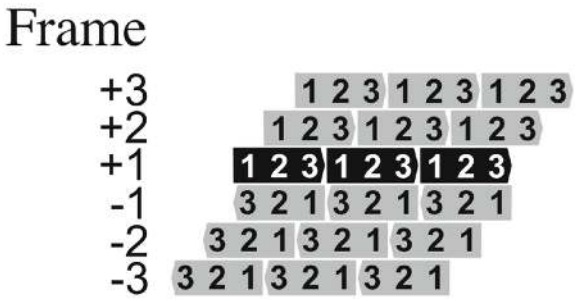


Figure: **Open Reading Frames**: Frame +1 is the ORF predicted in the database to encode a protein. +2 and +3 are the other two potential ORFs in the same strand and -1, -2, and -3 are the three potential ORFs in the antisense strand.

If a portion of a genome has been sequenced (e.g. 5′-ATCTAAAATGGGTGCC-3′), ORFs can be located by examining each of the three possible reading frames on each strand. In this sequence two out of three possible reading frames are entirely open, meaning that they do not contain a stop codon:

…A TCT AAA ATG GGT GCC…

…AT CTA AAA TGG GTG CC…

…ATC TAA AAT GGG TGC C…

Possible stop codons in DNA are “TGA”, “TAA”, and “TAG”. Thus, the last reading frame in this example contains a stop codon (TAA), unlike the first two.

Bacterial genomes display variation in size, even among strains of the same species. These microorganisms have very little noncoding or repetitive DNA, as the variation in their genome size usually reflects differences in gene repertoire. Some species, particularly bacterial parasites and symbionts, have undergone massive genome reduction and simply contain a subset of the genes present in their ancestors.

However, in free-living bacteria, such gene loss cannot explain the observed disparities in genome size because ancestral genomes would have had to contain improbably large numbers of genes. Surprisingly, a substantial fraction of the difference in gene contents in free-living bacteria is due to the presence of ORFans, that is, open reading frames (ORFs) that have no known homologs and are consequently of no known function.

The high numbers of ORFans in bacterial genomes indicate that, with the exception of those species with highly reduced genomes, much of the observed diversity in gene inventories does not result from either the loss of ancestral genes or the transfer from well-

characterized organisms (processes that result in a patchy distribution of orthologs but not in unique genes) or from recent duplications (which would likely yield homologs within the same or closely related genome).

Key Points

- Open reading frames are used as one piece of evidence to assist in gene prediction.
- If a portion of a genome has been sequenced, ORFs can be located by examining each of the three possible reading frames on each strand.
- Bacterial genomes display variation in size, even among strains of the same species.

Key Terms

- **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.
- **codons**: The genetic code is the set of rules by which information encoded within genetic material (DNA or mRNA sequences) is translated into proteins (amino acid sequences) by living cells. Biological decoding is accomplished by the ribosome, which links amino acids in an order specified by mRNA, using transfer RNA (tRNA) molecules to carry amino acids and to read the mRNA three nucleotides at a time. The genetic code is highly similar among all organisms, and can be expressed in a simple table with 64 entries.
- **open reading frame**: A sequence of DNA triplets, between the initiator and terminator codons, that can be transcribed into mRNA and later translated into protein.

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5.2D: Bioinformatic Analyses and Gene Distributions

Bioinformatics is the study of methods for storing, retrieving and analyzing biological data.

LEARNING OBJECTIVES

Describe the purposes and applications of bioinformatics

Key Takeaways

Bioinformatics is a branch of biological science dealing with the study of storing, retrieving and analyzing biological data like nucleic acid (DNA/RNA) and protein sequence, structure, function, pathways and genetic interactions. It generates new knowledge that is useful in such fields as drug design and development of new software tools. Bioinformatics also deals with algorithms, databases and information systems, web technologies, artificial intelligence and soft computing, information and computation theory, structural biology, software engineering, data mining, image processing, modeling and simulation, discrete mathematics, control and system theory, circuit theory, and statistics.

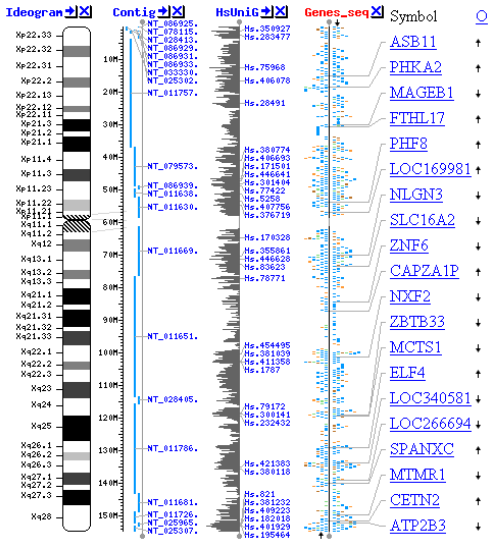


Figure: **Map of the human X chromosome**: Assembly of the human genome is one of the greatest achievements of bioinformatics

At the beginning of the "genomic revolution," the term bioinformatics refered to the creation and maintenance of a database to store biological information like nucleotide and amino acid sequences. Development of this type of database involved not only design issues but the development of complex interfaces whereby researchers could access existing data as well as submit new or revised data.

In order to study how normal cellular activities are altered in different disease states, the biological data must be combined to form a comprehensive picture of these activities. Therefore, the field of bioinformatics has evolved such that the most pressing task now involves the analysis and interpretation of various types of data. This includes nucleotide and amino acid sequences, protein domains and protein structures. The actual process of analyzing and interpreting data is referred to as computational biology. Important sub-disciplines within bioinformatics and computational biology include:

- the development of tools that enable efficient use of various types of information
- the development of new algorithms (mathematical formulas) and statistics with which to assess relationships among members of large data sets. For example, methods to locate a gene within a sequence (gene distributions), predict protein structure and/or function, and cluster protein sequences into families of related sequences.

The primary goal of bioinformatics is to increase the understanding of biological processes. What sets it apart from other approaches, however, is its focus on developing and applying computationally intensive techniques to achieve this goal. Examples include pattern recognition, data mining, machine learning algorithms, and visualization. Major research efforts in the field include

sequence alignment, gene finding, genome assembly, drug design, drug discovery, protein structure alignment, and the modeling of evolution.

Gene Ontology, or GO, is a major bioinformatics initiative to unify the representation of gene and gene product attributes across all species. More specifically, the project aims to:

- maintain and develop its controlled vocabulary of gene and gene product attributes
- annotate genes and gene products and assimilate and disseminate annotation data
- offer tools for easy access to all aspects of the data provided by the project

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Key Points

- The primary goal of bioinformatics is to increase the understanding of biological processes.
- Bioinformatics entails the creation and advancement of databases, algorithms, computational and statistical techniques and theory to solve problems arising from the management and analysis of biological data.
- Gene Ontology, or GO, is a major bioinformatics initiative to unify the representation of gene and gene product attributes across all species.

Key Terms

- **bioinformatics**: Bioinformatics is a branch of biological science which deals with the study of methods for storing, retrieving and analyzing biological data like nucleic acid (DNA/RNA) and protein sequence, structure, function, pathways and genetic interactions.
- **algorithms**: In mathematics and computer science, an algorithm is a step-by-step procedure for calculations. Algorithms are used for calculation, data processing, and automated reasoning.
- **gene ontology**: The Gene Ontology is a major bioinformatics initiative to unify the representation of gene and gene product attributes across all species.

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SECTION OVERVIEW

5.3: DNA Replication

5.3A: Basics of DNA Replication

5.3B: DNA Replication in Eukaryotes

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5.3A: Basics of DNA Replication

DNA replication uses a semi-conservative method that results in a double-stranded DNA with one parental strand and a new daughter strand.

LEARNING OBJECTIVES

Explain how the Meselson and Stahl experiment conclusively established that DNA replication is semi-conservative.

Key Takeaways

Basics of DNA Replication

Watson and Crick's discovery that DNA was a two-stranded double helix provided a hint as to how DNA is replicated. During cell division, each DNA molecule has to be perfectly copied to ensure identical DNA molecules to move to each of the two daughter cells. The double-stranded structure of DNA suggested that the two strands might separate during replication with each strand serving as a template from which the new complementary strand for each is copied, generating two double-stranded molecules from one.

Models of Replication

There were three models of replication possible from such a scheme: conservative, semi-conservative, and dispersive. In conservative replication, the two original DNA strands, known as the parental strands, would re-basepair with each other after being used as templates to synthesize new strands; and the two newly-synthesized strands, known as the daughter strands, would also basepair with each other; one of the two DNA molecules after replication would be "all-old" and the other would be "all-new". In semi-conservative replication, each of the two parental DNA strands would act as a template for new DNA strands to be synthesized, but after replication, each parental DNA strand would basepair with the complementary newly-synthesized strand just synthesized, and both double-stranded DNAs would include one parental or "old" strand and one daughter or "new" strand. In dispersive replication, after replication both copies of the new DNAs would somehow have alternating segments of parental DNA and newly-synthesized DNA on each of their two strands.

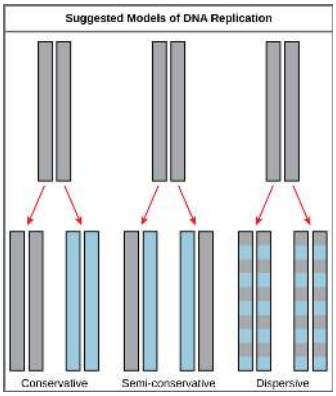


Figure: **Suggested Models of DNA Replication**: The three suggested models of DNA replication. Grey indicates the original parental DNA strands or segments and blue indicates newly-synthesized daughter DNA strands or segments.

To determine which model of replication was accurate, a seminal experiment was performed in 1958 by two researchers: Matthew Meselson and Franklin Stahl.

Meselson and Stahl

Meselson and Stahl were interested in understanding how DNA replicates. They grew *E. coli* for several generations in a medium containing a "heavy" isotope of nitrogen (^{15}N) that is incorporated into nitrogenous bases and, eventually, into the DNA. The *E. coli*culture was then shifted into medium containing the common "light" isotope of nitrogen (^{14}N) and allowed to grow for one generation. The cells were harvested and the DNA was isolated. The DNA was centrifuged at high speeds in an ultracentrifuge in a

tube in which a cesium chloride density gradient had been established. Some cells were allowed to grow for one more life cycle in ^{14}N and spun again.

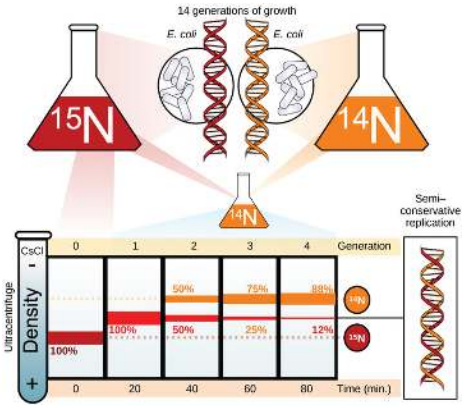


Figure: **Meselson and Stahl**: Meselson and Stahl experimented with E. coli grown first in heavy nitrogen (^{15}N) then in ligher nitrogen (^{14}N.) DNA grown in ^{15}N (red band) is heavier than DNA grown in ^{14}N (orange band) and sediments to a lower level in the cesium chloride density gradient in an ultracentrifuge. When DNA grown in ^{15}N is switched to media containing ^{14}N, after one round of cell division the DNA sediments halfway between the ^{15}N and ^{14}N levels, indicating that it now contains fifty percent ^{14}N and fifty percent ^{15}N.. In subsequent cell divisions, an increasing amount of DNA contains ^{14}N only. These data support the semi-conservative replication model.

During the density gradient ultracentrifugation, the DNA was loaded into a gradient (Meselson and Stahl used a gradient of cesium chloride salt, although other materials such as sucrose can also be used to create a gradient) and spun at high speeds of 50,000 to 60,000 rpm. In the ultracentrifuge tube, the cesium chloride salt created a density gradient, with the cesium chloride solution being more dense the farther down the tube you went. Under these circumstances, during the spin the DNA was pulled down the ultracentrifuge tube by centrifugal force until it arrived at the spot in the salt gradient where the DNA molecules' density matched that of the surrounding salt solution. At the point, the molecules stopped sedimenting and formed a stable band. By looking at the relative positions of bands of molecules run in the same gradients, you can determine the relative densities of different molecules. The molecules that form the lowest bands have the highest densities.

DNA from cells grown exclusively in ^{15}N produced a lower band than DNA from cells grown exclusively in ^{14}N. So DNA grown in ^{15}N had a higher density, as would be expected of a molecule with a heavier isotope of nitrogen incorporated into its nitrogenous bases. Meselson and Stahl noted that after one generation of growth in ^{14}N (after cells had been shifted from ^{15}N), the DNA molecules produced only single band intermediate in position in between DNA of cells grown exclusively in ^{15}N and DNA of cells grown exclusively in ^{14}N. This suggested either a semi-conservative or dispersive mode of replication. Conservative replication would have resulted in two bands; one representing the parental DNA still with exclusively ^{15}N in its nitrogenous bases and the other representing the daughter DNA with exclusively ^{14}N in its nitrogenous bases. The single band actually seen indicated that all the DNA molecules contained equal amounts of both ^{15}N and ^{14}N.

The DNA harvested from cells grown for two generations in ^{14}N formed two bands: one DNA band was at the intermediate position between ^{15}N and ^{14}N and the other corresponded to the band of exclusively ^{14}N DNA. These results could only be explained if DNA replicates in a semi-conservative manner. Dispersive replication would have resulted in exclusively a single band in each new generation, with the band slowly moving up closer to the height of the ^{14}N DNA band. Therefore, dispersive replication could also be ruled out.

Meselson and Stahl's results established that during DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are synthesized. The new strand will be complementary to the parental or "old" strand and the new strand will remain basepaired to the old strand. So each "daughter" DNA actually consists of one "old" DNA strand and one newly-synthesized strand. When two daughter DNA copies are formed, they have the identical sequences to one another and identical sequences to the original parental DNA, and the two daughter DNAs are divided equally into the two daughter cells, producing daughter cells that are genetically identical to one another and genetically identical to the parent cell.

Key Points

- There were three models suggested for DNA replication: conservative, semi-conservative, and dispersive.
- The conservative method of replication suggests that parental DNA remains together and newly-formed daughter strands are also together.
- The semi-conservative method of replication suggests that the two parental DNA strands serve as a template for new DNA and after replication, each double-stranded DNA contains one strand from the parental DNA and one new (daughter) strand.
- The dispersive method of replication suggests that, after replication, the two daughter DNAs have alternating segments of both parental and newly-synthesized DNA interspersed on both strands.
- Meselson and Stahl, using *E. coli* DNA made with two nitrogen istopes (^{14}N and ^{15}N) and density gradient centrifugation, determined that DNA replicated via the semi-conservative method of replication.

Key Terms

- **DNA replication**: a biological process occuring in all living organisms that is the basis for biological inheritance
- **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

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5.3B: DNA Replication in Eukaryotes

Learning Objectives

- Describe how DNA is replicated in eukaryotes

Because eukaryotic genomes are quite complex, DNA replication is a very complicated process that involves several enzymes and other proteins. It occurs in three main stages: initiation, elongation, and termination. DNA replication in eukaryotes occurs in three stages: initiation, elongation, and termination, which are aided by several enzymes.

Initiation

Eukaryotic DNA is bound to proteins known as histones to form structures called nucleosomes. During initiation, the DNA is made accessible to the proteins and enzymes involved in the replication process. There are specific chromosomal locations called origins of replication where replication begins. In some eukaryotes, like yeast, these locations are defined by having a specific sequence of basepairs to which the replication initiation proteins bind. In other eukaryotes, like humans, there does not appear to be a consensus sequence for their origins of replication. Instead, the replication initiation proteins might identify and bind to specific modifications to the nucleosomes in the origin region.

Certain proteins recognize and bind to the origin of replication and then allow the other proteins necessary for DNA replication to bind the same region. The first proteins to bind the DNA are said to "recruit" the other proteins. Two copies of an enzyme called helicase are among the proteins recruited to the origin. Each helicase unwinds and separates the DNA helix into single-stranded DNA. As the DNA opens up, Y-shaped structures called replication forks are formed. Because two helicases bind, two replication forks are formed at the origin of replication; these are extended in both directions as replication proceeds creating a replication bubble. There are multiple origins of replication on the eukaryotic chromosome which allow replication to occur simultaneously in hundreds to thousands of locations along each chromosome.

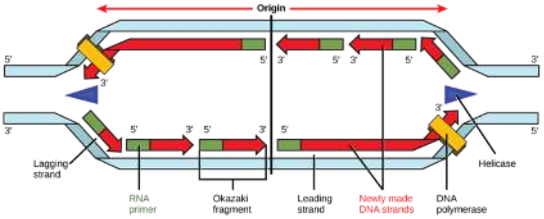


Figure: **Replication Fork Formation**: A replication fork is formed by the opening of the origin of replication; helicase separates the DNA strands. An RNA primer is synthesized by primase and is elongated by the DNA polymerase. On the leading strand, only a single RNA primer is needed, and DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches, each of which must start with its own RNA primer. The DNA fragments are joined by DNA ligase (not shown).

Elongation

During elongation, an enzyme called DNA polymerase adds DNA nucleotides to the 3′ end of the newly synthesized polynucleotide strand. The template strand specifies which of the four DNA nucleotides (A, T, C, or G) is added at each position along the new chain. Only the nucleotide complementary to the template nucleotide at that position is added to the new strand.

DNA polymerase contains a groove that allows it to bind to a single-stranded template DNA and travel one nucleotide at at time. For example, when DNA polymerase meets an adenosine nucleotide on the template strand, it adds a thymidine to the 3′ end of the newly synthesized strand, and then moves to the next nucleotide on the template strand. This process will continue until the DNA polymerase reaches the end of the template strand.

DNA polymerase cannot initiate new strand synthesis; it only adds new nucleotides at the 3′ end of an existing strand. All newly synthesized polynucleotide strands must be initiated by a specialized RNA polymerase called primase. Primase initiates polynucleotide synthesis and by creating a short RNA polynucleotide strand complementary to template DNA strand. This short stretch of RNA nucleotides is called the primer. Once RNA primer has been synthesized at the template DNA, primase exits, and DNA polymerase extends the new strand with nucleotides complementary to the template DNA.

Eventually, the RNA nucleotides in the primer are removed and replaced with DNA nucleotides. Once DNA replication is finished, the daughter molecules are made entirely of continuous DNA nucleotides, with no RNA portions.

The Leading and Lagging Strands

DNA polymerase can only synthesize new strands in the 5′ to 3′ direction. Therefore, the two newly-synthesized strands grow in opposite directions because the template strands at each replication fork are antiparallel. The "leading strand" is synthesized continuously toward the replication fork as helicase unwinds the template double-stranded DNA.

The "lagging strand" is synthesized in the direction away from the replication fork and away from the DNA helicase unwinds. This lagging strand is synthesized in pieces because the DNA polymerase can only synthesize in the 5′ to 3′ direction, and so it constantly encounters the previously-synthesized new strand. The pieces are called Okazaki fragments, and each fragment begins with its own RNA primer.

Termination

Eukaryotic chromosomes have multiple origins of replication, which initiate replication almost simultaneously. Each origin of replication forms a bubble of duplicated DNA on either side of the origin of replication. Eventually, the leading strand of one replication bubble reaches the lagging strand of another bubble, and

the lagging strand will reach the 5′ end of the previous Okazaki fragment in the same bubble.

DNA polymerase halts when it reaches a section of DNA template that has already been replicated. However, DNA polymerase cannot catalyze the formation of a phosphodiester bond between the two segments of the new DNA strand, and it drops off. These unattached sections of the sugar-phosphate backbone in an otherwise full-replicated DNA strand are called nicks.

Once all the template nucleotides have been replicated, the replication process is not yet over. RNA primers need to be replaced with DNA, and nicks in the sugar-phosphate backbone need to be connected.

The group of cellular enzymes that remove RNA primers include the proteins FEN1 (flap endonulcease 1) and RNase H. The enzymes FEN1 and RNase H remove RNA primers at the start of each leading strand and at the start of each Okazaki fragment, leaving gaps of unreplicated template DNA. Once the primers are removed, a free-floating DNA polymerase lands at the 3′ end of the preceding DNA fragment and extends the DNA over the gap. However, this creates new nicks (unconnected sugar-phosphate backbone).

In the final stage of DNA replication, the enyzme ligase joins the sugar-phosphate backbones at each nick site. After ligase has connected all nicks, the new strand is one long continuous DNA strand, and the daughter DNA molecule is complete.



This is a clip from a PBS production called "DNA: The Secret of Life." It details the latest research (as of 2005) concerning the process of DNA

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Key Points

- During initiation, proteins bind to the origin of replication while helicase unwinds the DNA helix and two replication forks are formed at the origin of replication.
- During elongation, a primer sequence is added with complementary RNA nucleotides, which are then replaced by DNA nucleotides.
- During elongation the leading strand is made continuously, while the lagging strand is made in pieces called Okazaki fragments.
- During termination, primers are removed and replaced with new DNA nucleotides and the backbone is sealed by DNA ligase.

Key Terms

- **origin of replication**: a particular sequence in a genome at which replication is initiated
- **leading strand**: the template strand of the DNA double helix that is oriented so that the replication fork moves along it in the 3′ to 5′ direction
- **lagging strand**: the strand of the template DNA double helix that is oriented so that the replication fork moves along it in a 5′ to 3′ manner

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SECTION OVERVIEW

5.4: Plasmids

Topic hierarchy

5.4A: Introduction to Plasmids

5.4B: Types of Plasmids and Their Biological Significance

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5.4A: Introduction to Plasmids

Learning Objectives

- Outline the utility of plasmids

The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952. A plasmid is a DNA molecule that is separate from, and can replicate independently of the chromosomal DNA. They are double-stranded and, in many cases, circular. Plasmids usually occur naturally in bacteria, but are sometimes found in archaea, and even in eukaryotic organisms (e.g., the 2-micrometre ring in *Saccharomyces cerevisiae*). Plasmid sizes vary from 1 to over 1,000 kbp. The number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances. Plasmids can be considered part of the mobilome because they are often associated with conjugation, a mechanism of horizontal gene transfer.

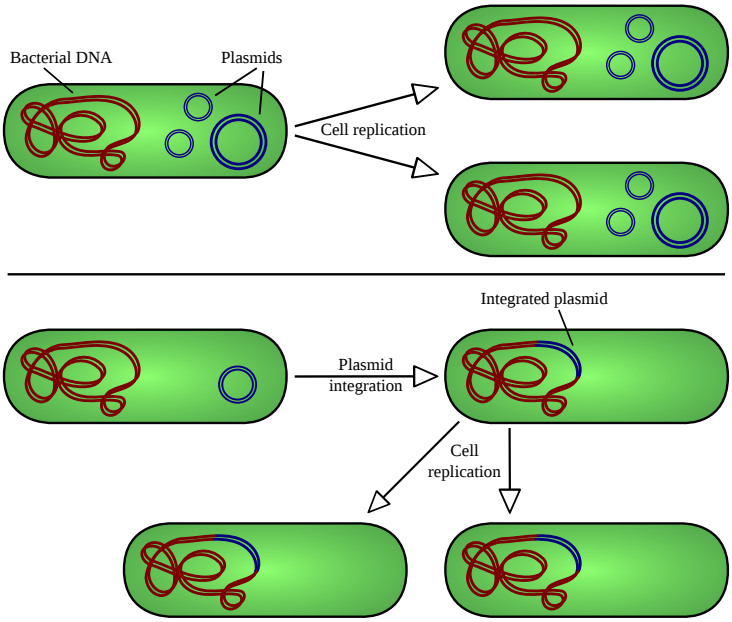


Figure: ***Step by step of cloning a gene using a plasmid****: This image shows a line drawing that compares the activity of non-integrating plasmids, on the top, with episomes, on the bottom, during cell division. The upper half of the image shows a bacterium with its chromosomal DNA and plasmids dividing into two identical bacteria, each with their chromosomal DNA and plasmids. The lower half of the image shows a bacterium with its chromosomal DNA, but with an episome. Next to this bacterium, we see the same bacterium, but after the episome has integrated into the chromosomal DNA and has become a part of it. This second bacterium now divides into two bacteria identical to it, each with an episome integrated into it.*

Plasmids are considered replicons. They can be found in all three major domains: Archaea, Bacteria, and Eukarya. Similar to viruses, plasmids are not considered by some to be a form of life. Unlike viruses, they are naked DNA and do not encode genes necessary to encase the genetic material for transfer to a new host, though some classes of plasmids encode the sex pilus necessary for their own transfer. Plasmid host-to-host transfer requires direct mechanical transfer by conjugation, or changes in incipient host gene expression allowing the intentional uptake of the genetic element by transformation. Microbial transformation with plasmid DNA is neither parasitic nor symbiotic in nature, because each implies the presence of an independent species living in a commensal or detrimental state with the host organism. Rather, plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or the proteins produced may act as toxins under similar circumstances. Plasmids can also provide bacteria with the ability to fix elemental nitrogen or to degrade recalcitrant organic compounds that provide an advantage when nutrients are scarce.

Key Points

- Plasmids can be found in all three major domains: Archaea, Bacteria, and Eukarya. Similar to viruses, plasmids are not considered by some to be a form of life.
- Plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state.
- Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or the proteins produced may act as toxins under similar circumstances.

Key Terms

- **plasmid**: A circle of double-stranded DNA that is separate from the chromosomes, which is found in bacteria and protozoa.
- **mobilome**: The entirety of the mobile (transposable) elements of a genome.
- **replicons**: a region of DNA or RNA, that replicates from a single origin of replication.

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5.4B: Types of Plasmids and Their Biological Significance

Plasmids are commonly used to multiply (make many copies of) or express particular genes.

LEARNING OBJECTIVES

Recognize the characteristics of, and thus the functions, of plasmids

Key Takeaways

Types of Plasmids

Plasmids used in genetic engineering are called vectors. Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to multiply (make many copies of) or express particular genes. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics. The gene is also inserted into a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments.

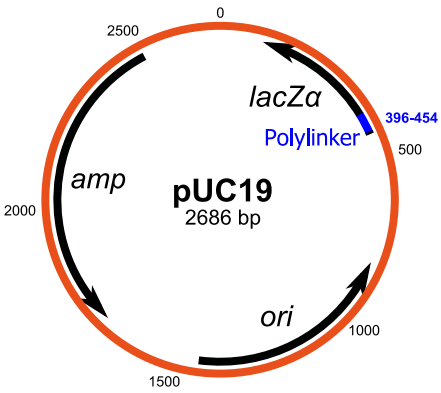


Figure: **A Plasmid Map of pUC19**: pUC19 is one of a series of plasmid cloning vectors created by Messing and co-workers in the University of California. The p in its name stands for plasmid and UC represents the University in which it was created. It is a circular double stranded DNA and has 2686 base pairs. pUC19 is one of the most widely used vector molecules as the recombinants, or the cells into which foreign DNA has been introduced, can be easily distinguished from the non-recombinants based on color differences of colonies on growth media. pUC18 is similar to pUC19, but the multiple cloning site region is reversed.

Next, the plasmids are inserted into bacteria by a process called transformation. Then, the bacteria are exposed to the particular antibiotics. Only bacteria that take up copies of the plasmid survive, since the plasmid makes them resistant. In particular, the protecting genes are expressed (used to make a protein) and the expressed protein breaks down the antibiotics. In this way, the antibiotics act as a filter, selecting only the modified bacteria. Finally, these bacteria can be grown in large amounts, harvested, and lysed (often using the alkaline lysis method) to isolate the plasmid of interest.

Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for; for example, insulin or even antibiotics.

One way of grouping plasmids is by their ability to transfer to other bacteria. Conjugative plasmids contain tra genes, which perform the complex process of conjugation, the transfer of plasmids to another bacterium. Non-conjugative plasmids are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids. An intermediate class of plasmids are mobilizable, and carry only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence. Plasmids are now being used to manipulate DNA, and may possibly be a tool for curing many diseases.

It is possible for plasmids of different types to coexist in a single cell. Several different plasmids have been found in E. coli. However, related plasmids are often incompatible, in the sense that only one of them survives in the cell line, due to the regulation

of vital plasmid functions. Thus, plasmids can be assigned into incompatibility groups.

Another way to classify plasmids is by function. There are five main classes:

- Fertility F-plasmids, which contain tra genes. They are capable of conjugation and result in the expression of sex pilli.
- Resistance plasmids, which contain genes that provide resistance against antibiotics or poisons. They were historically known as R-factors, before the nature of plasmids was understood.
- Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
- Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
- Virulence plasmids, which turn the bacterium into a pathogen.

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Key Points

- The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics, and into a multiple cloning site (MCS, or polylinker), allowing the easy insertion of DNA fragments.
- A major use of plasmids is to make large amounts of proteins. Bacterium can be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for; for example, insulin or even antibiotics.
- It is possible for plasmids of different types to coexist in a single cell. Several different plasmids have been found in E. coli. However, related plasmids are often incompatible, in the sense that only one of them survives in the cell line, due to the regulation of vital plasmid functions.

Key Terms

- **Col plasmids**: These plasmids contain genes that code for bacteriocins, proteins that can kill other bacteria.
- **F-plasmid**: Fertility F-plasmids contain tra genes and are capable of conjugation resulting in the expression of sex pilli.
- **Resistance plasmids**: These plasmids contain genes that provide resistance against antibiotics or poisons.

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SECTION OVERVIEW

5.5: RNA Synthesis - Transcription

Topic hierarchy

5.5A: Elongation and Termination in Eukaryotes

5.5B: The Promoter and the Transcription Machinery

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5.5A: Elongation and Termination in Eukaryotes

Elongation synthesizes pre-mRNA in a 5′ to 3′ direction, and termination occurs in response to termination sequences and signals.

LEARNING OBJECTIVES

Describe what is happening during transcription elongation and termination

Key Takeaways

Transcription through Nucleosomes

Following the formation of the pre-initiation complex, the polymerase is released from the other transcription factors, and elongation is allowed to proceed with the polymerase synthesizing RNA in the 5′ to 3′ direction. RNA Polymerase II (RNAPII) transcribes the major share of eukaryotic genes, so this section will mainly focus on how this specific polymerase accomplishes elongation and termination.

Although the enzymatic process of elongation is essentially the same in eukaryotes and prokaryotes, the eukaryotic DNA template is more complex. When eukaryotic cells are not dividing, their genes exist as a diffuse, but still extensively packaged and compacted mass of DNA and proteins called chromatin. The DNA is tightly packaged around charged histone proteins at repeated intervals. These DNA–histone complexes, collectively called nucleosomes, are regularly spaced and include 146 nucleotides of DNA wound twice around the eight histones in a nucleosome like thread around a spool.

For polynucleotide synthesis to occur, the transcription machinery needs to move histones out of the way every time it encounters a nucleosome. This is accomplished by a special protein dimer called FACT, which stands for “facilitates chromatin transcription.” FACT partially disassembles the nucleosome immediately ahead (upstream) of a transcribing RNA Polymerase II by removing two of the eight histones (a single dimer of H2A and H2B histones is removed.) This presumably sufficiently loosens the DNA wrapped around that nucleosome so that RNA Polymerase II can transcribe through it. FACT reassembles the nucleosome behind the RNA Polymerase II by returning the missing histones to it. RNA Polymerase II will continue to elongate the newly-synthesized RNA until transcription terminates.



The FACT protein dimer allows RNA Polymerase II to transcribe through packaged DNA: DNA in eukaryotes is packaged in nucleosomes, which consist of an octomer of 4 different histone proteins. When DNA is tightly wound twice around a nucleosome, RNA Polymerase II cannot access it for transcription. FACT removes two of the histones from the nucleosome immediately ahead of RNA Polymerase, loosening the packaging so that RNA Polymerase II can continue transcription. FACT also reassembles the nucleosome immediately behindd the RNA Polymerase by returning the missing histones.

Elongation

RNA Polymerase II is a complex of 12 protein subunits. Specific subunits within the protein allow RNA Polymerase II to act as its own helicase, sliding clamp, single-stranded DNA binding protein, as well as carry out other functions. Consequently, RNA Polymerase II does not need as many accessory proteins to catalyze the synthesis of new RNA strands during transcription elongation as DNA Polymerase does to catalyze the synthesis of new DNA strands during replication elongation.

However, RNA Polymerase II does need a large collection of accessory proteins to initiate transcription at gene promoters, but once the double-stranded DNA in the transcription start region has been unwound, the RNA Polymerase II has been positioned at the +1 initiation nucleotide, and has started catalyzing new RNA strand synthesis, RNA Polymerase II clears or “escapes” the promoter region and leaves most of the transcription initiation proteins behind.

All RNA Polymerases travel along the template DNA strand in the 3′ to 5′ direction and catalyze the synthesis of new RNA strands in the 5′ to 3′ direction, adding new nucleotides to the 3′ end of the growing RNA strand.

RNA Polymerases unwind the double stranded DNA ahead of them and allow the unwound DNA behind them to rewind. As a result, RNA strand synthesis occurs in a transcription bubble of about 25 unwound DNA basebairs. Only about 8 nucleotides of

newly-synthesized RNA remain basepaired to the template DNA. The rest of the RNA molecules falls off the template to allow the DNA behind it to rewind.

RNA Polymerases use the DNA strand below them as a template to direct which nucleotide to add to the 3′ end of the growing RNA strand at each point in the sequence. The RNA Polymerase travels along the template DNA one nucleotide at at time. Whichever RNA nucleotide is capable of basepairing to the template nucleotide below the RNA Polymerase is the next nucleotide to be added. Once the addition of a new nucleotide to the 3′ end of the growing strand has been catalyzed, the RNA Polymerase moves to the next DNA nucleotide on the template below it. This process continues until transcription termination occurs.

Termination



Transcription termination by RNA Polymerase II on a protein-encoding gene.: RNA Polymerase II has no specific signals that terminate its transcription. In the case of protein-encoding genes, a protein complex will bind to two locations on the growing pre-mRNA once the RNA Polymerase has transcribed past the end of the gene. CPSF in the complex will bind a AAUAAA sequence, and CstF in the complex will bind a GU-rich sequence (top figure). CPSF in the complex will cleave the pre-mRNA at a site between the two bound sequences, releasing the pre-mRNA (middle figure). Poly(A) Polymerase is a part of the same complex and will begin to add a poly-A tail to the pre-mRNA. At the same time, Xrn2 protein, which is an exonuclease, attacks the 5′ end of the RNA strand still associated with the RNA Polymerase. Xrn2 will start digesting the non-released portion of the newly synthesized RNA until Xrn2 reaches the RNA Polymerase, where it aids in displacing the RNA Polymerase from the template DNA strand. This terminates transcription at some random location downstream from the true end of the gene (bottom figure).

The termination of transcription is different for the three different eukaryotic RNA polymerases.

The ribosomal rRNA genes transcribed by RNA Polymerase I contain a specific sequence of basepairs (11 bp long in humans; 18 bp in mice) that is recognized by a termination protein called TTF-1 (Transcription Termination Factor for RNA Polymerase I.) This protein binds the DNA at its recognition sequence and blocks further transcription, causing the RNA Polymerase I to disengage from the template DNA strand and to release its newly-synthesized RNA.

The protein-encoding, structural RNA, and regulatory RNA genes transcribed by RNA Polymerse II lack any specific signals or sequences that direct RNA Polymerase II to terminate at specific locations. RNA Polymerase II can continue to transcribe RNA anywhere from a few bp to thousands of bp past the actual end of the gene. However, the transcript is cleaved at an internal site before RNA Polymerase II finishes transcribing. This releases the upstream portion of the transcript, which will serve as the initial RNA prior to further processing (the pre-mRNA in the case of protein-encoding genes.) This cleavage site is considered the “end” of the gene. The remainder of the transcript is digested by a 5′-exonuclease (called Xrn2 in humans) while it is still being transcribed by the RNA Polymerase II. When the 5′-exonulease “catches up” to RNA Polymerase II by digesting away all the overhanging RNA, it helps disengage the polymerase from its DNA template strand, finally terminating that round of transcription.

In the case of protein-encoding genes, the cleavage site which determines the “end” of the emerging pre-mRNA occurs between an upstream AAUAAA sequence and a downstream GU-rich sequence separated by about 40-60 nucleotides in the emerging RNA. Once both of these sequences have been transcribed, a protein called CPSF in humans binds the AAUAAA sequence and a protein called CstF in humans binds the GU-rich sequence. These two proteins form the base of a complicated protein complex that forms in this region before CPSF cleaves the nascent pre-mRNA at a site 10-30 nucleotides downstream from the AAUAAA site. The Poly(A) Polymerase enzyme which catalyzes the addition of a 3′ poly-A tail on the pre-mRNA is part of the complex that forms with CPSF and CstF.

The tRNA, 5S rRNA, and structural RNAs genes transcribed by RNA Polymerase III have a not-entirely-understood termination signal. The RNAs transcribed by RNA Polymerase III have a short stretch of four to seven U’s at their 3′ end. This somehow triggers RNA Polymerase III to both release the nascent RNA and disengage from the template DNA strand.

Key Points

- RNA polymerase II (RNAPII) transcribes the major share of eukaryotic genes.
- During elongation, the transcription machinery needs to move histones out of the way every time it encounters a nucleosome.

- Transcription elongation occurs in a bubble of unwound DNA, where the RNA Polymerase uses one strand of DNA as a template to catalyze the synthesis of a new RNA strand in the 5′ to 3′ direction.
- RNA Polymerase I and RNA Polymerase III terminate transcription in response to specific termination sequences in either the DNA being transcribed (RNA Polymerase I) or in the newly-synthesized RNA (RNA Polymerase III).
- RNA Polymerase II terminates transcription at random locations past the end of the gene being transcribed. The newly-synthesized RNA is cleaved at a sequence-specified location and released before transcription terminates.

Key Terms

- **nucleosome**: any of the subunits that repeat in chromatin; a coil of DNA surrounding a histone core
- **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
- **chromatin**: a complex of DNA, RNA, and proteins within the cell nucleus out of which chromosomes condense during cell division

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5.5B: The Promoter and the Transcription Machinery

Learning Objectives

- Describe the role of promoters in RNA transcription

Genes are organized to make the control of gene expression easier. The promoter region is immediately upstream of the coding sequence. This region can be short (only a few nucleotides in length) or quite long (hundreds of nucleotides long). The longer the promoter, the more available space for proteins to bind. This also adds more control to the transcription process. The length of the promoter is gene-specific and can differ dramatically between genes. Consequently, the level of control of gene expression can also differ quite dramatically between genes. The purpose of the promoter is to bind transcription factors that control the initiation of transcription.

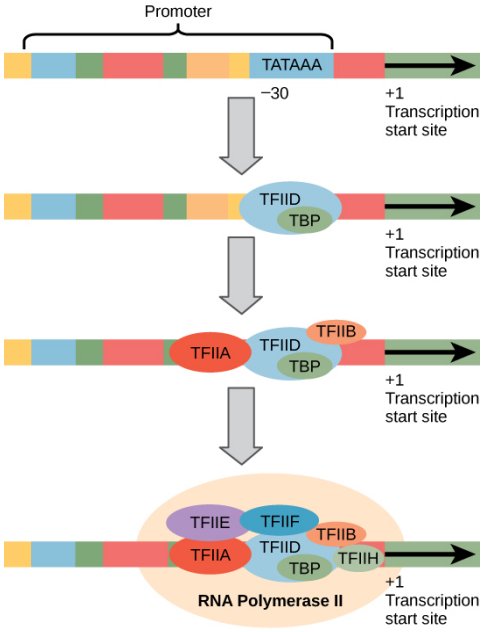


Figure: **Promoters**: A generalized promoter of a gene transcribed by RNA polymerase II is shown. Transcription factors recognize the promoter. RNA polymerase II then binds and forms the transcription initiation complex.

Within the promoter region, just upstream of the transcriptional start site, resides the TATA box. This box is simply a repeat of thymine and adenine dinucleotides (literally, TATA repeats). RNA polymerase binds to the transcription initiation complex, allowing transcription to occur. To initiate transcription, a transcription factor (TFIID) is the first to bind to the TATA box. Binding of TFIID recruits other transcription factors, including TFIIB, TFIIE, TFIIF, and TFIIH to the TATA box. Once this transcription initiation complex is assembled, RNA polymerase can bind to its upstream sequence. When bound along with the transcription factors, RNA polymerase is phosphorylated. This releases part of the protein from the DNA to activate the transcription initiation complex and places RNA polymerase in the correct orientation to begin transcription; DNA-bending protein brings the enhancer, which can be quite a distance from the gene, in contact with transcription factors and mediator proteins.

In addition to the general transcription factors, other transcription factors can bind to the promoter to regulate gene transcription. These transcription factors bind to the promoters of a specific set of genes. They are not general transcription factors that bind to every promoter complex, but are recruited to a specific sequence on the promoter of a specific gene. There are hundreds of transcription factors in a cell that each bind specifically to a particular DNA sequence motif. When transcription factors bind to the promoter just upstream of the encoded gene, they are referred to as cis-acting elements because they are on the same chromosome, just next to the gene. The region that a particular transcription factor binds to is called the transcription factor binding site. Transcription factors respond to environmental stimuli that cause the proteins to find their binding sites and initiate transcription of the gene that is needed.

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Key Points

- The purpose of the promoter is to bind transcription factors that control the initiation of transcription.
- The promoter region can be short or quite long; the longer the promoter is, the more available space for proteins to bind.
- To initiate transcription, a transcription factor (TFIID) binds to the TATA box, which causes other transcription factors to subsequently bind to the TATA box.
- Once the transcription initiation complex is assembled, RNA polymerase can bind to its upstream sequence and is then phosphorylated.
- Phosphorylation of RNA polymerase releases part of the protein from the DNA to activate the transcription initiation complex and places RNA polymerase in the correct orientation to begin transcription.
- Transcription factors respond to environmental stimuli that cause the proteins to find their binding sites and initiate transcription of the gene that is needed.

Key Terms

- **TATA box**: a DNA sequence (cis-regulatory element) found in the promoter region of genes in archaea and eukaryotes
- **transcription factor**: a protein that binds to specific DNA sequences, thereby controlling the flow (or transcription) of genetic information from DNA to mRNA
- **promoter**: the section of DNA that controls the initiation of RNA transcription

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SECTION OVERVIEW

5.6: Translation- Protein Synthesis

Topic hierarchy

5.6A: Processing of tRNAs and rRNAs

5.6B: The Protein Synthesis Machinery

5.6C: Prokaryotic Transcription and Translation Are Coupled

5.6D: The Incorporation of Nonstandard Amino Acids

5.6E: Unsticking Stuck Ribosomes

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5.6A: Processing of tRNAs and rRNAs

Learning Objectives

- Describe how pre-rRNAs and pre-tRNAs are processed into mature rRNAs and tRNAs.

The tRNAs and rRNAs are structural molecules that have roles in protein synthesis; however, these RNAs are not themselves translated. In eukaryotes, pre-rRNAs are transcribed, processed, and assembled into ribosomes in the nucleolus, while pre-tRNAs are transcribed and processed in the nucleus and then released into the cytoplasm where they are linked to free amino acids for protein synthesis.

Ribosomal RNA (rRNA)

The four rRNAs in eukaryotes are first transcribed as two long precursor molecules. One contains just the pre-rRNA that will be processed into the 5S rRNA; the other spans the 28S, 5.8S, and 18S rRNAs. Enzymes then cleave the precursors into subunits corresponding to each rRNA. In bacteria, there are only three rRNAs and all are transcribed in one long precursor molecule that is cleaved into the individual rRNAs. Some of the bases of pre-rRNAs are methylated for added stability. Mature rRNAs make up 50-60% of each ribosome. Some of a ribosome's RNA molecules are purely structural, whereas others have catalytic or binding activities.

The eukaryotic ribosome is composed of two subunits: a large subunit (60S) and a small subunit (40S). The 60S subunit is composed of the 28S rRNA, 5.8S rRNA, 5S rRNA, and 50 proteins. The 40S subunit is composed of the 18S rRNA and 33 proteins. The bacterial ribosome is composed of two similar subunits, with slightly different components. The bacterial large subunit is called the 50S subunit and is composed of the 23S rRNA, 5S rRNA, and 31 proteins, while the bacterial small subunit is called the 30S subunit and is composed of the 16S rRNA and 21 proteins.

The two subunits join to constitute a functioning ribosome that is capable of creating proteins.

Transfer RNA (tRNA)

Each different tRNA binds to a specific amino acid and transfers it to the ribosome. Mature tRNAs take on a three-dimensional structure through intramolecular basepairing to position the amino acid binding site at one end and the anticodon in an unbasepaired loop of nucleotides at the other end. The anticodon is a three-nucleotide sequence, unique to each different tRNA, that interacts with a messenger RNA (mRNA) codon through complementary base pairing.

There are different tRNAs for the 21 different amino acids. Most amino acids can be carried by more than one tRNA.

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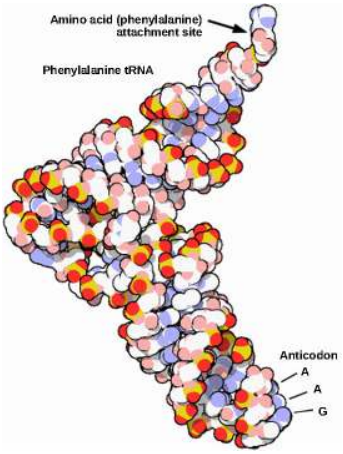


Figure: **Structure of tRNA**: This is a space-filling model of a tRNA molecule that adds the amino acid phenylalanine to a growing polypeptide chain. The anticodon AAG binds the codon UUC on the mRNA. The amino acid phenylalanine is attached to the other end of the tRNA.

In all organisms, tRNAs are transcribed in a pre-tRNA form that requires multiple processing steps before the mature tRNA is ready for use in translation. In bacteria, multiple tRNAs are often transcribed as a single RNA. The first step in their processing is the digestion of the RNA to release individual pre-tRNAs. In archaea and eukaryotes, each pre-tRNA is transcribed as a separate transcript.

The processing to convert the pre-tRNA to a mature tRNA involves five steps.

1. The 5′ end of the pre-tRNA, called the 5′ leader sequence, is cleaved off.

2. The 3′ end of the pre-tRNA is cleaved off.

3. In all eukaryote pre-tRNAs, but in only some bacterial and archaeal pre-tRNAs, a CCA sequence of nucleotides is added to the 3′ end of the pre-tRNA after the original 3′ end is trimmed off. Some bacteria and archaea pre-tRNAs already have the CCA encoded in their transcript immediately upstream of the 3′ cleavage site, so they don't need to add one. The CCA at the 3′ end of the mature tRNA will be the site at which the tRNA's amino acid will be added.

4. Multiple nucleotides in the pre-tRNA are chemically modified, altering their nitorgen bases. On average about 12 nucleotides are modified per tRNA. The most common modifications are the conversion of adenine (A) to pseudouridine (ψ), the conversion of adenine to inosine (I), and the conversion of uridine to dihydrouridine (D). But over 100 other modifications can occur.

5. A significant number of eukaryotic and archaeal pre-tRNAs have introns that have to be spliced out. Introns are rarer in bacterial pre-tRNAs, but do occur occasionally and are spliced out.

After processing, the mature pre-tRNA is ready to have its cognate amino acid attached. The cognate amino acid for a tRNA is the one specified by its anticodon. Attaching this amino acid is called charging the tRNA. In eukaryotes, the mature tRNA is generated in the nucleus, and then exported to the cytoplasm for charging.



Processing of a pre-tRNA.: A typical pre-tRNA undergoing processing steps to generate a mature tRNA ready to have its cognate amino acid attached. Nucleotides that are cleaved away are shown in green. Chemically-modified nucleotides are in yellow, as is the CAA trinucleotide that is added to the 3′ end of the pre-tRNA during processing. The anticodon nucleotides are shown in a lighter shade of red.

Key Points

- Ribosomal RNA (rRNA) is a structural molecule that makes up over half of the mass of a ribosome and aids in protein synthesis.
- Transfer RNA (tRNA) recognizes a codon on mRNA and brings the appropriate amino acid to that site.
- rRNAs are processed from larger pre-rRNAs by trimming the larger rRNAs down and methylating some of the nucleotides.
- tRNAs are processed from pre-tRNAs by trimming both ends of the pre-tRNA, adding a CCA trinucleotide to the 3′ end, if needed, removing any introns present, and chemically modified 12 nucleotides on average per tRNA.

Key Terms

- **anticodon**: a sequence of three nucleotides in transfer RNA that binds to the complementary triplet (codon) in messenger RNA, specifying an amino acid during protein synthesis

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5.6B: The Protein Synthesis Machinery

Protein synthesis, or translation of mRNA into protein, occurs with the help of ribosomes, tRNAs, and aminoacyl tRNA synthetases.

LEARNING OBJECTIVES

Explain the role played by ribosomes, tRNA, and aminoacyl tRNA synthetases in protein synthesis

Key Takeaways

The Protein Synthesis Machinery

In addition to the mRNA template, many molecules and macromolecules contribute to the process of translation. The composition of each component may vary across species. For instance, ribosomes may consist of different numbers of rRNAs and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to archaea to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors.

Ribosomes

A ribosome is a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides. In eukaryotes, the synthesis and assembly of rRNAs occurs in the nucleolus.

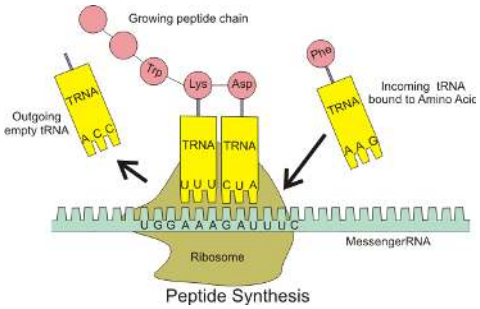


Figure: **The ribosome in action**: Structure and role of ribosomes during translation

Ribosomes exist in the cytoplasm in prokaryotes and in the cytoplasm and on rough endoplasmic reticulum membranes in eukaryotes. Mitochondria and chloroplasts also have their own ribosomes, and these look more similar to prokaryotic ribosomes (and have similar drug sensitivities) than the cytoplasmic ribosomes. Ribosomes dissociate into large and small subunits when they are not synthesizing proteins and reassociate during the initiation of translation.*E. coli* have a 30S small subunit and a 50S large subunit, for a total of 70S when assembled (recall that Svedberg units are not additive). Mammalian ribosomes have a small 40S subunit and a large 60S subunit, for a total of 80S. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs.

In bacteria, archaea, and eukaryotes, the intact ribosome has three binding sites that accomodate tRNAs: The A site, the P site, and the E site. Incoming aminoacy-tRNAs (a tRNA with an amino acid covalently attached is called an aminoacyl-tRNA) enter the ribosome at the A site. The peptidyl-tRNA carrying the growing polypeptide chain is held in the P site. The E site holds empty tRNAs just before they exit the ribosome.

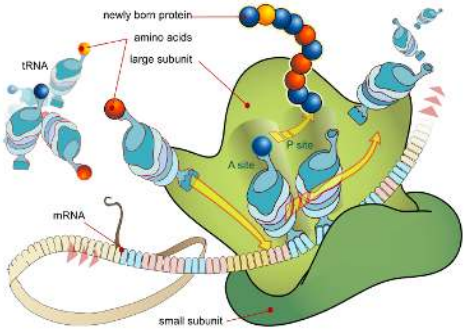


Figure: **Ribosome structure**: The large ribosomal subunit sits atop the small ribosomal subunit and the mRNA is threaded through a groove near the interface of the two subunits. The intact ribosome has three tRNA binding sites: the A site for incoming aminoacyl-tRNAs; the P site for the peptidyl-tRNA carrying the growing polypeptide chain; and the E site where empty tRNAs exit (not shown in this figure but immediately adjacent to the P site.)

Each mRNA molecule is simultaneously translated by many ribosomes, all reading the mRNA from 5′ to 3′ and synthesizing the polypeptide from the N terminus to the C terminus. The complete mRNA/poly-ribosome structure is called a polysome.

tRNAs in eukaryotes

The tRNA molecules are transcribed by RNA polymerase III. Depending on the species, 40 to 60 types of tRNAs exist in the cytoplasm. Specific tRNAs bind to codons on the mRNA template and add the corresponding amino acid to the polypeptide chain. (More accurately, the growing polypeptide chain is added to each new amino acid bound in by a tRNA.)

The transfer RNAs (tRNAs) are structural RNA molecules. In eukaryotes, tRNA mole are transcribed from tRNA genes by RNA polymerase III. Depending on the species, 40 to 60 types of tRNAs exist in the cytoplasm. Serving as adaptors, specific tRNAs bind to sequences on the mRNA template and add the corresponding amino acid to the polypeptide chain. (More accurately, the growing polypeptide chain is added to each new amino acid brought in by a tRNA.) Therefore, tRNAs are the molecules that actually “translate” the language of RNA into the language of proteins.

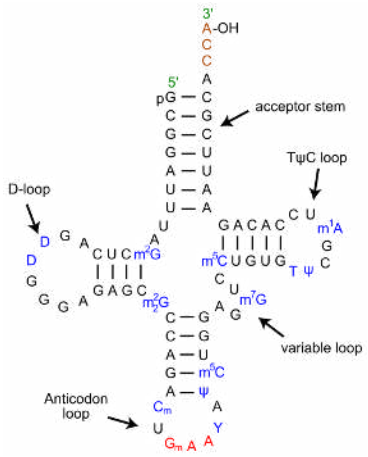


Figure: **The two-dimensional cloverleaf structure of a typical tRNA.**: All tRNAs, regardless of the species they come from or the amino acid they carry, self-basepair to produce a cloverleaf structure of four main stems and three main loops. The amino acid carried by the tRNA is covalently attached to the nucleotide at the 3′ end of the tRNA, known as the tRNA’s acceptor arm. The opposite end of the folded tRNA has the anticodon loop where the tRNA will basepair to the mRNA codon.

Of the 64 possible mRNA codons (triplet combinations of A, U, G, and C) three specify the termination of protein synthesis and 61 specify the addition of amino acids to the polypeptide chain. Of the three termination codons, one (UGA) can also be used to encode the 21st amino acid, selenocysteine, but only if the mRNA contains a specific sequence of nucleotides known as a SECIS sequence. Of the 61 non-termination codons, one codon (AUG) also encodes the initiation of translation.

Each tRNA polynucleotide chain folds up so that some internal sections basepair with other internal sections. If just diagrammed in two dimensions, the regions where basepairing occurs are called stems, and the regions where no basepairs form are called loops,

and the entire pattern of stems and loops that forms for a tRNA is called the “cloverleaf” structure. All tRNAs fold into very similar cloverleaf structures of four major stems and three major loops.

If viewed as a three-dimensional structure, all the basepaired regions of the tRNA are helical, and the tRNA folds into a L-shaped structure.

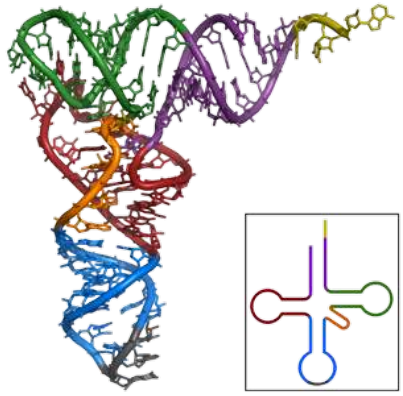


Figure: **The three dimensional shape taken by tRNAs.**: If viewed as a three-dimensional structure, all tRNAs are partially helical molecules that are vaguely L-shaped. The anticodon-containing loop is at one end of the molecule (in grey here) and the amino acid acceptor arm is at the other end of the molecule (in yellow here) past the bend of the “L”.

Each tRNA has a sequence of three nucleotides located in a loop at one end of the molecule that can basepair with an mRNA codon. This is called the tRNA’s anticodon. Each different tRNA has a different anticodon. When the tRNA anticodon basepairs with one of the mRNA codons, the tRNA will add an amino acid to a growing polypeptide chain or terminate translation, according to the genetic code. For instance, if the sequence CUA occurred on a mRNA template in the proper reading frame, it would bind a tRNA with an anticodon expressing the complementary sequence, GAU. The tRNA with this anticodon would be linked to the amino acid leucine.

Aminoacyl tRNA Synthetases

The process of pre-tRNA synthesis by RNA polymerase III only creates the RNA portion of the adaptor molecule. The corresponding amino acid must be added later, once the tRNA is processed and exported to the cytoplasm. Through the process of tRNA “charging,” each tRNA molecule is linked to its correct amino acid by a group of enzymes called aminoacyl tRNA synthetases. When an amino acid is covalently linked to a tRNA, the resulting complex is known as an aminoacyl-tRNA. At least one type of aminoacyl tRNA synthetase exists for each of the 21 amino acids; the exact number of aminoacyl tRNA synthetases varies by species. These enzymes first bind and hydrolyze ATP to catalyze the formation of a covalent bond between an amino acid and adenosine monophosphate (AMP); a pyrophosphate molecule is expelled in this reaction. This is called “activating” the amino acid. The same enzyme then catalyzes the attachment of the activated amino acid to the tRNA and the simultaneous release of AMP. After the correct amino acid covalently attached to the tRNA, it is released by the enzyme. The tRNA is said to be charged with its cognate amino acid. (the amino acid specified by its anticodon is a tRNA’s cognate amino acid.)

Key Points

- Ribosomes, macromolecular structures composed of rRNA and polypeptide chains, are formed of two subunits (in bacteria and archaea, 30S and 50S; in eukaryotes, 40S and 60S), that bring together mRNA and tRNAs to catalyze protein synthesis.
- Fully assembled ribosomes have three tRNA binding sites: an A site for incoming aminoacyl-tRNAs, a P site for peptidyl-tRNAs, and an E site where empty tRNAs exit.
- tRNAs (transfer ribonucleic acids), which serve to deliver the appropriate amino acid to the growing peptide chain, consist of a modified RNA chain with the appropriate amino acid covalently attached.
- tRNAs have a loop of unbasepaired nucleotides at one end of the molecule that contains three nucleotides that act as the anticodon that basepairs to the mRNA codon.
- Aminoacyl tRNA synthetases are enzymes that load the individual amino acids onto the tRNAs.

Key Terms

- **ribosome**: protein/mRNA complexes found in all cells that are involved in the production of proteins by translating messenger RNA

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5.6C: Prokaryotic Transcription and Translation Are Coupled

Learning Objectives

- Describe the events of prokaryotic transcription initiation

Overview of Prokaryotic Transcription

Prokaryotic transcription is the process in which messenger RNA transcripts of genetic material in prokaryotes are produced, to be translated for the production of proteins. Prokaryotic transcription occurs in the cytoplasm alongside translation. Prokaryotic transcription and translation can occur simultaneously. This is impossible in eukaryotes, where transcription occurs in a membrane-bound nucleus while translation occurs outside the nucleus in the cytoplasm. In prokaryotes genetic material is not enclosed in a membrane-enclosed nucleus and has access to ribosomes in the cytoplasm.

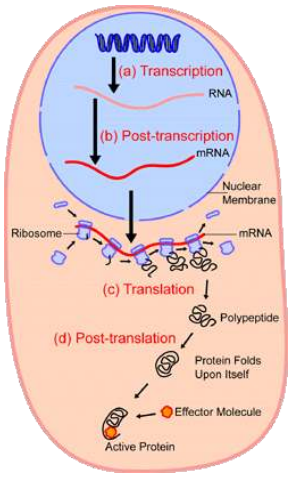


Figure: **Protein synthesis**: An overview of protein synthesis.Within the nucleus of the cell (light blue), genes (DNA, dark blue) are transcribed into RNA. This RNA is then subject to post-transcriptional modification and control, resulting in a mature mRNA (red) that is then transported out of the nucleus and into the cytoplasm (peach), where it undergoes translation into a protein. mRNA is translated by ribosomes (purple) that match the three-base codons of the mRNA to the three-base anti-codons of the appropriate tRNA. Newly synthesized proteins (black) are often further modified, such as by binding to an effector molecule (orange), to become fully active.

Transcription is controlled by a variety of regulators in prokaryotes. Many of these transcription factors are homodimers containing helix-turn-helix DNA-binding motifs.

Steps of Transcription Initiation

The following steps occur, in order, for transcription initiation:

- RNA polymerase (RNAP) binds to one of several specificity factors, σ, to form a holoenzyme. In this form, it can recognize and bind to specific promoter regions in the DNA. The -35 region and the -10 (“Pribnow box”) region comprise the basic prokaryotic promoter, and |T| stands for the terminator.
- The DNA on the template strand between the +1 site and the terminator is transcribed into RNA, which is then translated into protein. At this stage, the DNA is double-stranded (“closed”). This holoenzyme/wound-DNA structure is referred to as the closed complex.
- The DNA is unwound and becomes single-stranded (“open”) in the vicinity of the initiation site (defined as +1). This holoenzyme/unwound-DNA structure is called the open complex.
- The RNA polymerase transcribes the DNA (the beta subunit initiates the synthesis), but produces about 10 abortive (short, non-productive) transcripts which are unable to leave the RNA polymerase because the exit channel is blocked by the σ-factor.The σ-factor eventually dissociates from the holoenzyme, and elongation proceeds.

Additional Transcription Factors

Promoters can differ in “strength”; that is, how actively they promote transcription of their adjacent DNA sequence. Promoter strength is in many (but not all) cases, a matter of how tightly RNA polymerase and its associated accessory proteins bind to their respective DNA sequences. The more similar the sequences are to a consensus sequence, the stronger the binding is.

Additional transcription regulation comes from transcription factors that can affect the stability of the holoenzyme structure at initiation. Most transcripts originate using adenosine-5′-triphosphate (ATP) and, to a lesser extent, guanosine-5′-triphosphate (GTP) (purine nucleoside triphosphates) at the +1 site. Uridine-5′-triphosphate (UTP) and cytidine-5′-triphosphate (CTP) (pyrimidine nucleoside triphosphates) are disfavoured at the initiation site.

Two termination mechanisms are well known: *Intrinsic termination* (also called Rho-independent transcription termination) involves terminator sequences within the RNA that signal the RNA polymerase to stop. The terminator sequence is usually a palindromic sequence that forms a stem-loop hairpin structure that leads to the dissociation of the RNAP from the DNA template. *Rho-dependent termination* uses a termination factor called ρ factor(rho factor) which is a protein to stop RNA synthesis at specific sites. This protein binds at a rho utilisation site on the nascent RNA strand and runs along the mRNA towards the RNAP. A stem loop structure upstream of the terminator region pauses the RNAP, when ρ-factor reaches the RNAP, it causes RNAP to dissociate from the DNA, terminating transcription.

Key Points

- In prokaryotes genetic material is not enclosed in a membrane-enclosed nucleus and has access to ribosomes in the cytoplasm.
- Transcription is known to be controlled by a variety of regulators in prokaryotes. Many of these transcription factors are homodimers containing helix-turn-helix DNA -binding motifs.
- Additional transcription regulation comes from transcription factors that can affect the stability of the holoenzyme structure at initiation.

Key Terms

- **transcription**: The synthesis of RNA under the direction of DNA.

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5.6D: The Incorporation of Nonstandard Amino Acids

Aside from the 22 standard amino acids, there are many other amino acids that are called non-proteinogenic or non-standard.

LEARNING OBJECTIVES

Describe the process and function of posttranslational modification

Key Takeaways

Posttranslational modification (PTM) is the chemical modification of a protein after its translation. It is one of the later steps in protein biosynthesis, and thus gene expression, for many proteins. A protein (also called a polypeptide) is a chain of amino acids. During protein synthesis, 20 different amino acids can be incorporated to become a protein. After translation, the posttranslational modification of amino acids extends the range of functions of the protein by attaching it to other biochemical functional groups (such as acetate, phosphate, various lipids, and carbohydrates), changing the chemical nature of an amino acid (e.g., citrullination), or making structural changes (e.g., formation of disulfide bridges).

Also, enzymes may remove amino acids from the amino end of the protein, or cut the peptide chain in the middle. For instance, the peptide hormone insulin is cut twice after disulfide bonds are formed, and a propeptide is removed from the middle of the chain; the resulting protein consists of two polypeptide chains connected by disulfide bonds. Also, most nascent polypeptides start with the amino acid methionine because the “start” codon on mRNA also codes for this amino acid. This amino acid is usually taken off during post-translational modification.

Aside from the 22 standard amino acids, there are many other amino acids that are called non-proteinogenic or non-standard. Those either are not found in proteins (e.g., carnitine, GABA), or are not produced directly and in isolation by standard cellular machinery (e.g., hydroxyproline and selenomethionine).

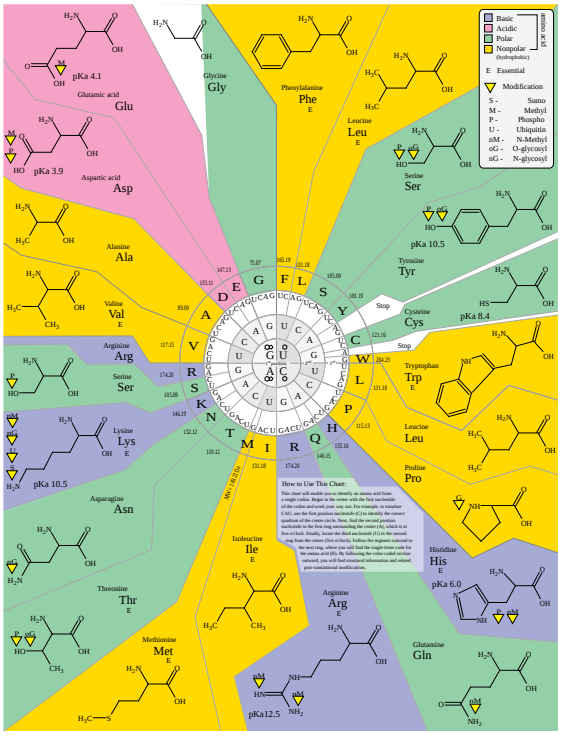


Figure: **Genetic code**: The genetic code diagram showing the amino acid residues as target of modification.

Non-standard amino acids that are found in proteins are formed by post-translational modification, which is modification after translation during protein synthesis. These modifications are often essential for the function or regulation of a protein. For example, the carboxylation of glutamate allows for better binding of calcium cations, and the hydroxylation of proline is critical for maintaining connective tissues.

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Another example is the formation of hypusine in the translation initiation factor EIF5A through modification of a lysine residue. Such modifications can also determine the localization of the protein. For instance, the addition of long hydrophobic groups can cause a protein to bind to a phospholipid membrane.

It is important to compare the structures of alanine and beta alanine. In alanine, the side-chain is a methyl group; in beta alanine, the side-chain contains a methylene group connected to an amino group, and the alpha carbon lacks an amino group. The two amino acids, therefore, have the same formulae but different structures.

Some nonstandard amino acids are not found in proteins. Examples include lanthionine, 2-aminoisobutyric acid, dehydroalanine, and the neurotransmitter gamma-aminobutyric acid. Nonstandard amino acids often occur as intermediates in the metabolic pathways for standard amino acids. For example, ornithine and citrulline occur in the urea cycle, which is part of amino acid catabolism. A rare exception to the dominance of α-amino acids in biology is the β-amino acid beta alanine (3-aminopropanoic acid), which is used in plants and microorganisms in the synthesis of pantothenic acid (vitamin B5), a component of coenzyme A.

Key Points

- During protein synthesis, 20 different amino acids can be incorporated to become a protein.
- Posttranslational modification of amino acids change the chemical nature of an amino acid (e.g., citrullination), or make structural changes (e.g., formation of disulfide bridges).
- Non-standard amino acids either are not found in proteins (e.g., carnitine, GABA), or are not produced directly and in isolation by standard cellular machinery.

Key Terms

- **Posttranslational modification**: the chemical modification of a protein after its translation. It is one of the later steps in protein biosynthesis, and thus gene expression, for many proteins.
- **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.
- **amino acid**: Any organic compound containing both an amino and a carboxylic acid functional group.

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5.6E: Unsticking Stuck Ribosomes

Aside from the 22 standard amino acids, there are many other amino acids that are called non-proteinogenic or non-standard.

LEARNING OBJECTIVES

Describe the process and function of posttranslational modification

Key Takeaways

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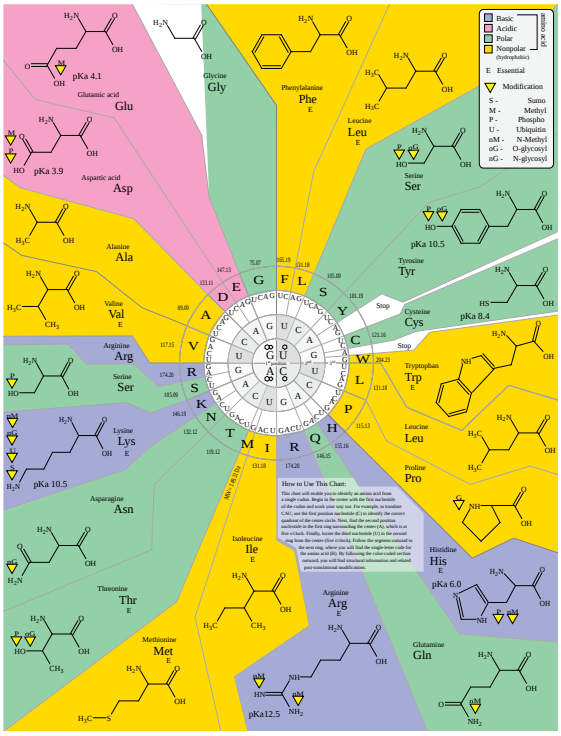


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Key Points

- During protein synthesis, 20 different amino acids can be incorporated to become a protein.
- Posttranslational modification of amino acids change the chemical nature of an amino acid (e.g., citrullination), or make structural changes (e.g., formation of disulfide bridges).
- Non-standard amino acids either are not found in proteins (e.g., carnitine, GABA), or are not produced directly and in isolation by standard cellular machinery.

Key Terms

- **Posttranslational modification**: the chemical modification of a protein after its translation. It is one of the later steps in protein biosynthesis, and thus gene expression, for many proteins.
- **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.
- **amino acid**: Any organic compound containing both an amino and a carboxylic acid functional group.

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SECTION OVERVIEW

5.7: Protein Modification, Folding, Secretion, and Degradation

Topic hierarchy

5.7A: mRNA Processing

5.7B: Denaturation and Protein Folding

5.7C: Protein Folding, Modification, and Targeting

5.7D: Regulating Protein Activity and Longevity

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5.7A: mRNA Processing

Eukaryotic pre-mRNA receives a 5′ cap and a 3′ poly (A) tail before introns are removed and the mRNA is considered ready for translation.

LEARNING OBJECTIVES

Outline the steps of pre-mRNA processing

Key Takeaways

Pre-mRNA Processing

The eukaryotic pre-mRNA undergoes extensive processing before it is ready to be translated. The additional steps involved in eukaryotic mRNA maturation create a molecule with a much longer half-life than a prokaryotic mRNA. Eukaryotic mRNAs last for several hours, whereas the typical *E. coli* mRNA lasts no more than five seconds.

Pre-mRNAs are first coated in RNA-stabilizing proteins; these protect the pre-mRNA from degradation while it is processed and exported out of the nucleus. The three most important steps of pre-mRNA processing are the addition of stabilizing and signaling factors at the 5′ and 3′ ends of the molecule, and the removal of intervening sequences that do not specify the appropriate amino acids. In rare cases, the mRNA transcript can be “edited” after it is transcribed.

5′ Capping

While the pre-mRNA is still being synthesized, a 7-methylguanosine cap is added to the 5′ end of the growing transcript by a 5′-to-5′ phosphate linkage. This moiety protects the nascent mRNA from degradation. In addition, initiation factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

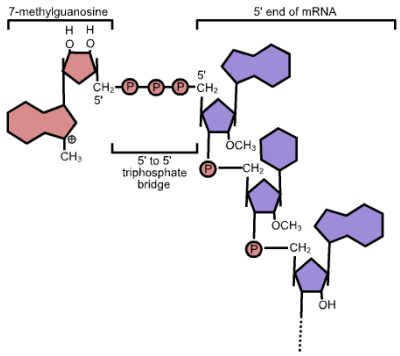


Figure: **5′ cap structure**: Capping of the pre-mRNA involves the addition of 7-methylguanosine (m^7G) to the 5′ end. The cap protects the 5′ end of the primary RNA transcript from attack by ribonucleases and is recognized by eukaryotic initiation factors involved in assembling the ribosome on the mature mRNA prior to initiating translation.

3′ Poly-A Tail

While RNA Polymerase II is still transcribing downstream of the proper end of a gene, the pre-mRNA is cleaved by an endonuclease-containing protein complex between an AAUAAA consensus sequence and a GU-rich sequence. This releases the functional pre-mRNA from the rest of the transcript, which is still attached to the RNA Polymerase. An enzyme called poly (A) polymerase (PAP) is part of the same protein complex that cleaves the pre-mRNA and it immediately adds a string of approximately 200 A nucleotides, called the poly (A) tail, to the 3′ end of the just-cleaved pre-mRNA. The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation.

image

Poly (A) Polymerase adds a 3′ poly (A) tail to the pre-mRNA.: The pre-mRNA is cleaved off the rest of the growing transcript before RNA Polymerase II has stopped transcribing. This cleavage is done by an endonuclease-containing protein complex that

binds to an AAUAAA sequence upstream of the cleavage site and to a GU-rich sequence downstream of the cut site. Immediately after the cleavage, Poly (A) Polymerase (PAP), which is also part of the protein complex, catalyzes the addition of up to 200 A nucleotides to the 3′ end of the just-cleaved pre-mRNA.

Pre-mRNA Splicing

Eukaryotic genes are composed of exons, which correspond to protein-coding sequences (*ex*-on signifies that they are *ex*pressed), and intervening sequences called introns (*int*-ron denotes their *int*ervening role), which may be involved in gene regulation, but are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins.

Discovery of Introns

The discovery of introns came as a surprise to researchers in the 1970s who expected that pre-mRNAs would specify protein sequences without further processing, as they had observed in prokaryotes. The genes of higher eukaryotes very often contain one or more introns. While these regions may correspond to regulatory sequences, the biological significance of having many introns or having very long introns in a gene is unclear. It is possible that introns slow down gene expression because it takes longer to transcribe pre-mRNAs with lots of introns. Alternatively, introns may be nonfunctional sequence remnants left over from the fusion of ancient genes throughout evolution. This is supported by the fact that separate exons often encode separate protein subunits or domains. For the most part, the sequences of introns can be mutated without ultimately affecting the protein product.

Intron Processing

All introns in a pre-mRNA must be completely and precisely removed before protein synthesis. If the process errs by even a single nucleotide, the reading frame of the rejoined exons would shift, and the resulting protein would be dysfunctional. The process of removing introns and reconnecting exons is called splicing. Introns are removed and degraded while the pre-mRNA is still in the nucleus. Splicing occurs by a sequence-specific mechanism that ensures introns will be removed and exons rejoined with the accuracy and precision of a single nucleotide. The splicing of pre-mRNAs is conducted by complexes of proteins and RNA molecules called spliceosomes.

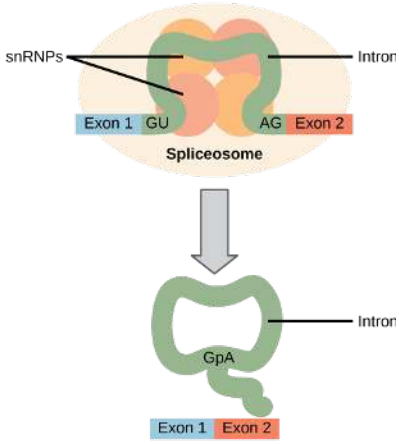


Figure: **Pre-mRNA splicing**: Pre-mRNA splicing involves the precise removal of introns from the primary RNA transcript. The splicing process is catalyzed by large complexes called spliceosomes. Each spliceosome is composed of five subunits called snRNPs. The spliceseome's actions result in the splicing together of the two exons and the release of the intron in a lariat form.

Each spliceosome is composed of five subunits called snRNPs (for small nuclear ribonucleoparticles, and pronounced "snurps".) Each snRNP is itself a complex of proteins and a special type of RNA found only in the nucleus called snRNAs (small nuclear RNAs). Spliceosomes recognize sequences at the 5′ end of the intron because introns always start with the nucleotides GU and they recognize sequences at the 3′ end of the intron because they always end with the nucleotides AG. The spliceosome cleaves the pre-mRNA's sugar phosphate backbone at the G that starts the intron and then covalently attaches that G to an internal A nucleotide within the intron. Then the spliceosme connects the 3′ end of the first exon to the 5′ end of the following exon, cleaving the 3′ end of the intron in the process. This results in the splicing together of the two exons and the release of the intron in a lariat form.

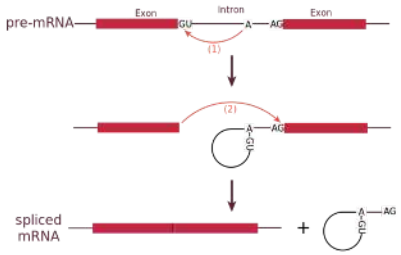


Figure: **Mechanism of pre-mRNA splicing.**: The snRNPs of the spliceosome were left out of this figure, but it shows the sites within the intron whose interactions are catalyzed by the spliceosome. Initially, the conserved G which starts an intron is cleaved from the 3′ end of the exon upstream to it and the G is covalently attached to an internal A within the intron. Then the 3′ end of the just-released exon is joined to the 5′ end of the next exon, cleaving the bond that attaches the 3′ end of the intron to its adjacent exon. This both joins the two exons and removes the intron in lariat form.

Key Points

- A 7-methylguanosine cap is added to the 5′ end of the pre-mRNA while elongation is still in progress. The 5′ cap protects the nascent mRNA from degradation and assists in ribosome binding during translation.
- A poly (A) tail is added to the 3′ end of the pre-mRNA once elongation is complete. The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation.
- Introns are removed from the pre-mRNA before the mRNA is exported to the cytoplasm.

Key Terms

- **intron**: a portion of a split gene that is included in pre-RNA transcripts but is removed during RNA processing and rapidly degraded
- **moiety**: a specific segment of a molecule
- **spliceosome**: a dynamic complex of RNA and protein subunits that removes introns from precursor mRNA

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5.7B: Denaturation and Protein Folding

Denaturation is a process in which proteins lose their shape and, therefore, their function because of changes in pH or temperature.

LEARNING OBJECTIVES

Discuss the process of protein denaturation

Key Takeaways

Each protein has its own unique sequence of amino acids and the interactions between these amino acids create a specify shape. This shape determines the protein's function, from digesting protein in the stomach to carrying oxygen in the blood.

Changing the Shape of a Protein

If the protein is subject to changes in temperature, pH, or exposure to chemicals, the internal interactions between the protein's amino acids can be altered, which in turn may alter the shape of the protein. Although the amino acid sequence (also known as the protein's primary structure) does not change, the protein's shape may change so much that it becomes dysfunctional, in which case the protein is considered denatured. Pepsin, the enzyme that breaks down protein in the stomach, only operates at a very low pH. At higher pHs pepsin's conformation, the way its polypeptide chain is folded up in three dimensions, begins to change. The stomach maintains a very low pH to ensure that pepsin continues to digest protein and does not denature.

Enzymes

Because almost all biochemical reactions require enzymes, and because almost all enzymes only work optimally within relatively narrow temperature and pH ranges, many homeostatic mechanisms regulate appropriate temperatures and pH so that the enzymes can maintain the shape of their active site.

Reversing Denaturation

It is often possible to reverse denaturation because the primary structure of the polypeptide, the covalent bonds holding the amino acids in their correct sequence, is intact. Once the denaturing agent is removed, the original interactions between amino acids return the protein to its original conformation and it can resume its function.

However, denaturation can be irreversible in extreme situations, like frying an egg. The heat from a pan denatures the albumin protein in the liquid egg white and it becomes insoluble. The protein in meat also denatures and becomes firm when cooked.



Figure: **Denaturing a protein is occasionally irreversible**: (Top) The protein albumin in raw and cooked egg white. (Bottom) A paperclip analogy visualizes the process: when cross-linked, paperclips ('amino acids') no longer move freely; their structure is rearranged and 'denatured'.

Chaperone proteins (or chaperonins) are helper proteins that provide favorable conditions for protein folding to take place. The chaperonins clump around the forming protein and prevent other polypeptide chains from aggregating. Once the target protein folds, the chaperonins disassociate.

Key Points

- Proteins change their shape when exposed to different pH or temperatures.
- The body strictly regulates pH and temperature to prevent proteins such as enzymes from denaturing.
- Some proteins can refold after denaturation while others cannot.
- Chaperone proteins help some proteins fold into the correct shape.

Key Terms

- **chaperonin**: proteins that provide favorable conditions for the correct folding of other proteins, thus preventing aggregation
- **denaturation**: the change of folding structure of a protein (and thus of physical properties) caused by heating, changes in pH, or exposure to certain chemicals

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5.7C: Protein Folding, Modification, and Targeting

In order to function, proteins must fold into the correct three-dimensional shape, and be targeted to the correct part of the cell.

LEARNING OBJECTIVES

Discuss how post-translational events affect the proper function of a protein

Key Takeaways

Protein Folding

After being translated from mRNA, all proteins start out on a ribosome as a linear sequence of amino acids. This linear sequence must "fold" during and after the synthesis so that the protein can acquire what is known as its native conformation. The native conformation of a protein is a stable three-dimensional structure that strongly determines a protein's biological function. When a protein loses its biological function as a result of a loss of three-dimensional structure, we say that the protein has undergone denaturation. Proteins can be denatured not only by heat, but also by extremes of pH; these two conditions affect the weak interactions and the hydrogen bonds that are responsible for a protein's three-dimensional structure. Even if a protein is properly specified by its corresponding mRNA, it could take on a completely dysfunctional shape if abnormal temperature or pH conditions prevent it from folding correctly. The denatured state of the protein does not equate with the unfolding of the protein and randomization of conformation. Actually, denatured proteins exist in a set of partially-folded states that are currently poorly understood. Many proteins fold spontaneously, but some proteins require helper molecules, called chaperones, to prevent them from aggregating during the complicated process of folding.

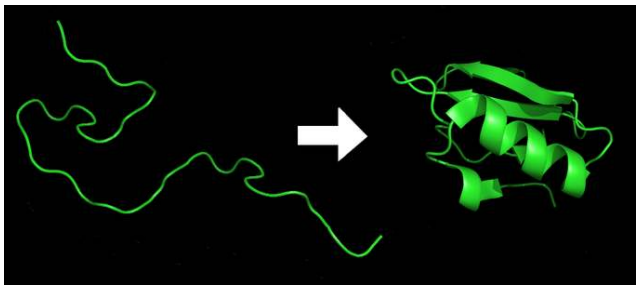


Figure: **Protein folding**: A protein starts as a linear sequence of amino acids, then folds into a 3-dimensional shape imbued with all the functional properties required inside the cell.

Protein Modification and Targeting

During and after translation, individual amino acids may be chemically modified and signal sequences may be appended to the protein. A signal sequence is a short tail of amino acids that directs a protein to a specific cellular compartment. These sequences at the amino end or the carboxyl end of the protein can be thought of as the protein's "train ticket" to its ultimate destination. Other cellular factors recognize each signal sequence and help transport the protein from the cytoplasm to its correct compartment. For instance, a specific sequence at the amino terminus will direct a protein to the mitochondria or chloroplasts (in plants). Once the protein reaches its cellular destination, the signal sequence is usually clipped off.

Misfolding

It is very important for proteins to achieve their native conformation since failure to do so may lead to serious problems in the accomplishment of its biological function. Defects in protein folding may be the molecular cause of a range of human genetic disorders. For example, cystic fibrosis is caused by defects in a membrane-bound protein called cystic fibrosis transmembrane conductance regulator (CFTR). This protein serves as a channel for chloride ions. The most common cystic fibrosis-causing mutation is the deletion of a Phe residue at position 508 in CFTR, which causes improper folding of the protein. Many of the disease-related mutations in collagen also cause defective folding.

A misfolded protein, known as prion, appears to be the agent of a number of rare degenerative brain diseases in mammals, like the mad cow disease. Related diseases include kuru and Creutzfeldt-Jakob. The diseases are sometimes referred to as spongiform

encephalopathies, so named because the brain becomes riddled with holes. Prion, the misfolded protein, is a normal constituent of brain tissue in all mammals, but its function is not yet known. Prions cannot reproduce independently and not considered living microoganisms. A complete understanding of prion diseases awaits new information about how prion protein affects brain function, as well as more detailed structural information about the protein. Therefore, improved understanding of protein folding may lead to new therapies for cystic fibrosis, Creutzfeldt-Jakob, and many other diseases.

Key Points

- Protein folding is a process in which a linear chain of amino acids attains a defined three-dimensional structure, but there is a possibility of forming misfolded or denatured proteins, which are often inactive.
- Proteins must also be located in the correct part of the cell in order to function correctly; therefore, a signal sequence is often attached to direct the protein to its proper location, which is removed after it attains its location.
- Protein misfolding is the cause of numerous diseases, such as mad cow disease, Creutzfeldt-Jakob disease, and cystic fibrosis.

Key Terms

- **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
- **chaperone**: a protein that assists the non-covalent folding/unfolding of other proteins

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5.7D: Regulating Protein Activity and Longevity

A cell can rapidly change the levels of proteins in response to the environment by adding specific chemical groups to alter gene regulation.

LEARNING OBJECTIVES

Explain how chemical modifications affect protein activity and longevity

Key Takeaways

Chemical Modifications, Protein Activity, and Longevity

Proteins can be chemically modified with the addition of methyl, phosphate, acetyl, and ubiquitin groups. The addition or removal of these groups from proteins regulates their activity or the length of time they exist in the cell. Sometimes these modifications can regulate where a protein is found in the cell; for example, in the nucleus, the cytoplasm, or attached to the plasma membrane.

Chemical modifications occur in response to external stimuli such as stress, the lack of nutrients, heat, or ultraviolet light exposure. These changes can alter protein function, epigenetic accessibility, transcription, mRNA stability, or translation; all resulting in changes in expression of various genes. This is an efficient way for the cell to rapidly change the abundance levels of specific proteins in response to the environment. Because proteins are involved in every stage of gene regulation, the phosphorylation of a protein (depending on the protein that is modified) can alter accessibility to the chromosome, can alter translation (by altering transcription factor binding or function), can change nuclear shuttling (by influencing modifications to the nuclear pore complex), can alter RNA stability (by binding or not binding to the RNA to regulate its stability), can modify translation (increase or decrease), or can change post-translational modifications (add or remove phosphates or other chemical modifications). All of these protein activities are affected by the phosphorylation process. The enzymes which are responsible for phosphorylation are known as protein kinases. The addition of a phosphate group to a protein can result in either activation or deactivation; it is protein dependent.

Another example of chemical modifications affecting protein activity include the addition or removal of methyl groups. Methyl groups are added to proteins via the process of methylation; this is the most common form of post-translational modification. The addition of methyl groups to a protein can result in protein-protein interactions that allows for transcriptional regulation, response to stress, protein repair, nuclear transport, and even differentiation processes. Methylation on side chain nitrogens is considered largely irreversible while methylation of the carboxyl groups is potentially reversible. Methylation in the proteins negates the negative charge on it and increases the hydrophobicity of the protein. Methylation on carboxylate side chains covers up a negative charge and adds hydrophobicity. The addition of this chemical group changes the property of the protein and, thus, affects it activity.

The addition of an ubiquitin group to a protein marks that protein for degradation. Ubiquitin acts like a flag indicating that the protein lifespan is complete. These proteins are moved to the proteasome, an organelle that functions to remove proteins to be degraded. One way to control gene expression is to alter the longevity of the protein: ubiquitination shortens a protein's lifespan.

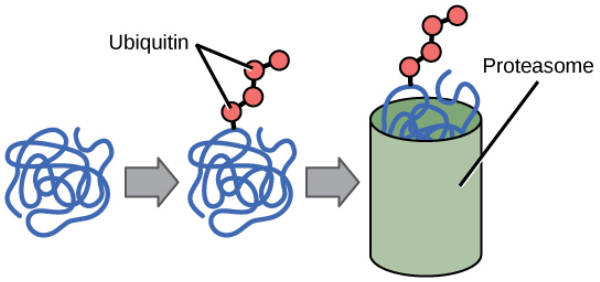


Figure: **Ubiquitin Tags**: Proteins with ubiquitin tags are marked for degradation within the proteasome.

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Key Points

- Proteins can be chemically modified by adding methyl, phosphate, acetyl, and ubiquitin groups.
- Protein longevity can be affected by altering stages of gene regulation, including but not limited to altering: accessibility to chromosomal DNA for transcription, rate of translation, nuclear shuttling, RNA stability, and post-translational modifications.
- Ubiquitin is added to a protein to mark it for degradation by the proteasome.

Key Terms

- **ubiquitin**: a small polypeptide present in the cells of all eukaryotes; it plays a part in modifying and degrading proteins
- **proteasome**: a complex protein, found in bacterial, archaeal and eukaryotic cells, that breaks down other proteins via proteolysis

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SECTION OVERVIEW

5.8: Archaeal Genetics

Topic hierarchy

5.8A: Chromosomes and DNA Replication in the Archaea

5.8B: Shared Features of Bacteria and Archaea

5.8C: Shared Features of Archaea and Eukaryotes

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5.8A: Chromosomes and DNA Replication in the Archaea

Archaea usually have a single circular chromosome.

LEARNING OBJECTIVES

Describe the characteristics of Archaeal chromosomes and their replication

Key Takeaways

Archaeal Genetics

Archaea usually have a single circular chromosome, the size of which may be as great as 5,751,492 base pairs in *Methanosarcina acetivorans*, which boasts the largest known archaean genome. One-tenth of this size is the tiny 490,885 base-pair genome of *Nanoarchaeum equitans*, which possesses the smallest archaean genome known; it is estimated to contain only 537 protein-encoding genes. Smaller independent pieces of DNA, called plasmids, are also found in archaea. Plasmids may be transferred between cells by physical contact, in a process that may be similar to bacterial conjugation.

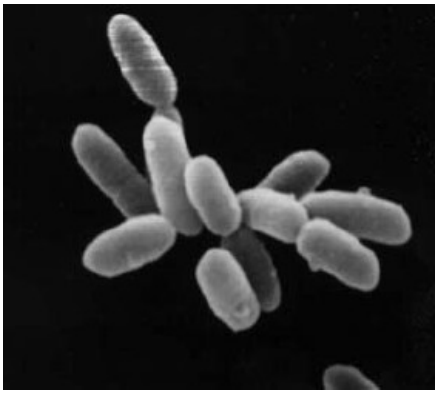


Figure: **Archaea**: Cluster of halobacterium (archaea)

Asexual Reproduction

The means of asexual reproduction that are used by Archaea include binary reproduction, multiple fission, fragmentation, or budding. The cell division process is controlled by the cell cycle; the chromosomes within the Archaea are replicated to produce two daughter chromosomes. Archaea typically have a single circular chromosome. The two daughter chromosomes are then separated and the cell divides. This process in Archaea appears to be similar to both bacterial and eukaryotic systems. The circular chromosomes contain multiple origins of replication, using DNA polymerases that resemble eukaryotic enzymes. However, the proteins involved that direct cell division are similar to those of bacterial systems.

DNA replication, similar in all systems, involves initiation, elongation, and termination. The replication of DNA, beginning at the origins of replication present on the circular chromosomes, requires initiator proteins. The recruitment of additional proteins by way of the initiator proteins allows the separation of the circular DNA and results in the formation of a bubble.

The DNA replication system in Archaea, similar to all systems, requires a free 3’OH group before synthesis is initiated. The primase used to synthesize a short RNA primer from the free 3’OH group varies in Archaea when compared to that of bacterial and eukaryotic systems. The primase used by archaea is a highly derived version of the RNA recognition motif (RRM). It is structurally similar to viral RNA dependent RNA polymerases, reverse transcriptases, cyclic nucleotide generating cyclases, and DNA polymerases involved in DNA replication and repair. Once the RNA primase has performed its job, DNA synthesis continues in a similar fashion by which the eukaryotic system and the DNA is replicated.

Key Points

- Archaea are genetically distinct from bacteria and eukaryotes, with up to 15% of the proteins encoded by any one archaeal genome being unique to the domain, although most of these unique genes have no known function.

- DNA replication in archaea requires a specific primase that shares similarities to the RNA recognition motif (RRM) and to viral RNA dependent RNA polymerases.
- The circular chromosomes of archaea contain multiple origins of replication for initiation of DNA synthesis.

Key Terms

- **chromosome**: A structure in the cell nucleus that contains DNA, histone protein, and other structural proteins.
- **eukaryote**: Any of the single-celled or multicellular organisms, of the taxonomic domain Eukaryota, whose cells contain at least one distinct nucleus.
- **bacteria**: A type, species, or strain of bacterium.

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5.8B: Shared Features of Bacteria and Archaea

Most of the metabolic pathways, which comprise the majority of an organism's genes, are common between Archaea and Bacteria.

LEARNING OBJECTIVES

Describe the evidence for the evolution of the Archaea from Bacteria

Key Takeaways



Figure: **Microbial Mats Around the Grand Prismatic Spring**: Thermophiles produce some of the bright colors of Grand Prismatic Spring, Yellowstone National Park

The relationship between the three domains is of central importance for understanding the origin of life. Most of the metabolic pathways, which comprise the majority of an organism 's genes, are common between Archaea and Bacteria, while most genes involved in genome expression are common between Archaea and Eukarya. Within prokaryotes, archaeal cell structure is most similar to that of Gram-positive bacteria, largely because both have a single lipid bilayer and usually contain a thick sacculus of varying chemical composition. In phylogenetic trees based upon different gene/ protein sequences of prokaryotic homologs, the archaeal homologs are more closely related to those of Gram-positive bacteria. Archaea and Gram-positive bacteria also share conserved indels in a number of important proteins, such as Hsp70 and glutamine synthetase I.

R.S. Gupta has proposed that the Archaea evolved from Gram-positive bacteria in response to antibiotic selection pressure. This is suggested by the observation that archaea are resistant to a wide variety of antibiotics that are primarily produced by Gram-positive bacteria, and that these antibiotics primarily act on the genes that distinguish Archaea from Bacteria. His proposal is that the selective pressure towards resistance generated by the Gram-positive antibiotics was eventually sufficient to cause extensive changes in many of the antibiotics' target genes, and that these strains represented the common ancestors of present-day Archaea. The evolution of Archaea in response to antibiotic selection, or any other competitive selective pressure, could also explain their adaptation to extreme environments (such as high temperature or acidity) as the result of a search for unoccupied niches to escape from antibiotic-producing organisms; Cavalier-Smith has made a similar suggestion. Gupta's proposal is also supported by other work investigating protein structural relationships and studies that suggest that Gram-positive bacteria may constitute the earliest branching lineages within the prokaryotes.

Key Points

- Within prokaryotes, archaeal cell structure is most similar to that of Gram-positive bacteria, largely because both have a single lipid bilayer and usually contain a thick sacculus of varying chemical composition.
- It has been proposed that the Archaea evolved from Gram-positive bacteria in response to antibiotic selection pressure.
- The evolution of Archaea in response to antibiotic selection, or any other competitive selective pressure, could also explain their adaptation to extreme environments (such as high temperature or acidity).

Key Terms

- **prokaryotes**: (/proʊkæri.oʊts/, pro-kah-ree-otes or /proʊkæriəts/, pro-kah-ree-əts) a group of organisms whose cells lack a cell nucleus (karyon), or any other membrane-bound organelles. Most prokaryotes are unicellular organisms, although a few such as myxobacteria have multicellular stages in their life cycles.
- **archaea**: a taxonomic domain of single-celled organisms lacking nuclei that are fundamentally from bacteria.

- **bacteria**: Bacteria constitute a large domain of prokaryotic microorganisms. Typically a few micrometres in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most habitats on the planet.

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5.8C: Shared Features of Archaea and Eukaryotes

Archaea possess genes and several metabolic pathways that are more closely related to those of eukaryotes than prokaryotes.

LEARNING OBJECTIVES

Describe the similarities between archaea, eukaryotes, and bacteria

Key Takeaways

The evolutionary relationship between *archaea* and *eukaryotes* remains unclear. Aside from the similarities in cell structure and function that are discussed below, many genetic trees group the two.

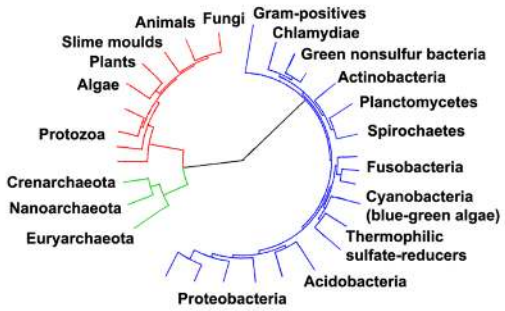


Figure: **Archaea and other domains**: Phylogenetic tree showing the relationship between the Archaea and other domains of life. Eukaryotes are colored red, archaea green and bacteria blue.

Complicating factors include claims that the relationship between eukaryotes and the archaeal phylum *Crenarchaeota* is closer than the relationship between the *Euryarchaeota*and the phylum *Crenarchaeota*, and the presence of archaean-like genes in certain bacteria, such as *Thermotoga maritima*, from horizontal gene transfer. The leading hypothesis is that the ancestor of the eukaryotes diverged early from the Archaea, and that eukaryotes arose through fusion of an archaean and eubacterium, which became the nucleus and cytoplasm. This explains various genetic similarities but runs into difficulties when it comes to explaining cell structure.

Despite this visual similarity to bacteria, archaea possess genes and several metabolic pathways that are more closely-related to those of eukaryotes, notably the enzymes involved in transcription and translation.

Archaea exhibit a great variety of chemical reactions in their metabolism and use many sources of energy. These reactions are classified into nutritional groups, depending on energy and carbon sources. Some archaea obtain energy from inorganic compounds such as sulfur or ammonia (they are lithotrophs). These include nitrifiers, methanogens and anaerobic methane oxidisers. In these reactions, one compound passes electrons to another (in a redox reaction), releasing energy to fuel the cell's activities. One compound acts as an electron donor and another as an electron acceptor. The energy released generates adenosine triphosphate (ATP) through chemiosmosis, in the same basic process that happens in the mitochondrion of eukaryotic cells.

The chromosomes replicate from multiple starting-points (origins of replication) using DNA polymerases that resemble the equivalent eukaryotic enzymes. However, the proteins that direct cell division, such as the protein FtsZ, which forms a contracting ring around the cell, and the components of the septum that is constructed across the center of the cell, are similar to their bacterial equivalents.

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Key Points

- Archaea exhibit a great variety of chemical reactions in their metabolism and use many sources of energy.
- The energy released generates adenosine triphosphate (ATP) through chemiosmosis, in the same basic process that happens in the mitochondrion of eukaryotic cells.
- The chromosomes replicate from multiple starting-points (origins of replication) using DNA polymerases that resemble the equivalent eukaryotic enzymes.

Key Terms

- **eukaryote**: Any of the single-celled or multicellular organisms, of the taxonomic domain Eukaryota, whose cells contain at least one distinct nucleus.

- **mitochondrion**: a spherical or ovoid organelle found in the cytoplasm of eukaryotic cells, contains genetic material separate from that of the host; it is responsible for the conversion of food to usable energy in the form of ATP
- **archaea**: a taxonomic domain of single-celled organisms lacking nuclei that are fundamentally from bacteria.
- **metabolism**: The complete set of chemical reactions that occur in living cells.

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SECTION OVERVIEW

5.9: Eukaryotic Genetics

Topic hierarchy

5.9A: The Role of the Cell Cycle

5.9B: The Relationship Between Genes and Proteins

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5.9A: The Role of the Cell Cycle

The cell cycle allows multiicellular organisms to grow and divide and single-celled organisms to reproduce.

LEARNING OBJECTIVES

Explain the role of the cell cycle in carrying out the cell's essential functions

Key Takeaways

Introduction: Cell Division and Reproduction

A human, as well as every sexually-reproducing organism, begins life as a fertilized egg or zygote. Trillions of cell divisions subsequently occur in a controlled manner to produce a complex, multicellular human. In other words, that original single cell is the ancestor of every other cell in the body. Once a being is fully grown, cell reproduction is still necessary to repair or regenerate tissues. For example, new blood and skin cells are constantly being produced. All multicellular organisms use cell division for growth and the maintenance and repair of cells and tissues. Cell division is tightly regulated because the occasional failure of regulation can have life-threatening consequences. Single-celled organisms use cell division as their method of reproduction.

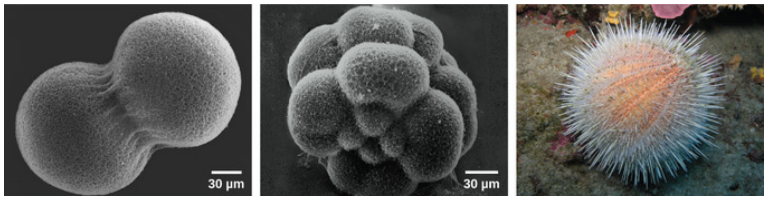


Figure: **Cell Division and Growth**: A sea urchin begins life as a single cell that (a) divides to form two cells, visible by scanning electron microscopy. After four rounds of cell division, (b) there are 16 cells, as seen in this SEM image. After many rounds of cell division, the individual develops into a complex, multicellular organism, as seen in this (c) mature sea urchin.

While there are a few cells in the body that do not undergo cell division, most somatic cells divide regularly. A somatic cell is a general term for a body cell: all human cells, except for the cells that produce eggs and sperm (which are referred to as germ cells), are somatic cells. Somatic cells contain two copies of each of their chromosomes (one copy received from each parent). Cells in the body replace themselves over the lifetime of a person. For example, the cells lining the gastrointestinal tract must be frequently replaced when constantly "worn off" by the movement of food through the gut. But what triggers a cell to divide and how does it prepare for and complete cell division?

The cell cycle is an ordered series of events involving cell growth and cell division that produces two new daughter cells. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and division that produces two identical (clone) cells. The cell cycle has two major phases: interphase and the mitotic phase. During interphase, the cell grows and DNA is replicated. During the mitotic phase, the replicated DNA and cytoplasmic contents are separated and the cell divides.

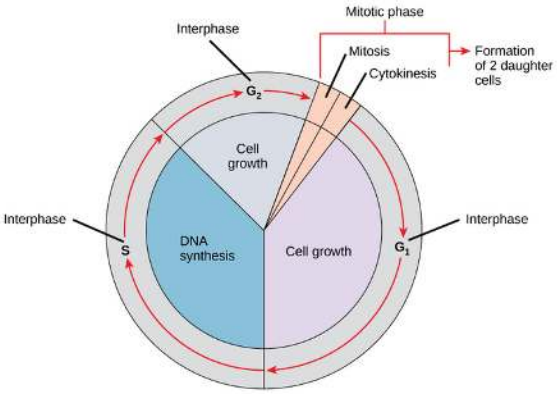


Figure: **The Cell Cycle**: The cell cycle consists of interphase and the mitotic phase. During interphase, the cell grows and the nuclear DNA is duplicated. Interphase is followed by the mitotic phase. During the mitotic phase, the duplicated chromosomes are segregated and distributed into daughter nuclei. The cytoplasm is usually divided as well, resulting in two daughter cells

Key Points

- All multicellular organisms use cell division for growth and the maintenance and repair of cells and tissues.
- Single-celled organisms use cell division as their method of reproduction.
- Somatic cells divide regularly; all human cells (except for the cells that produce eggs and sperm) are somatic cells.
- Somatic cells contain two copies of each of their chromosomes (one copy from each parent).
- The cell cycle has two major phases: interphase and the mitotic phase.
- During interphase, the cell grows and DNA is replicated; during the mitotic phase, the replicated DNA and cytoplasmic contents are separated and the cell divides.

Key Terms

- **somatic cell**: any normal body cell of an organism that is not involved in reproduction; a cell that is not on the germline
- **interphase**: the stage in the life cycle of a cell where the cell grows and DNA is replicated
- **mitotic phase**: replicated DNA and the cytoplasmic material are divided into two identical cells

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5.9B: The Relationship Between Genes and Proteins

Proteins, encoded by individual genes, orchestrate nearly every function of the cell.

LEARNING OBJECTIVES

Describe transcription and translation

Key Takeaways

Genes and Proteins

Since the rediscovery of Mendel's work in 1900, the definition of the gene has progressed from an abstract unit of heredity to a tangible molecular entity capable of replication, transcription, translation, and mutation. Genes are composed of DNA and are linearly arranged on chromosomes. Some genes encode structural and regulatory RNAs. There is increasing evidence from research that profiles the transcriptome of cells (the complete set all RNA transcripts present in a cell) that these may be the largest classes of RNAs produced by eukaryotic cells, far outnumbering the protein-encoding messenger RNAs (mRNAs), but the 20,000 protein-encoding genes typically found in animal cells, and the 30,o00 protein-encoding genes typically found in plant cells, nonetheless have huge impacts on cellular functioning.

Protein-encoding genes specify the sequences of amino acids, which are the building blocks of proteins. In turn, proteins are responsible for orchestrating nearly every function of the cell. Both protein-encoding genes and the proteins that are their gene products are absolutely essential to life as we know it.

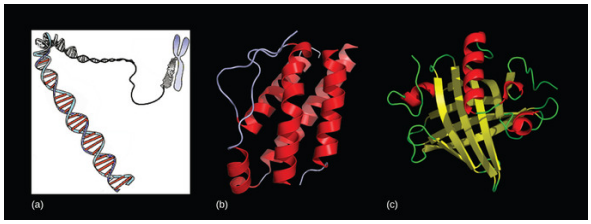


Figure: **Genes Encode Proteins**: Genes, which are carried on (a) chromosomes, are linearly-organized instructions for making the RNA and protein molecules that are necessary for all of processes of life. The (b) interleukin-2 protein and (c) alpha-2u-globulin protein are just two examples of the array of different molecular structures that are encoded by genes.

Replication, Transcription, and Translation are the three main processes used by all cells to maintain their genetic information and to convert the genetic information encoded in DNA into gene products, which are either RNAs or proteins, depending on the gene. In eukaryotic cells, or those cells that have a nucleus, replication and transcription take place within the nucleus while translation takes place outside of the nucleus in cytoplasm. In prokaryotic cells, or those cells that do not have a nucleus, all three processes occur in the cytoplasm.

Replication is the basis for biological inheritance. It copies a cell's DNA. The enzyme DNA polymerase copies a single parental double-stranded DNA molecule into two daughter double-stranded DNA molecules. Transcription makes RNA from DNA. The enzyme RNA polymerase creates an RNA molecule that is complementary to a gene-encoding stretch of DNA. Translation makes protein from mRNA. The ribosome generates a polypeptide chain of amino acids using mRNA as a template. The polypeptide chain folds up to become a protein.

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Key Points

- Genes are composed of DNA arranged on chromosomes.
- Some genes encode structural or regulatory RNAs. Other genes encode proteins.
- Replication copies DNA; transcription uses DNA to make complementary RNAs; translation uses mRNAs to make proteins.
- In eukaryotic cells, replication and transcription take place within the nucleus while translation takes place in the cytoplasm.
- In prokaryotic cells, replication, transcription, and translation occur in the cytoplasm.

Key Terms

- **DNA**: a biopolymer of deoxyribonucleic acids (a type of nucleic acid) that has four different chemical groups, called bases: adenine, guanine, cytosine, and thymine
- **messenger RNA**: Messenger RNA (mRNA) is a molecule of RNA that encodes a chemical “blueprint” for a protein product.
- **protein**: any of numerous large, complex naturally-produced molecules composed of one or more long chains of amino acids, in which the amino acid groups are held together by peptide bonds

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SECTION OVERVIEW

5.10: Genetic Transfer in Prokaryotes

Topic hierarchy

5.10A: Generalized Recombination and RecA

5.10B: Bacterial Transformation

5.10C: Bacterial Transduction

5.10D: Prokaryotic Reproduction

5.10E: Complementation

5.10F: Gene Transfer in Archaea

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5.10A: Generalized Recombination and RecA

In homologous recombination, a type of genetic recombination, nucleotide sequences are exchanged between two similar molecules of DNA.

LEARNING OBJECTIVES

Explain the process of homologous recombination in bacteria

Key Takeaways

Homologous recombination is a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA. It is most widely used by cells to accurately repair harmful breaks that occur on both strands of DNA, known as double-strand breaks. Homologous recombination also produces new combinations of DNA sequences during meiosis, the process by which eukaryotes make gamete cells, like sperm and egg cells in animals. These new combinations of DNA represent genetic variation in offspring, which in turn enables populations to adapt during the course of evolution. Homologous recombination is also used in horizontal gene transfer to exchange genetic material between different strains and species of bacteria and viruses.

Homologous recombination can vary among different organisms and cell types, but most forms involve the same basic steps. After a double-strand break occurs, sections of DNA around the 5′ ends of the break are cut away in a process called resection. In the strand invasion step that follows, an overhanging 3′ end of the broken DNA molecule then “invades” a similar or identical DNA molecule that is not broken. After strand invasion, one or two cross-shaped structures called Holliday junctions connect the two DNA molecules. Depending on how the two junctions are cut by enzymes, the type of homologous recombination that occurs in meiosis results in either chromosomal crossover or non-crossover. Homologous recombination that occurs during DNA repair tends to result in non-crossover products, in effect restoring the damaged DNA molecule as it existed before the double-strand break.

Homologous recombination is conserved across all three domains of life as well as viruses. Homologous recombination is also used in gene targeting, a technique for introducing genetic changes into target organisms.

Homologous recombination is a major DNA repair process in bacteria. It is also important for producing genetic diversity in bacterial populations. Homologous recombination has been most studied and is best understood for *Escherichia coli*. Double-strand DNA breaks in bacteria are repaired by the RecBCD pathway of homologous recombination. Breaks that occur on one of the two DNA strands, known as single-strand gaps, are thought to be repaired by the RecF pathway. Both the RecBCD and RecF pathways include a series of reactions known as branch migration, in which single DNA strands are exchanged between two intercrossed molecules of duplex DNA, and resolution, in which those two intercrossed molecules of DNA are cut apart and restored to their normal double-stranded state.

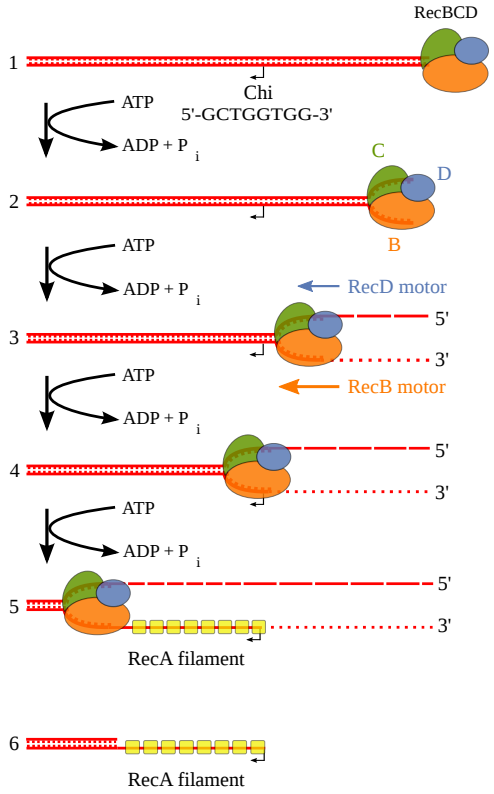


Figure: **Steps in the pre-synapsis phase of homologous recombination in bacteria**: Beginning of the RecBCD pathway. This model is based on reactions of DNA and RecBCD with Mg2+ ions in excess over ATP. Step 1: RecBCD binds to a DNA double strand break. Step 2: RecBCD initiates unwinding of the DNA duplex through ATP-dependent helicase activity. Step 3: RecBCD continues its unwinding and moves down the DNA duplex, cleaving the 3′ strand much more frequently than the 5′ strand. Step 4: RecBCD encounters a Chi sequence and stops digesting the 3′ strand; cleavage of the 5′ strand is significantly increased. Step 5: RecBCD loads RecA onto the 3′ strand. Step 6: RecBCD unbinds from the DNA duplex, leaving a RecA nucleoprotein filament on the 3′ tail.

The RecBCD pathway is the main recombination pathway used in bacteria to repair double-strand breaks in DNA. These double-strand breaks can be caused by UV light and other radiation, as well as chemical mutagens. Double-strand breaks may also arise by DNA replication through a single-strand nick or gap. Such a situation causes what is known as a collapsed replication fork and is fixed by several pathways of homologous recombination including the RecBCD pathway.

In this pathway, a three-subunit enzyme complex called RecBCD initiates recombination by binding to a blunt or nearly blunt end of a break in double-strand DNA. After RecBCD binds the DNA end, the RecB and RecD subunits begin unzipping the DNA duplex through helicase activity. The RecB subunit also has a nuclease domain, which cuts the single strand of DNA that emerges from the unzipping process. This unzipping continues until RecBCD encounters a specific nucleotide sequence (5′-GCTGGTGG-3′) known as a Chi site.

Upon encountering a Chi site, the activity of the RecBCD enzyme changes drastically. DNA unwinding pauses for a few seconds and then resumes at roughly half the initial speed. This is likely because the slower RecB helicase unwinds the DNA after Chi, rather than the faster RecD helicase, which unwinds the DNA before Chi. Recognition of the Chi site also changes the RecBCD enzyme so that it cuts the DNA strand with Chi and begins loading multiple RecA proteins onto the single-stranded DNA with the newly generated 3′ end. The resulting RecA-coated nucleoprotein filament then searches out similar sequences of DNA on a homologous chromosome. The search process induces stretching of the DNA duplex, which enhances homology recognition (a mechanism termed conformational proofreading). Upon finding such a sequence, the single-stranded nucleoprotein filament moves into the homologous recipient DNA duplex in a process called strand invasion. The invading 3′ overhang causes one of the strands of the recipient DNA duplex to be displaced, to form a D-loop. If the D-loop is cut, another swapping of strands forms a cross-shaped structure called a Holliday junction.Resolution of the Holliday junction by some combination of RuvABC or RecG can produce two recombinant DNA molecules with reciprocal genetic types, if the two interacting DNA molecules differ genetically.

Alternatively, the invading 3′ end near Chi can prime DNA synthesis and form a replication fork. This type of resolution produces only one type of recombinant (non-reciprocal).

Key Points

- Homologous recombination can vary among different organisms and cell types, but most forms involve the same basic steps.
- Homologous recombination is a major DNA repair process in bacteria. It is also important for producing genetic diversity in bacterial populations.
- Homologous recombination has been most studied and is best understood for *Escherichia coli*.

Key Terms

- **recombination**: The formation of genetic combinations in offspring that are not present in the parents
- **genetic**: Relating to genetics or genes.
- **homologous**: Showing a degree of correspondence or similarity.

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5.10B: Bacterial Transformation

Transformation is the direct uptake, incorporation and expression of exogenous genetic material from its surroundings.

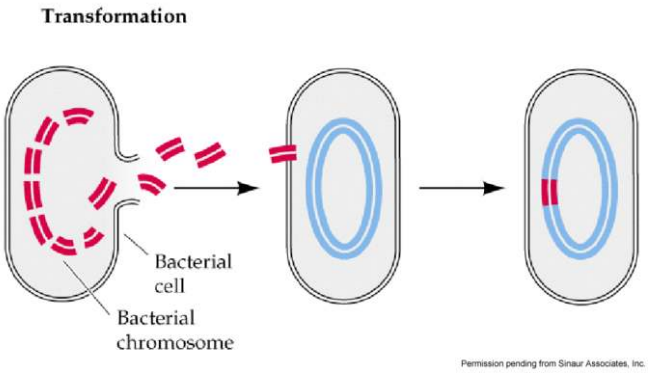
LEARNING OBJECTIVES

Differentiate between natural and artificial transformation

Key Takeaways

Genetic Alteration

In molecular biology, transformation is genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane(s).



Transformation: Illustration of bacterial transformation. DNA from dead cells gets cut into fragments and exits the cell. The free-floating DNA can then be picked up by competent cells. The exogenous DNA is incorporated into the host cell's chromosome via recombination.

NATURAL TRANSFORMATION

Transformation occurs naturally in some species of bacteria, but it can also be effected by artificial means in other cells. For transformation to happen, bacteria must be in a state of competence, which might occur as a time-limited response to environmental conditions such as starvation and cell density. Transformation is one of three processes by which exogenous genetic material may be introduced into a bacterial cell; the other two being conjugation (transfer of genetic material between two bacterial cells in direct contact), and transduction (injection of foreign DNA by a bacteriophage virus into the host bacterium).

"*Transformation*" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the term should be avoided for animal cells when describing introduction of exogenous genetic material. Introduction of foreign DNA into eukaryotic cells is often called "*transfection*".

Bacterial transformation may be referred to as a stable genetic change, brought about by the uptake of naked DNA (DNA without associated cells or proteins). Competence refers to the state of being able to take up exogenous DNA from the environment. There are two forms of competence: natural and artificial.

About 1% of bacterial species are capable of naturally taking up DNA under laboratory conditions; more may be able to take it up in their natural environments. DNA material can be transferred between different strains of bacteria in a process that is called horizontal gene transfer.

Some species, upon cell death, release their DNA to be taken up by other cells; however, transformation works best with DNA from closely-related species. These naturally-competent bacteria carry sets of genes that provide the protein machinery to bring DNA across the cell membrane(s). The transport of the exogeneous DNA into the cells may require proteins that are involved in the assembly of type IV pili and type II secretion system, as well as DNA translocase complex at the cytoplasmic membrane.

GRAM-POSITIVE AND GRAM-NEGATIVE DIFFERENCES

Due to the differences in structure of the cell envelope between Gram-positive and Gram-negative bacteria, there are some differences in the mechanisms of DNA uptake in these cells. However, most of them share common features that involve related proteins. The DNA first binds to the surface of the competent cells on a DNA receptor, and passes through the cytoplasmic membrane via DNA translocase. Only single-stranded DNA may pass through, one strand is therefore degraded by nucleases in the process, and the translocated single-stranded DNA may then be integrated into the bacterial chromosomes by a RecA-dependent process.

In Gram-negative cells, due to the presence of an extra membrane, the DNA requires the presence of a channel formed by secretins on the outer membrane. Pilin may be required for competence however, its role is uncertain. The uptake of DNA is generally non-sequence specific, although in some species the presence of specific DNA uptake sequences may facilitate efficient DNA uptake.

ARTIFICIAL TRANSFER

Artificial competence can be induced in laboratory procedures that involve making the cell passively permeable to DNA, by exposing it to conditions that do not normally occur in nature. Typically, the cells are incubated in a solution containing divalent cations; most commonly, calcium chloride solution under cold condition, which is then exposed to a pulse of heat shock. However, the mechanism of the uptake of DNA via chemically-induced competence in this calcium chloride transformation method is unclear.

The surface of bacteria such as E. coli is negatively-charged due to phospholipids and lipopolysaccharides on its cell surface, and the DNA is also negatively-charged. One function of the divalent cation therefore, would be to shield the charges by coordinating the phosphate groups and other negative charges, thereby allowing a DNA molecule to adhere to the cell surface. It is suggested that exposing the cells to divalent cations in cold condition may also change or weaken the cell surface structure of the cells making it more permeable to DNA. The heat-pulse is thought to create a thermal imbalance on either side of the cell membrane, which forces the DNA to enter the cells through either cell pores or the damaged cell wall.

Electroporation is another method of promoting competence. Using this method, the cells are briefly shocked with an electric field of 10-20 kV/cm which is thought to create holes in the cell membrane through which the plasmid DNA may enter. After the electric shock, the holes are rapidly closed by the cell's membrane-repair mechanisms.

O. T. Avery, et al. were first to demonstrate that "rough" colonies of S. pneumoniae could be transformed to "smooth" (capsule producing) colonies by addition of DNA extracts of the former to the latter, thus "transforming" them. (See Lederberg below)

1. Lederberg, Joshua (1994). The Transformation of Genetics by DNA: An Anniversary Celebration of AVERY, MACLEOD and MCCARTY(1944) in Anecdotal, Historical and Critical Commentaries on Genetics. The Rockfeller University, New York, New York 10021-6399. PMID 8150273.

Key Points

- Transformation results in the genetic alteration of the recipient cell.
- Exogenous DNA is taken up into the recipient cell from its surroundings through the cell membrane (s).
- Transformation occurs naturally in some species of bacteria, but it can also be affected by artificial means in other cells.

Key Terms

- **eukaryotic**: Having complex cells in which the genetic material is organized into membrane-bound nuclei.
- **transformation**: In molecular biology transformation is genetic alteration of a cell resulting from the direct uptake, incorporation and expression of exogenous genetic material (exogenous DNA) from its surroundings and taken up through the cell membrane(s).
- **expression**: Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product.
- **exogenous**: Produced or originating outside of an organism.
- **translocase**: An enzyme that assists in moving another molecule, usually across a membrane.

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5.10C: Bacterial Transduction

Transduction is the process by which DNA is transferred from one bacterium to another by a virus.

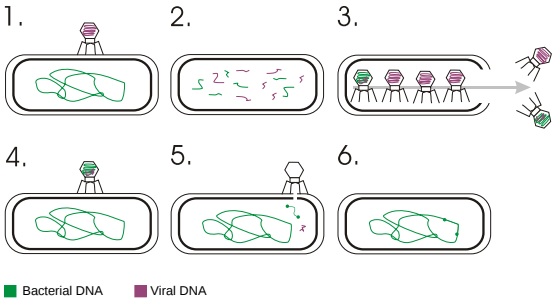
LEARNING OBJECTIVES

Differentiate between generalized and specialized transduction

Key Takeaways

Transduction

Transduction is the process by which DNA is transferred from one bacterium to another by a virus. It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector. Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNAase resistant (transformation is susceptible to DNAase). Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome.



Transduction: Transduction is the process by which DNA is transferred from one bacterium to another by a virus. It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector.

When bacteriophages (viruses that infect bacteria) infect a bacterial cell, their normal mode of reproduction is to harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat.

Transduction is especially important because it explains one mechanism by which antibiotic drugs become ineffective due to the transfer of antibiotic-resistance genes between bacteria. In addition, hopes to create medical methods of genetic modification of diseases such as Duchenne/Becker Muscular Dystrophy are based on these methodologies.

The Lytic Cycle and the Lysogenic Cycle

Transduction happens through either the lytic cycle or the lysogenic cycle. If the lysogenic cycle is adopted, the phage chromosome is integrated (by covalent bonds) into the bacterial chromosome, where it can remain dormant for thousands of generations. If the lysogen is induced (by UV light for example), the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles. The lytic cycle leads to the production of new phage particles which are released by lysis of the host.

Transduction is a method for transferring genetic material. The packaging of bacteriophage DNA has low fidelity and small pieces of bacterial DNA, together with the bacteriophage genome, may become packaged into the bacteriophage genome. At the same time, some phage genes are left behind in the bacterial chromosome.

There are generally three types of recombination events that can lead to this incorporation of bacterial DNA into the viral DNA, leading to two modes of recombination.

Generalized transduction is the process by which any bacterial gene may be transferred to another bacterium via a bacteriophage, and typically carries only bacterial DNA and no viral DNA. In essence, this is the packaging of bacterial DNA into a viral envelope. This may occur in two main ways, recombination and headful packaging.

If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell's machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA, the mistake will lead to generalized transduction.

If the virus replicates using "headful packaging," it attempts to fill the nucleocapsid with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion.

The new virus capsule, now loaded with part bacterial DNA, continues to infect another bacterial cell. This bacterial material may become recombined into another bacterium upon infection.

Fates of DNA Inserted into the Recipient Cell

When the new DNA is inserted into this recipient cell it can fall to one of three fates: the DNA will be absorbed by the cell and be recycled for spare parts; if the DNA was originally a plasmid, it will recirculate inside the new cell and become a plasmid again; if the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in conjugation. This type of recombination is random and the amount recombined depends on the size of the virus being used.

Specialized transduction is the process by which a restricted set of bacterial genes are transferred to another bacterium. The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome. Specialized transduction occurs when the prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to the prophage are included in the excised DNA. The excised DNA is then packaged into a new virus particle, which can then deliver the DNA to a new bacterium, where the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage.

When the partially encapsulated phage material infects another cell and becomes a "prophage" (is covalently bonded into the infected cell's chromosome), the partially coded prophage DNA is called a "heterogenote. " Example of specialized transduction is λ phages in *Escherichia coli*, which was discovered by Esther Lederberg.

Key Points

- Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNAase resistant.
- Transduction happens through either the lytic cycle or the lysogenic cycle.
- Transduction is especially important because it explains one mechanism by which antibiotic drugs become ineffective due to the transfer of antibiotic-resistance genes between bacteria.

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.
- **lysogenic cycle**: A form of viral reproduction involving the fusion of the nucleic acid of a bacteriophage with that of a host, followed by proliferation of the resulting prophage.
- **transduction**: Transduction is the process by which DNA is transferred from one bacterium to another by a virus.

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5.10D: Prokaryotic Reproduction

Prokaryotes reproduce asexually by binary fission; they can also exchange genetic material by transformation, transduction, and conjugation.

LEARNING OBJECTIVES

Distinguish among the types of reproduction in prokaryotes

Key Takeaways

Reproduction

Reproduction in prokaryotes is asexual and usually takes place by binary fission. The DNA of a prokaryote exists as as a single, circular chromosome. Prokaryotes do not undergo mitosis; rather the chromosome is replicated and the two resulting copies separate from one another, due to the growth of the cell. The prokaryote, now enlarged, is pinched inward at its equator and the two resulting cells, which are clones, separate. Binary fission does not provide an opportunity for genetic recombination or genetic diversity, but prokaryotes can share genes by three other mechanisms.

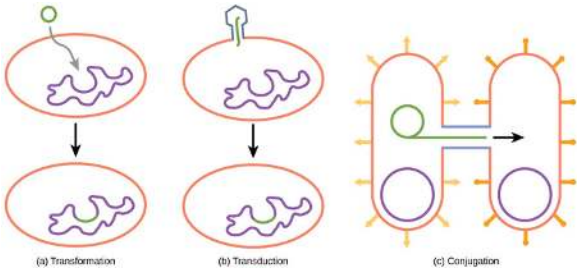


Figure: **Modes of prokaryote reproduction**: Besides binary fission, there are three other mechanisms by which prokaryotes can exchange DNA. In (a) transformation, the cell takes up prokaryotic DNA directly from the environment. The DNA may remain separate as plasmid DNA or be incorporated into the host genome. In (b) transduction, a bacteriophage injects DNA into the cell that contains a small fragment of DNA from a different prokaryote. In (c) conjugation, DNA is transferred from one cell to another via a mating bridge that connects the two cells after the pilus draws the two bacteria close enough to form the bridge.

In transformation, the prokaryote takes in DNA found in its environment that is shed by other prokaryotes. If a nonpathogenic bacterium takes up DNA for a toxin gene from a pathogen and incorporates the new DNA into its own chromosome, it, too, may become pathogenic. In transduction, bacteriophages, the viruses that infect bacteria, sometimes also move short pieces of chromosomal DNA from one bacterium to another. Transduction results in a recombinant organism. Archaea are not affected by bacteriophages, but instead have their own viruses that translocate genetic material from one individual to another. In conjugation, DNA is transferred from one prokaryote to another by means of a pilus, which brings the organisms into contact with one another. The DNA transferred can be in the form of a plasmid or as a hybrid, containing both plasmid and chromosomal DNA.

Reproduction can be very rapid: a few minutes for some species. This short generation time, coupled with mechanisms of genetic recombination and high rates of mutation, result in the rapid evolution of prokaryotes, allowing them to respond to environmental changes (such as the introduction of an antibiotic) very rapidly.

Key Points

- Binary fission is a type of reproduction in which the chromosome is replicated and the resultant prokaryote is an exact copy of the parental prokaryate, thus leaving no opportunity for genetic diversity.
- Transformation is a type of prokaryotic reproduction in which a prokaryote can take up DNA found within the environment that has originated from other prokaryotes.
- Transduction is a type of prokaryotic reproduction in which a prokaryote is infected by a virus which injects short pieces of chromosomal DNA from one bacterium to another.
- Conjugation is a type of prokaryotic reproduction in which DNA is transferred between prokaryotes by means of a pilus.

Key Terms

- **transformation**: the alteration of a bacterial cell caused by the transfer of DNA from another, especially if pathogenic
- **transduction**: horizontal gene transfer mechanism in prokaryotes where genes are transferred using a virus
- **binary fission**: the process whereby a cell divides asexually to produce two daughter cells
- **conjugation**: the temporary fusion of organisms, especially as part of sexual reproduction
- **pilus**: a hairlike appendage found on the cell surface of many bacteria

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5.10E: Complementation

Learning Objectives

- Explain the mechanism of genetic complementation

Complementation refers to a relationship between two different strains of an organism which both have homozygous recessive mutations that produce the same phenotype (for example, a change in wing structure in flies) but which do not reside on the same (homologous) gene.

These strains are true breeding for their mutation. If, when these strains are crossed with each other, some offspring show recovery of the wild-type phenotype, they are said to show "genetic complementation". When this occurs, each strain's haploid supplies a wild-type allele to "complement" the mutated allele of the other strain's haploid, causing the offspring to have heterozygous mutations in all related genes. Since the mutations are recessive, the offspring will display the wild-type phenotype.

A complementation test (sometimes called a "cis-trans" test) refers to this experiment, developed by American geneticist Edward B. Lewis. It answers the question: "Does a wild-type copy of gene X rescue the function of the mutant allele that is believed to define gene X?". If there is an allele with an observable phenotype whose function can be provided by a wild type genotype (i.e., the allele is recessive), one can ask whether the function that was lost because of the recessive allele can be provided by another mutant genotype. If not, the two alleles must be defective in the same gene. The beauty of this test is that the trait can serve as a read-out of gene function even without knowledge of what the gene is doing at a molecular level.

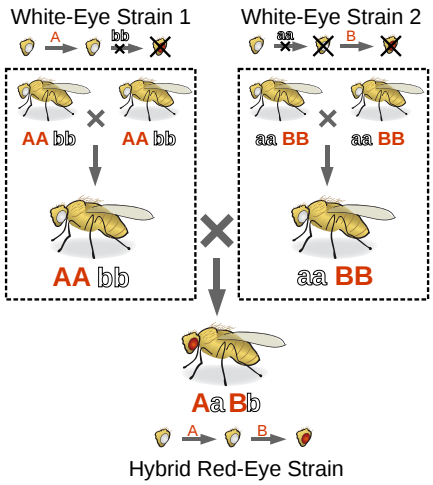


Figure: ***Complementation Test****: Example of a complementation test. Two strains of flies are white eyed because of two different autosomal recessive mutations which interrupt different steps in a single pigment-producing metabolic pathway. Flies from Strain 1 have complementary mutations to flies from Strain 2 because when they are crossed the offspring are able to complete the full metabolic pathway and thus have red eyes.*

Complementation arises because loss of function in genes responsible for different steps in the same metabolic pathway can give rise to the same phenotype. When strains are bred together, offspring inherit wildtype versions of each gene from either parent. Because the mutations are recessive, there is a recovery of function in that pathway, so offspring recover the wild-type phenotype. Thus, the test is used to decide if two independently derived recessive mutant phenotypes are caused by mutations in the same gene or in two different genes. If both parent strains have mutations in the same gene, no normal versions of the gene are inherited by the offspring; they express the same mutant phenotype and complementation has failed to occur.

In other words, if the combination of two haploid genomes containing different recessive mutations yields a mutant phenotype, then there are three possibilities: Mutations occur in the same gene; One mutation affects the expression of the other; One mutation may result in an inhibitory product. If the combination of two haploid genomes containing different recessive mutations yields the wild type phenotype, then the mutations must be in different genes.

Key Points

- A complementation test answers the question: “Does a wild-type copy of gene X rescue the function of the mutant allele that is believed to define gene X? “.
- Complementation arises because loss of function in genes responsible for different steps in the same metabolic pathway can give rise to the same phenotype.
- When strains are bred together, offspring inherit wildtype versions of each gene from either parent.

Key Terms

- **Complementation**: In genetics, complementation refers to a relationship between two different strains of an organism which both have homozygous recessive mutations that produce the same phenotype (for example, a change in wing structure in flies) but which do not reside on the same (homologous) gene.
- **mutation**: Any heritable change of the base-pair sequence of genetic material.
- **homozygous**: of an organism in which both copies of a given gene have the same allele

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5.10F: Gene Transfer in Archaea

Archaea are distinct from bacteria and eukaryotes, but genetic material can be transferred between them and between Archaea themselves.

LEARNING OBJECTIVES

Describe the mechanisms of gene transfer in Archaea

Key Takeaways

Key Points

- Archaea while being very different from eukaryotes and bacteria, there are many commonalities at the the genetic level between them.
- Horizontal gene transfer can explain the similarities between the genes found in the three domains of life and indeed there is evidence that horizontal gene transfer occurs with Archaea species.
- Archaea can be infected by double-stranded DNA viruses, which can account for gene transfers, as well like bacteria, Archaea may conjugate.

Key Terms

- **archaea**: a taxonomic domain of single-celled organisms lacking nuclei that are fundamentally from bacteria.
- **translation**: Translation is the communication of the meaning of a source-language text by means of an equivalent target-language text.
- **transcription**: Transcription is the process of creating a complementary RNA copy of a sequence of DNA. Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language that can be converted back and forth from DNA to RNA by the action of the correct enzymes.

Archaea are genetically distinct from bacteria and eukaryotes, but are poorly understood: many of the genes that Archaea encode are of unknown function. Transcription and translation in archaea resemble the same processes more closely in eukaryotes than in bacteria, with the archaean RNA polymerase and ribosomes being very close to their equivalents in eukaryotes.

Although Archaea only have one type of RNA polymerase, its structure and function in transcription is similar to that of the eukaryotic RNA polymerase II, with similar protein assemblies (the general transcription factors) directing the binding of the RNA polymerase to a gene's promoter. However, other archaean transcription factors are closer to those found in bacteria. Post-transcriptional modification is simpler than in eukaryotes, since most archaean genes lack introns, although there are many introns in their transfer RNA and ribosomal RNA genes, and introns may occur in a few protein-encoding genes. This is all to say there are many similarities in the genes shared between Archaea and the other domains of life, suggesting there was a transfer of genetic material between the domains of life. This phenomenon is described as horizontal gene transfer.

Horizontal gene transfer (HGT) refers to the transfer of genes between organisms in a manner other than traditional reproduction. Also termed lateral gene transfer, it contrasts with vertical transfer, the transmission of genes from the parental generation to offspring via sexual or asexual reproduction. HGT has been shown to be an important factor in the evolution of many organisms, including bacteria, plants and humans.

Archaea show high levels of horizontal gene transfer between lineages. Some researchers suggest that individuals can be grouped into species-like populations given highly similar genomes and infrequent gene transfer to/from cells with less-related genomes, as in the Archaea genus Ferroplasma. On the other hand, studies in Halorubrum found significant genetic transfer to/from less-related populations. These gene transfers are identified by sequencing the DNA of various Archaea species; through the similarities and differences of the DNA of the different types of Archaea it is determined if the gene was perfectly transferred or from a common ancestor. The elucidation of this can be controversial.

How genetic material can move from one Archaea to another is poorly understood. In bacteria the natural ways in which this occurs is through either bacterial conjugation or viral transfer, also known as transduction. Conjugation is where two (sometimes distantly related) bacteria transfer genetic material by direct contact. Transduction occurs when a virus "picks up" some DNA from its host and when infecting a new host, moves that genetic material to the new host. It is thought that conjugation can occur in Archaea,

though unlike bacteria the mechanism is not well understood. As well Archaea can be infected by viruses. In fact 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, including bottles, hooked rods, or teardrops. Taken together it is clear that gene transfer happens in Archaea, and probably is similar to horizontal gene transfer seen in the other domains of life.

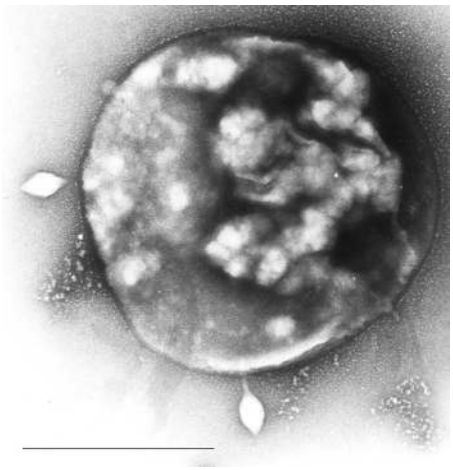


Figure: **Archaeal viral infection**: Cell of Sulfolobus infected by virus STSV1 observed under microscopy. Two spindle-shaped viruses were being released from the host cell. The strain of Sulfolobus and STSV1 (Sulfolobus tengchongensis Spindle-shaped Virus 1) were isolated by Xiaoyu Xiang and his colleagues 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.

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SECTION OVERVIEW

5.11: Genomics and Proteomics

Topic hierarchy

5.11A: Microarrays and the Transcriptome

5.11B: Proteomics

5.11C: Metabolomics

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5.11A: Microarrays and the Transcriptome

Learning Objectives

- Define the transcriptome

The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in one or a population of cells. The term can be applied to the total set of transcripts in a given organism, or to the specific subset of transcripts present in a particular cell type. Unlike the genome, which is roughly fixed for a given cell line (excluding mutations), the transcriptome can vary with external environmental conditions. Because it includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time, with the exception of mRNA degradation phenomena such as transcriptional attenuation.

Analysis of the Transcriptome

The study of transcriptomics, also referred to as expression profiling, examines the expression level of mRNAs in a given cell population, often using high-throughput techniques based on DNA microarray technology. A number of organism-specific transcriptome databases have been constructed and annotated to aid in the identification of genes that are differentially expressed in distinct cell populations.

DNA microarrays can provide a genome-wide method for comparison of the abundance of DNAs in the same samples.The DNA in spots can only be PCR products specific for individual genes. A DNA copy of RNA is made using the enzyme reverse transcriptase. Sequencing is now being used instead of gene arrays to quantify DNA levels, at least semi-quantitatively.

The transcriptomes of stem cells and cancer cells are of particular interest to researchers who seek to understand the processes of cellular differentiation and carcinogenesis. Analysis of the transcriptomes of human oocytes and embryos is used to understand the molecular mechanisms and signaling pathways controlling early embryonic development, and could theoretically be a powerful tool in making proper embryo selection during *in vitro* fertilization.

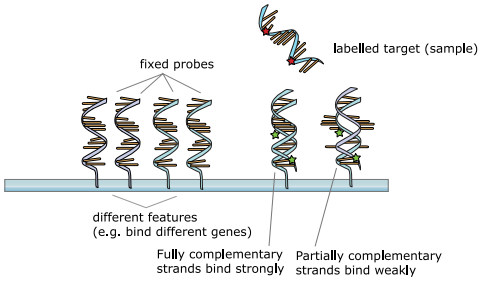


Figure: ***DNA microarray principle****: The core principle behind microarrays is hybridization between two DNA strands, the property of complementary nucleic acid sequences to specifically pair with each other by forming hydrogen bonds between complementary nucleotide base pairs.*

Key Points

- Unlike the genome, which is roughly fixed for a given cell line (excluding mutations), the transcriptome can vary with external environmental conditions.
- The transcriptome reflects the genes that are being actively expressed at any given time.
- DNA microarrays can provide a method for comparing on a genome-wide basis the abundance of DNAs in a specific sample.

Key Terms

- **transcriptome**: The complete set of messenger RNA molecules (transcripts) produced in a cell or a population of cells.
- **DNA microarray**: a collection of microscopic DNA spots attached to a solid surface forming an array; used to measure the expression levels of large numbers of genes simultaneously
- **PCR**: polymerase chain reaction

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5.11B: Proteomics

Learning Objectives

- Summarize the purpose of, and methods used for, proteomics

Proteomics is the large-scale study of proteins, particularly their structures and functions. The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system. This will vary with time and distinct requirements, or stresses, that a cell or organism undergoes.

While proteomics generally refers to the large-scale experimental analysis of proteins, it is often specifically used for protein purification and mass spectrometry. After genomics and transcriptomics, proteomics is considered the next step in the study of biological systems. It is much more complicated than genomics mostly because while an organism's genome is more or less constant, the proteome differs from cell to cell and from time to time. This is because distinct genes are expressed in distinct cell types. This means that even the basic set of proteins which are produced in a cell needs to be determined. In the past, this was done by mRNA analysis, but this was found not to correlate with protein content. It is now known that mRNA is not always translated into protein. The amount of protein produced for a given amount of mRNA depends on the gene it is transcribed from and the current physiological state of the cell.

Proteomics confirms the presence of the protein and provides a direct measure of the quantity present. Not only does the translation from mRNA cause differences, but many proteins are also subjected to a wide variety of chemical modifications after translation which are critical to the protein's function such as phosphorylation, ubiquitination, methylation, acetylation, glycosylation, oxidation, and nitrosylation. Some proteins undergo ALL of these modifications, often in time-dependent combinations, aptly illustrating the potential complexity one has to deal with when studying protein structure and function.

Proteomics typically gives us a better understanding of an organism than genomics. First, the level of transcription of a gene gives only a rough estimate of its level of expression into a protein. An mRNA produced in abundance may be degraded rapidly or translated inefficiently, resulting in a small amount of protein. Second, as mentioned above many proteins experience post-translational modifications that profoundly affect their activities. For example, some proteins are not active until they become phosphorylated. Third, many transcripts give rise to more than one protein through alternative splicing or alternative post-translational modifications. Fourth, many proteins form complexes with other proteins or RNA molecules. They only function in the presence of these other molecules. Finally, protein degradation rate plays an important role in protein content.

One way in which a particular protein can be studied is to develop an antibody which is specific to that modification. For example, there are antibodies that only recognize certain proteins when they are tyrosine-phosphorylated, known as phospho-specific antibodies. There are also antibodies specific to other modifications. These can be used to determine the set of proteins that have undergone the modification of interest. For more quantitative determinations of protein amounts, techniques such as ELISAs can be used.

Most proteins function in collaboration with other proteins. One goal of proteomics is to identify which proteins interact. This is especially useful in determining potential partners in cell signaling cascades. Several methods are available to probe protein–protein interactions. The traditional method is yeast two-hybrid analysis. New methods include protein microarrays, immunoaffinity, and chromatography followed by mass spectrometry, dual polarisation interferometry, Microscale Thermophoresis, and experimental methods such as phage display and computational methods.



Figure: ***Robotic preparation of MALDI mass spectrometry samples****: Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry. It allows for the analysis of biomolecules and large organic molecules which tend to be fragile and fragment when ionized by more conventional ionization methods.*

One of the most promising developments to come from the study of human genes and proteins has been the identification of potential new drugs for the treatment of disease. This relies on genome and proteome information to identify proteins associated with a disease, which computer software can then use as targets for new drugs. For example, if a certain protein is implicated in a disease, its 3-D structure provides the information to design drugs to interfere with the action of the protein. A molecule that fits the active site of an enzyme, but cannot be released by the enzyme, will inactivate the enzyme. Understanding the proteome, the structure and function of each protein and the complexities of protein–protein interactions will be critical for developing the most effective diagnostic techniques and disease treatments in the future. Moreover, an interesting use of proteomics is using specific protein biomarkers to diagnose disease. A number of techniques allow testing for proteins produced during a particular disease, which helps to diagnose the disease quickly.

Key Points

- The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system.
- The proteome varies with time and distinct requirements, or stresses, that a cell or organism undergoes.
- Proteomics typically gives us a better understanding of an organism than genomics.

Key Terms

- **proteomics**: The branch of molecular biology that studies the set of proteins expressed by the genome of an organism.
- **genomics**: The study of the complete genome of an organism.
- **mass spectrometry**: An analytical technique that measures the mass/charge ratio of the ions formed when a molecule or atom is ionized, vaporized, and introduced into a vacuum. Mass spectrometry may also involve breaking molecules into fragments – thus enabling its structure to be determined.

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5.11C: Metabolomics

Learning Objectives

- Review metabolomics

Metabolomics is the scientific study of chemical processes involving metabolites. The metabolome represents the collection of all metabolites, which are the end products of cellular processes, in a biological cell, tissue, organ, or organism. Thus, while mRNA gene expression data and proteomic analyses do not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology of that cell. One of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to give a more complete picture of living organisms.

History and Development

The idea that biological fluids reflect the health of an individual has existed for a long time. The term “metabolic profile” was introduced by Horning, et al. in 1971, after they demonstrated that gas chromatography- mass spectrometry (GC-MS;) could be used to measure compounds present in human urine and tissue extracts. GC-MS is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Concurrently, NMR spectroscopy, which was discovered in the 1940s, was also undergoing rapid advances. In 1974, Seeley et al. demonstrated the utility of using NMR to detect metabolites in unmodified biological samples. This first study on muscle tissue highlighted the value of NMR, in that it was determined that 90% of cellular ATP is complexed with magnesium. As sensitivity has improved with the evolution of higher magnetic field strengths and magic-angle spinning, NMR continues to be a leading analytical tool to investigate metabolism.



Figure: ***Gas Chromatography–mass spectrometry****: Gas chromatography–mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample.*

In 2005, the first metabolomics web database for characterizing human metabolites, METLIN, was developed in the Siuzdak laboratory at The Scripps Research Institute. METLIN contained over 10,000 metabolites and tandem mass spectral data. On January 23, 2007, the Human Metabolome Project, led by Dr. David Wishart of the University of Alberta, Canada, completed the first draft of the human metabolome, consisting of a database of approximately 2500 metabolites, 1200 drugs and 3500 food components.

As late as mid-2010, metabolomics was still considered an “emerging field”. Further, it was noted that further progress in the field was in large part the result of addressing otherwise “irresolvable technical challenges” through technical evolution of mass spectrometry instrumentation. The word was coined in analogy with transcriptomics and proteomics. Like the transcriptome and the proteome, the metabolome is dynamic, changing from second to second. Although the metabolome can be defined readily enough, it is not currently possible to analyse the entire range of metabolites by a single analytical method.

Metabolites are the intermediates and products of metabolism. Within the context of metabolomics, a metabolite is usually defined as any molecule less than 1 kDa in size. However, there are exceptions to this, depending on the sample and detection method. Macromolecules such as lipoproteins and albumin are reliably detected in NMR-based metabolomics studies of blood plasma. In plant-based metabolomics, it is common to refer to “primary metabolites,” which are directly involved in growth, development and

reproduction, and “secondary metabolites,” which are indirectly involved in growth, development and reproduction. In contrast, in human-based metabolomics it is more common to describe metabolites as being either endogenous (produced by the host organism) or exogenous. The metabolome forms a large network of metabolic reactions, where outputs from one enzymatic chemical reaction are inputs to other chemical reactions. Such systems have been described as hypercycles.

Separation methods: Gas chromatography, especially when interfaced with mass spectrometry (GC-MS), is one of the most widely used and powerful methods. It offers very high chromatographic resolution, but requires chemical derivatization for many biomolecules: only volatile chemicals can be analysed without derivatization.

Detection methods: Mass spectrometry (MS) is used to identify and to quantify metabolites after separation. Surface-based mass analysis has seen a resurgence in the past decade, with new MS technologies focused on increasing sensitivity, minimizing background, and reducing sample preparation.

Statistical methods: The data generated in metabolomics usually consist of measurements performed on subjects under various conditions. These measurements may be digitized spectra, or a list of metabolite levels. In its simplest form this generates a matrix with rows corresponding to subjects and columns corresponding to metabolite levels.

Key applications

- *Toxicity assessment/toxicology*. Metabolic profiling, especially of urine or blood plasma samples, can be used to detect the physiological changes caused by toxic insult of a chemical or mixture of chemicals. This is of particular relevance to pharmaceutical companies wanting to test the toxicity of potential drug candidates.
- *Functional genomics*. Metabolomics can be an excellent tool for determining the phenotype caused by a genetic manipulation, such as gene deletion or insertion. Sometimes this can be a sufficient goal in itself—for instance, to detect any phenotypic changes in a genetically-modified plant intended for human or animal consumption. More exciting is the prospect of predicting the function of unknown genes by comparison with the metabolic perturbations caused by deletion/insertion of known genes.

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Key Points

- The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes.
- Metabolites are the intermediates and products of metabolism.
- The metabolome forms a large network of metabolic reactions, where outputs from one enzymatic chemical reaction are inputs to other chemical reactions.
- NMR and Mass Spectroscopy are the most widely used techniques to identify metabolites.

Key Terms

- **metabolomics**: The study of the range of metabolites present in a person's body at normal times, and when suffering from specific diseases; may be useful as a diagnostic tool
- **mass spectrometry**: An analytical technique that measures the mass/charge ratio of the ions formed when a molecule or atom is ionized, vaporized, and introduced into a vacuum. Mass spectrometry may also involve breaking molecules into fragments – thus enabling its structure to be determined.
- **metabolite**: Any substance produced by, or taking part in, a metabolic reaction.

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SECTION OVERVIEW

5.12: Mutation

Topic hierarchy

5.12A: DNA Repair

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5.12A: DNA Repair

Most mistakes during replication are corrected by DNA polymerase during replication or by post-replication repair mechanisms.

LEARNING OBJECTIVES

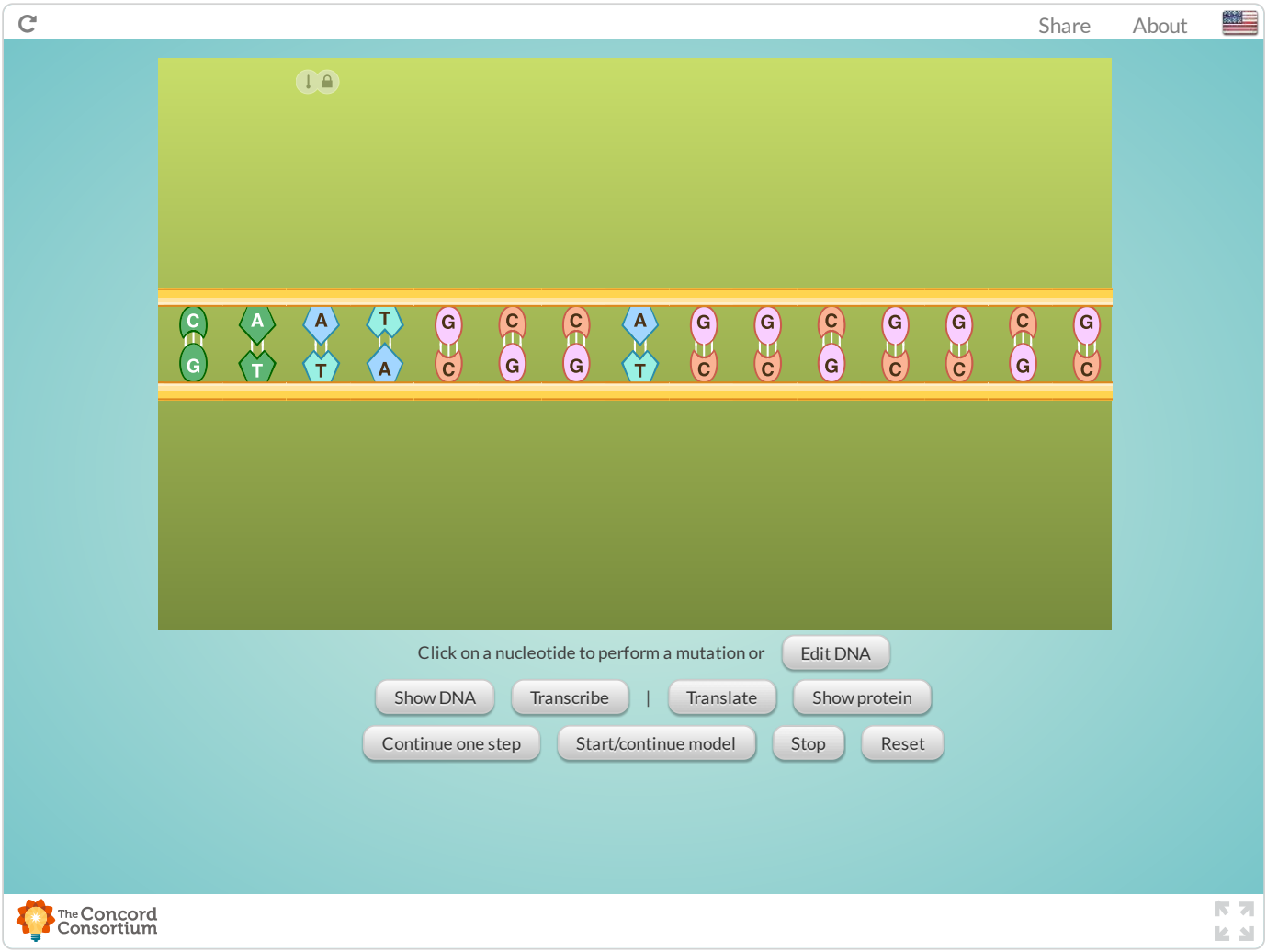
Explain how errors during replication are repaired

Key Takeaways

Errors during Replication

DNA replication is a highly accurate process, but mistakes can occasionally occur as when a DNA polymerase inserts a wrong base. Uncorrected mistakes may sometimes lead to serious consequences, such as cancer. Repair mechanisms can correct the mistakes, but in rare cases mistakes are not corrected, leading to mutations; in other cases, repair enzymes are themselves mutated or defective.

Mutations: In this interactive, you can "edit" a DNA strand and cause a mutation. Take a look at the effects!



Most of the mistakes during DNA replication are promptly corrected by DNA polymerase which proofreads the base that has just been added. In proofreading, the DNA pol reads the newly-added base before adding the next one so a correction can be made. The polymerase checks whether the newly-added base has paired correctly with the base in the template strand. If it is the correct base, the next nucleotide is added. If an incorrect base has been added, the enzyme makes a cut at the phosphodiester bond and releases the incorrect nucleotide. This is performed by the exonuclease action of DNA pol III. Once the incorrect nucleotide has been removed, a new one will be added again.

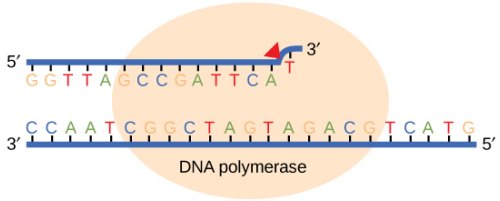


Figure: **DNA polymerase proofreading**: Proofreading by DNA polymerase corrects errors during replication.

Some errors are not corrected during replication, but are instead corrected after replication is completed; this type of repair is known as mismatch repair. The enzymes recognize the incorrectly-added nucleotide and excise it; this is then replaced by the correct base. If this remains uncorrected, it may lead to more permanent damage. How do mismatch repair enzymes recognize which of the two bases is the incorrect one? In *E. coli*, after replication, the nitrogenous base adenine acquires a methyl group; the parental DNA strand will have methyl groups, whereas the newly-synthesized strand lacks them. Thus, DNA polymerase is able to remove the incorrectly-incorporated bases from the newly-synthesized, non-methylated strand. In eukaryotes, the mechanism is not very well understood, but it is believed to involve recognition of unsealed nicks in the new strand, as well as a short-term continuing association of some of the replication proteins with the new daughter strand after replication has been completed.

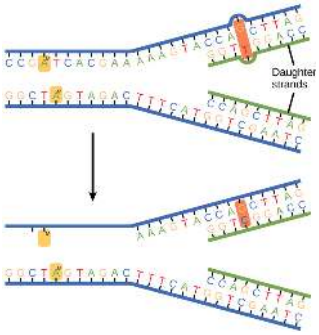


Figure: **Mismatch Repair**: In mismatch repair, the incorrectly-added base is detected after replication. The mismatch-repair proteins detect this base and remove it from the newly-synthesized strand by nuclease action. The gap is now filled with the correctly-paired base.

In another type of repair mechanism, nucleotide excision repair, enzymes replace incorrect bases by making a cut on both the 3′ and 5′ ends of the incorrect base. The segment of DNA is removed and replaced with the correctly-paired nucleotides by the action of DNA pol. Once the bases are filled in, the remaining gap is sealed with a phosphodiester linkage catalyzed by DNA ligase. This repair mechanism is often employed when UV exposure causes the formation of pyrimidine dimers.

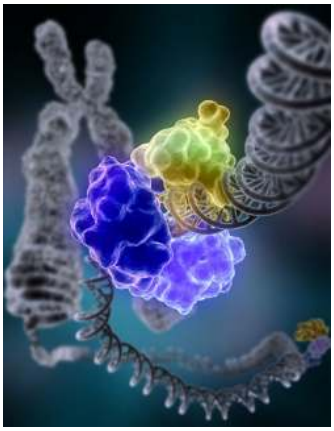


Figure: **DNA Ligase I Repairing Chromosomal Damage**: DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. A special enzyme, DNA ligase (shown here in color), encircles the double helix to repair a broken strand of DNA. DNA ligase is responsible for repairing the millions of DNA breaks generated during the normal course of a cell's life. Without molecules that can mend such breaks, cells can malfunction, die, or become cancerous. DNA ligases catalyse the crucial step of joining breaks in duplex DNA during DNA repair, replication and recombination, and require either Adenosine triphosphate (ATP) or Nicotinamide adenine dinucleotide (NAD+) as a cofactor.

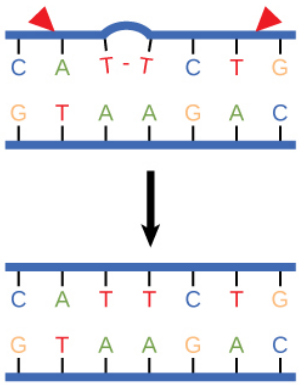


Figure: **Nucleotide Excision Repairs**: Nucleotide excision repairs thymine dimers. When exposed to UV, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced.

DNA Damage and Mutations

Errors during DNA replication are not the only reason why mutations arise in DNA. Mutations, variations in the nucleotide sequence of a genome, can also occur because of damage to DNA. Such mutations may be of two types: induced or spontaneous. Induced mutations are those that result from an exposure to chemicals, UV rays, X-rays, or some other environmental agent. Spontaneous mutations occur without any exposure to any environmental agent; they are a result of natural reactions taking place within the body.

Mutations may have a wide range of effects. Some mutations are not expressed; these are known as silent mutations. Point mutations are those mutations that affect a single base pair. The most common nucleotide mutations are substitutions, in which one base is replaced by another. These can be of two types: transitions or transversions. Transition substitution refers to a purine or pyrimidine being replaced by a base of the same kind; for example, a purine such as adenine may be replaced by the purine guanine. Transversion substitution refers to a purine being replaced by a pyrimidine or vice versa; for example, cytosine, a pyrimidine, is replaced by adenine, a purine. Mutations can also be the result of the addition of a base, known as an insertion, or the removal of a base, known as a deletion. Sometimes a piece of DNA from one chromosome may get translocated to another chromosome or to another region of the same chromosome.

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Key Points

- Mismatch repair enzymes recognize mis-incorporated bases, remove them from DNA, and replace them with the correct bases.
- In nucleotide excision repair, enzymes remove incorrect bases with a few surrounding bases, which are replaced with the correct bases with the help of a DNA polymerase and the template DNA.
- When replication mistakes are not corrected, they may result in mutations, which sometimes can have serious consequences.
- Point mutations, one base substituted for another, can be silent (no effect) or may have effects ranging from mild to severe.
- Mutations may also involve insertions (addition of a base), deletion (loss of a base), or translocation (movement of a DNA section to a new location on the same or another chromosome).

Key Terms

- **mismatch repair**: a system for recognizing and repairing some forms of DNA damage and erroneous insertion, deletion, or mis-incorporation of bases that can arise during DNA replication and recombination

- **nucleotide excision repair**: a DNA repair mechanism that corrects damage done by UV radiation, including thymine dimers and 6,4 photoproducts that cause bulky distortions in the DNA

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SECTION OVERVIEW

5.13: Genome Evolution

Topic hierarchy

5.13A: Gene Families

5.13B: Genomics and Biofuels

5.13C: Genome Reduction

5.13D: Pathogenicity Islands

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5.13A: Gene Families

Gene families are groups of functionally related genes arising from a duplicated gene.

LEARNING OBJECTIVES

Distinguish gene families from gene complexes

Key Takeaways

A gene family is a set of several similar genes, formed by duplication of a single original gene, that generally have similar biochemical functions. One such family are the genes for human haemoglobin subunits. The 10 genes are in two clusters on different chromosomes, called the α-globin and β-globin loci. Genes are categorized into families based on shared nucleotide or protein sequences. Phylogenetic techniques can be used as a more rigorous test. The positions of exons within the coding sequence can be used to infer common ancestry. Knowing the sequence of the protein encoded by a gene can allow researchers to apply methods that find similarities among protein sequences that provide more information than similarities or differences among DNA sequences. Furthermore, knowledge of the protein's secondary structure gives further information about ancestry, since the organization of secondary structural elements presumably would be conserved even if the amino acid sequence changes considerably.

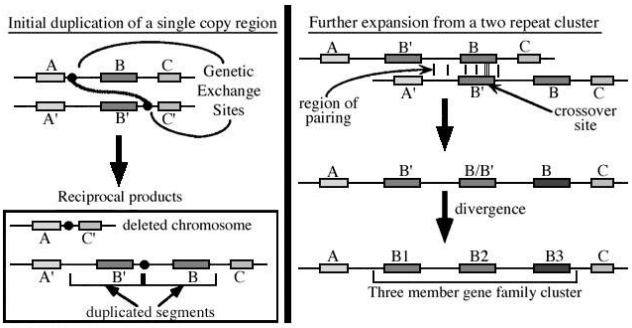


Figure: **Evolution of a Gene Family**: Unequal crossing over generates gene families. The left side illustrates an unequal crossing over event and the two products that are generated. One product is deleted and the other is duplicated for the same region. In this example, the duplicated region contains a second complete copy of a single gene (B). The right side illustrates a second round of unequal crossing over that can occur in a genome that is homozygous of the original duplicated chromosome. In this case, the crossover event has occurred between the two copies of the original gene. Only the duplicated product generated by this event is shown. Over time, the three copies of the B gene can diverge into three distinct functional units (B1, B2, and B3) of a gene family cluster.

These methods often rely upon predictions based upon the DNA sequence. If the genes of a gene family encode proteins, the term protein family is often used in an analogous manner to gene family. The expansion or contraction of gene families along a specific lineage can be due to chance or can be the result of natural selection. To distinguish between these two cases is often difficult in practice. Recent work uses a combination of statistical models and algorithmic techniques to detect gene families that are under the effect of natural selection.

In contrast, gene complexes are simply tightly linked groups of genes, often created via gene duplication (sometimes called segmental duplication if the duplicates remain side-by-side). Here, each gene has a similar though slightly diverged function.

Key Points

- Genes duplicate over evolutionary times. As they duplicate this can lead to families of related genes. Since they come from the same progenitor gene, they often have related biochemical functions.
- Gene families can expand and contract over evolutionary time scales.
- Gene complexes are groups of genes that work in a fashion similar to gene families; however, they need not arise from gene duplication.

Key Terms

- **phylogenetic**: Of or relating to the evolutionary development of organisms.
- **secondary structure**: The general three-dimensional structure of a biopolymer such as DNA or a protein.

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5.13B: Genomics and Biofuels

Learning Objectives

- Explain the process of creating new biofuels by using microbial genomics

Knowledge of the genomics of microorganisms is being used to find better ways to harness biofuels from algae and cyanobacteria. The primary sources of fuel today are coal, oil, wood, and other plant products, such as ethanol. Although plants are renewable resources, there is still a need to find more alternative renewable sources of energy to meet our population ‘s energy demands. The microbial world is one of the largest resources for genes that encode new enzymes and produce new organic compounds, and it remains largely untapped.

For microbial biomass breakdown, many candidates have already been identified. These include Clostridia species for their ability to degrade cellulose, and fungi that express genes associated with the decomposition of the most recalcitrant features of the plant cell wall, lignin, the phenolic “glue” that imbues the plant with structural integrity and pest resistance. The white rot fungus *Phanerochaete chrysosporium* produces unique extracellular oxidative enzymes that effectively degrade lignin by gaining access through the protective matrix surrounding the cellulose microfibrils of plant cell walls.

Another fungus, the yeast *Pichia stipitis*, ferments the five-carbon “wood sugar” xylose abundant in hardwoods and agricultural harvest residue. *Pichia*‘s recently-sequenced genome has revealed insights into the metabolic pathways responsible for this process, guiding efforts to optimize this capability in commercial production strains. Pathway engineering promises to produce a wider variety of organisms able to ferment the full repertoire of sugars derived from cellulose and hemicellulose and tolerate higher ethanol concentrations to optimize fuel yields. For instance, the hindgut contents of nature’s own bioreactor, the termite, has yielded more than 500 genes related to the enzymatic deconstruction of cellulose and hemicellulose.



Figure: ***Termites: Nature’s Bioreactors****: The hindgut of the termite has yielded more than 500 genes of microbes related to the enzymatic deconstruction of cellulose.*

Key Points

- Microorganisms can encode new enzymes and produce new organic compounds that can be used as biofuels.
- Genomic analysis of the fungus *Pichia* will allow optimization of its use in fermenting ethanol fuels.
- Analysis of the microbes in the hindgut of termites have found 500 genes that may be useful in enzymatic destruction of cellulose.
- Genetic markers have been used in forensic analysis, like in 2001 when the FBI used microbial genomics to determine a specific strain of anthrax that was found in several pieces of mail.
- Genomics is used in agriculture to develop plants with more desirable traits, such as drought and disease resistance.

Key Terms

- **renewable resource**: a natural resource such that it is replenished by natural processes at a rate comparable to its rate of consumption by humans or other users
- **biofuel**: any fuel that is obtained from a renewable biological resource

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5.13C: Genome Reduction

Learning Objectives

- Review genome reduction

Genome size is the total amount of DNA contained within one copy of a single genome. Over evolutionary times, genomes tend to increase in size due to the accumulation of duplication of the genome and an increase in genetic elements. The opposite or genome reduction also occurs. Genome reduction, also known as genome degradation, is the process by which a genome shrinks relative to its ancestor. Genomes fluctuate in size regularly; however, genome size reduction is most significant in bacteria.The most evolutionary significant cases of genome reduction may be the eukaryotic organelles that are derived from bacteria: the mitochondrion and plastid. These organelles are descended from endosymbionts, which can only survive within the host cell and which the host cell likewise needs for survival. Many mitochondria have less than 20 genes in their entire genome, whereas a free-living bacterium generally has at least 1000 genes. Many genes have been transferred to the host nucleus, while others have simply been lost and their function replaced by host processes.Other bacteria have become endosymbionts or obligate intracellular pathogens and have experienced extensive genome reduction as a result. This process seems to be dominated by genetic drift resulting from small population size, low recombination rates, and high mutation rates, as opposed to selection for smaller genomes. Some free-living marine bacterioplanktons also shows signs of genome reduction, which are hypothesized to be driven by natural selection.

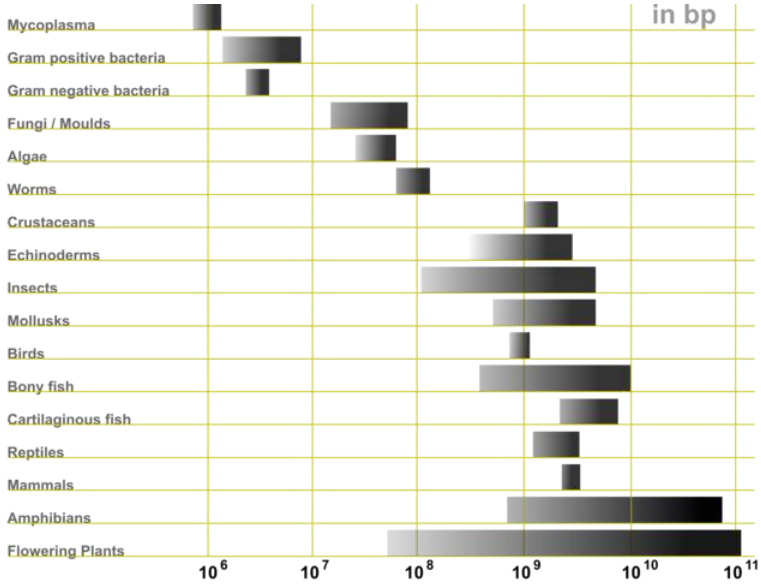


Figure: ***Variation in genome sizes****: A graph show the relative size of genomes, generally more "complex" organisms have larger genomes.*

Obligate endosymbiotic species are characterized by a complete inability to survive outside their host environment. These species have become a considerable threat to human health, as they are often highly capable of evading human immune systems and manipulating the host environment to acquire nutrients. A common explanation for these keen manipulative abilities is the compact and efficient genomic structure consistently found in obligate endosymbionts. This compact genome structure is the result of massive losses of extraneous DNA – an occurrence that is exclusively associated with the loss of a free-living stage. In fact, as much as 90% of the genetic material can be lost when a species makes the evolutionary transition from a free-living to obligate intracellular lifestyle. Common examples of species with reduced genomes include: *Buchnera aphidicola*, *Rickettsia prowazekii* and *Mycobacterium leprae*. One obligate endosymbiont of psyllid, *Candidatus Carsonella ruddii*, has the smallest genome currently known among cellular organisms at 160kb. It is important to note, however, that some obligate intracellular species have positive fitness effects on their hosts. The reductive evolution model has been proposed as an effort to define the genomic commonalities seen in all obligate endosymbionts. This model illustrates four general features of reduced genomes and obligate intracellular species: 'genome streamlining' resulting from relaxed selection on genes that are superfluous in the intracellular

environment; a bias towards deletions (rather than insertions), which heavily affects genes that have been disrupted by accumulation of mutations (pseudogenes); very little or no capability for acquiring new DNA; and considerable reduction of effective population size in endosymbiotic populations, particularly in species that rely on vertical transmission. Based on this model, it is clear that endosymbionts face different adaptive challenges than free-living species.

Key Points

- Genomes tend to increase in size over time, however exceptions occur.
- Genome reduction is observed in species that depend on a host for survival, the most extreme examples are eukaryotic organelles that have bacterial origins such as mitochondria.
- As an endosymbiont becomes dependent on its host, it becomes an obligate endosymbiont; during this time the genome is reduced due to deletions of genes not needed to live in the host. Parts of the genome can be transferred to the host as well, leading to further genome reduction.

Key Terms

- **genome**: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of basepairs.
- **endosymbiont**: An organism that lives within the body or cells of another organism.

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5.13D: Pathogenicity Islands

Learning Objectives

- Describe pathogenicity islands

Pathogenicity islands (PAIs) are a distinct class of genomic islands acquired by microorganisms through horizontal gene transfer. They are incorporated in the genome of pathogenic organisms, but are usually absent from those nonpathogenic organisms of the same or closely related species. These mobile genetic elements may range from 10-200 kb and encode genes which contribute to the virulence of the respective pathogen. Typical examples are adherence factors, toxins, iron uptake systems, invasion factors, and secretion systems. Pathogenicity islands are discrete genetic units flanked by direct repeats, insertion sequences or tRNA genes, which act as sites for recombination into the DNA. Cryptic mobility genes may also be present, indicating the provenance as transduction. One species of bacteria may have more than one PAI (i.e. Salmonella has at least 5). They are transferred through horizontal gene transfer events such as transfer by a plasmid, phage, or conjugative transposon.

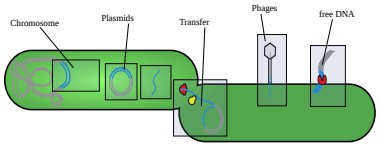


Figure: **Horizontal Transfer**: Pathogenicity islands are transferred horizontally, this details some of the ways that occurs.

Pathogenicity islands carry genes encoding one or more virulence factors, including, but not limited to, adhesins, toxins, or invasins. They may be located on a bacterial chromosome or may be transferred within a plasmid. The GC-content of pathogenicity islands often differs from that of the rest of the genome, potentially aiding in their detection within a given DNA sequence. PAIs are flanked by direct repeats; the sequence of bases at two ends of the inserted sequence is the same. They carry functional genes, such as integrases, transposases, or part of insertion sequences, to enable insertion into host DNA. PAIs are often associated with tRNA genes, which target sites for this integration event. They can be transferred as a single unit to new bacterial cells, thus conferring virulence to formerly benign strains.

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Key Points

- A host may have more than one pathogenicity island.
- Pathogenicity islands are transferred horizontally, through plasmids or transposons.

- The addition of a pathogenicity island to a non-invasive species can make the non-invasive species pathogenic.

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.
- **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.

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SECTION OVERVIEW

6.1: Tools of Genetic Engineering

Topic hierarchy

6.1A: Recombinant DNA Technology

6.1B: Selection

6.1C: Mutation

6.1D: Reproductive Cloning

6.1E: Basic Techniques to Manipulate Genetic Material (DNA and RNA)

6.1F: Molecular and Cellular Cloning

6.1G: Plasmids as Cloning Vectors

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6.1A: Recombinant DNA Technology

Molecular cloning permits the replication of a specific DNA sequence in a living microorganism.

LEARNING OBJECTIVES

Show some of the methods and uses of recombinant DNA

Key Takeaways

Recombinant DNA technology also referred to as molecular cloning is similar to polymerase chain reaction (PCR) in that it permits the replication of a specific DNA sequence. The fundamental difference between the two methods is that molecular cloning involves replication of the DNA in a living microorganism, while PCR replicates DNA in an *in vitro* solution, free of living cells.

In standard molecular cloning experiments, the cloning of any DNA fragment essentially involves seven steps:

1. Choice of host organism and cloning vector
2. Preparation of vector DNA
3. Preparation of DNA to be cloned
4. Creation of recombinant DNA
5. Introduction of recombinant DNA into host organism
6. Selection of organisms containing recombinant DNA
7. Screening for clones with desired DNA inserts and biological properties

Although a very large number of host organisms and molecular cloning vectors are in use, the great majority of molecular cloning experiments begin with a laboratory strain of the bacterium *E. coli* (*Escherichia coli*) and a plasmid cloning vector. *E. coli* and plasmid vectors are in common use because they are technically sophisticated, versatile, widely available, and offer rapid growth of recombinant organisms with minimal equipment. The cloning vector is treated with a restriction endonuclease to cleave the DNA at the site where foreign DNA will be inserted. The restriction enzyme is chosen to generate a configuration at the cleavage site that is compatible with that at the ends of the foreign DNA.

Typically, this is done by cleaving the vector DNA and foreign DNA with the same restriction enzyme, for example *EcoRI*. Most modern vectors contain a variety of convenient cleavage sites that are unique within the vector molecule (so that the vector can only be cleaved at a single site) and is located within a gene (frequently beta-galactosidase) whose inactivation can be used to distinguish recombinant from non-recombinant organisms at a later step in the process. To improve the ratio of recombinant to non-recombinant organisms, the cleaved vector may be treated with an enzyme (alkaline phosphatase) that dephosphorylates the vector ends. Vector molecules with dephosphorylated ends are unable to replicate, and replication can only be restored if foreign DNA is integrated into the cleavage site.

For cloning of genomic DNA, the DNA to be cloned is extracted from the organism of interest. Polymerase chain reaction (PCR) methods are often used for amplification of specific DNA or RNA (RT-PCR) sequences prior to molecular cloning. The purified DNA is then treated with a restriction enzyme to generate fragments with ends capable of being linked to those of the vector. If necessary, short double-stranded segments of DNA (linkers) containing desired restriction sites may be added to create end structures that are compatible with the vector. The creation of recombinant DNA is in many ways the simplest step of the molecular cloning process. DNA prepared from the vector and foreign source are simply mixed together at appropriate concentrations and exposed to an enzyme (DNA ligase) that covalently links the ends together. This joining reaction is often termed ligation. The resulting DNA mixture containing randomly joined ends is then ready for introduction into the host organism. The DNA mixture, previously manipulated *in vitro*, is moved back into a living cell, referred to as the host organism. The methods used to get DNA into cells are varied, and the name applied to this step in the molecular cloning process will often depend upon the experimental method that is chosen (e.g. transformation, transduction, transfection, electroporation).

When microorganisms are able to take up and replicate DNA from their local environment, the process is termed transformation, and cells that are in a physiological state such that they can take up DNA are said to be competent. When bacterial cells are used as host organisms, the selectable marker is usually a gene that confers resistance to an antibiotic that would otherwise kill the cells, typically ampicillin. Cells harboring the vector will survive when exposed to the antibiotic, while those that have failed to take up

vector sequences will die. Modern bacterial cloning vectors (e.g. pUC19) use the blue-white screening system to distinguish colonies (clones) of transgenic cells from those that contain the parental vector.

In these vectors, foreign DNA is inserted into a sequence that encodes an essential part of beta-galactosidase, an enzyme whose activity results in formation of a blue-colored colony on the culture medium that is used for this work. Insertion of the foreign DNA into the beta-galactosidase coding sequence disables the function of the enzyme, so that colonies containing recombinant plasmids remain colorless (white). Therefore, recombinant clones are easily identified.



Figure: **Blue White screen**: The blue-white screen is a screening technique that allows for the detection of successful ligations in vector-based gene cloning. DNA of interest is ligated into a vector. The vector is then transformed into competent cell (bacteria). The competent cells are grown in the presence of X-gal. If the ligation was successful, the bacterial colony will be white; if not, the colony will be blue. This technique allows for the quick and easy detection of successful ligation.

Key Points

- Although a very large number of host organisms and molecular cloning vectors are in use, the great majority of molecular cloning experiments begin with a laboratory strain of the bacterium *E. coli* (*Escherichia coli*) and a plasmid cloning vector.
- *E. coli* and plasmid vectors are in common use because they are technically sophisticated, versatile, widely available, and offer rapid growth of recombinant organisms with minimal equipment.
- Modern bacterial cloning vectors (e.g. pUC19) use the blue-white screening system to distinguish colonies (clones) of transgenic cells from those that contain the parental vector.

Key Terms

- **polymerase chain reaction**: A technique in molecular biology for creating multiple copies of DNA from a sample; used in genetic fingerprinting etc.
- **molecular cloning**: a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms.
- **restriction enzyme**: An endonuclease that catalyzes double-strand cleavage of DNA containing a specific sequence.

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6.1B: Selection

A selectable marker is usually a gene that confers resistance to an antibiotic that would otherwise kill the cells.

LEARNING OBJECTIVES

Identify the purpose of selection in genetic engineering

Key Takeaways

Scientists who do experimental genetics employ artificial selection experiments that permit the survival of organisms with user-defined phenotypes. Artificial selection is widely used in the field of microbial genetics, especially molecular cloning.

DNA recombination has been used to create gene replacements, deletions, insertions, inversions. Gene cloning and gene/protein tagging is also common. For gene replacements or deletions, usually a cassette encoding a drug-resistance gene is made by PCR.

Molecular cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. The use of the word cloning refers to the fact that the method involves the replication of a single DNA molecule starting from a single living cell to generate a large population of cells containing identical DNA molecules. Molecular cloning generally uses DNA sequences from two different organisms: the species that is the source of the DNA to be cloned, and the species that will serve as the living host for replication of the recombinant DNA. Molecular cloning methods are central to many contemporary areas of modern biology and medicine.

In a conventional molecular cloning experiment, the DNA to be cloned is obtained from an organism of interest. It is then treated with enzymes in the test tube to generate smaller DNA fragments. Subsequently, these fragments are then combined with vector DNA to generate recombinant DNA molecules. The recombinant DNA is then introduced into a host organism (typically an easy-to-grow, benign, laboratory strain of *E. coli* bacteria). This will generate a population of organisms in which recombinant DNA molecules are replicated along with the host DNA. Because they contain foreign DNA fragments, these are transgenic or genetically-modified microorganisms (GMO). This process takes advantage of the fact that a single bacterial cell can be induced to take up and replicate a single recombinant DNA molecule. This single cell can then be expanded exponentially to generate a large amount of bacteria, each of which contain copies of the original recombinant molecule. Thus, both the resulting bacterial population, and the recombinant DNA molecule, are commonly referred to as “clones”. Strictly speaking, recombinant DNA refers to DNA molecules, while molecular cloning refers to the experimental methods used to assemble them.

Molecular cloning takes advantage of the fact that the chemical structure of DNA is fundamentally the same in all living organisms. Therefore, if any segment of DNA from any organism is inserted into a DNA segment containing the molecular sequences required for DNA replication, and the resulting recombinant DNA is introduced into the organism from which the replication sequences were obtained, then the foreign DNA will be replicated along with the host cell’s DNA in the transgenic organism.

Molecular cloning is similar to polymerase chain reaction (PCR) in that it permits the replication of a specific DNA sequence. The fundamental difference between the two methods is that molecular cloning involves replication of the DNA in a living microorganism, while PCR replicates DNA in an in vitro solution, free of living cells. Whichever method is used, the introduction of recombinant DNA into the chosen host organism is usually a low efficiency process; that is, only a small fraction of the cells will actually take up DNA. Experimental scientists deal with this issue through a step of artificial genetic selection, in which cells that have not taken up DNA are selectively killed, and only those cells that can actively replicate DNA containing the selectable marker gene encoded by the vector are able to survive. When bacterial cells are used as host organisms, the selectable marker is usually a gene that confers resistance to an antibiotic that would otherwise kill the cells, typically ampicillin. Cells harboring the vector will survive when exposed to the antibiotic, while those that fail to take up vector sequences die. When mammalian cells (e.g. human or mouse cells) are used, a similar strategy is used, except that the marker gene (in this case typically encoded as part of the kanMX cassette) confers resistance to the antibiotic Geneticin.

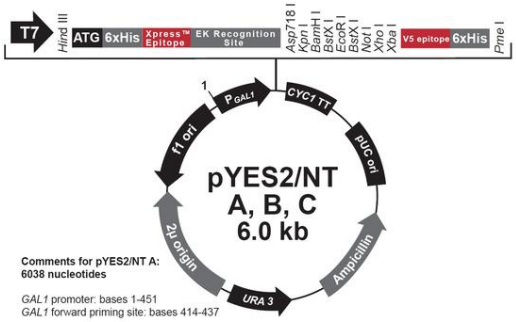


Figure: **Plasmid vector map**: This vector confers amplicillin resistance.

Key Points

- Recombinant DNA is introduced into the organism from which the replication sequences were obtained, then the foreign DNA will be replicated along with the host cell's DNA in the transgenic organism.
- Artificial genetic selection is the process in which cells that have not taken up DNA are selectively killed, and only those cells that can actively replicate DNA containing the selectable marker gene encoded by the vector are able to survive.
- When bacterial cells are used as host organisms, the selectable marker is usually a gene that confers resistance to an antibiotic that would otherwise kill the cells, typically ampicillin.

Key Terms

- **molecular cloning**: a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms.
- **PCR**: polymerase chain reaction

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6.1C: Mutation

Mutations are accidental changes in a genomic sequence of DNA; this includes the DNA sequence of a cell's genome or the DNA or RNA sequence.

LEARNING OBJECTIVES

Explain genetic manipulation through mutations

Key Takeaways

In molecular biology and genetics, mutations are accidental changes in a genomic sequence of DNA: the DNA sequence of a cell's genome or the DNA or RNA sequence in some viruses. These random sequences can be defined as sudden and spontaneous changes in the cell. Mutations are caused by radiation, viruses, transposons, and mutagenic chemicals. They are also caused by errors that occur during meiosis or DNA replication. They can also be induced by the organism itself, through cellular processes such as hypermutation.

Site-directed mutagenesis, also called site-specific mutagenesis or oligonucleotide-directed mutagenesis, is a molecular biology technique often used in biomolecular engineering in which a mutation is created at a defined site in a DNA molecule. In general, this form of mutagenesis requires that the wild type gene sequence be known. It is commonly used in protein engineering.

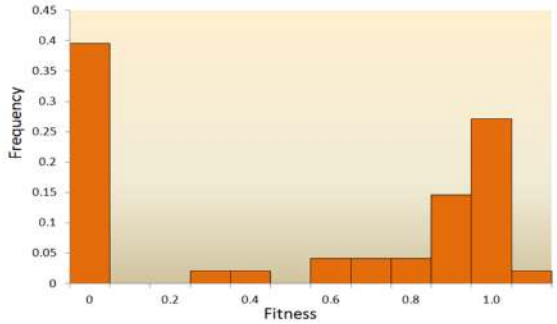


Figure: **The distribution of fitness effects**: The distribution of fitness effects of mutations in vesicular stomatitis virus. In this experiment, random mutations were introduced into the virus by site-directed mutagenesis. The fitness of each mutant was compared with the ancestral type. A fitness of zero, less than one, one, more than one, respectively, indicates that mutations are lethal, deleterious, neutral, and advantageous.

The basic procedure requires the synthesis of a short DNA primer. This synthetic primer contains the desired mutation and is complementary to the template DNA around the mutation site so it can hybridize with the DNA in the gene of interest. The mutation may be a single base change (a point mutation), multiple base changes, deletion, or insertion. The single-stranded primer is then extended using a DNA polymerase, which copies the rest of the gene. The copied gene contains the mutated site. It is then introduced into a host cell as a vector and cloned. Finally, mutants are selected.

The original method using single-primer extension was inefficient due to a lower yield of mutants. The resulting mixture may contain both the original unmutated template as well as the mutant strand, producing a mix population of mutant and non-mutant progenies. The mutants may also be counter-selected due to presence of a mismatch repair system which favors the methylated template DNA. Many approaches have since been developed to improve the efficiency of mutagenesis.

Key Points

- Mutations are caused by radiation, viruses, transposons, and mutagenic chemicals. They are also caused by errors that occur during meiosis or DNA replication.
- Site-directed mutagenesis, also called site-specific mutagenesis or oligonucleotide-directed mutagenesis, is a molecular biology technique often used in biomolecular engineering in which a mutation is created at a defined site in a DNA molecule.
- The basic procedure requires the synthesis of a short DNA primer. This synthetic primer contains the desired mutation. It is complementary to the template DNA around the mutation site so it can hybridize with the DNA in the gene of interest.

Key Terms

- **site-directed mutagenesis**: Also called site-specific mutagenesis or oligonucleotide-directed mutagenesis, is a molecular biology technique often used in biomolecular engineering in which a mutation is created at a defined site in a DNA molecule.
- **mutation**: Any heritable change of the base-pair sequence of genetic material.

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6.1D: Reproductive Cloning

Reproductive cloning, possible through artificially-induced asexual reproduction, is a method used to make a clone of an entire organism.

LEARNING OBJECTIVES

Differentiate reproductive cloning from cellular and molecular cloning

Key Takeaways

Reproductive Cloning

Reproductive cloning is a method used to make a clone or an identical copy of an entire multicellular organism. Most multicellular organisms undergo reproduction by sexual means, which involves genetic hybridization of two individuals (parents), making it impossible to generate an identical copy or clone of either parent. Recent advances in biotechnology have made it possible to artificially induce asexual reproduction of mammals in the laboratory.

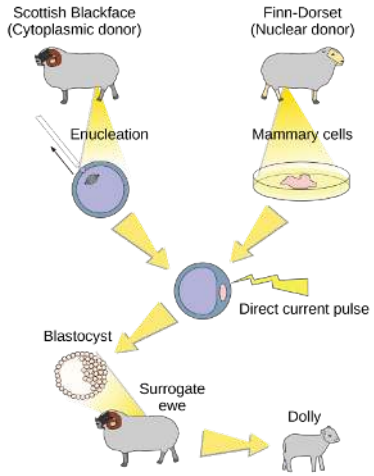


Figure: **Reproductive Cloning of Dolly, the Sheep**: Dolly the sheep was the first mammal to be cloned. To create Dolly, the nucleus was removed from a donor egg cell. The nucleus from a second sheep was then introduced into the cell, which was allowed to divide to the blastocyst stage before being implanted in a surrogate mother.

Parthenogenesis, or “virgin birth,” occurs when an embryo grows and develops without the fertilization of the egg occurring; this is a form of asexual reproduction. An example of parthenogenesis occurs in species in which the female lays an egg. If the egg is fertilized, it is a diploid egg and the individual develops into a female; if the egg is not fertilized, it remains a haploid egg and develops into a male. The unfertilized egg is called a parthenogenic, or virgin, egg. Some insects and reptiles lay parthenogenic eggs that can develop into adults.

Sexual reproduction requires two cells; when the haploid egg and sperm cells fuse, a diploid zygote results. The zygote nucleus contains the genetic information to produce a new individual. However, early embryonic development requires the cytoplasmic material contained in the egg cell. This idea forms the basis for reproductive cloning. If the haploid nucleus of an egg cell is replaced with a diploid nucleus from the cell of any individual of the same species (called a donor), it will become a zygote that is genetically identical to the donor. Somatic cell nuclear transfer is the technique of transferring a diploid nucleus into an enucleated egg. It can be used for either therapeutic cloning or reproductive cloning.

The first cloned animal was Dolly, a sheep who was born in 1996. The success rate of reproductive cloning at the time was very low. Dolly lived for seven years and died of respiratory complications. There is speculation that because the cell DNA belongs to an older individual, the age of the DNA may affect the life expectancy of a cloned individual. Since Dolly, several animals (e.g. horses, bulls, and goats) have been successfully cloned, although these individuals often exhibit facial, limb, and cardiac abnormalities. There have been attempts at producing cloned human embryos as sources of embryonic stem cells. Sometimes referred to as cloning for therapeutic purposes, the technique produces stem cells that attempt to remedy detrimental diseases or

defects (unlike reproductive cloning, which aims to reproduce an organism). Still, therapeutic cloning efforts have met with resistance because of bioethical considerations.

Key Points

- A form of asexual reproduction, parthenogenesis, occurs when an embryo grows and develops without the fertilization of the egg.
- In reproductive cloning, if the haploid nucleus of an egg cell is replaced with a diploid nucleus from the cell of an individual of the same species, it will become a zygote that is genetically identical to the donor.
- Reproductive cloning has become successful, but still has limitations as cloned individuals often exhibit facial, limb, and cardiac abnormalities.
- Therapeutic cloning, the cloning of human embryos as a source of embryonic stem cells, has been attempted in order to produce cells that can be used to treat detrimental diseases or defects.

Key Terms

- **clone**: a living organism produced asexually from a single ancestor, to which it is genetically identical
- **stem cell**: a primal undifferentiated cell from which a variety of other cells can develop through the process of cellular differentiation
- **parthenogenesis**: a form of asexual reproduction where growth and development of embryos occur without fertilization

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6.1E: Basic Techniques to Manipulate Genetic Material (DNA and RNA)

Basic techniques used in genetic material manipulation include extraction, gel electrophoresis, PCR, and blotting methods.

LEARNING OBJECTIVES

Distinguish among the basic techniques used to manipulate DNA and RNA

Key Takeaways

Basic Techniques to Manipulate Genetic Material (DNA and RNA)

To understand the basic techniques used to work with nucleic acids, remember that nucleic acids are macromolecules made of nucleotides (a sugar, a phosphate, and a nitrogenous base) linked by phosphodiester bonds. The phosphate groups on these molecules each have a net negative charge. An entire set of DNA molecules in the nucleus is called the genome. DNA has two complementary strands linked by hydrogen bonds between the paired bases. The two strands can be separated by exposure to high temperatures (DNA denaturation) and can be reannealed by cooling. The DNA can be replicated by the DNA polymerase enzyme. Unlike DNA, which is located in the nucleus of eukaryotic cells, RNA molecules leave the nucleus. The most common type of RNA that is analyzed is the messenger RNA (mRNA) because it represents the protein -coding genes that are actively expressed.

DNA and RNA Extraction

To study or manipulate nucleic acids, the DNA or RNA must first be isolated or extracted from the cells. This can be done through various techniques. Most nucleic acid extraction techniques involve steps to break open the cell and use enzymatic reactions to destroy all macromolecules that are not desired (such as degradation of unwanted molecules and separation from the DNA sample). Cells are broken using a lysis buffer (a solution that is mostly a detergent); lysis means “to split.” These enzymes break apart lipid molecules in the membranes of the cell and the nucleus. Macromolecules are inactivated using enzymes such as proteases that break down proteins, and ribonucleases (RNAses) that break down RNA. The DNA is then precipitated using alcohol. Human genomic DNA is usually visible as a gelatinous, white mass. Samples can be stored at –80°C for years.

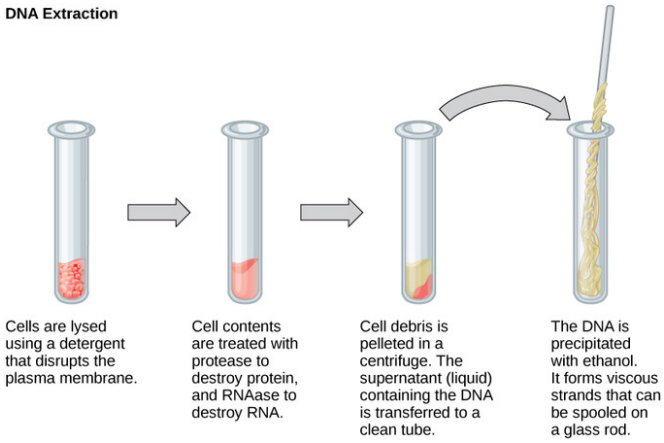


Figure: **DNA Extraction**: This diagram shows the basic method used for extraction of DNA.

RNA analysis is performed to study gene expression patterns in cells. RNA is naturally very unstable because RNAses are commonly present in nature and very difficult to inactivate. Similar to DNA, RNA extraction involves the use of various buffers and enzymes to inactivate macromolecules and preserve the RNA.

Gel Electrophoresis

Because nucleic acids are negatively-charged ions at neutral or basic pH in an aqueous environment, they can be mobilized by an electric field. Gel electrophoresis is a technique used to separate molecules on the basis of size using this charge and may be separated as whole chromosomes or fragments. The nucleic acids are loaded into a slot near the negative electrode of a porous gel matrix and pulled toward the positive electrode at the opposite end of the gel. Smaller molecules move through the pores in the gel faster than larger molecules; this difference in the rate of migration separates the fragments on the basis of size. There are

molecular-weight standard samples that can be run alongside the molecules to provide a size comparison. Nucleic acids in a gel matrix can be observed using various fluorescent or colored dyes. Distinct nucleic acid fragments appear as bands at specific distances from the top of the gel (the negative electrode end) on the basis of their size.

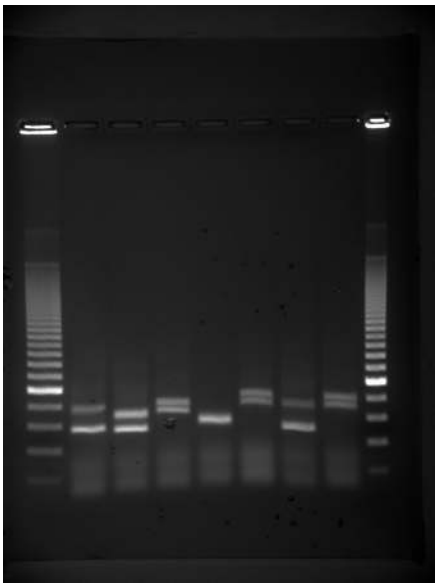


Figure: **Gel Electrophoresis**: Shown are DNA fragments from seven samples run on a gel, stained with a fluorescent dye, and viewed under UV light.

Amplification of Nucleic Acid Fragments by Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a technique used to amplify specific regions of DNA for further analysis. PCR is used for many purposes in laboratories, such as the cloning of gene fragments to analyze genetic diseases, identification of contaminant foreign DNA in a sample, and the amplification of DNA for sequencing. More practical applications include the determination of paternity and detection of genetic diseases.

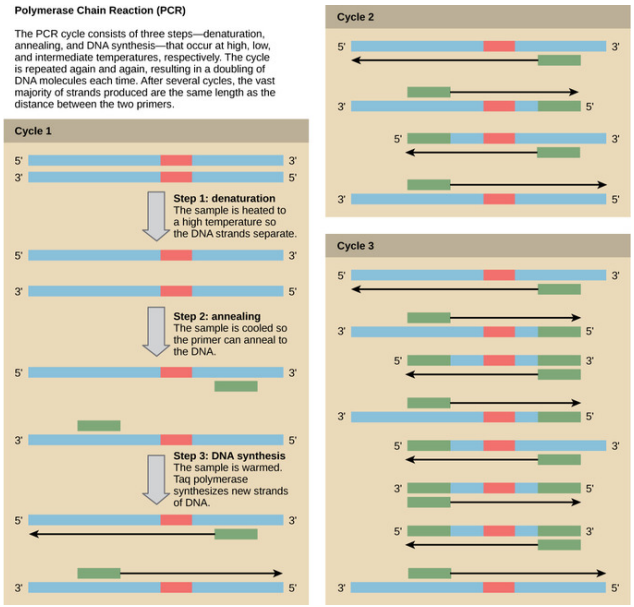


Figure: **PCR Amplification**: Polymerase chain reaction, or PCR, is used to amplify a specific sequence of DNA. Primers—short pieces of DNA complementary to each end of the target sequence—are combined with genomic DNA, Taq polymerase, and deoxynucleotides. Taq polymerase is a DNA polymerase isolated from the thermostable bacterium Thermus aquaticus that is able to withstand the high temperatures used in PCR. Thermus aquaticus grows in the Lower Geyser Basin of Yellowstone National Park. Reverse transcriptase PCR (RT-PCR) is similar to PCR, but cDNA is made from an RNA template before PCR begins.

DNA fragments can also be amplified from an RNA template in a process called reverse transcriptase PCR (RT-PCR). The first step is to recreate the original DNA template strand (called cDNA) by applying DNA nucleotides to the mRNA. This process is called reverse transcription. This requires the presence of an enzyme called reverse transcriptase. After the cDNA is made, regular PCR can be used to amplify it.

Hybridization, Southern Blotting, and Northern Blotting

Nucleic acid samples, such as fragmented genomic DNA and RNA extracts, can be probed for the presence of certain sequences. Short DNA fragments called probes are designed and labeled with radioactive or fluorescent dyes to aid detection. Gel electrophoresis separates the nucleic acid fragments according to their size. The fragments in the gel are then transferred onto a nylon membrane in a procedure called blotting. The nucleic acid fragments that are bound to the surface of the membrane can then be probed with specific radioactively- or fluorescently-labeled probe sequences. When DNA is transferred to a nylon membrane, the technique is called Southern blotting; when RNA is transferred to a nylon membrane, it is called northern blotting. Southern blots are used to detect the presence of certain DNA sequences in a given genome, and northern blots are used to detect gene expression.

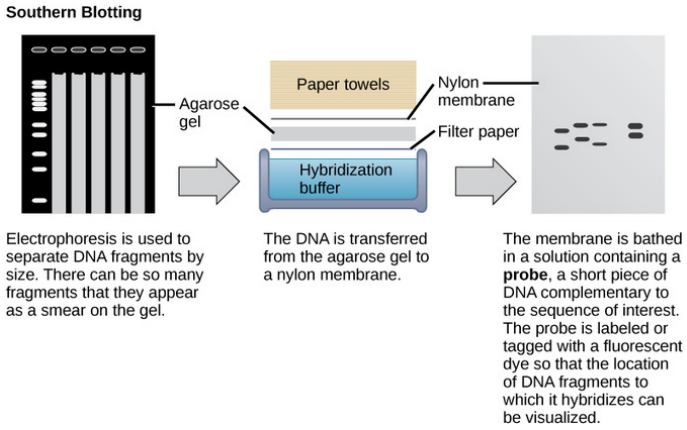


Figure: **Blotting Techniques**: Southern blotting is used to find a particular sequence in a sample of DNA. DNA fragments are separated on a gel, transferred to a nylon membrane, and incubated with a DNA probe complementary to the sequence of interest. Northern blotting is similar to Southern blotting, but RNA is run on the gel instead of DNA. In western blotting, proteins are run on a gel and detected using antibodies.

Key Points

- The first step to study or work with nucleic acids includes the isolation or extraction of DNA or RNA from cells.
- Gel electrophoresis depends on the negatively-charged ions present on nucleic acids at neutral or basic pH to separate molecules on the basis of size.
- Specific regions of DNA can be amplified through the use of polymerase chain reaction for further analysis.
- Southern blotting involves the transfer of DNA to a nylon membrane, while northern blotting is the transfer of RNA to a nylon membrane; these techniques allow samples to be probed for the presence of certain sequences.

Key Terms

- **denaturation**: the change of folding structure of a protein (and thus of physical properties) caused by heating, changes in pH, or exposure to certain chemicals
- **electrophoresis**: a method for the separation and analysis of large molecules, such as proteins or nucleic acids, by migrating a colloidal solution of them through a gel under the influence of an electric field
- **polymerase chain reaction**: a technique in molecular biology for creating multiple copies of DNA from a sample

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6.1F: Molecular and Cellular Cloning

Molecular cloning reproduces the desired regions or fragments of a genome, enabling the manipulation and study of genes.

LEARNING OBJECTIVES

Describe the process of molecular cloning

Key Takeaways

Molecular Cloning

In general, the word “cloning” means the creation of a perfect replica; however, in biology, the re-creation of a whole organism is referred to as “reproductive cloning.” Long before attempts were made to clone an entire organism, researchers learned how to reproduce desired regions or fragments of the genome, a process that is referred to as molecular cloning.

Cloning small fragments of the genome allows for the manipulation and study of specific genes (and their protein products) or noncoding regions in isolation. A plasmid (also called a vector) is a small circular DNA molecule that replicates independently of the chromosomal DNA. In cloning, the plasmid molecules can be used to provide a “folder” in which to insert a desired DNA fragment. Plasmids are usually introduced into a bacterial host for proliferation. In the bacterial context, the fragment of DNA from the human genome (or the genome of another organism that is being studied) is referred to as foreign DNA (or a transgene) to differentiate it from the DNA of the bacterium, which is called the host DNA.

Plasmids occur naturally in bacterial populations (such as *Escherichia coli*) and have genes that can contribute favorable traits to the organism such as antibiotic resistance (the ability to be unaffected by antibiotics). Plasmids have been repurposed and engineered as vectors for molecular cloning and the large-scale production of important reagents such as insulin and human growth hormone. An important feature of plasmid vectors is the ease with which a foreign DNA fragment can be introduced via the multiple cloning site (MCS). The MCS is a short DNA sequence containing multiple sites that can be cut with different commonly-available restriction endonucleases. Restriction endonucleases recognize specific DNA sequences and cut them in a predictable manner; they are naturally produced by bacteria as a defense mechanism against foreign DNA. Many restriction endonucleases make staggered cuts in the two strands of DNA, such that the cut ends have a 2- or 4-base single-stranded overhang. Because these overhangs are capable of annealing with complementary overhangs, these are called “sticky ends.” Addition of an enzyme called DNA ligase permanently joins the DNA fragments via phosphodiester bonds. In this way, any DNA fragment generated by restriction endonuclease cleavage can be spliced between the two ends of a plasmid DNA that has been cut with the same restriction endonuclease.

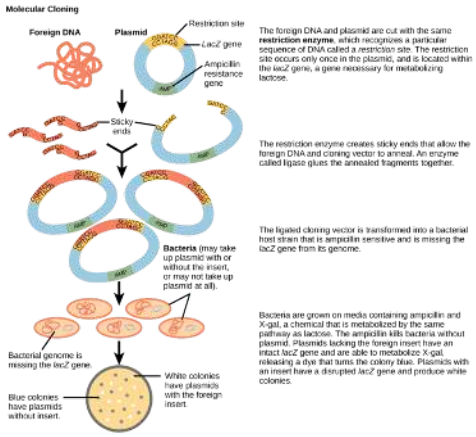


Figure: **Molecular Cloning**: This diagram shows the steps involved in molecular cloning, where regions or fragments of a genome are reproduced to allow the study or manipulation of genes and their protein products.

Recombinant DNA Molecules

Plasmids with foreign DNA inserted into them are called recombinant DNA molecules because they are created artificially and do not occur in nature. They are also called chimeric molecules because the origin of different parts of the molecules can be traced

back to different species of biological organisms or even to chemical synthesis. Proteins that are expressed from recombinant DNA molecules are called recombinant proteins. Not all recombinant plasmids are capable of expressing genes. The recombinant DNA may need to be moved into a different vector (or host) that is better designed for gene expression. Plasmids may also be engineered to express proteins only when stimulated by certain environmental factors so that scientists can control the expression of the recombinant proteins.

Cellular Cloning

Unicellular organisms, such as bacteria and yeast, naturally produce clones of themselves when they replicate asexually by binary fission; this is known as cellular cloning. The nuclear DNA duplicates by the process of mitosis, which creates an exact replica of the genetic material.

Key Points

- Cloning small fragments of a genome allows specific genes, their protein products, and non-coding regions to be studied in isolation.
- A plasmid, also known as a vector, is a small circular DNA molecule that replicates independently of the chromosomal DNA; it can be used to provide a “folder” in which to insert a desired DNA fragment.
- Recombinant DNA molecules are plasmids with foreign DNA inserted into them; they are created artificially as they do not occur in nature.
- Bacteria and yeast naturally produce clones of themselves when they replicate asexually through cellular cloning.

Key Terms

- **recombinant DNA**: DNA that has been engineered by splicing together fragments of DNA from multiple species and introduced into the cells of a host
- **molecular cloning**: a biological method that creates many identical DNA molecules and directs their replication within a host organism
- **plasmid**: a circle of double-stranded DNA that is separate from the chromosomes, which is found in bacteria and protozoa

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6.1G: Plasmids as Cloning Vectors

Plasmids can be used as cloning vectors, allowing the insertion of exogenous DNA into a bacterial target.

LEARNING OBJECTIVES

Illustrate how plasmids can be used as cloning vectors

Key Takeaways

Vectors

In molecular biology, a vector is a DNA molecule used as a vehicle to transfer foreign genetic material into another cell. The four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes. All engineered vectors have an origin of replication, a multi-cloning site, and a selectable marker. The vector itself is generally a DNA sequence that consists of an insert (transgene) and a larger sequence, which serves as the "backbone" of the vector. The purpose of a vector that transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. Vectors called expression vectors (expression constructs) express the transgene in the target cell, and they generally have a promoter sequence that drives expression of the transgene.

Plasmids

Plasmids are double-stranded, generally circular DNA sequences capable of automatically replicating in a host cell. Plasmid vectors minimally consist of the transgene insert and an origin of replication, which allows for semi-independent replication of the plasmid in the host. Modern plasmids generally have many more features, notably a "multiple cloning site"—with nucleotide overhangs for insertion of an insert—and multiple restriction enzyme consensus sites on either side of the insert. Plasmids may be conjugative/transmissible or non-conjugative. Conjugative plasmids mediate DNA transfer through conjugation and therefore spread rapidly among the bacterial cells of a population. Nonconjugative plasmids do not mediate DNA through conjugation.

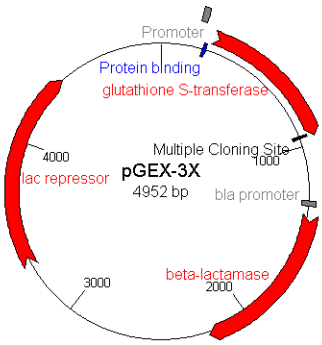


Figure: **Bacterial Cloning Vector**: The pGEX-3x plasmid is a popular cloning vector. The various elements of the plasmid are labelled.

Transcription

Transcription is a necessary component in all vectors. The purpose of a vector is to multiply the insert, although expression vectors also drive the translation of the multiplied insert. Even stable expression is determined by stable transcription, which depends on promoters in the vector. However, expression vectors have a two expression patterns: constitutive (consistent expression) or inducible (expression only under certain conditions or chemicals). Expression is based on different promoter activities, not post-transcriptional activities, meaning these two different types of expression vectors depend on different types of promoters. Expression vectors require translation of the vector's insert, thus requiring more components than simpler transcription-only vectors.

Expression vectors require sequences that encode for:

- A polyadenylation tail at the end of the transcribed pre-mRNA: This protects the mRNA from exonucleases and ensures transcriptional and translational termination and stabilizes mRNA production.

- Minimal UTR length: UTRs contain specific characteristics that may impede transcription or translation, so the shortest UTRs are encoded for in optimal expression vectors.
- Kozak sequence: a vector should encode for a Kozak sequence in the mRNA, which assembles the ribosome for translation of the mRNA.

The above conditions are necessary for expression vectors in eukaryotes, not prokaryotes.

Modern vectors may encompass additional features besides the transgene insert and a backbone:

- Promoter: a necessary component for all vectors, used to drive transcription of the vector's transgene.
- Genetic markers: Genetic markers for viral vectors allow for confirmation that the vector has integrated with the host genomic DNA.
- Antibiotic resistance: Vectors with antibiotic-resistance allow for survival of cells that have taken up the vector in growth media containing antibiotics through antibiotic selection.
- Epitope: A vector containing a sequence for a specific epitope that is incorporated into the expressed protein. Allows for antibody identification of cells expressing the target protein.
- Reporter genes: Some vectors may contain a reporter gene that allow for identification of plasmid that contains inserted DNA sequence. An example is lacZ-α which codes for the N-terminus fragment of β-galactosidase, an enzyme that digests galactose.
- Targeting sequence: Expression vectors may include encoding for a targeting sequence in the finished protein that directs the expressed protein to a specific organelle in the cell or specific location such as the periplasmic space of bacteria.
- Protein purification tags: Some expression vectors include proteins or peptide sequences that allows for easier purification of the expressed protein. Examples include polyhistidine-tag, glutathione-S-transferase, and maltose binding protein. Some of these tags may also allow for increased solubility of the target protein. The target protein is fused to the protein tag, but a protease cleavage site positioned in the polypeptide linker region between the protein and the tag allows the tag to be removed later.

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Key Points

- All engineered vectors have an origin of replication, a multi- cloning site, and a selectable marker.
- Expression vectors (expression constructs) express the transgene in the target cell, and they have a promoter sequence that drives expression of the transgene.

- Transcription is needed for a plasmid to function, without the proper sequences to transcribe parts of a plasmid it will not be expressed or even maintained in host cells.
- Vectors can have many additional sequences that can be used for downstream applications—purification of proteins encoded by the plasmid and expressing proteins targeted to be exported or to a certain compartment of the cell.

Key Terms

- **Kozak sequence**: a sequence which occurs on eukaryotic mRNA and has the consensus (gcc)gccRccAUGG. The Kozak consensus sequence plays a major role in the initiation of the translation process. The sequence was named after the person who brought it to prominence, Marilyn Kozak.
- **transcription**: The synthesis of RNA under the direction of DNA.
- **polyadenylation**: The formation of a polyadenylate, especially that of a nucleic acid

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SECTION OVERVIEW

6.2: Cloning Techniques

Topic hierarchy

6.2A: Putting Foreign DNA into Cells

6.2B: Obtaining DNA

6.2C: Hosts for Cloning Vectors

6.2D: Shuttle Vectors and Expression Vectors

6.2E: Bacteriophage Lambda as a Cloning Vector

6.2F: Vectors for Genomic Cloning and Sequencing

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6.2A: Putting Foreign DNA into Cells

The methods used to get DNA into cells are varied (e.g., transformation, transduction, transfection, and electroporation).

LEARNING OBJECTIVES

Describe the methods of introducing foreign DNA into cells

Key Takeaways

The DNA mixture, previously manipulated in vitro, is moved back into a living cell, referred to as the host organism. The methods used to get DNA into cells are varied, and the name applied to this step in the molecular cloning process will often depend upon the experimental method that is chosen (e.g., transformation, transduction, transfection, electroporation).

When microorganisms are able to take up and replicate DNA from their local environment, the process is termed transformation, and cells that are in a physiological state such that they can take up DNA, are said to be competent. In mammalian cell culture, the analogous process of introducing DNA into cells is commonly termed transfection. Both transformation and transfection usually require preparation of the cells through a special growth regime and chemical treatment process that will vary with the specific species and cell types that are used.

Electroporation uses high voltage electrical pulses to translocate DNA across the cell membrane (and cell wall, if present). In contrast, transduction involves the packaging of DNA into virus-derived particles, and using these virus-like particles to introduce the encapsulated DNA into the cell through a process resembling viral infection. Although electroporation and transduction are highly specialized methods, they may be the most efficient methods to move DNA into cells.

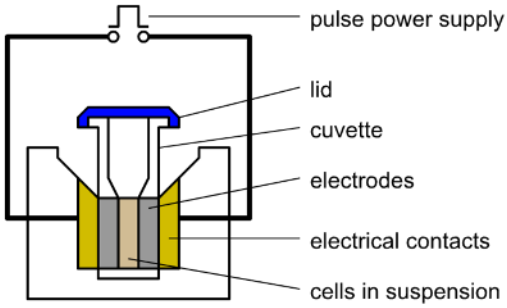


Figure: **Electroporation**: Diagram of the major components of an electroporator with cuvette loaded.

Whichever method is used, the introduction of recombinant DNA into the chosen host organism is usually a low efficiency process; that is, only a small fraction of the cells will actually take up DNA. Experimental scientists deal with this issue through a step of artificial genetic selection, in which cells that have not taken up DNA are selectively killed, and only those cells that can actively replicate DNA containing the selectable marker gene encoded by the vector are able to survive.

When bacterial cells are used as host organisms, the selectable marker is usually a gene that confers resistance to an antibiotic that would otherwise kill the cells, typically ampicillin. Cells harboring the vector will survive when exposed to the antibiotic, while those that have failed to take up vector sequences will die. When mammalian cells (e.g., human or mouse cells) are used, a similar strategy is used, except that the marker gene (in this case typically encoded as part of the kanMX cassette) confers resistance to the antibiotic Geneticin.

Key Points

- When microorganisms are able to take up and replicate DNA from their local environment, the process is termed transformation.
- In mammalian cell culture, the analogous process of introducing DNA into cells is commonly termed transfection.
- Electroporation uses high voltage electrical pulses to translocate DNA across the cell membrane (and cell wall, if present).

Key Terms

- **transformation**: The alteration of a bacterial cell caused by the transfer of DNA from another, especially if pathogenic.
- **microorganisms**: A microorganism or microbe is a microscopic organism that comprises either a single cell (unicellular), cell clusters, or multicellular relatively complex organisms.

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6.2B: Obtaining DNA

When cloning genomic DNA, the DNA to be cloned is extracted from the organism of interest.

LEARNING OBJECTIVES

Explain the methods of obtaining DNA for molecular cloning experiments and the process of creating a recombinant DNA molecule

Key Takeaways

Molecular cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms. The word cloning in this context refers to the fact that the method involves the replication of a single DNA molecule starting from a single living cell to generate a large population of cells containing identical DNA molecules. Molecular cloning generally uses DNA sequences from two different organisms: the species that is the source of the DNA to be cloned, and the species that will serve as the living host for replication of the recombinant DNA.

For cloning of genomic DNA, the DNA to be cloned is extracted from the organism of interest. Virtually any tissue source can be used (even tissues from extinct animals) as long as the DNA is not extensively degraded. The DNA is then purified using simple methods to remove contaminating proteins (extraction with phenol), RNA (ribonuclease) and smaller molecules (precipitation and/or chromatography). Polymerase chain reaction (PCR) methods are often used for amplification of specific DNA or RNA (by a process known as Reverse-Transcription or RT-PCR) sequences prior to molecular cloning using primers or short DNA sequences specific for the region of interest. DNA for cloning experiments may also be obtained from RNA using reverse transcriptase (complementary DNA or cDNA cloning), or in the form of synthetic DNA (artificial gene synthesis). cDNA cloning is usually used to obtain clones representative of the mRNA population of the cells of interest, while synthetic DNA is used to obtain any precise sequence defined by the designer.

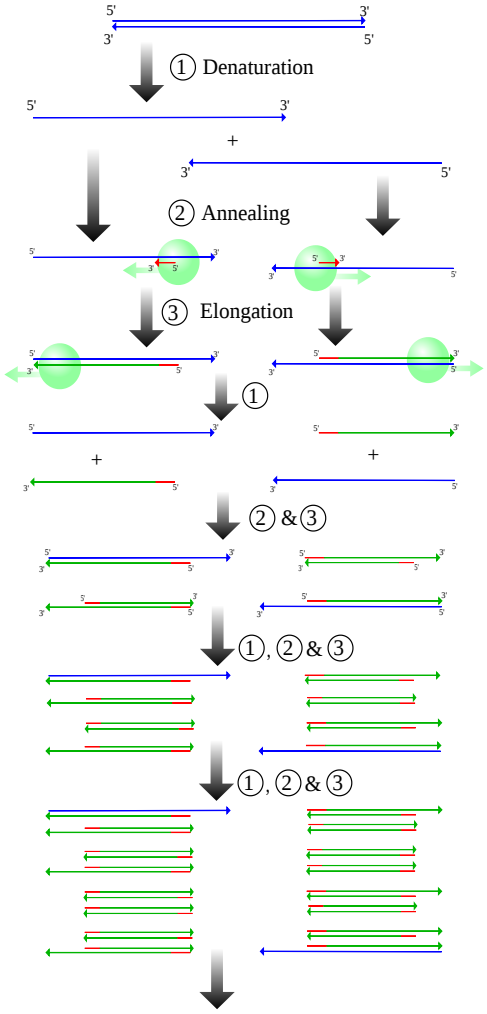


Figure: **The Steps of PCR**: This illustrates a PCR reaction to demonstrate how amplification leads to the exponential growth of a short product flanked by the primers. 1. Denaturing at 96°C. 2. Annealing at 68°C. 3. Elongation at 72°C. The first cycle is complete. The two resulting DNA strands make up the template DNA for the next cycle, thus doubling the amount of DNA duplicated for each new cycle.

Although a very large number of host organisms and molecular cloning vectors are used, the great majority of molecular cloning experiments begin with a laboratory strain of the bacterium E. coli (Escherichia coli) and a plasmid cloning vector. E. coli and plasmid vectors are in common use because they are technically sophisticated, versatile, widely available and offer rapid growth of recombinant organisms with minimal equipment. If the DNA to be cloned is exceptionally large (hundreds of thousands to millions of base pairs), then a bacterial artificial chromosome or yeast artificial chromosome vector is often chosen.

The cloning vector is treated with a restriction endonuclease to cleave the DNA at the site where foreign DNA will be inserted. The restriction enzyme is chosen to generate a configuration at the cleavage site that is compatible with that at the ends of the foreign DNA. Typically, this is done by cleaving the vector DNA and foreign DNA with the same restriction enzyme. Most modern vectors contain a variety of convenient cleavage sites that are unique within the vector molecule (so that the vector can only be cleaved at a single site) and are not located within the gene of interest to be cloned.

The creation of recombinant DNA is in many ways the simplest step of the molecular cloning process. DNA prepared from the vector and foreign source are treated with restriction enzymes to generate fragments with ends capable of being linked to those of the vector and they are simply mixed together at appropriate concentrations and exposed to an enzyme (DNA ligase) that covalently links the ends together. This joining reaction is often termed ligation. The resulting DNA mixture containing randomly

joined ends is then ready for introduction into the host organism for amplification (a process known as transformation). In mammalian cell culture, the analogous process of introducing DNA into cells is commonly known as transfection. Both transformation and transfection usually require preparation of the cells through a special growth regimen and chemical treatment process that will vary with the specific species and cell types that are used. Whichever method is used, the introduction of recombinant DNA into the chosen host organism is usually a low efficiency process; that is, only a small fraction of the cells will actually take up DNA. When bacterial cells are used as host organisms, the selectable marker is usually a gene that confers resistance to an antibiotic, typically ampicillin, that would otherwise kill the cells. Cells harboring the cloning vector will survive when exposed to the antibiotic, while those that have failed to take up cloning vector will die. The former can therefore be amplified and screened for the presence of the gene of interest in the cloning vector by restriction digest analysis.

Key Points

- The cloning vector is treated with a restriction endonuclease to cleave the DNA at the site where foreign DNA will be inserted.
- DNA for cloning experiments may also be obtained from RNA using reverse transcriptase (complementary DNA or cDNA cloning), or in the form of synthetic DNA (artificial gene synthesis).
- The creation of recombinant DNA is in many ways the simplest step of the molecular cloning process.

Key Terms

- **DNA**: A biopolymer of deoxyribonucleic acids (a type of nucleic acid) that has four different chemical groups, called bases: adenine, guanine, cytosine, and thymine.
- **cloning**: The production of a cloned embryo by transplanting the nucleus of a somatic cell into an ovum.
- **cloning vector**: A cloning vector is a small piece of DNA, taken from a virus, a plasmid, or the cell of a higher organism, into which a foreign DNA fragment can be inserted.

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6.2C: Hosts for Cloning Vectors

The majority of molecular cloning experiments begin with a laboratory strain of the bacterium *E. coli* (*Escherichia coli*) as the host.

LEARNING OBJECTIVES

Describe the features of a typical cloning vector

Key Takeaways

A very large number of host organisms and molecular cloning vectors are in use, but the great majority of molecular cloning experiments begin with a laboratory strain of the bacterium *E. coli* (*Escherichia coli*) and a plasmid cloning vector. *E. coli* and plasmid vectors are in common use because they are technically sophisticated, versatile, widely available, and offer rapid growth of recombinant organisms with minimal equipment. If the DNA to be cloned is exceptionally large (hundreds of thousands to millions of base pairs), then a bacterial artificial chromosome or yeast artificial chromosome vector is often chosen.

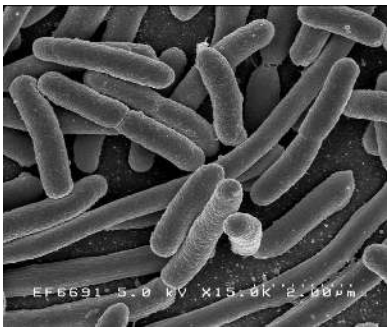


Figure: **Classification of E. coli**: Domain: Bacteria, Kingdom: Eubacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Enterobacteriales, Family: Enterobacteriaceae, Genus: Escherichia, Species: E. coli.

Specialized applications may call for specialized host-vector systems. For example, if the experimentalists wish to harvest a particular protein from the recombinant organism, then an expression vector is chosen that contains appropriate signals for transcription and translation in the desired host organism. Alternatively, if replication of the DNA in different species is desired (for example transfer of DNA from bacteria to plants), then a multiple host range vector (also termed shuttle vector) may be selected. In practice, however, specialized molecular cloning experiments usually begin with cloning into a bacterial plasmid, followed by subcloning into a specialized vector.

Whatever combination of host and vector are used, the vector almost always contains four DNA segments that are critically important to its function and experimental utility–(1) an origin of DNA replication is necessary for the vector (and recombinant sequences linked to it) to replicate inside the host organism, (2) one or more unique restriction endonuclease recognition sites that serves as sites where foreign DNA may be introduced, (3) a selectable genetic marker gene that can be used to enable the survival of cells that have taken up vector sequences, and (4) an additional gene that can be used for screening which cells contain foreign DNA.

Key Points

- *E. coli* and plasmid vectors are in common use because they are technically sophisticated, versatile, widely available, and offer rapid growth of recombinant organisms with minimal equipment.
- If the DNA to be cloned is exceptionally large, then a bacterial artificial chromosome or yeast artificial chromosome vector is often chosen.
- Specialized applications may call for specialized host -vector systems.

Key Terms

- **Escherichia coli**: Escherichia coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms).
- **plasmid**: A circle of double-stranded DNA that is separate from the chromosomes, which is found in bacteria and protozoa.

- **molecular cloning**: a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms.

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6.2D: Shuttle Vectors and Expression Vectors

An expression vector is generally a plasmid that is used to introduce a specific gene into a target cell.

LEARNING OBJECTIVES

Explain the structure and function of shuttle and expression vectors

Key Takeaways

An expression vector, otherwise known as an expression construct, is generally a plasmid that is used to introduce a specific gene into a target cell. Once the expression vector is inside the cell, the protein that is encoded by the gene is produced by the cellular-transcription and translation machinery ribosomal complexes. The plasmid is frequently engineered to contain regulatory sequences that act as enhancer and promoter regions and lead to efficient transcription of the gene carried on the expression vector. The goal of a well-designed expression vector is the production of large amounts of stable messenger RNA, and in extension, proteins. Expression vectors are basic tools for biotechnology and the production of proteins such as insulin, which is important for the treatment of diabetes.

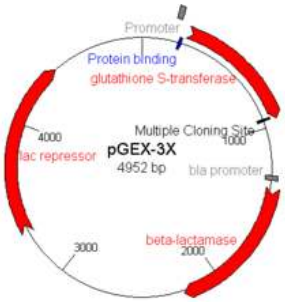


Figure: **The pGEX-3x Plasmid**: The pGEX-3x plasmid is a popular cloning vector. Please note the presence of a multiple cloning site, a promoter, a repressor, and a selectable marker.

After expression of the gene product, the purification of the protein is required; but since the vector is introduced to a host cell, the protein of interest should be purified from the proteins of the host cell. Therefore, to make the purification process easy, the cloned gene should have a tag. This tag could be histidine (His) tag or any other marker peptide.

Expression vectors are used for molecular biology techniques such as site-directed mutagenesis. Cloning vectors, which are very similar to expression vectors, involve the same process of introducing a new gene into a plasmid, but the plasmid is then added into bacteria for replication purposes. In general, DNA vectors that are used in many molecular-biology gene-cloning experiments need not result in the expression of a protein.

Expression vectors must have expression signals such as a strong promoter, a strong termination codon, adjustment of the distance between the promoter and the cloned gene, and the insertion of a transcription termination sequence and a PTIS (portable translation initiation sequence).

A shuttle vector is a vector that can propagate in two different host species, hence, inserted DNA can be tested or manipulated in two different cell types. The main advantage of these vectors is that they can be manipulated in E. coli and then used in a system which is more difficult or slower to use.

Shuttle vectors can be used in both eukaryotes and prokaryotes. Shuttle vectors are frequently used to quickly make multiple copies of the gene in E. coli (amplification). They can also be used for in vitro experiments and modifications such as mutagenesis and PCR. One of the most common types of shuttle vectors is the yeast shuttle vector that contains components allowing for the replication and selection in both E. coli cells and yeast cells. The E. coli component of a yeast shuttle vector includes an origin of replication and a selectable marker, such as an antibiotic resistance like beta lactamase. The yeast component of a yeast shuttle vector includes an autonomously replicating sequence (ARS), a yeast centromere (CEN), and a yeast selectable marker.

Key Points

- The plasmid is frequently engineered to contain regulatory sequences that act as enhancer and promoter regions and lead to efficient transcription of the gene carried on the expression vector.
- Expression vectors must have expression signals such as a strong promoter, a strong termination codon, adjustment of the distance between the promoter and the cloned gene, and the insertion of a transcription termination sequence and a portable translation initiation sequence.
- Expression vectors are used for molecular biology techniques such as site-directed mutagenesis.

Key Terms

- **plasmid**: A circle of double-stranded DNA that is separate from the chromosomes, which is found in bacteria and protozoa.
- **expression vector**: An expression vector, otherwise known as an expression construct, is generally a plasmid that is used to introduce a specific gene into a target cell.
- **transcription**: The synthesis of RNA under the direction of DNA.

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6.2E: Bacteriophage Lambda as a Cloning Vector

Enterobacteria phage λ (lambda phage, coliphage λ) is a bacterial virus that infects the bacterial species Escherichia coli.

LEARNING OBJECTIVES

Describe the life cycle of lambda phage

Key Takeaways

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), 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.

Lambda phage has been of major importance in the study of specialized transduction.

Specialized transduction is the process by which a restricted set of bacterial genes are transferred to another bacterium. The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome. Specialized transduction occurs when the prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to the prophage are included in the excised DNA. The excised DNA is then packaged into a new virus particle, which delivers the DNA to a new bacterium where the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage. When the partially encapsulated phage material infects another cell and becomes a "prophage" (is covalently bonded into the infected cell's chromosome), the partially coded prophage DNA is called a "heterogenote. "

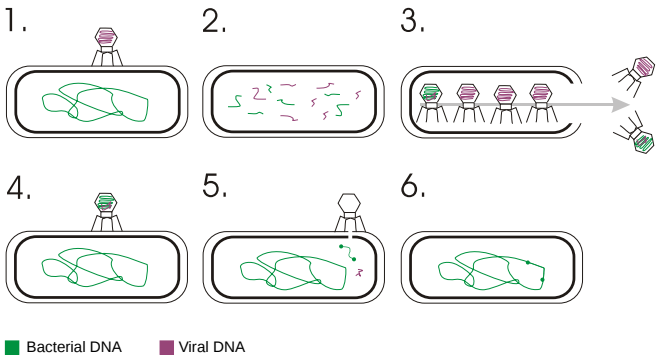


Figure: **Transduction**: Transduction is the process by which DNA is transferred from one bacterium to another by a virus.It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector.

Key Points

- Lambda phage consists of a virus particle including a head (also known as a capsid), tail and tail fibers.
- Specialized transduction is the process by which a restricted set of bacterial genes are transferred to another bacterium.
- The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome.

Key Terms

- **transduction**: Transduction is the process by which DNA is transferred from one bacterium to another by a virus.
- **lysogeny**: the process by which a bacteriophage incorporates its nucleic acids into a host bacterium

- **Lambda phage**: 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.

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6.2F: Vectors for Genomic Cloning and Sequencing

In molecular biology, a vector is a DNA molecule used as a vehicle to transfer foreign genetic material into another cell.

LEARNING OBJECTIVES

Differentiate between expression vectors and transcription vectors

Key Takeaways

In molecular biology, a vector is a DNA molecule used as a vehicle to transfer foreign genetic material into another cell. The four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes. Common to all engineered vectors are an origin of replication, a multicloning site, and a selectable marker.

The vector itself is generally a DNA sequence that consists of an insert (transgene) and a larger sequence that serves as the "backbone" of the vector. The purpose of a vector which transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. Vectors called expression vectors (expression constructs) are specifically for the expression of the transgene in the target cell, and generally have a promoter sequence that drives expression of the transgene. Simpler vectors called transcription vectors are only capable of being transcribed but not translated: they can be replicated in a target cell but not expressed, unlike expression vectors. Transcription vectors are used to amplify their insert.

Insertion of a vector into the target cell is usually called transformation for bacterial cells, and transfection for eukaryotic cells, although the insertion of a viral vector is often called transduction.

Plasmids are double-stranded generally circular DNA sequences that are capable of automatically replicating in a host cell. Plasmid vectors minimalistically consist of an origin of replication that allows for semi-independent replication of the plasmid in the host and also the transgene insert. Modern plasmids generally have many more features, notably including a "multiple cloning site" which includes nucleotide overhangs for insertion of an insert, and multiple restriction enzyme consensus sites to either side of the insert.

In the case of plasmids utilized as transcription vectors, incubating bacteria with plasmids generates hundreds or thousands of copies of the vector within the bacteria in hours. The vectors can be extracted from the bacteria, and the multiple cloning sites can be cut by restriction enzymes to excise the hundredfold or thousandfold amplified insert. These plasmid transcription vectors characteristically lack crucial sequences that code for polyadenylation sequences and translation termination sequences in translated mRNAs, making protein expression from transcription vectors impossible.

Plasmids may be conjugative / transmissible and non-conjugative. Conjugative vectors mediate DNA transfer through conjugation and therefore spread rapidly among the bacterial cells of a population, such as the F plasmid, as well as many R and some col plasmids. Non-conjugative vectors do not mediate DNA through conjugation, such as many R and col plasmids.

Viral vectors are generally genetically-engineered viruses carrying modified viral DNA or RNA that has been rendered noninfectious, but still contain viral promoters and also the transgene. This allows for the translation of the transgene through a viral promoter. However, because viral vectors are frequently lacking infectious sequences, they require helper viruses or packaging lines for large-scale transfection. Viral vectors are often designed for permanent incorporation of the insert into the host genome, and thus leave distinct genetic markers in the host genome after incorporating the transgene. For example, retroviruses leave a characteristic retroviral integration pattern after insertion that is detectable and indicates that the viral vector has incorporated into the host genome.

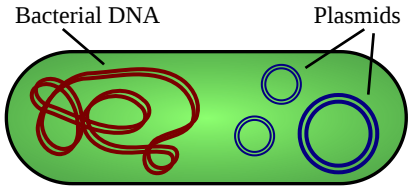


Figure: **A Plasmid**

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Key Points

- The vector itself is generally a DNA sequence that consists of an insert (transgene) and a larger sequence that serves as the “backbone” of the vector.
- The four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes.
- Common to all engineered vectors are an origin of replication, a multicloning site, and a selectable marker.

Key Terms

- **vector**: A carrier of a disease-causing agent.
- **plasmids**: Plasmids are double-stranded generally circular DNA sequences that are capable of automatically replicating in a host cell.
- **chromosomes**: A chromosome is an organized structure of DNA and protein found in cells. It is a single piece of coiled DNA containing many genes, regulatory elements, and other nucleotide sequences.

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SECTION OVERVIEW

6.3: Bioinformatics

Topic hierarchy

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6.3B: Annotating Genomes

6.3C: Homologs, Orthologs, and Paralogs

6.3D: Synthesizing DNA

6.3E: Amplifying DNA - The Polymerase Chain Reaction

6.3F: DNA Sequencing Based on Sanger Dideoxynucleotides

6.3G: Metagenomics

6.3H: Reporter Fusions

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6.3A: Strategies Used in Sequencing Projects

The strategies used for sequencing genomes include the Sanger method, shotgun sequencing, pairwise end, and next-generation sequencing.

LEARNING OBJECTIVES

Compare the different strategies used for whole-genome sequencing: Sanger method, shotgun sequencing, pairwise-end sequencing, and next-generation sequencing

Key Takeaways

Strategies Used in Sequencing Projects

The basic sequencing technique used in all modern day sequencing projects is the chain termination method (also known as the dideoxy method), which was developed by Fred Sanger in the 1970s. The chain termination method involves DNA replication of a single-stranded template with the use of a primer and a regular deoxynucleotide (dNTP), which is a monomer, or a single unit, of DNA. The primer and dNTP are mixed with a small proportion of fluorescently-labeled dideoxynucleotides (ddNTPs). The ddNTPs are monomers that are missing a hydroxyl group (–OH) at the site at which another nucleotide usually attaches to form a chain. Each ddNTP is labeled with a different color of fluorophore. Every time a ddNTP is incorporated in the growing complementary strand, it terminates the process of DNA replication, which results in multiple short strands of replicated DNA that are each terminated at a different point during replication. When the reaction mixture is processed by gel electrophoresis after being separated into single strands, the multiple, newly-replicated DNA strands form a ladder due to their differing sizes. Because the ddNTPs are fluorescently labeled, each band on the gel reflects the size of the DNA strand and the ddNTP that terminated the reaction. The different colors of the fluorophore-labeled ddNTPs help identify the ddNTP incorporated at that position. Reading the gel on the basis of the color of each band on the ladder produces the sequence of the template strand.

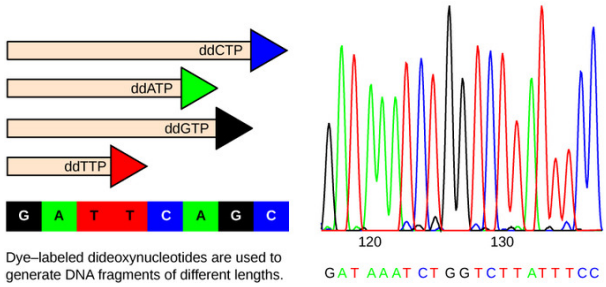


Figure: **Sanger's Method**: Frederick Sanger's dideoxy chain termination method uses dideoxynucleotides, in which the DNA fragment can be terminated at different points. The DNA is separated on the basis of size, and these bands, based on the size of the fragments, can be read.

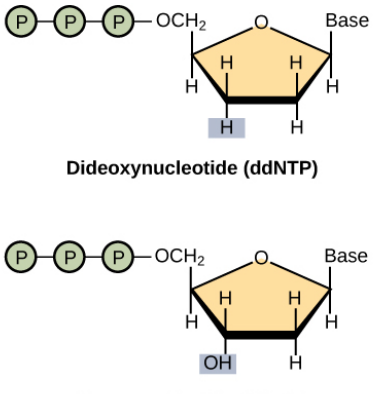


Figure: **Structure of a Dideoxynucleotide**: A dideoxynucleotide is similar in structure to a deoxynucleotide, but is missing the 3′ hydroxyl group (indicated by the box). When a dideoxynucleotide is incorporated into a DNA strand, DNA synthesis stops.

Early Strategies: Shotgun Sequencing and Pair-Wise End Sequencing

In the shotgun sequencing method, several copies of a DNA fragment are cut randomly into many smaller pieces (somewhat like what happens to a round shot cartridge when fired from a shotgun). All of the segments are then sequenced using the chain-sequencing method. Then, with the help of a computer, the fragments are analyzed to see where their sequences overlap. By matching overlapping sequences at the end of each fragment, the entire DNA sequence can be reformed. A larger sequence that is assembled from overlapping shorter sequences is called a contig. As an analogy, consider that someone has four copies of a landscape photograph that you have never seen before and know nothing about how it should appear. The person then rips up each photograph with their hands, so that different size pieces are present from each copy. The person then mixes all of the pieces together and asks you to reconstruct the photograph. In one of the smaller pieces you see a mountain. In a larger piece, you see that the same mountain is behind a lake. A third fragment shows only the lake, but it reveals that there is a cabin on the shore of the lake. Therefore, from looking at the overlapping information in these three fragments, you know that the picture contains a mountain behind a lake that has a cabin on its shore. This is the principle behind reconstructing entire DNA sequences using shotgun sequencing.

Originally, shotgun sequencing only analyzed one end of each fragment for overlaps. This was sufficient for sequencing small genomes. However, the desire to sequence larger genomes, such as that of a human, led to the development of double-barrel shotgun sequencing, more formally known as pairwise-end sequencing. In pairwise-end sequencing, both ends of each fragment are analyzed for overlap. Pairwise-end sequencing is, therefore, more cumbersome than shotgun sequencing, but it is easier to reconstruct the sequence because there is more available information.

Next-generation Sequencing

Since 2005, automated sequencing techniques used by laboratories are under the umbrella of next-generation sequencing, which is a group of automated techniques used for rapid DNA sequencing. These automated, low-cost sequencers can generate sequences of hundreds of thousands or millions of short fragments (25 to 500 base pairs) in the span of one day. Sophisticated software is used to manage the cumbersome process of putting all the fragments in order.

Key Points

- The Sanger method is a basic sequencing technique that uses fluorescently-labeled dideoxynucleotides (ddNTPs) during DNA replication which results in multiple short strands of replicated DNA that terminate at different points, based on where the ddNTP was incorporated.
- Shotgun sequencing is a method that randomly cuts DNA fragments into smaller pieces and then, with the help of a computer, takes the DNA fragments, analyzes them for overlapping sequences, and reassembles the entire DNA sequence.
- Pairwise-end sequencing is a type of shotgun sequencing which is used for larger genomes and analyzes both ends of the DNA fragments for overlap.
- Next-generation sequencing is a type of sequencing which is automated and relies on sophisticated software for rapid DNA sequencing.

Key Terms

- **fluorophore**: a molecule or functional group which is capable of fluorescence
- **contig**: a set of overlapping DNA segments, derived from a single source of genetic material, from which the complete sequence may be deduced
- **dideoxynucleotide**: any nucleotide formed from a deoxynucleotide by loss of an a second hydroxyl group from the deoxyribose group

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6.3B: Annotating Genomes

Genome annotation is the identification and understanding of the genetic elements of a sequenced genome.

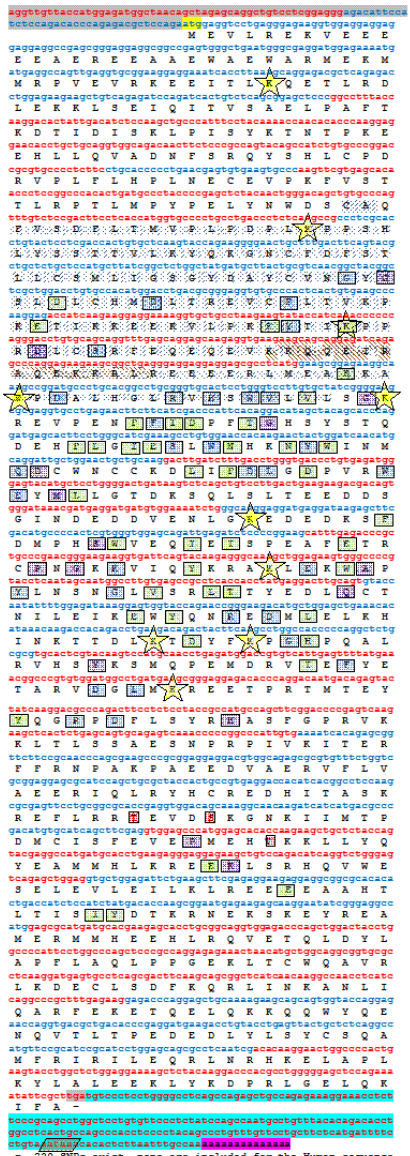
LEARNING OBJECTIVES

Define genome annotation

Key Takeaways

Genome projects are scientific endeavors that ultimately aim to determine the complete genome sequence of an organism (be it an animal, a plant, a fungus, a bacterium, an archaean, a protist, or a virus). They annotate protein-coding genes and other important genome-encoded features. The genome sequence of an organism includes the collective DNA sequences of each chromosome in the organism. For a bacterium containing a single chromosome, a genome project will aim to map the sequence of that chromosome.

Once a genome is sequenced, it needs to be annotated to make sense of it. An annotation (irrespective of the context) is a note added by way of explanation or commentary. Since the 1980's, molecular biology and bioinformatics have created the need for DNA annotation. DNA annotation or genome annotation is the process of identifying the locations of genes and all of the coding regions in a genome and determining what those genes do.



- 230 SNPs exist, none are included for the Human sequence.
- 4 isoforms present, the table below indicates which isoform form from the splicing of different exon numbers:



[1]

- The domain containing Transglutaminase-like family and Nuclear localization sequence are shaded in with a positive slope and negative slope, respectively[2].
- Amino acids that were predicted by Phosphosite to be possible phosphorylation sites are shaded in red.
- Highly conserved amino acids are shaded in blue.
- Conservation of strong amino acids are shaded in green.
- Conservation of weak amino acids are shaded in purple.

NOTE: these results are based on CLUSTALW of all 20 orthologs

[3]Letters in star represents amino acid with high probability for sumoylation.

One alternative poly-A signal was obtained in this mRNA sequence, however, [4]8 total poly-A signals were obtained when the complete genomic sequence [introns and exons was analyzed]. The following positions were where signals were obtained when NC_000016.9, Homo sapiens chromosome 16, GRCh37 primary reference assembly, was obtained from NCBI; 333, 4598, 15655, 15849, 17699, 18806, 20829, and 22379. In must be noted that these predictions do not contain a G/U rich element downstream which might limit the poly-A addition. Also, 7 of the positions 333, 4598, 15655, 15849, 17699, 18806, and 20829, are located within the introns of mRNA, hence, they may cause a defect in the amino acid sequence.

[1] Isoform information obtained from GeneCard, http://www.genecards.org/cgi-bin/carddisp.pl?gene=CCDC135&search=ccdc135
[2] Domain information obtained from Kegg, http://www.genome.jp/kegg/
[3] http://www.abgent.com/tools/sumoplot, with scores above 0.67.
[4] Polyadq output, http://rulai.cshl.org/cgi-bin/tools/polyadq/polyadq4.cgi

https://bio.libretexts.org/@go/page/105735

Figure: **Genome Annotation**: Here a small region of genome is annotated, with various elements identified. The annotation of an entire genome would entail a similar in depth analysis of thousand even millions of such DNA sequences.

Genome annotation is the process of attaching biological information to sequences. It consists of two main steps: identifying elements on the genome, a process called gene prediction, and attaching biological information to these elements. Automatic annotation tools try to perform all of this by computer analysis, as opposed to manual annotation (a.k.a. curation) which involves human expertise. Ideally, these approaches co-exist and complement each other in the same annotation pipeline (process). The basic level of annotation is using BLAST for finding similarities, and then annotating genomes based on that. However, nowadays more and more additional information is added to the annotation platform. The additional information allows manual annotators to deconvolute discrepancies between genes that are given the same annotation. Some databases use genome context information, similarity scores, experimental data, and integrations of other resources to provide genome annotations through their Subsystems approach. Other databases rely on both curated data sources as well as a range of different software tools in their automated genome annotation pipeline.

Structural annotation consists of the identification of genomic elements: ORFs and their localization, gene structure, coding regions, and the location of regulatory motifs. Functional annotation consists of attaching biological information to genomic elements: biochemical function, biological function, involved regulation and interactions, and expression.

These steps may involve both biological experiments and in silico analysis. Proteogenomics based approaches utilize information from expressed proteins, often derived from mass spectrometry, to improve genomics annotations. A variety of software tools have been developed to permit scientists to view and share genome annotations. Genome annotation is the next major challenge for the Human Genome Project, now that the genome sequences of human and several model organisms are largely complete. Identifying the locations of genes and other genetic control elements is often described as defining the biological “parts list” for the assembly and normal operation of an organism. Scientists are still at an early stage in the process of delineating this parts list and in understanding how all the parts “fit together. ”

Key Points

- Once a genome is sequenced, all of the sequencings must be analyzed to understand what they mean.
- Critical to annotation is the identification of the genes in a genome, the structure of the genes, and the proteins they encode.
- Once a genome is annotated, further work is done to understand how all the annotated regions interact with each other.

Key Terms

- **BLAST**: In bioinformatics, Basic Local Alignment Search Tool, or BLAST, is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences.
- **in silico**: In computer simulation or in virtual reality

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6.3C: Homologs, Orthologs, and Paralogs

Learning Objectives

- Distinguish homologs, orthologs and paralogs

Homology forms the basis of organization for comparative biology. A homologous trait is often called a homolog (also spelled homologue). In genetics, the term “homolog” is used both to refer to a homologous protein and to the gene (DNA sequence) encoding it. As with anatomical structures, homology between protein or DNA sequences is defined in terms of shared ancestry. Two segments of DNA can have shared ancestry because of either a speciation event (orthologs) or a duplication event (paralogs). Homology among proteins or DNA is often incorrectly concluded on the basis of sequence similarity. The terms “percent homology” and “sequence similarity” are often used interchangeably. As with anatomical structures, high sequence similarity might occur because of convergent evolution, or, as with shorter sequences, because of chance. Such sequences are similar, but not homologous. Sequence regions that are homologous are also called conserved. This is not to be confused with conservation in amino acid sequences in which the amino acid at a specific position has been substituted with a different one with functionally equivalent physicochemical properties. One can, however, refer to partial homology where a fraction of the sequences compared (are presumed to) share descent, while the rest does not. For example, partial homology may result from a gene fusion event.

```
AAB24882        TYHMCQFHCRYVNNHSGEKLYECNERSKAFSCPSHLQCHKRRQIGEKTHEHNQCGKAFPT 60
AAB24881        --------------------YECNQCGKAFAQHSSLKCHYRTHIGEKPYECNQCGKAFSK 40
                                     ****: .***:  * *:** * :****.:* *******..

AAB24882        PSHLQYHERTHTGEKPYECHQCGQAFKKCSLLQRHKRTHTGEKPYE-CNQCGKAFAQ- 116
AAB24881        HSHLQCHKRTHTGEKPYECNQCGKAFSQHGLLQRHKRTHTGEKPYMNVINMVKPLHNS 98
                 **** *:***********:***:**.: .***************    :  *.: :
```

Figure: **Example of Homologous DNA**: This is the sequence alignment of a homologous protein from two different species The “*” represent a conserved amino acid in the two proteins.

Homologous sequences are orthologous if they were separated by a speciation event: when a species diverges into two separate species, the copies of a single gene in the two resulting species are said to be orthologous. Orthologs, or orthologous genes, are genes in different species that originated by vertical descent from a single gene of the last common ancestor. For instance, the plant Flu regulatory protein is present both in *Arabidopsis* (multicellular higher plant) and *Chlamydomonas* (single cell green algae). The *Chlamydomonas* version is more complex: it crosses the membrane twice rather than once, contains additional domains, and undergoes alternative splicing. However, it can fully substitute the much simpler *Arabidopsis* protein, if transferred from algae to plant genome by means of gene engineering. Significant sequence similarity and shared functional domains indicate that these two genes are orthologous genes, inherited from the shared ancestor. Orthologous sequences provide useful information in taxonomic classification and phylogenetic studies of organisms. The pattern of genetic divergence can be used to trace the relatedness of organisms. Two organisms that are very closely related are likely to display very similar DNA sequences between two orthologs. Conversely, an organism that is further removed evolutionarily from another organism is likely to display a greater divergence in the sequence of the orthologs being studied.

Homologous sequences are paralogous if they were separated by a gene duplication event: if a gene in an organism is duplicated to occupy two different positions in the same genome, then the two copies are paralogous. Paralogous genes often belong to the same species, but this is not necessary. For example, the hemoglobin gene of humans and the myoglobin gene of chimpanzees are paralogs. Paralogs can be split into in-paralogs (paralogous pairs that arose after a speciation event) and out-paralogs (paralogous pairs that arose before a speciation event). Between species out-paralogs are pairs of paralogs that exist between two organisms due to duplication before speciation. Within species out-paralogs are pairs of paralogs that exist in the same organism, but whose duplication event happened after speciation. Paralogs typically have the same or similar function, but sometimes do not. Due to lack of the original selective pressure upon one copy of the duplicated gene, this copy is free to mutate and acquire new functions. Paralogous sequences provide useful insight into the way genomes evolve. The genes encoding myoglobin and hemoglobin are considered to be ancient paralogs. Similarly, the four known classes of hemoglobins (hemoglobin A, hemoglobin A2, hemoglobin B, and hemoglobin F) are paralogs of each other. While each of these proteins serves the same basic function of oxygen transport, they have already diverged slightly in function: fetal hemoglobin (hemoglobin F) has a higher affinity for oxygen than adult hemoglobin. However, function is not always conserved. Human angiogenin diverged from ribonuclease, for example, and while the two paralogs remain similar in tertiary structure, their functions within the cell are now quite different.

Key Points

- A homologous gene (or homolog) is a gene inherited in two species from a common ancestor. While homologous genes can be similar in sequence, similar sequences are not necessarily homologous.
- Orthologous are homologous genes where a gene diverges after a speciation event, but the gene and its main function are conserved.
- If a gene is duplicated in a species, the resulting duplicated genes are paralogs of each other, even though over time they might become different in sequence composition and function.

Key Terms

- **conserved**: In biology, conserved sequences are similar or identical sequences that occur within nucleic acid sequences (such as RNA and DNA sequences), protein sequences, protein structures.
- **selective pressure**: Any cause that reduces reproductive success in a proportion of a population, potentially exerts evolutionary pressure or selection pressure.

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6.3D: Synthesizing DNA

Learning Objectives

- Outline the methods and uses of DNA synthesis

To understand bacterial genetics, the underlying genetic material (i.e. DNA) must be understood. DNA must be synthesized to study genes, the sequence of genomes, and many other studies. This occurs in two fashions, by polymerase chain reaction (PCR) which is enzymatic and chemical synthesis. PCR is covered in another atom. Here we will focus on chemical synthesis of DNA, which is also known as oligonucleotide synthesis.

Oligonucleotide synthesis is the chemical synthesis of relatively short fragments of nucleic acids, both DNA and RNA with a defined chemical structure (sequence). The technique is extremely useful in current laboratory practice because it provides a rapid and inexpensive access to custom-made oligonucleotides of the desired sequence. Whereas enzymes synthesize DNA and RNA in a 5′ to 3′ direction, chemical oligonucleotide synthesis is carried out in the opposite, 3′ to 5′ direction.

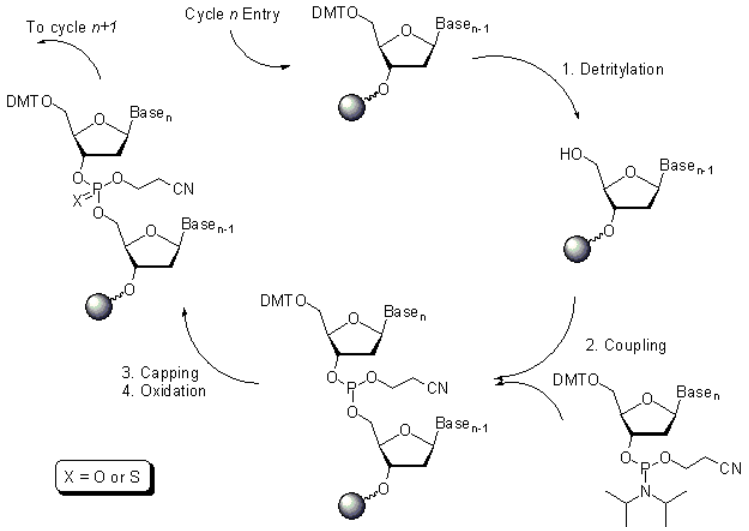


Figure: **Oligosynthesis.**: The complex chemical reactions that are needed to couple one nucleotide to another are outlined here.

Currently, the process is implemented as solid -phase synthesis using phosphoramidite method and phosphoramidite building blocks derived from protected 2′-deoxynucleosides (dA, dC, dG, and T), ribonucleosides (A, C, G, and U), or chemically modified nucleosides, e.g. LNA. To obtain the desired oligonucleotide, the building blocks are sequentially coupled to the growing oligonucleotide chain in the order required by the sequence of the product. The process has been fully automated since the late 1970's. Upon the completion of the chain assembly, the product is released from the solid phase to solution, deprotected, and collected. The occurrence of side reactions sets practical limits for the length of synthetic oligonucleotides (up to about 200 nucleotide residues) because the number of errors accumulates with the length of the oligonucleotide being synthesized. Products are often isolated by HPLC to obtain the desired oligonucleotides in high purity. Typically, synthetic oligonucleotides are single-stranded DNA or RNA molecules around 15–25 bases in length. Oligonucleotides find a variety of applications in molecular biology and medicine. They are most commonly used as antisense oligonucleotides, small interfering RNA, primers for DNA sequencing and amplification, probes for detecting complementary DNA or RNA via molecular hybridization, tools for the targeted introduction of mutations and restriction sites, and for the synthesis of artificial genes.

A further application of oligosynthesis is to make artificial genes. Artificial gene synthesis is the process of synthesizing a gene in vitro without the need for initial template DNA samples. The main method is currently by oligonucleotide synthesis (also used for other applications) from digital genetic sequences and subsequent annealing of the resultant fragments. In contrast, natural DNA replication requires existing DNA templates for synthesizing new DNA.

Key Points

- DNA and RNA are at their essence chemical structures, and as such complex chemical reactions can be used to synthesize them.
- There are enzymatic ways to amplify DNA, notably PCR, while DNA sequences can be chemically synthesized by a process known as oligosynthesis.
- Oligosynthesis can be used to make artificial genes, which allows scientists to design and synthesis novel gene products, without relying a template of a gene found in nature.

Key Terms

- **phosphoramidite**: Any of a class of organic compounds formally derived from a phosphite by replacing a >P-O-R with a >P-N<R2 group; used in the synthesis of nucleic acids, etc.
- **HPLC**: High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying and purifying the individual components of the mixture.

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6.3E: Amplifying DNA - The Polymerase Chain Reaction

Learning Objectives

- Illustrate the applications, components and steps of PCR

The polymerase chain reaction (PCR) is a biochemical technology in molecular biology used to amplify a single, or a few copies, of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Applications

Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications including the following:

- DNA cloning for sequencing; DNA-based phylogeny, or functional analysis of genes
- The diagnosis of hereditary diseases
- The identification of genetic fingerprints (used in forensic sciences and paternity testing)
- The detection and diagnosis of infectious diseases

The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region, along with a DNA polymerase (after which the method is named) are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.

Components

PCR is used to amplify a specific region of a DNA strand (the DNA target). Most PCR methods typically amplify DNA fragments of up to ~10 kilo base pairs (kb), although some techniques allow for amplification of fragments up to 40 kb in size. The reaction produces a limited amount of final amplified product that is governed by the available reagents in the reaction, and the feedback-inhibition of the reaction products. A basic PCR set up requires the following components and reagents:

- DNA template that contains the DNA region (target) to be amplified
- Two primers that are complementary to the 3′ (three prime) ends of each of the sense and anti-sense strand of the DNA target
- Taq polymerase or another DNA polymerase with a temperature optimum at around 70 °C
- Deoxynucleoside triphosphates (dNTPs; nucleotides containing triphosphate groups), the building-blocks from which the DNA polymerase synthesizes a new DNA strand
- Buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.Divalent cations, magnesium or manganese ions; generally Mg^{2+}
- Monovalent cation potassium ions

Typically, PCR consists of a series of 20-40 repeated temperature changes, called cycles, with each cycle commonly consisting of two to three discrete temperature steps, usually three. The temperatures used, and the length of time they are applied in each cycle, depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers.

Steps

The following are the steps of PCR:

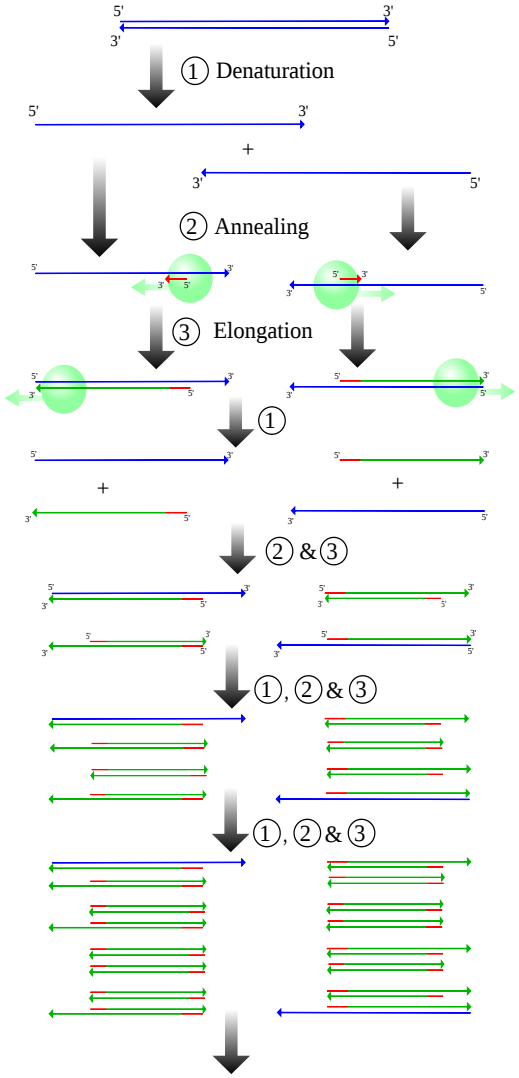


Figure: **The Steps of PCR**: This illustrates a PCR reaction to demonstrate how amplification leads to the exponential growth of a short product flanked by the primers. 1. Denaturing at 96°C. 2. Annealing at 68°C. 3. Elongation at 72°C. The first cycle is complete. The two resulting DNA strands make up the template DNA for the next cycle, thus doubling the amount of DNA duplicated for each new cycle.

1. Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 94-98°C. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.
2. Annealing step: The reaction temperature is lowered to 50-65°C for 20-40 seconds allowing annealing of the primers to the single-stranded DNA template.
3. Extension/elongation step: The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75-80°C, and commonly a temperature of 72°C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5′ to 3′ direction, condensing the 5′-phosphate group of the dNTPs with the 3′-hydroxyl group at the end of the nascent (extending) DNA strand.

After elongation, the cycle goes back to step one, usually for 20-40 cycles. Under optimum conditions (i.e., if there are no limitations due to limiting substrates or reagents) at each extension step, the amount of DNA target is doubled, leading to exponential (geometric) amplification of the specific DNA fragment.

Key Points

- PCR is used to amplify a specific region of DNA.
- PCR typically consists of three steps: denaturation, annealing, and elongation.
- The amplified DNA can be used for many purposes, such as identifying different genes and species of bacteria.

Key Terms

- **annealing**: Annealing, in genetics, means for complementary sequences of single-stranded DNA or RNA to pair by hydrogen bonds to form a double-stranded polynucleotide. The term is often used to describe the binding of a DNA probe, or the binding of a primer to a DNA strand during a polymerase chain reaction (PCR). The term is also often used to describe the reformation (renaturation) of complementary strands that were separated by heat (thermally denatured). Proteins such as RAD52 can help DNA anneal.

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6.3F: DNA Sequencing Based on Sanger Dideoxynucleotides

Learning Objectives

- Recall dideoxynucleotide sequencing

Sanger sequencing, also known as chain-termination sequencing, refers to a method of DNA sequencing developed by Frederick Sanger in 1977. This method is based on amplification of the DNA fragment to be sequenced by DNA polymerase and incorporation of modified nucleotides – specifically, dideoxynucleotides (ddNTPs).

The classical chain-termination method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleotidetriphosphates (dNTPs), and modified nucleotides (dideoxyNTPs) that terminate DNA strand elongation. These chain-terminating nucleotides lack a 3′-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a ddNTP is incorporated. The ddNTPs may be radioactively or fluorescently labelled for detection in automated sequencing machines.The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP). Following rounds of template DNA extension from the bound primer, the resulting DNA fragments are heat denatured and separated by size using gel electrophoresis. This is frequently performed using a denaturing polyacrylamide-urea gel with each of the four reactions run in one of four individual lanes (lanes A, T, G, C). The DNA bands may then be visualized by autoradiography or UV light and the DNA sequence can be directly read off the X-ray film or gel image.

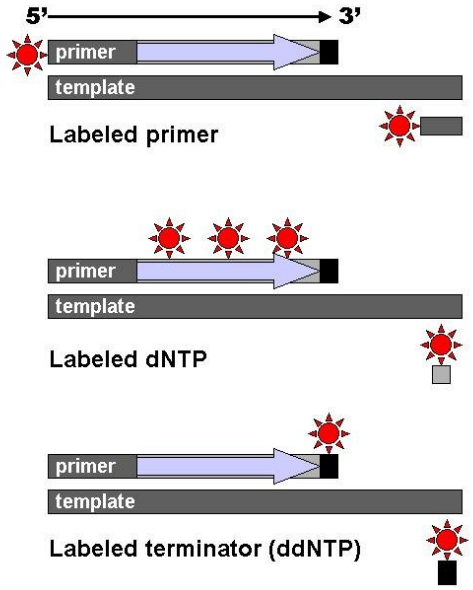


Figure: **Sanger sequencing**: Different types of Sanger sequencing, all of which depend on the sequence being stopped by a terminating dideoxynucleotide (black bars).

Technical variations of chain-termination sequencing include tagging with nucleotides containing radioactive phosphorus for radiolabelling, or using a primer labeled at the 5′ end with a fluorescent dye. Dye-primer sequencing facilitates reading in an optical system for faster and more economical analysis and automation. The later development by Leroy Hood and coworkers of fluorescently labeled ddNTPs and primers set the stage for automated, high-throughput DNA sequencing. Chain-termination methods have greatly simplified DNA sequencing. More recently, dye-terminator sequencing has been developed. Dye-terminator sequencing utilizes labelling of the chain terminator ddNTPs, which permits sequencing in a single reaction, rather than four

reactions as in the labelled-primer method. In dye-terminator sequencing, each of the four dideoxynucleotide chain terminators is labelled with fluorescent dyes, each of which emit light at different wavelengths.

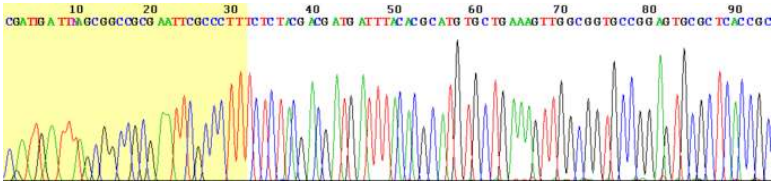


Figure: **Chromatograph**: This is an example of the output of a Sanger sequencing read using fluorescently labelled dye-terminators. The four DNA bases are represented by different colours which are interpreted by the software to give the DNA sequence above.

Automated DNA-sequencing instruments (DNA sequencers) can sequence up to 384 DNA samples in a single batch (run) in up to 24 runs a day. DNA sequencers carry out capillary electrophoresis for size separation, detection and recording of dye fluorescence, and data output as fluorescent peak trace chromatograms. Automation has lead to the sequencing of entire genomes.

Key Points

- The lack of the second deoxy group on an dNTP making it ddNTP, stops the incorporation of further nucleotides, this termination creates DNA lengths stopped at every nucleotide, this is central to further identifying each nucleotide.
- Different labels can be used, ddNTPS, dNTPs and primers can all be labelled with radioactivity and fluorescently.
- Using fluorescent labels, dideoxy sequencing can be automated allowing high-throughput methods which have been utilized to sequence entire genomes.

Key Terms

- **chromatogram**: The visual output from a chromatograph. Usually a graphical display or histogram.
- **dideoxynucleotide**: Any nucleotide formed from a deoxynucleotide by loss of an a second hydroxy group from the deoxyribose group

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6.3G: Metagenomics

Metagenomics is the study of genetic material derived from environmental samples.

LEARNING OBJECTIVES

Summarize the utility of metagenomics

Key Takeaways

Metagenomics

Metagenomics is the study of metagenomes; genetic material recovered directly from environmental samples. The broad field may also be referred to as environmental genomics, ecogenomics or community genomics. While traditional microbiology and microbial genome sequencing and genomics rely upon cultivated clonal cultures, early environmental gene sequencing cloned specific genes (often the 16S rRNA gene) to produce a profile of diversity in a natural sample. Such work revealed that the vast majority of microbial biodiversity had been missed by cultivation-based methods. Recent studies use “shotgun” Sanger sequencing or massively parallel pyrosequencing to get largely unbiased samples of all genes from all the members of the sampled communities. Due to its ability to reveal the previously hidden diversity of microscopic life, metagenomics offers a powerful lens for viewing the microbial world that has the potential to revolutionize understanding of the entire living world.

Conventional Sequencing Studies

Conventional sequencing begins with a culture of identical cells as a source of DNA. However, early metagenomic studies revealed that there are probably large groups of microorganisms in many environments that cannot be cultured and thus cannot be sequenced. These early studies focused on 16S ribosomal RNA sequences which are relatively short, often conserved within a species, and generally different between species. Many 16S rRNA sequences have been found which do not belong to any known cultured species, indicating that there are numerous non-isolated organisms. These surveys of ribosomal RNA (rRNA) genes taken directly from the environment revealed that cultivation based methods find less than 1% of the bacterial and archaeal species in a sample.

Shotgun Metagenomics

Advances in bioinformatics, refinements of DNA amplification, and the proliferation of computational power have greatly aided the analysis of DNA sequences recovered from environmental samples, This allows the adaptation of shotgun sequencing to metagenomic samples. The approach, used to sequence many cultured microorganisms and the human genome, randomly shears DNA, sequences many short sequences, and reconstructs them into a consensus sequence. Shotgun sequencing and screens of clone libraries reveal genes present in environmental samples. This provides information both on which organisms are present and what metabolic processes are possible in the community. This can be helpful in understanding the ecology of a community, particularly if multiple samples are compared to each other.

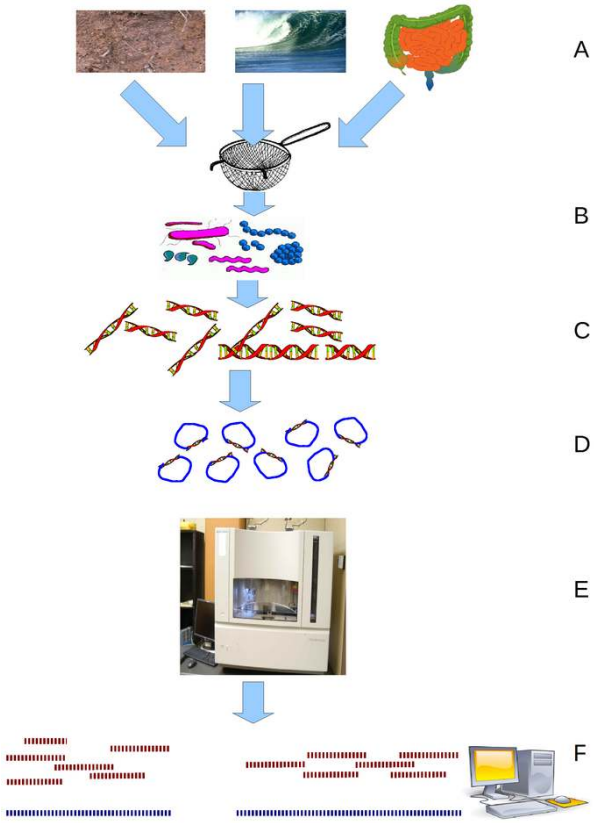


Figure: **Environmental Shotgun Sequencing (ESS)**: (A) sampling from habitat; (B) filtering particles, typically by size; (C) Lysis and DNA extraction; (D) cloning and library construction; (E) sequencing the clones; (F) sequence assembly into contigs and scaffolds

Shotgun metagenomics is also capable of sequencing nearly complete microbial genomes directly from the environment. As the collection of DNA from an environment is largely uncontrolled, the most abundant organisms in an environmental sample are most highly represented in the resulting sequence data. To achieve the high coverage needed to fully resolve the genomes of under-represented community members, large samples are needed. On the other hand, the random nature of shotgun sequencing ensures that many of these organisms, which would otherwise go unnoticed using traditional culturing techniques, will be represented by at least some small sequence segments.

High-Throughput Sequencing

The first metagenomic studies conducted using high-throughput sequencing used massively parallel 454 pyrosequencing. Two other technologies commonly applied to environmental sampling are the Illumina Genome Analyzer II and the Applied Biosystems SOLiD system. These techniques for sequencing DNA generate shorter fragments than Sanger sequencing; 454 pyrosequencing typically produces ~400 bp reads, Illumina and SOLiD produce 25-75 bp reads. These read lengths are significantly shorter than the typical Sanger sequencing read length of ~750 bp.

However, this limitation is compensated for by the much larger number of sequence reads. Pyrosequenced metagenomes generate 200–500 megabases, while Illumina platforms generate around 20–50 gigabases. An additional advantage to short read sequencing is that this technique does not require cloning the DNA before sequencing, removing one of the main biases in environmental sampling. As most short-read assembly software was not designed for metagenomic applications, specialized methods have been developed to utilize mate-read data in metagenomic assembly. From these studies the microbial fauna that might reside in a sample of soil, even on the surface of a keyboard, can be more accurately and efficiently identified.

Key Points

- While previous work needed cultivation of single microbes before they could be sequenced and identified, metagenomics attempts to more completely identify many of the microbes that inhabit a given environmental location.

- The first attempts at metagenomics was to sequence one gene from a sample. The changes in that one gene helped determine the microbial diversity in a sample.
- High-throughput sequencing allows the complete sequencing and assembly of entire genomes of the microbes that inhabit a given environment, giving unprecedented depth into understanding the microbial diversity of the world around us.

Key Terms

- **solid**: SOLiD (Sequencing by Oligonucleotide Ligation and Detection) is a next-generation DNA sequencing technology developed by Life Technologies and has been commercially available since 2008. This next generation technology generates hundreds of millions to billions of small sequence reads at one time.
- **gigabase**: One billion bases (nucleotides) as a unit of length of a nucleic acid
- **pyrosequencing**: A technique used to sequence DNA using chemiluminescent enzymatic reactions

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6.3H: Reporter Fusions

A reporter fusion is the hybrid of a gene or portion of a gene with a tractable marker.

LEARNING OBJECTIVES

Explain reporter fusions

Key Takeaways

In molecular biology, a reporter gene (often simply reporter) is a gene that researchers attach to a regulatory sequence of another gene of interest in bacteria, cell culture, animals, or plants. Certain genes are chosen as reporters because the characteristics they confer on organisms expressing them are easily identified and measured, or because they are selectable markers. Reporter genes are often used as an indication of whether a certain gene has been taken up by or expressed in the cell or organism population.

To introduce a reporter gene into an organism, scientists place the reporter gene and the gene of interest in the same DNA construct to be inserted into the cell or organism. For bacteria or prokaryotic cells in culture, this is usually in the form of a circular DNA molecule called a plasmid. It is important to use a reporter gene that is not natively expressed in the cell or organism under study, since the expression of the reporter is being used as a marker for successful uptake of the gene of interest. Commonly used reporter genes that induce visually identifiable characteristics usually involve fluorescent and luminescent proteins. Examples include the gene that encodes jellyfish green fluorescent protein (GFP), which causes cells that express it to glow green under blue light, the enzyme luciferase, which catalyzes a reaction with luciferin to produce light, and the red fluorescent protein from the gene dsRed. A common reporter in bacteria is the E. coli lacZ gene, which encodes the protein beta-galactosidase. This enzyme causes bacteria expressing the gene to appear blue when grown on a medium that contains the substrate analog X-gal. An example of a selectable-marker which is also a reporter in bacteria is the chloramphenicol acetyltransferase (CAT) gene, which confers resistance to the antibiotic chloramphenicol.

Reporter genes can also be used to assay for the expression of the gene of interest, which may produce a protein that has little obvious or immediate effect on the cell culture or organism. In these cases the reporter is directly attached to the gene of interest to create a gene fusion. The two genes are under the same promoter elements and are transcribed into a single messenger RNA molecule. The mRNA is then translated into protein. In these cases it is important that both proteins be able to properly fold into their active conformations and interact with their substrates despite being fused. In building the DNA construct, a segment of DNA coding for a flexible polypeptide linker region is usually included so that the reporter and the gene product will only minimally interfere with one another. This is often done with GFP. The resulting protein-GFP hybrid transcribed from the reporter construct now has a protein attached to GFP. In the case of GFP which fluorescence one can deduce that the attached protein is wherever the fluorescence is. This allows a researched to determine where in a cell a protein may be localized in a cell.

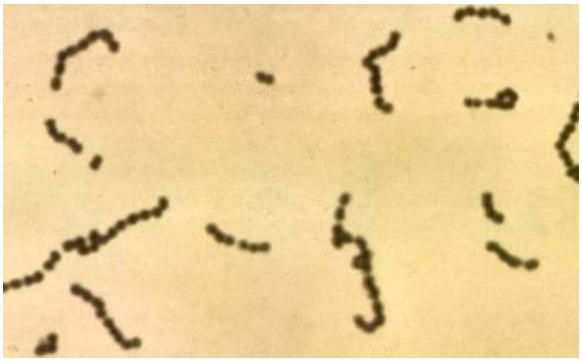


Figure: **Streptococci**: Light microscopy view of streptococci, a non-sporulating lactic acid bacteria.

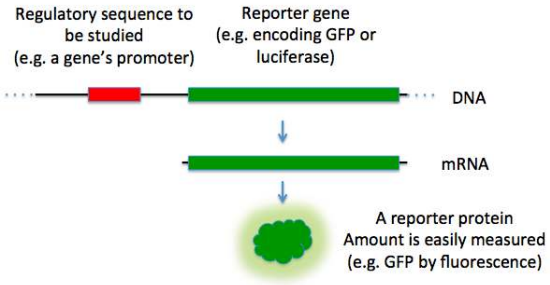


Figure: **Introducing a reporter gene into a cell**: In molecular biology, a reporter gene (often simply reporter) is a gene that researchers attach to a regulatory sequence of another gene of interest in bacteria, cell culture, animals, or plants

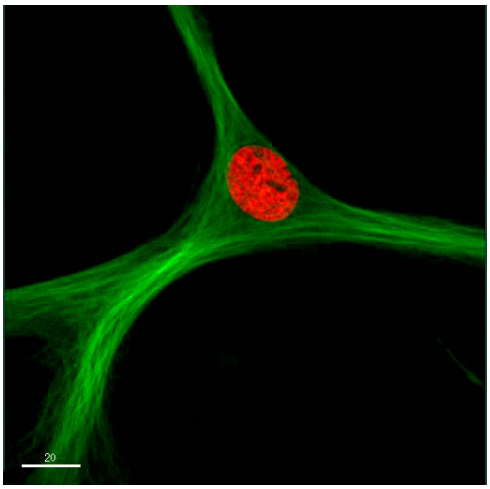


Figure: **GFP fusion proteins**: A human mesenchymal stem cell. In this cell a microtubule protein is fused to GFP (green) while a histone protein is fused to RFP (red). As you can see the localization of the fused protein can now be determined using fluorescent reporter fusions.

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Key Points

- A reporter construct allows the study of gene ‘s function and localization of a gene product.
- The promoter reporter constructs allow a protein to be expressed under the control of a target gene.
- Reporter fusions can fuse a protein of interest to a protein with a property of interest, therefore allowing the tagged protein to be further studied.

Key Terms

- **substrate analog**: Substrate analogs (substrate state analogues), are chemical compounds with a chemical structure that resemble the substrate molecule in an enzyme-catalyzed chemical reaction.
- **luminescent**: Emitting light by luminescence.

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SECTION OVERVIEW

6.4: Environmental Genomics

Topic hierarchy

6.4A: Environmental Metagenomics

6.4B: Detecting Uncultured Microorganisms

6.4C: Viral Genomes in Nature

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6.4A: Environmental Metagenomics

Environmental Metagenomics

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Introduction

Metagenomics can be defined as the techniques and procedures that are used for the culture-independent analysis of the total genomic content of microorganisms living in a certain environment [2]. It has many useful applications with very promising potential in both medical and environmental microbiology. The most common use of metagenomics in environmental microbiology is studying the diversity of microbial communities in particular environments through the analysis of rRNA genes and how these communities change in response to changes in physical and chemical properties of these environments [3].

Metagenomics also provides an opportunity to obtain and identify novel enzymes with industrial applications from extreme environments where unculturable extremophiles live. In such circumstances, functional metagenomics enables the isolation of genes coding for extremozymes, enzymes that are capable of being catalytically active in extreme conditions, or genes that will allow for better understanding of the mechanisms that make such organisms resistant to extreme environmental conditions [4].

Metagenomics has special importance when it comes to studying soil microbiology. It is estimated that the number of distinct microorganisms in 1 gram of soil exceeds the number of microbial species cultured so far [5]. Therefore, metagenomics seems to be the ideal culture-independent technique for unraveling the biodiversity of soil microorganisms and to study how this biodiversity is affected with continuously changing conditions.

Sequencing technologies and metagenomics

Recently, taxonomic profiling, characterization, and analysis of microbial communities are being mostly performed using different next-generation sequencing (NGS) platforms. Metagenomic samples are high-throughput, short-read sequences, and the cost is relatively decreasing. In addition, these platforms are advantageous, avoiding the need for cloning of DNA fragments [6].

Recent advances in NGS technologies were developed to suit various numbers of applications, cost, and capabilities [7]. The most commonly used platforms are the 454 Life Sciences (Roche) and Illumina systems (Solexa) [8]. The 454 sequencing technology, which was the first commercially available next-generation technology, is based on the pyrosequencing technique. It provides high throughput and relatively cheap analysis [9]. During the sequencing reaction in this technique, nucleotide incorporation into the growing chain is detected by the capture of the released pyrophosphate, which is converted into a light through an enzymatic reaction. Different nucleotides are sequentially added into each nucleotide incorporation event; therefore the light signal can be attributed to a specific nucleotide. Finally, the light signals are converted into sequence information. In the 454 pyrosequencer, the DNA fragments are amplified after being fixed on beads in a water-oil emulsion [10]. Pyrosequencing has been employed widely in the analysis of microbial diversity in many environments including marine environments [11] and different soil environments [12, 13].

Illumina sequencing technology relies on the use of fluorescently labeled reversible terminator nucleotides. Instead of being chemically modified to prevent further DNA synthesis (dideoxynucleotides) which is the case with Sanger sequencing, the terminator nucleotides are attached with blocking group that can be removed from the nitrogen base in a single step. DNA synthesis takes place on a chip where primers are attached. After each cycle, the dyes attached to each nucleotide are excited by laser followed by scanning of the incorporated bases. In order for the next synthesis cycle to proceed, the blocking group and the dye are first removed by a chemical reaction. Illumina sequencing platform was successfully used to study microbial diversity in many environments [14, 15, 16].

In addition to the abovementioned technologies, recently developed sequencing technologies are available and being employed in metagenomic studies. These include SOLiD 5500 W Series developed by Applied Biosystems, single-molecule real-time (SMRT) DNA sequencing from Pacific Biosciences, and Ion Torrent semiconductor sequencing [8]. More innovative technologies are being developed that could be of great use for metagenomic studies in the near future. Strand sequencing technologies, currently being developed by Oxford Nanopore technologies, enable the sequencing of intact DNA strand that passes through a protein nanopore

[17]. Irys Technology, developed by BioNano Genomics, represents one of the very promising new technologies in genomics era [8].

The metagenomic approaches

Metagenomics research strategy starts with selecting a proper ecological or biological environment of interest that hosts a wide variety of microbial communities which may have potential biotechnological applications. Environments that attract metagenomic researchers are mainly those characterized with extreme conditions or unique environmental conditions. These include environments with highly acidic or alkaline pH; high metal concentrations, pressures, or radiation; and high salinity or extreme temperatures [4].

Metagenomic analysis starts with isolating genomic DNA that represents the whole community in the soil sample, constructing a DNA library from the isolated DNA, and screening the available library for a target gene. It is important here to select the DNA extraction method that will provide enough yield and DNA that represents the diversity of the whole microbial community in the target environment. This is still one of the most challenging steps of metagenomic analysis. The chemical and physical characteristics of soils are very wide and complex, depending on the type of the soil examined, that will make it difficult to develop a reference method for DNA extraction from soils. Besides, soils contain many substances that are co-extracted with genomic DNA and harbor inhibitory effects on the downstream processing of the extracted DNA. Examples include humic and fulvic acids [18]. Therefore, optimization and comparison between different extraction methods are usually required for each type of soil [19, 20, 21, 22].

A DNA library is then constructed from the genomic DNA isolated from the target environment. This is performed by fragmenting the isolated DNA into fragments with appropriate sizes that would allow for their cloning. This is performed by either using restriction enzyme digestion or mechanical shearing. DNA fragments obtained from such processes are cloned into the proper cloning vector. Plasmid vectors are used for small DNA fragments, and the libraries generated are called small-insert genomic libraries. Large inserts are cloning into cosmid or fosmid vectors which can hold inserts up to 40 kb in size or BAC vector which can carry inserts with sizes that exceed 40 Kb [23].

DNA libraries are usually constructed in a microorganism that is well-studied and is easy to manipulate inside the laboratory such as *Escherichia coli*. In case there is a need for expressing the genes within the DNA inserts in other microorganisms, shuttle vectors are used to transfer the libraries into a proper host [24].

Finally, a screening assay is applied to search for a gene of a particular function, and the gene product is functionally analyzed. There are two different metagenomic strategies that are commonly used in research. The first one is focused on the use of marker genes such as the ribosomal genes 16S rRNA [25] and 18S rRNA [26] to study the composition of the microbial community in a certain environment or specific protein-coding gene with medical or industrial importance [27, 28, 29]. Such a strategy is called targeted metagenomics. The second approach is the shotgun metagenomics, in which a wide coverage of genomic DNA sequences is achieved using high-throughput next-generation sequencing to assess the entire taxonomic structure or functional potential of microbial communities [30].

The most challenging aspect of the screening process in metagenomics is the analysis of a huge amount of sequence data that are generated from the constructed library. A wide range of bioinformatic tools has been developed over the years to help analyze the metagenomic data and compare it to available online databases.

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6.4B: Detecting Uncultured Microorganisms

Using metagenomics, the microbial constituents of the world can be identified by culturing each individual species.

LEARNING OBJECTIVES

Recognize the methods used to detect uncultured microorganisms

Key Takeaways

Identification of bacteria in the laboratory is particularly relevant in medicine, where the correct treatment is determined by the bacterial species causing an infection. Consequently, the need to identify human pathogens was a major impetus for the development of techniques to identify bacteria.

Early studies have shown that the microbial life around us in the air, sea, and soil is very diverse and only a small fraction of the species are known. One limitation of identifying human pathogens or conventional sequencing begins with a culture of identical cells as a source of DNA. However, early metagenomic studies revealed that there are probably large groups of microorganisms in many environments that cannot be cultured and thus cannot be sequenced. These early studies focused on 16S ribosomal RNA sequences which are relatively short, often conserved within a species, and generally different between species. Many 16S rRNA sequences have been found which do not belong to any known cultured species, indicating that there are numerous non-isolated organisms out there. These surveys of ribosomal RNA (rRNA) genes taken directly from the environment revealed that cultivation based methods find less than 1% of the bacterial and archaeal species in a sample.

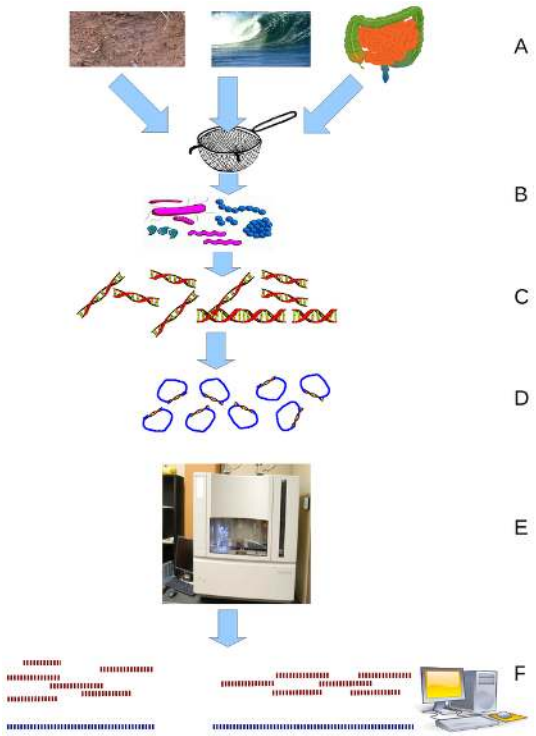


Figure: **Environmental Shotgun Sequencing (ESS)**: (A) sampling from habitat; (B) filtering particles, typically by size; (C) Lysis and DNA extraction; (D) cloning and library construction; (E) sequencing the clones; (F) sequence assembly into contigs and scaffolds

The discovery of such diversity led to the field of metagenomics, which is the study of metagenomes, genetic material recovered directly from environmental samples. Rather than culturing a microbe, this approach takes a sample and identifies the different species in it by sequencing all the species simultaneously. However, recovery of DNA sequences longer than a few thousand base pairs from environmental samples was very difficult until recent advances in molecular biological techniques. More specifically, the construction of libraries in bacterial artificial chromosomes (BACs) provided better vectors for molecular cloning.

Advances in bioinformatics, refinements of DNA amplification, and the proliferation of computational power have greatly aided the analysis of DNA sequences recovered from environmental samples. These advances have allowed the adaptation of shotgun sequencing to metagenomic samples. The approach, used to sequence many cultured microorganisms and the human genome, randomly shears DNA, sequences many short sequences, and reconstructs them into a consensus sequence.

Shotgun sequencing and screens of clone libraries reveal genes present in environmental samples. This can be helpful in understanding the ecology of a community, particularly if multiple samples are compared to each other. This was further followed by high-throughput sequencing which did the same process as the shotgun sequencing but at a much bigger scale in terms of the amount of DNA that could sequenced from one sample. This provides information both on which organisms are present and what metabolic processes are possible in the community. Using metagenomics, and the resultant sequencing of uncultured microbes, metagenomics has the potential to advance knowledge in a wide variety of fields. It can also be applied to solve practical challenges in medicine, engineering, agriculture, and sustainability.

Key Points

- Many organisms that can cause diseases are not culturable, so a unicellular culture cannot be obtained.
- Initial studies of microbial fauna showed that they are more diverse than expected and contained many microbes that were not culturable.
- Advances in molecular biological techniques allow the sequencing of all or at least many of the genomes of microbes found in a sample.

Key Terms

- **shotgun sequencing**: A DNA sequencing technique in which a large number of small fragments of a long DNA strand are generated at random, sequenced, and reassembled to form a sequence of the original strand.
- **culturable**: Able to be cultured (grown in a suitable environment).
- **high-throughput sequencing**: Technologies that parallelize the sequencing process, producing thousands or millions of sequences at once.

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6.4C: Viral Genomes in Nature

Viruses are the most abundant biological entity on earth, they outnumber all other lifeforms on earth combined.

LEARNING OBJECTIVES

Recognize viral genomic diversity in nature

Key Takeaways

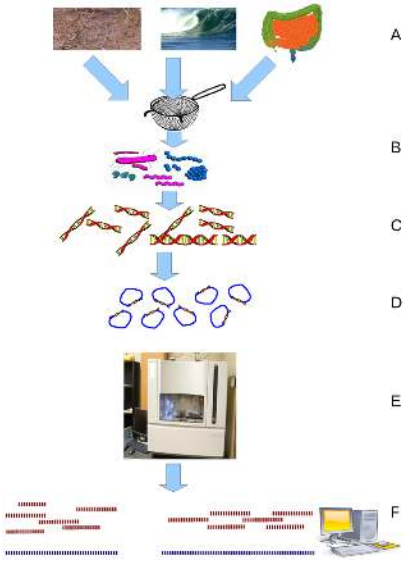


Figure: **Environmental Shotgun Sequencing (ESS)**: (A) sampling from habitat; (B) filtering particles, typically by size; (C) Lysis and DNA extraction; (D) cloning and library construction; (E) sequencing the clones; (F) sequence assembly into contigs and scaffolds

Viruses are by far the most abundant biological entities on earth and they outnumber all the others put together. They infect all types of cellular life including animals, plants, bacteria, and fungi. However, different types of viruses can infect only a limited range of hosts and many are species-specific. Some, such as smallpox virus for example, can infect only one species – in this case humans, and are said to have a narrow host range. Other viruses, such as rabies virus, can infect different species of mammals and are said to have a broad range. The viruses that infect plants are harmless to animals, and most viruses that infect other animals are harmless to humans. The host range of some bacteriophages is limited to a single strain of bacteria and they can be used to trace the source of outbreaks of infections by a method called phage typing.

Metagenomic sequencing is particularly useful in the study of viral communities. As viruses lack a shared universal phylogenetic marker (as 16S RNA for bacteria and archaea, and 18S RNA for eukarya), the only way to access the genetic diversity of the viral community from an environmental sample is through metagenomics. Viral metagenomes (also called viromes) should therefore provide more and more information about viral diversity and evolution. In 2002, Mya Breitbart, Forest Rohwer, and colleagues used environmental shotgun sequencing to show that 200 liters of seawater contains over 5,000 different viruses. Subsequent studies showed that there are more than a thousand viral species in human stool and possibly a million different viruses per kilogram of marine sediment, including many bacteriophages. Essentially all of the viruses in these studies were new species.

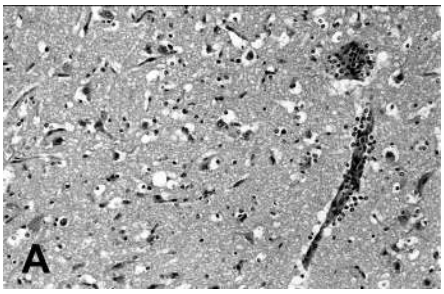


Figure: **Phocine distermper encephalitis**: This is a section from a harbor seal cerebral cortex. The white areas are tissue lesions caused a phocine distermper viral infection.

To understand the complex diversity of viruses a further look at viruses in aquatic environments shows, a teaspoon of seawater contains about one million viruses. They 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 three 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 phocine distemper virus. Many other viruses, including caliciviruses, herpesviruses, adenoviruses, and parvoviruses circulate in marine mammal populations.

Viruses are an important natural means of transferring genes between different species, which increases genetic diversity and drives evolution. It is thought that viruses played a central role in the early evolution, before the diversification of bacteria, archaea, and eukaryotes and at the time of the last universal common ancestor of life on earth. Viruses are still one of the largest reservoirs of unexplored genetic diversity on earth.

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Key Points

- Viruses infect all types of cellular life including animals, plants, bacteria, and fungi.
- Metagenomic sequencing has identified that there are an enormous number of species of virus in nature, showing that a a teaspoon of water has a million species of viruses.
- Viruses kill about 20% of the microbes that constitute the oceans microbial fauna, each day. This viral-driven biological turn over is needed for for life on earth to continue.
- Viruses can transfer genetic material from one species to another, and as such viruses may be a main driving force of evolution.

Key Terms

- **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.
- **stool**: Feces; excrement.

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CHAPTER OVERVIEW

7: Environmental application

7.1: Genomics and Biofuels

7.2: Microbial Bioremediation

7.2A: Microbial Ore Leaching

7.2B: Petroleum Biodegradation

7.2C: The Degradation of Synthetic Chemicals in Soils and Water

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7.1: Genomics and Biofuels

Learning Objectives

- Explain the process of creating new biofuels by using microbial genomics

Knowledge of the genomics of microorganisms is being used to find better ways to harness biofuels from algae and cyanobacteria. The primary sources of fuel today are coal, oil, wood, and other plant products, such as ethanol. Although plants are renewable resources, there is still a need to find more alternative renewable sources of energy to meet our population ‘s energy demands. The microbial world is one of the largest resources for genes that encode new enzymes and produce new organic compounds, and it remains largely untapped.

For microbial biomass breakdown, many candidates have already been identified. These include Clostridia species for their ability to degrade cellulose, and fungi that express genes associated with the decomposition of the most recalcitrant features of the plant cell wall, lignin, the phenolic “glue” that imbues the plant with structural integrity and pest resistance. The white rot fungus *Phanerochaete chrysosporium* produces unique extracellular oxidative enzymes that effectively degrade lignin by gaining access through the protective matrix surrounding the cellulose microfibrils of plant cell walls.

Another fungus, the yeast *Pichia stipitis*, ferments the five-carbon “wood sugar” xylose abundant in hardwoods and agricultural harvest residue. *Pichia*‘s recently-sequenced genome has revealed insights into the metabolic pathways responsible for this process, guiding efforts to optimize this capability in commercial production strains. Pathway engineering promises to produce a wider variety of organisms able to ferment the full repertoire of sugars derived from cellulose and hemicellulose and tolerate higher ethanol concentrations to optimize fuel yields. For instance, the hindgut contents of nature’s own bioreactor, the termite, has yielded more than 500 genes related to the enzymatic deconstruction of cellulose and hemicellulose.



Figure: ***Termites: Nature’s Bioreactors****: The hindgut of the termite has yielded more than 500 genes of microbes related to the enzymatic deconstruction of cellulose.*

Key Points

- Microorganisms can encode new enzymes and produce new organic compounds that can be used as biofuels.
- Genomic analysis of the fungus *Pichia* will allow optimization of its use in fermenting ethanol fuels.
- Analysis of the microbes in the hindgut of termites have found 500 genes that may be useful in enzymatic destruction of cellulose.
- Genetic markers have been used in forensic analysis, like in 2001 when the FBI used microbial genomics to determine a specific strain of anthrax that was found in several pieces of mail.
- Genomics is used in agriculture to develop plants with more desirable traits, such as drought and disease resistance.

Key Terms

- **renewable resource**: a natural resource such that it is replenished by natural processes at a rate comparable to its rate of consumption by humans or other users
- **biofuel**: any fuel that is obtained from a renewable biological resource

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SECTION OVERVIEW

7.2: Microbial Bioremediation

Topic hierarchy

7.2A: Microbial Ore Leaching

7.2B: Petroleum Biodegradation

7.2C: The Degradation of Synthetic Chemicals in Soils and Water

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7.2A: Microbial Ore Leaching

Learning Objectives

- Assess the advantages of microbial ore leaching

Microbial ore leaching (bioleaching) is the process of extracting metals from ores with the use of microorganisms. This method is used to recover many different precious metals like copper, lead, zinc, gold, silver, and nickel. Microorganisms are used because they can:

- lower the production costs.
- cause less environmental pollution in comparison to the traditional leaching methods.
- very efficiently extract metals when their concentration in the ore is low.

The Leaching Process

Bacteria perform the key reaction of regenerating the major ore oxidizer which in most cases is ferric iron as well as further ore oxidation. The reaction is performed at the bacterial cell membrane. In the process, free electrons are generated and used for the reduction of oxygen to water which produces energy in the bacterial cell.

Ores, like pyrite (FeS_2), are first oxidized by ferric iron (Fe^{3+}) to thiosulfate ($S_2O_3^{2-}$) in the absence of bacteria.

In the first step, disulfide is spontaneously oxidized to thiosulfate by ferric iron (Fe^{3+}), which in turn is reduced to give ferrous iron (Fe^{2+}):

(1) FeS2+6Fe3++3H2O⟶7Fe2++S2O2−3+6H+spontaneousFeS2+6Fe3++3H2O⟶7Fe2++S2O32−+6H+spontaneous

Bacteria are added in the second step and recover Fe^{3+} from ferrous iron (Fe^{2+}) which is then reused in the first step of leaching:

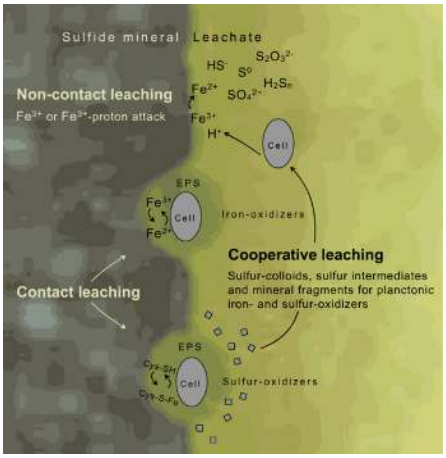


Figure: **Sulfide mineral bacterial leaching**: Bacterial cells oxidizing the ferrous iron back to ferric iron while using slightly different contact mechanisms with the metal.

(2) 4Fe2++O2+4H+⟶4Fe3++2H2O(iron oxidizers)4Fe2++O2+4H+⟶4Fe3++2H2O(iron oxidizers)

Thiosulfate is also oxidized by bacteria to give sulfate:

(3) S2O2−3+2O2+H2O⟶2SO2−4+2H+(sulfur oxidizers)S2O32−+2O2+H2O⟶2SO42−+2H+(sulfur oxidizers)

The ferric iron produced in reaction (2) oxidized more sulfide as in reaction (1), closing the cycle and given the net reaction:

(4) 2FeS2+7O2+2H2O⟶2Fe2++4SO2−4+4H+2FeS2+7O2+2H2O⟶2Fe2++4SO42−+4H+

The net products of the reaction are soluble ferrous sulfate and sulfuric acid.

The microbial oxidation process occurs at the cell membrane of the bacteria. The electrons pass into the cells and are used in biochemical processes to produce energy for the bacteria while reducing oxygen to water. The critical reaction is the oxidation of sulfide by ferric iron. The main role of the bacterial step is the regeneration of this reactant.

Copper leaching has a very similar mechanism.

Microorganisms Capable of Ore Leaching

Bioleaching reactions industrially are performed by many bacterial species that can oxidize ferrous iron and sulfur. An example of such species is *Acidithiobacillus ferroxidans*. Some fungi species (*Aspergillus niger* and *Penicillium simplicissimum*) have also been shown to have the ability to dissolute heavy metals. When fungi are used, the leaching mechanism is different. The fungi use the acids that they produce in their metabolic reactions to dissolve the metal.

In general, bioleaching is cleaner and safer for the environment than chemical processing. However environmental pollution with toxic products, like sulfuric acid from the pyrite leaching, and heavy metals is still possible. Another drawback of microbial leaching is the slow rate at which microbes work.

Key Points

- Bioleaching is cheaper than chemical extraction, safer for the environment, and more efficient in extracting metals with low concentration in ores.
- It is performed by iron and sulfide oxidizing bacteria or acid producing fungus.
- Bacteria recycle the major leaching reagent, like ferric iron, and perform further oxidation steps while gaining energy from the electron transfer.

Key Terms

- **ore leaching**: The process of recovering metals from ores by using a number of different techniques.

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7.2B: Petroleum Biodegradation

Learning Objectives

- Show how microbes biodegrade petroleum waste

Biodegradation is the process in which living organisms, most often microorganisms, break down material into simpler components. Such material is usually organic matter that could be dissolved into chemical elements by organisms that possess the metabolic pathways to perform the reactions. Some microorganisms produce enzymes that can degrade a variety of chemical compounds, including hydrocarbons like oil.

Petroleum (crude oil) is a liquid fossil fuel. It is a product of decaying organic matter, such as algae and zooplankton. It is one of the major energy sources in the world, and is also used by the chemical industry to manufacture a large number of consumer products. However, oil drilling or transportation can cause accidents that lead to contamination of the environment. Oil spills in marine environments are especially damaging because they cannot be contained and can spread over huge areas. The aromatic compounds in oil are toxic to living organisms and such spills can render havoc in an ecosystem. Natural seepages from unexplored oil sources is another source of contamination.



Figure: **The Deepwater Horizon Oil Spill**: This spill was the largest in the history of the oil industry and spread over huge areas.

In the environment, such spills are naturally cleaned by microorganisms that can break down the oil. The dominant group of such bacteria are the hydrocarbonoclastic bacteria (HCB). The concentration of these bacteria increases significantly in areas of oil spill. One of the best studied representative of this group is Alcanivorax borkumensis; it's also the only one to have its genome sequenced. This species contains individual genes responsible for breaking down certain alkanes into harmless products. It also possesses genes to direct the production of a layer of biosurfactant around the cell to enhance the oil emulsification. The addition of nitrogen and phosphorus to the Alcanivorax environment increases its growth rate. However, the addition of these nutrients in natural environments to improve the cleanup of oil spills is not desirable, since it can have an overall negative impact on the ecosystem.

Aside from hydrocarbons, crude oil contains additional toxic compounds, such as pyridine. These are degraded by representatives of other genera such as Micrococcus and Rhodococcus. Oil tarballs are biodegraded slowly by species from the genera Chromobacterium, Micrococcus, Bacillus, Pseudomonas, Candida, Saccharomyces and others. In the clean up of the Deepwater Horizon oil spill, genetically modified microorganisms were used, but some scientists suspect they might have caused health issues for people in the affected areas.

Key Points

- Biodegradation is the process of breaking down material in simpler components by living organisms, most often microorganisms.
- Oil spills occur due to accidents in the industry as a result of extraction or transportation. Since such spills spread over great areas and have deleterious effects on living organisms. It is important to use environmentally friendly mechanisms for their cleanup.
- There are many microorganisms that can break down petroleum, the most prominent being hydrocarbonoclastic bacteria. A representative of this group is Alcanivorax borkumensis, and its genome contains genes that code for the degradation of alkanes.

Key Terms

- **hydrocarbons**: Organic compounds made only of carbon and hydrogen. Examples include alkanes, alkenes, and aromatic hydrocarbons.
- **emulsification**: The process of forming a mixture of substances that are nonmixable under normal conditions.
- **tarball**: A blob of petroleum oil.

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7.2C: The Degradation of Synthetic Chemicals in Soils and Water

Learning Objectives

- Distinguish bioremediation of soil vs. water

Our planet is contaminated with many chemicals that are toxic to living organisms. These chemicals are products or byproducts from different industries. Examples of such contaminants are polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), chloroethenes, and pharmaceutical substances. These pollutants can cause dangerous contamination of the air, soil, and water. Microorganisms play a major role in eliminating such pollutants from the environment.

Bioremediation of Soil

Soil is a major reservoir of microorganisms with each gram containing about one billion microbes. Microbes are used for bioremediation *in situ* of contaminated soil. Microorganisms that can remove contaminants from the environment are called bioremediators. Contaminations are most often caused by a mixture of pollutants and the best strategy for cleanup is to use a cocktail of different species since each one of them will be optimized for the degradation of a specific toxic compound.

The microbe activity is usually monitored on such sites to ensure optimal conditions for bacterial growth and hence degradation. Decomposition of the toxic substances can be performed both in the presence (aerobically) and absence (anaerobically) of oxygen. The limiting factor for bioremediaton in soils is the bioavailability of the contaminant agents.

Bioremediation of Water

The treatment of sewage water is a critical process to assure the purification of wastewater that will prevent chemical and microbiological pollution of the environment, especially for the drinking water supplies. An important part of this process is the biological step which involves the activity of living organisms to clean the water from organic matter. This happens in the secondary step of sewage purification. Microorganisms substantially lessen the concentration of nutrients which if released in the environment can lead to undesirable overgrowth of microorganisms and algae.



Figure: **Sewage Treatment Plant**: Wastewater treatment plants are critical in lowering the levels of pollutants in the environment.

They also have the potential to clean the water from toxic components. The degradation is performed in the anaerobic, aerobic, and composting steps. Anaerobic digestion by bacteria is allowed to procede for almost two weeks to guarantee enough time to complete the process. During this time, the organic matter undergoes four different enzymatic transformations: *hydrolysis*, *acidogenesis*, *acetogenesis* and *methanogenesis*. The final products are water, carbon dioxide, and methane. In the aerobic step, oxygen is added into the system and the organic matter is converted to carbon dioxide. In the composting step, additional carbon sources are added to aid the final steps of degradation.

In recent years, advances in genomics, proteomics, and bioinformatics studies of environmental microorganisms have revealed a tremendous potential in metabolic pathways.

Such studies showed that *Burkholderia xenovorans* LB400 and *Rhodococcus* sp. strain RHA1, have evolved pathways to degrade aromatic compounds, which are some of the toughest contaminants to eliminate. The bacteria have genes coding for *deoxygenases* to open the aromatic ring structures of these chemicals.

Hydrocarbons and their derivatives were long believed to be degraded only in the presence of oxygen. This would often lead to oxygen depletion in environments heavily polluted with oil. Studies in recent years have proven that there are numerous anaerobic

bacterial species capable of decomposing this group of pollutants. A common feature among these bacteria is that they possess *reductive dehalogenases*.

Bacterial species are assisted in the process of degradation by fungi (e.g., *Aspergillus* sp.), protozoa, and representatives of Archaea. The primary role of fungi is in secreting numerous extracellular enzymes that break down complex molecules into their components and make them readily available to the bacterial community.

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Key Points

- Bacteria decompose organic matter by producing a number of different enzymes for more general reactions such as hydrolysis, acetogenesis and methanogenesis, and highly specific enzymes such as deoxygenases that can break aromatic compounds.
- Bacterial species are assisted in the process of degradation by fungi (e.g., Aspergillus sp.), protozoa, and representatives of Archaea.
- The recent advances in genomics, proteomics, and bioinformatics fields allow new insights into the tremendous metabolic potential of microorganisms.

Key Terms

- **bioremediation**: The use of biological organisms, usually microorganisms, to remove contaminants, especially from polluted water.
- **bioavailability**: The amount of matter that is physicochemically accesible for degradation by microorganisms.

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CHAPTER OVERVIEW

8: Environmental Microbiomes

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8.2: The Ocean Microbiome and Marine Life
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8.1: Environmental Nutrient Cycling and Human Health

Environmental Nutrient Cycling and Human Health

The importance of microorganisms is unquestionable in regard to how nutrients circulate throughout each ecosystem. There are direct and indirect links between Earth's ecosystems and human health, though like other microbial networks, they are sometimes unfathomably complex.

Climate change may be the most obvious association between environmental and human health, however, the solution to balance is not one likely easily achieved. Each type of ecosystem is unique, and reflects different changes to anthropogenic activity. Global soil microbiomes and organic foliar litter have great impact on worldwide biogeochemical cycling, plant health, and bioremediation, where environmental changes can disrupt microbial taxonomic distribution and functional profiles (Albright et al., 2020, Naylor et al., 2020). The ocean microbiome is vast considering it is the largest ecosystem on the planet, and plays a tremendous part in biogeochemical cycling, ecosystem dynamics, and response to climate change(Moran, 2015, Acinas et al., 2019, Marz et al., 2021). Also, since much of the ocean microbiome is uncharted, it could also serve as a major untapped reservoir for novel and progressive biosynthetic products (Paoli et al., 2021). Other aquatic environments such rivers, lakes, wetlands, and freshwater systems and their interaction with sediments and plants greatly influence carbon and other nutrient cycles in their respective ecosystems (Amado and Roland, 2017, Avila et al., 2019, Trevathan-Tackett et al., 2021).



In a cyclic manner, climate change can also greatly affect microbiome dynamics of large ecosystems like glaciers, tundra, permafrost, and even dry deserts, which can further exacerbate disturbances in that region and beyond. (Hamilton et al., 2013, Tripathi et al., 2019, Vigneron et al., 2019, Hough et al., 2020, Ray et al., 2020). These environments harbor several dormant microorganisms that can produce greenhouse gases like carbon dioxide and methane, and if they become metabolically active en masse, this could drastically increase contribution to climate change (Feng et al., 2020). Not only can ecosystem biodiversity be affected, but these changes can impact human society and health. Thus, it is important to consider analytical strategies to better understand global change so future actions can be coordinated to mitigate any negative consequences, and evaluating microbiomes may be part of the solution.

Global Change and the Soil Microbiome: A Human-Health Perspective

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The importance of the gut and the soil microbiomes as determinants of human and ecosystem health, respectively, is gaining rapid acceptation in the medical and ecological literatures. This suggests that there is a wealth of highly transferable knowledge about the microbial ecology of human and non-human ecosystems that is currently being generated in parallel, but mostly in isolation from one another. I suggest that effectively sharing this knowledge could greatly help at more efficiently understanding and restoring human health and the functioning of ecosystems, which are currently under wide-spread pressure. I illustrate this by comparing the effects of nitrogen deposition on ecosystem carbon sequestration with unhealthy dietary habits and human disease. The deposition of N, a key nutrient for plant growth, may increase carbon sequestration (equivalent to obesity) through several mechanisms,

including a reduction in the ability of soil microbes to process organic matter, which some argue could help mitigate climate change. However, this usually results in a degradation of ecosystem health and, thus, cannot represent a real solution. Similarly, human obesity is linked to an alteration of the composition and functioning of microbial communities inhabiting the gut, which is often attributed to unhealthy dietary habits, including ingesting high amounts of simple sugars and processed foods. Finally, I advocate for the explicit recognition of the many commonalities between the functioning of the gut and ecosystems and a broader multidisciplinary collaboration among experts in ecology and human health, including the engineering of soil microbial communities designed *ad-hoc* to restore ecosystem health.

Nitrogen Deposition and Carbon Sequestration in a Changing Climate

It has been widely proposed that atmospheric nitrogen (N) deposition could help mitigate climate change by increasing the rates of carbon (C) sequestration in terrestrial ecosystems (Knorr et al., 2005; Reich et al., 2006; Yue et al., 2016). Two commonly observed responses are typically proposed as mechanisms: first, a greater amount of N usually implies a higher capacity for plant growth, which would result in a greater amount of C retained within the system (Magnani et al., 2007; de Vries et al., 2009; Laubhann et al., 2009). Of course, for this to be true, it is necessary that the increase in the rates of C uptake and accumulation exceed the C emission rates, whatever the main route by which the latter happens, including plant and/or microbial respiration and changes in fire dynamics due to an excess of biomass accumulation (Dezi et al., 2010; Fenn et al., 2010). The second main mechanism is linked to a reduction in decomposition rates, particularly of recalcitrant organic matter, which would, therefore, accumulate within the system (Knorr et al., 2005; Waldrop and Zak, 2006). Otherwise, this accumulated C may be lost to the atmosphere in the form of CO_2 after being respired by soil microorganisms (Janssens et al., 2010). Of course, the relative importance of these mechanisms depend on how plant communities and soil microorganisms respond, directly and indirectly, to the additional inputs of N which, in any case, usually ends up resulting in a disruption of the interaction between these two key components of the ecosystem (Liu et al., 2014).

The Need for a New Perspective

In this article, I will adopt a human health perspective, hardly used in the discipline of global change ecology, to substantiate why atmospheric N deposition cannot represent a positive (i.e., healthy) alternative to mitigate climate change. In the medical literature, it is now widely recognized that human beings are like ecosystems (in fact, some consider us as living ecosystems) in which the eukaryotic cells that form part of our bodies and the prokaryotic cells that live in and on us are deeply interconnected, whereas the enormous importance of our microbiome to human health is also increasingly gaining acceptation (Bengmark, 1998; Berendsen et al., 2012; Ha et al., 2014; Alivisatos et al., 2015; Tilg and Adolph, 2015; Blaser, 2016; Blaser et al., 2016). The fact that many modern diseases, including conditions of the nervous and circulatory systems, skin and heart and allergies (including atopic dermatitis and food allergies), are directly caused by alterations in the microbial communities that live in our interior and exterior is also gaining rapid acceptation (Ha et al., 2014; Tilg and Adolph, 2015; Chang et al., 2016; Tang and Lodge, 2016). In this sense, the word ecosystem is widely used in the current literature of integrative medicine and gastroenterology. However, the opposite does not frequently happen in ecology [i.e., (cautiously) comparing ecosystems with the human body], despite the wealth of knowledge in the medical and human health literature that we, as ecologists, could apply in, for example, issues related to understanding the functioning (i.e., metabolism) of ecosystems and plant-soil-microbe interactions subjected to human pressure (Berendsen et al., 2012; Blaser et al., 2016; Table 1; Figure 1). Therefore, I will finally defend the need to approach problems in ecology from a more multidisciplinary, fresher perspective.

Characteristics associated with a healthy gut	Impacts associated with an unhealthy diet	References	Characteristics of healthy soils and ecosystems	Impacts associated with nitrogen deposition	References
Higher bacterial diversity and abundance that are able to metabolize more food sources and provide essential molecules such as vitamins, hormones, etc.	Functionally and compositionally impoverished gut communities	Thomas et al., 2014	Higher microbial diversity and abundance	Functionally and compositionally impoverished soil communities	Leff et al., 2015; Berg et al., 2017
Protection against disease through the stimulation of the immune system. More longevous individuals	Disease (obesity, diabetes, depression, inflammation etc.). People die younger	Berendsen et al., 2012; Ha et al., 2014; Thomas et al., 2014; Alivisatos et al., 2015; Tilg and Adolph, 2015; Xu et al., 2015; Blaser, 2016	Higher stability and resistance to disturbance	Die-back due to long-term N toxicity, altered fire regimes, pest outbreaks, less tolerance to frost, heat waves, etc.	Bobbink et al., 2010
Lean phenotype	Obese phenotype	Joyce and Gahan, 2014	Rapid processing, transformation and stabilization of litter inputs in the long-term soil pool	Accumulation of intact and partially decomposed leaf litter (i.e., carbon accumulation)	Knorr et al., 2005
High-energy feeling	Low-energy feeling	Umu et al., 2013	Higher ecosystem functionality and supply of key services such as air and water purification, food resources for pollinators, protections against extreme events such as floods, heat waves, etc.	Lower functionality and reduced supply of key services	Costanza et al., 1997; Jones et al., 2014
Well-trained immune system. No medication required. This is also associated with a smaller social cost	Weak immune system. Medication is very often required to treat diverse conditions. Development of drug-resistant strains. Higher social cost	Round and Mazmanian, 2009; Blaser, 2016	High ability to self-regenerate. No or very little management required. No extra cost involved	Management required, including applying herbicides, weeding, etc. High monetary cost	Costanza et al., 1997; Chiquoine et al., 2016
Higher Bacteroidetes to Firmicutes ratio	Lower Bacteroidetes to Firmicutes ratio	Mathur and Barlow, 2015	Higher fungal to bacterial biomass and activity ratio. Higher abundance and diversity of mutualistic mycorrhizal strains	Lower fungal to bacterial ratio. Less mutualistic mycorrhizal strains	Frey et al., 2004; Treseder, 2004; Waldrop et al., 2004; de Vries et al., 2006; Wallenstein et al., 2006

Table 1. The importance of the gut and the soil microbiomes as determinants of human and ecosystem health, respectively, is gaining rapid acceptation in the medical and ecological literatures.

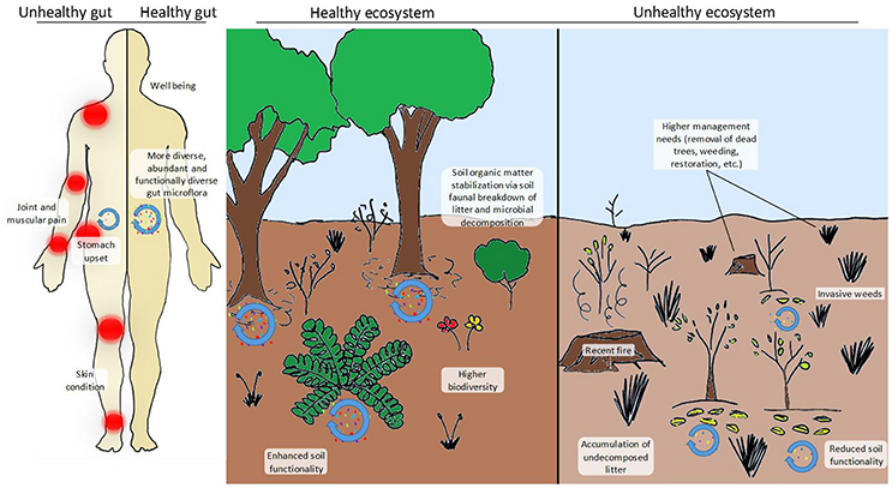


Figure 1. Comparison between a healthy and an unhealthy gut (left-hand side panel) and a healthy and an unhealthy ecosystem (right-hand side panel). In the case of the human individual, his/her gut microbiota is less diverse ("red" microbes are completely absent), less abundant and contains more pathogenic taxa ("yellow" microbes). The individual with the healthy gut has a more functional and more abundant microbiota that provides him/her with essential nutrients, hormones, amino acids, etc. and stimulates his/her immune system. A healthy gut is also less prone to become infected by pathogens and can process toxic compounds (i.e., detoxify) more easily. The disturbed (i.e., unhealthy) ecosystem shown here has recently been affected by a devastating fire fuelled by the accumulation of N-loving exotic grasses that have altered the natural fire dynamics of the system. Facilitated by the altered fire dynamics, the system has also become chronically dominated by weedy grasses, therefore requiring intensive (and costly) management practices (dead tree removal, weeding and restoration), also posing a threat to nearby human populations and their properties. Biodiversity (in terms of microbial, faunal and plant communities) is remarkably higher in the healthy ecosystem, which also has a higher potential to process organic matter inputs and stabilize them in the long-term soil pool. In contrast, undecomposed or partially decomposed litter accumulates on the functionally impoverished soil of the unhealthy ecosystem. This pattern is in agreement with reported observations of higher soil carbon sequestration under increased nitrogen deposition scenarios due to the inhibition of soil enzymes and the reduction of microbial biomass but poses relevant questions such as: Is this type of N deposition-induced carbon sequestration desirable? And, does it really represent a long-term (or even short-term) solution?

Why Nitrogen Deposition Cannot be the Solution to Climate Change

The reason why I think that a temporary, N deposition-induced increase in the rate of C sequestration will not contribute to mitigating climate change in the long term is equivalent to the reason of those that argue that an increase in obesity rates in human

populations derived from a diet rich in simple sugars and processed food and the consequent alteration of their microbiome will not successfully and permanently solve any public health problem of today's societies. Ingesting large amounts of simple sugars, processed foods, sugary drinks and saturated fats is definitely better than starving, but that does not mean that it is a healthy practice. And the same happens with N deposition and C sequestration. In ecosystems where N is still a limiting nutrient, which is quite common worldwide (LeBauer and Treseder, 2008), an increase in the availability of N can increase ecosystem productivity to levels comparable to human obesity (Tian et al., 2016), but that does not mean that the ecosystem is healthier and, therefore, that this will result in a long-term benefit (Bobbink et al., 2010; Jones et al., 2014). In this sense, a healthy ecosystem may be defined here as a highly multifunctional ecosystem that can maintain an adequate supply of services, at least as compared to a previously defined reference state.

In medicine, the term dysbiosis refers to changes in the composition of the microbiome that are not beneficial to the individuals, including a loss of abundance and diversity of beneficial microorganisms and increased number of pathogens, and that result in the development of a condition (Ha et al., 2014; Tilg and Adolph, 2015). This term could also be used to describe ecosystems that are dysfunctional due to alterations of their microbial communities. In this sense, it has been repeatedly shown through experimental studies and meta-analyses that increased N deposition is typically associated with changes in soil microbial communities (usually related to a decrease in abundance and biodiversity; Treseder, 2004, 2008; Ramirez et al., 2010; Zeng et al., 2015), reduced ecosystem functionality (alterations of energy metabolism; Waldrop and Zak, 2006; Treseder, 2008; Liu et al., 2014) and short- to mid-term increases in C sequestration, especially in aboveground biomass, but also in the soil and roots (comparable to obesity, as previously mentioned; Xia and Wan, 2008; Yue et al., 2016). Given that metabolic disorders and obesity in humans are clearly associated with a deterioration in the health status of individuals that may even result in cases of fatality due to chronic diseases, sudden death or, quite commonly in the natural world, to increased sensitivity to other environmental stresses (Mathur and Barlow, 2015; Monteiro et al., 2015), I think that we would do well to be cautious when we consider, perhaps naively, the potential benefits of a N that, after all, is the result of the atmospheric pollution derived from our activities (Gruber and Galloway, 2008).

The "Deceptively Simple" Solution

The connections between human health, disease, and the microbiome, especially in the case of the gut, are becoming increasingly apparent and are attracting the public attention, especially because of the high social cost of unhealthy dietary habits and lifestyles and the "deceptively simple" solution of the problem (Mathur and Barlow, 2015; Tilg and Adolph, 2015; Blaser, 2016). In the case of both people and ecosystems, (i) ensuring a healthy supply of nutrients derived from the breakdown and cycling of unprocessed food/organic matter, (ii) minimizing the use of antibiotics (particularly those associated with the livestock industry in the case of ecosystems; Park and Choi, 2008) and chemicals (including herbicides and pesticides in the case of ecosystems) that destroy the microbiome, unless this is strictly necessary, and (iii) promoting practices that favor the system's ability to self-regenerate, something that living systems do wonderfully well, and that increase its resilience against pathogens and extreme events could be part of the solution, if not all, of the problem.

Of course, there are opportunities to aid in the recovery of our damaged and degraded ecosystems as well as there are possibilities to recover the lost or damaged intestinal flora (Brudnak, 2002; Sheth et al., 2016). This can be achieved by the use of properly designed probiotics or fecal transplants or, in the case of ecosystems, inocula assembled in the lab from pure cultures or soil samples obtained in the field from healthy ecosystems (Bowker, 2007; Chiquoine et al., 2016; Wubs et al., 2016) in conjunction with a balanced nutrient supply (i.e., organic matter inputs, the equivalent to prebiotics; Mathur and Barlow, 2015; Sheth et al., 2016). In this sense, the concept of synbiotics (i.e., synchronous administration of probiotics and prebiotics) could represent a particularly promising benchmark borrowed from the human health literature to successfully restore degraded ecosystems (Tang and Lodge, 2016) and, thus, the human probiotics industry has an opportunity to play a key role in this development.

Concluding Remarks

Recognizing and understanding the similarities and deep connections between the gut and the belowground world, where roots are the equivalent to our gut and the rhizosphere is the gut microflora (Berendsen et al., 2012) can help us advance the understanding of ecosystems by leaps and bounds through the search of similar microbial indicators of disease (e.g., Bacteroidetes to Firmicutes ratio in humans; Mathur and Barlow, 2015) and, therefore, to implement quick and successful measures in ecosystem management rather than relying, perhaps naively, on that the very same thing that caused climate change (i.e., pollutant emissions to the atmosphere) will also be part of the solution. From here, I advocate for the development of a new field of research that specifically

aims at recognizing and make practical use of the profound links between the functioning of the gut and the ecosystems that extend beyond our bodies and that benefits from a truly multidisciplinary collaboration among experts in the areas of global change ecology and human health.

Author Contributions

The author confirms being the sole contributor of this work and approved it for publication.

Conflict of Interest Statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Info Hotspot

Click on the boxes to learn more about the importance of microbiomes in creating and maintaining homeostatic balance between humans and the environment.

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- Which ecosystems have the greatest impact on global nutrient cycling?
- Which microbiome is an under-explored source for the discovery of biosynthetic products?
- How could global warming's effect on permafrost and tundra further increase greenhouse gases?
- What are the pros and cons of nitrogen deposition to counteract climate change?
- What are some suggested solutions to improve environmental and human health concerning microbiomes?

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8.2: The Ocean Microbiome and Marine Life

The Ocean Microbiome and Marine Life

The ocean is teeming with life, both macro and micro, where their adaptation and success are dependent on both a local and global scale. Oceanic microorganisms have major roles in nutrient cycling, ecological interactions, and influence and respond to environmental changes (Doney et al., 2012). Temperature is the strongest factor that determines microbial community composition, which parallels depth stratification, and these communities can serve as indicators for anthropogenically-induced climate changes (Sunagawa et al., 2015). It is predicted that carbon fixation by microbial primary producers will decrease which will have downstream effects on ocean microbiome structure and overall trophic level interactions (Moran, 2015).

While free-living marine microorganisms can provide relevant information and predictive models about the ocean, those host-associated microbes can also give environmental insight into the largest ecosystem on the planet.

Marine Animal Microbiomes: Toward Understanding Host–Microbiome Interactions in a Changing Ocean

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All animals on Earth form associations with microorganisms, including protists, bacteria, archaea, fungi, and viruses. In the ocean, animal–microbial relationships were historically explored in single host–symbiont systems. However, new explorations into the diversity of microorganisms associating with diverse marine animal hosts is moving the field into studies that address interactions between the animal host and a more multi-member microbiome. The potential for microbiomes to influence the health, physiology, behavior, and ecology of marine animals could alter current understandings of how marine animals adapt to change, and especially the growing climate-related and anthropogenic-induced changes already impacting the ocean environment. This review explores the nature of marine animal–microbiome relationships and interactions, and possible factors that may shift associations from symbiotic to dissociated states. I present a brief review of current microbiome research and opportunities, using examples of select marine animals that span diverse phyla within the Animalia, including systems that are more and less developed for symbiosis research, including two represented in my own research program. Lastly, I consider challenges and emerging solutions for moving these and other study systems into a more detailed understanding of host–microbiome interactions within a changing ocean.

Introduction

Marine animals are the icons of life in the oceans. They represent about two million species (Mora et al., 2011) and include a wide range of body designs, from the highly simplistic sponges lacking true tissues and organs to the complex vertebrates containing specialized tissues and organs, such as fish and marine mammals, with some iconic representatives presented in Figure 1. The bodies of marine animals span several orders of magnitude in size, from the abundant planktonic copepod (1–2 mm) to the highly mobile blue whale (30 m), the largest animal on Earth. Marine animals are key members of ocean ecosystems and serve as both prey of and predators for other animals within the complex ocean food web. In contrast to terrestrial animals, marine animals have developed strategies for osmoregulation as well as highly specialized approaches for maintaining homeostasis within diverse temperature, oxygen and pressure gradients of the ocean (Graham, 1990; Knoll and Carroll, 1999). Marine animals also possess sophisticated specializations and functions that promote their success on or within their benthic or pelagic habitats, including specializations for living or enduring depths (outlined in Figure 1) that vary widely in factors such as light availability, access to food and predator exposure.

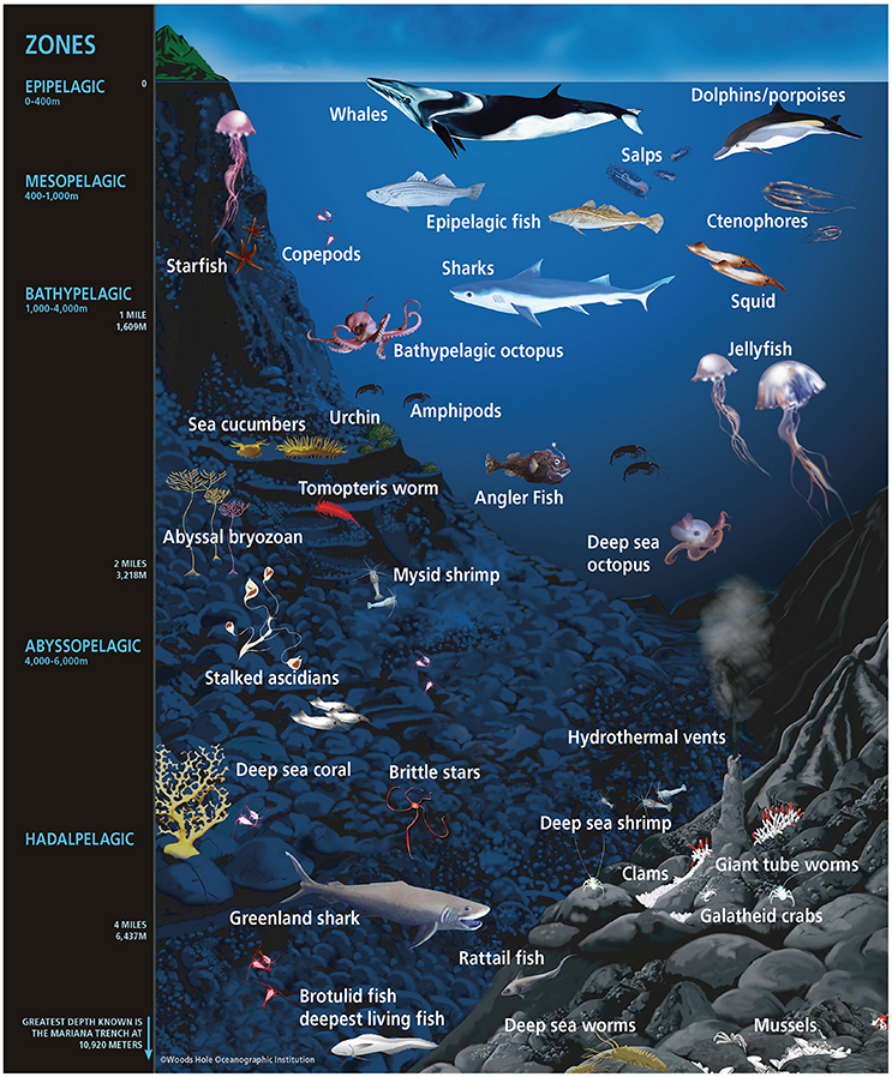


Figure 1. Illustration and common names of representative ocean animal life within their approximate depth-defined ecological habitats. Microorganisms exist on the surfaces and within the tissues and organs of the diverse life inhabiting the ocean, across all ocean habitats. Animals are not drawn to scale.

Marine animals share the sea with a vast diversity of microorganisms, including protists, bacteria, archaea, fungi, and viruses which comprise millions of cells in each milliliter of the 1.3 billion km^3 of water comprising the oceans (Eakins and Sharman, 2010). These microorganisms are several micrometers or smaller in size, but collectively their roles in oxygen production, nutrient cycling, and organic matter degradation provide critical functions to the oceans and Earth (Arrigo, 2005; Falkowski et al., 2008). Microorganisms that associate with marine animals are part of the animal's microbiome, or collection of microorganisms that reside on or within the animal. Some of the microorganisms comprising the microbiomes of marine animals are thought to originate from this surrounding supply of seawater-associated cells (e.g., Nussbaumer et al., 2006), while other cells appear to have strict inheritance patterns, passed on through generations from the host (Sharp et al., 2007).

Over the past two decades, the widespread application of genomic and more integrative microbiological approaches have advanced our understanding of animal microbiomes (reviewed within McFall-Ngai et al., 2013). Symbiotic relationships between microorganisms and marine animals have been studied for decades, but technological advancements are providing new insights into the sheer diversity of microbial life in association with animals in the sea (Smith, 2001; Douglas, 2010). For example, reef-building corals are acknowledged as the icons of animal–microbial symbiosis in the sea, with corals hosting photosynthetic symbionts that make critical contributions to host nutrition (Muscatine et al., 1981). New reports of diverse protists, bacteria, archaea, and viruses in association with corals provide insights into the role of these cells for fulfilling diverse functional processes within the different niches of the coral host (reviewed within Thompson et al., 2015; Bourne et al., 2016). In fact, for many

terrestrial animals, new reports of microbial symbioses provide insights into the variety of genetic and biochemical interactions and the ways that microorganisms contribute to animal health, behavior, and ecology (e.g., Ley, 2010; Cho and Blaser, 2012).

Understanding the microbiomes of marine animals is a growing research area within the field of marine science. Currently, the science is heavily focused on identifying consistent or "core" microbial members of the microbiome (Shade and Handelsman, 2012). After first gaining an understanding of "who's there" generally using diversity-based surveys targeting the small subunit (SSU) ribosomal RNA (rRNA) gene, these microbiomes are then often examined as a whole or in smaller units to understand the function of the cells, the nature of the associations and ultimately gain insight into the role of the microbiome in animal health, physiology, ecology, and behavior (Ezenwa et al., 2012; McFall-Ngai et al., 2013). Additionally, the ocean environment is changing at unprecedented rates due to climate-related and anthropogenic-induced impacts (Halpern et al., 2008; Doney et al., 2012), and the microbiome is also being investigated for its possible role as a sentinel of a changing host (Ainsworth and Gates, 2016).

How environmental changes and animal life history events affect the microbiomes of marine animals is growing area of research, and there is an emerging focus on better understanding interactions between the animal, microbiome, and ocean environment, including the elements that may define their exchanges (e.g., Meron et al., 2011; Lesser et al., 2016; Webster et al., 2016). Therefore, this review considers the symbiosis and dissociated stages of animal–microbiome associations, and discusses factors and causes that may alter interactions between animals and their microbiome. Next, this review discusses current research examining animal–microbiome relationships and interactions, by focusing on select systems that represent diverse marine animal phyla and which span the range of being more to less developed for microbiome research. Two of these systems, corals and marine mammals, are represented in my own research program. Lastly, this review concludes with a discussion of challenges in marine animal–microbiome research and opportunities available to further advance knowledge of animal–microbiome interactions in the ocean.

Conceptual Model of Factors Contributing to Host-Microbiome Interactions

Host–microbiome dynamics are generally described as falling into two main categories: symbiosis, in which the organisms are involved in a normal metabolic and immune signaling interactions, and secondly dysbiosis, in which the relationship or interactions are heavily altered, possibly related to a major stress or infection event. While host–microbiome symbiosis and dysbiosis has been mostly considered in humans and humanized models (Hamdi et al., 2011; Nicholson et al., 2012; Scharschmidt and Fischbach, 2013), many of the same concepts are applicable to organisms in the sea (Egan and Gardiner, 2016), and are being explored in various systems (discussed below). The exact factors and mechanisms tipping the scale between symbiosis and dysbiosis will probably vary with complexity of the host anatomy and immune functioning (e.g., simplistic sponges and corals compared to more complex fish and sharks) as well as with the complexity of interactions that may occur between the members of the microbiome.

A normal animal–microbiome relationship in the ocean could be referred to as a "symbiotic" state, although the exact nature of the relationship may vary for each cell in the association. For example, cells residing on the surface or within the gut cavity of an animal are physically associated, yet do not share as intimate of an association as those microbes residing intracellularly with the host's cells. This normal symbiotic state is subject to a variety of environmental fluctuations, which are generally defined by the characteristics of the habitat (Figure 1). For example, in the ocean's upper photic zone, animals are exposed to variations in temperature and light, and host–symbiont interactions, especially in ectothermal animals, could alter on cycles such as seasons that generally control the temperature and light environment. Normal fluctuations in animal-specific patterns could also alter host–microbiome relationships. For example, changes in diet, possibly due to short-term prey availability, can alter gut microbiota and host–microbiome metabolic exchanges in other systems (e.g., David et al., 2014), and similar diet trends may also affect marine animals. Stress is another factor more complex animals encounter on a daily basis (e.g., squid, crabs, fish), which could be related to social/territorial encounters or chasing or fleeing from prey, and the short-term production of stress hormones such as cortisol can influence host–microbiome relationships (e.g., Moloney et al., 2014).

There are also normal animal life events that occur on longer time frames or that are more drastic in scope, such as animal development, aging, and reproduction. In non-marine animals, these factors have been shown to cause alterations in animal-microbial relationships (e.g., Heintz and Mair, 2014). These changes can be drastic enough to cause a state of "altered symbiosis" that could extend for short or longer term. For example, the gut microbiome of women generally becomes altered during pregnancy (Koren et al., 2012). Events resulting in normal animal stress may also lead to a more altered symbiotic state, for example if social conflict was more chronic, perhaps due to the pressures of a particular habitat. Data from humans and humanized models suggests that the microbial community and associated genes do fluctuate with the normal variations and animal life events, and both may be

considered “healthy” fluctuations (Nicholson et al., 2012). However, how these fluctuations affect exchanges between the host and microbiome is much less understood.

If symbiosis and altered symbiosis are considered as normal host–microbiome variations throughout an organism’s life, dysbiosis is the breakdown in the relationship, generally related to one or more major stressors, and can greatly alter host health and lead to a disease state (Holmes et al., 2011). The stressor may come from an external source, such as a pollutant, infective agent, or a longer-term natural environmental change—and there are probably countless other factors that could fit this category (Figure 2). For example, one of the most visible signs of host–microbiome dysbiosis is with scleractinian corals, whose relationship with unicellular algae breaks down after long-term yet small increases in seawater temperature, causing the coral to become “bleached” (Brown, 1997). In humanized models, major stressors such as malnutrition are related to less physically visible changes in innate immunity, which are linked to microbial ecology (Hashimoto et al., 2012). Understanding the relationship between symbiosis, dysbiosis and host health and functioning are general topics of research in most host–microbiome studies, but the environmental changes occurring in the ocean environment have made this area of research more pressing for marine animals. Overall, the concepts behind the model presented in Figure 2, as well as variations of this model, are generally driving much of the current research examining animal-microbial relationships in the ocean.

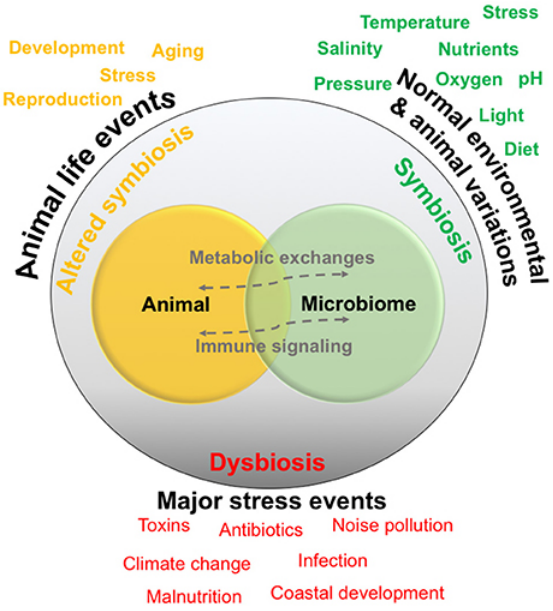


Figure 2. Conceptual diagram of a host-microbiome relationship. Relationships are generally thought to exist in a symbiotic state, and are normally exposed to environmental and animal-specific factors that may cause natural variations. Some events may change the relationship into a functioning but altered symbiotic state, whereas extreme stress events may cause dysbiosis or a breakdown of the relationship and interactions.

Overview of Diverse and Emerging Animal-Microbiome Study Systems

The microbiomes of diverse marine animals are currently under study, from simplistic organisms including sponges (e.g., Webster et al., 2010) and ctenophores (Daniels and Breitbart, 2012) to more complex organisms such as sea squirts (Blasiak et al., 2014) and sharks (Givens et al., 2015). Below I present some of the current study systems that represent a diverse cross-section of marine animal phyla, and trends of research in these systems including focus on symbiosis and dysbiosis. The organisms are generally presented in order from increasing to decreasing knowledge about the host-microbiome relationship.

The relationship between the Hawaiian bobtail squid *Euprymna scolopes* (phylum Mollusca) and the bioluminescent bacterium *Vibrio fisheri* (also recognized as *Aliivibrio fisheri*) is one of the best studied symbiotic relationships in the sea and is a choice system for general symbiosis research (Figures 3A,B). The *E. scolopes*-*V. fisheri* relationship has provided insight into fundamental processes in animal-microbial symbioses, and especially biochemical interactions and signaling between the host and bacterium (McFall-Ngai, 2000, 2014). Much of this research focuses on establishment of the symbiosis, with less focus on dysbiosis. Additionally, because *V. fisheri* exists in the light organ, these studies have been primarily limited to this one isolated relationship, with the remainder of the squid’s microbiome virtually unstudied (but see Barbieri et al., 2001; Collins et al., 2012). The *E. scolopes*–*V. fisheri* system offers simplicity for the study of host–microbial interactions and numerous helpful developments in

animal husbandry, genomic tools, and experimental design that could be applied to ask more comprehensive questions about squid–microbiome interactions, including the conditions leading to dysbiosis of relationships.

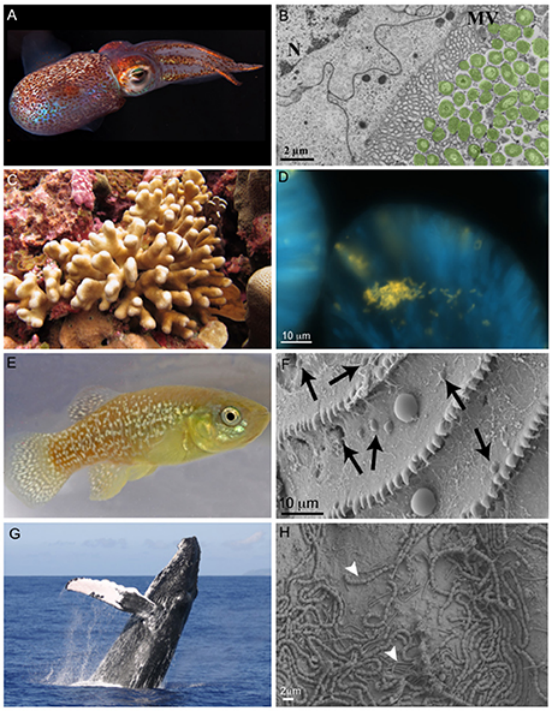


Figure 3. Photographs of marine animals and their associated microbiomes from select study systems. Photographs include: the Hawaiian bobtail squid Euprymna scolopes (A) and a transmission electron micrograph of Vibrio fisheri cells associating with dense microvilli (MV) and in proximity to the epithelial nucleus (N) within the light organ (B); the reef-building coral Stylophora pistillata (C) and a microscopy image of Endozoicomonas cells (probed yellow using in situ hybridization) within the tentacles of a S. pistillata host (D); the Atlantic killifish (Fundulus heteroclitus) (E) and a scanning electron microscopy (SEM) image of the surface and scales of the fish, with arrows pointing to bacterial-sized cells and larger cells (which are not noted) are presumably phytoplankton (F); a humpback whale (Megaptera novaeangliae) breaching (G) and a scanning electron microscopy image of a humpback's skin surface associated bacteria, with arrows indicating two different cell morphologies (H). Photographs (A,B) were produced by M. McFall-Ngai and were previously published photographs (McFall-Ngai, 2014), (C,H) were previously published by the author Neave et al. (2016) and Apprill et al. (2014), photograph (D) was taken by Liping Xun and photograph (E) by Evan D'Alessandro.

Similar to *E. scolope*, the gutless marine oligochaete worm *Olavius algarvensis* (phylum Annelida) is another relatively well-studied marine host to microbes. One major difference is that it has been studied within the context of a larger consortium of microorganisms compared to *E. scolope*. These 3 cm long worms reside within shallow marine sediments of the Mediterranean Sea. The worms do not contain a mouth or a digestive or excretory system, but are instead nourished with the help of a suite of extracellular bacterial endosymbionts that reside upon coordinated use of sulfur present in the environment (Dubilier et al., 2001). This system has benefited from some of the most sophisticated 'omics and visualization tools (Woyke et al., 2006). For example, multi-labeled probing has improved visualization of the microbiome (Schimak et al., 2016) and transcriptomics and proteomics have been applied to examine host–microbiome interactions, including energy transfer between the host and microbes (Kleiner et al., 2012) and recognition of the consortia by the worm's innate immune system (Wippler et al., 2016). The major strength of this system is that it does offer the ability to study host–microbiome interactions with a low diversity microbial consortium, and it also offers a number of host and microbial genomic resources (e.g., Woyke et al., 2006; Ruehland et al., 2008). Dysbiosis has not been heavily investigated in this system, and given the growing knowledge of host–microbial interactions, *O. algarvensis* could be an imperative animal for dysbiosis research.

As mentioned above, corals (phylum Cnidaria) (Figure 3C) are one of the most common examples of an animal host whose symbiosis with microalgae can turn to dysbiosis, and is visibly detected as bleaching. Coral microbiomes have been examined in a variety of studies, which demonstrate how variations in the ocean environment, most notably temperature, light, and inorganic nutrients, affect the abundance and performance of the microalgal symbionts, as well as calcification and physiology of the host (Dubinsky and Jokiel, 1994; Anthony et al., 2008). Studies have also suggested that resident bacteria, archaea, and fungi

additionally contribute to nutrient and organic matter cycling within the coral, with viruses also possibly playing a role in structuring the composition of these members, thus providing one of the first glimpses at a multi-domain marine animal symbiosis (reviewed in Bourne et al., 2016). The gammaproteobacterium *Endozoicomonas* is emerging as a central member of the coral's microbiome, with flexibility in its lifestyle (Figure 3D) (Neave et al., 2016, 2017). Ocean disturbances including elevated temperature and ocean acidification have been shown to disrupt the coral's associated bacteria (Thurber et al., 2009; Meron et al., 2011), including relationships with *Endozoicomonas* (Morrow et al., 2015). However, some members of this microbiome appear to be stable across large environmental gradients (Hernandez-Agreda et al., 2016). In addition to nutrition, the microbiome plays a role in coral health and stress. Temperature and light stress to corals can result in overproduction of reactive oxygen species (ROS), which can be detrimental to *Symbiodinium* and result in bleaching, but the associated bacteria have also recently been shown to contribute extracellular ROS (Diaz et al., 2016; Zhang et al., 2016), which could play a signaling role with the host or within the microbiome. Given the recent mass bleaching occurring on reefs (Hughes et al., 2017), corals will likely continue to be a useful and popular system for symbiosis and dysbiosis research. There are number of resources available to further promote study of the coral microbiome, including integrated databases (Franklin et al., 2012; Madin et al., 2016), a growing number of host and microbial genomes (Shinzato et al., 2011; Bayer et al., 2012; Neave et al., 2017), and laboratory amendable "model" systems (Weis et al., 2008; Baumgarten et al., 2015).

Sponges (phylum Porifera) are common members of the ocean's diverse benthic habitats and their abundance and ability to filter large volumes of seawater have led to the awareness that these organisms play critical roles in influencing benthic and pelagic processes in the ocean (Bell, 2008). They are one of the oldest lineages of animals, and have a relatively simple body plan that commonly associates with bacteria, archaea, algal protists, fungi, and viruses (reviewed within Webster and Thomas, 2016). Sponge microbiomes are composed of specialists and generalists, and complexity of their microbiome appears to be shaped by host phylogeny (Thomas et al., 2016). Studies have shown that the sponge microbiome contributes to nitrogen cycling in the oceans, especially through the oxidation of ammonia by archaea and bacteria (Bayer et al., 2008; Radax et al., 2012). Most recently, microbial symbionts of tropical sponges were shown to produce and store polyphosphate granules (Zhang et al., 2015), perhaps enabling the host to survive periods of phosphate depletion in oligotrophic marine environments (Colman, 2015). The microbiomes of some sponge species do appear to change in community structure in response to changing environmental conditions, including temperature (Simister et al., 2012a) and ocean acidification (Morrow et al., 2015; Ribes et al., 2016), as well as synergistic impacts (Lesser et al., 2016). Understanding the effect of these altered host–microbiome interactions on sponge growth and ecology are topics for further research. As such, there are a number of resources to support research on sponges including a curated database of sponge–microbial sequences (Simister et al., 2012b), cultivated microbial isolates and sponge cell cultures from some species (Taylor et al., 2007) to facilitate investigations.

Atlantic killifish, (*Fundulus* spp., Phylum Chordata) (Figure 3E) are one of the most abundant estuarine fishes in North America, and are related to other families with more global distributions in coastal areas (Fritz et al., 1975; Lotrich, 1975). The killifish have a broad North American geographic distribution yet limited subpopulation movement, and thus the Atlantic killifish have become a useful field-residing model species for examining biological and ecological responses to natural environment conditions (salinity, oxygen, pH, and temperature) as well as chemical pollutants (Burnett et al., 2007). While the killifish microbiome (Figure 3F) has not been extensively studied, there is work examining the influence of pollutants on the skin and mucus of the fish, which suggests that this skin microbial community is relatively resistant to change (Larsen et al., 2015). Populations of the fish offer a unique host genetic resistance to toxicity (Hahn et al., 2004), and it is possible that this resistance is also facilitated by features of the microbiome. The Atlantic killifish appear to be an ideal study species for microbiome investigations and especially the response of the host–microbiome symbiosis to changing ocean conditions. Specifically, the killifish can be maintained in laboratory aquaria, they are hardy and amendable to experimental manipulation, and spawning material can be acquired for developmental (Burnett et al., 2007).

The microbiomes of marine mammals (phylum Chordata) (Figure 3G) have recently been investigated and offer a comparative study system to terrestrial mammals (reviewed within Nelson et al., 2015). Marine mammals are often viewed as sentinel species of the ocean, because they appear to rapidly respond to ocean conditions, disturbances, and pathogens similarly to humans (Bossart, 2011). Several studies have examined the skin (Figure 3H), gut and respiratory microbiomes of diverse marine mammal species, and describe species-specific relationships (Johnson et al., 2009; Apprill et al., 2014; Bik et al., 2016). Connections between the community composition of the microbiome and animal health (Apprill et al., 2014) and diet (Nelson et al., 2013; Sanders et al., 2015) have been made, and more detailed studies are needed to understand these specific connections. While there are very limited

resources available for studying host–microbiome interactions in marine mammals, there are some animals in captivity as well as well-studied populations that will heighten investigations of host–microbiome symbiosis and dysbiosis in these sentinel species.

Challenges and Emerging Solutions to Studying Animal–Microbiome Interactions

A number of the systems highlighted above are currently examining animal–microbiome interactions, but these are generally most developed in systems such as *O. algarvensis* that offer lower complexity microbiomes, or within the single host–symbiont relationship between *E. scolopes* and *V. fisheri*. As such, a major challenge to the field is exploring host–microbiome interactions within the context of a diverse microbiome, and especially if the microbiome includes members such a uncharacterized protists, fungi, and viruses, which have generally not been described in most marine animal systems. Therefore, a through description of the microbiome is a first necessity, but this still presents many challenges on a variety of levels. For example, amplifying or shotgun sequencing microbial DNA with the presence of abundant host cells often requires optimization or high sequencing output (e.g., Rocha et al., 2014; Weber et al., 2017). Taxonomic databases generally contain few microbial sequences from many of these animals, and therefore simple tasks such as assigning taxonomy can be challenging. Developing animal-specific databases (Simister et al., 2012b), which include the next-generation supplied sequences generally not available in curated taxonomic databases, could help alleviate this problem. There are also a number of new tools for metagenomics-based analysis, including advancements in binning genomes from complex samples (Kang et al., 2015; Graham et al., 2017) as well as new visualization methods for comparing genomes (Eren et al., 2015; Wagner et al., 2017). A challenging issue that has received less attention is how to gain information from unknown genes and gene families, which can make up over half of the environmental microbial genomes. Algorithms utilizing gene function predictions do provide some assistance with this problem (Mi et al., 2015), and these tools may improve as more environmental microbial genomes are available. Lastly, computational tools are emerging to facilitate identifying associations between host genetic variation and microbiome composition (Lynch et al., 2016).

Once some of these hurdles are overcome and a comprehensive view of the microbiome is available, researchers can then explore the nature of the host–microbiome relationship. Visualization using a variety of different microscopy-based techniques is a powerful tool to recognize the physical relationship between a host and the microbiome, as well as the organization of cells within the microbiome. Electron microscopy provides the most detailed information about this organization, but this is less useful for complex microbiomes because taxonomically distinct microbial cells with similar appearances cannot be distinguished. Fluorescent *in situ* hybridization (FISH), and especially using a multi-taxonomic, simultaneous probing technique such as Combinatorial Labeling and Spectral Imaging FISH (CLASI-FISH) (Valm et al., 2011) can provide significant insight into host–microbe and microbe–microbe interactions. FISH techniques do require optimization for some animal systems, such as corals that possess autofluorescent host tissues (Wada et al., 2016). Visualization techniques can also be paired with isotope probing, to provide opportunities to trace the transfer of specific molecules between the host and microbiome, as well as within the microbiome using Nano-SIMS and Nano-SIP approaches (Musat et al., 2016). There have also been many recent instrumental and database advances in the field of metabolomics (Beisken et al., 2015), and this approach is beginning to be applied to examine host–microbiome interactions (Gomez et al., 2015; Sogin et al., 2016). An understanding of specific microbial metabolites will help facilitate targeted investigations of how these products affect the host nutritional and immune systems.

Lastly, experimental manipulation is a challenge to the study of host–microbial interactions in the ocean. Studying the animals in their natural environment is the most ideal approach because it ensures that the surrounding seawater microbial community is maintained. However, natural experiments are only as helpful as the natural variability in the host-microbe system, and generally only afford the opportunity to study events such as seasonality, animal growth or other life history events. Artificial systems such as aquaria or mesocosms offer opportunities to manipulate environmental conditions or expose the animal to antibiotics or other molecules that are difficult to dose in the wild. However, not all animals are ideal for these systems (e.g., large whales, hydrothermal vent worms), and it can be challenging to reproduce some environmental conditions. Advances in aquaria design that offer consistency in environmental conditions and the ability to manipulate complex environmental interactions, such as the Australian Institute of Marine Science's National Sea Simulator, provide opportunities to conduct more realistic experiments. As the need to understand how host–microbiome interactions will alter with the forecasted changes in ocean temperature and pH, facilities such as this will become critical to animal–microbiome research in the ocean.

While studies of marine animal–microbiome interactions are certainly plagued by a number of challenges, the future is also very bright for this emerging field. Many of the new bioinformatics and methodological advancements now available to marine biologists stem from the biomedical field, and thus marine animal microbiome research, as well as other environmental-based

fields, are profiting from the elevation in microbiome research funding and attention. There could also be growing interest in using marine animals as models for examining resilience, promoted by the fact that alterations in the ocean conditions are often outpacing those in terrestrial environments. Given the phylogenetic breath of animals in the ocean, coupled with the many diverse ocean environments, there is certainly a wealth of research opportunities available to study host–microbiome interactions in the ocean.

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The author confirms being the sole contributor of this work and approved it for publication.

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Conflict of Interest Statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Studying Ocean Microbiomes Data

Prevalence of certain marine topics appearing in combination with the term holobiont in the title, abstract, and/or keywords of scientific publications from 1990 to February 2021 (N = 1269). Bars indicate the percentage of publications that contain terms related to a certain marine topic (cnidarians, corals, sponges, etc.). It is important to note that the different topics may overlap in several publications. For example, many publications treat of the topics of coral and sponge holobionts together. Data retrieved from the curated citation and abstract database Scopus on the date 10/03/2021. (Stevenne et al., 2021)

Check your Understanding

- Which environmental factor is the biggest driver of change for the community composition of free-living oceanic microbes?
- What factors affect the normal symbiotic state between marine host and their microbiome?
- Describe one well-studied marine animal-microbial symbiosis and its importance.
- What are some challenges to studying marine host-associated microbiomes?

Media Attributions

- Figure 1 – Illustration and common names of representative ocean animal life within their approximate depth-defined ecological habitats by Apprill, 2017. Licensed under the terms of the Creative Commons Attribution License (CC BY).
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- Figure 3 – Photographs of marine animals and their associated microbiomes from select study systems by Apprill, 2017. Licensed under the terms of the Creative Commons Attribution License (CC BY).

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8.3: Soil Microbiomes

Soil Microbiomes

The terrestrial landscape on Earth harbors countless microbes that have major responsibilities in shaping their surrounding environment. One gram of soil can contain up to 10 billion microorganisms and consist of thousands of different species. Each ecosystem has unique soil properties that cultivate a diverse array of microbial communities, which are primarily composed of bacteria, however archaea, protists, fungi, viruses, and other microscopic organisms can be found in varying abundances too. The composition and health of the surrounding environment and its macro inhabitants are dependent on their soil borne microbial partners, and a disturbance in the balance of the soil microbiome can have far-reaching effects (Omotayo and Babalola, 2021).

Environmental and agricultural sustainability is important in a number of regards, especially maintaining biodiversity, plant and animal health, ecosystem homeostasis, and even human health. Anthropogenic effects on the climate and environment have become abundantly apparent, and it is imperative to adjust mindsets to become more environmentally conscious, not only to preserve aspects of nature, but to accommodate an increasing human population. The soil is foundational to agricultural practices, and therefore food production, and so a better understanding of various soil microbiomes could serve to benefit future applications and practices (Tosi et al., 2020). One possibility, is to become less reliant on synthetic nitrogen fertilizers for soil, and utilize symbiotic nitrogen-fixing bacteria as sources for plant nitrogen.



Microbe to Microbiome: A Paradigm Shift in the Application of Microorganisms for Sustainable Agriculture

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Light, water and healthy soil are three essential natural resources required for agricultural productivity. Industrialization of agriculture has resulted in intensification of cropping practices using enormous amounts of chemical pesticides and fertilizers that damage these natural resources. Therefore, there is a need to embrace agriculture practices that do not depend on greater use of fertilizers and water to meet the growing demand of global food requirements. Plants and soil harbor millions of microorganisms, which collectively form a microbial community known as the microbiome. An effective microbiome can offer benefits to its host, including plant growth promotion, nutrient use efficiency, and control of pests and phytopathogens. Therefore, there is an immediate need to bring functional potential of plant-associated microbiome and its innovation into crop production. In addition to that, new scientific methodologies that can track the nutrient flux through the plant, its resident microbiome and surrounding soil, will offer new opportunities for the design of more efficient microbial consortia design. It is now increasingly acknowledged that the diversity of a microbial inoculum is as important as its plant growth promoting ability. Not surprisingly, outcomes from such plant and soil microbiome studies have resulted in a paradigm shift away from single, specific soil microbes to a more holistic microbiome approach for enhancing crop productivity and the restoration of soil health. Herein, we have reviewed this paradigm shift and discussed various aspects of benign microbiome-based approaches for sustainable agriculture.

Introduction

The health of soil plays an essential role in the ability of plants to produce food, fuel, and fiber for a growing world population. To keep pace, the total area of cultivated land worldwide has increased over 500% in the last five decades with a 700% increase in fertilizer use and a several-fold increase in pesticide use (Banerjee et al., 2019). In addition to being the world's largest agricultural producers and exporters, the EU, Brazil, United States, and China also are some of the world's largest pesticide users – each using 827 million, 831 million, 1.2 billion, and 3.9 billion pounds of pesticides, respectively, in 2016 (Donley, 2019). However, these numbers are not sustainable from either a supply-chain or environmental perspective. Thus, because natural resources are limited and their overuse pollutes the environment, the continued use of fertilizers and water to meet the demand of future global food requirements is not sustainable. Of relevance here is that agricultural intensification with high resource use and low crop diversity can negatively affect soil- and plant-associated microbiota (the so-called "phytobiome") with subsequent impacts on critical ecosystem services (Matson et al., 1997).

There is growing evidence that aboveground plant diversity supports belowground microbial biodiversity, primarily through root exudation and rhizo-deposition (Bais et al., 2006; Eisenhauer et al., 2017; Morella et al., 2020). These more simple carbohydrates released into the soil primarily feed bacteria (Gunina and Kuzyakov, 2015) and are the most abundant near the root surface and diffuse along a gradient as distance from the root increases (Gao et al., 2011). The microbial composition is more abundant and complex in the rhizosphere, the narrow zone surrounding plant roots, with up to 10^9 cells per gram in typical rhizospheric soil, comprising up to 10^6 taxa (Lakshmanan et al., 2017). The more complex carbohydrates (e.g., lignin, cellulose) are largely degraded by decomposer fungi that break down these recalcitrant compounds into forms that can be used by other microbes. This conversion is largely decoupled from conventional agricultural practices, wherein the organic matter content is often lost to the system (Craven and Ray, 2019), and the carbon flux is at least partially unregulated in this regard. Again, defining nutrient fluxes with techniques like Stable Isotope Labeling (SIP) holds great potential to define and construct resilient, functioning and beneficial microbiomes that can contribute to future holistic agriculture. Thus, applying an efficient and diverse soil microbiome backed by these new technologies can facilitate and promote sustainable agriculture and can effectively contribute to meet the triple requirements of economic, social and environmental sustainability (Ray and Craven, 2016).

Historically, microorganisms that promote plant growth and nutrient acquisition have been used largely as single strains in agriculture to offset such fertilizer inputs as nitrogen and phosphorous. However, studies of natural populations suggest that groups of microbes with distinct function niches play pivotal roles in adhering and desorbing inorganic nutrients to physical surfaces, as well as breaking down organic residues and incorporating them into the soil (Lakshmanan et al., 2014; Finkel et al., 2017; Kumar and Dubey, 2020). Conceptually, such observations support the idea of the microbiome as a second genome or an extended genome of the plant (Vandenkoornhuyse et al., 2015). It is now evident that improving plant performance in a sustainable manner is beyond the binary interaction between a specific microbe or a consortium of beneficial microbes and a targeted host plant. This is a much more complex set of interactions than previously thought that requires modeling for improving predictable outcomes. In this review, we will highlight the current state of the art for the incorporation of specific plant growth-promoting microorganisms and discuss the principles and management practices for next-generation, microbiome-based approaches for sustainable agriculture.

Application of Beneficial Microbes in Sustainable Agriculture: Past, Present and Future

Since the early 1800s, the United States Department of Agriculture has recommended the use of certain rhizobacteria to improve nitrogen fertility in leguminous crops (Schneider, 1892). Since that time, a great deal of research has been conducted on this relationship between legumes and these bacteria, now termed rhizobia, that inhabit unique structures, the nodules, that form on the roots. Rhizobia infecting these nodules are now capable of "biological nitrogen fixation," whereby di-nitrogen is fixed into forms that can be used by the plant. Symbiotically, the bacteria trade these nitrogenous compounds to the host plant in exchange for photosynthetically derived carbon. Despite these limited applications, much remains to be learned regarding both the functional and taxonomic diversity of these symbiotic bacteria and their host plants, the role they play in the global nitrogen cycle, and ultimately, how they can best be harnessed for improving plant productivity. This is particularly true for marginal lands that are not suited for row crop production but will need to be incorporated into global food and forage production approaches moving forward. Further, such degraded lands must but regenerated with the goal of restoring soil health and productivity. Any successful endeavor in this regard must include a characterization of the soil microbiome, both taxonomically and functionally. Attempts currently are underway to fix nitrogen in such non-legumes as wheat, corn and other staple crops that produce the bulk of human food by engineering symbiotic relationships using synthetic biology approaches (Rogers and Oldroyd, 2014; Ryu et al., 2020). Such

approaches would significantly impact global food supplies, and may function adequately to reduce the arable land required to meet productivity goals.

Plant growth-promoting microbes not only play critical and diverse roles in growth promotion *per se*, but also in improving various aspects of plant resilience against a wide array of biotic and abiotic stresses (Arnold et al., 2003; Sun et al., 2010; Agler et al., 2016; Azad and Kaminskyj, 2016; Singh, 2016; Oleńska et al., 2020; Rai et al., 2020). In this context, researchers globally have worked over the last several decades on plant growth-promoting microorganisms, such as root-associated mycorrhizal fungi, across a broad range of crops and encompassing a wide range of agro-climatic conditions. For perspective, Brundrett and Tedersoo (2018) recently reviewed 135 years of mycorrhizal research and reported that merely 8% of the vascular plants are non-mycorrhizal, suggesting that plant families associating with mycorrhizae have been very successful over the evolution of the plant kingdom.

Traditionally, agricultural application of beneficial microorganisms involves a few types of well-characterized microbes, such as mycorrhizal fungi or rhizobia bacteria, for which the mechanisms underlying the plant growth promotion effects are well understood. Further, most of these studies focused solely on the ability of the applied microorganisms to facilitate such specific plant growth-promoting traits as phosphate solubilization, nitrogen fixation, ACC deaminase production (Sarkar et al., 2018), siderophore production, biofilm formation, plant hormone production, biotic, and abiotic stress tolerance or resistance, among others (Weyens et al., 2009; Bhattacharyya and Jha, 2012; Singh et al., 2019). While these beneficial microorganisms can impart considerable benefits to plant growth and fitness, they are typically documented in simple, one-on-one studies, often conducted in sterile soils in greenhouse conditions. As a consequence, the effects found in such simplified conditions often fail to translate to more complex field situations (Chutia et al., 2007; Nicot et al., 2011; Parnell et al., 2016). Soil in field plots have more complex microbial environments that are presumably adapted to the local eco-environment.

In recent years, next-generation sequencing has revolutionized our understanding of microbial community composition and function, and together with improved culturing methodologies has greatly facilitated the use of biologicals in the field (Schweitzer et al., 2008; Panke-Buisse et al., 2014; Mueller and Sachs, 2015). Specifically, metagenomics-based approaches have uncovered vast, previously unrecognized populations of microbes that may have new or enhanced properties that could be used for agriculture, bioremediation, and human health. For example, comparative analyses of rhizosphere metagenomes from resistant and susceptible tomato plants enabled the identification and assembly of a flavobacterial genome that was far more abundant in the resistant plant rhizosphere microbiome than in that of the susceptible plants. Such findings certainly reveal a role for native microbiota in protecting plants from phytopathogens, and pave a way forward for the development of probiotics to ameliorate plant diseases akin to human health (Kwak et al., 2018). In another study, a 16S rRNA gene amplicon sequencing analysis of maize root microbiome led to the identification of bacteria that promote growth under low temperature conditions (Beirinckx et al., 2020). Additionally, principles of consortium design that rely on cross-talk, cross-feeding and/or substrate channeling between different microorganisms offer new opportunities for “intelligent” consortia design (Calvo et al., 2014; Vorholt et al., 2017; Paredes et al., 2018). We propose that the manipulation of the plant microbiome holds tremendous potential for agricultural improvement (Table 1). Through recent years of research, it is elucidated how microbes worked in nature before, and how decades of chemical fertilizer use have silenced their ability to improve plant fitness and soil health. Therefore, designing a microbial consortium that carefully weighs and evaluates the relationship between inoculants and the resident microbiome would substantially improve the plant growth-promoting potential and resilience of agricultural biologicals to boost plant growth. In this review, we will discuss the key considerations that would improve the likelihood of microbial products to improve crop yield, decrease disease severity and/or ameliorate abiotic stress response. Further, it is likely that such considerations would reduce the inconsistency between the performances of beneficial microbes from controlled greenhouse conditions and more natural environments.

Category	Salient findings	Reference
Plant growth promotion	Chilling temperatures critically affects growth of Maize in N. hemisphere. This study reported enrichment of *Comamonadaceae* and the *Pseudomonadaceae* in the root endosphere of maize grown under chilling conditions. Additionally two bacterial strains were identified from the root endosphere that could boost maize growth under chilling conditions.	Beirinckx et al., 2020
	A root endophytic bacteria Sphingomonas sp. Cra20 improved growth of Arabidopsis thaliana under drought stress by stimulating the growth of lateral roots and root hairs. Additionally, the relative abundance of Sphingomonas increased in the rhizosphere bacterial community in the water-deficit treatment, suggesting the role of Sphingomonas sp. Cra20 in alleviating drought induced stress.	Luo et al., 2019
	A community-based culture collection (CBC) approach was undertaken to isolate bacteria from the stalks and rhizosphere of Sugarcane. Subsequently, a synthetic community was designed by cross-referencing the CBC with the sugarcane microbiome profile that comprised of highly abundant bacterial groups from roots and stalks. The synthetic community could successfully improve the biomass of sugarcane, and was found to displace the native rhizosphere bacteria community.	Armanhi et al., 2018
	Willows (Salix spp.) were grown in gamma-irradiated petroleum-contaminated soils. Plants were inoculated with contaminated rhizosphere soil from a willow that grew well, or with contaminated bulk soil in which the plants had died. Willows inoculated with bulk soil performed better than those inoculated with rhizosphere soil. Microbiomes of different treatments were divergent at the beginning, but had converged at the end of the study, suggesting lasting effect of inoculated microbiome on plant growth, but not on the rhizosphere microbiome.	Yergeau et al., 2015
Plant defense response	Microbiome structure of Banana endosphere in the roots and shoot tips were investigated during plant growth and wilting processes. The keystone bacterial species belonging to the family *Enterobacteriaceae* family were isolated and further engineered to express ACC deaminase. Plants inoculated with engineered *Enterobacteriaceae* strains increased resistance to the Fusarium wilt disease. The findings illustrate that the keystone species in the banana microbiome plays functional role in the wilt resistance.	Liu et al., 2019
	Composts represent a sustainable way to suppress diseases and improve plant growth. Compost derived microbial communities enriched in the rhizosphere of Tomato were analyzed for antifungal activity against soil-borne fungal pathogens. Subsequently, microbial synthetic communities (SynComs) were designed with an overarching aim to improve plant fitness. SynComs were found to promote tomato growth as well as suppressed Fusarium wilt symptoms in controlled conditions.	Tsolakidou et al., 2019
	Tomato variety Hawaii 7996 is resistant to the soil-borne pathogen *Ralstonia solanacearum*, whereas the Moneymaker variety is susceptible to the pathogen. Rhizosphere microbiome analysis revealed clear differences in community profile of these two varieties. Transplantation of rhizosphere microbiota from resistant plants suppressed disease symptoms in susceptible plants. Additionally, a flavobacterium strain isolated from resistant plant rhizosphere microbiome was found to suppress R. solanacearum in susceptible plants.	Kwak et al., 2018
	A simplified synthetic bacterial community based on maize rhizosphere microbiome was designed representing most dominant phyla to study their functional attributes in maize seedlings. This synthetic community inhibited the phytopathogenic fungus *Fusarium verticillioides*, both *in planta* and *in vitro*. This study indicates how community profile information can be utilized to design beneficial microbial consortia for improving plant fitness and productivity	Niu et al., 2017
Abiotic stress response and nutrient use efficiency	Recognition of microbes by plant immune system mediated by phosphate stress was investigated. A representative synthetic bacterial community (SynCom) was designed that comprised of 35 bacteria isolated from the roots of Brassicaceae. SynCom enhanced the activity of PHR1, the master transcriptional regulator of the PSR, in Arabidopsis thaliana grown under limited phosphate. Additionally PHR1, repressed plant's immune system in phosphorous starved regime, validating plant's prioritization of nutritional stress over defense.	Castrillo et al., 2017
	Nitrogen-use efficiency of indica varieties of rice is superior to that of japonica varieties. Root microbiome analysis of these two varieties revealed that microbiota of indica and japonica highly distinct. Further, it was found that this distinctness was associated with a rice nitrate transporter and sensor NRT1.1B. Based on microbiome analysis, microbial synthetic communities (SynComs) were designed. It was found that indica-enriched SynCom improved rice growth in organic nitrogen conditions compared with a japonica-enriched SynCom.	Zhang et al., 2019
	Root associated microbiome of drought-sensitive pepper plant (*Capsicum annuum* L.) were analyzed focusing on role of microbes conferring plant growth under water limitation. Subsequently pepper root associated culturable bacteria were isolated and evaluated for plant growth promotion and drought tolerance abilities. The composition of the cultivable community associated to rhizosphere and root surrounding soil fractions shared a high similarity. Most of these isolates were able to promote plant growth and alleviate drought-induced stress with enhanced abilities observed in *Bacillus* and the *Rhizobacteria* strains.	Marasco et al., 2012
	Community profiling microbiome associated with superior halo-tolerant seepweed Suaeda salsa revealed that rhizospheric and endophytic bacterial community were enriched in genes responsible for salt stress acclimatization. This suggest that S. salsa preferentially recruit halotolerant taxa to confront soil salinity. Based on root endosphere core microbiota, halotolerant bacterial and fungal strains belonging to Pseudomonadales and Montagnulaceae were isolated. It was demonstrated that these core microbiome members were successfully able to improve growth and salt tolerance in the non-host rice plant.	Yuan et al., 2016

Table 1. List of recent publications in plant and soil microbiome focusing on plant fitness and productivity.

Microbes for Plant Growth Promotion: A Reductionist Approach

Sustainable agriculture primarily focuses on reducing the dependency of plants on chemical fertilizers and improving their ability to grow on marginal soil types. For such purposes, individual microorganisms for plant growth-promotion have largely focused on those that facilitate growth and development by enhancing acquisition of nutrient resources from the environment, including fixed nitrogen, iron and phosphate, or modulating growth by altering plant hormone levels (Figure 1) (Hayat et al., 2010). Another approach aimed at reducing yield losses to disease relies on microbes that decrease or prevent the deleterious effects of plant pathogens by several different mechanisms (Glick, 2012), i.e., by acting as a biocontrol agent. Microbe-based plant growth-promoting products, more popularly marketed as biofertilizer, has been commercially available in many countries since the 1950s (Timmusk et al., 2017). Application of such plant growth-promoting microbes in agricultural context and more specifically as inoculants has been nicely reviewed by Souza et al. (2015). However, under certain cases, the results obtained in the laboratory could not be reproduced in the field primarily due to the presence of many crop species and crop varieties, variable environmental conditions between fields, (Timmusk et al., 2017; Saad et al., 2020), occasionally due to the low quality of the inocula, and their inability to compete with the indigenous population. In that context, it is important to consider the fact that there is always greater likelihood of success by introducing mixed cultures of compatible microorganisms, rather than single, pure cultures. This is simply because each strain in the multi-strain consortium can compete effectively with the indigenous rhizosphere population and enhance plant growth with its partners. For example, sequential inoculation of nitrogen fixing bacterium *Azotobacter vinelandii*, followed by plant growth-promoting root-endophytic fungus *Serendipita indica* demonstrated better growth in rice (Dabral et al., 2020). Dual inoculation of *S. indica* and *Mycolicibacterium* strains boosted the beneficial effects in tomato (del Barrio-Duque et al., 2019) and that of arbuscular mycorrhizal fungus with plant growth-promoting bacteria *Bacillus subtilis* demonstrated better growth in wheat (Yadav et al., 2020) as compared to the singly inoculated plants. There also are numerous other reports that showed two strains used in a consortium promoted plant growth in a more effective manner (Nadeem et al., 2013; Fatnassi et al., 2015; Priyadharsini and Muthukumar, 2016). Nevertheless, to unlock the full potential of soil microbes for such nutrient cycling as nitrogen or phosphorus and providing plant protection against biotic and abiotic stress microbiomes, it is necessary to develop strategies to comprehend the functional capabilities of soil microbial communities. Irrespective of the approach, persistence is the first and foremost principle underlying the design of a successful microbial consortium for conferring plant growth promotion. This is not surprising, as the survival and activity of microbes in any soil system face a monumental task of competing with the myriad of microbes naturally adapted to that same soil. Thus, in addition to establishment of a compatible interaction with the host, a successful microbial inoculant has to subsequently compete and persist in the context of indigenous microbes as well as local abiotic conditions (Finkel et al., 2017). It has been reported that bacterial inoculations can persist in soil up to 7 weeks, but whether this inoculum also can provide plant growth benefits is not clear (Schreiter et al., 2014). While persistence or resilience of any microbial inoculum is more dependent on biotic components of a specific soil type, their persistence can be improved by inoculating crops with consortia rather than single strains (Verbruggen et al., 2012; Nemergut et al., 2013). Thus, it can arguably be stated that the diversity of a microbial inoculum, in addition to its plant growth-promoting traits, is critical for enhancing productivity and longevity (Cordero and Polz, 2014).

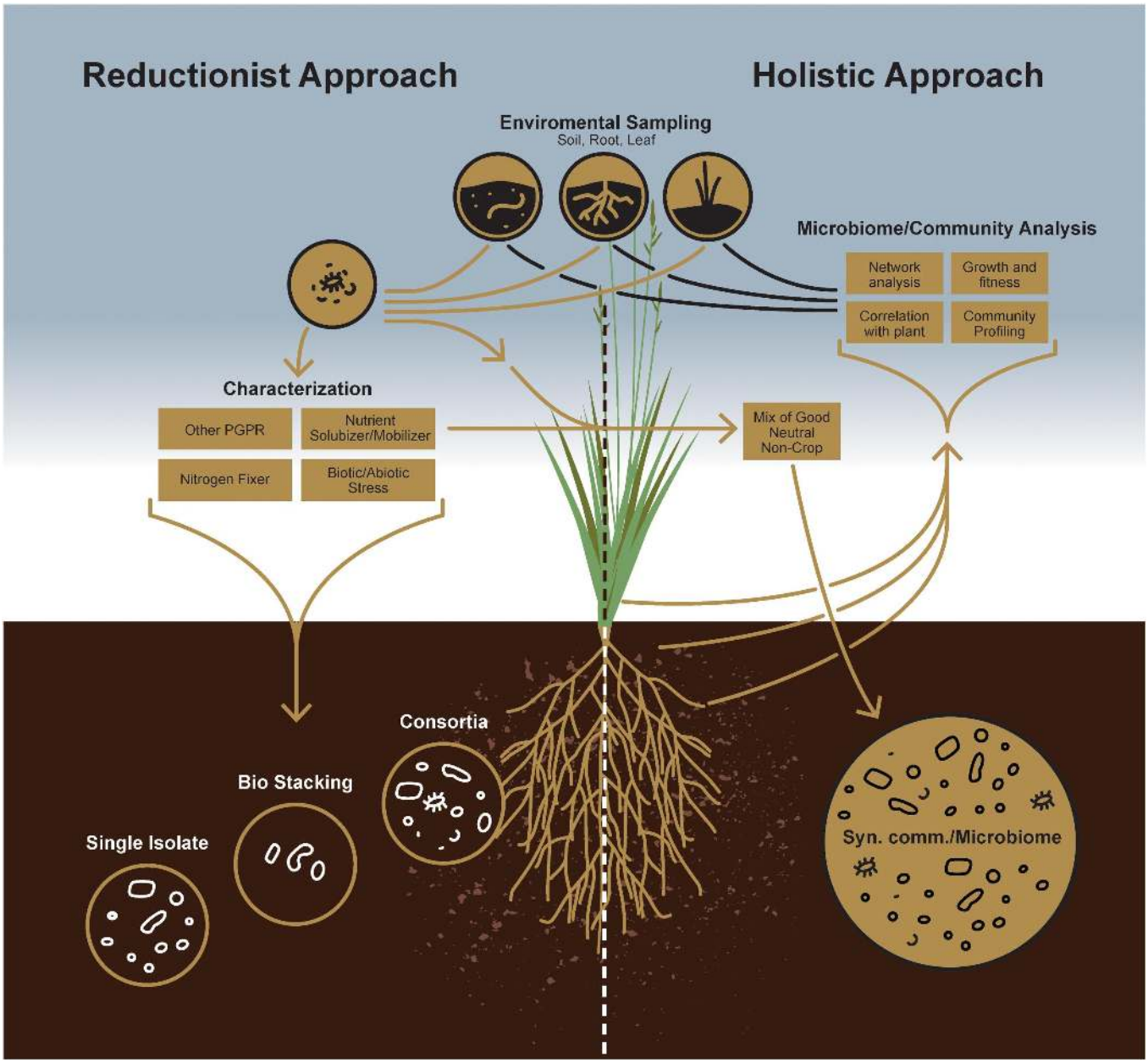


Figure 1. A schematic comparison between individual microorganism-based reductionist approach and microbial community-based holistic approach.

To improve the likelihood of success for such a management strategy, *a priori* knowledge of indigenous microbial populations competing with the introduced plant growth-promoting agent(s) is critical. While a reductionist approach can define the currency of individual plant-microbe interactions, the concepts of microbial community survival and functioning require, a more holistic, microbiome-based approach empowered by next-generation sequencing technology to study plant-microbe interactions at the community level (Figure 2). Indeed, this will enable researchers to design more robust, synthetic microbial consortia capable of reliably enhancing agricultural productivity.

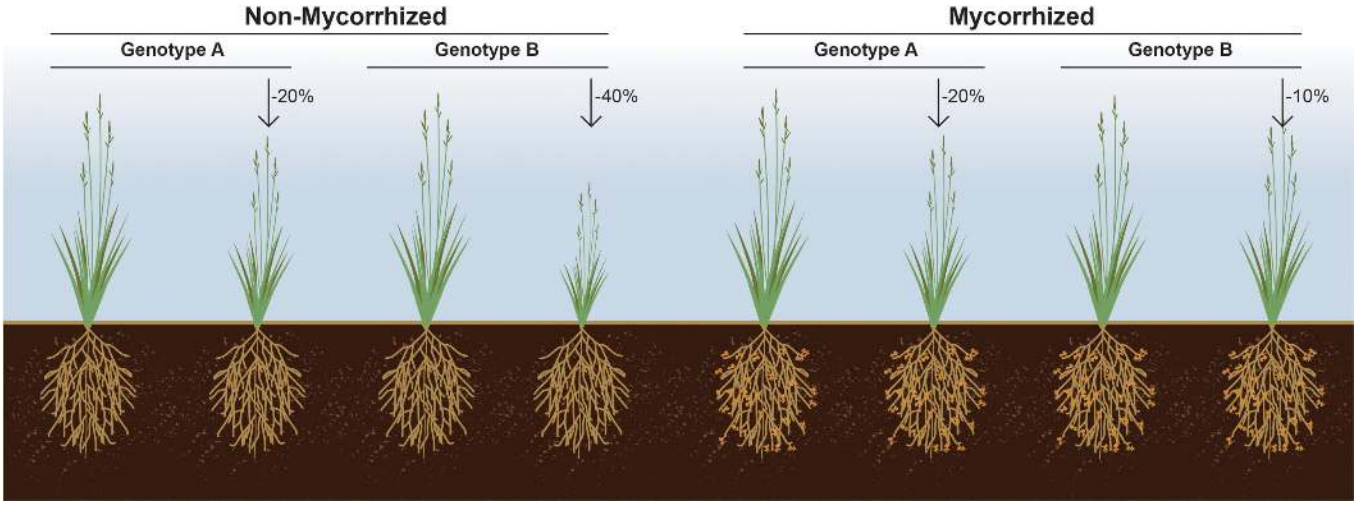


Figure 2. Responsiveness as the% gain in plant fitness attribute in response to symbiosis over un-colonized cohorts. This figure illustrates a hypothetical situation wherein genotype A loses less biomass (–20%) in response to soil nutrient limitation than does genotype B (–40%). However, if genotype B for its inherently associated rhizosphere microbiome responds optimally to a mycorrhizal symbiont, then it may be that it loses the least biomass (–10%) due to soil nutrient limitation, if the symbiont is present.% denotes loss in biomass due to soil nutrient limitation.

Microbiomes for Plant Growth Promotion: The Holistic Approach

Soil is a vastly heterogeneous growth medium, providing a wide spectrum of ecological niches for microorganisms that enable diverse strains to coexist and form complex microbial communities. When the earliest plants extended their roots into primordial soils, they encountered a habitat already teeming with bacterial and fungal life (Bulgarelli et al., 2013; Kemen, 2014). Since that early time, plants have interacted with rhizosphere microbes, evolving strategies to forge beneficial alliances with some while keeping others at bay. Such early associations certainly had consequences on plant growth and development. Therefore, a more holistic approach is needed to understand better these microbes and the roles they play in the overall health of plant and soil (Figure 1). Again, recent advances in next-generation sequencing technology and the decreasing costs associated with that technology now allow us to evaluate how microbial populations fluctuate in both space and time or to identify core microbiomes that appear conserved among host genotypes or species (Sergaki et al., 2018). Thus, although culture-independent methods have contributed tremendously to our understanding of plant-associated fungal and bacterial community structures, the study of microbiome functions remains challenging because of the inherent noise of plant-associated microbial communities. It is now well known that there are core sets of microbes that, depending on the host, are recognized as keystone taxa that consistently associate with healthy plants (Banerjee et al., 2018). Consequently, researchers working with specific plant-microbe interactions have increasingly acknowledged the mitigating impact these larger microbial communities have on individual plant-microbe outcomes for plant growth promotion or fitness. Now, plant-associated fungal and bacterial stains from various plant species are being isolated, which will provide in the near future an inestimable resource for assembling taxonomically defined microbial communities with increasing complexity. Therefore, it is now imperative to take advantage of this knowledge to design consortia of microbes to maintain a sustainable rhizosphere community, with key functional properties that include plant protection, nutrient acquisition, and alleviating biotic and abiotic stress responses. From that perspective, synthetic community (SynCom) approaches can provide functional and mechanistic insights into how plants regulate their microbiomes (Figure 1). Not surprisingly, recent culture-independent analyses thus have paved the way for developing SynComs more often (Bodenhausen et al., 2014; Armanhi et al., 2018; Carlström et al., 2019).

Mycorrhizal fungi, at least the arbuscular type, were early symbiotic partners of most land plant species, improving nutritional conditions through soil exploration and pathogen resistance of host plants (Klironomos et al., 2000). In reward for the essential physiological services, they receive ca. 20% of net photosynthetic products from plants (HoÈgberg et al., 2001). Other mycorrhizal systems may have different nutritional benefits and costs, as has been proposed for the serendipitous system (Craven and Ray, 2019). Additionally, third-party partners can modulate the outcome of the tripartite interaction, such as the case of mycorrhizal helper bacteria (Frey-Klett et al., 2007), fungal endobacteria (Bonfante and Desirò, 2017; Bonfante et al., 2019) like *Candidatus Moeniiplasma glomeromycotorum* within the spores and hyphae of Glomeromycotina (Naito et al., 2017), *Rhizobium radiobacter* within *Serendipita indica* (Guo et al., 2017), and N_2-fixing endobacteria *Pseudomonas stutzeri* inside basidiomycetes yeast

endophyte *Rhodotorula mucilaginosa* (Paul et al., 2020). Hence, it is imperative to consider the composition and functioning of these microbe–microbe interactions to understand plant–microbiome associations in a holistic manner.

Principles and Management of Rhizosphere Microbiomes for Sustainable Agriculture

Competence and Resilience of the Rhizosphere Microbiome: Impact of Introduced Microbes on Native Microbiomes

In 1904, the German agronomist and plant physiologist Lorenz Hiltner coined the term *rhizosphere* (Hartmann et al., 2008) to describe the area around a plant root inhabited by a unique population of microorganisms. Since then, numerous studies have been undertaken to decipher the interplay between plants and rhizosphere microorganisms, encompassing a wide variety of plant growth-promoting bacteria, fungi, insects, protozoans, viruses, etc. (Marschner, 2012; McNear, 2013). The majority of these studies have traditionally followed a simple principle for maximizing successful host infection by pre-inoculation onto the targeted crop of choice to provide a competitive advantage for a desired microbe. Conceptually, this increases the relative abundance of a given beneficial microbe in the rhizosphere, at least temporarily, to achieve the desired benefit. Such studies typically take place in a controlled, artificial condition, such as a defined growth medium in a greenhouse, where competition from a native rhizosphere community is relatively low or non-existent. As mentioned above, this approach occasionally has failed once field application is attempted or the benefits are dramatically reduced in amplitude and/or endurance.

As an example, Lekberg and Helgason (2018) conducted a literature survey of research papers published on mycorrhizal functioning spanning a 30-year period (1987–2017). The most striking finding of this survey was that less than 5% of the work scientifically manipulated mycorrhizal abundance in the field. While we are not arguing the merit of greenhouse-based studies where the number of variables can be controlled and accounted for, yield gains in field conditions will continue to be modest with such an approach. Rhizosphere competence must be evaluated in a field situation if the true power of this approach is to be realized.

Over the last few decades, mycorrhiza-based bio-fertilizers containing one or several species of fungi were developed in forestry and agriculture (Jeffries and Rhodes, 1987; Baraza et al., 2016; Igiehon and Babalola, 2017). These inoculants are generally effective in plant growth promotion under controlled lab and greenhouse conditions. However, few targeted efforts have been made to measure interactions between the introduced microbe(s) and the native mycorrhizal community, let alone the more complex rhizosphere microbiome (Svenningsen et al., 2018; Turrini et al., 2018). To optimize outcomes from these interactions, targeted research must be undertaken to understand how such mycorrhiza-based biofertilizer integrate themselves within the context of the native microbiome.

Integration of Rhizosphere Microbiomes in Plant-Microbe-Nutrient Relationships

The soil microbial community often assists plants by weathering minerals from rock surfaces and degrading recalcitrant soil organic matter whereby soil microbes break down soluble and insoluble organic matter and convert it into inorganic, plant-available forms. Soil organic matter turnover is thus considered a net positive, as it liberates the nutrients locked up in organic matter. For this reason, conventional farming has always relied heavily on soil tillage, along with such other intensive agricultural practices as usage of inorganic fertilizers, herbicides and pesticides. However, it is already clear that such practices have negative consequences on the functional diversity of soil microbiomes. Long-term chemical fertilization has been shown to dramatically decrease the soil pH, which leads to a decrease in bacterial diversity and other changes in microbial community structure (Sun et al., 2015). This was well documented in the work of Kumar et al. (2017), who showed that long-term application of high doses of inorganic nitrogenous fertilizers severely reduces relative abundance, diversity and structure of diazotrophs, which play a key role in converting atmospheric N_2 to plant-available ammonium.

As mentioned above, soil bacterial communities play a pivotal role in soil organic matter decomposition. In particular, soil carbon and nitrogen are critical factors for bacteria that rely on soil organic C and N decomposition to obtain energy (Chen et al., 2014; Wild et al., 2014; Tian et al., 2018). Further, different types of soil C selectively manipulate soil microbial community composition, resulting in changes in such belowground ecosystem functions as decomposition and nutrient transfer and creating feedbacks that may affect overall plant growth and productivity (Orwin et al., 2006). For example, bacteria belonging to the genera *Chloroflexi*, *Nitrospirae*, and *Planctomycetes* preferentially feed on recalcitrant organic C, whereas *Proteobacteria* and *Bacteroidetes* prefer labile organic C present in the soil (Nie et al., 2018). For this reason, amending the soil with such organic fertilizers as compost or manure contributes to higher microbial diversity and biomass compared to mineral-fertilized soils, which in turn positively impacts soil health (Schmid et al., 2018; Banerjee et al., 2019). Unfortunately, only a few agroecosystem experiments exist that compare

organic and conventional management strategies over an extended period for evaluation of impact on soil health and restoration (Raupp et al., 2006; Khatoon et al., 2020). Hartmann et al. (2015) took a metagenomics approach to assess microbial diversity of soil in response to more than 20 years of continuous organic and conventional farming. Not surprisingly, they found that organic farming increased richness, decreased evenness, and shifted the structure of the soil microbiota when compared with conventionally managed soils under mineral fertilization (Hartman et al., 2018; Li et al., 2020b). There also are reports of significant alterations in the microbial community composition of both summer maize and winter wheat in response to increased nitrogen fertilization dose (Wang et al., 2018; Li et al., 2020a). Clearly, a better understanding of the interactions between the soil microbiome and conventional agricultural practices is crucial for the development of sustainable management of soil fertility and crop production.

Managing the Rhizosphere Microbiome to Induce Disease Suppression in Soil

Disease suppressive soils were originally defined by Baker and Cook (1974) as "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil." Disease suppressive soils are the best examples of microbiome-mediated protection of plants against root infections by soil-borne pathogens. Such disease-suppressive soils have been described for various soil-borne pathogens, including fungi, bacteria, oomycetes, and nematodes (Mazzola, 2007; Kwak et al., 2018). To date, several microbial genera have been proposed as key players in disease suppressiveness of soils, but the complexity of the microbiome, as well as the underlying mechanisms and microbial traits, remain elusive for most disease suppressive soils (Toyota and Shirai, 2018).

Recently, Carrión et al. (2019) showed that upon pathogen invasion, members of the *Chitinophagaceae* and *Flavobacteriaceae* became enriched within the plant endosphere. They proposed that this bacterial population shift led to the induction of enzymatic activities associated with fungal cell-wall degradation, as well as secondary metabolite biosynthesis, all aimed at accelerating and augmenting the plant defense response(s). Although the disease suppressive abilities of certain soils can be at least partially attributed to their physico-chemical properties, the capacity of a soil to suppress disease progression is more often attributed to agri-management practices and crop rotation (Weller et al., 2002). In classic studies by Gerlagh (1968) and Shipton et al. (1973), the authors have shown soil to become disease suppressive after mono-culturing wheat over time. More recently, a comparative metatranscriptome analysis of wheat rhizosphere microbiome grown in fields suppressive and non-suppressive to the plant pathogen *R. solani* AG8 clearly revealed distinct dominant taxa in these two soil types. Additionally, suppressive samples showed greater expression of polyketide cyclase, terpenoid biosynthesis, and cold shock proteins (Hayden et al., 2018). While development of probiotics for the human gut microbiome has already been an established field of research, the use of probiotics that comprises naturally occurring bacterial antagonists and competitors that suppress pathogens has recently emerged as a promising strategy for disease suppression in soil. A study on application of probiotic consortia that comprised predefined *Pseudomonas* species reported suppression of the bacterial plant pathogen *Ralstonia solanacearum* in the tomato rhizosphere microbiome (Hu et al., 2016). In another study, amendment of *Metarhizium*, an insect-pathogenic fungus that is commonly employed as biological control agents against crop pests, in the rhizosphere of common bean (*Phaseolus vulgaris*) significantly increased the relative abundance of plant growth promoting such taxa as *Bradyrhizobium*, *Flavobacterium*, *Chaetomium*, and *Trichoderma* while suppressing the root rot disease symptoms *Fusarium solani* (Barelli et al., 2020). Soil suppressive properties are mostly derived from the biological functions of soils. Therefore, elucidation of microbial functions in suppressive soils by a next-generation sequencing approach will facilitate the development of effective, consistent and durable disease management tools.

Impact of Agriculture Management Practices on the Soil Microbiome

One important context for plant-microbe interactions is soil structure, as it can vary greatly depending on land-use history, plant species composition and successional stage (Erktan et al., 2016). Besides playing pivotal roles in soil organic matter decomposition, carbon cycling, nutrient mobilization, etc., saprotrophic fungi also are involved in creating soil structure through the secretion of extracellular compounds and physical binding of soil via hyphal networks (Bergmann et al., 2016). Interestingly, studies on the impact of tillage on the soil fungal communities have shown mixed results. Reports in no-till systems have varied from increased ratios of fungal to bacterial biomass (Acosta-Martínez et al., 2010) to decreased ratios (Mbuthia et al., 2015), as well as no change at all (Mathew et al., 2012). More recent studies have shown that soil fungal communities are negatively impacted by tillage, as they typically would be responsible for degrading crop residue left on the surface with no-till (Yin et al., 2017). More specifically, soil bacterial communities were primarily found to be structured by tillage, whereas soil fungal

communities responded mainly to management type with additional effects by tillage (Hartman et al., 2018). Additionally, it is acknowledged that organically managed systems increased taxonomic and phylogenetic richness, diversity and heterogeneity of the soil microbiota when compared with conventional farming systems (Lupatini et al., 2017). In a simple definition, organic farming system consists of low-input agro-ecosystem farms in which plant productivity and ecosystem functionality are based on the natural availability of plant nutrients (Lammerts van Bueren et al., 2002). A study aimed at comparing the soil microbiome in conventional and organic farming systems in central Europe revealed no major differences among the main phyla of bacteria between the two farming styles (Armalytė et al., 2019), whereas another study that investigated the effects of 12 years of organic farming on soil microbiomes in northern China reported shifting of the community composition of dominant phyla and significant alterations of functional groups associated with ammonia oxidation, denitrification and phosphorus recycling when compared to conventional farming systems (Ding et al., 2019).

In addition to tillage, crop rotation also plays a pivotal role in increasing belowground microbial diversity compared to intensive mono-cropping practices. Although the United States Department of Agriculture has advocated [via the Conservation Reserve Program (CRP)] crop rotation to improve eroded land as early as 1985 (Allen and Vandever, 2005), its benefit on soil health has only been recognized recently. Several studies reported increases in such soil quality parameters as organic matter content, microbial biomass and respiration under crop rotation management when compared with a mono-cropping system (Campbell et al., 1991; Luce et al., 2013). A meta-analysis of 122 studies that examined crop rotation revealed similar findings, namely that adding one or more crops in rotation to a monoculture substantially increased the soil microbial biomass along with increases in total soil C and N, respectively (McDaniel et al., 2014). In another study, soil microbial communities of corn and switchgrass in mono-cropping systems when compared with mixed prairie grasses demonstrated that bacterial and fungal biomass, especially arbuscular mycorrhizal fungi, were higher in plots with mixed prairie grasses (Jesus et al., 2016). A 16S amplicon-based metagenomic analysis of an almost 20-year-old field trial in Bernburg, Germany revealed a significant effect of tillage practice and the preceding crop on prokaryotic community structures (Babin et al., 2019)

Cover crops are typically unharvested crops planted between cash crops that augment C provisioning to the soil system not only via unharvested residues, but also as root exudates that can support many rhizosphere microbes during the active growing season of the cover crop. Other benefits attributed to cover cropping include improved N fertility by incorporating legumes as a cover crop, reduced soil compaction via deep-rooted plants, and reduced erosion by keeping a plant and its root system in the field year round (Fernandez et al., 2016). Of various crop rotation management practices, those that include cover crops sustain soil quality and productivity by enhancing soil C, N and microbial biomass (Kim et al., 2020), making them a cornerstone for sustainable agroecosystems. Nonetheless, very few studies have assessed the relationship between cover crop stands and their associated belowground microbial communities. Early research in unfertilized grasslands demonstrated that fungal communities respond positively to plant-derived C inputs, suggesting that inclusion of cover crops in a rotation may promote fungal community development (Denef et al., 2009). More recently, a field study tested this hypothesis by specifically examining the impact on soil microbial communities of eight fall-sown cover crop species grown singly and in multispecies mixtures following a spring oats (*Avena sativa* L.) cropping season and found that certain cover crops selectively favored particular microbial functional groups. Arbuscular mycorrhizal fungi were more abundant beneath oat and cereal rye (*Secale cereale* L.) cover crops, while non-AM fungi were positively associated with hairy vetch (*Vicia villosa* L.) (Finney et al., 2017). Beyond positively affecting soil C and increasing the diversity of such beneficial fungi as arbuscular mycorrhiza, clover as a cover crop is often reported to suppress the relative abundance of pathogenic fungi (Benitez et al., 2016). Contrarily, in a 2-year field study, cover crops reportedly increased overall phylogenetic diversity of fungi but did not change the relative abundance of saprophytes, symbionts or pathogens, implying that cover cropping does not always appear to contribute to functional changes in the fungal community (Schmidt et al., 2019).

Reassessment of Plant Responsiveness to Symbiosis

It is now increasingly evident that plants employ fine-tuned mechanisms to shape the structure and function of their microbiome, with different genotypes of the same plant species growing in the same soil yet associating with distinct microbial communities (Berendsen et al., 2012). This is demonstrated in the findings of Bazghaleh et al. (2015), who clearly demonstrated the importance of intraspecific host variation in the association of chickpea cultivars with AM and non-AM fungi. Therefore, specific traits of a plant that modulate its microbiome should be considered as a trait for plant breeding (Wallenstein, 2017).

Despite the obvious importance of beneficial microorganisms for plant growth and fitness, and the impact of plant genotype on shaping their microbiome composition, plant germplasm is typically screened in the absence of microbes, and the selection of best

breeding lines made solely based on the interaction between plant genotype and performance under various abiotic factors. We propose that an *a priori* examination of the interaction between a plant genotype(s) and the symbiotic microbes upon which it likely depends is an important factor in the selection of plant breeding lines. It seems very likely that a subset of rejected germplasm could outperform others, but only when coupled with a beneficial microbe or microbiome (Figure 2). Arguably, current breeding and selection efforts most likely result in decoupling of the soil microbiome from plant fitness. As a result, modern varieties may have lost their ability to support diverse microbiomes and thus, fail to gain the most from these interactions (Wallenstein, 2017).

It is now acknowledged that transitioning from a highly intensive mono-cropping system to a more diversified cropping system consisting of multiple host genotypes leads to increased bacterial and fungal diversity (Calderon et al., 2016). Hence, future plant breeding efforts should incorporate plant characteristics that are related to microbiome diversity. For example, efforts focusing on manipulating plant root exudates likely play a critical role in selective recruitment of the rhizosphere microbiome (Bakker et al., 2012). In support of this notion, it has been shown that plants can select which microbial populations receive the lion's share of root exudates, demonstrating a capacity by the host to refine its microbial composition. Hence, an unbiased screening of plant genotypes for responsiveness in the presence of a beneficial microbe or microbiome can set forth a new and potentially transformative paradigm in selecting microbes for plant growth promotion (Figure 2).

Significance of Mycorrhizas: A Critical Component of Healthy Soil Rhizospheres

Mycorrhizae are mutualistic associations between soil fungi and plant roots that gradually evolved to be reciprocally beneficial to both partners (Brundrett, 2002). The benefits are generally assumed to involve an exchange of photosynthetically derived carbon from the host plant in exchange for soil nutrients provided by the foraging mycorrhiza. While likely true of arum-type arbuscular types of mycorrhizae, there are other types that can derive carbon from organic matter in the soil, or even "steal" it from one host plant to supply to another (Allen and Allen, 1991). A recent study has reported that in contrast to *Arum maculatum*, in which carbon is entirely derived from photo-assimilation, the green leaves of *Paris quadrifolia* contain a striking 50% carbon of fungal origin. Such partial mycoheterotrophy could thus potentially be widespread among the roughly 100,000 plant species that are known to develop a Paris-type AM, with far-reaching implications for our understanding of C trading in plant-microbe communities (Giesemann et al., 2019). Exactly what the mycorrhiza gains from this interaction is still under debate, but benefits may involve a safe haven from the open, more competitive soil space and a second, more reliable carbon source (Sapp, 2004).

Mycorrhizae not only shape plant communities, but they also affect the functional diversity of their cohabitants in the rhizospheric microbiome. The mycelium of mycorrhizal fungi transports plant-derived carbon into the soil in the form of sugars, amino acids and polyols to help sustain the microbiome (Tarkka et al., 2018). More recent studies focusing on soil microbial ecology revealed that mycorrhizal fungi mediate many diverse interactions within the soil "mycorrhizosphere," including pathogens and mutualists that fix atmospheric nitrogen, take up phosphorus, produce vitamins, and/or protect against antagonists (Buée et al., 2009; Tedersoo et al., 2020). The "ectomycorrhizosphere," which forms a very specific interface between soil and many trees, hosts a large and diverse community of microorganisms that likely play roles in mineral weathering and solubilization processes (Uroz et al., 2007). This carbon-rich mycorrhizosphere also supports large communities of root-associated microorganisms that further accelerate weathering of minerals by excreting organic acids, phenolic compounds, protons, and siderophores (Drever and Vance, 1994; Illmer et al., 1995).

Similarly, the extraradical hyphae of arbuscular mycorrhiza provide a direct pathway for the translocation of photosynthetically derived carbon to the soil, leading to the development of nutrient-rich niches for other soil microorganisms, particularly bacteria. A quantitative real-time PCR method detected significantly higher 16S rDNA abundance in both the bulk and the rhizosphere soils of zucchini (*Cucurbita pepo* L.) inoculated with *Acaulospora laevis* and *Glomus mosseae* (Qin et al., 2014). Additionally, arbuscular mycorrhizae have been reported to increase the relative abundance of *Firmicutes*, *Streptomycetes*, *Comamonadaceae*, and *Oxalobacteraceae* inhabiting the mycorrhizosphere (Offre et al., 2007; Nuccio et al., 2013). While there is clear evidence that microbial communities in the rhizosphere function cohesively with their mycorrhizal partner in nutrient mobilization from soil minerals, nitrogen cycling and protection of plants against root pathogens, such bidirectional synergy is not always universal. There are reports that indicate suppressive effects of bacterial communities on mycorrhizal functioning and vice versa. While one study reported (Svenningsen et al., 2018) that soil with a higher abundance of *Acidobacteria* suppresses the normal functioning of extra-radical mycelium in arbuscular mycorrhizae, another study found that *Glomus intraradices* and *Glomus mosseae* suppressed most of the associated soil microbial community (Welc et al., 2010).

A Novel Type of Endophytic Symbiont: The *Serendipitaceae*

A diverse group of fungi in the Basidiomycota, the *Serendipitaceae* (formerly Sebacinales Group B) (Oberwinkler et al., 2014) encompasses endophytes and lineages that repeatedly evolved ericoid, orchid and ectomycorrhizal types. Accordingly, in many natural ecosystems these fungi form mycorrhizal symbioses with an astounding variety of host plants – every mycorrhizal type, in fact, except for arbuscular. Previous research performed in our lab with a strain of this group, *Serendipita vermifera*, demonstrated plant growth-promoting properties in a variety of plants (Ghimire and Craven, 2011; Ray et al., 2015; Ray and Craven, 2016; Ray et al., 2020). Unfortunately, the agronomic utility of these fungi is hampered by the paucity of strains available, the large majority isolated from Australian orchids. We have begun to address this constraint by isolating the first North American strain of *Serendipita*, named *Serendipita vermifera* subsp. *bescii* NFPB0129, from the roots of a switchgrass plant in Ardmore, Oklahoma (Craven and Ray, 2017; Ray et al., 2018).

As mentioned above, soil organic matter has a tremendous influence on the biological, chemical, and physical properties of soils, making it a vital component of healthy agricultural systems. Whether a natural soil or an agricultural one, the release of the nutrients locked within SOM requires decomposers, primarily insects, fungi, and bacteria, to secrete organic acids and enzymes that can loosen and break down the cellulose and the recalcitrant lignin into nutritive forms that can be used by other microbes and plants. Unlike arbuscular mycorrhizae, which exchange inorganic, mineralized nutrients mined from the soil for carbon derived from host photosynthesis, members of the *Serendipitaceae* studied thus far have a complete arsenal of carbohydrate-active enzymes (CAZymes), representing approximately 4% of the entire gene set and rivaling the more well-studied saprophytic white and brown wood rotters, and much more than other symbiotic fungi. Additionally, genome analysis of *S. bescii* and *S. vermifera* suggests that *Serendipitaceae* fungi have the metabolic capacity to assimilate N from organic forms of N-containing compounds (Ray et al., 2019). We hypothesize that this carbohydrate-degrading enzyme complement endows these *Serendipitaceae* fungi with saprotrophic abilities (Craven and Ray, 2019). Unlike free-living decomposers that maintain a solitary lifestyle, seeking only dead or dying plant tissues as their source of subsistence, *Serendipitaceae* fungi seem to maintain a largely symbiotic lifestyle with the roots of living host plants. It currently is unclear whether there is expression of CAZymes while strains of *Serendipita* are in symbiosis with host plants, and if so, whether there is spatial or temporal separation from more mutualistic traits. Still, the capacity of some strains to form mycorrhizal relationships with orchids, where the seeds require carbon from the fungus for germination and often well into the plant's lifespan, suggests that these *Serendipitaceae* symbionts may be less of a carbon cost to their host plant. Presumably, this saved carbon could potentially be used for other symbiotic relationships or developmental processes. In any case, these intriguing fungi and their seemingly unlimited host range provide a novel symbiosis that could be used in a broad variety of cropping systems.

Conclusion

Soil-dwelling microorganisms are critical components of soil health, itself a determinant of plant productivity and stress tolerance. Deploying microbes to improve agriculture productivity is an extremely attractive approach that is non-transgenic and can be viewed collectively as the extended plant genome. Because these same microbes can contribute to restoring soil health and productivity, they have a bright future in low-input, sustainable agriculture that extends beyond more classically defined plant-microbe symbioses.

Author Contributions

PR and KC conceived and planned the overall idea of the review manuscript. PR, VL, JL, and KC wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Hotspot Quiz

Check your Understanding

- What roles does the soil microbiome have in the movement towards sustainable agriculture?
- What is a rhizosphere?
- What type of soil fungi are associated with promoting plant health as well as the soil microbiome? How do they do so?
- What are the challenges with studying plant growth promoting microorganisms?
- Explain the difference between a reductionist vs. holistic approach for improving plant growth with microorganisms.
- What types of agricultural practices can alter the soil microbiome?
- How could the use of 'probiotics' suppress disease-causing soil microorganisms?

Media Attributions

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8.4: Plant Microbiomes

Plant Microbiomes

Plant health is influenced by a variety of environmental factors, and though the soil content (including the microbial community present) is a major element, the distinct plant microbiome serves an important role. Similar to organ systems in the human body (e.g. skin, gut, etc.), the components of various plants (e.g. internal tissues, leaves, roots, etc.) can have unique microbial communities that contribute to its respective and overall vitality. A better understanding of the corresponding roles of plant microbiomes can be combined in a synergistic effort with other environmental microbiomes to maintain and promote sustainable agricultural practices, ecosystem biodiversity, and research model studies.



Plant microbiome–an account of the factors that shape community composition and diversity

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Abstract

Plants live in association with diverse microorganisms, collectively called the microbiome. These microbes live either inside (endosphere) or outside (episphere) of plant tissues. Microbes play important roles in the ecology and physiology of plants. Significant progress has been made in revealing structure and dynamics of plant microbiome in the last few years. Various factors related to host, microbes as well as environment influence the community composition and diversity of plant microbiome. This review aimed to provide a general account of the factors (host, microbe and environment) that drive the microbial community composition in plant. First, we gave an overview of the aboveground and belowground plant microbiomes. Next, we discussed which host factors are involved in variation in plants followed by importance of microbe-microbe interactions and the elements of environment that influence composition and community structuring of plant microbiomes.

Introduction

A diverse kind of microorganisms associated with a higher organism (human, animal, plants etc.) is together defined as its microbiome. All higher organisms examined to date, including plants, insects, fish, rats, apes, and humans, harbor microbiomes [1,2]. Research on the human microbiome has progressed very quickly. Recently, researchers have also paid much attention to elucidating the composition and functions of plant and soil microbiomes. It is now believed that plants are not separate entities, but rather they live in association with a large variety of microbes. These microbes live either inside (endosphere) or outside (episphere) of plant tissues. Among these microorganisms, bacteria and fungi are predominant. About a few thousand bacterial and fungal taxa have been reported from plant tissues [3,4]. They play important roles such as increased nutrient availability, uptake by plants and increased plant stress tolerance. Thus, plant fitness (growth and survival) is the result of physical and physiological functions of the plant *per se* as well as the associated microbiome, which together are known as a plant holobiont [5].

The study of the association of plants with microorganisms precedes that of the animal and human microbiomes, notably the roles of microbes in nitrogen and phosphorus uptake. The most notable examples are plant root-arbuscular mycorrhizal (AM) and

legume-rhizobial symbioses, both of which greatly influence the ability of roots to uptake various nutrients from the soil. Some of these microbes cannot survive in the absence of the plant host (the 'obligate symbionts' including viruses, some bacteria and fungi), which provides space, oxygen, proteins, and carbohydrates to the microorganisms. The association of AM fungi with plants has been known since 1842, and over 80 % of land plants are found associated with them [6]. It is thought that AM fungi helped in the domestication of plants [7]. Traditionally, culturable microbes have been used for plant-microbe interaction studies with the enormous unculturable microbes remain uninvestigated and consequently, our knowledge of the roles of these unculturable microbes remains largely unknown.

Unraveling the types and outcomes of plant-microbe interactions has received considerable interest among ecologists, evolutionary biologists, plant biologists, and agronomists [4,8,9]. Recent developments in meta-omics and the establishment of large collections of microorganisms have dramatically increased our knowledge of the plant microbiome composition and diversity. The sequencing of marker genes of entire microbial communities, referred to as metagenomics, sheds light on the phylogenetic diversity of the microbiomes of plants. It also adds to the knowledge of the major biotic and abiotic factors responsible for shaping plant microbiome community assemblages [8].

However, our understanding on the roles of microbiomes, with respect to their impact on plant ecology and physiology, is still far from complete, and we are at the beginning of knowing their functions [10]. The outcome of this improved knowledge will have significant bearings on a variety of experiments and applications, such as development of biofertilizer and biopesticides for sustainable agricultural production with less reliance on agrochemicals, while augmenting yield and nutritional value [11].

In this review, we will present an account of recent studies and prospects of studying the plant microbiome. Firstly, we will present an overview of aboveground and belowground plant microbiomes. Next, we will discuss the factors driving the composition and community structuring of plant microbiomes. We will not address plant pathogenic microbes, although inclusion of this fraction of microbiome would make sense from a broader and more holistic viewpoint.

Rhizosphere microbiome

The rhizosphere comprises the 1–10 mm zone of soil immediately surrounding the roots that is under the influence of the plant through its deposition of root exudates, mucilage and dead plant cells [12]. A diverse array of organisms specialize in living in the rhizosphere, including bacteria, fungi, oomycetes, nematodes, algae, protozoa, viruses, and archaea [13]. The most frequently studied beneficial rhizosphere organisms are mycorrhizae, rhizobium bacteria, plant growth promoting rhizobacteria (PGPR), and biocontrol microbes. Gans, Wolinsky [14] projected that one gram of soil could harbor more than a million distinct bacterial genomes. İnceoğlu, Al-Soud [15] reported 55,121 OTUs (operational taxonomic unites) from the potato rhizosphere. Among the prokaryotes in the rhizosphere, the most frequent bacteria are within the Acidobacteria, Proteobacteria, Planctomycetes, Actinobacteria, Bacteroidetes, and Firmicutes [3,16]. In some studies, no significant differences were reported in the microbial community composition between the bulk soil (soil not attached to the plant root) and rhizosphere soil [17,18]. Certain bacterial groups (e. g. Actinobacteria, Xanthomonadaceae) are less abundant in the rhizosphere than in nearby bulk soil [3].

Mycorrhizal fungi are abundant members of the rhizosphere community, and have been found in over 200,000 plant species, and are estimated to associate with over 80 % of all plants [19]. These mycorrhizae–root associations play profound roles in land ecosystems by regulating nutrient and carbon cycles. Mycorrhizae are integral to plant health because they provide up to 80 % of N and P requirements. In return, the fungi obtain carbohydrates and lipids from host plants [20]. Recent studies of arbuscular mycorrhizal fungi using sequencing technologies show greater between-species and within-species diversity than previously known [21].

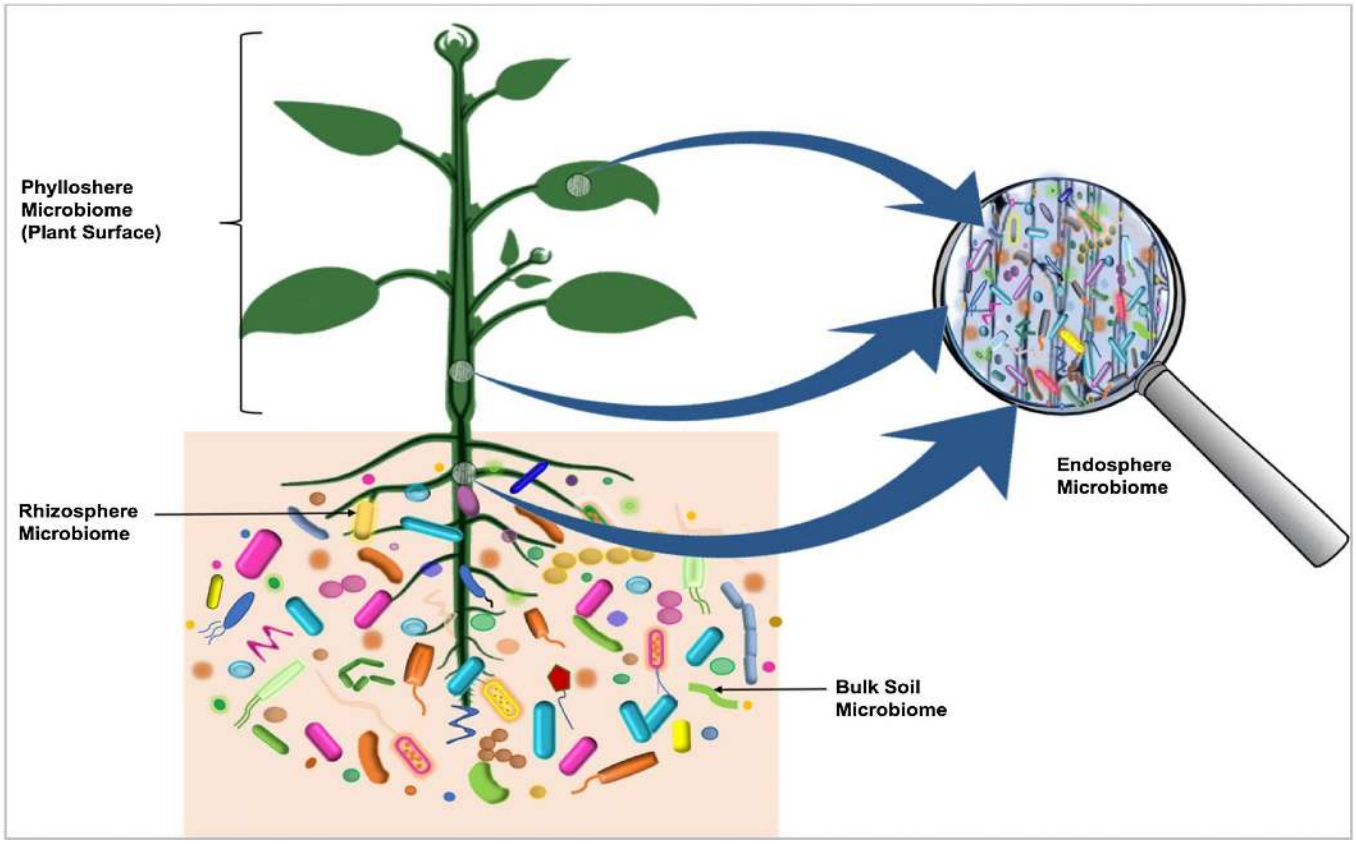
Phyllosphere microbiome

The aerial surface of a plant (stem, leaf, flower, fruit) is called the phyllosphere and is considered comparatively nutrient poor when compared to the rhizosphere and endosphere. The environment in the phyllosphere is more dynamic than the rhizosphere and endosphere environments. Microbial colonizers are subjected to diurnal and seasonal fluctuations of heat, moisture, and radiation. In addition, these environmental elements affect plant physiology (such as photosynthesis, respiration, water uptake etc.) and indirectly influence microbiome composition. Rain and wind also cause temporal variation to the phyllosphere microbiome [22]. Overall, there remains high species richness in phyllosphere communities. Fungal communities are highly variable in the phyllosphere of temperate regions and are more diverse than in tropical regions [23]. There can be up to 10^7 microbes per cm^2 present on leaf surfaces of plants, and thus the bacterial population of the phyllosphere on a global scale is estimated to be 10^{26}

cells [24]. The population size of the fungal phyllosphere is likely to be smaller [25]. Phyllosphere microbes from different plants appear to be somewhat similar at high levels of taxa, but at the lower levels taxa there remain significant differences. This indicates that microorganisms may need finely tuned metabolic adjustment to survive in phyllosphere environment [24]. Proteobacteria seems to be the dominant colonizers, with Bacteroidetes and Actinobacteria also predominant in phyllospheres [26]. Although there are similarities between the rhizosphere and soil microbial communities, very low similarity has been reported between phyllosphere communities and those in open air [27].

Endosphere microbiome

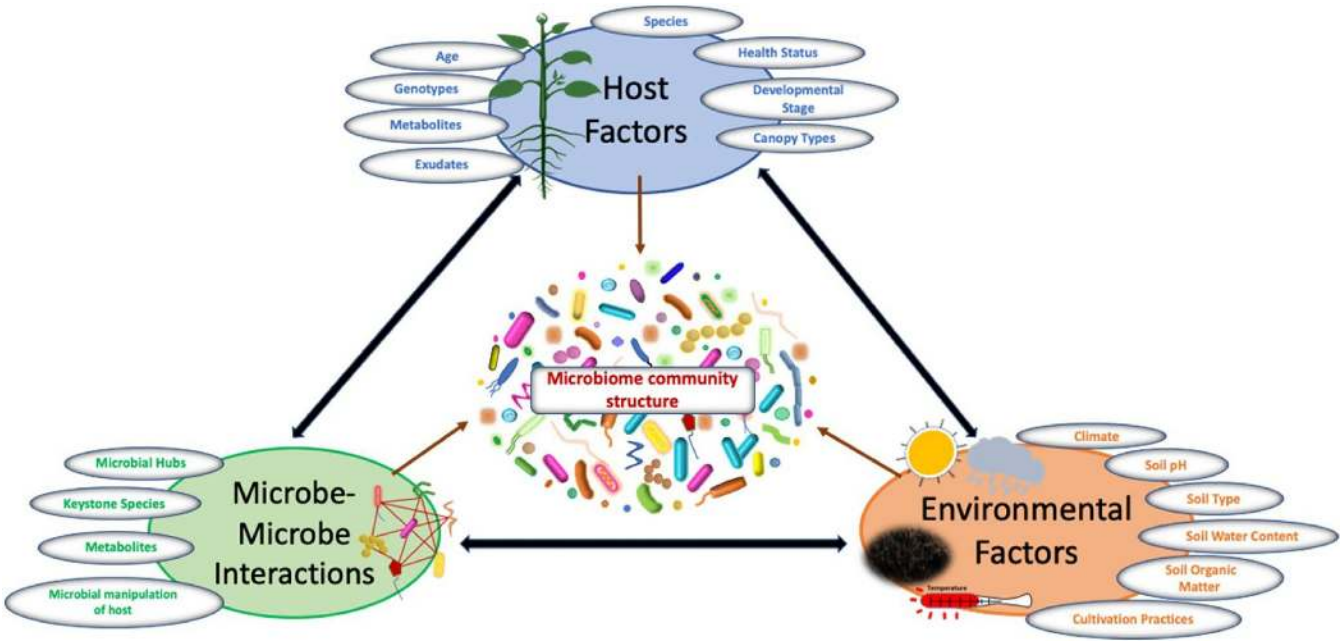
Some microorganisms, such as endophytes, penetrate and occupy the plant internal tissues, forming the endospheric microbiome (Fig. 1). The AM and other endophytic fungi are the dominant colonizers of the endosphere [28]. Bacteria, and to some degree Archaea, are important members of endosphere communities. Some of these endophytic microbes interact with their host and provide obvious benefits to plants [[29], [30], [31]]. Unlike the rhizosphere and the rhizoplane, the endospheres harbor highly specific microbial communities. The root endophytic community can be very distinct from that of the adjacent soil community. In general, diversity of the endophytic community is lower than the diversity of the microbial community outside the plant [18]. The identity and diversity of the endophytic microbiome of above-and below-ground tissues may also differ within the plant [28].



The ‘Plant Microbiome’ consists of diverse microbial communities on the outside surface and in internal tissues of the host plant. The rhizosphere, endosphere, and phyllosphere constitute the major compartments of plant microbiome. The soil microbiome is an important source of the plant microbiome.

Drivers of plant microbiome composition

Plant microbiome structure is influenced by complex interactions between hosts, microbes, and associated environmental factors such as climate, soil, cultivation practices etc. (Fig. 2). Below, we provide an assessment of current knowledge of these factors, providing insight to plant-microbe interactions in a broader-sense.



A simple schematic representation of various factors that shape microbiome community structures. The host, microbe, and environment all factors are interconnected and together build the microbiome community in plants.

Host factors that influence plant microbiome community composition

Plant species

The identity of the host plant has a significant influence on the identity of its microbiome. Different plant species growing adjacent to one another can harbor distinct microbiomes. A comparative survey of root microbiomes in maize, sorghum, and wheat showed different community composition among these plants [32]. Samad et al. [33] investigated the microbiome compositions of roots and rhizospheres using 16S rRNA gene from grapevines and some weed species growing in the same field, and their findings suggested that these species hosted significantly different microbiomes in the roots and rhizosphere, with the more pronounced difference in the root communities. Plants that are distantly-related phylogenetically show greater variation in associated microbiome compositions, suggesting a role of plant phylogeny in structuring root microbiomes [32]. Plant species also influences the identity and diversity of endophytic communities. Manter et al. [34] reported differences in endophytic community composition in potato and *Eucalyptus* plants. The most abundant bacterial root endophytes in potato were rare or absent in *Eucalyptus* and vice-versa [34] suggesting that the host plant selects its endophytic microbes. Analysis of endophytic fungi of three native Australian *Nicotiana* species revealed that they are host-specific but not plant organ- or host location-specific [28]. In addition to the rhizospheric and endophytic microbiomes, phyllosphere community composition also depends on plant identity [24]. Kembel et al. [35] showed that the leaf microbiome community is highly correlated with plant evolutionary relatedness similar to the endospheric microbiome.

The effects of host plant species in recruiting microbes from the surrounding environment indicate that plants have evolved traits that govern root microbiome assemblages [36]. For example, endosphere, rhizosphere community composition are correlated with host taxonomy [36]. Xiao et al. [37] found that the rhizosphere and root microbiomes are mostly influenced by soil type, and the nodule and root endophytes are influenced by plant species. Differential microbiome assembly in different plant species is attributed to variation in plant resource consumption [36]. Plant traits such as leaf permeability, wettability and topography and physicochemical properties, cuticle chemistry, root exudates, antibiotic production, and inherent plant immunity to invasion by microbes may also to play a role.

Plant genotypes

Evidence suggests a difference in microbe community composition between genotypes of a particular species [17,26,38]. Genetics of the host is one of the factors that shape the plant-microbiome structure. For example, OTUs in three different potato varieties were cultivar-specific [36]. Similarly, cultivar-dependent effects have been reported for the bacterial communities in young potato rhizospheres [15]. Peiffer et al. [39] demonstrated that OTU richness and β-diversity are influenced by plant genotypes in maize.

Bulgarelli et al. [38] reported that genotype contributed to about 6% of the variation of the microbiome composition in the rhizosphere region. A larger influence of host genotype on community composition has been reported [40]. Genotype-dependent microbiome community structuring has been reported for sweet potato, wheat, pea, and oat [9,41]. Bacteria such as *Acinetobacter*, *Chryseobacterium*, *Pseudomonas*, *Sphingobium*, and *Stenotrophomonas* were more abundant in low-starch cultivars than those having high-starch contents [41]. The rhizosphere communities of different genetic clones of wild-type and transgenic lines have been reported to be distinct in *Populus* [42]. Within-species genetic variability can influence microbiome composition in leaf tissues [43]. Wagner et al. [44] conducted an extensive field experiment to unravel drivers of community composition of bacteria associated with leaves and roots of *Boechera stricta*. Their findings suggested that the host genotype influences leaf community, but the root microbiome was variable at different collection sites.

Agler et al. [45] proposed that host genotype influences on keystone microbes, which then pass these effects onto the total microbiome by changing microbe–microbe interactions and altering plant fitness [3]. The host specificity of plant microbiome could also be attributed to the nutrient preferences of plants [46]. However, whether the observed host influences are heritable, how strong the effect is, and whether these associations will be actionable for plant breeding is yet to be established.

Plant organ

Different plant tissues host distinct microbiome communities. Edwards et al. [47] demonstrated that each surface and internal tissue of plants may harbor distinct microbial communities and that the role of tissue-type was greater than host type and the microbiome of the soil. This may be because the adaptation strategies of various tissues may affect the microbes in colonizing them for community composition. For instance, surface tissues are exposed to constant fluctuations of weather and have relatively poor nutritional status compared to the root or internal tissues. Therefore, microbes colonizing the leaf surface need to be adapted in these conditions [25]. Other studies found very little or no effect of plant organ in community composition of fungi and bacteria [28].

Study with agaves determined that prokaryotes are largely influenced by the plant compartment, whereas the rhizosphere, the phyllosphere, and endosphere communities are clearly different from each other and from adjacent soils [48]. Many studies have reported that fungal communities show a different pattern, where the biogeography of host were the major influencing factor [48]. This may be explained by the dispersal limitation in fungi [49], because fungi are eukaryotes like plants and animals, but bacteria are different in this respect.

Plant age and developmental stage

In the cases of interactions between plants and pathogens, ontogenic resistance (age-related resistance) is widely reported and correlated with plant developmental stage [50]. Symbiosis research has also indicated that plant age and developmental stage are important factors affecting microbial communities [51]. Analysis of the bacterial rhizosphere community of *Arabidopsis* revealed that the seedling stage selects distinct microbiomes at developmental time points. Plants produce mixtures of compounds and specific phytochemicals in the root exudates. Some of these chemicals are indeed distinct at plant developmental stages and appear to shape microbiome community assemblages [52]. The effect of the plant age on microbiome using DGGE fingerprint analysis revealed that it significantly influences bacterial community composition of all groups investigated for all three sweet potato cultivars [41]. The effect of plant age on the composition of bacterial microbiome in the rhizosphere has been demonstrated in potato, maize and soybean [15,53,54]. Age-related microbiome differentiation may be associated with root growth, physiology, root architecture, root morphology, root exudate, and its composition [51,52]. However, further research is needed on the identity and effect of root exudates at different plant developmental stages to determine how plants communicate in the rhizosphere. This knowledge might offer a basis for augmenting agricultural crops by the application of rhizosphere microbes.

Plant canopy type

Plant canopy type also influences microbiome community composition. For example, bacterial communities in sugar maple leaf samples are correlated with canopy composition [55]. The microbial migration through rain runoff may be an important factor for variation in microbial colonization in different canopy types. Canopy structure influences the composition of endophytic community but not the rhizospheric community, indicating less effect of rain runoff, and there may be other mechanisms such as soil factors or some unknown factors involved in this variation [55].

Plant immunity and signaling

Plant health status may influence microbiome composition. Plants employ two layers of defense against pathogens: pattern-triggered immunity (PTI), which is triggered by conserved molecular structures such as microbe/pathogen-associated molecular patterns (MAMPs/PAMPs), and damage-associated molecular patterns, which are recognized by plasma membrane-localized pattern recognition receptors [56]. It is unknown whether plants recognize non-pathogenic microbes in the similar way as they recognize pathogens and modulate their response. When plants are challenged with herbivory or pathogens they release hormones and exogenous volatiles that alter the composition of root exudates (for review, see [57]), and these in turn modify the microbiome community. Aphid infestation and pathogenic microbial infection increaseed populations of the non-pathogenic rhizobacterium *Bacillus subtilis* in the sweet pepper rhizosphere [58]. In *Arabidopsis thaliana* plants infected by *Pseudomonas syringae*, the expression of root malate transporter is altered, indicating a change in secretion of malic acid that increased the number of the beneficial rhizobacterium *Bacillus subtilis* [59,60]. Cucumber roots infected by *Fusarium oxysporum* f. sp. *cucumerinum* exhibited augmented secretion of fumaric and citric acid, which led to the formation of biofilms (aggregates of living bacteria in a slimy extracellular polymeric substances) of *Bacillus amyloliquefaciens*. Most investigations, however, have focused on one-to-one interactions (plant-microbe), although in reality, plants are subjected to attack by numerous microbial pathogens and insect pests. Therefore, it would be interesting to see how multiple herbivory and/or pathogens modify the community composition of plant microbiomes. Recent studies have reported a change in rhizosphere microbiome community composition as influenced by specific compounds such as sugars, sugar alcohols, or mixtures of various chemical compounds in root exudates [52]. Plants use various strategies in response to pathogenic infection and insect attack. One of them is activation of defense responses in roots, which may influence microbiome composition in the rhizosphere and roots [61]. When aphids feed on foliage both SAR (Systemic Acquired Resistance) and ISR (Induced Systemic Resistance) signaling are activated throughout the plants, which elicits sweet pepper plants to attract *B. subtilis* in the rhizosphere [58]. Again, increased JA signaling in plant either by injury or exogenously in *Medicago truncatula* caused higher colonization of beneficial mycorrhizae [62]. Different root and phyllosphere endophyte microbial communities have been reported when altered SA signaling was induced [63]. Plants lacking in jasmonate-mediated defense have shown more diverse epiphytic colonization [64]. It is evident from these studies that the role of plant defense systems on the microbial composition are inconstant, and that SAR is an important factor in regulating some bacterial community composition. Chemical signals released by plants for example, flavonoids, activate varied responses in plant rhizosphere microbiomes [65]. Branching in mycorrhizal hyphae is affected by strigolactones, which enhance and promote seed germination by parasitic plants [66].

Plant derived compounds

A diverse array of antimicrobial compounds are produced in plants [67]. Some of them, such as different alkaloids, phenolics, and terpenoids, are common in plants. Some are specific to particular groups [68], for instance, Brassicales produce glucosinolates. It was found that transgenic *Arabidopsis* producing an exogenous glucosinolate had different microbiomes in the rhizosphere and root tissues [69]. Voges et al. 2018 reported a significant role of plant derived coumarins in structuring the rhizosphere community. They suggested that iron-mobilizing coumarins are involved in redox reactions that can mobilize ferric iron and generate reactive oxygen species (ROS) with detrimental effects on microbial proliferation and thus selectively inhibit certain microbial growth while allowing proliferation other more beneficial partners. Triterpenoid saponins, which are known as avenacins, are found in oat (*Avena strigosa*), and have antifungal properties [70]. Oat plants lacking avenacin production attracted different culturable fungi in roots [71] and were more vulnerable to pathogenic infections than wild-type oat [72]. Interestingly, however, a recent comprehensive analysis of the rhizosphere community of these two genotypes reported little difference between the fungal communities. The effect of avenacins on the Amoebozoa and Alveolata was profound but has not been reported for bacterial communities [73]. This revealed that a small difference in plant genotype might exert multifaceted and unpredicted effects on the plant microbiome composition and diversity. These plants derived compound may affect microbiome assembly in different ways. For example, root exudates may be specific for host plant and can modulate rhizosphere community as well as selects specific root microbiome and thus contributing host specific plant microbiome. Also, antimicrobial compound may selectively enhance microbial growth by restricting certain microbes which is a kind of ‘balance’ in mutualism.

Microbial factors in shaping plant microbiome structure

Microorganisms play important roles in shaping microbial community structures in plants. However, our knowledge on how microorganisms influence microbiome structuring is limited.

Microbial manipulation of hosts

Microorganisms can affect host plants, for example host root exudations, which in turn affect the permeability of roots and root metabolism. Some microbes in soil where the plant if growing can also absorb certain compounds in root exudates and excrete other compounds. Soil microbes can produce compounds that affect plant signaling and metabolism, which lead to production of microbe-derived compounds in plants. Some microbes produce antibiotics (e.g., penicillin and polymyxin) which increase the exudation of organic materials, altered cell permeability, and increased leakage [74] and results in a variable microbiome assembly.

Microbe-microbe interaction

The extent to which microbe–microbe interactions can play roles in the microbiome composition is not well understood. The outcome of microbe-microbe interactions could be explained as cooperation, parasitism, and competition. In cooperation, at least one species benefits, while others are not harmed. When both species benefit, the term mutualism is used, whereas, when one partner benefits while the other is not affected, the term commensalism is used. In contrast, parasitism and competition are harmful for at least one species [75].

We know from recent studies that microbial communities harbor highly connected taxa called keystone taxa [76]. These taxa independently or in a group show a substantial effect on microbiome composition and functions regardless of their spatial and temporal dynamics. They play a unique and vital role on microbiomes, and their absence could cause a significant alterations in microbiome composition and functioning [76]. They use various strategies to impact on host microbiome. For instance, they might cause changes in intermediate or effector groups which in turn regulate microbiome community composition and functioning [77]. Production of a secondary metabolite (2,4-diacetyl phloroglucinol) was reported for some strains of *Pseudomonas fluorescens* that suppress *Gaeumannomyces graminis* var. *tritici*, responsible for wheat take-all [78]. Similarly, many fungi (e.g. *Trichoderma* and bacteria (e.g. *Bacillus*) produce various secondary metabolites that suppress microbial growth [79,80]. The keystone taxa may produce bacteriocins to shift microbiota structure selectively. Again, by synergism, keystone taxa may alter the abundance of their partners, and influence community structure and performance. For example, certain species of *Burkholderia* are symbiotic with arbuscular mycorrhizae and may change abundance and community composition of AM fungi, thereby influencing plant community richness, diversity, and production [19]. Agler et al. [45] studied the roles of microorganisms (bacteria, fungi and oomycetes) in the community composition of phyllosphere microbiomes of Arabidopsis thaliana using a systems biology approach. They described an interkingdom interactions network with a profound influence on community structure. They identified a few taxa, termed “microbial hubs’, which are highly interlinked and have a significant impact on communities. They used two “hub” microbes (Albugo, an oomycete pathogen and Dioszegia, a basidiomycete yeast) in detail. Albugo strongly affected epiphytic and endophytic bacterial colonization. Many symbiotic microbes (including pathogens) produce effector proteins to suppress, activate, or alter host defense mechanisms [81], and some can entirely reprogram the host metabolism [82]. These host adjustments can lead to alterations of microbiome composition because some microorganisms and not others can benefit from altered conditions. Actually, the niches of some microorganisms is dependent on others. For instance, primary colonizers can aid subsequent colonizers against hazardous abiotic factors [83] or can enhance the competitive ability of following colonizers by producing secondary metabolic compounds [84]. There can be direct microbe-microbe interactions, such as the hyper-parasitism (parasite of parasite) of primary colonizers [85] and opportunists that utilize host’s compromised plant defenses to colonize them [86]. Such phenomena point out why some colonizers can affect the development of microbes on the host even if they may be distantly related [84] and highlight a crucial functions of such interactions in shaping microbiome composition and structure.

One of the major strategies by which PGPR augments plant growth is by its influence on rhizosphere microbes. For example, *Pseudomonas* sp. DSMZ 13134 alters the composition of dominant bacteria in barley roots [87]. In some cases, however, the effects of PGPRs on resident microbiome may not be prominent. For example, no substantial changes in rhizosphere community were noticed after the application of *Bacillus amyloliquefaciens* FZB42 [88]. Supporting results have also been reported in soybean with the application of *B. amylolique faciens* BNM122 [89]. The recent investigation on the effect of *B. amyloli quefaciens* on the lettuce microbiome using 454-amplicon sequencing revealed no or only transient and minor effects in the rhizosphere zone [90]. Interestingly, a decrease in the bacterial number was reported. In the field only 55 % of the inoculated bacterial DNA could be traced after a month [91]. The effects of *Bacillus subtilis* strain PTS-394 on the rhizosphere microbiome has been examined by metagenomic profiling. Similar to the results above for *B. amyloliquefaciens* FZB42 [90,91], only a minor effect on the composition was reported. However, up until now, the impact of *Bacillus* PGPR on other plant microbiota, such as fungi, has not

been investigated, and investigation on this could reveal a more general effect of inoculated bacteria on resident microbiota and thus on host physiology and ecology.

Environmental factors as drivers of plant microbiome assembly

Environmental elements, such as soils, climatic conditions, geography, farming activities, and plant domestication, could result in the differences of plant associated microbial community composition [92]. A change in an environmental component results in plant phenotypic changes (e.g., [93], which consequently also change the assemblage of distinct microbiomes harboring plant compartments [94]. Plants grown under controlled conditions provide specific environments for microorganisms. For instance, when lettuce was grown under a glasshouse, a distinct bacterial signature was found from those grown in open fields [95]. Whitaker, Reynolds et al. [96] reported the community composition of endophytic fungi local environment (i.e., site), but not by host ecotype, pointing that environmental factors are major drivers of the endophytic mycobiome of switchgrass.

Soil

Plants recruit root microbes mainly from the soils where they grow. Various soil factors viz. soil types, soil pH, and the C/N ratio, as well as available P and K are frequently reported to be the determinants of root microbial community composition by affecting plant growth and immunity [8,9]. Innumerable studies using high throughput sequencing have proved that soil type is a major factor for root microbiome structure, which is evident from the differential initial microbial inocula present in different soil types [18,47]. Dombrowski et al. [97] collected the arctic-alpine *Arabis alpine* samples from the native location as well as from those grown under controlled conditions and investigated the root microbiome by 16S rRNA amplicon sequencing analysis. They reported that soil type and length of time the plants remained in the soil are the most important drivers, causing variation of up to 15 % of root microbiota. In addition, in the same soil, the root microbiome of perennial *A. alpina* was similar to *A. thaliana* and *Cardamine hirsuta*, the annual relatives of *A. alpina*. The root microbiome communities are strongly influenced by the composition of the soil microbiome close to roots. A strong correlation between the soil and root bacterial communities in *A. thaliana* has been reported by various authors [3,18]. Similarly, the structure of fungal communities is influenced more predominately by soil type than by host plant [98]. The type of soil influenced the rhizosphere bacterial microbiota composition in lettuce [99], oak [16], *Arabidopsis* [3], and maize [100].

Several studies have shown that environmental variability, such as soil pH, C: N ratio, soil carbon, water content and biogeography may influence the microbiota composition [39]. Lauber et al. [101] described that the impact of soil pH on total community composition was obvious even at a very high taxonomic level. Analyses revealed that pH has substantial correlation with the structure of these microbiome phyla in all soil types studied. The effects of soil pH on soil bacterial community composition has also been reported in other studies using various methods [102]. Zarraonaindia et al. [103] reported that the composition of soil and root microbiome of grapevine significantly influenced by soil pH and C:N ratio but leaf- and grape-associated microbiota were mostly influenced by soil carbon. Hartman, Richardson et al. [104] reported from their study that pH was the most important factor in predicting the alteration of soil bacterial communities, and they detected changes in phylum-level abundances across the pH levels. However, contrasting results have been reported by Fierer et al. [105], where they noted soil carbon was more important than soil pH for Bacteroidetes, Betaproteobacteria, and Acidobacteria. This difference of the findings was possibly linked to sample sizes and number of soil types investigated as well as to differences in methodologies. The exact mechanism(s) of the role of soil pH on microbial community composition and diversity is unknown. Two general explanations have been given [101]. Firstly, soil pH may indirectly alter bacterial community structure with its influences on soil characteristics. Secondly, soil pH may directly impose a physiological limit on soil bacteria, changing outcomes of competition or reducing survival of taxa intolerable to condition. In addition, we hypothesize that soil pH may alter plant microbiome by its influence on plant growth and physiology, which also may influence soil microbiome composition. Other soil properties, for example soil temperature and contaminants in the soil, also influence microbiome composition. Heat disturbance of soil results in a shift in rhizobacterial microbiome composition [106]. Heat disturbances due to natural wildfires can cause a decrease in microbial activity and significant alterations in microbial communities [107]. Increasing petroleum hydrocarbon contamination levels results in the alteration of willow microbiome structure. These alterations were less extreme in the rhizosphere and plant tissues, but they were prominent in the bulk soil. These could be because plants provide more controlled conditions and shield microbes against an enhanced contamination gradient [108].

Cultivation practices

Land use and cultivation practices are the most important causes of declines in biodiversity, leading to undesirable consequences for the environment [109]. Changes in the vegetation influence the diversity and structure of soil microbiomes. Agricultural activities do not necessarily have negative consequences in soil bacterial community diversity and structure, but they may have positive or neutral feedback (effect not perceived) [110]. For example, the intensity of land use (LUI) has been reported to influence the pattern of bacterial communities. Estendorfer et al. [111] found that under low LUI, there remains a strong interaction between plants and adjacent soil. In contrast, no influence of LUI on microbial diversity has been found in the rhizosphere, which indicates that plant species have much more influence on the rhizosphere community than soil properties do [112]. However, Suleiman et al. [113] reported that plants may have robust core microbiome compositions that are less prone to alteration due to variation of land use, soil type or edaphic factors. It was found that microbiome of in relatively untouched deciduous forest and long-term mowed grassland soils were comparable, although there were significant differences in soil properties and vegetation [114], Continuous cultivation causes changes in soil properties, which in turn might affect the soil microbiome communities. As per the reports of Allison and Martiny [115], there are three potential impacts caused by land disturbance such as the microbial structure might be affected, it might be changed but quickly return to the original composition (resilient), or might remain unchanged.

Climatic variables

Climate is an important driver of plant and soil microbiome composition. The role of climate in influencing plant microbiome community composition has to date been largely unheeded or found to be of little importance [116]. A recent study, however, across various ecosystems in Britain showed that rainfall and temperature gradients are two major climatic factors in shaping the bacterial community composition in plants [117]. Researchers de Vries et al. [118] reported that precipitation is an important driver of soil microbial community composition. The biomass of fungi and bacteria has been reported to increase with increasing mean annual precipitation (MAP), and the effect was more prominent for fungi, resulting in comparatively higher fungal abundance (increased F/B ratio) under higher precipitation levels. The above trends could be linked to higher soil organic matter contents in higher rainfall areas. In particular, in uplands where harsh climatic conditions prevail, a higher organic matter build-up is noticeable, which leads to fungi-dominated microbiome communities [119].

Conclusions and future perspective

The factors that influence microbiome assemblages and dynamics in plant and soil are now better understood, and research in this aspect is increasing. However, our knowledge on the underlying mechanism(s) of microbiome assemblages and how they influence the host plants is still lacking. Connecting the microbiome composition and diversity to their function is a great challenge for future research. For instance, to what extent could we use and manipulate the plant microbiome to boost sustainable agricultural production and environmental protection? We now know that that host genetic factor has a significant influence on microbiome diversity and structure, indicating that breeding and trait selection provide opportunities to select for desired microbiomes [45]. To have a more profound and broader knowledge on plant microbiome, there is a need to integrate novel molecular approaches (e.g., meta-omics), ecological models (e.g., food web theory, assembly of communities, or coexistence theory), and recent bioinformatics and statistical advances with a view to correlating community assemblages with ecological functions.

In the future, more emphasis should be placed on identifying the underlying mechanisms that drive microbiome community composition and assembly. We need to know the contributions of (1) the microbe-microbe interactions, (2) soil and other environmental variable and (3) various host traits in shaping community structure. Future research will direct toward solving some pertinent questions. For example, how stable are the drivers of microbiome community? To what extent do agricultural practices affect the microbiome of plant species? How predictable are these divers? With high throughput technologies, such as next-generation sequencing and metagenomics, we can begin to study endophyte microbiomes across hosts, environmental conditions, and at different time points and focus on the mechanisms of the plant-endophyte association.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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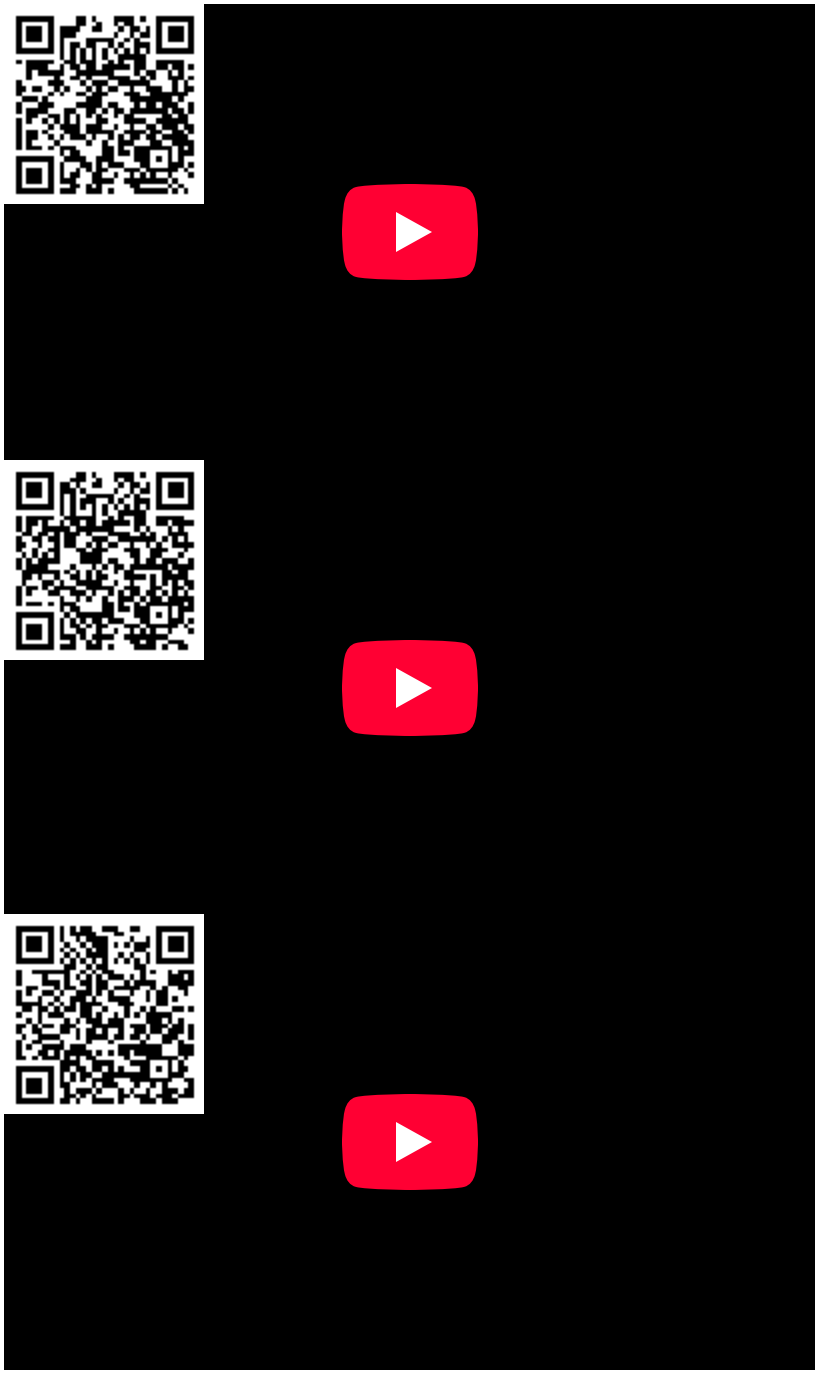
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Hotspot Quiz

Check your Understanding

- What are the various types of plant-associated microbiomes and how does each affect plant health?

- How are the compositions of plant microbiomes influenced by the environment?
- What aspects of the host plant can shape its microbiome?
- In what ways can the plant microbiome be governed by microbe-microbe interactions?

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8.5: Pollution and Bioremediation

Pollution and Bioremediation

Achieving sustainable life, for humans, animals, and the environment, requires a plan of action to mitigate anthropogenically-induced damage and develop future practices to maintain planetary homeostasis. One of the biggest threats to ourselves and the environment is the buildup of human waste and pollution (e.g. plastics, oil, synthetic products, etc.) that take a tremendously long time to naturally breakdown. With the human population predicted to continually climb, continuing the same destructive practices will only result in more waste generation in a fraction of the time that it takes for decomposition. One alternative means to solve the pollution problem is to use specialized microbiomes for bioremediation. That is, a specially designed microbial consortia could clean up (i.e. breakdown and assimilate) various toxic molecules much more quickly. However, this will take time to understand how and which type of microbes could perform these tasks and if there are any associated (negative) effects.

Alternative Strategies for Microbial Remediation of Pollutants via Synthetic Biology

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Continuous contamination of the environment with xenobiotics and related recalcitrant compounds has emerged as a serious pollution threat. Bioremediation is the key to eliminating persistent contaminants from the environment. Traditional bioremediation processes show limitations, therefore it is necessary to discover new bioremediation technologies for better results. In this review we provide an outlook of alternative strategies for bioremediation via synthetic biology, including exploring the prerequisites for analysis of research data for developing synthetic biological models of microbial bioremediation. Moreover, cell coordination in synthetic microbial community, cell signaling, and quorum sensing as engineered for enhanced bioremediation strategies are described, along with promising gene editing tools for obtaining the host with target gene sequences responsible for the degradation of recalcitrant compounds. The synthetic genetic circuit and two-component regulatory system (TCRS)-based microbial biosensors for detection and bioremediation are also briefly explained. These developments are expected to increase the efficiency of bioremediation strategies for best results.

Introduction

The remediation processes aided by microorganisms present at the various contaminated scenarios constitute bioremediation (Basu et al., 2018; Kumar et al., 2019). Microbial remediation uses multiple metabolic pathways responsible for enzyme production (Sharma B. et al., 2018; Dangi et al., 2019). These enzymes mainly take part in the degradation pathways of xenobiotics (Junghare et al., 2019). There are different customary methods for bioremediation, primarily based on the site of bioremediation, *in* and *ex situ* (Tomei and Daugulis, 2013). *In situ* is applied to the site to minimize soil disturbance. This method is mostly adopted due to less expenditure from avoiding excavation and transport of contaminated soil (Khan et al., 2004). According to Khan et al., 2004 less disruption in *in situ* bioremediation causes less dust dispersion and hence better degradation (Joshi et al., 2016) of contaminant. Bioaugmentation, bioventing, biosparging, and engineered *in situ* bioremediation are main *in situ* bioremediation methods (Azubuike et al., 2016). *Ex situ* bioremediation methods are solid phase system (composting, landfarming, and biopiling) and slurry phase system (bioreactors) (Kumar et al., 2011). Transportation of soil to accelerate microbial degradation are done by solid and slurry phase systems, whereby treatments of domestic, industrial, and organic waste are done by *ex situ* bioremediation (Juwarkar et al., 2010). These traditional bioremediation methods take time and consume much cost expenditure, giving less result output. Traditional bioremediation (Duarte et al., 2017) processes showed the above limitations of extra time taking, less removal or dissimilation of pollutants, (Bharagava et al., 2019) disturbance to nature delicacy such as more land coverage for a long time, and a foul smell in the environment (Dangi et al., 2019; Kumar, 2019). Therefore, researchers are eager to discover new bioremediation technologies for best results. Dvoøák et al. (2017) described bioremediation via synthetic biology for boosting bioremediation strategies. This approach can catch the catabolic (Jacquiod et al., 2014) and metabolic complexities for reviewing the potential of the microbial population synthetically. The preliminary information for developing synthetic microbial models for bioremediation can be obtained by mining genes from the databases (Fajardo et al., 2019). The computer logics involvement can determine the microbial cell interactions with recalcitrant compounds (Kim et al., 2015). These strategies can together grasp the natural metabolic potential of microorganisms to transform into novel biological entities of interest (Dangi et al., 2019). Furthermore, the regulation of metabolic pathways (Alves et al., 2018) in a controlled manner can also be achieved for bioremediation processes (Rochfort,

2005). This transition via synthetic biology application (Figure 1) for remediation purposes would improve the bioremediation processes via the involvement of potent (Zhu et al., 2017) dissimilating particular contaminants (Trigo et al., 2008). Synthetic biological systems mediate cellular modulations for efficient functioning and working of existing processes. They permit the modification of cellular processes *viz.* metabolic pathway acting for a particular chemical compound. The advancement of synthetic biology for bioremediation of various contaminants is attaining the focus of scientists and researchers. For instance, a sustainable synthetic microbial community's establishment for bioremediation is being investigated. Microbial interactions and quorum sensing within communities are vastly studied for application in the area of bioremediation with synthetic biology applications. Achievement of the synthetic genetic circuit of *Pseudomonas putida* proved to be the golden gadget for degradation studies. Besides this, genome editing by CRISPR-Cas, TALEN, and ZFNs adds knowledge for reviewing the progression in bioremediation studies. Synthetic microbial biosensors and metabolic engineering of cellular processes for utilization and detection of contaminant residues will remediate the environment from persistent recalcitrant pollutants. This review is focused on the above mentioned strategies and their elements (Figure 2) applicable for bioremediation purposes and research.

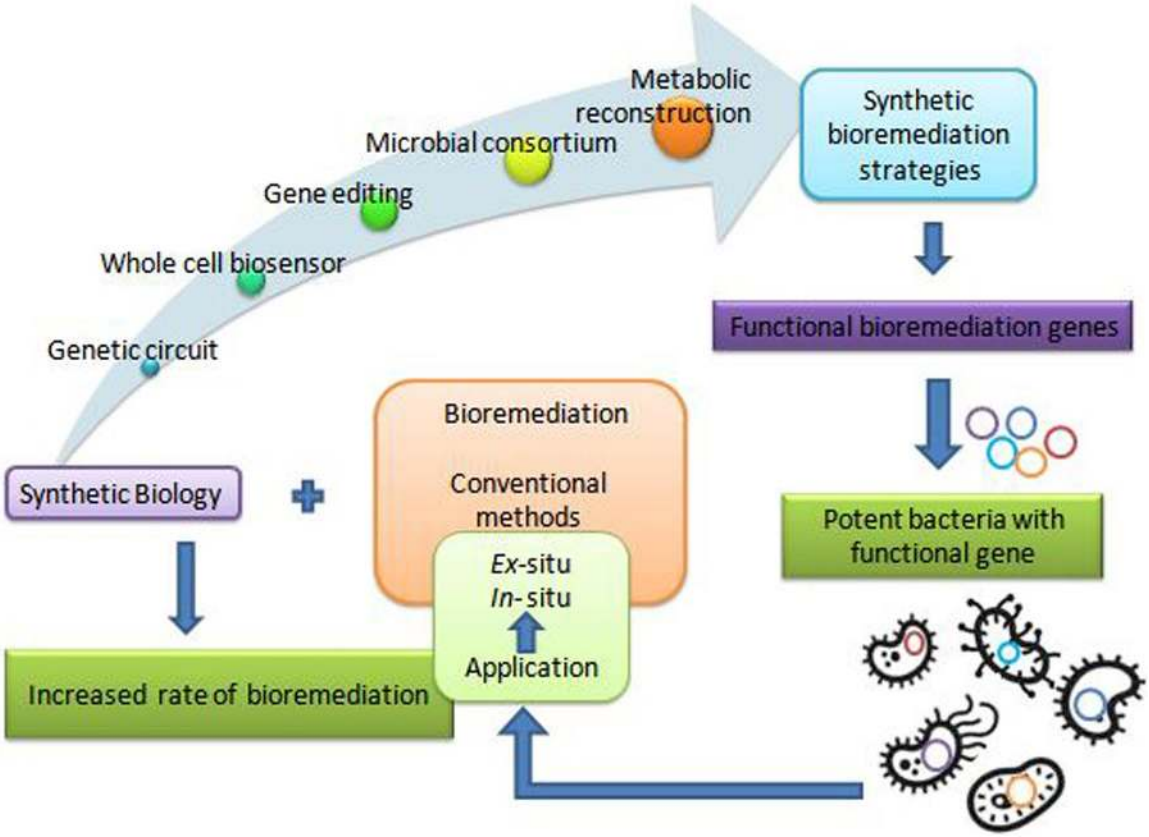


Figure 1. The strategies of synthetic biology applicable for bioremediation.

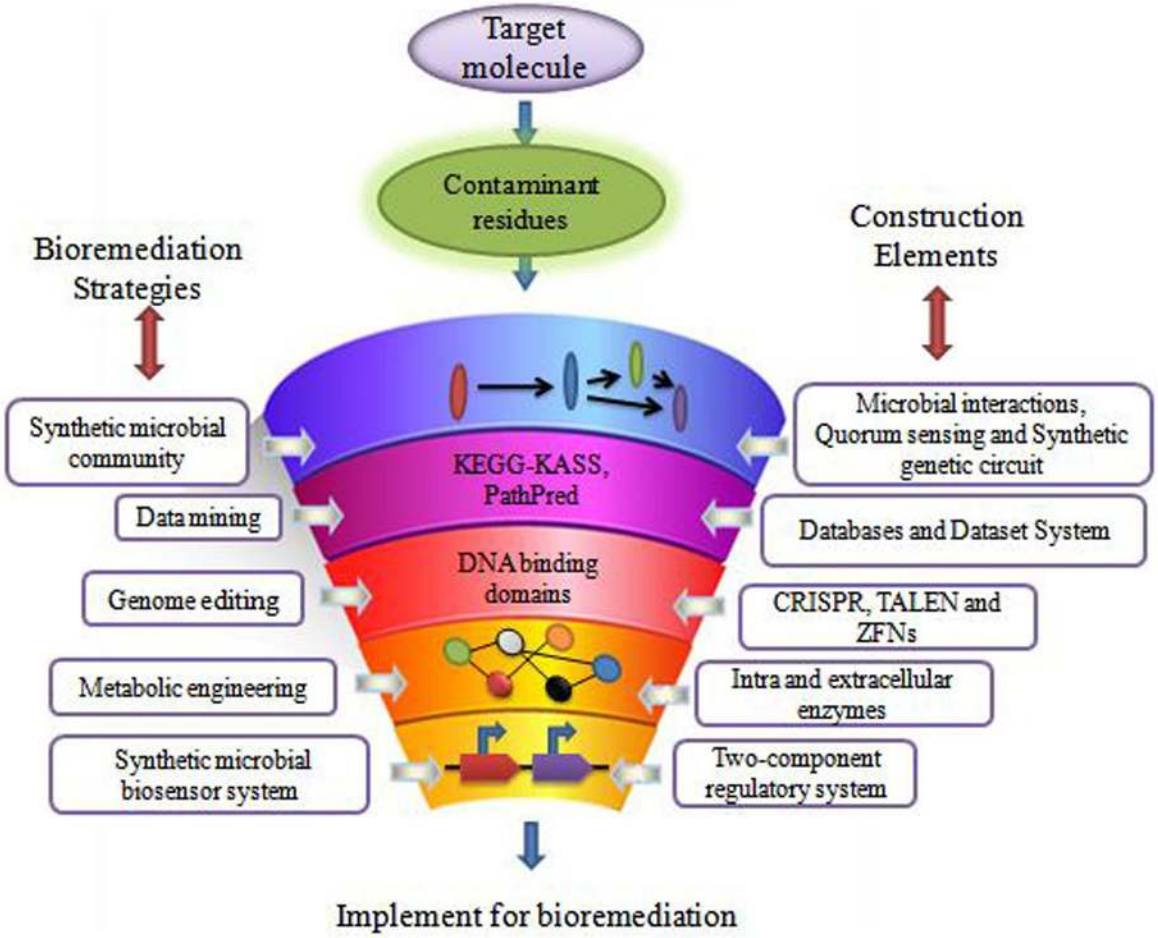


Figure 2. The components and their construction elements of synthetic biology for bioremediation studies.

Metabolic Reconstruction for Designing Synthetic Models

A computational platform is utilized for the reconstruction of cellular metabolism (Agapakis et al., 2012) via metabolic pathway analysis (MPA) (Banerjee et al., 2016). MPA mathematically represents the reactions of metabolism. This method is based on stoichiometric balance reactions so as to propose steady-state metabolic flux during cellular growth. The stoichiometry matrix imposes constraints of flux, making the consumption and production of the compound at a steady state (Bordbar et al., 2014). The maximum and minimum flux of a reaction can also be determined by providing the topper and least bound. This helps to define the extent of permissible flux supply (Richelle et al., 2016; Rawls et al., 2019). The next step is defining the objective according to the biological problem to be studied. This objective mathematically represents the reactions responsible for the phenotype appearance. The mathematical reactions and phenotype are combined with linear equations and solved by computational algorithms such as COBRA Toolbox11 and Matlab toolbox (Orth et al., 2010; Bordel, 2014). FBA is fundamentally simple, having immense applications in studying gaps, physiology, and genomes via systems biology approach (Kim et al., 2015; Hellweger et al., 2016). These gaps are missing metabolic reactions, making the genome partially known. FBA uses computational algorithms that can predict missing reactions *viz.* OptKnock and OptCom, which can knock out the genes responsible for producing the desired compound (Biggs et al., 2015). These approaches are beneficial for constructing microbial communities for bioremediation of particular contaminants (Khandelwal et al., 2013). However, MPA is the most challenging method when metabolic information is incomplete, making it difficult to obtain a real model (Covert et al., 2001). But this method shows cellular functions in the dynamic community, and thus is very useful for the prediction and exchange of metabolic flux in communities of microorganisms (Khandelwal et al., 2013). Recently, a metabolic model has been constructed by using two *Geobacter* species with parameterized electron transfer and metabolic exchange to characterize syntrophic growth dynamics. Such a system may have useful applications in the field of bioremediation and degradation of particular contaminants (Butler et al., 2010). A computational platform is also needed for better prediction of engineered genetic pathways for community dynamics. A graph-based tool Metabolic Tinker was developed by McClymont and Soyer to identify thermodynamically feasible biochemical routes for compounds deterioration

(Johns et al., 2016). This may be applied to identify the routes for degradation of recalcitrant compounds by microbial consortia. These computational tools are utilized along with omics (Kim et al., 2014; El Amrani et al., 2015) and biological data for desired output (Berger et al., 2013) and toxicity prediction (i.e., META-CASETOX System) (Peijnenburg and Damborský, 2012). These are also applied for functional gene identification and their profile analysis, PCR analysis and drug discovery, etc (Dangi et al., 2019). Computer-aided drug discovery and development (CADDD) is used effectively with chemical and biological aspects, i.e., chemical structures accounting the biological role and its activity via ligand-based drug design, structure-based drug design, quantitative structure-property relationships, and quantitative structure-activity (Kapetanovic, 2008). Furthermore, Table 1 depicts similar methodologies applicable to bioremediation studies. De Jong (2002) analyzed the multicellular feedback control strategy in a bacterial consortium (Bruneel et al., 2011) to define the robustness conceivable under desired conditions.

S.no.	Purpose	Approach	Methodology	References
1.	Degradation study	Functional gene identification for bioremediation	PCR (Polymerase Chain Reaction) product sequence analysis	Jagwani et al., 2018
2.	Cell behavior study	Whole-cell simulation	A computer model for bacterial cell in response to the contaminated environment	O'Brien et al., 2015; Panigrahi et al., 2019
3.	Toxicity of chemicals	Analysis of chemical and biological properties	*In silico* toxicology (IST) protocols for toxicity assessment; QSAR (Quantitative Structure Activity Relationship) model	Pavan and Worth, 2006; Myatt et al., 2018
4.	Identification of functional bioremediating microbe	Target identification	Protein structure prediction, Protein – protein interaction (PPIs)	Shukla, 2017
5.	Remediation of textile dyes	Interaction of protein-ligand	Molecular docking	Sridhar and Chandra, 2014; Kumar et al., 2016
6.	Bioremediation of toxic pollutants	Structure prediction	Biodegradability evaluation and simulation system	Das et al., 2016

Table 1. The methodologies applied for bioremediation research.

They utilized an ordinary differential equations (ODE)-based model and agent-based simulation on a consortium (Hawley et al., 2014; Atashgahi et al., 2018) of interacting species population for increasing the efficacy of the proposed feedback control strategy. The application of bioinformatics (Arora and Bae, 2014) resources is a prerequisite dimension for obtaining the data to begin the microbial bioremediation studies of recalcitrant compounds (Gong et al., 2012; Ofaim et al., 2019). This involves the information related to the degradation of xenobiotics by microbes and their pathways for dissimilation (Dao et al., 2019; Salam and Ishaq, 2019; Thelusmond et al., 2019; Wei et al., 2019). The data related to end products and intermediate metabolites released throughout degradation pathways can also be retrieved (Dvoøák et al., 2017). An extended information source linked to degradation is MetaRouter, allowing data (Singh and Gothalwal, 2018) for life sciences laboratories to explore degradation possibilities of recalcitrant compounds (Mohanta et al., 2015). The information on oxygenic degradation of xenobiotics can be retrieved from OxDBase, a biodegradative oxygenase database (Chakraborty et al., 2014; Shah et al., 2018). Oxygenase is a class of enzyme which transfers the oxygen molecule for oxidizing the chemical compound. They play a role in the degradation of organic compounds by aromatic ring cleavage (Jadeja et al., 2014). OxDBase is very particular in providing knowledge of oxygenases-catalyzed reactions, and is a powerful tool applicable to bioremediation studies (Singh, 2018). Bioconversion and biodegradation of persistent and toxic xenobiotics (Desai et al., 2010; Bao et al., 2017) compounds catalyzed by oxygenases decrease the compound sustainability and toxicity in the environment (Kües, 2015; Kondo, 2017). Therefore, OxDBase is very helpful in acknowledging the degradation processes involved in bioremediation (Shah et al., 2012). The transcriptional characterization of genes responsible for the biodegradation and biodissimilation of a particular compound has great significance in proposing molecular methodologies. This can be done by the Bionemo (Biodegradation Network Molecular Biology) database (Libis et al., 2016a). Bionemo contains the entries for sequences of genes coding for biodegradation (Carbajosa and Cases, 2010). It also links the gene transcription and its regulation (Libis et al., 2016b). The data retrieved from Bionemo can be used for designing cloning experiments and primers (Arora and Shi, 2010). Garg et al. (2014) used eMolecules and the EAWAG-BBD PPS database for the prediction of pathways involved in the biodegradation of 1-naphthyl-N-methyl carbamate. These above findings empower the researchers to analyze and establish what prerequisites must be fulfilled for developing synthetic bioremediation models.

Designing the Synthetic Microbial Communities

Recent advancements in the field of synthetic biology for environmental issues have shown a great impact. The use of GMOs in environmental biotechnology for remediation (Malla et al., 2018) of toxic compounds, xenobiotics, and pesticidal compounds are being done. To design a synthetic community, it is important to understand natural microbial communities (Schloss and Handelsman, 2008). In a natural community, it is difficult to find out which species are actually taking part in bioremediation (Großkopf and Soyer, 2014). Thus, a synthetic microbial community is a promising method for constructing an artificial microbial community with function-specific species for bioremediation purposes. These communities may act as a model system for the study of functional, ecological, and structural characteristics in a controlled manner. Großkopf and Soyer (2014), defined synthetic

community by the culturing of two microbial species under well-defined conditions on the basis of interaction and function (Bruggeman and Westerhoff, 2007). These factors determine the dynamics and structure of the community. It is based upon the identification of processes and patterns engaged in by bacterial species. These microbial interaction patterns are metabolism-driven and responsible for community interaction (Wintermute and Silver, 2010). Social-based microbial interactions (i.e., mutualism, cooperation, and competition, etc.) and the total outcome of these interactions between two microbial populations can be +/+ and −/+ or +/−, respectively (Foster and Bell, 2012). It is said that community structure and function majorly depends on cooperation. The effect of cooperation on community dynamics is determined by engineered cooperation resulting in the synthetic community. Engineered cooperation between two microbial strains (Singh et al., 2016) can be done by manipulation of environmental conditions, i.e., knocking the genes out and in Zuroff et al. (2013). Beyond this, other interaction patterns have been analyzed with engineered microbial species in the synthetic community. Such an application of engineered interaction is highly recognizable in bioremediation strategies (Sharma, 2012). Synthetic biology provides greater potential for the sustainable existence of microorganisms (Dellagnezze et al., 2014) acting together in a large population. Thus, synthetic microbial communities are proved as a key strategy for the bioremediation of contaminants, i.e., pesticides, petroleum (Kachienga et al., 2018), oil spill, acid drainage (Serrano and Leiva, 2017), etc. For building the synthetic microbial communities, the engineered interspecies and intraspecies interactions can make cellular functions robust and enhance the capabilities of microbial consortia in various contaminated scenarios. Quorum sensing is a bacterial signaling mechanism, which is a density-dependent phenomenon via cell-cell communication and population level behavior. The signaling is done by the release and reception of chemical compounds by microbial candidates in a population. This leads to multicellular behavior (Obst, 2007), offering engineerable tasks to design function specific synthetic communities. These synthetic models can also be exploited to obtain a rational design that can lose the function when subjected to competition with other species in the natural environment. With the evolution of genomic constituents and gene transfer, the possibility of the gradual extinction of genetic circuits is present. Thus, strategies are required to maintain the robustness of the synthetic community, achieved via the synthetic models by the development of synergistic and cooperative properties that reduce instability and loss of function (Johns et al., 2016). A recent study by Coyte et al. (2015) suggests that competition among species is significant in determining the stability of communities, acting as a limiting factor in the stability of the synthetic community. Thus, these dynamics must be accelerated in order to design particular function specific synthetic communities for bioremediation purposes (Coyte et al., 2015).

Genetic and Metabolic Engineering

Enríquez (2016) said that genome editing is an umbrella term that refers to “scientific technological advances that enable rational genetic engineering at a local (gene) or global (genome) level to facilitate precise insertion, removal, or substitution of fragments of Deoxyribonucleic acid (“DNA”) molecules, comprising one or more nucleotides into the cell(s) of an organism’s genome.” Transcription-activators like effector nucleases (TALEN), clustered regularly interspaced short palindromic repeats (CRISPR-Cas), and zinc finger nucleases (ZFNs) are major gene editing tools used (Table 2). The most efficient and simple technique of gene editing has been described as CRISPR-Cas (Kanchiswamy et al., 2016). These tools can boost the process of bioremediation. TALEN has a DNA-binding modular which is sequence-specific for the host genome (Utturkar et al., 2013). TALEN binding to DNA creates a double stranded break (DSB) in the target sequence and leaves sticky ends for stability. Similarly, ZFNs is also a DNA-binding domain composed of 30 amino acids. It introduces DSB at the target site of the host genome by the Fok1 cleavage domain. A new sense of using hybrid nucleases containing TALENs and ZFNs nucleases came to act for solving the molecular complications. The CRISPR-Cas system, on the other hand, has unique action properties of high sequence specificity and multiplex gene editing. This unique property is derived from bacteria *Streptococcus pyogenes* as immunity against the virus. The CRISPR-Cas system consists of crisper derived RNA (crRNA) and trans acting antisense RNA (trcRNA) joined by guide RNA (gRNA). gRNA directs the Cas9 enzyme to introduce DSB in the target DNA sequence by recognizing it. These gene editing tools create the knock-in and knock-out and are under processing for implementation in bioremediation studies (Kumar V. et al., 2018). Recent reports indicate though that the CRISPR-Cas system is mostly adopted and performed by researchers in model organisms i.e., *Pseudomonas* (Karimi et al., 2015; Nogales et al., 2020) or *Escherichia coli* (Chen et al., 2018; Marshall et al., 2018; Pontrelli et al., 2018). Nowadays, the new insights toward CRISPR tool kits and designing gRNA for expression of function-specific genes related to remediation in non-model organisms (i.e., *Rhodococcus rube*r TH, *Comamonas testosteroni* and *Achromobacter* sp. HZ01) are also suggested in the field of bioremediation (Mougiakos et al., 2016; Jusiak et al., 2016; Hong et al., 2017; Stein et al., 2018; Tang et al., 2018; Liang et al., 2020). For gene editing and metabolic engineering, the contaminant-inhabited bacteria are the most suitable candidates because they are used to survive and harbor in stress conditions of various toxic, recalcitrant and non-

degradable xenobiotics, as discussed above. Moreover, understanding metabolic pathways seems to be important in studying the microbial bioremediation (Plewniak et al., 2018), i.e., bioremediation of toxic pollutants by the haloalkane dehalogenases production pathway and decontamination of pyrethroid from the soil via the biodegradation pathway of fenpropathrin studied in *Bacillus* sp. DG-02 (Chen et al., 2014). Metabolic engineering leads to modification of the existing pathway for the betterment of the bioremediation process (Michel et al., 2007). This approach majorly covers the study of microbial enzymes, i.e., oxidases, esterases, monooxygenases, oxidoreductases, phenoloxidases involved at various degradation steps (Figure 3A; Mónica and Jaime, 2019; Mujawar et al., 2019). Enzyme-based bioremediation has many advantages because it is an eco-friendly process. The genetic approach increases the perspective of getting recombinant enzymes. There are research reports of extracellular enzymes having a role in enzymatic bioremediation. For instance, arsenic bioremediation (Andres and Bertin, 2016; Choe and Sheppard, 2016; Akhter et al., 2017; Biswas et al., 2019) (bioaccumulation and biotransformation) is achieved *via* arsenite oxidase coded by *aioA* gene of *Klebsiella pneumonia* (Mujawar et al., 2019); enzymes released by white rot fungi degrade PAHs (polycyclic aromatic hydrocarbon) (Zhao and Poh, 2008; Košnár et al., 2019), dyes, TNT (2,4,6- Trinitrotoluene) and PCBs (polychlorinated biphenyls) (Gupte et al., 2016; Kutateladze et al., 2018; Sadańoski et al., 2018). Esterase D enzyme acts on β-endosulfan (organochlorine pesticide), producing simpler compounds (Mehr et al., 2017; Chandra et al., 2019). *LiPs* encoding hemoproteins from *Phanerochaete chrysosporium* degrade PAHs. However, incomplete or partial degradation of contaminants lead to simpler non-toxic degradable compounds which can be consumed by microbes (Kumavath and Satyanarayana, 2014) as intermediates in biological pathways or substrate, i.e., *LiP* (lignin peroxidase) dissimilate benzopyrene to three compounds of quinine, namely 1,6-quinone, 6,12- quinine and 3,6- quinine (Gupta and Pathak, 2020). Furthermore, *MnP* (Manganese peroxidase) oxidizes organic compounds in the presence of Mn(II) (Xu et al., 2018; Singh et al., 2019). Laccase, MFO (mixed function oxidases), glutathione S transferase, cytochrome P_{450} also acts in biodegradation of recalcitrant compounds (Singh, 2019; Boudh et al., 2019). Catechol 1,2-dioxygenase (intracellular enzyme) from *Pseudomonas* NP-6 dissimilate catechol to muconate compounds (Guzik et al., 2011). Also, enzyme immobilization (Cavalca et al., 2013; Sharma B. et al., 2018) increases the half-life, stability, and enzyme activity at a notable level. The enzymatic bioremediation is an elementary, expeditious, and environmental friendly method for microbial removal and degradation of persistent xenobiotics compounds (Sharma B. et al., 2018). Isolation and characterization of microorganisms with enzymatic capabilities have been done with the limitation of less productivity (Rayu et al., 2012). Organophosphates (OP) and organochlorines (OC), major constituents of insecticides, accumulate in the agricultural soil (Panelli et al., 2017) and reach the water bodies via agricultural run-off. Effective bioremediation of γ-hexachlorocyclohexane (OC) and methyl parathion (OP) has been reported by genetically engineered microorganisms (Gong et al., 2016). Moreover, bioremediation of organophosphates and pyrethroids has been experimented with using genetically modified *P. putida* KT2440 (Zuo et al., 2015). With the advent of metabolic engineering, the catabolism and degradation of various persistent compounds has been reported. The degradation pathways of methyl parathion and γ-hexachlorocyclohexane in *Sphingobium japonicum* and *Pseudomonas* sp. WBC-3 witnessed the bioremediation strategy (Liu et al., 2005; Miyazaki et al., 2006). Furthermore, 1, 2, 3-trichloropropane, a persistent constituent of fumigant, is dissimilated into the environment (Techtmann and Hazen, 2016) via heterologous catabolism by the assembly of three enzymes from two different microorganisms in *E. coli* (Dvorak et al., 2014). A metabolic pathway (Bertin et al., 2011) degrading organophosphorus and paraoxon is engineered by inserting the organophosphorus hydrolase gene (opd) and pnp operon encoding enzymes that convert p-nitrophenol into β-ketoadipate in *P. putida* (de la Pena Mattozzi et al., 2006). A study showed *pobA* and *chcpca* gene clusters of *Rhodococcus opacus* R7 take part in the bioremediation of naphthenic acid; more specifically, expression *aliA1* gene codes for fatty acid CoA ligase for degrading long chains of linear as well as alicyclic naphthenic acid (Zampolli et al., 2020). To minimize the accumulation, the above-mentioned strategy is attained using microbes for partial or complete mineralization of persistent compounds (Miyazaki et al., 2006).

Features	Gene editing tools			References
	CRISPR	TALEN	ZFNs	
System	Adaptive immune system	Pathogenic *Xanthomonas*	Gene expression system	Kumar N. M. et al., 2018
Specificity	crRNA	TALE Domain	Zn finger Domain	Jaiswal et al., 2019b
Cleavage	Cas9	*Fok*I nuclease	Nuclease	Kumar V. et al., 2018; Jaiswal et al., 2019b
Nucleases per target per experiment	Single or more sgRNA; singleCas9	Single TALEN pair	Single ZFN pair	Hamilton et al., 2019
Activity	High	High	Moderate	Shanmugam et al., 2019
Designing and screening	Easy	Difficult	Difficult	Dangi et al., 2019
Multiple gene editing	Suitable	Not suitable	Not suitable	Sinha et al., 2019

Table 2. Comparative features of CRISPR, TALE, and ZFNs.

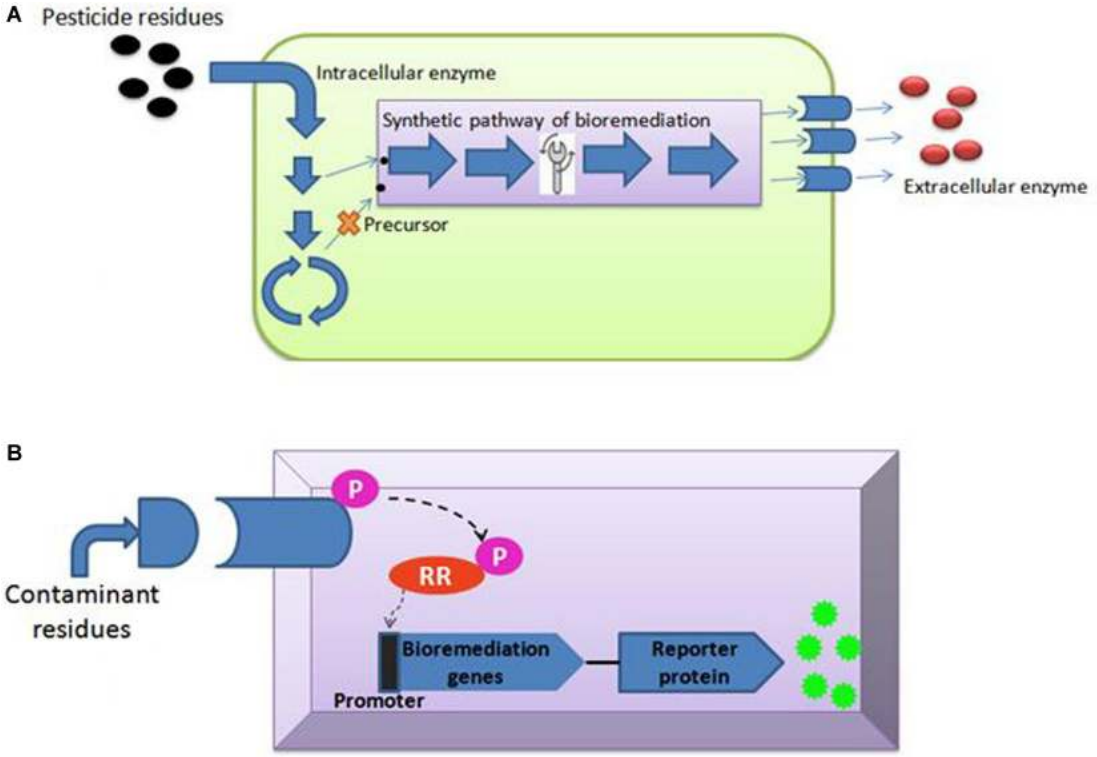


Figure 3. Schematic presentation (A) intracellular and extracellular enzymes production; (B) TCRS based biosensor.

Synthetic Genetic Circuit and Microbial Biosensor

The synthetic genetic circuit requires chassis for implantation. The *P. putida* is a HVB (Host Vector Biosafety) strain recognized as safe by the Recombinant DNA Advisory Committee. It is also referred to as GRAS (Generally Recognized as Safe) to release in the environment. It is ideal for the next generation of synthetic biology chassis panel because it can withstand high intolerant changing conditions including temperature, pH, solvents, toxins, osmotic, and oxidative stress. Also, *P. putida* has versatile metabolism and low nutrient requirements (Pabo and Nekludova, 2000). These qualities make this organism the best bacterial model for environmental bioremediation applications (Tanveer et al., 2018). Recently, the *P. putida* synthetic genetic circuit has been established for the designing of promoter genes and expression of the gene responsible for the degradation of persistent compounds (Adams, 2016). An extension of synthetic biology is the integration of genome with reporter system, and synthetic promoters of *P. putida* may be developed for synthetic bioremediation pathways. Elmore et al. use serine integrases for synthetic genetic circuit development. Microbial cells have the advantage of a cellular system, which controls cell growth and response to external factors like temperature, light, pH, and oxygen (Tropel and Van Der Meer, 2004). The external environment of microbes inhabiting the contaminated site will respond to concentrations of various persistent compounds present (Ray et al., 2018; Antonacci and Scognamiglio, 2019). Whole cell biosensors for checking the presence, detection and biodegradation potential of xenobiotics compounds (pharmaceutical residues, pesticides, paraffin, PAHs and PCBs, etc.) present (Adhikari, 2019) in environmental samples are attaining attention (Wynn et al., 2017; Heng et al., 2018; Patel et al., 2019). The reporter proteins acting microbe makes a color signal at the detection of particular contaminants *via* transducer (Zhang and Liu, 2016). A biosensor aiming for detection

and bioremediation purposes must have enhanced contact between microbe and contaminant (Dhar et al., 2019). This helps the bacterium to adjust their cellular pathways in response to external environmental conditions and encodes the genes for utilizing the recalcitrant compounds as substrate (Bilal and Iqbal, 2019; Skinder et al., 2020). Synthetic biology strategies are feasible for removing a particular toxic compound because the genetic circuits can be developed against the exogenous environmental toxin (Checa et al., 2012; Tay et al., 2017). The synthetic genetic circuits are assembled via a two-component regulatory system (TCRS) in bacteria (Futagami et al., 2014; Uluşeker et al., 2017). This system acts upon environmental change, and thereby, cells respond to these changes. A prokaryotic TCRS has histidine kinase (HK) and response regulator (RR). The HK is a sensor domain with an extracellular loop present as an integral membrane protein. HK also has a transmitter domain in the last cytoplasmic transmembrane, which is a highly conserved domain. Histidine phosphotransfer (DHp) and catalytic ATP-binding domain (CA) acts for molecular recognition of RR and ATP hydrolysis. The transmitter domain transmits the signal from periplasm to cytoplasm via PAS (Periodic circadian proteins, Aryl hydrocarbon nuclear translocator proteins, and single minded proteins), HAMP (HKs, Adenyltatecyclases, Methyltransferases, and Phosphodiesterases) and GAF (cGMP-specific phosphodiesterases, adenylyl cyclases, and formate hydrogenases) (Casino et al., 2010). Thus, HK senses the external environmental change and adds phosphate to conserved histidine. The HK also regulates RR by phosphorylating the aspartate residues. This promotes the promoter (Figure 3B) binding to activate the gene expression or vice-versa (Ravikumar et al., 2017). Therefore, TCRS-based synthetic biology application for biosensor development for cell-mediated detection and bioremediation can prove to be a new advancement.

Ecological Safety and Risk Assessment

The scientists and researchers are performing the experimental setup to study the bioremediation (Yergeau et al., 2012) potential against various pollutants like oil spill, plastics, synthetic dyes, organic hydrocarbons (Yadav et al., 2015), pesticides (Jaiswal et al., 2019a), heavy metals (Hemmat-Jou et al., 2018; Lebrazi and Fikri-Benbrahim, 2018), and other xenobiotics, etc (Rucká et al., 2017; Paniagua-Michel and Fathepure, 2018; Wu et al., 2020). Considering that bioremediation is performed in an open environment rather than in a closed fermentation tank, the ecological safety of bioremediation performing bacteria must be considered. Economic safety is justified by the metabolic aptness (Gillan et al., 2015) of microorganisms as compared to other traditional physical and chemical bioremediation methods. Besides, regulation for using genetically and metabolically modified bacteria is released to evaluate the possible risks (Khudur et al., 2019). The risk assessment is mainly done by regulatory agencies, i.e., Organization for Economic Cooperation and Development (OECD) at the application level for environmental safety (Russo et al., 2019; Alam and Murad, 2020; Pastor-Jáuregui et al., 2020). The possible risks are gene contamination in the native member of microbial consortium, leading to mislaying of the natural trait (Mills et al., 2019; Pineda et al., 2019; Rycroft et al., 2019). The competitiveness between natural and genetically modified species can give rise to selection pressure on non-target microflora (Kumar N. M. et al., 2018; Mohapatra et al., 2019). Moreover, environment and ecosystem risk assessments infer unpredictable and adverse effects, as discussed above (Cervelli et al., 2016; van Dorst et al., 2020). Particularly, the ecological risk assessment behind addition of GEMs (Genetically Engineered Microorganisms) (Benjamin et al., 2019; Ahankoub et al., 2020) to the native environment rather than a laboratory (Fernandez et al., 2019) is done mainly because of unnecessary delivery of antibiotic resistance marker along with recombinant genome of interest (Davison, 2002; Nora et al., 2019), and unintentional uptake or transfer of induced genes to other microorganisms (French et al., 2019; Janssen and Stucki, 2020). This phenomenon is definitely disturbing the microbial native genome entity (Gangar et al., 2019; Petsas and Vagi, 2019). Another aspect to consider is the change in microbial metabolism (Okino-Delgado et al., 2019), which may release uncertain toxic compounds into the environment, indirectly affecting (negatively) microbial candidates in this context (Myhr and Traavik, 2002). Under the TSCA (Toxic Substances Control Act) (Gardner and Gunsch, 2020), the Office of Pollution Prevention and Toxics (OPPT) programs (Pietro-Souza et al., 2020) of the United States Environmental Protection Agency (Leong and Chang, 2020; Saxena et al., 2020) monitors the environmental and health risks and releases premanufacture legal notice for the accreditation of field research outlines and prototypes (McPartland and McKernan, 2017; Khan et al., 2020). A magnificent example is given by University of Tennessee. In 1995, they gave application and suggested the risk evaluation of microbial bioremediation agents (mainly *Pseudomonas fluorescens* HK44) on the environment and health (Sayler et al., 1999; Khan et al., 2016; Sharma J. K. et al., 2018; El Zanfaly, 2019). Remarkably, the literature survey points toward a biowar weapon for humanity (Gómez-Tatay and Hernández-Andreu, 2019; Wang and Zhang, 2019), stating that gene editing tools left in bad hands could mislead ethical and moral duties (Khan, 2019; Thakur et al., 2019; Sharma et al., 2020).

Conclusion and Future Perspectives

The microbial bioremediation process for removal and detoxification of contaminants from the environment has now emerged as the best option. Synthetic biology is addressing the decontamination and remediation strategies for xenobiotics and related compounds from the environment. It has been observed that the requisite for understanding existing metabolic pathways is a must for developing synthetic models of bioremediation. Moreover, genomics reconstruction methods (Luo et al., 2014; Marco and Abram, 2019) and technologies made a solid platform for bioremediation studies. Satisfactory progress has been witnessed in the field of bioremediation among various contaminants with the role of specific genes and enzymes applicable via synthetic biology methodologies. Therefore, it is concluded that microbial synthetic biology remediation strategies not only increase the efficiency of microbial bioremediation processes for a particular contaminant, but also provide the best methodologies for researchers.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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True or False?

Check your Understanding

- Explain bioremediation and the roles that microorganisms can have in this process.
- What is synthetic biology and how can it be applied for bioremediation?
- What are some important considerations and challenges for designing a synthetic microbiome?
- Compare the major techniques for genetic and metabolic engineering.
- How could microbial biosensors contribute to bioremediation efforts?
- What aspects of ecological safety and risk assessment must be addressed for application of synthetic microbiomes for bioremediation in open environments? Why?

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8.6: Forensic Microbiomes

Forensic Microbiomes

Characterizing human and environmental microbiomes is not only important to maintain host and ecological health, but can have other far-reaching applications such as forensic science. Determination of unexplained situations and phenomenon can be quite challenging, and with advances in microbiomics, there is a possibility to generate sufficient data and evidence to provide viable answers and solutions.

Forensic Applications of Microbiomics: A Review

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The rise of microbiomics and metagenomics has been driven by advances in genomic sequencing technology, improved microbial sampling methods, and fast-evolving approaches in bioinformatics. Humans are a host to diverse microbial communities in and on their bodies, which continuously interact with and alter the surrounding environments. Since information relating to these interactions can be extracted by analyzing human and environmental microbial profiles, they have the potential to be relevant to forensics. In this review, we analyzed over 100 papers describing forensic microbiome applications with emphasis on geolocation, personal identification, trace evidence, manner and cause of death, and inference of the postmortem interval (PMI). We found that although the field is in its infancy, utilizing microbiome and metagenome signatures has the potential to enhance the forensic toolkit. However, many of the studies suffer from limited sample sizes and model accuracies, and unrealistic environmental settings, leaving the full potential of microbiomics to forensics unexplored. It is unlikely that the information that can currently be elucidated from microbiomics can be used by law enforcement. Nonetheless, the research to overcome these challenges is ongoing, and it is foreseeable that microbiome-based evidence could contribute to forensic investigations in the future.

Introduction

For over 100 years, microbiology has played a relatively diminutive role in forensic science (MacCallum and Hastings, 1899). In the early 1990s, the sequencing of amplified viral DNA was used to support a case alleging the transmission of Human Immunodeficiency Virus from a dentist to several patients in Florida, United States (Smith and Waterman, 1992). The emergence of PCR-mediated genotyping of bacteria was considered to be a valuable forthcoming tool in forensics—e.g., van Belkum (1994) suggested that forensic science would soon be a major area for the application of PCR-mediated genotyping due to the rapidity of technological advances at the time (van Belkum, 1994). In the mid-1990s, fungal and pollen spore analyses were also developed, allowing investigators to differentiate between soil types, which in turn allowed linking substrate items to particular sites (Bruce and Dettmann, 1996; Bryant and Mildenhall, 1998). However, it was not until the early 2000s and the rise of bioterrorism that *microbial forensics*—the "scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime, or inadvertent microorganism/toxin release for attribution purposes"—emerged in response to the new threat (Budowle et al., 2003; Carter et al., 2017).

Many forensic applications have been limited to individual taxa analyses, and *microbial forensics* has, historically, been constrained by a lack of available and cost-effective sequencing technologies (Berglund et al., 2011; Kuiper, 2016). This approach has changed dramatically in the last decade as advances in genomic sequencing technology, and new methods for processing complex community datasets (and often low biomass samples) have led to the advent of a new field of microbiomics. The science and study of the microbiome (Statnikov et al., 2013; Capasso et al., 2019) combined with metagenomics (all genomes from a sample) have enhanced the development of the microbial forensic toolkit (Clarke et al., 2017; Hampton-Marcell et al., 2017).

As of March 2019, the conviction rate for homicides in England and Wales (United Kingdom) was only 79% (Office for National Statistics, 2019), slightly higher than in the US (~70%) (Bureau for Justice Statistics., 2019). Across the globe, there is also a high prevalence of wrongful convictions and often insufficient evidence to convict a perpetrator of a crime (Sangero and Halpert, 2007; Ingemann-Hansen et al., 2008; LaPorte, 2017; Walsh et al., 2017). According to the Innocence Project, a national litigation and public policy organization dedicated to exonerating wrongfully convicted individuals, to date, 375 people in the United States have been exonerated by DNA testing, including 21 who served time on death row (Innocent Project, 2020). There is thereby a strong interest from the public, lawmakers, and the law enforcement system to augment and expand the forensic toolkit, including molecular methods. Microorganisms are abundant in and on the human body (microbial cells can outnumber or equal the total

number of human somatic cells) (Noel et al., 2014; Sender et al., 2016; Vázquez-Baeza et al., 2018), in surrounding environments, and on objects associated with a crime (Desmond et al., 2018; Oliveira and Amorim, 2018). A growing body of evidence suggests that forensically relevant microbial profiles could be used as evidence or, at the very least, complement traditional investigative methods (Metcalf et al., 2017; Schmedes et al., 2017; Richardson et al., 2019; Phan et al., 2020). This use of microbial profiles as evidence is done using computational tools that are being developed alongside new approaches in bioinformatics, processing tools, and refined protocols. However, since the field is still in its infancy (Goudarzi et al., 2016; Komaroff, 2018) and historically underfunded (Morgan and Levin, 2019), there is much uncertainty as to the true potential of microbiomic tools in forensics.

In this review, we provide an overview of past, current, and future potential applications of microbiomics in forensics. Specifically, we will discuss the six most comprehensively researched themes (Figure 1): including geolocation (e.g., substrate analysis and different spatial dimensions and the power of machine learning), personal identification, biological sex determination, trace evidence, manner and cause of death (e.g., death by drowning), PMI, and other applications (e.g., localization through animal microbiomes).

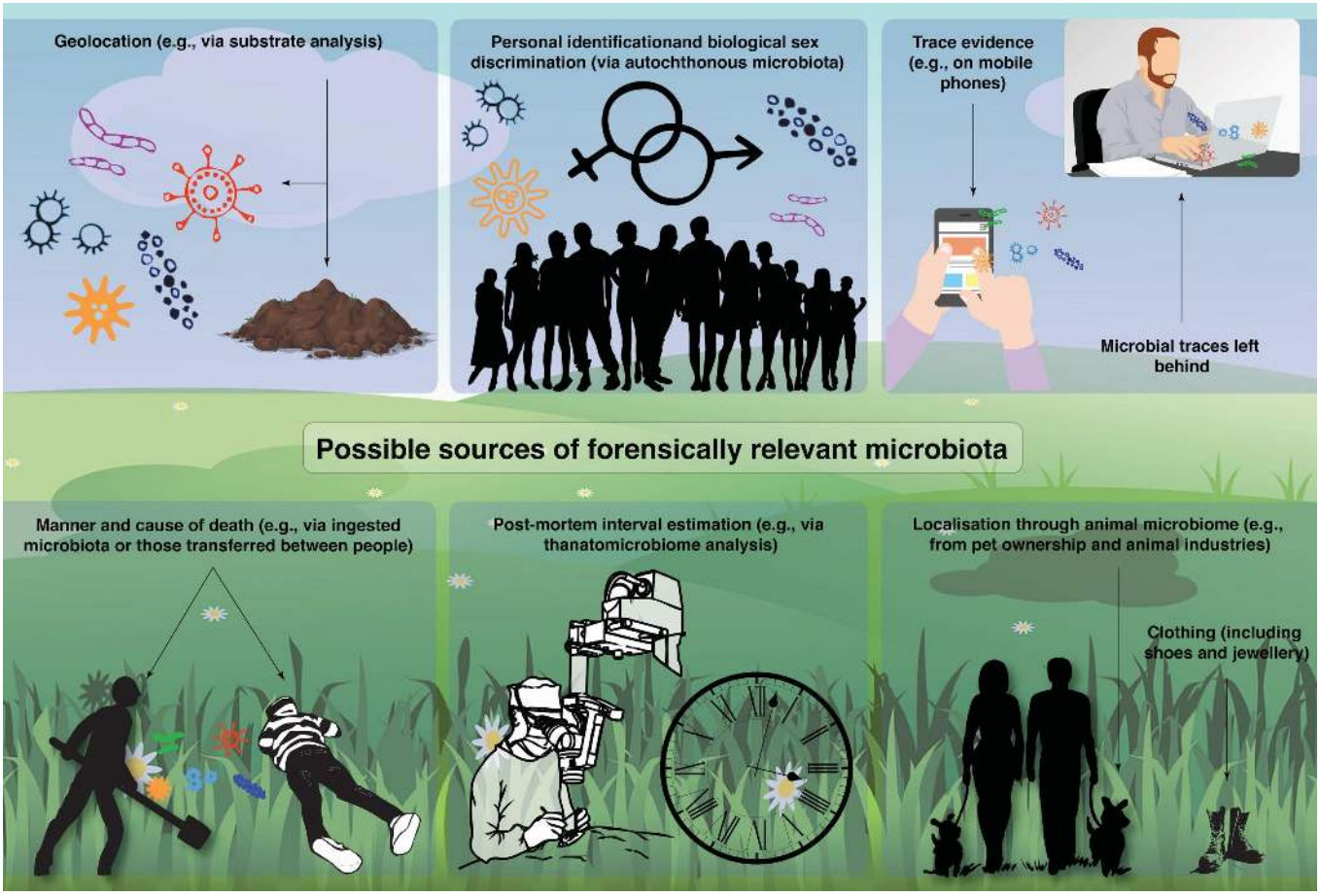


Figure 1. A summary of possible sources of forensically relevant microbiota identified by the literature review.

Major Themes in the Forensic Microbiology Literature

Geolocation

In the past few years, intensive work has been carried out to characterize environmental microbiome, particularly in urban environments and transit systems. These studies have demonstrated that unique community profiles may exist in certain areas of a city (Afshinnekoo et al., 2015; Rosenfeld et al., 2016), as well as “molecular echoes” of environmental events, and even a forensic capacity for geospatial microbiomic data (MetaSUB International Consortium, 2016; Danko et al., 2019a). In the following, we focus on two leading aspects of geolocalization.

Substrate Analysis

The potential of analyzing microbial profiles from the soil is increasingly being recognized in forensic microbiological research. Both the rhizosphere and bulk soil microbiomes exhibit a high level of heterogeneity between different sites. As such, with methodological refinement, soil microbiome samples could provide valuable biogeographic data to localize the origin of the soil sample. Another potential application is the acquisition of information to help determine the provenance of an item(s) associated with a crime.

Habtom et al. (2019) demonstrated distance-decay relationships between microbial samples from the soil at a local scale (25–1,000 m) (n = 5 sites, n = 2–4 soil types, and five replications). The results showed that the greater the distance between the samples, the more they differed, suggesting that both soil type and geographic location are important factors in determining microbial community composition. Indeed, patch discrimination using the soil microbiome has previously been demonstrated (Macdonald et al., 2011), and Jesmok et al. (2016) correctly classified 95.4% of soil bacterial profiles to their location of origin using various methods including abundance charts, non-metric multidimensional scaling, analysis of similarity, and k-nearest neighbor. However, this was a feasibility study with a modest sample size (n = 19). Further studies with larger sample sizes and replications are needed to explore the full potential of this approach.

Evaluating the microbial communities in an assemblage of soil samples (e.g., soils from a crime scene or alibi site and other intermediary sites) could be useful in forensics. Samples originating from a mixture of different soil substrates have been correctly differentiated by using a combination of Ribosomal Intergenic Spacer Analysis and 16S rRNA gene sequencing (Demanèche et al., 2017). Recent evidence suggests that 18S rRNA gene sequencing can also provide greater discriminatory power over traditional Mid-Infrared spectroscopy at fine scales for eukaryotic species (Young et al., 2015). Furthermore, Sanachai et al. (2016) demonstrated that the site origin of soil, obtained from the sole of a shoe, could be elucidated by comparing the similarity of soil bacterial 16S rDNA profiles acquired by the denaturing gradient gel electrophoresis technique.

Despite the potential in this field, further limitations need to be identified and addressed. For example, Pasternak et al. (2019) identified several potentially limiting factors to consider when interpreting the results of microbiomic analyses. For instance, soil samples are incredibly complex and highly heterogeneous even at short spatial scales, which presents a major issue to using these in a forensic context, and microbiomes exhibit a high level of physical, chemical, and biological diversity in both space and time. Pasternak et al. (2013) showed that actinobacterial fingerprints significantly differed between two seasons (summer and winter) at the same sites—implying that temporally-associated issues could arise. Keet et al. (2019) have also pointed out that soil microbiome composition can change as a result of abiotic soil conditions and plant community patterns—which poses a considerable challenge to the accuracy of results.

Metagenomic analysis of gravesoil and the soil around human and non-human animal cadavers has been undertaken for forensic purposes. Carter et al. (2015) investigated microbial community succession in soils associated with swine cadavers across two seasons (summer and winter). They demonstrated that postmortem microbial communities changed in specific and reproducible ways, but decomposition effects on soil microbial communities differed significantly between seasons. The authors suggest that the ecological succession of microbial communities will be useful for forensic investigations, but future research should aim to gain a greater understanding of seasonality on decomposition. The sample size for this study was not explicitly stated, but according to the ordination plots, it appears to be modest ($n \leq 10$ per treatment). Therefore, results should be interpreted with caution.

Adserias-Garriga et al. (2017) investigated daily thanatomicrobiome changes in the soil as an approach to estimate PMI. They collected soil samples from around human cadavers (n = 1 male and n = 2 females) and demonstrated successional changes on a daily basis. Rapid growth of *Firmicutes* was observed from the bloat stage to advance decay (<5% relative abundance at day 1 to 75% relative abundance day 12), and the authors proposed a *Firmicutes* growth curve to estimate PMI. However, the authors state that the growth curve results may only apply under Tennessee summer conditions and that confirmatory research is needed using a larger number of cadavers and under different environmental conditions. The results do, however, corroborate those of Finley et al. (2016), who evaluated microbial communities associated with gravesoil human cadavers (n = 18). The researchers allowed the cadavers to decompose over a range of decomposition time periods (3–303 days) and showed increases in the relative abundance of *Firmicutes* in surface bodies over the decomposition period (from ~10% at 0–3 months to ~40% at 7–9 months).

Singh et al. (2018) investigated the spatial (0, 1, and 5 m) dynamics of human cadaver decomposition on soil bacterial community structure. They collected soil samples from each spatial buffer (n = 14 for the 0 m, n = 17 for both 1 and 5 m) and observed

evidence of a predictable response to cadaver decomposition that varied over space. Bacterial community composition (beta diversity) at 0 m was significantly different from the 1 and 5 m communities, whereas there were no significant differences between the 1 and 5 m communities. The researchers also found that bacterial alpha diversity was significantly lower in the 0 m samples, suggesting that the additional nutrient input from the cadavers may reduce bacterial alpha diversity. This study provides additional spatial-compositional insights to complement the growing body of knowledge in this area of forensic microbiome applications.

Soil microbiome analysis has the potential to be used in forensics; however, additional research is required to validate the sensitivity and reproducibility of results (Young et al., 2017). Overall, to gain a greater understanding of both spatial and temporal dynamics associated with the microbiome and to develop techniques to mitigate similar pitfalls, further microbiome surveys are essential.

Different Spatial Dimensions and the Power of Machine Learning

The growing interest in sampling and predicting environmental microbiome profiles at different spatial scales and orientations (e.g., between households, cities, states, and altitudes) to provide information on the location and provenance of people and objects resulted in the development of a multitude of approaches. Chase et al. (2016) identified the three cities where nine offices were located with 85% accuracy based on analyzing office microbiome samples using sampling plates, and although this study suffers from a small sample size (n = 3 per office), it demonstrates potential with further refinement. Lax et al. (2014) analyzed samples from household occupants (n = 1625 from 18 participants in 10 houses) and their built environments. The authors matched feet microbiome samples to the house with 82.9% accuracy—a relatively low degree of accuracy from an evidentiary perspective but demonstrating the potential of such methods for fine-scale biolocalization. Walker and Datta (2019) analyzed whole-genome sequenced microbiota sampled from 12 cities in seven different countries as part of the 2018 CAMDA MetaSUB Forensic Challenge. The CAMDA dataset (n = 30) included three mystery samples. The authors applied machine learning techniques to identify the geographical provenance of the microbiome samples. Up to 90% of the samples were correctly classified, demonstrating the potential of machine learning applications to biogeography, although further evidence is necessary to employ these applications in an evidentiary context. In a related study, Ryan (2019) applied a random forest classifier built on a dataset of 311 city microbiome samples. Their method correctly classified 83.3% of the mystery samples. Grantham et al. (2019) presented a different algorithm for predicting the geolocation of fungal samples from dust (n = 1300) in the United States using deep neural network classifiers. Applied to a global dataset of samples from 28 countries, the authors state that their algorithms make “good point predictions” with >50% of the geolocation errors under 100 km for US-wide analysis and nearly 90% classification accuracy of a sample’s country of origin for the global analysis. This particular field, combining microbiomics and machine learning, is in its infancy, and future studies would benefit from larger sample sizes and improved classification accuracy before such approaches can be used with confidence in a forensic context.

Another important spatial factor to consider is that microbiome compositions do not only differ in horizontal space. Skin microbiomes have also been shown to differ between humans living in high and low altitudes. For example, Zeng et al. (2017) collected skin microbiome samples from humans (n = 99) and pigs (n = 82) in Tibet. They found enrichments of several bacterial taxa (e.g., *Arthrobacter* sp., *Paenibacillus* sp., and *Carnobacterium* sp.) in samples collected from higher altitudes. Alpha diversity was also significantly lower in skin samples collected from individuals living at higher altitudes. This suggests a potential future route to determine geolocation based on altitudinal parameters via the analysis of skin microbiome samples in the future—although here too, methodological refinement will be essential. Furthermore, understanding how skin microbiomes may fluctuate throughout the life course will also be an essential factor to consider.

Overall, all the models prioritize classification over prediction abilities. To enable real-time prediction of geographical coordinates from sampling data, increasing the sample sizes geographically and temporally, and developing more rigorous methods is essential.

Personal Identification

A growing body of evidence suggests that human individuals may be uniquely identified based on stable autochthonous (i.e., native to a given environment) microbial profiles. This could have a substantial impact on forensic science—for example, in situations where the investigator cannot retrieve sufficient amounts of human DNA (i.e., from human somatic and germ cells). Yet it is unknown whether the variation in microbial communities between people is sufficient to identify individuals within large populations uniquely or stable enough to place them over time.

To answer some of these questions, Franzosa et al. (2015) tested different body site-specific microbial profiles and attempted to match them with 25–105 microbiome profiles during the person's first and second visits to the sampling site. The authors reported that these profiles were useful in distinguishing individuals at the initial sampling time point and that 30% of the individuals were still uniquely identified several months later. In this study, gut microbiome samples were used to pinpoint 80% of individuals (n = 120) up to a year later. These results are encouraging—particularly in shorter timescales—however, they still suffer from relatively high variability. As such, greater improvements, e.g., in methods and sampling effort, will be needed before such approaches can be useful in a forensic setting.

High resolution melting analysis that targeted the 16S rRNA gene from oral swab samples have also been used to demonstrate its potential in distinguishing between individuals (Wang et al., 2019), albeit with a very small sample size in this study (n = 5). Schmedes et al. (2018) demonstrated accurate identification of individuals (n = 12) based on skin swab samples from different body sites (n = 14). They achieved 97% accuracy by sampling shirts and 96% accuracy using palm samples based on 1-nearest neighbor classification on nucleotide diversity of the bacterial genome. In another recent study, the researchers utilized a similar approach to identify individuals (n = 51). They analyzed microbiome samples collected from three different body sites—the manubrium (i.e., the upper-most segment of the sternum), the palmar surface of the hand, and the ball of the foot (Woerner et al., 2019). The researchers achieved 100% classification accuracy when conditioned on a maximum nearest neighbor distance for diversity, suggesting strong potential should these results be replicable in studies with much larger sample sizes.

Watanabe et al. (2018) suggested that minor taxa are one of the key factors for distinguishing between individuals. Their study analyzed microbiome samples (n = 66) from individuals (n = 11) over 2 years and achieved 85% accuracy in distinguishing individuals. They also used the same analytical methods to classify publicly available skin microbiome samples from individuals (n = 89) with a 78% identification accuracy. However, this level of accuracy is unlikely to be sufficient for forensic applications. The authors suggested that although personal identification is possible, the estimation of the accuracy decreases for larger cohorts due to increments of similar microbiome patterns. Overall, the use of microbiomics as a forensic tool to determine personal identification shows potential and technological viability and might be useful in situations where the investigator is unable to retrieve sufficient amounts of human DNA. Nonetheless, the findings fall short of the burden of proof. Improvements in the model's sensitivity and specificity are required, and a methodology to address potential contamination issues. Furthermore, a better understanding of the microbial dynamics across time and space is essential for the findings to have a forensics value.

Biological Sex Determination

Recent evidence supports another contribution of microbiomics toward personal identification –the discrimination of biological sex, which could be useful where sufficient quantities of human DNA are unable to be retrieved. For example, airborne bacteria communities have previously been characterized in indoor environments (Chan et al., 2009). Luongo et al. (2017) investigated airborne bacterial and fungal diversity (i.e., constituents of the "aerobiome") from different University dormitory rooms (n = 91). They used machine learning techniques and were able to predict the biological sex of room occupants with 79% accuracy based on relative abundances of the microbiota. Curiously, rooms occupied by males exhibited higher relative abundances of the microbiota. The authors suggested that it could be because males may shed more biological particles or use fewer cosmetic barriers such as skin lotions.

Biological sex-related differences in the human thanatomicrobiome—the microbial communities colonizing organs following death (*thanatos*, Greek for death) (Zhou and Bian, 2018)—have also been demonstrated by Bell et al. (2018). The authors compared amplicon signatures (using the 16S rRNA gene V1-2 and V4 regions) in the corpse heart tissue of 10 individuals and discovered key differences between males (n = 6) and females (n = 4). For example, *Streptococcus* sp. was found exclusively in male heart tissues, whereas females had a significantly higher prevalence of *Pseudomonas* sp. With refinement, such an approach could help to determine the biological sex of a corpse and the provenance of body parts.

In a study by Tridico et al. (2014), the authors "readily distinguished" male (n = 3) and female (n = 4) subjects based on the analysis of their pubic hair microbiomes. They identified *Lactobacillus* spp. that were unique to female participants. They also suggested that pubic hair is relatively insulated from the environment and colonized with niche-specific microbiota, which could be useful in forensic investigations. Unfortunately, the modest sample size of this study limits the conclusions that can be drawn from it. Nonetheless, the findings were supported by another small study by Williams and Gibson (2017), who identified individuals (n = 9) and their biological sex from pubic hair microbiota with an error ratio of 0.027 ± 0.058 and 0.017 ± 0.052, respectively.

However, the sample sizes for all these studies are modest, and as such, further validation studies with larger sample sizes are needed before reliable conclusions can be drawn.

Interestingly, Phan et al. (2020) analyzed skin microbiome samples from both genders (n = 45) and found that the absence of the bacterial genus *Alloiococcus* could be useful in predicting female biological sex. The study showed a correlation between certain bacterial species and personal characteristics (e.g., biological sex). They specifically explored the presence/absence of microbiota from fingermarks left behind on surfaces and achieved a relatively modest 67% sex prediction accuracy using leave-one-out cross-validation analysis. Improvements in sample sizes and machine learning accuracy are necessary to explore the potential of this approach further. Additional research into whether certain bacteria (and other microorganisms) are distinct to females or simply related to external factors (such as cosmetic products on hands) would also be necessary.

Trace Evidence

There is an increasing interest in studying forensically relevant microbial profiles left behind on objects and surfaces. For instance, several studies showed that there is often a high level of bacterial presence on personal objects such as mobile phones (Koroglu et al., 2015; Kõljalg et al., 2017; Koscova et al., 2018; Kurli et al., 2018). Furthermore, human-associated items such as shoes and mobile phones have been shown to support distinct microbiomes (Lax et al., 2015; Coil et al., 2019).

Meadow et al. (2014) investigated the potential utility of mobile phones as "personal microbiome sensors." They selected 17 individuals and collected three samples (the cell phone's touch surface, their index finger, and their thumb). They demonstrated that bacterial communities sampled from mobile phones were more similar to their owners than other people. They found that about 22% of the taxa on participants' fingers were also found on their phones, whereas only 17% were shared with other people's phones. An individual's index finger shared approximately 5% more OTUs with their mobile phone than with everyone else's mobile phone in the study. Furthermore, 82% of the OTUs were shared between a person's index finger and their phone. Although promising, here again, the sample size and accuracy of results need to be increased in future studies.

Kodama et al. (2019) found that postmortem skin microbiomes could be associated with personal objects with a high degree of accuracy. Several of the objects in the study were associated with 100% accuracy (i.e., medical devices, eyeglasses, bottles, and steering wheels), whereas objects like computer devices, remote controls, and cell phones were associated with over 67% accuracy, suggesting that with refinement, skin microbiome samples could be reliably linked to objects at the scene. Furthermore, studies have found that the postmortem skin microbiomes were stable and similar to antemortem skin microbiomes for up to 60 h postmortem (Kodama et al., 2019).

Salzmann et al. (2019) investigated the microbial profiles of different bodily fluids (n = 22). They identified source-specific microbial signatures from various bodily fluids. For example, the phyla *Proteobacteria* was associated with skin and semen sources, whereas *Firmicutes* showed a higher prevalence in saliva and vaginal secretions. Dobay et al. (2019) suggest that even when body fluid is exposed to indoor conditions for 30 days, samples continue to harbor body-site-specific microbial signatures. Hanssen et al. (2017) also demonstrated promising results, albeit with a small sample size (n = 6), for the microbially-mediated classification of body fluids. They performed pattern recognition by fitting a linear discriminant analysis model using Principal Component scores and were able to classify saliva samples in 94% of the cases.

Neckovic et al. (2020) recently investigated the potential transfer of skin microbiomes between individuals and substrates (i.e., allochthonous microbiota). They found that skin microbiota has been reliably transferred through direct contact, that is, between individuals shaking hands. Microbiota also transmits through indirect contact, as demonstrated by individuals rubbing a substrate and then swapping substrates with another person. The authors suggested that such analysis could be useful to corroborate sexual assault cases or other contact-related crimes. They also suggested that further research should consider the relative surface area of contact, pressure, friction, and the duration of the contact.

Manner and Cause of Death

The 'manner of death' is a determination made by an expert following an investigation (e.g., a coroner, the police, or a medical examiner). Five manners of death are generally considered: natural, accidental, suicide, homicide, and undetermined (Advenier et al., 2016). Lutz et al. (2019) recently collected microbiome samples from 265 corpses from Finland, Italy, and the United States. The inspected cadavers differed in the manners of death: accidental death (n = 88), natural death (n = 106), homicide (n = 23), and suicide (n = 45). Their results suggested that *Lactobacillus*, *Enterobacteriaceae*, *Sediminibacterium*, *and Rhizobiales* were

associated with different manners of death. With further research, these associations could be developed into predictive markers that help to determine the manner of death. However, as noted by the authors, *Sediminibacterium* and *Rhizobiales* bacteria may also represent environmental contamination, which needs to be controlled, and further validation through controlled experiments is needed to improve the reliability of their approach to determine the manner of death.

The potential of this microbiomics approach to determining the manner of death was corroborated in a recent study by Zhang et al. (2019) who, by obtaining samples during routine death investigations at the Wayne County Medical Examiner's Office (Detroit, Michigan, United States), found different biomarkers associated with the manner of death. In this study, *Xanthomonadaceae* was more prevalent in cases related to hospital deaths, whereas *Actinomyces* sp. tended to be more prevalent in suicide cases. Increasing the numbers of samples generally increased the accuracy of the models. The authors cautioned that the prediction accuracy depends on the machine learning methods used and the number of anatomical sites analyzed. The authors suggest this study provides baseline information, and it could be possible to use machine learning to develop reference databases that allow microbially-mediated manner of death predictions in the future.

Kaszubinski et al. (2020) modeled beta-dispersion to test for manner and cause of death association using a microbiome data set of n = 188 postmortem cases (five body sites per case). The researchers demonstrated that beta-dispersion and demographic data could distinguish among manner and cause of death. In particular, they found that cardiovascular disease and drug-related deaths were correctly classified in 79% of cases. They found that binary logistic regression models were most effective at improving model success. This was an improvement over multinomial logistic regression models, which confirmed the manner and cause of death assessment only 62% of the time. The results of this study show promise for using postmortem microbiomes to indicate the manner of death. However, as the researchers' highlight, sample sizes need to be greater. Moreover, the development of large databases will likely be required to train models with high success rates prior to being used in practical forensic contexts.

In terms of cause of death (i.e., the disease or injury that produces physiological disruption in the body leading to death), researchers such as Christoffersen (2015) have investigated the importance of microbiological testing. Studying autopsy results (n = 42), the author reported that the cause of death could be determined in 42% of the cases via microbiological analysis. The study highlighted factors indicative of a microbiologically related cause of death, such as a raised CRP measurements. Raised CRPs have also been implicated in SIDS as a cause of death (Rambaud et al., 1999; Szydlowski et al., 2013) and even for astronauts returning from space (Garrett-Bakelman et al., 2019). Deadly bacterial infections, such as infection or sepsis, may also occur following neonatal circumcision (Elhaik, 2016, 2019).

A specific forensic microbiome application for determining the cause of death is the diagnosis of 'death by drowning,' which is one of the leading causes of unnatural deaths worldwide (Domínguez et al., 2018; Cenderadewi et al., 2019). Analyzing the presence of diatoms (single-celled algae) has been the 'gold standard' for well over a decade; however, its reliability has been questioned (Kakizaki et al., 2009; Huys et al., 2012). Several studies have provided support for death by drowning diagnoses by designing real-time PCR assays with primers to detect bacterial species associated with aquatic environments, such as *Aeromonas* spp. (Aoyagi et al., 2009; Uchiyama et al., 2012; Rutty et al., 2015; Voloshynovych et al., 2019). These studies provided support for this cause of death diagnosis based on relatively high detection rates of microbiota, for example, 84% (n = 32), 75% (n = 20), and 84% (n = 43)—although to strengthen the cause of death diagnoses, the accuracy levels, and sample sizes could again be much improved. It has also been suggested that bioluminescent bacteria may be biomarkers for death by drowning in seawater. For example, Kakizaki et al. (2009) developed a simple assay targeting the 16S rRNA gene to identify bioluminescent colonies such as *Vibrio fischeri* and *Vibrio harveyi*. More recently, Lee et al. (2017) analyzed microbiome composition and pulmonary surfactant protein (SP-A) expression to develop a marker for diagnosis of death by drowning. They analyzed microbiota and histological appearance of both drowned and postmortem groups of experimental rats, comparing freshwater vs. marine water treatments. The authors found that 5513 and 5480 OTUs were unique to marine and freshwater, respectively. They also found that expression levels of SP-A were higher in the lungs of drowning victims compared to postmortem submersion. These findings could have important forensic value (e.g., determining both the type of environment and the timing of death) and demonstrate good potential for future applications. Marella et al. (2019) point out that other studies have focused on the presence of fecal bacteria, coliforms, and streptococcal bacteria to help determine the cause of death by drowning. These bacteria are sampled from the femoral artery and vein and the right and left ventricles. Fecal bacteria are considered to be always present in subjects who drowned compared with those with other cause of death diagnoses (Lucci et al., 2008; Marella et al., 2019). For example, Lucci and Cirnelli (2007) found fecal streptococcal presence in 100% of the freshwater drowning cases they studied (n = 22) and coliforms present in 90.91%. In

this study, the control subjects (n = 30) uniformly showed an absence of fecal bacteria. In a later study, Lucci et al. (2008) assessed if the presence of these bacteria in the drowning medium could be detected in victims submerged after death. The researchers collected samples from drowned victims (n = 5 freshwater and n = 5 in seawater) and victims who were submerged after death (n = 3). Coliforms and streptococci were detected in all drowned victims but not in those submerged after death. These findings suggest that fecal coliforms and streptococci could be used as markers of drowning. However, the minuscule sample sizes must be interpreted with caution and increased considerably in future studies.

Postmortem Interval

The Thanatomicrobiome

Determining the PMI (the time elapsed since a person has died) is often an essential part of a criminal investigation. To improve PMI prediction accuracy, researchers have begun examining the thanatomicrobiome (Javan et al., 2016; Burcham et al., 2019). Postmortem, these communities overwhelm the immune system allowing for subsequent colonization (Javan et al., 2019). Preliminary studies suggest that these microbial communities may undergo important successional changes in organs that could aid in determining the PMI (Adserias-Garriga et al., 2017).

Early studies on model animals suggest that this is feasible. For over a 48-day period of decomposition, Metcalf et al. (2013) aimed to uncover a “microbial clock” to provide an estimate of PMI by sequencing the 16S rRNA gene for bacterial and archaeal communities and the 18S rRNA gene for microeukaryotes. Their model provided reliable PMI estimates (±3 days) (n = 223). However, the study was conducted in controlled conditions using experimental mouse models—thereby necessitating a degree of caution when extrapolating the data to ‘real-life’ situations. Another study investigated the decomposition of pig cadavers. Their model predicted the PMI within 2–3 h of the time of death with 94.4% accuracy (Pechal et al., 2014), demonstrating promise with further methodological refinement. Pechal et al. (2018) carried out a large-scale study of body microbiome samples (n = 188) that found postmortem microbiomes were stable, reflecting antemortem microbiomes 24–48 h after death. The researchers also found that specific bacterial taxa were important in predicting health status. For example, *Haemophilus* and *Fusobacterium* were twice as abundant in healthy individuals, whereas *Rothia* was 0.09 times more abundant in heart disease cases. With further development, this could be used to indicate the state of human health during clinical investigations into a range of deaths, from chronic and natural to sudden and violent (Pechal et al., 2018). It is important to note that, although appropriate at the time, the bioinformatics approach used to process OTUs and to make functional predictions (e.g., QIIME 1.8 and PICRUSt 1) is now considered to be outdated. Furthermore, Amplicon Sequence Variants (ASV) may provide a richer taxonomic picture (Callahan et al., 2017).

Studying human subjects, Johnson et al. (2016) sampled the skin microbiome of decomposing human cadavers and developed an algorithm to estimate PMI. The authors achieved low error rates for skins samples and a PMI estimation accuracy of ±2 days (n = 144 from 21 cadavers), a substantial improvement compared to prior efforts (e.g., via entomological analysis). Belk et al. (2018) used 16S rRNA amplicon sequencing and found that creating models with the class or phylum taxonomic levels provided the most accurate predictions of PMI. This finding corroborated the study by Johnson et al. (2016) and illustrated its potential usefulness for forensics.

Another study using 454 pyrosequencing to determine abundances and diversity of the postmortem microbiome in several key organs such as the brain, heart, liver, and spleen found varying PMIs ranging from 29.5 to 240 h (Can et al., 2014). This study revealed that the most abundant taxa in postmortem microbial communities were the anaerobic, spore-forming Firmicute bacteria, *Clostridium* sp. Javan et al. (2017) confirmed that *Clostridium* sp. dominated at long PMIs, adding evidence to support the use of microbiomics in PMI determination in the future.

Localization Through Animal Microbiomes

Several studies have shown that animals from different taxonomic groups and environments possess unique microbial profiles. For example, Tibetan chickens *Gallus gallus*, Chinese Rhesus macaques *Macaca mulatta*, and plateau sheep *Ovis* spp. have unique gut microbiomes (Zhou et al., 2016; Huang et al., 2017; Zhao et al., 2018) shaped by genetic, geographical, and altitudinal factors. It has been demonstrated that the skin microbiomes of Estrildid finches, amphibians, bats, cetaceans, and dogs *Canis lupus familiaris* are unique (McKenzie et al., 2012; Avena et al., 2016; Erwin et al., 2017; Torres et al., 2017; Engel et al., 2018; Russo et al., 2018). Interestingly, Song et al. (2013) found that humans share microbial communities with their dogs.

With further investigations and methodological refinement, such capabilities point to the potential feasibility of linking a person with a site based on shared microorganisms with animals. Although further studies are needed, there is potential for forensic pathways to associate trace microbial profiles obtained from other species (unique to the given species) to a given environment and/or occupation (e.g., animal industries) or to pet ownership. For example, non-human animal-specific microbiota could potentially be detected on the body or clothing of a suspect or victim, which may be useful in the absence of sufficient animal DNA (i.e., from somatic and germ cells) evidence. This profile could then conceivably be traced to the point of contact with an animal or animal-based environments such as equine stables, pet shops, or zoos, thus complementing other traditional forensic evidence. However, this approach is mostly theoretical at the moment, and future research will be needed to test its feasibility.

Discussion

As of today, microbiome-based forensics are almost absent from criminal investigations and courts. To explain why this is so, we may divide the different possible applications of microbial forensics into two groups: first, reconstruction issues such as the cause of death and PMI, which ask “what happened?” and help elucidate the circumstances of the crime; and second, comparison issues such as geolocation and personal identification, which ask “how similar are these two DNA profiles?” and may (dis)connect a suspect from an object or a place (e.g., murder weapon or crime scene). Reconstruction applications for forensic use are easier to develop since competing propositions are usually well-defined and limited in number; if sufficient research is invested in ascertaining the microbial characteristics associated with each combination of possible environmental, spatial and temporal conditions, then reconstruction becomes straight-forward. For example, if a cadaver is found buried at a depth of 1 m in a desert in summertime, and the temporal succession of the gut microbial community for these environmental conditions has previously been established, then PMI can be inferred with a high degree of accuracy and certainty. Thus, reconstruction microbiomics can readily pass the *Daubert* standard set by the US supreme court (Daubert v. Merrell Dow Pharmaceuticals Inc, 1993) to be recognized as admissible evidence bearing sufficient scientific foundation, including general acceptance in the scientific community, known and acceptable rate of error, and so on.

Comparison microbiomic tools, on the other hand, may provide greater benefit to the criminal investigation but are harder to develop to a level that would satisfy the *Daubert* standard. The most beneficial way to employ such tools would be in a “one-to-many” configuration, similar to forensic human DNA analysis: a DNA profile from trace evidence is compared to all the profiles (from known persons and locations) in a database, and if it exists in the database, its frequency in the relevant population (e.g., of soils) is calculated to enumerate the probability of encountering this profile by chance (i.e., originating from a location or person unrelated to the crime). At this time, however, there are hardly any relevant forensic databases of microbiomes that can be compared to trace evidence. In their absence, the only way to proceed in a forensic context is in a “one-to-one” configuration. For each criminal case, questioned samples (e.g., from a suspect’s shoe) are compared to context samples from the crime scene, alibi area, and other relevant sites. This approach provides less benefit to the investigation because it can only give conclusions of exclusion, that two samples do not share the same origin, or relative conclusions, such as “sample A is more similar to sample B than it is to sample C.” Inclusionary conclusions such as “sample A is very similar to sample B; the probability of a different sample, from another place, person or time, being this similar to sample B at random is 1 in X Million” is impossible without either an extensive database (which is costly to build and maintain) or thorough theoretical knowledge (which we do not have yet) regarding the factors that shape microbiomes. However, even for “one-to-one” analyses, we can provide a statistical evaluation of the evidence (Habtom et al., 2019) based on the Likelihood Ratio framework as recommended by the European Network of Forensic Science Institutes (Willis et al., 2015).

There are three more major hurdles to forensic tools of comparative microbiomics. The first one involves samples of mixed origins, containing substrates or DNA from different locations or persons. DNA analysis of mixtures is difficult even with simple human DNA profiles, and certainly more so with microbiomic DNA profiles which are far more complex. It is often impossible to tell with certainty which, or even how many, disparate microbiomes are present in the mixture, let alone accurately infer the DNA profile of each one. The famed 2016 report of the US President’s Council of Advisors on Science and Technology [President’s Council of Advisors on Science and Technology (US), 2016] found that the prevalent subjective analysis of complex human DNA mixtures by forensic experts is not a reliable methodology. Consequently, several computer programs were developed to interpret complex human DNA mixtures in an objective manner, and these are slowly being validated and accepted for routine forensic use. Software for objective analysis of microbiomic DNA mixtures may be built on this basis, but these are still years in the future. The second major hurdle is temporal variation. Contrary to human DNA, which can remain unchanged for years, microbial communities (both

on the body and deposited as trace evidence) can fluctuate over time, often in correlation with changes in environmental parameters like moisture and pH (e.g., Pasternak et al., 2013). In cases where two samples for comparison are obtained at different times when markedly different environmental conditions prevail, mitigating the temporal changes in community structure is needed before analysis can ensue. So far, only a few studies have addressed this topic experimentally, mainly by applying various carbon sources to force the different microbial communities to "converge" (Pasternak et al., 2019), but so far with limited success. The third, and perhaps most challenging hurdle, is the problem of DNA transfer. In the past decade, human DNA evidence gained widespread credibility and acceptance in the courts so that the identification of a DNA profile from trace evidence as originating from a specific person is rarely disputed nowadays. Instead, it is becoming more and more common for the defense to challenge the method of deposition of the DNA, suggesting that it reached the crime scene by a legitimate activity (before or after the crime occurred) or by DNA transfer (e.g., when the innocent suspect shook the hand of the real perpetrator). This hurdle can sometimes be overcome by using the likelihood-ratio approach with activity-level propositions (Mayuoni-Kirshenbaum et al., 2020); however, similar to the former hurdles, this one is also very much still an open question, and it will take more time, effort, and research before microbiomics is ready to be employed and accepted within the legal system.

Conclusion

Over the last decade, advances in genomic sequencing and bioinformatics have given rise to microbiomics, which fructified in a growing compendium of tools seeking to explore the panoply of microorganisms present in our bodies and environment. The evidence examined in this review indicates that microbiomics could be a forensically relevant and promising discipline with a multitude of applications—from determining substrate provenance and acquiring trace evidence to identifying individuals and estimating PMI. These advances may allow various microbiomic data, like those obtained from thanatomicrobiome analysis, to be used by forensic scientists to address questions related to criminal investigations, or at least be used alongside other forensic methods.

Throughout their life-course, humans and their microbiomes undergo complex interactions and co-adaptation processes involving nutrient intake and resulting in the production of decomposition products such as metabolites. Following a person's death, these interactions change dramatically, and the microbiome composition and dynamics fluctuate accordingly. Understanding these colonizations and fluctuations represent major conceptual, methodological, and computational challenges—as do antemortem microbial dynamics. Related microbiome-based research in a forensics context and greater exploration of fungal and viral communities may also lead to an important enhancement in the forensic toolkit in the future.

Many challenges remain to overcome, such as contamination issues, modest study sample sizes, model over-specification and misspecification, prediction accuracies of machine learning techniques, understanding complex spatial and temporal variations in environmental microbiome dynamics, as well as risks and ethical concerns (Shamarina et al., 2017). Notably, even human DNA-based evidence, which is far better understood, is not error-proof, as indicated by the Phantom of Heilbronn case (Daniel and van Oorschot, 2011). Moreover, the vast majority of published work used 16S or targeted sequencing approaches, which have known limitation for taxonomic resolution and could likely benefit from metagenomics methods (Ogilvie and Jones, 2015; McIntyre et al., 2017) and/or methods that utilize longer reads (Danko et al., 2019b; Foox et al., 2020). Also, more field-based testing and deployment of these sequencing methods could benefit from rigorous, titrated standards for ensuring accuracy (McIntyre et al., 2019). Given this, many of the applications reported in the literature should be considered proof of concepts rather than full-fledged forensic applications. Nonetheless, as Ogilvie and Jones (2015, p. 1) have summarized: "*it is clear that we remain in a period of discovery and revelation, as new methods and technologies begin to provide [a] deeper understanding of the inherent ecological characteristics of this [microbial] ecosystem.*"

Author Contributions

EE initiated the study. JR carried out the review. EE, JR, ZP, and CM wrote the manuscript. All authors approved the manuscript.

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Conflict of Interest

EE consults the DNA Diagnostics Center. CM is a co-founder of Biotia, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Abbreviations

PCR, polymerase chain reaction; MetaSUB, metagenomics and metadesign of subways and urban biomes; PRISMA, preferred reporting items for systematic reviews and meta-analyses; CAMDA, critical assessment of massive data analysis; PMI, postmortem interval; OUT, operational taxonomic unit; CRP, C-reactive protein; SIDS, sudden infant death syndrome.

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Quick Quiz

Check your Understanding

- What types of microbiomes can be analyzed in regards to geolocation for forensic science?
- How could microbiome analytical techniques be improved for personal identification?
- How are various microbiomes different between biological sexes?
- What are some common objects that can have microbiomes applicable for trace evidence?
- What is the thanatomicrobiome and how can it be used to determine postmortem interval?
- What are the major difficulties with comparative microbiomes concerning forensic applications?

Media Attributions

- Figure 1 – A summary of possible sources of forensically relevant microbiota identified by the literature review by Robinson et al., 2021 licensed under the terms of the Creative Commons Attribution License (CC BY).

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CHAPTER OVERVIEW

9: Case study

9.1: Case Study - Environment

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9.1: Case Study - Environment

Case study #2 – The Land is Sick

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Part I – Background and Problem

Coffee production in South America has drastically decreased in the 2021-23 growing seasons. Specifically, the major farms in Columbia and Brazil which collectively contribute over half of each countries coffee (both specialty and commercial) production, have had severe crop failures. As a result, the price of a cup of coffee has noticeably increased, putting financial strain on suppliers and businesses.

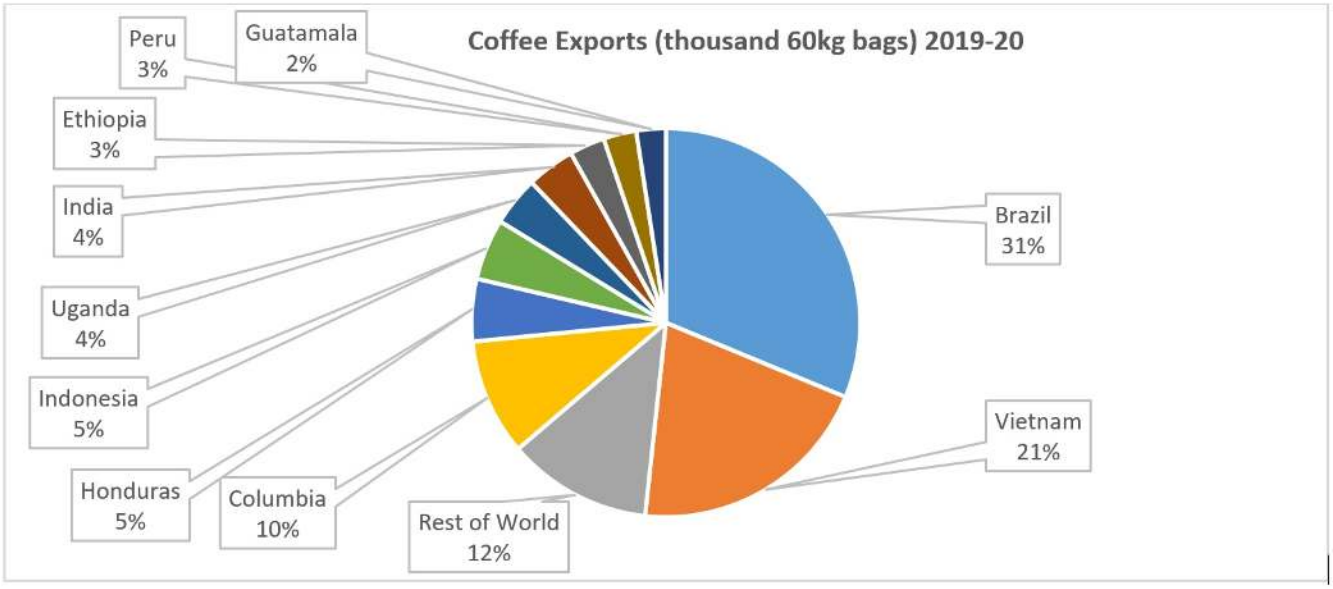


Figure 1. Coffee exports by country 2019-20. Source: International Coffee Organization

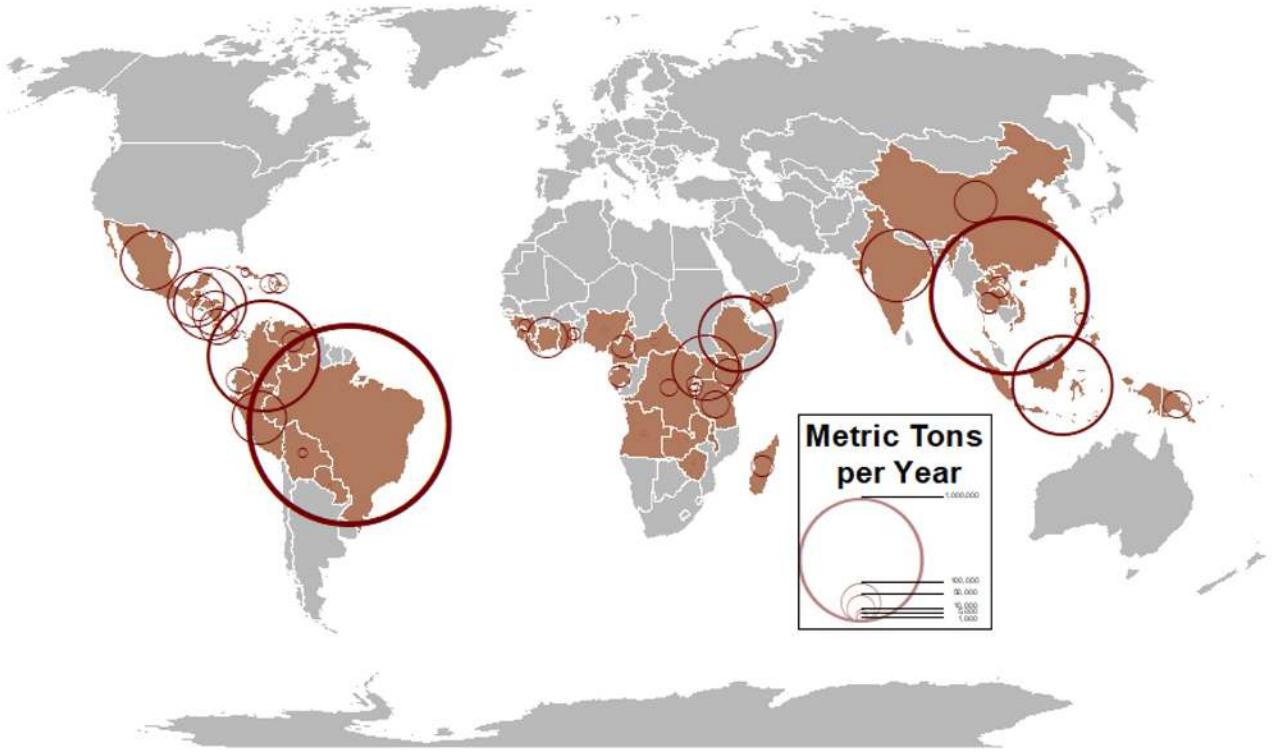


Figure 2. A world map of countries by coffee production, 2019.

Interestingly, the few years prior to this decline, coffee production was at an all-time high, with more coffee in the market than demanded. This dropped the price of coffee, and in many cases original farmers were not fairly compensated.

The International Coffee Organization (ICO) has tasked a team of researchers to identify potential problems to South American coffee plant failure and provide quick and efficient resolution.

Article 1 – The Bacterial Microbiome of *Meloidogyne*-Based Disease Complex in Coffee and Tomato

Article 2 – A review of three major fungal diseases of *Coffea Arabica* L. in the rainforests of Ethiopia and progress in breeding for resistance in Kenya

Article 3 – Structure and Dynamics of the Gut Bacterial Community Across the Developmental Stages of the Coffee Berry Borer, *Hypothenemus hampei*

Video 1 – The biggest threats to the coffee industry

Video 2 – #1 Specialty coffee and the price crisis | Producer Crossover 2019

Questions

1. As a scientific researcher, what are some factors you would take into consideration when addressing this problem?
2. What type of environmental microbiomes could be implicated in coffee plant crop failure and why?
3. Should other coffee farms in countries besides those in South America be worried about similar failure in their crops? Why or why not?

Attributions

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Case study #2 – The Land is Sick

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Part II – Approach, Implementation, Reasoning

Upon initial inspection, the unhealthy coffee plants exhibited yellowing of leaves, appearance of red-brown lesions, abnormal shape, damaged coffee berries, and little new growth or production. Researchers decided to take samples of healthy and unhealthy leaves and berries, as well as soil and root samples.

Article 1 – Prokaryotic diversity in the rhizosphere of organic, intensive, and transitional coffee farms in Brazil

Article 2 – A metagenomics approach in the evaluation of the soil microbiome in coffee plantations under organic and conventional production in tropical agroecosystems

Video 1 – #2 Producer Crossover 2019

Video 2 – How countries farm and make coffee differently

Over the next year, other plantations in Columbia and Central American begin to experience similar declines in coffee plant health. Farmers were questioned about any changes in practices, and while there have been some adjustments do to the fluctuating coffee prices, traditional farming techniques have remained the same for the most part in smaller plantations. Larger plantations with intensive farming made more changes due to trader suggestions on improving yields including application of different fertilizers and hiring less experienced workers.

Questions

1. What type of analytical techniques do you think were or should be performed on the samples taken?
2. How could the observed symptoms of the unhealthy plants be connected to an associated microbiome?
3. How could human intervention exacerbate or ameliorate this situation?

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Part III – Discussion

Analysis of the negatively affected plantations' soil showed a decline in plant growth promoting bacteria and mycorrhizae and an increase in bacterial and fungal pathogens like *Pseudomonas syringae* and *Cercospora coffeicola*.

Over the next few years (2022-24) coffee plants still struggle to grow in Central and South America. Other parts of the world, such as Vietnam and other Asian countries, are seeing a decline in crop health also, further contributing to global coffee market troubles. Many of these farms switched from shade-grown to intense sun-grown coffee to try to meet needs for demand. Larger corporations and roasters have purchased many of the smaller family farms to try to recoup losses and implement 'new and improved' growing strategies for increased production. This included switching to primarily sun-grown coffee, and application of large amounts of synthetic nitrogen fertilizer.



Figure 1. Shade grown coffee in Guatemala



Figure 2. Sun-grown coffee plantation in Brazil

Video 1- #3 The future of the price crisis

Video 2 – How coffee destroys the environment

Article 1 – Root endophytes of coffee (*Coffea Arabica*): Variation across climatic gradients and relationships with functional traits

Article 2 – Brazilian Coffee Production and the Future Microbiome and Mycotoxin Profile Considering the Climate Change Scenario

Article 3 – Effects of environmental factors on microbiota of fruits and soil of *Coffea arabica* in Brazil

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Questions

1. What factors could cause these changes in the soil microbiomes?
2. Why do you think coffee plantations across the globe are beginning to fail as well?
3. How does natural biodiversity benefit ecosystem health on both a micro and macro scale?

Attributions

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Part IV – Resolution

In 2030, most varieties of coffee are extinct, and only small amounts of coffee are produced on shade grown farms further from the equator. It appears that coffee plantations were severely impacted by increasing global temperatures, which altered ecosystem dynamics and promoted pathogen and pest invasion. Fungi, bacteria, and arthropods devastated already struggling coffee farms and with a major switch to sun-grown coffee, soil microbiomes were depleted and disease spread rapidly through monoculture crops. This switchover was prompted by an energy drink corporation, KAPOW!, which wanted to corner the caffeine market and boost production, though their expertise in growing coffee was lacking and the excessive use of synthetic fertilizers on already sun baked land which requires much more watering further doomed plantations. At least they now produce a cheap "coffee" flavored energy drink. It tastes terrible.

Article 1 – Coffee Microbiota and Its Potential Use in Sustainable Crop Management. A Review

Article 2 – Soil fungal communities differ between shaded and sun-intensive coffee plantations in El Salvador

Article 3 – One health relationships between human, animal, and environmental microbiomes: A mini-review

Video 1 – Teaching coffee farmers about the birds and the bees

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Questions

1. In what ways are environmental microbiomes impacted by changing environmental factors and how can this promote diseases within an ecosystem?
2. How could various environmental microbiomes be utilized to improve sustainable practices and technology?
3. How are environmental and human microbiomes interconnected?

Attributions

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- 9: Case study - *Undeclared*

- 9.1: Case Study - Environment - *CC BY 4.0*
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