INTRO TO BIOTECHNOLOGY



Andre Walther
Cedar Crest College

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Introduction to Biotechnology

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1: INTRODUCTION TO SCIENCE

1.1: Definition and a Short History of Biotechnology
1.2: The Process of Science

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1.1: Definition and a Short History of Biotechnology

Overview of biotechnology

Biotechnology is a multidisciplinary field that combines biology and technology in order to develop products and processes that improve the quality of life and the environment. It involves the manipulation of living organisms or their components, using them to solve problems and produce useful products. Biotechnology plays an essential role in our everyday life, from the production of medicine, to the food we eat, to the clothes we wear. It applications range of from agriculture to medicine, energy production, chemical processing, industrial manufacturing, and bioremediation.

Learning Objectives

By the end of this section, you will be able to:

- list some of the areas of biotechnology
- outline some of the major events in the history of biotechnology
- describe the interdisciplinary nature of biotechnology

Areas of Biotechnology

Biotechnology profoundly impacts our everyday lives. Its broad scope encompasses several key areas that contribute to the quality of human life worldwide. Some of the areas of biotechnology in use today are:

1. **Medical and Health Biotechnology ("Red Biotechnology)**
2. **Agricultural Biotechnology ("Green Biotechnology)**
3. **Food Biotechnology ("Yellow" Biotechnology**
4. **Industrial and Manufacturing Biotechnology ("White Biotechnology)**
5. **Marine Biotechnology ("Blue" Biotechnology)**
6. **Environmental Biotechnology ("Gray" Biotechnology)**
7. **Land Management Biotechnology ("Brown" Biotechnology)**
8. **Bio-defense Biotechnology ("Black Biotechnology)**

Many of these areas will be discussed in subsequent chapters in this textbook.

Brief History of Biotechnology

Throughout history, humans have used biology to manipulate living organisms, from altering bacteria to enhance fermentation, to the selective breeding of plants or animals to improve desired traits. Such careful manipulation of their biology has created the modern organisms we see and use today. If one were to compare the modern apple with its ancestral counterpart, one would be shocked at the difference. Ancestral apples were small and tart (Figure 1.1.1). Through selective breeding principles, the modern apple is significantly larger and sweeter. These breeding principles are an example of how biotechnology can be used to change an organism.



Figure 1.1.1: Wild Siberian crabapples are very similar to the early apples that were eaten by humans. Biotechnology, using selective breeding, has increased the size of apples and their sweetness and produced the modern varieties enjoyed today. (Siberian crabapple by Jackie Diectrich; CC-BY-NC 3.0).

The identification of bacteria and their role in fermentation and disease, together with technological advancements in biology, such as the development of pure bacterial cultures, paved the way for the development of modern biotechnology. With the discovery of DNA and its role in heredity and genetics, new means for manipulating living organisms became available. In 1973, the first **genetically-modified organism (GMO)** was created by Stanley Cohen and Herbert Boyer, who used biotechnology procedures to introduce foreign DNA into a bacterial genome. In 1977, Herbert Boyer and Keiichi Itakura succeeded in inducing genetically-modified bacteria to produce the first human protein (i.e., somatostatin). Their recombinant somatostatin was found to be identical to its natural counterpart and their studies marked the start of the biotechnology industry. One year later, the first synthetic "human" insulin was produced using genetically-engineered *E. coli* bacteria. In 1982, Humulin®, human insulin used for diabetes, was the first medication developed using biotechnology and the first recombinant pharmaceutical approved by the FDA for commercial use in the United States (Figure 1.1.2).



Figure 1.1.2: A vial of the first Humulin® solution developed by Genentech and licensed to Eli Lilly (Humulin R by National Museum of American History; CC0)

The importance of biotechnology and its creation of Humulin® cannot be understated. Prior to the advent of biotechnology, scientists relied either on chemically synthesized human therapeutics made in the laboratory, or on a microbe or animal that naturally produced the desired compound. Prior to 1982, Type I diabetics used insulin extracted from cows and pigs. These animal-derived insulin therapies induced minor allergic reactions in some patients. Furthermore, fears emerged that demand for

insulin would soon outstrip the supply from pigs and cows. Through biotechnology and its creation of Humulin®, diabetic patients now had the option to use genetically-engineered insulin that was cheaper, safer, and could be mass produced.

The 1970s were marked by the development of the first commercial method for sequencing DNA. Developed by Frederick Sanger in 1977, the "Sanger method", or dideoxy sequencing, revolutionized biotechnology by allowing scientists to determine the precise order of nucleotides in DNA. In the following decades, advancements in sequencing technologies, such as the development of automated sequencing machines in the 1980s and the introduction of next-generation sequencing (NGS) in the early 2000s, exponentially increased sequencing speed and reduced costs for those working in the biotechnology field. The Human Genome Project, completed in 2003, marked a monumental achievement, providing a complete map of the human genome and ushering in a new era of genomics. Today, sequencing technologies continue to evolve, with techniques enabling real-time sequencing and expanding our understanding of genetics and its applications in medicine, agriculture, and beyond.

The 1980s was a decade of significant breakthroughs in the production of more advanced and refined GMOs. The first patent for a GMO was issued in 1980 to Ananda Chakrabarty, who engineered *Pseudomonas sp.* bacteria to breakdown crude oil spills as a means of bioremediation. Their patent became the first one to be granted to a living organism and paved the way for future biotechnology patents on modified plants, animals, and other organisms. This GMO would later be used in the 1989 cleanup of the Exxon oil spill at Prince William Sound, Alaska. In 1981, the first genetically-modified animal, also known as a **transgenic animal,** was produced in mice. Two years later, genetic modifications using biotechnology were being applied to sheep, pigs, and cattle, significantly impacting the livestock industry. In 1983, the first transgenic plant, a tobacco plant resistant to antibiotics, was introduced to the agriculture market. Two years later, Agracetus received the first patent for its genetically-modified cotton. Since then, GMOs have become widespread in many research fields and commercial markets. In 2020, genetically-modified plants accounted for about 55 percent of the total harvested cropland in the United States. Most of the corn, soybeans and cotton planted in the US have been genetically-modified either to resist pest or to be resistant to herbicides (Figure 1.1.3). Today, genetically modifying flies, fish, or mice are a common and essential tool in research labs.

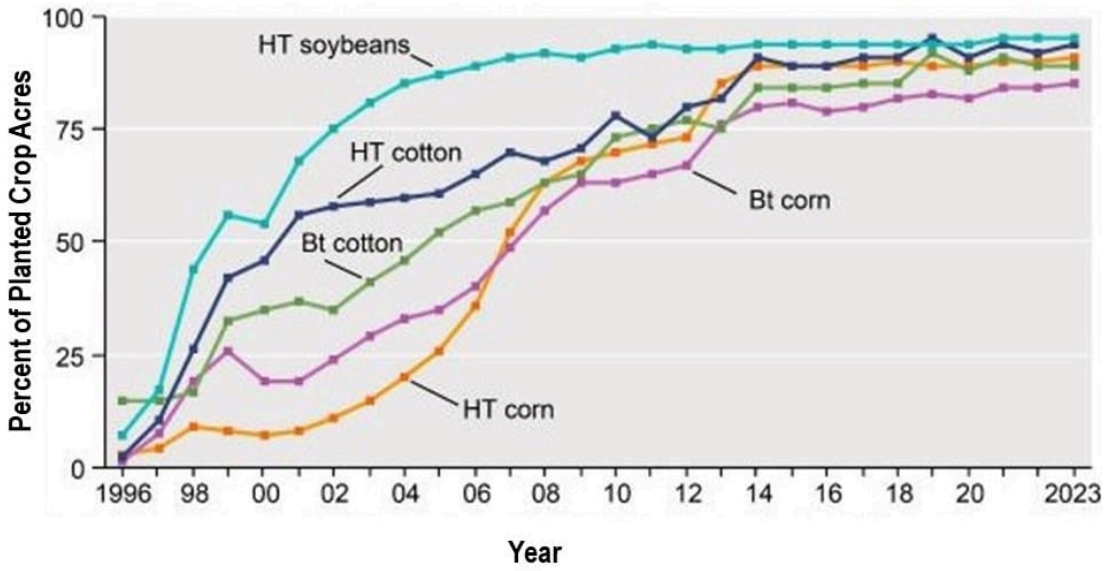


Figure 1.1.3: Adoption of genetically-modified crops in the United States from 1996 to 2023. The percentage of acres planted with crops (i.e. percent of planted crop acres) as a function of time (in years) was plotted for Herbicide-Tolerant (HT) soybeans (teal squares), cotton (blue squares), and corn (orange squares). Insect resistant varieties containing genes from the soil bacterium *Bacillus thuringiensis* (Bt) were also plotted (Bt-corn, purple squares; Bt-cotton, green squares). The graph shows increases over time in the percent of planted crop acres for all HT- and Bt-varieties from 1996 to 2010, with the percentage of acres planted leveling off to close to 100% from 2010 to 2023. (Genetically modified crops by Patricia Zuk, CC BY 4.0; adapted from Adoption of genetically engineered crops by USDA Economic Research Service, Public Domain)

Advancements in biotechnology are currently growing at an exponential rate with improvements in techniques, such as the evolution of sequencing methods mentioned above, or creation of new methodologies constantly occurring.

The Interdisciplinary Nature of Biotechnology

Biotechnology exemplifies the interdisciplinary nature of science by its merging of diverse scientific and engineering fields

to address complex biological challenges. At its core, biotechnology integrates molecular biology, cell biology, genetics, and microbiology with other life science areas, like virology, in order to understand and manipulate living organisms at the molecular level. Chemistry and biochemistry play crucial roles in understanding the biochemical pathways and interactions within cells. Physics provides advanced techniques like X-ray crystallography and nuclear magnetic resonance, which are essential for elucidating the structures of biomolecules. Engineering principles are applied to the design of bioprocesses, bioreactors, and medical devices. Additionally, computer science and bio-informatics are indispensable for managing and analyzing the vast amounts of data generated in biotechnology research, enabling the modeling of biological systems and the development of algorithms for gene sequencing and drug design. This synergy of disciplines fosters innovation, leading to advancements in healthcare, agriculture, environmental protection, and industrial processes, ultimately improving quality of life and sustainability.

Key Concepts

Biotechnology has been used for breeding livestock and crops long before people understood the scientific basis of this field. A better understanding of cellular and molecular biology and rapid advances in new technologies has led to the growth of biotechnology. Today, biotechnology is an interdisciplinary field incorporating numerous STEM fields, including biology, biochemistry, physics, engineering, and computer science.

Some important concepts to remember are:

- there are numerous areas of biotechnology, including medical, agricultural, food, and industrial
- genetically-modified organisms (GMOs) were first created using biotechnology in 1973
- bacteria were the first GMOs to be created and used in biotechnology
- the first synthetic drug to be made through biotechnology was human insulin
- the first patented GMO was issued in 1980
- GMO animals are often called transgenic animals

Glossary

Biodiversity - the variety of life on Earth, including all living organisms, ecosystems, and genetic diversity

Bioremediation - the use of living organisms, such as bacteria, fungi, or plants, to clean up and remove environmental contaminants from soil, water, and air

Biotechnology - the use of biological systems, organisms, or derivatives to develop or create useful products and technologies

Genetic engineering - the direct modification of an organism's DNA using biotechnology; often done to introduce new traits, to improve resistance to disease, or to produce beneficial products

Genetically-modified organism (GMO) - any organism whose DNA has been altered using genetic engineering techniques

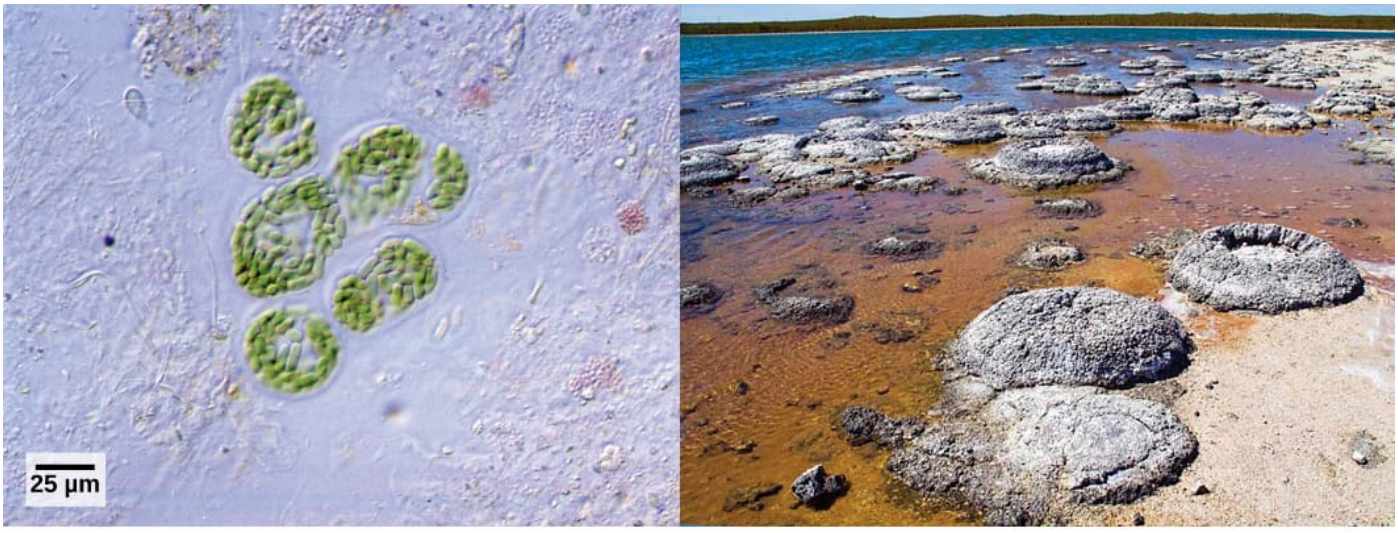
Interdisciplinary - the integration and combination of knowledge, methods, and perspectives from two or more different disciplines or fields of study

Transgenic - an alternate term used for a GMO; often used to refer to genetically-modified animals

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1.2: The Process of Science

Like geology, physics, and chemistry, biology is a science that gathers knowledge about the natural world. Specifically, biology is the study of life. The discoveries of biology are made by a community of researchers who work individually and together using agreed-on methods. In this sense, biology, like all sciences is a social enterprise like politics or the arts.



(a) (b)

Figure 1.2.1**:** Formerly called blue-green algae, the (a) cyanobacteria seen through a light microscope are some of Earth's oldest life forms. These (b) stromatolites along the shores of Lake Thetis in Western Australia are ancient structures formed by the layering of cyanobacteria in shallow waters. (credit a: modification of work by NASA; scale-bar data from Matt Russell; credit b: modification of work by Ruth Ellison)

The methods of science include careful observation, record keeping, logical and mathematical reasoning, experimentation, and submitting conclusions to the scrutiny of others. Science also requires considerable imagination and creativity; a well-designed experiment is commonly described as elegant, or beautiful. Like politics, science has considerable practical implications and some science is dedicated to practical applications, such as the prevention of disease (Figure 1.2.2). Other science proceeds largely motivated by curiosity. Whatever its goal, there is no doubt that science, including biology, has transformed human existence and will continue to do so.

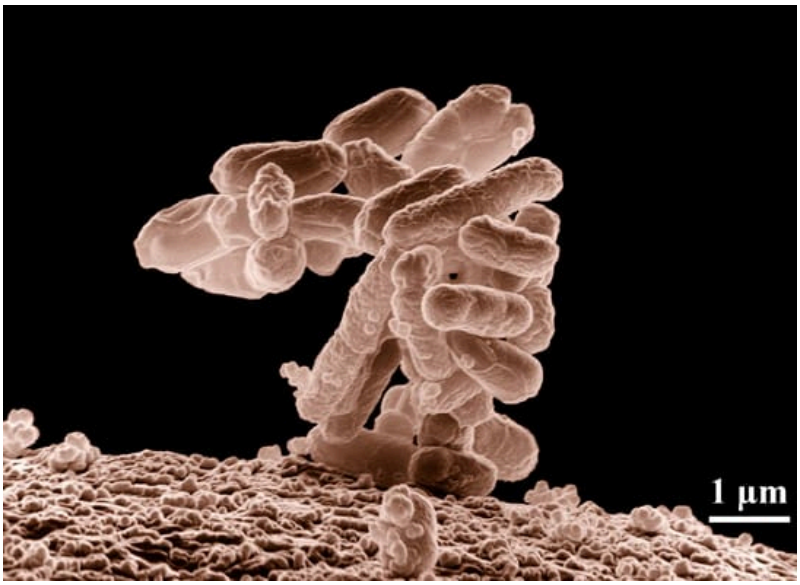


Figure 1.2.2**:** Biologists may choose to study *Escherichia coli* (*E. coli*), a bacterium that is a normal resident of our digestive tracts but which is also sometimes responsible for disease outbreaks. In this micrograph, the bacterium is visualized using a scanning electron microscope and digital colorization. (credit: Eric Erbe; digital colorization by Christopher Pooley, USDA-ARS)

The Nature of Science

Biology is a science, but what exactly is science? What does the study of biology share with other scientific disciplines? Science (from the Latin s*cientia,* meaning "knowledge") can be defined as knowledge about the natural world.

Science is a very specific way of learning, or knowing, about the world. The history of the past 500 years demonstrates that science is a very powerful way of knowing about the world; it is largely responsible for the technological revolutions that have taken place during this time. There are however, areas of knowledge and human experience that the methods of science cannot be applied to. These include such things as answering purely moral questions, aesthetic questions, or what can be generally categorized as spiritual questions. Science has cannot investigate these areas because they are outside the realm of material phenomena, the phenomena of matter and energy, and cannot be observed and measured.

The scientific method is a method of research with defined steps that include experiments and careful observation. The steps of the scientific method will be examined in detail later, but one of the most important aspects of this method is the testing of hypotheses. A hypothesis is a suggested explanation for an event, which can be tested. Hypotheses, or tentative explanations, are generally produced within the context of a scientific theory. A scientific theory is a generally accepted, thoroughly tested and confirmed explanation for a set of observations or phenomena. Scientific theory is the foundation of scientific knowledge. In addition, in many scientific disciplines (less so in biology) there are scientific laws, often expressed in mathematical formulas, which describe how elements of nature will behave under certain specific conditions. There is not an evolution of hypotheses through theories to laws as if they represented some increase in certainty about the world. Hypotheses are the day-to-day material that scientists work with and they are developed within the context of theories. Laws are concise descriptions of parts of the world that are amenable to formulaic or mathematical description.

Natural Sciences

What would you expect to see in a museum of natural sciences? Frogs? Plants? Dinosaur skeletons? Exhibits about how the brain functions? A planetarium? Gems and minerals? Or maybe all of the above? Science includes such diverse fields as astronomy, biology, computer sciences, geology, logic, physics, chemistry, and mathematics (Figure 1.2.3). However, those fields of science related to the physical world and its phenomena and processes are considered natural sciences. Thus, a museum of natural sciences might contain any of the items listed above.

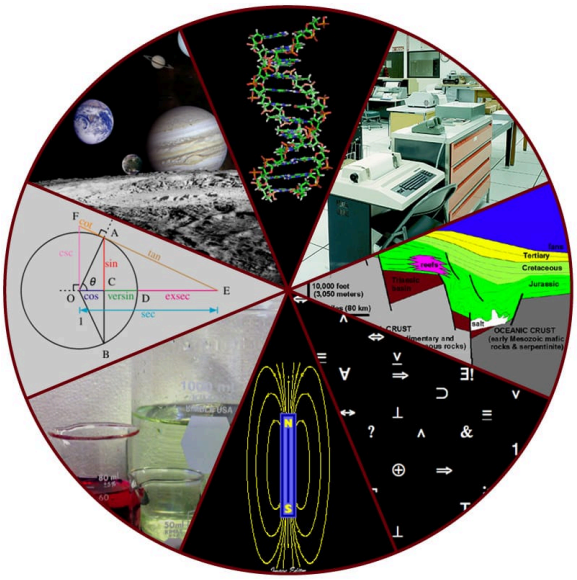


Figure 1.2.3**:** Some fields of science include astronomy, biology, computer science, geology, logic, physics, chemistry, and mathematics. (credit: "Image Editor"/Flickr)

There is no complete agreement when it comes to defining what the natural sciences include. For some experts, the natural sciences are astronomy, biology, chemistry, earth science, and physics. Other scholars choose to divide natural sciences into life sciences, which study living things and include biology, and physical sciences, which study nonliving matter and include astronomy, physics, and chemistry. Some disciplines such as biophysics and biochemistry build on two sciences and are interdisciplinary.

Scientific Inquiry

One thing is common to all forms of science: an ultimate goal “to know.” Curiosity and inquiry are the driving forces for the development of science. Scientists seek to understand the world and the way it operates. Two methods of logical thinking are used: inductive reasoning and deductive reasoning.

Inductive reasoning is a form of logical thinking that uses related observations to arrive at a general conclusion. This type of reasoning is common in descriptive science. A life scientist such as a biologist makes observations and records them. These data can be qualitative (descriptive) or quantitative (consisting of numbers), and the raw data can be supplemented with drawings, pictures, photos, or videos. From many observations, the scientist can infer conclusions (inductions) based on evidence. Inductive reasoning involves formulating generalizations inferred from careful observation and the analysis of a large amount of data. Brain studies often work this way. Many brains are observed while people are doing a task. The part of the brain that lights up, indicating activity, is then demonstrated to be the part controlling the response to that task.

Deductive reasoning or deduction is the type of logic used in hypothesis-based science. In deductive reasoning, the pattern of thinking moves in the opposite direction as compared to inductive reasoning. Deductive reasoning is a form of logical thinking that uses a general principle or law to forecast specific results. From those general principles, a scientist can extrapolate and predict the specific results that would be valid as long as the general principles are valid. For example, a prediction would be that if the climate is becoming warmer in a region, the distribution of plants and animals should change. Comparisons have been made between distributions in the past and the present, and the many changes that have been found are consistent with a warming climate. Finding the change in distribution is evidence that the climate change conclusion is a valid one.

Both types of logical thinking are related to the two main pathways of scientific study: descriptive science and hypothesis-based science. Descriptive (or discovery) science aims to observe, explore, and discover, while hypothesis-based science begins with a specific question or problem and a potential answer or solution that can be tested. The boundary between these two forms of study is often blurred, because most scientific endeavors combine both approaches. Observations lead to questions, questions lead to forming a hypothesis as a possible answer to those questions, and then the hypothesis is tested. Thus, descriptive science and hypothesis-based science are in continuous dialogue.

Hypothesis Testing

Biologists study the living world by posing questions about it and seeking science-based responses. This approach is common to other sciences as well and is often referred to as the scientific method. The scientific method was used even in ancient times, but it was first documented by England's Sir Francis Bacon (1561–1626) (Figure 1.2.4), who set up inductive methods for scientific inquiry. The scientific method is not exclusively used by biologists but can be applied to almost anything as a logical problem-solving method.



Figure 1.2.4: Sir Francis Bacon is credited with being the first to document the scientific method.

The scientific process typically starts with an observation (often a problem to be solved) that leads to a question. Let's think about a simple problem that starts with an observation and apply the scientific method to solve the problem. One Monday morning, a student arrives at class and quickly discovers that the classroom is too warm. That is an observation that also describes a problem: the classroom is too warm. The student then asks a question: "Why is the classroom so warm?"

Recall that a hypothesis is a suggested explanation that can be tested. To solve a problem, several hypotheses may be proposed. For example, one hypothesis might be, "The classroom is warm because no one turned on the air conditioning." But there could be other responses to the question, and therefore other hypotheses may be proposed. A second hypothesis might be, "The classroom is warm because there is a power failure, and so the air conditioning doesn't work."

Once a hypothesis has been selected, a prediction may be made. A prediction is similar to a hypothesis but it typically has the format "If . . . then . . . ." For example, the prediction for the first hypothesis might be, "*If* the student turns on the air conditioning, *then* the classroom will no longer be too warm."

A hypothesis must be testable to ensure that it is valid. For example, a hypothesis that depends on what a bear thinks is not testable, because it can never be known what a bear thinks. It should also be falsifiable, meaning that it can be disproven by experimental results. An example of an unfalsifiable hypothesis is "Botticelli's *Birth of Venus* is beautiful." There is no experiment that might show this statement to be false. To test a hypothesis, a researcher will conduct one or more experiments designed to eliminate one or more of the hypotheses. This is important. A hypothesis can be disproven, or eliminated, but it can never be proven. Science does not deal in proofs like mathematics. If an experiment fails to disprove a hypothesis, then we find support for that explanation, but this is not to say that down the road a better explanation will not be found, or a more carefully designed experiment will be found to falsify the hypothesis.

Each experiment will have one or more variables and one or more controls. A variable is any part of the experiment that can vary or change during the experiment. A control is a part of the experiment that does not change. Look for the variables and controls in the example that follows. As a simple example, an experiment might be conducted to test the hypothesis that phosphate limits the growth of algae in freshwater ponds. A series of artificial ponds are filled with water and half of them are treated by adding phosphate each week, while the other half are treated by adding a salt that is known not to be used by algae. The variable here is the phosphate (or lack of phosphate), the experimental or treatment cases are the ponds with added phosphate and the control

ponds are those with something inert added, such as the salt. Just adding something is also a control against the possibility that adding extra matter to the pond has an effect. If the treated ponds show lesser growth of algae, then we have found support for our hypothesis. If they do not, then we reject our hypothesis. Be aware that rejecting one hypothesis does not determine whether or not the other hypotheses can be accepted; it simply eliminates one hypothesis that is not valid (Figure 1.2.5). Using the scientific method, the hypotheses that are inconsistent with experimental data are rejected.

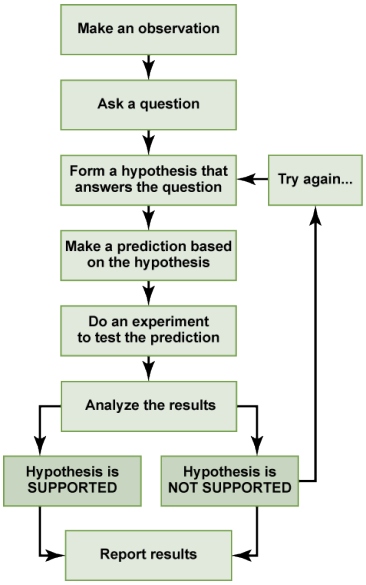


Figure 1.2.5: The scientific method is a series of defined steps that include experiments and careful observation. If a hypothesis is not supported by data, a new hypothesis can be proposed.

Example 1.2.1

In the example below, the scientific method is used to solve an everyday problem. Which part in the example below is the hypothesis? Which is the prediction? Based on the results of the experiment, is the hypothesis supported? If it is not supported, propose some alternative hypotheses.

1. My toaster doesn't toast my bread.
2. Why doesn't my toaster work?
3. There is something wrong with the electrical outlet.
4. If something is wrong with the outlet, my coffeemaker also won't work when plugged into it.
5. I plug my coffeemaker into the outlet.
6. My coffeemaker works.

Solution

The hypothesis is #3 (there is something wrong with the electrical outlet), and the prediction is #4 (if something is wrong with the outlet, then the coffeemaker also won't work when plugged into the outlet). The original hypothesis is not supported, as the coffee maker works when plugged into the outlet. Alternative hypotheses may include (1) the toaster might be broken or (2) the toaster wasn't turned on.

In practice, the scientific method is not as rigid and structured as it might at first appear. Sometimes an experiment leads to conclusions that favor a change in approach; often, an experiment brings entirely new scientific questions to the puzzle. Many times, science does not operate in a linear fashion; instead, scientists continually draw inferences and make generalizations, finding patterns as their research proceeds. Scientific reasoning is more complex than the scientific method alone suggests.

Basic and Applied Science

The scientific community has been debating for the last few decades about the value of different types of science. Is it valuable to pursue science for the sake of simply gaining knowledge, or does scientific knowledge only have worth if we can apply it to

solving a specific problem or bettering our lives? This question focuses on the differences between two types of science: basic science and applied science.

Basic science or “pure” science seeks to expand knowledge regardless of the short-term application of that knowledge. It is not focused on developing a product or a service of immediate public or commercial value. The immediate goal of basic science is knowledge for knowledge’s sake, though this does not mean that in the end it may not result in an application.

In contrast, applied science or “technology,” aims to use science to solve real-world problems, making it possible, for example, to improve a crop yield, find a cure for a particular disease, or save animals threatened by a natural disaster. In applied science, the problem is usually defined for the researcher.

Some individuals may perceive applied science as “useful” and basic science as “useless.” A question these people might pose to a scientist advocating knowledge acquisition would be, “What for?” A careful look at the history of science, however, reveals that basic knowledge has resulted in many remarkable applications of great value. Many scientists think that a basic understanding of science is necessary before an application is developed; therefore, applied science relies on the results generated through basic science. Other scientists think that it is time to move on from basic science and instead to find solutions to actual problems. Both approaches are valid. It is true that there are problems that demand immediate attention; however, few solutions would be found without the help of the knowledge generated through basic science.

One example of how basic and applied science can work together to solve practical problems occurred after the discovery of DNA structure led to an understanding of the molecular mechanisms governing DNA replication. Strands of DNA, unique in every human, are found in our cells, where they provide the instructions necessary for life. During DNA replication, new copies of DNA are made, shortly before a cell divides to form new cells. Understanding the mechanisms of DNA replication enabled scientists to develop laboratory techniques that are now used to identify genetic diseases, pinpoint individuals who were at a crime scene, and determine paternity. Without basic science, it is unlikely that applied science would exist.

Another example of the link between basic and applied research is the Human Genome Project, a study in which each human chromosome was analyzed and mapped to determine the precise sequence of DNA subunits and the exact location of each gene. (The gene is the basic unit of heredity; an individual’s complete collection of genes is his or her genome.) Other organisms have also been studied as part of this project to gain a better understanding of human chromosomes. The Human Genome Project (Figure 1.2.6) relied on basic research carried out with non-human organisms and, later, with the human genome. An important end goal eventually became using the data for applied research seeking cures for genetically related diseases.

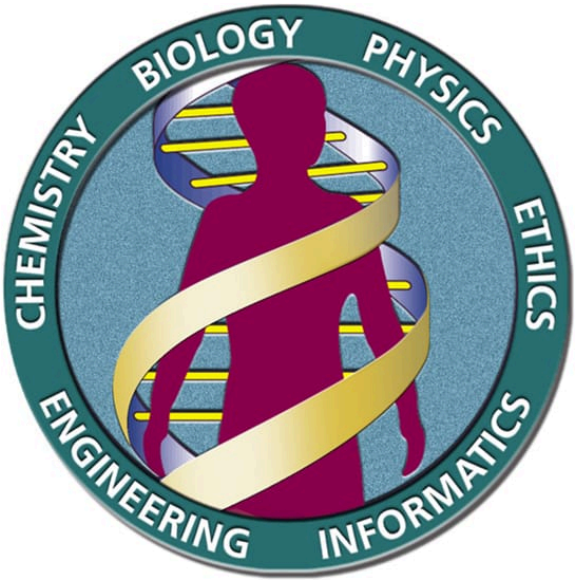


Figure 1.2.6: The Human Genome Project was a 13-year collaborative effort among researchers working in several different fields of science. The project was completed in 2003. (credit: the U.S. Department of Energy Genome Programs)

While research efforts in both basic science and applied science are usually carefully planned, it is important to note that some discoveries are made by serendipity, that is, by means of a fortunate accident or a lucky surprise. Penicillin was discovered when biologist Alexander Fleming accidentally left a petri dish of *Staphylococcus* bacteria open. An unwanted mold grew, killing the bacteria. The mold turned out to be *Penicillium*, and a new antibiotic was discovered. Even in the highly organized world of

science, luck—when combined with an observant, curious mind—can lead to unexpected breakthroughs.

Reporting Scientific Work

Whether scientific research is basic science or applied science, scientists must share their findings for other researchers to expand and build upon their discoveries. Communication and collaboration within and between sub disciplines of science are key to the advancement of knowledge in science. For this reason, an important aspect of a scientist's work is disseminating results and communicating with peers. Scientists can share results by presenting them at a scientific meeting or conference, but this approach can reach only the limited few who are present. Instead, most scientists present their results in peer-reviewed articles that are published in scientific journals. Peer-reviewed articles are scientific papers that are reviewed, usually anonymously by a scientist's colleagues, or peers. These colleagues are qualified individuals, often experts in the same research area, who judge whether or not the scientist's work is suitable for publication. The process of peer review helps to ensure that the research described in a scientific paper or grant proposal is original, significant, logical, and thorough. Grant proposals, which are requests for research funding, are also subject to peer review. Scientists publish their work so other scientists can reproduce their experiments under similar or different conditions to expand on the findings. The experimental results must be consistent with the findings of other scientists.

There are many journals and the popular press that do not use a peer-review system. A large number of online open-access journals, journals with articles available without cost, are now available many of which use rigorous peer-review systems, but some of which do not. Results of any studies published in these forums without peer review are not reliable and should not form the basis for other scientific work. In one exception, journals may allow a researcher to cite a personal communication from another researcher about unpublished results with the cited author's permission.

Summary

Biology is the science that studies living organisms and their interactions with one another and their environments. Science attempts to describe and understand the nature of the universe in whole or in part. Science has many fields; those fields related to the physical world and its phenomena are considered natural sciences.

A hypothesis is a tentative explanation for an observation. A scientific theory is a well-tested and consistently verified explanation for a set of observations or phenomena. A scientific law is a description, often in the form of a mathematical formula, of the behavior of an aspect of nature under certain circumstances. Two types of logical reasoning are used in science. Inductive reasoning uses results to produce general scientific principles. Deductive reasoning is a form of logical thinking that predicts results by applying general principles. The common thread throughout scientific research is the use of the scientific method. Scientists present their results in peer-reviewed scientific papers published in scientific journals.

Science can be basic or applied. The main goal of basic science is to expand knowledge without any expectation of short-term practical application of that knowledge. The primary goal of applied research, however, is to solve practical problems.

Glossary

atomic number - the number of protons in an atom

cation - a positive ion formed by losing electrons

chemical bond - an interaction between two or more of the same or different elements that results in the formation of molecules

covalent bond - a type of strong bond between two or more of the same or different elements; forms when electrons are shared between elements

electron - a negatively charged particle that resides outside of the nucleus in the electron orbital; lacks functional mass and has a charge of –1

electron transfer - the movement of electrons from one element to another

element - one of 118 unique substances that cannot be broken down into smaller substances and retain the characteristic of that substance; each element has a specified number of protons and unique properties

hydrogen bond - a weak bond between partially positively charged hydrogen atoms and partially negatively charged elements or molecules

ion - an atom or compound that does not contain equal numbers of protons and electrons, and therefore has a net charge

ionic bond - a chemical bond that forms between ions of opposite charges

isotope - one or more forms of an element that have different numbers of neutrons

mass number - the number of protons plus neutrons in an atom

matter - anything that has mass and occupies space

neutron - a particle with no charge that resides in the nucleus of an atom; has a mass of 1

nonpolar covalent bond - a type of covalent bond that forms between atoms when electrons are shared equally between atoms, resulting in no regions with partial charges as in polar covalent bonds

nucleus - (chemistry) the dense center of an atom made up of protons and (except in the case of a hydrogen atom) neutrons

octet rule - states that the outermost shell of an element with a low atomic number can hold eight electrons

periodic table of elements - an organizational chart of elements, indicating the atomic number and mass number of each element; also provides key information about the properties of elements

polar covalent bond - a type of covalent bond in which electrons are pulled toward one atom and away from another, resulting in slightly positive and slightly negative charged regions of the molecule

proton - a positively charged particle that resides in the nucleus of an atom; has a mass of 1 and a charge of +1

radioactive isotope - an isotope that spontaneously emits particles or energy to form a more stable element

van der Waals interaction - a weak attraction or interaction between molecules caused by slightly positively charged or slightly negatively charged atoms

Contributors and Attributions

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2: THE MOLECULES OF LIFE

2.1: The Building Blocks of Molecules
2.2: Water
2.3: Macromolecules
2.4: Carbohydrates
2.5: Lipids
2.6: Nucleic Acids
2.7: Proteins

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2.1: The Building Blocks of Molecules

At its most fundamental level, life is made up of matter. Matter occupies space and has mass. All matter is composed of elements, substances that cannot be broken down or transformed chemically into other substances. Each element is made of atoms, each with a constant number of protons and unique properties. A total of 118 elements have been defined; however, only 92 occur naturally, and fewer than 30 are found in living cells. The remaining 26 elements are unstable and, therefore, do not exist for very long or are theoretical and have yet to be detected.

Each element is designated by its chemical symbol (such as H, N, O, C, and Na), and possesses unique properties. These unique properties allow elements to combine and to bond with each other in specific ways.

Atoms

An atom is the smallest component of an element that retains all of the chemical properties of that element. For example, one hydrogen atom has all of the properties of the element hydrogen, such as it exists as a gas at room temperature, and it bonds with oxygen to create a water molecule. Hydrogen atoms cannot be broken down into anything smaller while still retaining the properties of hydrogen. If a hydrogen atom were broken down into subatomic particles, it would no longer have the properties of hydrogen.

At the most basic level, all organisms are made of a combination of elements. They contain atoms that combine together to form molecules. In multicellular organisms, such as animals, molecules can interact to form cells that combine to form tissues, which make up organs. These combinations continue until entire multicellular organisms are formed.

All atoms contain protons, electrons, and neutrons (Figure 2.1.1). The only exception is hydrogen (H), which is made of one proton and one electron. A proton is a positively charged particle that resides in the nucleus (the core of the atom) of an atom and has a mass of 1 and a charge of +1. An electron is a negatively charged particle that travels in the space around the nucleus. In other words, it resides outside of the nucleus. It has a negligible mass and has a charge of –1.

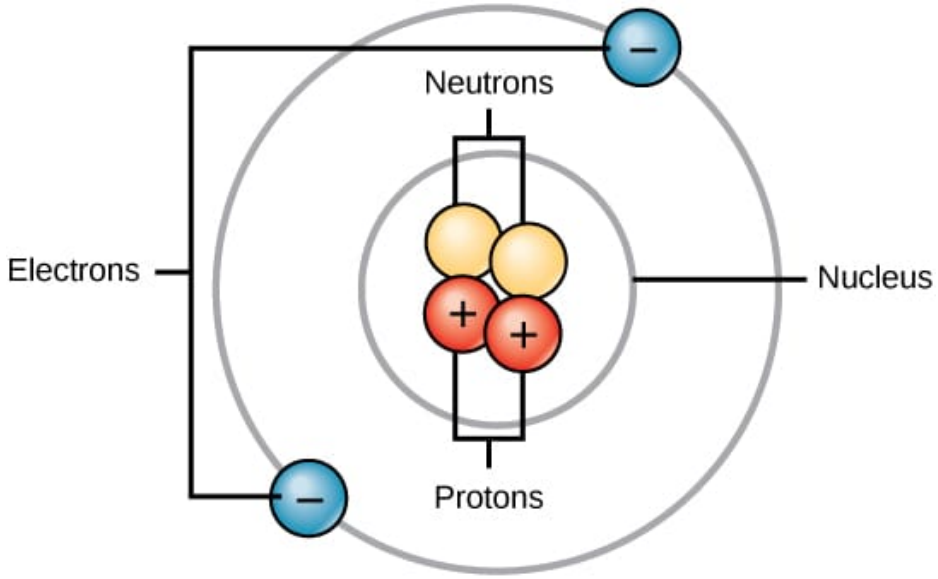


Figure 2.1.1**:** Atoms are made up of protons and neutrons located within the nucleus, and electrons surrounding the nucleus.

Neutrons, like protons, reside in the nucleus of an atom. They have a mass of 1 and no charge. The positive (protons) and negative (electrons) charges balance each other in a neutral atom, which has a net zero charge.

Because protons and neutrons each have a mass of 1, the mass of an atom is equal to the number of protons and neutrons of that atom. The number of electrons does not factor into the overall mass, because their mass is so small.

As stated earlier, each element has its own unique properties. Each contains a different number of protons and neutrons, giving it its own atomic number and mass number. The atomic number of an element is equal to the number of protons that element contains. The mass number, or atomic mass, is the number of protons plus the number of neutrons of that element. Therefore, it is possible to determine the number of neutrons by subtracting the atomic number from the mass number.

These numbers provide information about the elements and how they will react when combined. Different elements have different melting and boiling points, and are in different states (liquid, solid, or gas) at room temperature. They also combine in different ways. Some form specific types of bonds, whereas others do not. How they combine is based on the number of electrons present. Because of these characteristics, the elements are arranged into the periodic table of elements, a chart of the elements that includes the atomic number and relative atomic mass of each element. The periodic table also provides key information about the properties of elements (Figure 2.1.2)—often indicated by color-coding. The arrangement of the table also shows how the electrons in each element are organized and provides important details about how atoms will react with each other to form molecules.

Isotopes are different forms of the same element that have the same number of protons, but a different number of neutrons. Some elements, such as carbon, potassium, and uranium, have naturally occurring isotopes. Carbon-12, the most common isotope of carbon, contains six protons and six neutrons. Therefore, it has a mass number of 12 (six protons and six neutrons) and an atomic number of 6 (which makes it carbon). Carbon-14 contains six protons and eight neutrons. Therefore, it has a mass number of 14 (six protons and eight neutrons) and an atomic number of 6, meaning it is still the element carbon. These two alternate forms of carbon are isotopes. Some isotopes are unstable and will lose protons, other subatomic particles, or energy to form more stable elements. These are called radioactive isotopes or radioisotopes.

ART CONNECTION

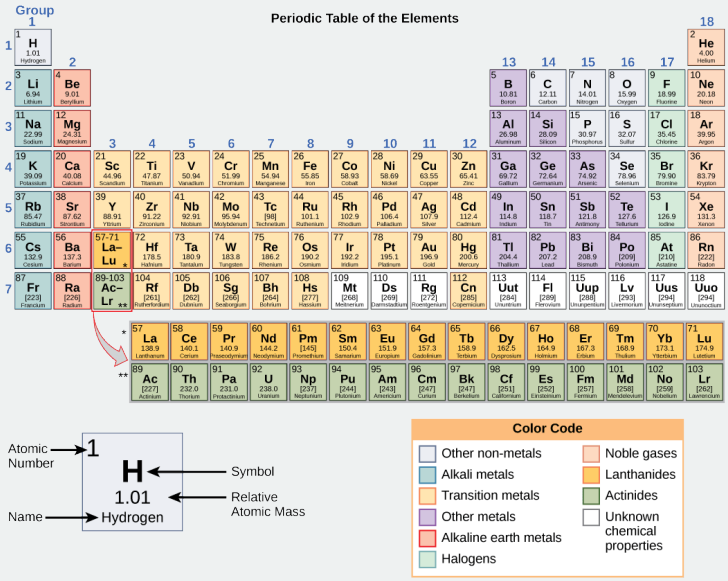


Figure 2.1.2: Arranged in columns and rows based on the characteristics of the elements, the periodic table provides key information about the elements and how they might interact with each other to form molecules. Most periodic tables provide a key or legend to the information they contain.

How many neutrons do (K) potassium-39 and potassium-40 have, respectively?

EVOLUTION IN ACTION: Carbon Dating

Carbon-14 (^{14}C) is a naturally occurring radioisotope that is created in the atmosphere by cosmic rays. This is a continuous process, so more ^{14}C is always being created. As a living organism develops, the relative level of ^{14}C in its body is equal to the concentration of ^{14}C in the atmosphere. When an organism dies, it is no longer ingesting ^{14}C, so the ratio will decline. ^{14}C decays to ^{14}N by a process called beta decay; it gives off energy in this slow process.

After approximately 5,730 years, only one-half of the starting concentration of ^{14}C will have been converted to ^{14}N. The time it takes for half of the original concentration of an isotope to decay to its more stable form is called its half-life. Because the half-life of ^{14}C is long, it is used to age formerly living objects, such as fossils. Using the ratio of the ^{14}C concentration found in an object to the amount of ^{14}C detected in the atmosphere, the amount of the isotope that has not yet decayed can be determined. Based on this amount, the age of the fossil can be calculated to about 50,000 years (Figure 2.1.3). Isotopes with longer half-lives, such as potassium-40, are used to calculate the ages of older fossils. Through the use of carbon dating, scientists can reconstruct the ecology and biogeography of organisms living within the past 50,000 years.



Figure 2.1.3: The age of remains that contain carbon and are less than about 50,000 years old, such as this pygmy mammoth, can be determined using carbon dating. (credit: Bill Faulkner/NPS)

CONCEPT IN ACTION

To learn more about atoms and isotopes, and how you can tell one isotope from another, visit this site and run the simulation.

Chemical Bonds

How elements interact with one another depends on how their electrons are arranged and how many openings for electrons exist at the outermost region where electrons are present in an atom. Electrons exist at energy levels that form shells around the nucleus. The closest shell can hold up to two electrons. The closest shell to the nucleus is always filled first, before any other shell can be filled. Hydrogen has one electron; therefore, it has only one spot occupied within the lowest shell. Helium has two electrons; therefore, it can completely fill the lowest shell with its two electrons. If you look at the periodic table, you will see that hydrogen and helium are the only two elements in the first row. This is because they only have electrons in their first shell. Hydrogen and helium are the only two elements that have the lowest shell and no other shells.

The second and third energy levels can hold up to eight electrons. The eight electrons are arranged in four pairs and one position in each pair is filled with an electron before any pairs are completed.

Looking at the periodic table again (Figure 2.1.2), you will notice that there are seven rows. These rows correspond to the number of shells that the elements within that row have. The elements within a particular row have increasing numbers of electrons as the columns proceed from left to right. Although each element has the same number of shells, not all of the shells are completely filled with electrons. If you look at the second row of the periodic table, you will find lithium (Li), beryllium (Be), boron (B), carbon (C), nitrogen (N), oxygen (O), fluorine (F), and neon (Ne). These all have electrons that occupy only the first and second shells. Lithium has only one electron in its outermost shell, beryllium has two electrons, boron has three, and so on, until the entire shell is filled with eight electrons, as is the case with neon.

Not all elements have enough electrons to fill their outermost shells, but an atom is at its most stable when all of the electron

positions in the outermost shell are filled. Because of these vacancies in the outermost shells, we see the formation of chemical bonds, or interactions between two or more of the same or different elements that result in the formation of molecules. To achieve greater stability, atoms will tend to completely fill their outer shells and will bond with other elements to accomplish this goal by sharing electrons, accepting electrons from another atom, or donating electrons to another atom. Because the outermost shells of the elements with low atomic numbers (up to calcium, with atomic number 20) can hold eight electrons, this is referred to as the octet rule. An element can donate, accept, or share electrons with other elements to fill its outer shell and satisfy the octet rule.

When an atom does not contain equal numbers of protons and electrons, it is called an ion. Because the number of electrons does not equal the number of protons, each ion has a net charge. Positive ions are formed by losing electrons and are called cations. Negative ions are formed by gaining electrons and are called anions.

For example, sodium only has one electron in its outermost shell. It takes less energy for sodium to donate that one electron than it does to accept seven more electrons to fill the outer shell. If sodium loses an electron, it now has 11 protons and only 10 electrons, leaving it with an overall charge of +1. It is now called a sodium ion.

The chlorine atom has seven electrons in its outer shell. Again, it is more energy-efficient for chlorine to gain one electron than to lose seven. Therefore, it tends to gain an electron to create an ion with 17 protons and 18 electrons, giving it a net negative (–1) charge. It is now called a chloride ion. This movement of electrons from one element to another is referred to as electron transfer. As Figure 2.1.4 illustrates, a sodium atom (Na) only has one electron in its outermost shell, whereas a chlorine atom (Cl) has seven electrons in its outermost shell. A sodium atom will donate its one electron to empty its shell, and a chlorine atom will accept that electron to fill its shell, becoming chloride. Both ions now satisfy the octet rule and have complete outermost shells. Because the number of electrons is no longer equal to the number of protons, each is now an ion and has a +1 (sodium) or –1 (chloride) charge.

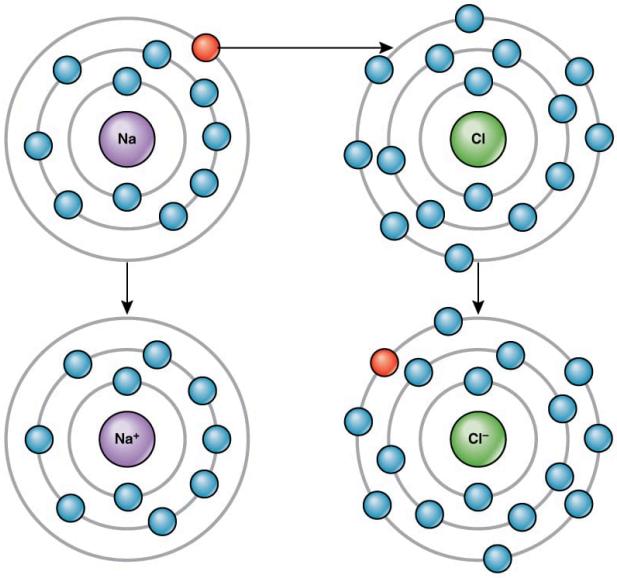


Figure 2.1.4**:** Elements tend to fill their outermost shells with electrons. To do this, they can either donate or accept electrons from other elements.

Ionic Bonds

There are four types of bonds or interactions: ionic, covalent, hydrogen bonds, and van der Waals interactions. Ionic and covalent bonds are strong interactions that require a larger energy input to break apart. When an element donates an electron from its outer shell, as in the sodium atom example above, a positive ion is formed. The element accepting the electron is now negatively charged. Because positive and negative charges attract, these ions stay together and form an ionic bond, or a bond between ions. The elements bond together with the electron from one element staying predominantly with the other element. When Na^+ and Cl^- ions combine to produce NaCl, an electron from a sodium atom stays with the other seven from the chlorine atom, and the sodium and chloride ions attract each other in a lattice of ions with a net zero charge.

Covalent Bonds

Another type of strong chemical bond between two or more atoms is a covalent bond. These bonds form when an electron is shared between two elements and are the strongest and most common form of chemical bond in living organisms. Covalent bonds form between the elements that make up the biological molecules in our cells. Unlike ionic bonds, covalent bonds do not dissociate in water.

The hydrogen and oxygen atoms that combine to form water molecules are bound together by covalent bonds. The electron from the hydrogen atom divides its time between the outer shell of the hydrogen atom and the incomplete outer shell of the oxygen atom. To completely fill the outer shell of an oxygen atom, two electrons from two hydrogen atoms are needed, hence the subscript "2" in H_2O. The electrons are shared between the atoms, dividing their time between them to "fill" the outer shell of each. This sharing is a lower energy state for all of the atoms involved than if they existed without their outer shells filled.

There are two types of covalent bonds: polar and nonpolar. Nonpolar covalent bonds form between two atoms of the same element or between different elements that share the electrons equally. For example, an oxygen atom can bond with another oxygen atom to fill their outer shells. This association is nonpolar because the electrons will be equally distributed between each oxygen atom. Two covalent bonds form between the two oxygen atoms because oxygen requires two shared electrons to fill its outermost shell. Nitrogen atoms will form three covalent bonds (also called triple covalent) between two atoms of nitrogen because each nitrogen atom needs three electrons to fill its outermost shell. Another example of a nonpolar covalent bond is found in the methane (CH_4) molecule. The carbon atom has four electrons in its outermost shell and needs four more to fill it. It gets these four from four hydrogen atoms, each atom providing one. These elements all share the electrons equally, creating four nonpolar covalent bonds (Figure 2.1.5).

In a polar covalent bond, the electrons shared by the atoms spend more time closer to one nucleus than to the other nucleus. Because of the unequal distribution of electrons between the different nuclei, a slightly positive (δ+) or slightly negative (δ–) charge develops. The covalent bonds between hydrogen and oxygen atoms in water are polar covalent bonds. The shared electrons spend more time near the oxygen nucleus, giving it a small negative charge, than they spend near the hydrogen nuclei, giving these molecules a small positive charge.

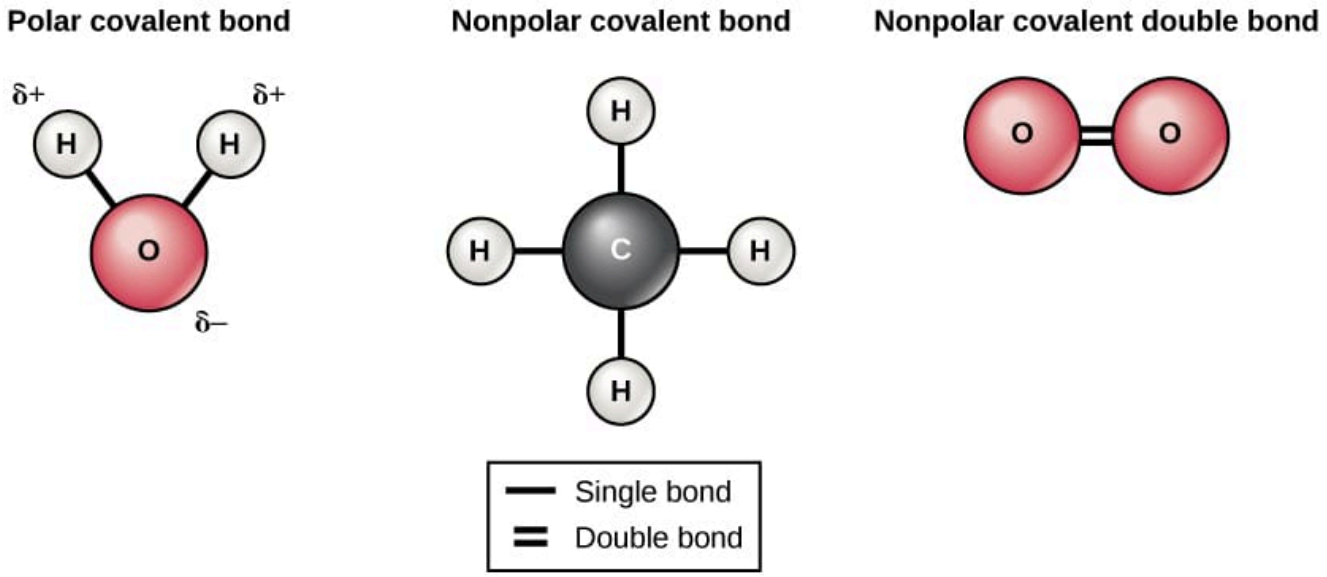


Figure 2.1.5**:** The water molecule (left) depicts a polar bond with a slightly positive charge on the hydrogen atoms and a slightly negative charge on the oxygen. Examples of nonpolar bonds include methane (middle) and oxygen (right).

Hydrogen Bonds

Ionic and covalent bonds are strong bonds that require considerable energy to break. However, not all bonds between elements are ionic or covalent bonds. Weaker bonds can also form. These are attractions that occur between positive and negative charges that do not require much energy to break. Two weak bonds that occur frequently are hydrogen bonds and van der Waals interactions. These bonds give rise to the unique properties of water and the unique structures of DNA and proteins.

When polar covalent bonds containing a hydrogen atom form, the hydrogen atom in that bond has a slightly positive charge. This is because the shared electron is pulled more strongly toward the other element and away from the hydrogen nucleus. Because the hydrogen atom is slightly positive (δ+), it will be attracted to neighboring negative partial charges (δ–). When this happens, a weak interaction occurs between the δ+ charge of the hydrogen atom of one molecule and the δ– charge of the other molecule. This interaction is called a hydrogen bond. This type of bond is common; for example, the liquid nature of water is caused by the hydrogen bonds between water molecules (Figure 2.1.6). Hydrogen bonds give water the unique properties that sustain life. If it were not for hydrogen bonding, water would be a gas rather than a liquid at room temperature.

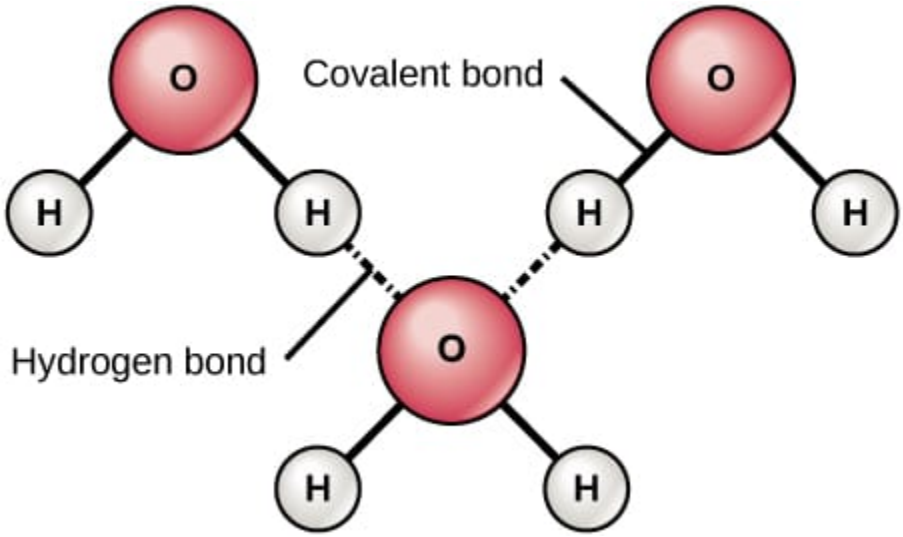


Figure 2.1.6: Hydrogen bonds form between slightly positive (δ+) and slightly negative (δ–) charges of polar covalent molecules, such as water.

Hydrogen bonds can form between different molecules and they do not always have to include a water molecule. Hydrogen atoms in polar bonds within any molecule can form bonds with other adjacent molecules. For example, hydrogen bonds hold together two long strands of DNA to give the DNA molecule its characteristic double-stranded structure. Hydrogen bonds are also responsible for some of the three-dimensional structure of proteins.

van der Waals Interactions

Like hydrogen bonds, van der Waals interactions are weak attractions or interactions between molecules. They occur between polar, covalently bound, atoms in different molecules. Some of these weak attractions are caused by temporary partial charges formed when electrons move around a nucleus. These weak interactions between molecules are important in biological systems.

Summary

Matter is anything that occupies space and has mass. It is made up of atoms of different elements. All of the 92 elements that occur naturally have unique qualities that allow them to combine in various ways to create compounds or molecules. Atoms, which consist of protons, neutrons, and electrons, are the smallest units of an element that retain all of the properties of that element. Electrons can be donated or shared between atoms to create bonds, including ionic, covalent, and hydrogen bonds, as well as van der Waals interactions.

Art Connections

Figure 2.1.2: How many neutrons do (K) potassium-39 and potassium-40 have, respectively?

Answer

Potassium-39 has twenty neutrons. Potassium-40 has twenty one neutrons.

Glossary

atomic number - the number of protons in an atom

cation - a positive ion formed by losing electrons

chemical bond - an interaction between two or more of the same or different elements that results in the formation of molecules

covalent bond - a type of strong bond between two or more of the same or different elements; forms when electrons are shared between elements

electron - a negatively charged particle that resides outside of the nucleus in the electron orbital; lacks functional mass and has a charge of –1

electron transfer - the movement of electrons from one element to another

element - one of 118 unique substances that cannot be broken down into smaller substances and retain the characteristic of that substance; each element has a specified number of protons and unique properties

hydrogen bond - a weak bond between partially positively charged hydrogen atoms and partially negatively charged elements or molecules

ion - an atom or compound that does not contain equal numbers of protons and electrons, and therefore has a net charge

ionic bond - a chemical bond that forms between ions of opposite charges

isotope - one or more forms of an element that have different numbers of neutrons

mass number - the number of protons plus neutrons in an atom

matter - anything that has mass and occupies space

neutron - a particle with no charge that resides in the nucleus of an atom; has a mass of 1

nonpolar covalent bond - a type of covalent bond that forms between atoms when electrons are shared equally between atoms, resulting in no regions with partial charges as in polar covalent bonds

nucleus - (chemistry) the dense center of an atom made up of protons and (except in the case of a hydrogen atom) neutrons

octet rule - states that the outermost shell of an element with a low atomic number can hold eight electrons

periodic table of elements - an organizational chart of elements, indicating the atomic number and mass number of each element; also provides key information about the properties of elements

polar covalent bond - a type of covalent bond in which electrons are pulled toward one atom and away from another, resulting in slightly positive and slightly negative charged regions of the molecule

proton - a positively charged particle that resides in the nucleus of an atom; has a mass of 1 and a charge of +1

radioactive isotope - an isotope that spontaneously emits particles or energy to form a more stable element

van der Waals interaction - a weak attraction or interaction between molecules caused by slightly positively charged or slightly negatively charged atoms

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2.2: Water

Biotech Focus

Strategies for Cleaner Water

Industrial activities produces a large amount of wastewater containing pollutants such as pesticides or industrial dyes. Using biotechnology, wastewater treatment can be more efficient, sustainable and cost effective. Several strategies have been developed, using microorganisms, enzymes, or plants specifically engineered to break down pollutants and contaminants, ultimately making the water safer for consumption.

For more information about the different approaches to treat water, read Biological wastewater treatment technology: Advancement and drawbacks

Introduction

Do you ever wonder why scientists spend time looking for water on other planets? It is because water is essential to life; even minute traces of it on another planet can indicate that life could or did exist on that planet. Water is one of the more abundant molecules in living cells. Approximately 60–70 percent of the human body is made up of water. It is critical our viability by serving many essential functions (Figure 2.2.1). In humans, water forms saliva and keeps mucosal membranes moist. It allows the body's cells to grow, reproduce and survive. Water flushes out wastes through urination. It provides lubrication to joints. It is needed by the brain to manufacture hormones and neurotransmitters and acts as a shock absorber for the brain, spinal cord, and fetus. Without water, life simply would not exist.

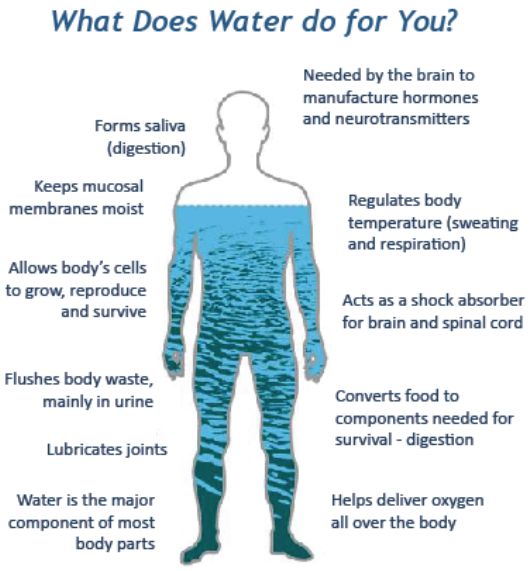


Figure 2.2.1: The functions of water. Water is the major component of most body parts and serves a number of essential functions like keeping membrane moist, flushing wastes from the body, lubricating joints, and regulates body temperature. (The water in you: What water does for your body by USGS.gov, public domain)

Water has several important properties, including:

1. **it is polar**
2. **it has the ability to form hydrogen bonds**
3. **it is a solvent**
4. **it has high cohesion**
5. **it has high adhesion**

6. **it moderates temperature**

Learning Objectives

Water is one of the most abundant molecules on Earth. By the end of this section, you will be able to:

- Describe the properties of water that are critical to maintaining life
- Explain why water is an excellent solvent
- Provide examples of water's cohesive and adhesive properties
- Discuss the role of acids, bases, and buffers in homeostasis

The Polarity of Water & Hydrogen Bonding

The bonds between the oxygen (O) and hydrogen (H) atoms in the water molecule are known as **covalent bonds.** *Electrons are shared between two atoms in a covalent bond*. Oxygen has 6 electrons in its outermost shell and hydrogen has only 1 electron. As a result, oxygen shares each of its outer electrons with a hydrogen atom. While there is no overall charge to a water molecule, the shared electrons are found more closely associated with the center of the oxygen atom than they are with the center of a hydrogen atom. This results in "unequal sharing" of electrons within the covalent bond and produces a slight positive charge on each hydrogen atom and a slight negative charge on the oxygen atom. Because of these charges, the slightly positive hydrogen atoms repel each other and form the unique shape (Figure 2.2.2).

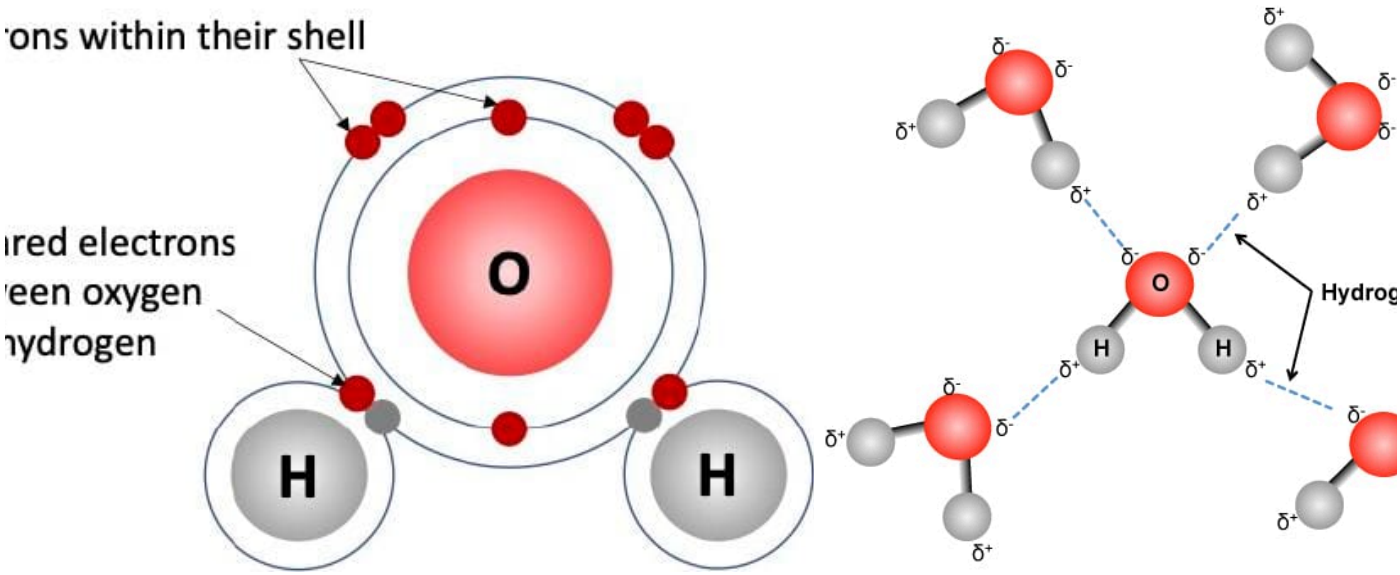


Figure 2.2.2: Left image: The electron configuration in a molecule of water produces polar covalent bonds. The two hydrogen electrons are "pulled" closer to the center of the oxygen atom, making the hydrogen atoms slightly positive in charge and the oxygen atom slightly negative. This "unequal sharing" of electrons is known as a polar covalent bond. (Electron sharing by Kareen Martin, CC BY-NC 4.0)
Right image: Hydrogen bonds form and reform between the partial positive end of one water molecule (i.e., the hydrogen atom) and the partial negative charge of an adjacent water molecule (i.e. the oxygen atom.) The partial charge in a polar bond is denoted with the Greek letter "delta". A molecule of water can interact with 4 hydrogen bonds with 4 other molecules of water. (Hydrogen bonds by Kareen Martin, CC BY-NC 4.0)

As a result of the positive and negative charges in the different parts of the water molecule, each water molecule attracts other water molecules (Figure 2.2.2). These attractions create **hydrogen bonds** between water molecules. Hydrogen bonds are weak interactions that form between *a hydrogen with a partial positive charge of one molecule and another atom with a partial negative charge*. The partial charge in a polar bond is denoted with the Greek letter "delta". Hydrogen bonds can break and reform easily giving water's fluidity.

Concept in Action

Animation: How polarity makes water behave strangely

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Water is an Excellent Solvent

The slight positive and negative charges of water molecules enables them to easily dissolve ionic compounds and polar molecules through their formation of hydrogen bonds with surrounding water molecules. A compound that dissolves in water is referred to as **hydrophilic** ("water-loving"). Because nonpolar molecules do not have partial positive or partial negative charges, they are not attracted to water molecules, explaining why oil separates when added to water. Or vinegar, a polar substance, does not mix with olive oil (a non-polar substance) as seen in Figure 2.2.3. These nonpolar compounds are called **hydrophobic** ("water-fearing").



Figure 2.2.3: Balsamic vinegar (black), a polar compound and olive oil (yellow), a non polar one, do not mix. (Plate with vinegar by Tim Ereneta, CC BY-NC 2.0)

Because water is capable of dissolving another substance, it is considered to be a **solvent**. The compounds dissolved in a solvent are called **solutes**. When polar and ionic compounds are dissolved in water, hydrogen bonds will form between the charged compounds and adjacent water molecules, resulting in the compound becoming surrounded by a layer of water molecules. This layer is called a **hydration shell** and it serves to keep solutes separated or dispersed in the water. For example, when table salt (NaCl) is dissolved in water (Figure 2.2.4), the sodium and chloride ions separate, or dissociate, in the water, and hydration shells form around each ion. Each positively-charged sodium ion is surrounded by negatively-charged oxygen atoms in water molecules. Each negatively-charged chloride ion is surrounded by positively-charged hydrogen atoms.

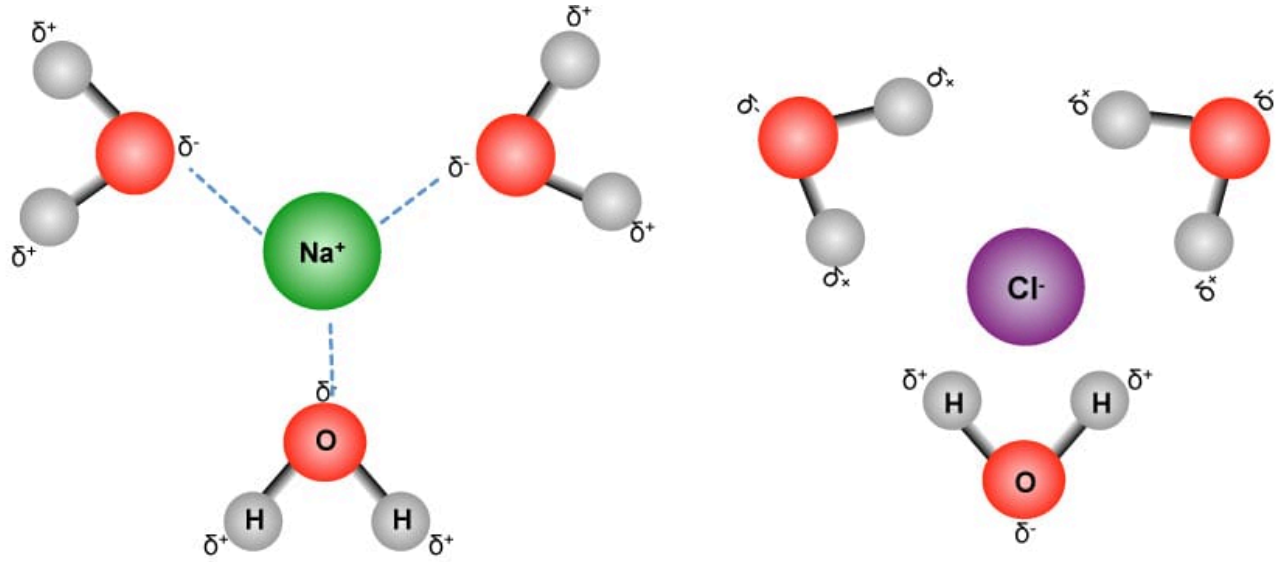


Figure 2.2.4: When table salt (NaCl) is mixed in water, each ion becomes surrounded by a hydration shell of water molecules. (Hydration shell by Kareen Martin, CC BY-NC 4.0)

The polarity of the water molecule makes it an effective solvent and is important in its many roles in living systems. However, one common mistake or misconception about water is that it dissolves everything because it is the “universal solvent." Water has the ability to dissolve many substances but the term “universal solvent" is misleading. *Water is able to dissolve other polar molecules and ions but cannot dissolve non-polar compounds.*

Water Moderates Temperature

Temperature is defined as a measure of the motion, or the kinetic energy of molecules. There is a direct relationship between kinetic energy and temperature. As kinetic energy increases, temperature increases. However, water is able to moderate changes in temperature within organisms and in the environment. This is because water has a high **specific heat capacity. Specific heat** is defined as the *amount of energy it takes to raise 1 gram of water by 1 degree Celsius*. Water's high specific heat capacity means that it takes a lot of energy to raise the temperature of water by a single degree, and it is the result of hydrogen bonding. Increasing kinetic energy disrupts the hydrogen bonds found between water molecules. Because these bonds can break and re-form rapidly, water can absorb large increases in energy before its temperature will change. In fact, water has the highest specific heat capacity of any liquid.

As energy input continues, the balance between hydrogen-bond formation and destruction swings toward the destruction side. More bonds are broken than are formed. This process results in the release of individual water molecules in gas form in a process called **evaporation**. As with specific heat capacity, it takes a significant amount of energy to convert liquid water to gaseous water (i.e. steam). This is because water has a **high heat of vaporization**. Evaporation of sweat, which is 90 percent water, allows for cooling of an organism, because breaking hydrogen bonds requires an input of energy and takes heat away from the body.

As kinetic energy decreases and temperatures drop, less energy is present to break the hydrogen bonds between water molecules. At a specific temperature, these hydrogen bonds will remain intact and begin to form a rigid, lattice-like structure as the water solidifies into ice (Figure 2.2.5). When water freezes, the distance between the water molecules increases, making ice less dense then liquid water. This means that ice floats (Figure 2.2.5). In lakes, ponds, and oceans, ice will form on the surface of the water, creating an insulating barrier to protect the animal and plant life beneath from freezing in the water. If this did not happen, plants and animals living in water would freeze in a block of ice and could not move freely, making life in cold temperatures difficult or impossible.

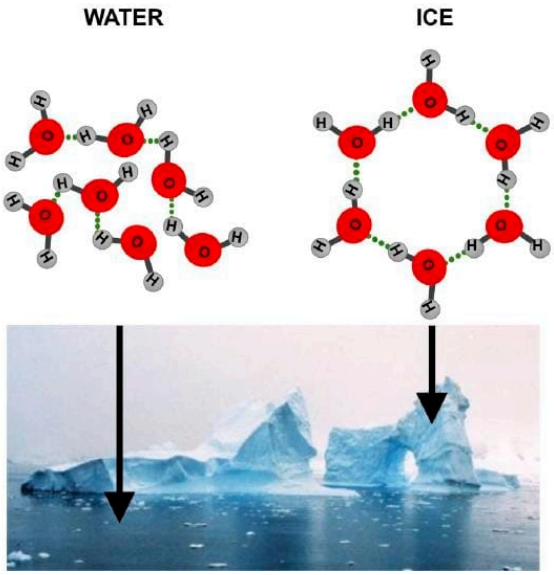


Figure 2.2.5: Hydrogen bonding makes ice less dense than liquid water. The lattice structure of ice, seen on the right side, makes it less dense than the freely flowing molecules of liquid water, (left side) enabling it to float on water. (Icebergs by Ben-Zin, public domain, Water and Ice Structure by Kareen Martin, CC BY-NC 4.0)

Water Is "Sticky" (Cohesion and Adhesion)

Water has both high cohesion and high adhesion. In **cohesion**, water molecules at a liquid-air interface are attracted to each other (because of hydrogen bonding), keeping the molecules close together and well organized (Figure 2.2.6). The hydrogen bonds found between water molecules at the surface are stronger than those below them, giving rise to **surface tension**, the capacity of a substance to withstand rupture when placed under tension or stress. Because of cohesion and surface tension, it is possible to “float” a steel needle on top of a glass of water if the needle is placed gently and does not break the surface tension. This is possible even though the needle is denser than the water. Small insects such as the water strider can walk on water because their weight is not enough to break the surface tension. (Figure 2.2.6).

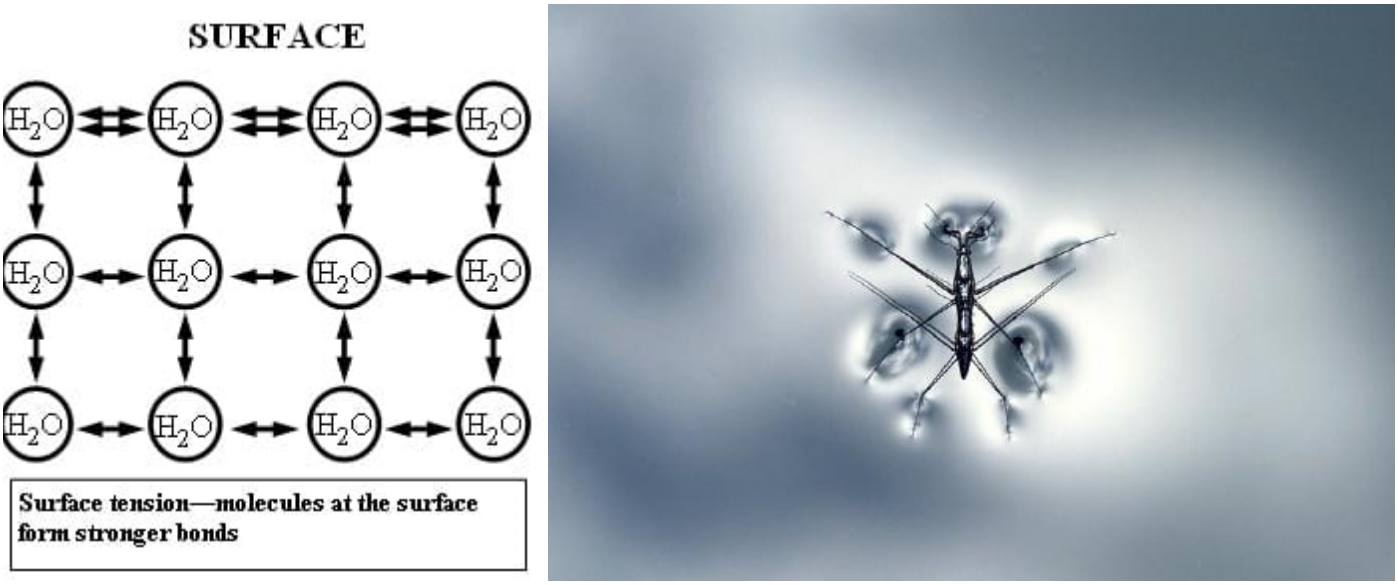


Figure 2.2.6: Left image - surface tension in water is due to cohesion (i.e. attraction) between water molecules at the surface of the water as adjacent water molecules form strong hydrogen bonds between one another. (Surface tension by USGS.gov, public domain)
Right image - a "water strider" is able to walk on top of water due to a combination of high surface tension of water and long, hydrophobic legs to help them stay above water. (Water strider by Unsplash, CCO)

Water also exhibits **adhesion**, which is the attraction of *two different molecules to one another*. This can be observed when water

"climbs" up a straw placed in a glass of water. Because of adhesion, the water molecules are attracted to the plastic of the straw and form bonds with it. It is important to distinguish that adhesion and cohesion are related to one another *but are not the same thing*. Adhesion takes place because of the interaction between water molecules and another molecule; cohesion takes place because of the interaction between water molecules. Cohesive and adhesive forces are important for sustaining life. For example, because of these forces, water can flow up from the roots to the tops of plants to feed the plant (Figure 2.2.7).



Figure 2.2.7: Left: Cohesion between water molecules allows a drop to form and cohesion results in the drop clinging to the tip of a leaf. Right: With the help of adhesion and cohesion, water can work its way all the way up to the branches and leaves at the top of trees. (Cohesion and Adhesion by Kareen Martin, CC BY-NC 4.0)

Buffers, pH, Acids, and Bases

The **pH** of a solution is a measure of its acidity or basicity and is determined by the concentration of hydrogen ions in that solution. The overall concentration of hydrogen ions is inversely related to its pH. The more H+ present, the lower the pH the more acidic the solution is; conversely, the fewer hydrogen ions, the higher the pH and the more basic (i.e. alkaline) the solution is. Acidity and basicity is measured using the **pH scale** (Figure 2.2.8). The pH scale ranges from 0 to 14. A change of one unit on the pH scale represents a change in the concentration of hydrogen ions by a factor of 10; a change in two units represents a change in the hydrogen ion concentration by a factor of 100. Thus, small changes in pH represent large changes in the concentrations of hydrogen ions. Pure water is neutral. It is neither acidic nor basic, and has a pH of 7.0. Anything below 7.0 (from 0.0 to 6.9) is acidic, and anything above 7.0 (from 7.1 to 14.0) is alkaline. Most cells operate within a very narrow window of the pH scale, typically ranging only from 7.2 to 7.6.

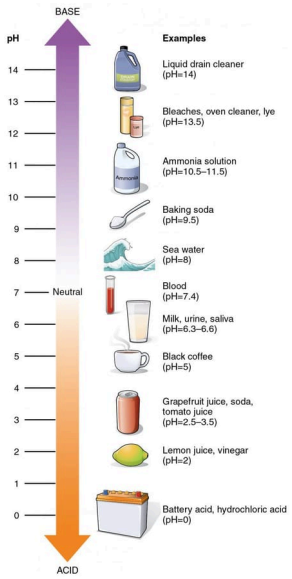


Figure 2.2.8: The pH scale ranges from 0 to 14, and most solutions fall within this range. Anything below 7.0 is acidic, and anything above 7.0 is alkaline, or basic. The pH values of common substances like milk, saliva, coffee and blood are shown. (pH Scale by OpenStax College, CC BY 3.0)

An **acid** is a substance that donates hydrogen ions (H+) and lowers pH. (Figure 2.2.9). The stronger the acid, the more readily it donates H+. For example, hydrochloric acid and lemon juice are very acidic and readily give up H+ when added to water. Conversely, a **base** is a substance that accepts H+ ions and raises pH. A base can also be defined as a substance that donates hydroxide ions (OH–). The OH– ions from the base combine with H+ from the acid to produce water, which raises a substance's pH. Sodium hydroxide (NaOH) and many household cleaners are very alkaline and give up OH– rapidly when placed in water, thereby raising the pH.

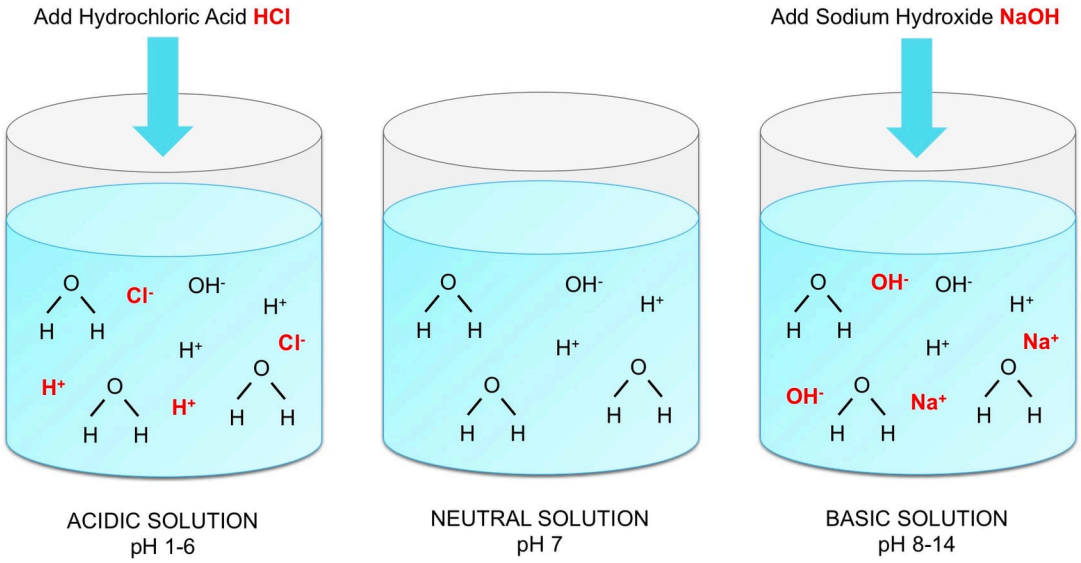


Figure 2.2.9: Left image - in an acidic solution (e.g. hydrochloric acid/HCl), ionic dissociation gives rise to an excess of H^+. The pH of an acidic solution ranges between 1 and 6. Center image - in a neutral solution, water molecules dissociate into an equal number of hydrogen ions (H^+) and hydroxide ions (OH^-) and recombine to reform water. The resulting pH is 7.0. Right image - in a basic solution (e.g. sodium hydroxide/ NaOH), ionic dissociation gives rise to an excess of OH^- that can combine with H^+ to produce water molecules. As a result, the number of H^+ decreases and the pH of the solution becomes basic. The pH of a basic solution ranges comprised between 8 and 14. (pH Solutions by Kareen Martin, CC BY-NC 4.0)

Organisms can maintain the pH of their cells and fluids within a relatively narrow range using buffers. A **buffer** is a solution that resists pH changes when small amounts of acid or base are added. In other words, buffers readily absorb excess H+ or

OH–, maintaining the pH of the organism at a desired level. **Carbon dioxide (CO_2)** is part of a prominent buffer system used by many organisms called the **carbonic acid-bicarbonate buffer system**. This buffer system involves **carbonic acid (H_2CO_3)** and **bicarbonate (HCO_3^-) anion** (Figure 2.2.10). If too a solution has too much H+, bicarbonate will combine with the H+ to create carbonic acid and limit the decrease in pH of the solution. Likewise, if too much OH– is introduced into a solution, carbonic acid will rapidly dissociate into bicarbonate and H+ ions. The H+ ions can combine with the OH– ions, limiting the increase in pH.

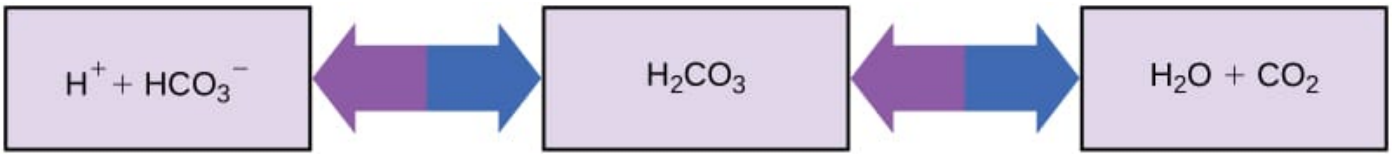


Figure 2.2.10: Carbonic acid acts as a buffer. Water and carbon dioxide (CO_2) combine to form carbonic acid (H_2CO_3). Carbonic acid dissociates to form H+ and bicarbonate ions (HCO_3^-). When an acid is added to a fluid, HCO_3^- react with the excess H^+ to form carbonic acid and prevent a drop in pH. When a base is added, carbonic acid donates H+ to neutralize the excess OH- and form more carbonic acid (modified from Buffer by CNX OpenStax; CC BY 4.0).

Many organisms rely upon this buffering system to maintain the pH of their blood plasma (and tissue fluids) between 7.2 and 7.4. If the blood becomes too acidic, the organism will increase its respiration rate to remove more carbon dioxide and decrease the concentration of carbonic acid. This will decrease the amount of H+ in the blood produced by carbonic acid dissociation. If the blood becomes too basic, the organism can slow its breathing rate, increasing carbonic acid levels and lowering pH through the production of more H+.

Key Concepts

Water has many properties that are critical to maintaining life. These properties of water are intimately connected to the biochemical and physical processes performed by living organisms, and life would be very different if these properties were altered, if it could exist at all.

Some important concepts to remember:

- Water is a polar molecule, allowing for the formation of hydrogen bonds with other molecules, including other water molecules
- Water is an excellent solvent. Its hydrogen bonds allow ions and other polar molecules to dissolve in water
- Water stabilizes temperature. The hydrogen bonds between water molecules cause the water to have a high heat capacity, meaning it takes considerable added heat to raise its temperature.
- The evaporation of water takes considerable amounts of energy; this allows organisms to cool their internal temperatures
- Water is less dense when in solid form; this means that ice floats
- The attraction of water molecules to one another at the surface of a volume of water is called cohesion; cohesion produces surface tension
- The attraction between a water molecule and another molecule is called adhesion
- The pH of a solution is a measure of hydrogen ion concentration
- Acids and bases can change pH values, but buffers tend to moderate the changes they cause

Glossary

Acid - a substance that increases the concentration of hydrogen ions (H⃟) in a solution (i.e. a H+ donor)

Adhesion - the attraction between molecules of different substances; caused by intermolecular forces such as hydrogen bonding, van der Waals forces, or dipole interactions

Base - a substance that increases the concentration of hydroxide ions (OH⃟) or accepts protons (H⃟) in a solution (i.e. an OH- donor)

Buffer - a solution that resists changes in pH when small amounts of acid or base are added; helps to maintain a stable pH in biological and chemical systems

Cohesion - the attraction between molecules of the same substance due to intermolecular forces like hydrogen bonding, van der Waals forces, or dipole interactions

Evaporation - the process by which a liquid turns into a gas at a temperature below its boiling point; it occurs at the surface of the liquid, where molecules with sufficient kinetic energy escape into the air as vapor

Hydrophilic - a substance that is attracted to and dissolves in water

Hydrophobic - a substance that repels water and does not dissolve in it

Kinetic energy - the energy of motion

pH scale - a measurement system used to express how acidic or basic a solution is; ranges from 0 to 14

Solute - a substance that dissolves in a solvent

Solvent - a solution that dissolves a solute

Surface tension - a property of liquids that causes the surface to act like a membrane; a result of strong cohesive attraction between water molecules at the surface of the liquid

Temperature - a measure of the average kinetic energy of the particles in a substance

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2.3: Macromolecules

Biotech Focus

The study of how macromolecules are made can be difficult. Initial studies in this area were made possible through radiolabeling. In **radiolabeling**, radioactive isotopes, like carbon-14 (^{14}C), sulfur-35 (^{35}S) and phosphorus-32 (^{32}P), are added to cells so that these isotopes are incorporated into the macromolecules being made. For DNA synthesis, researchers will rely upon ^{32}P incorporation into nucleotides. Protein synthesis uses ^{35}S, while carbohydrate synthesis can be studied using ^{14}C. Thanks to its sensitivity, radiolabeling allows researchers to track, quantify, and analyze the behavior of macromolecules in biological systems.

Introduction

A **macromolecule** is a large, complex molecule that is essential to the viability and function of cells. There are four major classes of biological macromolecules, **carbohydrates, lipids, proteins, and nucleic acids**, and each is an important component of the cell. Combined, these molecules make up the majority of a cell's dry mass. They perform a wide variety of functions, including energy storage, structural support, cellular communication, and genetic information transfer. Understanding the structure of a macromolecule is key to comprehending complex biological processes, from cellular metabolism, to the transfer of genetic information.



Figure 2.3.1: Portrait of a man made out of fruits and vegetables by Giuseppe Arcimboldo. (Arcimboldo, Public Domain)

Concept in Action

Video: Macromolecules

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Learning Objectives

By the end of this section, you will be able to:

- Describe the relationship between a monomer and a polymer
- Understand the synthesis of macromolecules
- Explain dehydration and hydrolysis reactions

Synthesis of macromolecules

Biological macromolecules are large molecules, necessary for life, that are built from smaller organic molecules. Biological macromolecules are organic, meaning they contain carbon and are bound to hydrogen, and may contain oxygen, nitrogen, and additional minor elements. Most (but not all) biological macromolecules are polymers. A **polymer** is a molecule made up of smaller subunits, called **monomers**. Think of a polymer as being like a necklace, a series of beads strung together. Typically all the monomers in a polymer tend to be the same, or at least very similar to each other, linked to one another over and over again to build up the larger macromolecule. The monomer used depends on the macromolecule. Table 2.3.1 outlines the types of monomers used to construct a carbohydrate, protein, and nucleic acid.

Table 2.3.1: Types of monomer forming organic molecules

Monomer	Polymer (Macromolecule)
"*Bead*" 	"*Necklace*" 
Monosaccharide	Carbohydrate
Amino Acid	Protein
Nucleotide	Nucleic Acid (DNA and RNA)

Monomers combine with each to make the polymer through the formation of covalent bonds. In doing so, the monomers release water molecules as byproducts. This type of reaction is known as **dehydration synthesis**, which means “to put together while losing water.” When performed in a test tube, the release of water results in condensation on the side of the test tube. As such, dehydration synthesis reactions are also known as condensation reactions.

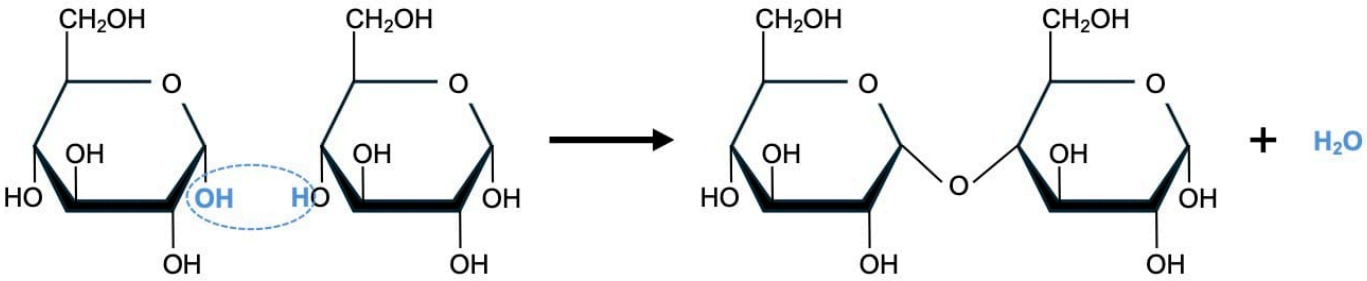


Figure 2.3.2: Dehydration synthesis reactions create bonds through the removal of a water molecule. In the dehydration synthesis reaction depicted above, two molecules of glucose combine together to form the disaccharide maltose. A hydroxyl (OH) is removed from carbon 1 of one glucose and combines with a hydrogen (H) removed from carbon 4 of the second glucose, to create a molecule water (dotted circle). A new covalent bond forms between the two glucose molecules. Additional monomers may join by the same process, growing the chain and forming a polymer. (Dehydration synthesis by Kareen Martin; CC BY 4.0)

In a dehydration synthesis reaction (Figure 2.3.2), the hydrogen of one monomer combines with the hydroxyl group of another monomer, releasing a molecule of water. At the same time, the monomers share electrons and form covalent bonds. As additional monomers join using dehydration synthesis, the growing chain of repeating monomers forms the polymer. Since dehydration reactions involve the formation of new bonds, they require an input of energy. Different types of monomers can combine in many

configurations, giving rise to a diverse group of macromolecules. Even one kind of monomer can combine in a variety of ways to form several different polymers: for example, glucose monomers are the constituents of starch, glycogen, and cellulose.

Because polymers form through the removal of water molecules, water is required to break them down. The bonds found within a polymer can be broken through a reaction called **hydrolysis**, which means "to split with water". The hydrolysis reaction is a reaction in which a water molecule is added to a bond, resulting in the breaking of the bond (Figure 2.3.3); one product of the reaction gains a hydrogen atom (H+) and the other gains a hydroxyl molecule (OH–). Because hydrolysis reactions break bonds, they will release energy.

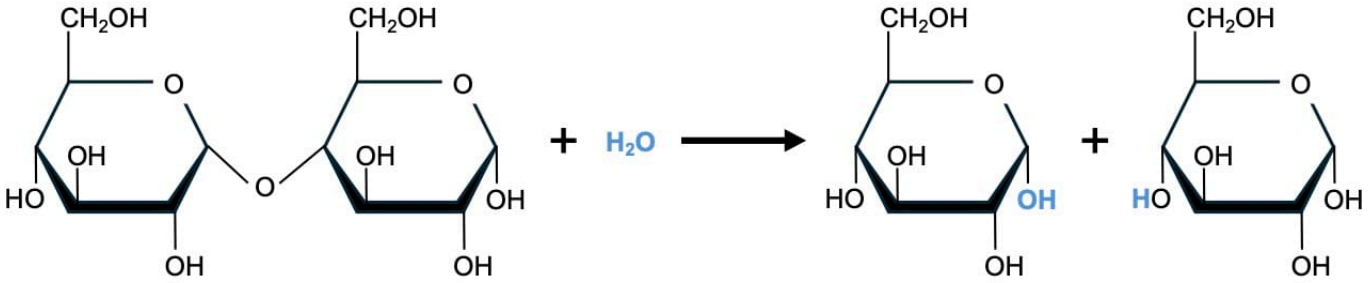


Figure 2.3.3: Hydrolysis reactions break bonds through the addition of a water molecule. In the hydrolysis reaction shown here, the addition of water breaks down the disaccharide maltose to form two glucose monomers. One of the new molecules gains a hydrogen atom, while the other gains a hydroxyl (-OH) group. Note that this reaction is the reverse of the dehydration synthesis reaction. (Hydrolysis by Kareen Martin; modified from Synthesis of Biological Macromolecules; CC BY 4.0)

Dehydration and hydrolysis reactions are catalyzed, or "sped up," by specific **enzymes**. In biological organisms, these enzymes are specific to the macromolecule. For instance, the carbohydrates amylose, sucrose, lactose, and maltose are broken down by the enzymes amylase, sucrase, lactase, and maltase, respectively. DNA polymers are created by a class of enzymes called DNA polymerases. A discussion of each of the four biological macromolecules, how they are made and how they are degraded, can be found in the remaining pages of this chapter.

Key Concepts

Biological macromolecules are important cellular components and perform a wide array of functions necessary for the survival and growth of living organisms.

Some important concepts to remember are:

- a polymer is a large macromolecule made up of subunits called monomers
- the monomers of a polymer are joined together through a dehydration synthesis reaction, which removes a molecule water to form the bond
- a monomer can be removed from a polymer through a hydrolysis reaction, which breaks a bond through the addition of a water molecule
- the four major classes of biological macromolecules are carbohydrates, lipids, proteins, and nucleic acids
- most biological macromolecules can be considered polymers

Glossary

Catalyst - a substance that speeds up chemical reactions without being consumed, often refers to enzymes in biological systems

Dehydration - to remove water

Dehydration synthesis - a chemical reaction in which two molecules combine to form a larger molecule, while removing a water molecule (H_2O); also known as a condensation reaction

Enzyme - a biological catalyst that speeds up a chemical reaction; not consumed by the chemical reaction

Hydrolysis - to break with water ("hydro" = water; "lysis" = to break)

Macromolecule - a large molecule; often used interchangeably with polymer

Monomer - a small molecule that when combined with one another forms a polymer

Polymer - a large molecule made up of repeating units called monomers

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2.4: Carbohydrates

Biotech Focus

Carbohydrates are not only important components of foods, but they are essential macromolecules that perform a range of critical biological functions in cells. In addition to providing structure to cells (in plants) and being a source of cellular energy (in all cells), some carbohydrates have been approved for the treatment of various diseases. However, their use is very limited. To learn why and read about the future potential of carbohydrates as medicine, check out the article Exploring Carbohydrates for Therapeutics: A Review on Future Directions.

Introduction

Carbohydrates play essential roles in the human body as well as in other organisms. They are an important source of energy to cells and also provide structural support.

Learning Objectives

By the end of this section, you will be able to:

- Discuss the role of carbohydrates in cells and in the extracellular materials of animals and plants
- Explain the basic composition of a carbohydrate
- List the major types of carbohydrates
- Explain how monosaccharides are classified
- Explain the major forms of monosaccharides
- Explain the different types of common monosaccharides, disaccharides, and polysaccharides
- Explain the difference between a dehydration synthesis reaction and a hydrolysis reaction

Molecular Structures

Carbohydrates are biological molecules made of carbon, hydrogen, and oxygen. This composition gives carbohydrates their name: they are made up of carbon (carbo-) plus water (-hydrate). Carbohydrates can be represented by the stoichiometric formula $\mathbf{(CH_2O)_n}$, where n is the number of carbons in the molecule. In other words, there is as number of carbon atoms in a carbohydrate molecule equals that of oxygen atoms and there are twice as many hydrogen atoms as carbon.

Carbohydrates can be classified into three categories:

1. **monosaccharides**
2. **disaccharides**
3. **polysaccharides**

Monosaccharides

Monosaccharides (mono- = “one”; sacchar- = “sugar”) are the simplest form of a carbohydrate and are often referred to as **simple sugars**. They typically range in size from three to seven carbons (Figure 2.4.1). Most monosaccharide names end with the suffix "-ose" (e.g. glucose). They can be classified in several ways, one of which is based on the number of carbons.

Examples of carbohydrates classified using this method are:

- **triose sugars** (three carbons)
- **pentose sugars** (five carbons)
- **hexose sugars** (six carbons)

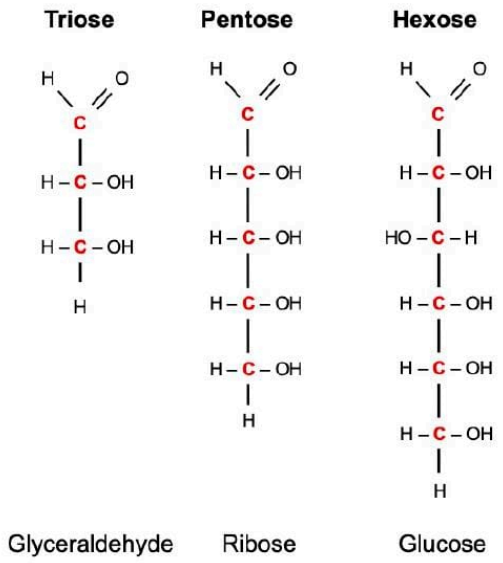


Figure 2.4.1: Monosaccharides can be classified based on the number of carbons in the backbone. The triose sugar, glyceraldehyde, ($C_3H_6O_3$) has three carbons (carbons are highlighted in red). The pentose sugar, ribose, ($C_5H_{10}O_5$) has five sugars. The hexose sugar, glucose, ($C_6H_{12}O_6$) is made of 6 carbons. (Monosaccharides by Kareen Martin; CC BY 4.0)

A monosaccharide is composed of carbon, oxygen, and hydrogen atoms. Most of the oxygen atoms in the monosaccharide can be found in hydroxyl (OH) groups. However, one oxygen atom is found as part of a functional group called a **carbonyl (C=O)** (Figure 2.4.2). The position of the carbonyl group is another way to classify a monosaccharide.

Using this method, there are two types of monosaccharides:

1. **Aldose:** the carbonyl (C=O) is located at the end of the sugar, forming an **aldehyde** group
2. **Ketose:** The carbonyl (C=O) is located within the molecule, forming a **ketone** group

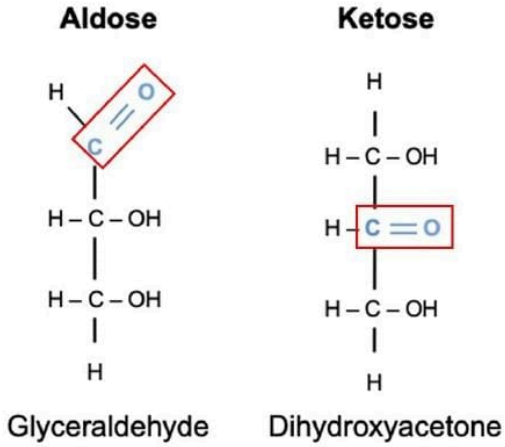


Figure 2.4.2: Monosaccharides can be classified based on the position of their carbonyl group (C=O) in the carbon chain (red boxes). Aldose sugars (e.g. glyceraldehyde) have a carbonyl group at the end of the carbon chain, and ketose sugars (e.g. dihydroxyacetone) have a carbonyl group in the middle of the carbon chain. (Aldose vs Ketose by Kareen Martin; CC BY 4.0)

The most common monosaccharides found in cells are the hexose sugars **glucose, fructose, and galactose** and the pentose sugars **ribose and deoxyribose.** Both ribose and deoxyribose are found in nucleic acids. Galactose can be found as part of lactose (i.e. milk sugar) and fructose is a component of the disaccharide in sucrose, which is found in fruit. Glucose is an important source of energy to eukaryotic cells. During cellular respiration, energy is released from glucose, and that energy is used to help

make adenosine triphosphate (ATP). Plants synthesize glucose using carbon dioxide and water, and glucose in turn is used for energy requirements for the plant. In plants and certain algae excess glucose is stored as starch that is broken down by humans and other animals that feed on these materials. In animals and certain fungi, excess glucose is stored as glycogen.

The chemical formula for glucose, galactose, and fructose is $C_6H_{12}O_6$. While their chemical formulae are identical, their structures are different (Figure 2.4.3). Compounds that have the same molecular formulae, but have different structures are known as **structural isomers.** Therefore, *glucose, galactose and fructose are structural isomers of one another.*

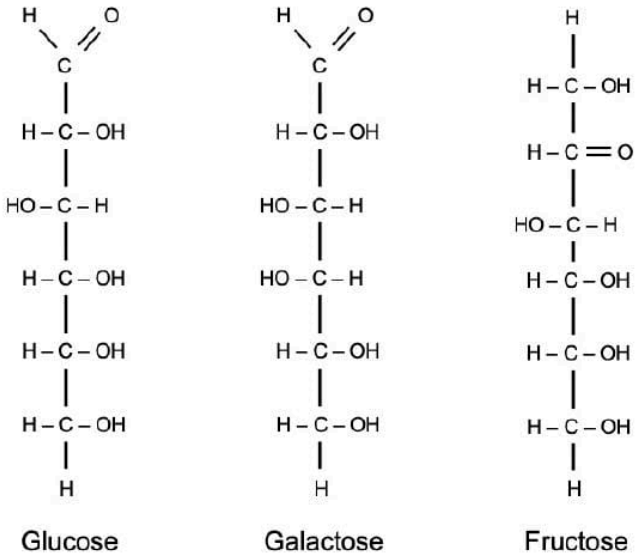


Figure 2.4.3: Hexose sugars. Glucose, galactose, and fructose are all hexose sugars with the same chemical formula ($C_6H_{12}O_6$). They are known as structural isomers, meaning they have the same chemical formula but a different arrangement of atoms. (Structural Isomers of Glucose by Kareen Martin; CC BY 4.0)

When placed into aqueous solutions, monosaccharides form **ring-shaped molecules** (Figure 2.4.4). The ring form of a monosaccharide is more stable than its linear counterpart. This is because the reactive carbonyl function group found in the linear form is no longer exposed and becomes "trapped" in the ring.

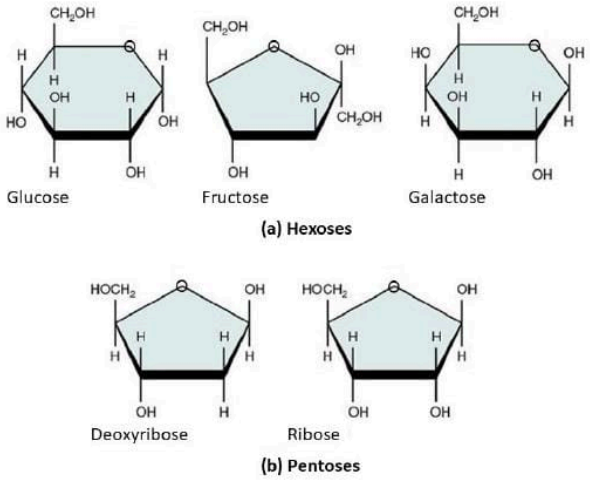


Figure 2.4.4: Monosaccharides can be found in ring forms. The ring forms of the hexose sugars glucose, fructose, and galactose are shown at the top of the figure and the pentose sugars deoxyribose and ribose are shown below. (Carbohydrate Ring Forms by Kareen Martin; CC BY 4.0)

The linear molecule is converted into a ring form when the hydroxyl group on the fifth carbon reacts with the carbonyl group on the first carbon. Numbering of the carbons in the resulting ring-shaped monosaccharide begins with the carbon to the right of

the oxygen and proceeds in a clock-wise fashion (Figure 2.4.5).

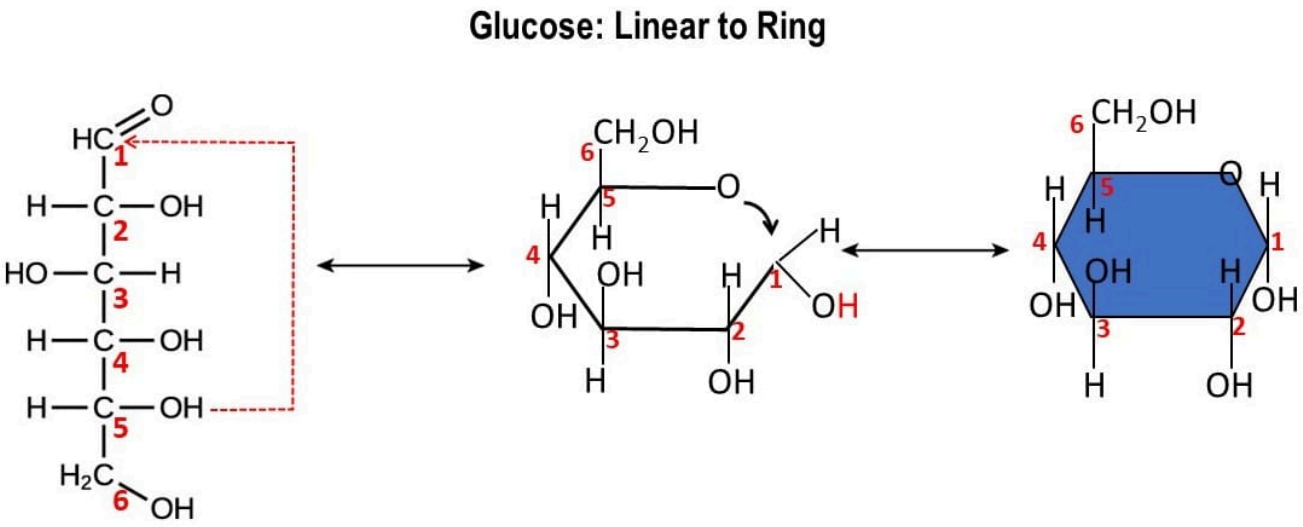


Figure 2.4.5: Transformation of linear glucose to the ring form. Conversion of a linear glucose molecule to its ring form occurs when the hydroxyl group at the 5th carbon reacts with the carbonyl group on the 1st carbon (left image, dotted red arrow). The linear form begins to convert to its ring conformation, with a bond forming between the 5th and 1st carbon (middle image, arrow). The hydrogen from the 5th carbon (shown in red) bonds to the oxygen at the 1st carbon to form a hydroxyl group. The ring formation of glucose has a specific numbering scheme with the 1st carbon located to the right of the oxygen (right image). (Glucose Linear to Ring by Patricia Zuk, CC BY 4.0)

Glucose and galactose in their ring structure, differ in the orientation of the hydroxyl group (OH) at the fourth carbon, with glucose having this functional group located below the plane of the ring. Furthermore, glucose can have two different arrangements of the hydroxyl group around the first carbon. If the hydroxyl group at carbon 1 found is below the plane of the ring, it is said to be in the **alpha (α) position**, and if it is above the plane, it is said to be in the **beta (ß) position** (Figure 2.4.6). The beta-form is more stable than the alpha form and is typically found in large polymers like cellulose, whereas the alpha form can be found in plant starches like amylose. Fructose, with its unique shape does not have alpha and beta conformations.

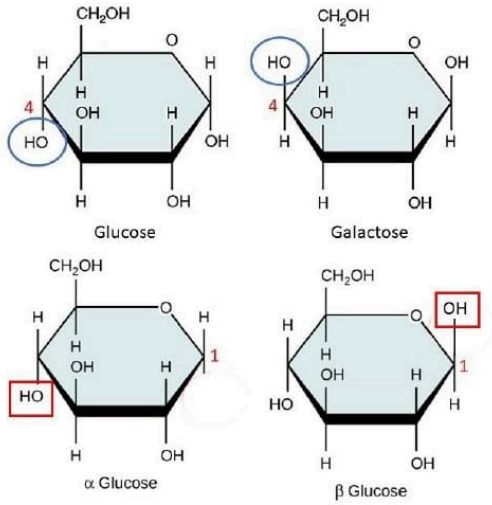


Figure 2.4.6: Monosaccharide conformations. Glucose and galactose can be distinguished using the hydroxyl groups located at carbon four (blue circles). The alpha and beta forms of glucose can be distinguished using the hydroxyl groups located at carbon one (red squares). (Glucose and Galactose Forms by Kareen Martin, CC BY 4.0)

Disaccharides

The joining of two monosaccharides forms a **disaccharide** (di- = “two”; sacchar- = “sugar”). Disaccharides are formed through **dehydration synthesis** and result in the formation of a **glycosidic bond** between the two monosaccharide subunits. During this process, the hydroxyl group (–OH) of one monosaccharide combines with a hydrogen atom of another monosaccharide, releasing a molecule of water (H_2O) and forming a covalent bond between atoms in the two sugar molecules (Figure 2.4.7).

Common disaccharides are:

- **sucrose** - formed by joining glucose to fructose
- **lactose** - formed by joining glucose to galactose
- **maltose** - formed by joining glucose to glucose

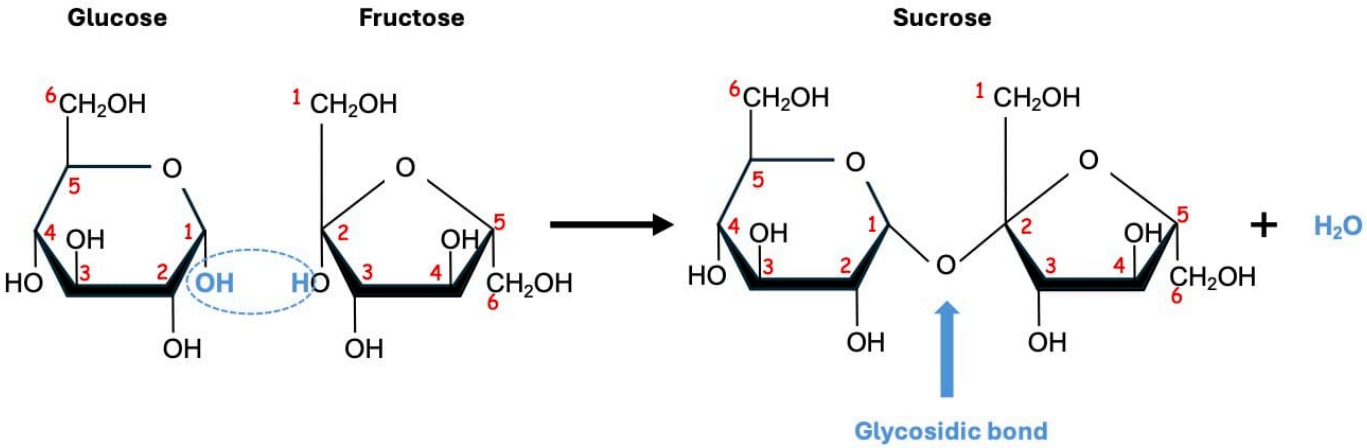


Figure 2.4.7: Sucrose is a disaccharide made of a monomer of glucose and a monomer of fructose. Sucrose is formed through a dehydration reaction that forms a glycosidic linkage between carbon 1 in glucose and carbon 2 in fructose. The components of the water molecule removed to make the bond is shown with a dotted circle. (Sucrose Formation by Kareen Martin; CC BY 4.0)

The breakdown of these disaccharides is done through a **hydrolysis reaction** and will yield glucose plus either galactose or fructose. In cells, this hydrolytic breakdown is catalyzed by enzymes. For example, the enzyme **sucrase** catalyzes the breakdown of sucrose into glucose and fructose Figure 2.4.8).

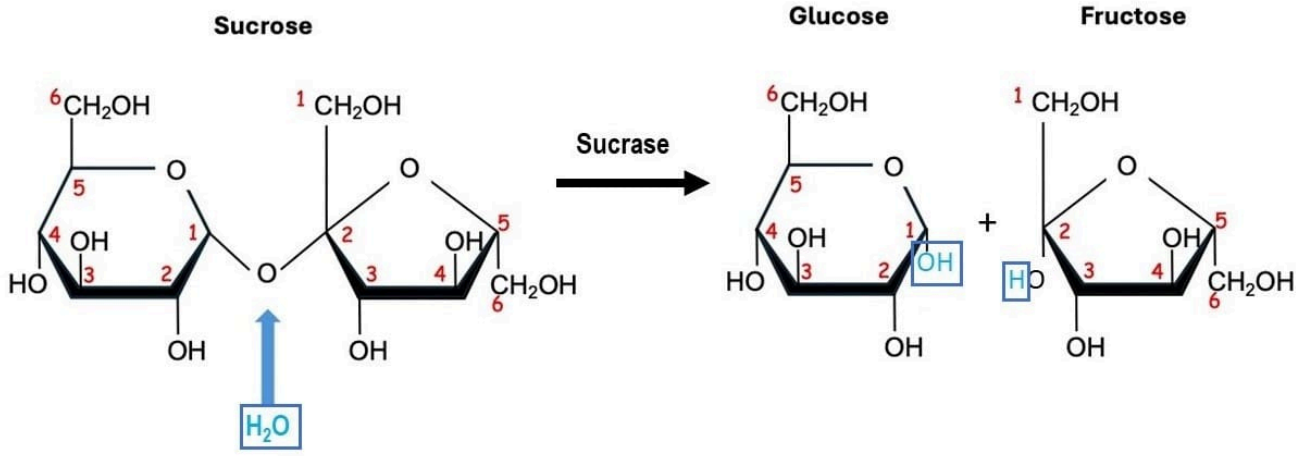


Figure 2.4.8: Sucrose breakdown by sucrase. The enzyme sucrase catalyzes the hydrolytic breakdown of sucrose into the monomers glucose and fructose. The location of the incorporated water molecule is shown with blue boxes. (Sucrose Breakdown by Patricia Zuk, CC BY 4.0)

Polysaccharides

The joining of three of more monosaccharides creates a **polysaccharide** (poly- = “many”). Each polysaccharide is made of either a few or hundreds of monosaccharides with each joined through a dehydration synthesis reaction. Unlike simple sugars, polysaccharides are not soluble in water and do not taste very sweet. This is because the majority of polysaccharides found in living organisms are made of glucose monomers. Starch, glycogen, cellulose, and chitin are examples of polysaccharides. Starch and glycogen are considered **storage polysaccharides** owing to their roles in energy utilization. Cellulose and chitin are examples of **structural polysaccharides** that are incorporated into the structure of the organism.

Starch is the stored form of glucose made by plants and can be found in two forms: **amylose** and **amylopectin** (Figure 2.4.9). Amylose is the simplest of these two forms with the alpha form of glucose linked by glycosidic bonds found between the first carbon of one glucose monomer and the fourth carbon of the next monomer (i.e. 1-4 glycosidic linkages). This creates a starch with a helical (i.e. coiled) conformation. Amylopectin has regions of coils, in addition to branch points that are formed by glycosidic bonds that form between the first carbon of one glucose monomer and the sixth carbon of another (i.e. 1-6 glycosidic linkages). Excess glucose in plants is stored as starch in different plant parts, including the roots and seeds. The starch that is consumed by animals is broken down into smaller molecules, such as glucose. The cells can then utilize the glucose.

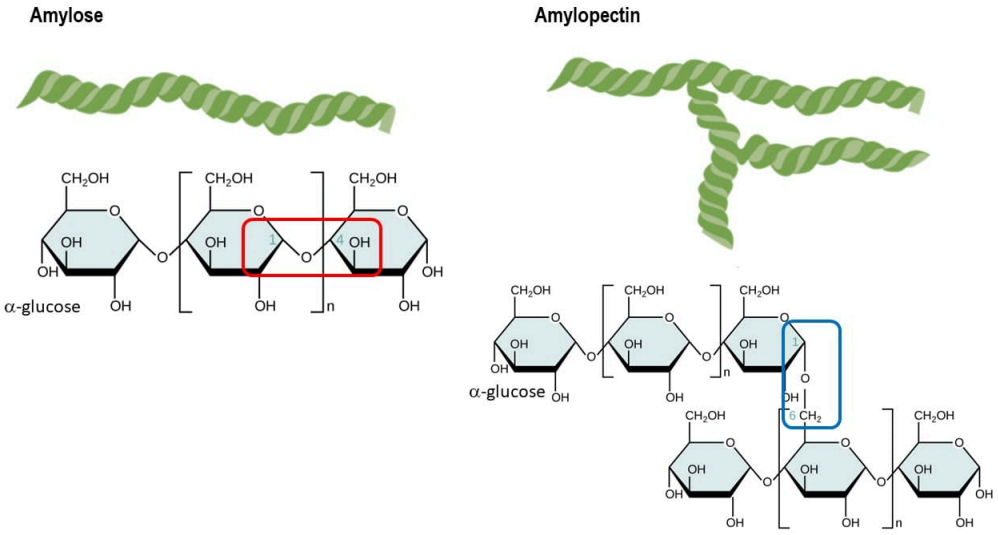


Figure 2.4.9: Amylose and amylopectin. The starch molecules amylose and amylopectin are composed of α-glucose subunits. Amylose (left image) is a simple, coiled starch made of α-glucose monomers joined through 1-4 glycosidic linkages (red box). Amylopectin (right image) is made of α-glucose monomers joined through 1-4 glycosidic linkages, in addition to 1-6 glycosidic linkages (blue box). The 1-6 glycosidic linkages produce a branch point in this starch. (Amylose and Amylopectin by Patricia Zuk, CC BY 4.0; modified from Carbohydrates, CC BY-SA 4.0)

The main polysaccharide of vertebrates is **glycogen.** Glycogen is a complex, branched polysaccharide whose monomers are the beta form of glucose (Figure 2.4.10) Glycogen is the animal equivalent of starch. Branch points are the result of 1-6 glycosidic bonds, with the coiled sections being the result of 1-4 glycosidic bonds. Excess glucose is converted into glycogen by the cells of the liver and skeletal muscle. When glucose levels in an organism drop, glycogen is broken down to release glucose.

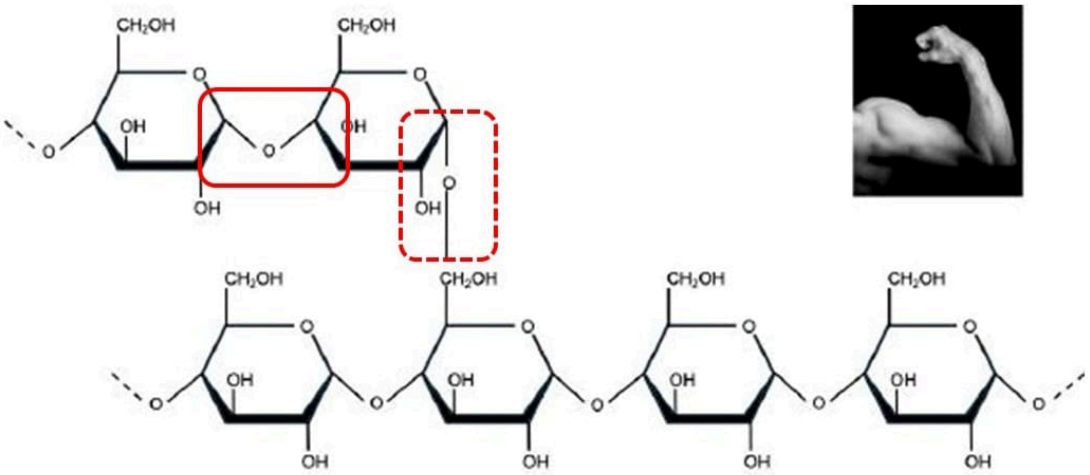


Figure 2.4.10: Glycogen structure. Glycogen is a highly branched molecule made of ß-glucose monomers connected by 1-4 glycosidic linkages (solid ed box) and 1-6 glycosidic linkages (dotted red box). The 1-6 glycosidic linkage will produce a branch point in the molecule. Glycogen is the animal equivalent of starch and usually stored in liver and muscle cells. (Glycogen Structure by Kareen Martin; CC BY 4.0)

Cellulose is one of the most abundant natural biopolymers. The cell walls of plants are mostly made of cellulose, which provides structural support to the cell. Wood and paper are mostly cellulosic in nature. The chemical formula for cellulose is $(C_6H_{10}O_5)_n$, where n is the number of glucose monomers. Cellulose is made up of glucose monomers that are linked by 1-4 glycosidic bonds. (Figure 2.4.11). While this is the same glycosidic linkage found in starch, *in cellulose the glucose monomer is the beta form* and every other beta-glucose monomer in cellulose is flipped in its orientation. This allows for the creation of extremely long chains of glucose that are rigid and have a high tensile strength—two parameters that are important to plant cells.

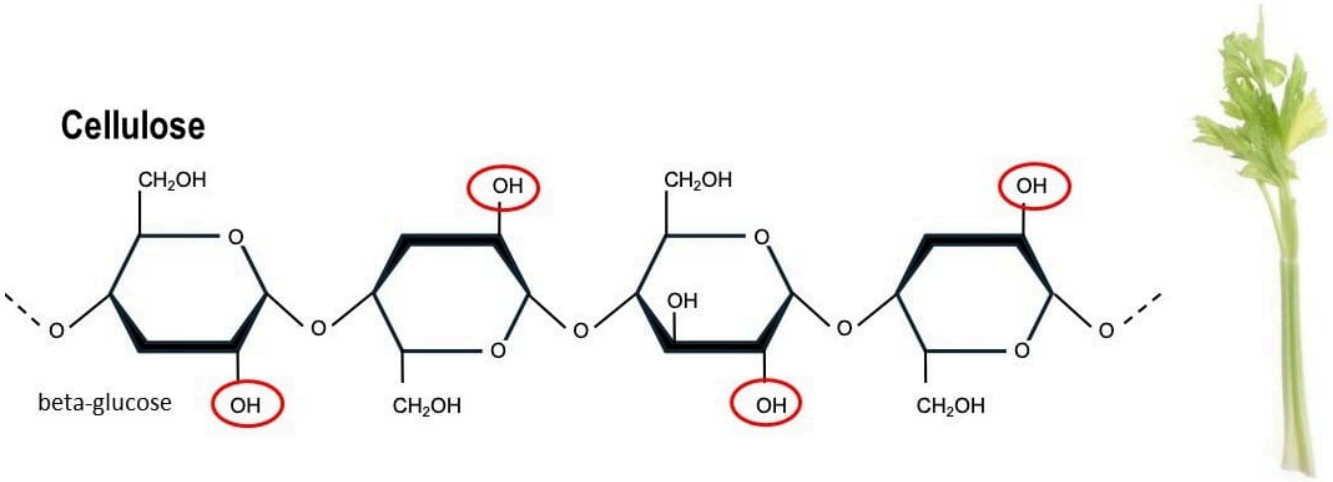


Figure 2.4.11: Cellulose structure. In cellulose, glucose monomers (beta form) are linked in unbranched chains (left image). Because of the way the beta-glucose subunits are joined, every glucose monomer is flipped relative to the next one (OH groups at carbon 3 shown circled in red). Cellulose is found in the cell walls of plants and gives them rigidity as seen in celery (right image). (Cellulose Structure by Kareen Martin; CC BY 4.0)

Cellulose passing through the digestive system is called dietary fiber. While the glucose-glucose bonds in cellulose cannot be broken down by human digestive enzymes, herbivores such as cows, buffalos, and horses are able to digest grass that is rich in cellulose and use it as a food source. In these animals, certain species of bacteria reside in the digestive system and secrete the enzyme **cellulase.** The appendix in some ruminants also contains bacteria that break down cellulose. Cellulases can break down cellulose into glucose monomers that can be used as an energy source by the animal.

Carbohydrates serve other functions in different animals. Arthropods, such as insects, spiders, and crabs, have an outer skeleton, called the **exoskeleton**, which protects their internal body parts. This exoskeleton is made of the biological macromolecule **chitin**, which is a nitrogenous carbohydrate. Chitin is made of repeating units of **N-acetylglucosamine (GlcNAc)**, a modified sugar derived from glucose and containing nitrogen. (Figure 2.4.12).

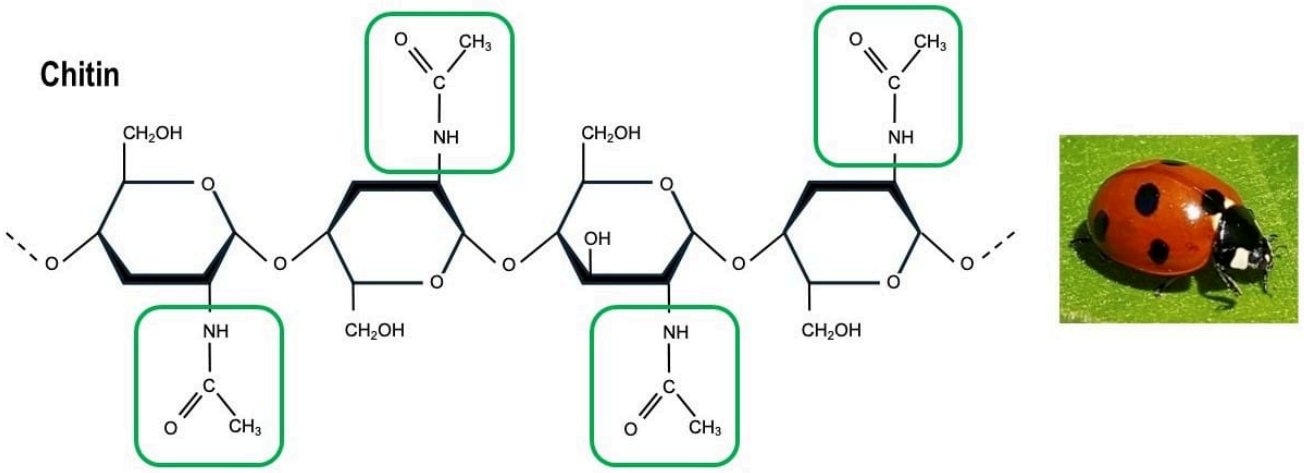


Figure 2.4.12: Chitin structure. Chitin is a polysaccharide made of repeating units of N-acetylglucosamine (GlcNAc). N-acetylglucosamine is a modified glucose monomer that has an amine and acetyl group (shown in the green box) bound to the 2nd carbon. Insects like the ladybug shown on the right, have a hard outer exoskeleton made of chitin. (Chitin Structure by Kareen Martin; CC BY 4.0)

While the polysaccharides discussed in this page are all built of glucose monomers, differences in their molecular structure allow for the different functions of energy storage (starch and glycogen) and structural support and protection (cellulose and chitin).

Key Concepts

Carbohydrates are a group of macromolecules that are a vital energy source for the cell and provide structural support to plant cells, fungi, and all of the arthropods that include lobsters, crabs, shrimp, insects, and spiders.

Some important concepts to remember are:

- Carbohydrates are classified as monosaccharides, disaccharides, and polysaccharides depending on the number of monomers in the molecule
- The monomer of a carbohydrate is the monosaccharide
- Monosaccharides are linked together by glycosidic bonds that are formed as a result of dehydration synthesis reactions
- Disaccharides are formed by joining two monosaccharides by a glycosidic bond
- Polysaccharides are formed by joining numerous monosaccharides by glycosidic bonds
- Glucose, galactose, and fructose are common monosaccharides
- Common disaccharides include lactose, maltose, and sucrose
- Starch and glycogen are storage polysaccharides, made of glucose and found in plants and animals, respectively.
- Cellulose and chitin are structural polysaccharides

Glossary

Carbohydrate - a macromolecule composed of carbon, hydrogen, and oxygen; primarily used for energy storage and structural functions.

Cellulose - a structural polysaccharide found in the cell walls of plants; composed of glucose molecules linked by glycosidic bonds; indigestible by humans but important as dietary fiber.

Disaccharide - a carbohydrate composed of two monosaccharides linked by a glycosidic bond, such as sucrose, lactose, and maltose.

Fiber - a type of carbohydrate that is not digested by human enzymes but aids in digestion and overall health; found in plant-based foods.

Fructose - a monosaccharide found in fruits and honey; often referred to as fruit sugar.

Glucose - a monosaccharide that serves as the primary source of energy for cells; often referred to in humans as as blood sugar.

Glycogen - a polysaccharide that serves as the primary storage form of glucose in animals and humans; stored mainly in the liver and muscles.

Glycosidic bond - a covalent bond that links carbohydrate molecules together; formed between the hydroxyl groups of two sugars.

Lactose - a disaccharide composed of glucose and galactose; commonly found in milk and dairy products.

Monosaccharide - the simplest form of carbohydrate, consisting of a single sugar unit, such as glucose, fructose, or galactose.

Polymer - a large molecule made up of repeating units called monomers

Polysaccharide - a complex carbohydrate composed of multiple monosaccharide units, such as starch, cellulose, and glycogen.

Starch - a polysaccharide found in plants that serves as their main form of energy storage; examples include amylose and amylopectin.

Sucrose - a disaccharide made up of glucose and fructose; commonly known as table sugar.

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2.5: Lipids

Biotech Focus

Statins are among the most widely-prescribed medications in the United States due to their ability to decrease LDL-cholesterol and lower the risk of developing cardiovascular diseases. Their development started with the works of Drs. Brown and Goldstein on the underlying mechanisms of cholesterol metabolism, for which they were awarded the 1985 Nobel Prize in Physiology or Medicine. Their findings eventually led to the discovery of the first statins by the Japanese biochemist Akira Endo and the subsequent development of statin drugs.

Learn more about the history, mechanism, and side effects of statins by watching the video below.

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

Introduction

Lipids include a diverse group of compounds that are largely non-polar in nature. This is because they are hydrocarbons that include mostly non-polar carbon–carbon or carbon–hydrogen bonds. Non-polar molecules are hydrophobic ("water fearing"), or insoluble in water. Lipids perform many different functions in a cell. Cells store energy for long-term use in the form of fats. Lipids also provide insulation from the environment for plants and animals. For example, they help keep aquatic birds and mammals dry when forming a protective layer over fur or feathers because of their water-repellant hydrophobic nature. Lipids are also the building blocks of many hormones and are an important constituent of all cellular membranes.

Lipids include the following:

1. **triglycerides (fats, oils)**
2. **phospholipids**
3. **steroids (cholesterol, bile salts, hormones)**
4. **fatty acids (omega family)**
5. **fat-soluble vitamins**
6. **eicanosoids (prostaglandins, leukotrienes)**
7. **lipoproteins (HDL, LDL)**
8. **waxes**
9. **pigments**

Learning Objectives

Lipids are a diverse group of non-polar macromolecules. By the end of this section, you will be able to:

- Describe the four major types of lipids
- Explain the role of fats in storing energy
- Differentiate between saturated and unsaturated fatty acids
- Describe phospholipids and their role in cells
- Define the basic structure of a steroid and some functions of steroids
- Explain the how cholesterol helps to maintain the fluid nature of the plasma membrane



Figure 2.5.1: The hydrophobic lipids found in the fur of aquatic mammals, such as this river otter, protect them from the elements by repelling water.

Triglycerides: Fats and Oils

A **triglyceride** molecule consists of two main components: **glycerol and fatty acids**. A solid triglyceride is referred to as a fat. A liquid triglyceride is known as an oil

- **Glycerol** is an organic compound (alcohol) with three carbons, five hydrogens, and three hydroxyl (OH) groups.
- **Fatty acids** have a long chain of hydrocarbons to which a carboxyl group is attached, hence the name “fatty acid.” The number of carbons in the fatty acid may range from 4 to 36; most common are those containing 12–18 carbons.

To form a triglyceride, three fatty acids attach to the glycerol molecule through three separate dehydration synthesis reactions. The bond that forms between a fatty acid and the glycerol is called an **ester linkage or ester bond** (Figure 2.5.2). To form an ester bond, a hydrogen from the glycerol's hydroxyl group and a hydroxyl group from the fatty acid's carboxyl group are removed to form a molecule of water. During triglyceride synthesis a total of three water molecules are formed and released and three ester bonds are created.

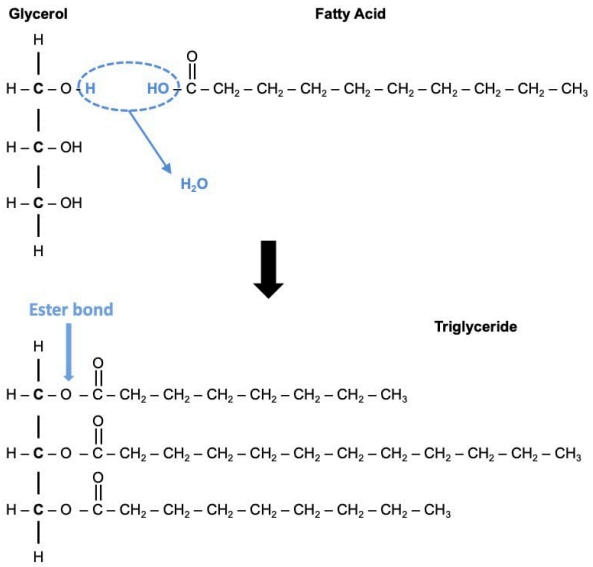


Figure 2.5.2: The triglyceride. A triglyceride is composed of three fatty acids and a glycerol backbone. Glycerol is a 3-carbon molecule with each carbon bound to a hydroxyl group. A fatty acid is a long chain of carbon and hydrogen with a carboxylic acid (-COOH) on one end. In triglyceride synthesis, the hydroxyl group (OH) fatty acid's carboxyl group combines with a hydrogen (H) from the glycerol's hydroxyl group (dotted circle), forming a molecule of water and resulting in the formation of an ester bond between the fatty acid and the glycerol (see arrow). Two more fatty acids bind to the remaining two hydroxyl groups of the glycerol. A total of three water molecules are formed. (Triglyceride Formation by Kareen Martin; CC BY 4.0)

The three fatty acids in the triglyceride may be similar to or different from one another. Some fatty acids have common names that specify their origin. For example, palmitic acid, is a fatty acid derived from the palm tree. Arachidic acid is derived from *Arachis hypogea*, the scientific name for peanuts. Fatty acids can be classified as saturated and unsaturated. In a **saturated fatty acid**, all carbons in the hydrocarbon chain are linked to each other through single bonds. Because of this, saturated fatty acids are said to be "saturated" with hydrogen; in other words, the number of hydrogen atoms attached to the carbon skeleton is maximized and no more hydrogen may be added to the carbon atoms of the chain. The saturated fatty acid is a long, linear molecule. Stearic acid is an example of a saturated fatty acid (Figure 2.5.3).

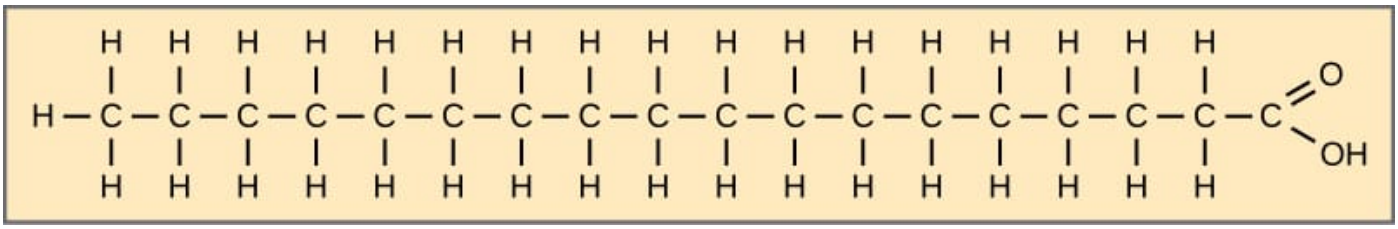


Figure 2.5.3: The saturated fatty acid. Stearic acid is a saturated fatty acid with an 18-carbon chain. All bonds between the carbons are single bonds. (Stearic acid by Openstax; CC BY 4.0)

In an **unsaturated fatty acid**, the hydrocarbon chain contains one or multiple carbon-carbon double bonds. In other words, carbons are not saturated with hydrogens. Oleic acid is an example of an unsaturated fatty acid (Figure 2.5.4). If there is one double bond in the molecule, then it is known as a **monounsaturated fatty acid** (e.g., found in olive oil), and if there is more than one double bond, then it is known as a **polyunsaturated fatty acid** (e.g., found in canola oil). Only one of the three fatty acids needs to be unsaturated for the triglyceride to be classified as unsaturated.

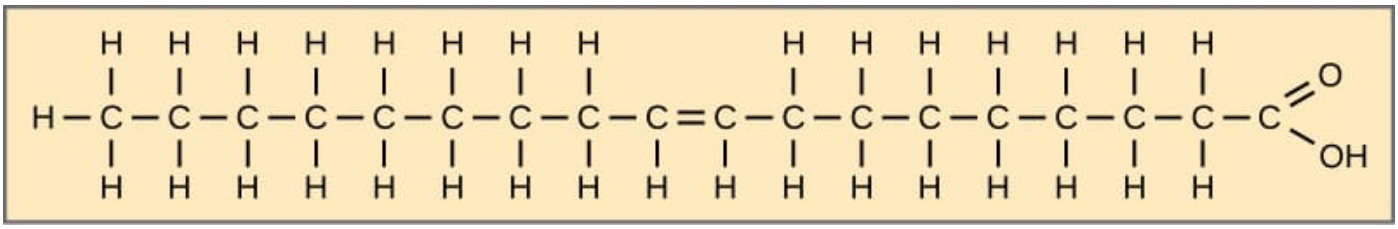


Figure 2.5.4: The unsaturated fatty acid. Oleic acid is an unsaturated fatty acid with an 18-carbon chain. It has one double bond between the 9th and 10th carbons. (Oleic acid by Openstax; CC BY 4.0)

The position of the hydrogen atoms around the carbon-carbon double bond can be used to further classify the unsaturated fatty acid as either ***cis*** or ***trans***. If hydrogen atoms are present in the same plane at this bond (i.e. on the same side of the double bond), the unsaturated fatty acid is called a ***cis* fatty acid** (Figure 2.5.5). The *cis* double bond causes a bend or a "kink" in the fatty acid. This bend prevents triglycerides from packing tightly on top of one another and results in a liquid triglyceride at room temperature, otherwise known as an **oil** (Figure 2.5.5). Olive oil, corn oil, canola oil, and cod liver oil are examples of *cis* unsaturated fats. If the hydrogen atoms at the double bond are on two different planes (i.e. across the double bond), the fatty acid is called a ***trans* fatty acid**. Because of the position of these hydrogen atoms, the *trans* fatty acid is a long, straight molecule. A triglyceride made of trans fatty acids or saturated fatty acids tend to be solid at room temperature because these long, straight fatty acids can be densely packed on top of one another. A solid triglyceride is called a **fat**. Examples of animal fats are stearic acid and palmitic acid (common in meat) and the fat butyric acid found in butter. Mammals store fats in specialized cells called adipocytes, where globules of fat occupy most of the cell's volume.

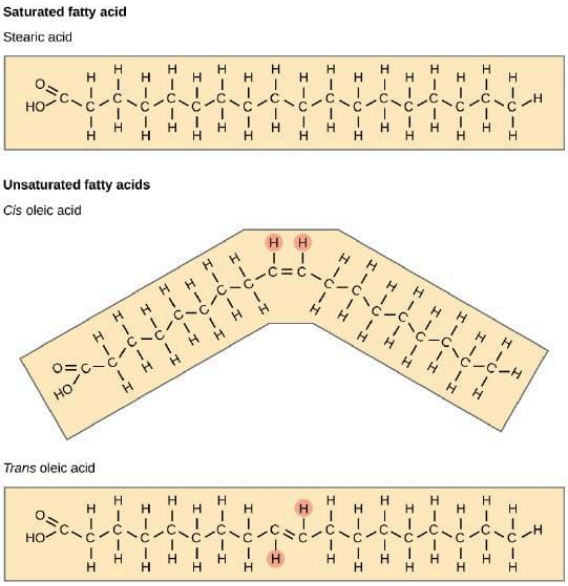


Figure 2.5.5: Comparison of saturated and unsaturated fatty acids. The saturated fatty acid stearic acid has an 18-carbon chain with a carboxyl group (-COOH) at the left end. All bonds between the carbons are single bonds. Oleic acid is made from stearic acid through the creation of a carbon-carbon double bond between the 9th and 10th carbons. In cis-oleic acid, the hydrogen atoms are on the same side of the double bond causing a kink in the chain. In trans-oleic acid, the hydrogen atoms are on opposite side of the double bond and the fatty acid is linear. (Comparison of saturated and unsaturated fatty acids by Openstax; CC BY 4.0)

In the food industry, oils are **artificially hydrogenated** to increase their shelf-life and give them a semi-solid consistency that desirable for many processed foods. In hydrogenation, hydrogen gas is bubbled through polyunsaturated oils to solidify them. During this hydrogenation process, some double bonds of the *cis* conformation in the hydrocarbon chain may be converted to the *trans* conformation. Margarine, some types of peanut butter, and shortening are examples of artificially hydrogenated *trans* fats. Recent studies have shown that an increase in *trans* fats in the human diet may lead to an increase in levels of low-density lipoproteins (LDL), or "bad" cholesterol, which in turn may lead to plaque deposition in the arteries, resulting in heart disease. Many fast food restaurants have recently banned the use of *trans* fats, and food labels are required to display the trans fat content.

Like carbohydrates, fats (i.e. triglycerides) have received a lot of bad publicity. It is true that eating an excess of fried foods and other "fatty" foods leads to weight gain. However, fats do have important functions. Many vitamins are fat soluble, and fats serve as a long-term storage form of fatty acids: a source of energy. They also provide insulation for the body. Therefore, "healthy" fats in moderate amounts should be consumed on a regular basis.

Phospholipids

Phospholipids are modified triglycerides. Like triglycerides, they are composed of non-polar fatty acid chains attached to a glycerol backbone. However, instead of three fatty acids, the phospholipid has only two fatty acids, with the third carbon of the glycerol bound to a **phosphate group** (Figure 2.5.6). This phosphate group is modified with a functional group that may be charged or polar in nature. The functional group determines the chemical and physical properties of the phospholipid and can range from something as simple as a hydroxyl group (e.g. phosphatidic acid) to more complex compounds like carbon rings (e.g. phosphatidylinositol).

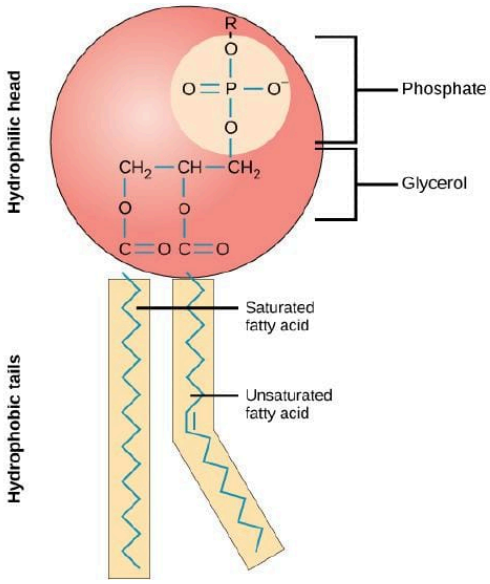


Figure 2.5.6: The phospholipid. A phospholipid is a molecule with two fatty acids (i.e., the hydrophobic tails) and a modified phosphate group attached to a glycerol backbone. The phosphate group may be modified by the addition of charged or polar functional groups (indicated with an 'R'). The glycerol and phosphate group are called the hydrophilic head group. (Phospholipid structure by Openstax; CC BY 4.0)

The phospholipid is comprised of both hydrophobic and hydrophilic regions. The modified phosphate group bound to the glycerol backbone is often called the **polar head group** of the phospholipid and the two fatty acids are called the **hydrophobic tails**. The hydrophobic and hydrophilic nature of the phospholipid makes it an **amphipathic** molecule. It also means that when added to an aqueous environment, phospholipids will spontaneously arrange themselves into a **phospholipid bilayer,** with the hydrophobic fatty acids tails facing away from water (towards the inside of the bilayer) and the hydrophilic head groups facing out towards the aqueous environment (Figure 2.5.7). In a cell, the phospholipid bilayer makes up the majority of the plasma membrane and they are responsible for its dynamic nature.

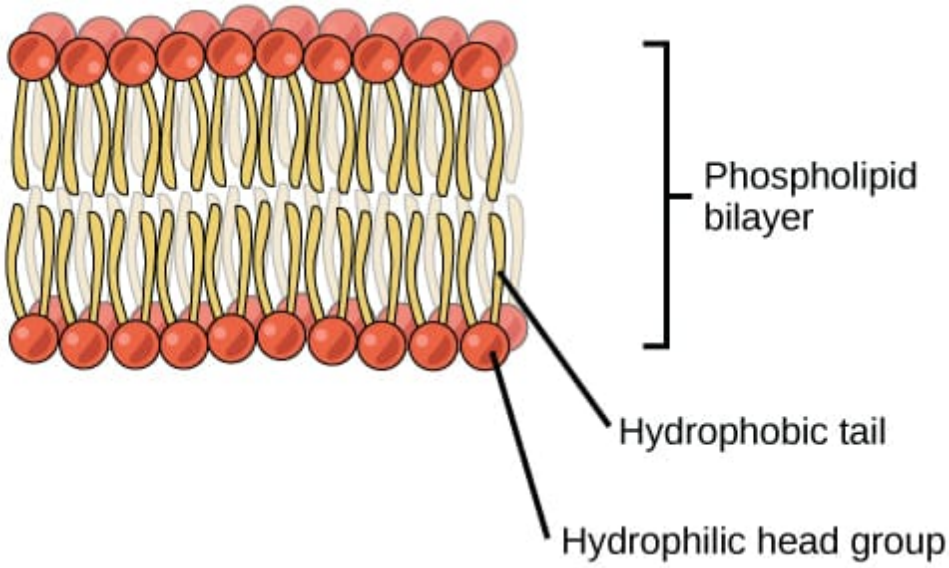


Figure 2.5.7: The phospholipid bilayer. The phospholipids of the phospholipid bilayer orient themselves so that the hydrophilic head groups face the aqueous solution and the hydrophobic tails face inward. (Phospholipid bilayer by Openstax; CC BY 4.0)

Steroids

A **steroid** is a lipid with a fused ring structure. Although they do not resemble the other lipids, they are grouped with them because they are also hydrophobic and insoluble in water. All steroids have four linked carbon rings and are modified with specific function groups that determine their function (Figure 2.5.8).

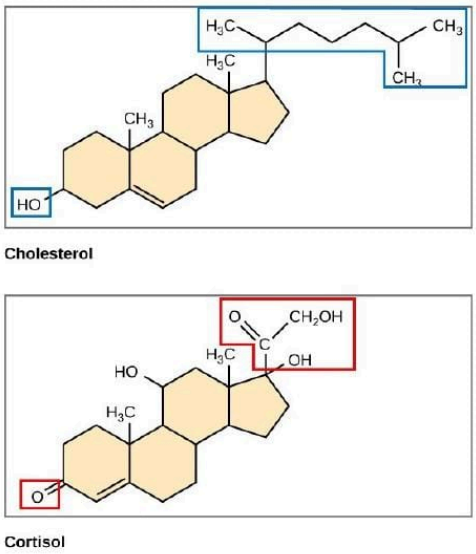


Figure 2.5.8: The steroid. Steroids such as cholesterol (top) and cortisol (bottom) are composed of four fused hydrocarbon rings modified through the addition of specific functional groups (indicated with the boxes). (Steroid structure by Openstax; CC BY 4.0; modified by Patricia Zuk)

The most common steroid is **cholesterol**. As with all steroids, cholesterol is made of four fused carbon rings, giving the molecule rigidity. A hydrocarbon chain fused to one ring makes the molecule mostly non-polar and a hydroxyl group at the other end makes the molecule slightly polar. As such, cholesterol is an amphipathic molecule. Cholesterol is mainly synthesized by animals in the cells of the liver or it can be ingested through food. Although cholesterol is often spoken of in negative terms by lay people, it is necessary for proper functioning of the body. For example, cholesterol is a component of the phospholipid bilayer and is the precursor to many steroid hormones such as testosterone, estradiol, cortisol, and aldosterone. It is also the precursor to Vitamin D and to bile salts, which help in the emulsification and absorption of fats.

Fatty Acids

Essential fatty acids are fatty acids that are not synthesized by an organism but must be obtained through the diet. There are two main types of essential fatty acids: **omega-3 and omega-6**. Omega-3 fatty acids (e.g. alpha-linoleic acid) are a type of polyunsaturated fat that have their first double bond between the third and fourth carbon from the end of the fatty acid chain (Figure 2.5.9). Examples of omega-3 fatty acids are alpha-linoleic acid (ALA) and docosahexaenoic acid (DHA). Omega-6 fatty acids are also a type of polyunsaturated fat. The omega-6 fatty acid has its first double bond at the sixth carbon from the end of the fatty acid chain.

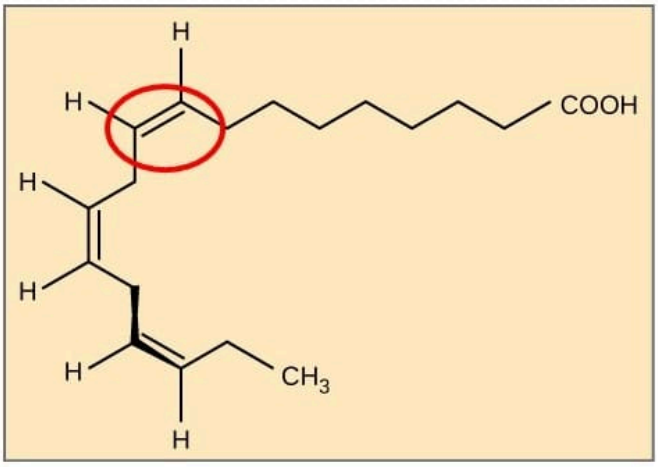


Figure 2.5.9: The omega-3 fatty acid. Omega-3 fatty acids have a double bond located at the third carbon from the end of the hydrocarbon chain (circled). (Omega-3 structure by Openstax; CC BY 4.0; modified by Patricia Zuk)

Salmon, trout, and tuna are good sources of omega-3 fatty acids. Research shows that omega-3 fatty acids are important in brain function and normal growth and development. They may also prevent heart disease, decrease inflammation, and reduce the risk of cancer.

Key Concepts

Lipids are a class of macromolecules that are nonpolar and hydrophobic in nature. Major types include triglycerides, phospholipids, fatty acids, and steroids.

Some major concepts to remember are:

- Fats and oils are a stored form of energy and are also known as triacylglycerols or triglycerides.
- Triglycerides are made up of three fatty acids bound to a glycerol.
- Fatty acids may be unsaturated or saturated, depending on the presence or absence of double bonds in the hydrocarbon chain. If only single bonds are present, they are known as saturated fatty acids. Unsaturated fatty acids may have one or more double bonds in the hydrocarbon chain.
- Phospholipids have a glycerol backbone to which two fatty acid chains and a phosphate-containing group are attached. Phospholipids form the bilayers that make up the plasma membrane.
- Steroids are made of four fused carbon rings modified through the addition of functional groups.
- Cholesterol is the most basic type of steroid
- Modifications to cholesterol result in the production of the steroid hormones (e.g. sex hormones, aldosterone, cortisol)
- Omega-3 and omega-6 fatty acids are known as essential fatty acids

Glossary

Amphipathic - a molecule that has both hydrophilic and hydrophobic regions

Cis - a term used in chemistry and biochemistry to describe the arrangement of atoms or functional groups in a molecule, such as an unsaturated fatty acid

Cis isomer - an isomer that has similar or identical functional groups positioned on the same side of a double bond

Cholesterol - a lipid made of fused carbon rings bound to unique functional groups; a precursor for steroid hormones and bile

Eicosanoids - a group of signaling molecules derived from fatty acids; include the prostaglandins and leukotrienes; involved in inflammation and immunity

Essential fatty acid - a fatty acid that must be obtained through the diet

Fat - a triglyceride that is solid at room temperature

Fatty acid - a hydrocarbon chain with a carboxyl (-COOH) group at one end; can be be saturated or unsaturated

Glycerol - a three-carbon molecule that serves as the backbone of triglycerides and phospholipids

Hydrogenation - a chemical process in which hydrogen is added to unsaturated fats or other organic compounds

Hydrophobic - a property of molecules that repel water; characteristic of the fatty acid tails in lipids

Lipid Bilayer - a double-layered structure formed by phospholipids in aqueous environments, making up the fundamental structure of cell membranes

Lipoprotein - a complex of lipids and proteins that transports fats through the bloodstream, including HDL (high-density lipoprotein) and LDL (low-density lipoprotein)

Oil - a triglyceride that is liquid at room temperature

Omega fatty acid - a type of polyunsaturated fatty acid that play essential roles in human health; categorized based on the position of the first double bond from the methyl (CH_3) end of the hydrocarbon chain

Phospholipid - a modified triglyceride made of two fatty acids "tails" and a polar "head group" attached to a glycerol backbone

Pigment - a lipid that absorbs specific wavelengths of light

Polar head group - the hydrophilic (water-attracting) part of an amphipathic molecule, such as a phospholipid

Polymer - a large molecule made up of repeating units called monomers

Saturated fatty acid - a fatty acid in which all carbons in the hydrocarbon chain are linked to each other through single bonds

Steroid - a lipid made of four fused carbon rings modified with specific functional groups

Trans - a term used in chemistry and biochemistry to describe the arrangement of atoms or functional groups in a molecule, such as an unsaturated fatty acid

Trans isomer - an isomer that has similar or identical functional groups positioned on the opposite side of a double bond

Trans fat - a fat containing artificially modified unsaturated fatty acids with a trans configuration; often found in processed foods and linked to health risks

Triglyceride - a lipid made of three fatty acids attached to a glycerol backbone

Unsaturated fatty acid - a fatty acid in which carbon-carbon double bonds can be found

Wax - a type of lipid that is highly water-resistant and protects various structures in plants, animals and microbes; made of long-chain fatty acids bound to long-chain alcohols rather than glycerol

Attribution

Figures taken from Open Stax Biology 3.3 Lipids

- Figure 2.5.1
- Figure 2.5.3
- Figure 2.5.4
- Figure 2.5.5
- Figure 2.5.6
- Figure 2.5.7
- Figure 2.5.8
- Figure 2.5.9

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2.6: Nucleic Acids

Biotech Focus



Figure 2.6.1: Mary Maynard Daly. (CCO)

Marie Maynard Daly's early research provided key insights into how DNA is structured and regulated within the cell nucleus. She particularly identified the four nucleic acid bases of DNA (adenine, guanine, thymine and cytosine) leading the way to understanding the structure of DNA. She continued to make significant contributions to science by identifying histones, the proteins around which DNA is wrapped within cells and by researching the relationship between cholesterol and heart disease among others.

If you would like to know more, read: Marie Maynard Daly: A National Historic Chemical Landmark

Introduction

Nucleic acids are the most important macromolecules for the continuity of life. They carry the genetic blueprint of a cell and carry instructions for the functioning of the cell. The two main types of nucleic acids are **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**.

Learning Objectives

By the end of this section, you will be able to:

- Describe the structure of nucleic acids and define the two types of nucleic acids
- Explain the structure and role of DNA
- Explain the structure and roles of RNA

DNA and RNA are Nucleic Acids

DNA is the genetic material found in all living organisms, ranging from single-celled bacteria to multi-cellular mammals. In eukaryotes, the majority of DNA is found in the nucleus, with smaller amounts found in the organelles, such as the chloroplasts, and mitochondria. In prokaryotes, the DNA is not enclosed in a membranous envelope but is found in a section of the cytoplasm called the **nucleoid**. More about the structure and function of DNA can be found in Chapter 3.1 DNA Structure.

The other type of nucleic acid, **RNA**, plays critical roles in the gene expression and its regulation. In eukaryotic cells, because the DNA molecules never leave the nucleus, an intermediary nucleic acid is needed to communicate with the rest of the cell. This intermediary is RNA. There are several kinds of RNA, with more being discovered. The most studied RNA types are mRNA, tRNA, and rRNA - all of which play key roles in protein synthesis. For more about the the types of RNA and their functions, go to Chapter 3.3 Transcription of RNA.

Nucleotides are the Building Blocks of DNA and RNA

DNA and RNA are polymers known as **polynucleotides.** The building blocks of DNA and RNA are monomers called **nucleotides**.

Each nucleotide is made up of three components (Figure 2.6.2):

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

To build a nucleotide, the pentose sugar is linked to a specific nitrogenous base at its first carbon and to one or more phosphate groups at its fifth carbon. The base and sugar linked together are called a **nucleoside**. Adding the phosphate group(s) creates a nucleotide.

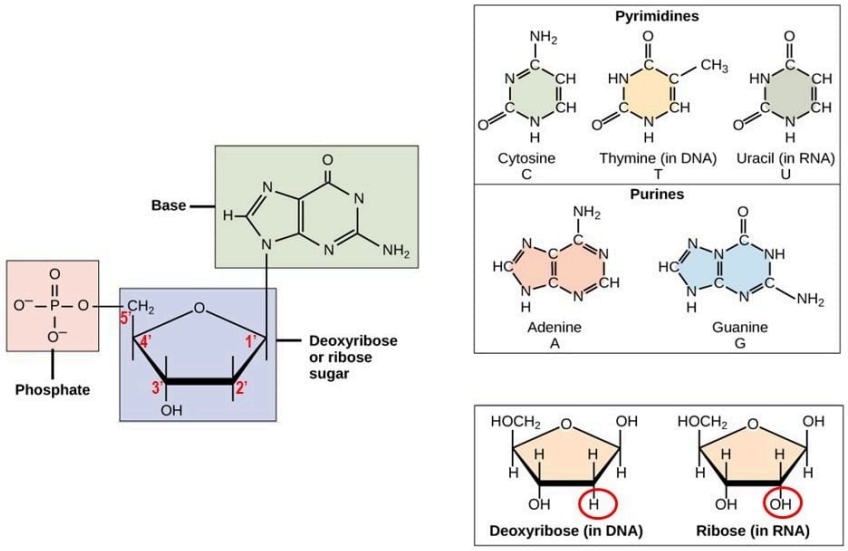


Figure 2.6.2: Nucleotide structure. Left image: a nucleotide is made up a nitrogenous base (green box), a pentose sugar (blue box), and one or more phosphate groups (pink box). Carbon residues in the pentose are numbered clockwise from the oxygen as 1 "prime" (1′) through 5 "prime" (5′) . The base attaches to the 1′ carbon of the pentose sugar, and the phosphate is attached to the 5′ carbon. Two types of pentose sugars are found in nucleotides, deoxyribose (DNA) and ribose (RNA). Top right image: the nitrogenous bases are classified as either purines and pyrimidines. The purine bases, adenine and guanine and the pyrimidine base, cytosine, are found in DNA and RNA. The pyrimidine base thymine is found only in DNA while the pyrimidine, uracil, is only found in RNA. Bottom right image: deoxyribose is similar in structure to ribose, but it has an H instead of an OH at the 2′ position. (Nucleotide Structure by Patricia Zuk, CC BY 4.0; modified from Molecular structure of a nucleotide by Openstax, CC BY-NC 4.0)

The nitrogenous bases are organic molecules containing carbon and nitrogen. Because of the presence of an amino group with the potential of binding an extra hydrogen, the nitrogenous base is capable of decreasing the hydrogen ion concentration in its environment, thus acting as a base. The nitrogenous bases of nucleic acids are **adenine (A), guanine (G) cytosine (C), thymine (T), and uracil**. These nitrogenous bases are classified as either **purines** or **pyrimidines**. The primary structure of a purine (i.e. adenine and guanine) is two carbon-nitrogen rings, while the pyrimidine (cytosine, thymine, uracil) has a single carbon-nitrogen ring (Figure 2.6.2). The nitrogenous base is specific to the nucleic acid. DNA contains A, T, G, and C, whereas RNA contains A, U, G, and C.

The pentose sugar in DNA is called **deoxyribose**, and in RNA, the sugar is **ribose**. The carbon atoms of the sugar are numbered clockwise from the oxygen within the ring. They are numbered from 1′ to 5′ (e.g. 1′ is read as “one prime”). The use of the term "prime" to number the carbons in the pentose sugar is meant to distinguish between these carbons and those found in the rings

of the nitrogenous base. While the overall structure of deoxyribose and ribose are the same, there is an important difference - *deoxyribose lacks an oxygen at the 2' carbon*.

DNA and RNA nucleotides are named based on the nitrogenous base, the type of sugar and the number of phosphate groups. For example, *adenosine triphosphate (ATP)* is an RNA nucleotide with an adenine base and three phosphate groups attached to the ribose sugar. The DNA nucleotide counterpart would be *deoxynucleotide triphosphate (dATP)*. If these nucleotides have only one phosphate group bound to the pentose sugar, they would be named *adenosine monophosphate (AMP)* and deoxynucleotide monophosphate (dAMP), respectively. Table 2.6.1 below summarizes the nomenclature for DNA and RNA nucleotides.

Table 2.6.1: Nucleotide Nomenclature

Nucleoside Name	Number of phosphates	Abbreviation	Name
adenosine/deoxyadenosine	1 2 3	AMP/dAMP ADP/dADP ATP/dATP	adenosine monophosphate/deoxyadenosine monophosphate adenosine diphosphate/deoxyadenosine diphosphate adenosine triphosphate/deoxyadenosine diphosphate
guanosine/deoxyguanosine	1 2 3	GMP/dGMP GDP/dGDP GTP/dGTP	guanosine monophosphate/deoxyguanosine monophosphate guanosine diphosphate/deoxyguanosine diphosphate guanosine triphosphate/deoxyguanosine triphosphate
cytidine/deoxycytidine	1 2 3	CMP/dCMP CDP/dCDP CTP/dCTP	cytidine monophosphate/deoxycytidine monophosphate cytidine diphosphate/deoxycytidine diphosphate cytidine triphosphate/deoxycytidine triphosphate
deoxythymidine (found in DNA only)	1 2 3	dTMP dTDP dTTP	deoxythymidine monophosphate deoxythymidine diphosphate deoxythymidine triphosphate
uridine (found in RNA only)	1 2 3	UMP UDP UTP	uridine monophosphate uridine diphosphate uridine triphosphate

Phosphodiester Bonds Join Nucleotides

Nucleotides join to one another through **phosphodiester bonds** (Figure 2.6.3). In phosphodiester bond formation, the hydroxyl on the 3' carbon of one nucleotide forms a bond with the phosphate group closest to the pentose sugar in another nucleotide. When the diphosphate or triphosphate forms of the nucleotides are used in this reaction, water is not released and it is not considered a

dehydration synthesis reaction. However, if a monophosphate nucleotide is used, water is removed through dehydration synthesis. When forming DNA and RNA, the phosphodiester bond forms in a specific direction. Specifically, the bond forms between the phosphate group(s) of an incoming nucleotide and the 3' hydroxyl of the existing polynucleotide chain. Because of this, one end of a nucleic acid has an exposed phosphate group and is named the "5' end" while the other end has a 3' hydroxyl and is called the "3' end". The phosphodiester bond is said to form "*in the 5' to 3' direction*".

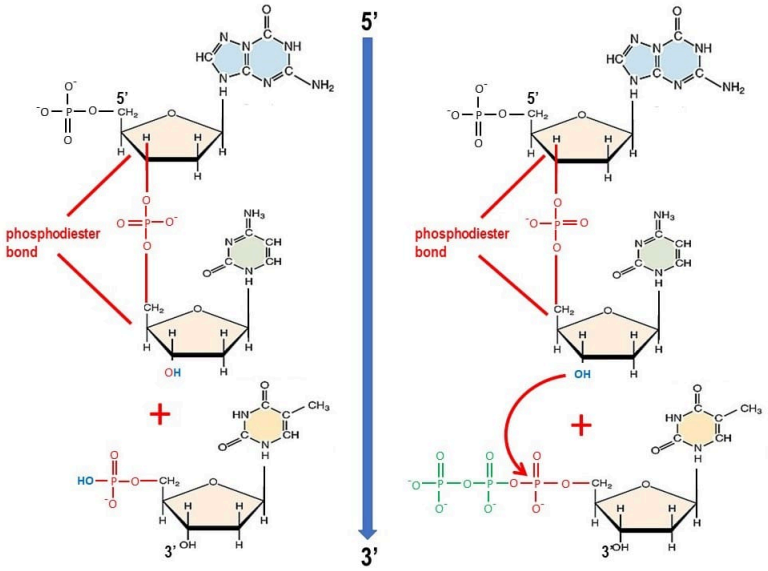


Figure 2.6.3: The phosphodiester bond forms in a specific direction. Left image: the addition of the monophosphate form of the nucleotide (bottom) to a nucleotide chain (top) via a phosphodiester bond. Bond formation involves the removal of a water molecule (atoms shown in blue). Right image: the addition of the triphosphate form (bottom) to the nucleotide chain (top). The phosphodiester bond forms as a result of a "nucleophilic attack" by the 3' OH group (blue) on the first phosphate group of the incoming nucleotide. This attack removes the remaining two phosphate groups (green). The atoms involved in the phosphodiester bond are shown in red. The direction of phosphodiester bond formation from 5' to 3' is indicated with an arrow. (Phosphodiester bond by Patricia Zuk, CC BY 4.0)

DNA is Double-Helix Structure

DNA exists as a **double-helix** (Figure 2.6.4), consisting of two long strands of DNA polynucleotides that twist like a "spiral staircase". In the DNA helix, the sugars and phosphates are found on the outside of the helix, not unlike the rails of the spiral staircase. The nitrogenous bases project to the interior of the helix, like the steps. The two polynucleotide strands of the helix run in opposite directions, or **anti-parallel** to one another, meaning that the 5′ end of one strand will be next to the 3′ end of the other strand. The bases from each nucleotide chain pair up with one another and are bound to each other by hydrogen bonds. This base pairing is restricted in the DNA helix and is known as **complementary base pairing**. In this pairing scheme, *purines pair with pyrimidines*. Specifically, adenine (A, the purine) pairs with thymine (T, the pyrimidine) through the formation of two hydrogen bonds and guanine (G, the purine) pairs with cytosine (C, the pyrimidine) through the formation of three hydrogen bonds. What results is a DNA helix in which the two DNA strands are complementary to each other. This complementary base pairing has implications for cellular processes like DNA replication. During DNA replication, each of the DNA strands are "copied" to produce two new DNA helices. However, the term copy is misleading, as a identical copy is not made rather a complementary copy is. For example, if the sequence of one strand is AATTGGCC, the complementary strand would have the sequence TTAACCGG. For more information about DNA replication, read Chapter 3.2 Replication of DNA.

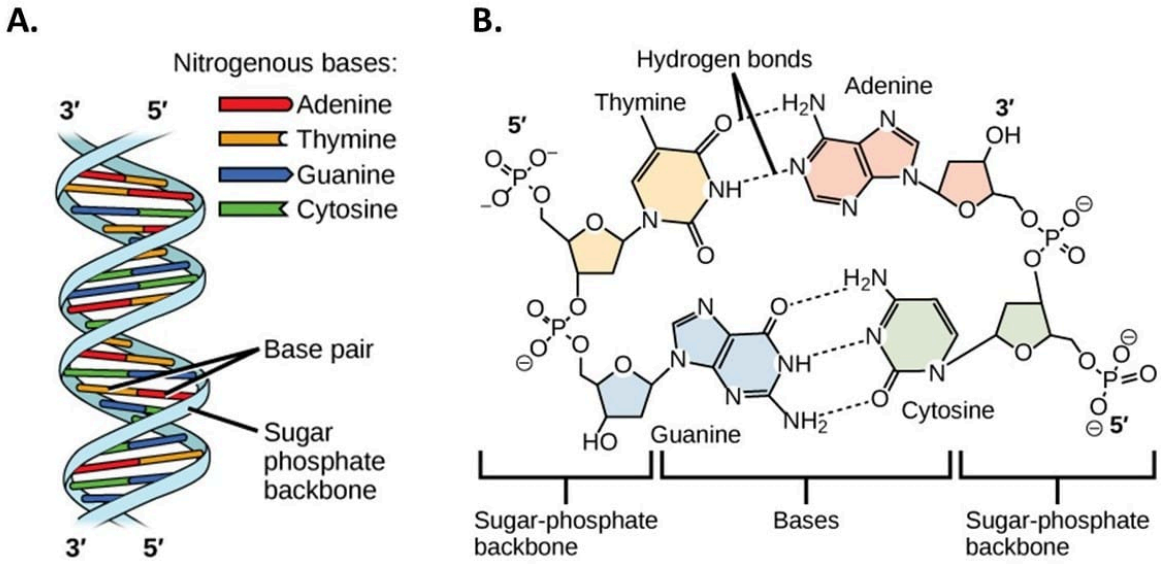


Figure 2.6.4: The DNA Helix. (A) Two phosphate-sugar backbones (curved blue lines) run on the outside of the DNA helix with the bases (adenine, thymine, guanine, cytosine) projecting to the inside. The two DNA strands run "anti-parallel" to one another. (B) Each base from one strand interacts with a "complementary" base from the opposing strand via hydrogen bonding (dotted black lines); adenine pairs with thymine and cytosine pairs with guanine. (DNA Helix by Jerome Walker and Denis Myts, Concepts of Biology; CC BY-SA 4.0)

Comparison between DNA and RNA

Like DNA, RNA is a polynucleotide chain made up of nucleotides joined by phosphodiester bonds that form in the 5' to 3' direction. However, RNA does differ from DNA in several aspects. Table 2.6.2 below outlines these differences.

Table 2.6.2: Differences between DNA and RNA

	DNA	RNA
Function	Stores and carries genetic information	Transfers genetic information; involved in protein synthesis
Types	One type	Several types
Location	Mostly in the nucleus; some in organelles	Nucleus and cytoplasm
Structure	Double-stranded; forms a helix	Usually single-stranded
Pentose Sugar	Deoxyribose (lacks an oxygen at the 2' carbon)	Ribose (has an oxygen at the 2' carbon
Pyrimidine bases	Cytosine, thymine	Cytosine, uracil
Purine bases	Adenine, guanine	Adenine, guanine

Key Concepts

DNA and RNA comprise the two types of nucleic acids found in cells. DNA provides the code for a cell's activities, while RNA is involved in translating that code into proteins that carry out these activities.

Some important concepts to remember:

- Both DNA and RNA are made from nucleotides
- Nucleotides contain a five-carbon sugar backbone, one to three phosphate groups, and a nitrogenous base.
- Nucleotides are joined to one another by a phosphodiester bond.
- Phosphodiester bonds form in the 5' to 3' direction.

- The bases of nucleic acids are adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U).
- The bases of DNA are A, G, C, and T; the bases of RNA are A, G, C, U.
- Bases are classified as purines (A, G) or pyrimidines (C, T, U)
- The nitrogen bases A pairs with its complementary base T (or U in RNA); the base C pairs with its complement G
- DNA is found as a double-stranded helix, made of two "anti-parallel" DNA strands
- Most RNA is single-stranded

Glossary

Adenine (A) - a nitrogenous base found in DNA and RNA; pairs with thymine (T) in DNA and uracil (U) in RNA.

Anti-parallel - the orientation of the two strands of DNA in opposite directions, one running 5' to 3' and the other 3' to 5'.

Complementary base pairing - the specific hydrogen bonding between complementary nitrogenous bases in nucleic acids (A-T/U, C-G).

Cytosine (C) - a nitrogenous base found in DNA and RNA; pairs with guanine (G).

Deoxyribonucleic Acid (DNA) - a double-stranded nucleic acid that carries genetic information and directs cellular functions.

Deoxyribose - the five-carbon sugar found in DNA; lacks an oxygen atom at the 2' carbon.

Double helix - the spiral-shaped structure of DNA, consisting of two complementary strands.

Guanine (G) - a nitrogenous base found in DNA and RNA; pairs with cytosine (C).

Hydrogen bond - a weak bond found between the positively-charged hydrogen of one molecule and a negatively-charge atom of another molecule (usually O or N); holds complementary nitrogenous bases together in DNA and RNA.

Nitrogenous base - an organic molecule found bound to the pentose sugar of a nucleic acid.

Nucleic Acid - a macromolecule composed of nucleotides, essential for storing and transmitting genetic information (DNA and RNA).

Nucleotide - the basic building block of nucleic acids, consisting of a nitrogenous base, a sugar, and a phosphate group.

Pentose sugar - a 5-carbon sugar.

Phosphate group - a functional group that links nucleotides together in the sugar-phosphate backbone of nucleic acids.

Polymer - a large molecule made up of repeating units called monomers

Purines - a category of nitrogenous bases with a double-ring structure; adenine (A) and guanine (G).

Pyrimidines - a category of nitrogenous bases with a single-ring structure; cytosine (C), thymine (T), and uracil (U).

Ribonucleic Acid (RNA) - a single-stranded nucleic acid involved in protein synthesis and gene expression.

Ribose - the five-carbon sugar found in RNA; has an oxygen atom at the 2' carbon.

Sugar-Phosphate backbone - the repeating structure of sugar and phosphate groups that forms the framework of DNA and RNA strands.

Thymine (T) - a nitrogenous base found in DNA; pairs with adenine (A); replaced by uracil (U) in RNA.

Uracil (U) - a nitrogenous base found only in RNA; pairs with adenine (A) instead of thymine.

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2.7: Proteins

Biotech Focus



Figure 2.7.1: Rosalyn Yalow in her laboratory. (Rosalyn Yalow; CCO)

In 1977, Rosalyn Sussman Yalow received the Nobel prize in medicine for the development of the **radioimmunoassay (RIA)**. An RIA is a highly sensitive method that uses radioactive-labeled antibodies to measure very low concentration of specific proteins. For example, RIA is used to detect narcotic drugs, viral antigens, hormones, early stage of cancer. RIA can be used for the early diagnosis of diseases such as thyroid disorders, diabetes, and various cancers, enabling timely interventions and treatment plans.

If you would like to read more about Rosalyn Sussman Yalow and her research, you can click on the following link: Rosalyn Yalow and radioimmunoassay.

Introduction

Proteins are one of the most abundant organic molecules in living systems. In the adult human, proteins make up 12 to 18% of the body's mass. Each cell in a living system may contain thousands of proteins, each with a unique function. Their structures, like their functions, vary greatly. They are all, however, polymers of **amino acids**, arranged in a linear sequence that is determined by the genetic code. To learn how the genetic code specifies amino acid sequence, go to Chapter 3.4: Translation of the polypeptide.

Learning Objectives

By the end of this section, you will be able to:

- Describe the functions proteins perform in the cell and in tissues
- Discuss the relationship between amino acids and proteins
- Explain the four levels of protein organization
- Describe the ways in which protein shape and function are linked

Types and Functions of Proteins

There are a wide variety of proteins that serve a diverse set of cellular functions. Some of these are shown in Table 2.7.1.

Table 2.7.1: Protein Types and Functions

Type	Examples	Functions
Catalytic (e.g. enzymes)	Amylase, DNA polymerase, hexokinase	• Help in digestion of food by catabolizing nutrients into monomeric units • Catalyze metabolic reactions • Synthesize molecular compounds • Repair damage • Produce energy
Transport	Hemoglobin, albumin	• Carry substances throughout the organism
Structural	Actin, tubulin, keratin	• Form the structural framework of cells and tissues
Regulatory (e.g. hormones, neurotransmitters, growth factors, receptors)	Insulin, substance P, epidermal growth factor (EGF), EGF receptor	• Coordinate the activity of different organ systems • Detect signals and trigger cellular responses • Form cell signaling pathways
Immunological	Immunoglobulins, interferons	• Protect the organism from foreign pathogens and cancerous cells
Contractile	Actin, myosin	• Mediate muscle contraction
Storage	Legume storage proteins, egg white (albumin)	• Provide nourishment in early development

Enzymes, which are produced by living cells, are **catalysts** in biochemical reactions. A catalyst is a substance that speeds up a chemical reaction without being consumed by that reaction. Because they increase the rate of a reaction, enzymes are considered to be **biologic catalysts**. Each enzyme is specific for the **substrate** (a reactant that binds to an enzyme) that it acts on. Enzymes catabolize a wide variety of chemical reactions, including catabolic (i.e. decomposition), anabolic (i.e. synthesis), substitution, and redox reactions. An example of an enzyme is salivary amylase, which hydrolyzes its substrate amylose, a component of starch.

Hormones are chemical-signaling molecules, usually small proteins or steroids, secreted by endocrine cells, that act to control or regulate specific physiological processes, including growth, development, metabolism, and reproduction. For example, insulin is a protein hormone that helps to regulate the blood glucose level.

Hormones (and other signaling molecules) act by binding to proteins called **receptors**. Receptors are proteins found either on the surface of a cell or inside of a cell. The receptor binds to a specific molecule called a **ligand**. Once bound the receptor triggers a biological response inside of the cell. Examples of ligands include hormones, neurotransmitters, growth factors, and drugs.

Amino Acids are the Building Blocks of Proteins

The building block of a protein is the **amino acid** (Figure 2.7.2). The name "amino acid" is derived from the fact that they contain an amine group and a carboxyl acid group in their basic structure. Each amino acid consists of a central carbon atom, also known as the **alpha (α) carbon**, bonded to the following:

1. a **carboxyl group (COOH)** that will donate protons when the amino acid is in an aqueous solution
2. an **amino group (NH2)** that will receive proteins from the environment
3. a **hydrogen atom**
4. a **"side chain" or "R group"** that distinguishes one amino acid from another. For each amino acid, the R group (or side chain) is different (Figure).

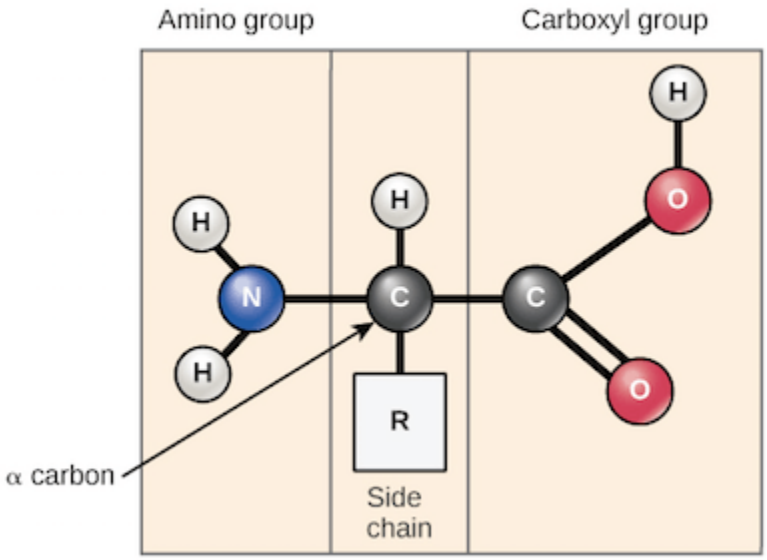


Figure 2.7.2: The amino acid. An amino acid has an alpha carbon to which an amino group, a carboxyl group, a hydrogen, and a side chain are attached. The side chain varies for different amino acids, and is designated with an “R”.

All proteins are made up using the same 20 types of amino acids arranged in a unique sequence according to the genetic code. When in an aqueous environment, the amino acid has a slightly positively charged end (the amine group) and a slightly negatively charged end (the carboxyl group). However, it is the R group that determines the overall physical and chemical characteristic of the amino acid.

Based on the R chain, amino acids are classified into the following groups (Figure 2.7.3):

- **non-polar (hydrophobic)** - have side chains that are non-polar and uncharged; amino acids such as valine and leucine
- **polar (hydrophilic) -** have side chains that are polar and uncharged; amino acids such as serine, threonine, arginine, glutamine
- **electrically charged acidic** - have side chains that are positively charged at physiological pH; amino acids glutamic acid and aspartic acid
- **electrically charged basic** have side chains that are negatively charged at physiological pH; amino acids histidine, lysine, arginine

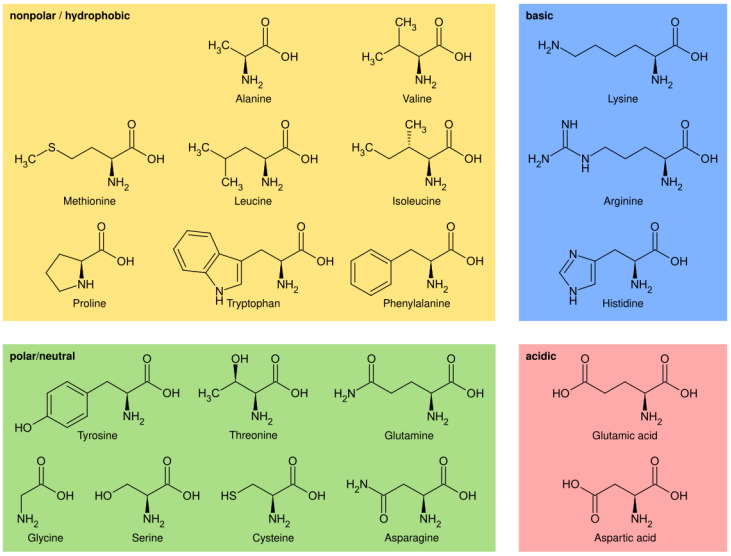


Figure 2.7.3: The 20 amino acids found in proteins each have different R groups (side chain) that determine whether they are non-polar, polar, acidic, or basic. Nonpolar (or hydrophobic) amino acids include glycine, alanine, phenylalanine, and tyrosine. Polar (or hydrophilic) amino acids are uncharged (i.e., neutral) and include serine, threonine, and cysteine. The three positively-charged amino acids are lysine, arginine, and histidine. The negatively-charged amino acids are aspartic acid (i.e., aspartate) and glutamic acid (i.e., glutamate). (Amino acid types; by isk, CC0 1.0)

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

Individual amino acids are linked together through a covalent bond called a **peptide bond.** Formation of the peptide bond results in the production of a water molecule and is a **dehydration synthesis reaction**. To form a peptide bond, the hydroxyl from the carboxyl group of one amino acid and the hydrogen from the amino group of the incoming amino acid combine, forming a molecule of water (Figure 2.7.4).

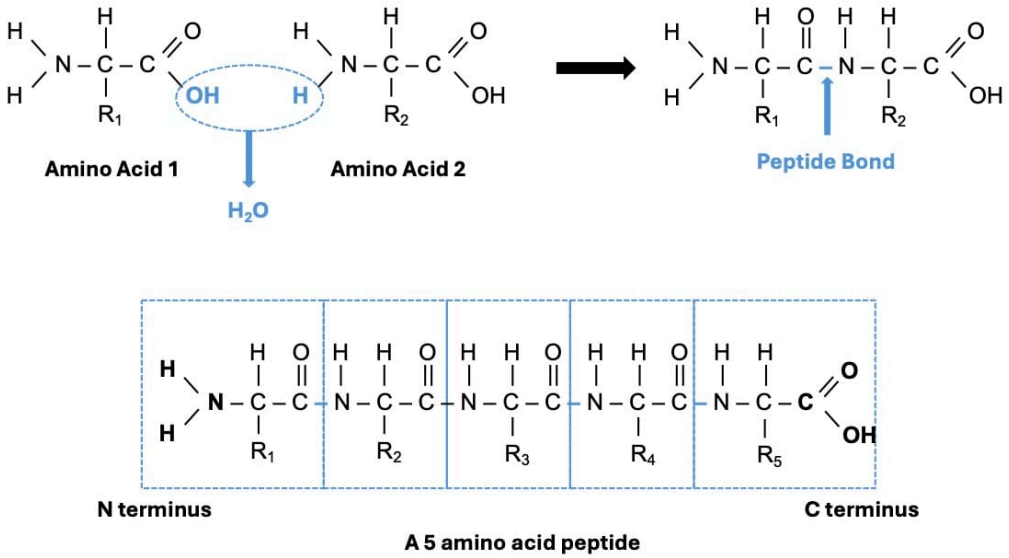


Figure 2.7.4: Peptide bond formation. Top image: Peptide bond formation is a dehydration synthesis reaction. When the peptide bond forms, the carbon from the carboxyl group of the first amino acid becomes attached to the nitrogen from the amino group of the second amino acid. The OH from the carboxyl group and an H from the amino group form a molecule of water (dotted circle). Bottom image: a chain of 5 amino acids bound by peptide bonds form a short peptide. Since peptide bonds are formed between carboxyl and amino group, the peptide starts with an amino group, called N-terminus, and ends with a carboxyl group called C-terminus. (Peptide bond formation by Kareen Martin; CC BY 4.0)

The joining of two amino acids through a peptide bond produces a **dipeptide**. The joining of three amino acids produces a

tripeptide. Joining from 4 to 9 amino acids results in a **peptide**. Joining more than 10 amino acids produces a **polypeptide**. A polypeptide can be relatively small (e.g. about 50 amino acids) or could be comprised of thousands of amino acids. . While the terms polypeptide and protein are sometimes used interchangeably, a polypeptide is technically a polymer of amino acids, whereas the term protein is used for a polypeptide or polypeptides that have been folded into a unique three-dimensional structure. In addition, after protein synthesis (i.e. translation), most proteins have been modified through **post-translational modifications** of the polypeptide chain. They may undergo cleavage, phosphorylation, or may require the addition of other chemical groups. Only after these modifications and folding is complete is the protein functional.

Protein Structure

The shape of a protein is critical to its function. For example, an enzyme binds a substrate at a site known as the active site. If the shape of the active site is altered because of changes in protein structure, the enzyme may be unable to bind to the substrate. Protein structure is described using four levels of conformation:

1. **primary** - the amino acid sequence of the polypeptide strand
2. **secondary** - results in regions of alpha-helical coils and beta-pleated sheets
3. **tertiary** - the 3D shape of the protein
4. **quaternary** - produced through the interaction between more than two polypeptide chains

Primary Structure

The **primary structure** of a protein is the specific order of amino acids found in the polypeptide. These amino acids are linked together via peptide bonds. Each polypeptide has a free amino group at one end. This end is called the **N terminus**, or the **amino terminus.** The other end of the polypeptide has a free carboxyl group, which is known as the **C terminus**, or the **carboxy terminus** (Figure 2.7.5).

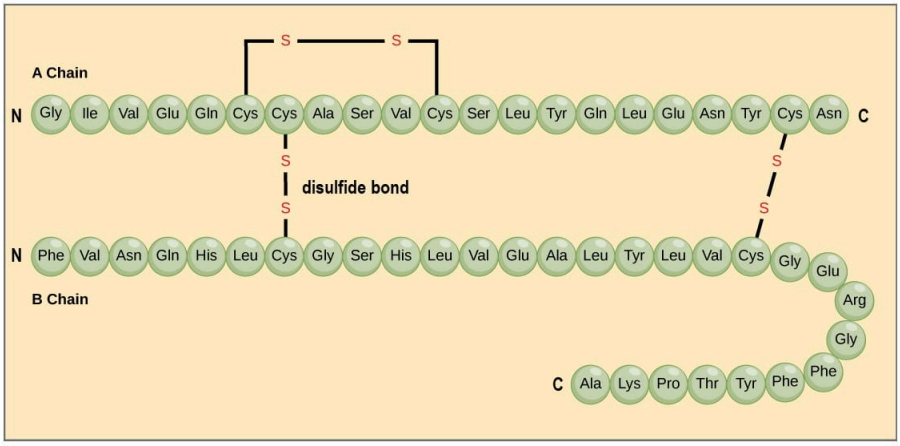


Figure 2.7.5: The hormone insulin. Insulin is a protein hormone made of two peptide chains (A and B) linked by disulfide bonds (black lines). Each amino acid is indicated by its three-letter abbreviation. The A chain is 21 amino acids long with an N terminal amino acid (N) of glycine (Gly) and a C terminal amino acid (C) of asparagine (Asn). The B chain is 30 amino acids long and has the amino acid phenylalanine (Phe) as the N terminus amino acid and the amino acid alanine (Ala) as the C-terminus amino acid. (Insulin by Patricia Zuk, CC BY 4.0; modified from Primary structure of proteins by Openstax, CC BY-NC 4.0)

The unique sequence found in the primary structure is determined by the gene encoding the protein. A change in the gene's DNA sequence may lead to a change in the amino acid sequence of the protein. A change in just one amino acid in the primary structure can ultimately affect the protein's overall structure and function. Sickle cell anemia (see Figure 2.7.9 below) is a disease caused by a single amino acid change in the primary structure of a hemoglobin polypeptide.

Secondary Structure

Local folding of the polypeptide in certain regions gives rise to the **secondary structure** of the protein. The most common secondary structures in proteins are the **α-helix** and **β-pleated** sheet (Figure 2.7.6). An α-helix coils like a spring and is produced when a hydrogen bond forms between the oxygen atom in the carbonyl group (C=O) of one amino acid and a hydrogen atom (H) in the amide (N=H) group of another amino acid that is four amino acids farther along the chain. Every helical turn in an α-helix has 3.6 amino acids. The R groups (the variant groups) of the polypeptide protrude out from the α-helix chain. In the β-pleated sheet, sections of the polypeptide lie top of one another together and form "pleats". These pleats are formed by hydrogen bonding between the carbonyl group in one section of the polypeptide and the amide groups of another section lying adjacent to it. The R groups extend above and below the folds of the pleat.

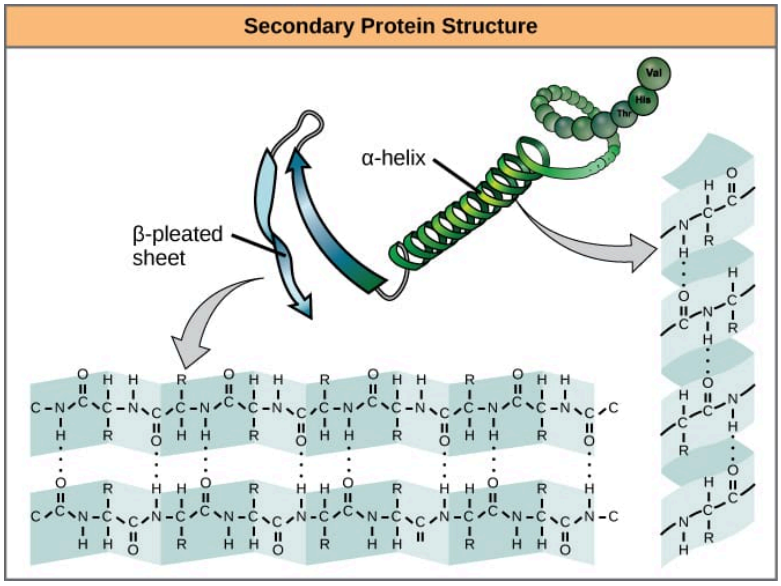


Figure 2.7.6: Secondary protein structure. The α-helix and ß-pleated sheet make up the secondary structure of a protein. Both form because of hydrogen bonding between carbonyl (C=O) and amide (N=H) groups in the polypeptide backbone. Certain amino acids will form an α-helix, while others will form a ß-pleated sheet. In an α-helix, hydrogen bonding occurs between the carbonyl group of one amino acid and the amide group of the amino acid that occurs four residues later. In a beta-pleated sheet, hydrogen bonding occurs between these two functional groups that are found on different sections of the polypeptide.

Tertiary Structure

Proteins have different shapes; some proteins are globular whereas others are fibrous in nature. The folding of the protein into a specific 3-dimensional shape produces its **tertiary structure**. Tertiary structure is primarily due to *interactions between the R groups of the amino acids* that make up the protein.

The R group interactions that contribute to tertiary structure include (Figure 2.7.7):

1. **hydrogen bonding**
2. **ionic bonding** - form between basic and acidic R groups.
3. **hydrophobic interactions** - occur between non-polar, hydrophobic R groups; hydrophobic interactions result in the positioning of hydrophobic amino acids in the interior of the protein, leaving hydrophilic amino acids on the outside to interact with surrounding water molecules.
4. **Disulfide bonds** - occur between the sulfur atoms of cysteine amino acids in the presence of oxygen; stabilizes the 3D structure of the protein

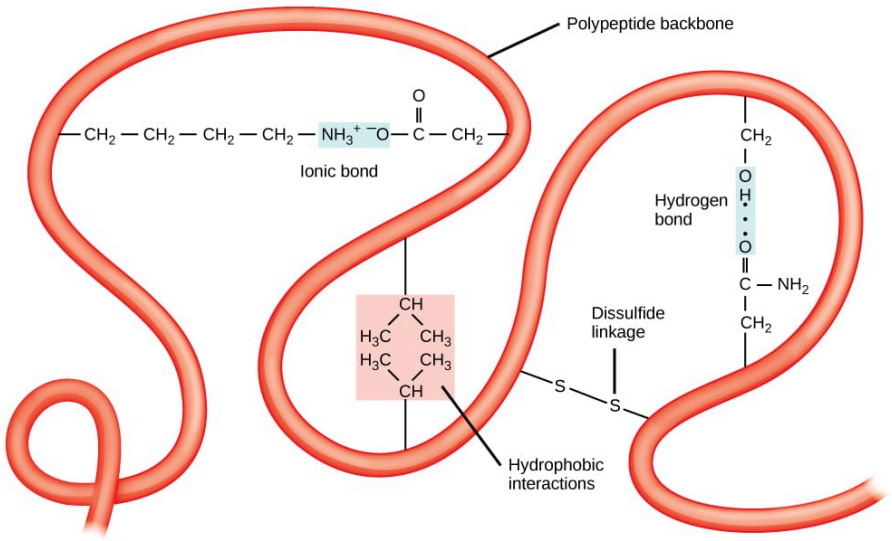


Figure 2.7.7: Protein tertiary structure. The tertiary structure of a protein is determined by a variety of chemical interactions that occur between the R groups of specific amino acids. These interactions include hydrophobic interactions, ionic bonding, hydrogen bonding and disulfide linkages.

All of these interactions combined together determine the final tertiary structure (i.e. the three-dimensional shape) of the protein. Protein shape is critical to its function. When a protein loses its tertiary structure, it may no longer be functional. Changes in temperature, pH, and exposure to chemicals may lead to permanent changes in the shape of the protein, leading to loss of function. The loss of "natural" shape is known as **denaturation.**

Quaternary Structure

Some proteins are formed from several polypeptides, also known as **subunits**, and the interaction of these subunits is the **quaternary structure** of the protein. Weak interactions between the subunits, such as hydrogen bonds, help to stabilize the overall structure.

The four levels of protein structure (primary, secondary, tertiary, and quaternary) are illustrated below in Figure 2.7.8.

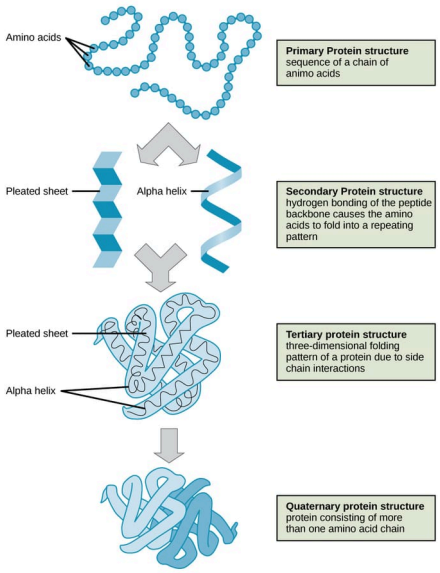


Figure 2.7.8: The four levels of protein structure. The primary structure is the amino acid sequence. Secondary structure is a regular folding pattern due to hydrogen bonding. Two types of secondary structure are shown: a ß pleated sheet, which is flat with regular ripples, and an α-helix, which coils like a spring. Tertiary structure is the three-dimensional folding pattern of the protein due to interactions between amino acid side chains. Quaternary structure is the interaction of two or more polypeptide chains.

Concept in Action

Video: Protein Structure and Function

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

Protein Structure and Disease: Sickle Cell Anemia

A functional protein requires that each of its structural levels is correct. Changes to one level of structure can impact another. In sickle cell anemia, the **hemoglobin ß chain** has a single amino acid substitution, causing a change in protein structure and function and leading the disease known as **sickle cell anemia**. **Hemoglobin**, a transport protein that carries oxygen in the blood, is comprised of four polypeptide chains: two α chains and two ß chains. In sickle cell, a genetic mutation changes the hydrophobic amino acid, valine, in the primary structure of the ß chain to the charged and acidic amino acid, glutamic acid (Figure 2.7.9). This change in the primary structure results in changes to remaining structural levels. The tertiary structure of the ß chain subunits are altered and this affects how these subunit assemble with their α hemoglobin partners. The abnormal quaternary structure results in hemoglobin with a lower oxygen-carrying capacity.

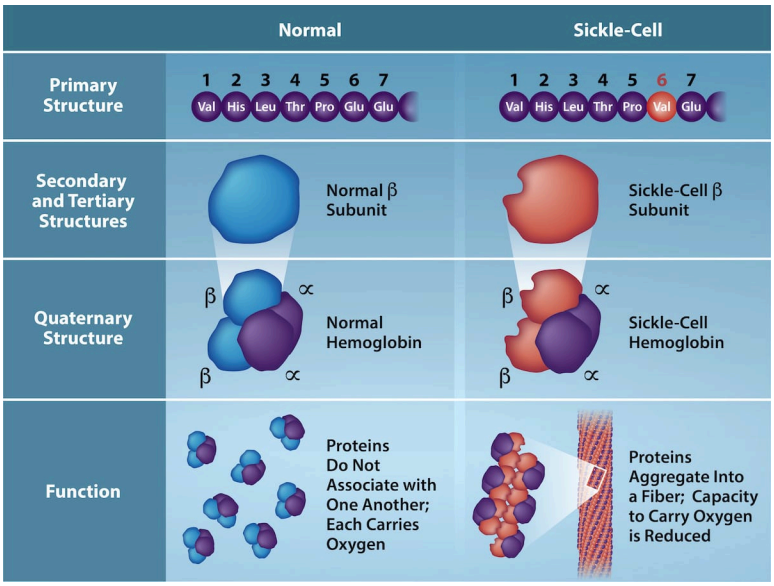


Figure 2.7.9: Hemoglobin structure and sickle-cell anemia. The ß chain of hemoglobin is 147 residues in length, yet a single amino acid substitution leads to sickle cell anemia. In the normal ß hemoglobin polypeptide, the amino acid at position six is glutamate. In sickle cell, this glutamate is replaced by a valine. This produces abnormal secondary and tertiary structures. When the abnormal ß hemoglobin combines with its α hemoglobin partners to produce the resulting quaternary structure is altered. The abnormal hemoglobin proteins aggregate into fibers that causes the red blood cells to be rigid and have a crescent shape. These red blood cells tend to clog capillaries and decrease the flow of blood through tissues. (Hemoglobin and Sickle Cell by Kareen Martin, CC BY 4.0; adapted from Sickle Cell Anemia by BruceBlaus, CC BY-SA 4.0)

Furthermore, once the oxygen is offloaded to the tissues, the hemoglobin molecules aggregate with one another to form long fibers. This results in the distortion of red blood cells, which become stiffer and assume a crescent or “sickle” shape, clogging arteries (Figure 2.7.10). This can lead to myriad serious health problems such as breathlessness, dizziness, headaches, and abdominal pain for those affected by this disease.

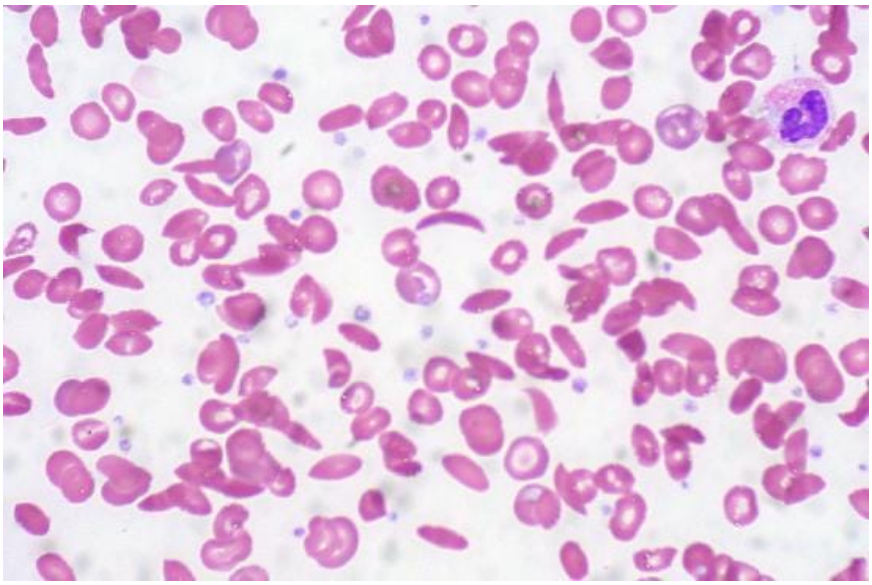


Figure 2.7.10: In this blood smear, visualized at 535x magnification using bright field microscopy, sickle cells are crescent shaped, while normal cells are disc-shaped. (Sickle Cell Anemia - Peripheral blood smear by Ed Uthman, CC BY 2.0)

Denaturation and Protein Folding

Each protein has its own unique shape that is produced and maintained by chemical interactions. If the protein is subject to changes in temperature, pH, or exposure to chemicals, these interactions may be disrupted, causing a change in protein structure and shape. Disruptions in secondary to quaternary structure may result, with the polypeptide maintaining only its primary structure. This loss of "natural structure" is called **denaturation**. Denaturation is often reversible because the primary structure of the polypeptide is conserved. If the denaturing agent is removed, the protein can resume its additional structural levels and function. However, sometimes denaturation is irreversible, leading to a permanent loss of function. One example of irreversible protein denaturation is when an egg is fried. The albumin protein in the liquid egg white is denatured when placed in a hot pan. Not all proteins are denatured at high temperatures; for instance, bacteria that survive in hot springs have proteins that function at temperatures close to boiling. The stomach is also very acidic, has a low pH, and denatures proteins as part of the digestion process; however, the digestive enzymes of the stomach retain their activity under these conditions.

Protein folding is critical to its function. It was originally thought that the proteins themselves were responsible for the folding process. Only recently was it found that often they receive assistance in the folding process from protein helpers known as **chaperones** (or chaperonins) that associate with the target protein during the folding process. They act by preventing aggregation of polypeptides that make up the complete protein structure, and they disassociate from the protein once the target protein is folded.

Key Concepts

Proteins are a class of macromolecules that perform a diverse range of functions for the cell. They help in metabolism by providing structural support and by acting as enzymes, carriers, or hormones.

Some important concepts to remember:

- The building blocks of proteins (monomers) are amino acids.
- Each amino acid has a central carbon that is linked to an amino group, a carboxyl group, a hydrogen atom, and an R group or side chain.
- There are 20 commonly occurring amino acids, each of which differs in the R group.
- Each amino acid is linked to its neighbors by a peptide bond.
- A long chain of amino acids is known as a polypeptide.
- Proteins are organized at four levels: primary, secondary, tertiary, and (optional) quaternary.

1. The primary structure is the unique sequence of amino acids.
2. The local folding of the polypeptide to form structures such as the α helix and β-pleated sheet constitutes the secondary structure.
3. The overall three-dimensional structure is the tertiary structure.
4. When two or more polypeptides combine to form the complete protein structure, the configuration is known as the quaternary structure of a protein.

- Protein shape and function are intricately linked; any change in shape caused by changes in temperature or pH may lead to protein denaturation and a loss in function.

Glossary

Alpha helix (α helix) - a common secondary structure of proteins; characterized by a coiled, helical shape; stabilized by hydrogen bonds

Amino acid - the building blocks of proteins; consist of a central carbon, an amino group, a carboxyl group, and a side chain (R group)

Beta-pleated sheet (ß-pleated sheet) - a secondary structure in proteins where sections of the polypeptide lie parallel or antiparallel to one another; held together by hydrogen bonds

Catalyst - a compound that speeds up a chemical reaction without being consumed by the reaction

Chaperone proteins - proteins that assist in the proper folding of other proteins, preventing aggregation or mis-folding

Denaturation - the process by which a protein loses its native shape due to external stress, such as heat, pH changes, or chemicals; often results in loss of function

Disulfide Bond - a strong covalent bond formed between sulfur atoms in cysteine residues, stabilizing protein structure

Enzyme - a protein that acts as a biological catalyst to speed up chemical reactions without being consumed.

Essential amino acids - amino acids that must be obtained from the diet because the body cannot synthesize them

Hydrophobic interactions - interactions between R groups of amino acids that place non-polar amino acids in the interior of a protein; one of the interactions that result in a protein's tertiary structure

Peptide bond - a covalent bond linking amino acids together in a polypeptide chain; formed through dehydration synthesis

Polymer - a large molecule made up of repeating units called monomers

Polypeptide - a long chain of amino acids linked by peptide bonds; can be processed to form a protein

Primary structure - the linear sequence of amino acids in a polypeptide chain

Protein - a large, complex molecule made up of several amino acids joined together through peptide bonds

Protein folding - the process by which a polypeptide folds into its functional three-dimensional structure; produces the tertiary structure of a protein

Quaternary structure - the level of protein structure formed by the interaction of multiple polypeptide subunits

Secondary structure - the local folding of a polypeptide into structures such as alpha helices and beta sheets; stabilized by hydrogen bonds

Tertiary structure - the overall three-dimensional shape of a polypeptide, determined by interactions between amino acid side chains

Transport protein - a protein that helps move substances across membranes or within the bloodstream, such as hemoglobin

Attribution

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- Figure 2.7.2
- Figure 2.7.6
- Figure 2.7.7
- Figure 2.7.8

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3: CELLS AND VIRUSES

3.1: Themes and Concepts of Biology

3.2: Prokaryotic Cells

3.3: Eukaryotic Cells

3.4: Viruses

3.4.1: Viral Evolution, Morphology, and Classification

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3.4.4: Key Terms

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3.1: Themes and Concepts of Biology

Biology is the science that studies life. What exactly is life? This may sound like a silly question with an obvious answer, but it is not easy to define life. For example, a branch of biology called virology studies viruses, which exhibit some of the characteristics of living entities but lack others. It turns out that although viruses can attack living organisms, cause diseases, and even reproduce, they do not meet the criteria that biologists use to define life.

From its earliest beginnings, biology has wrestled with four questions: What are the shared properties that make something “alive”? How do those various living things function? When faced with the remarkable diversity of life, how do we organize the different kinds of organisms so that we can better understand them? And, finally—what biologists ultimately seek to understand—how did this diversity arise and how is it continuing? As new organisms are discovered every day, biologists continue to seek answers to these and other questions.

Properties of Life

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

Order

Organisms are highly organized structures that consist of one or more cells. Even very simple, single-celled organisms are remarkably complex. Inside each cell, atoms make up molecules. These in turn make up cell components or organelles. Multicellular organisms, which may consist of millions of individual cells, have an advantage over single-celled organisms in that their cells can be specialized to perform specific functions, and even sacrificed in certain situations for the good of the organism as a whole. How these specialized cells come together to form organs such as the heart, lung, or skin in organisms like the toad shown in Figure 3.1.1 will be discussed later.



Figure 3.1.1**:** A toad represents a highly organized structure consisting of cells, tissues, organs, and organ systems. (credit: "Ivengo(RUS)"/Wikimedia Commons)

Sensitivity or Response to Stimuli

Organisms respond to diverse stimuli. For example, plants can bend toward a source of light or respond to touch (Figure 3.1.2). Even tiny bacteria can move toward or away from chemicals (a process called chemotaxis) or light (phototaxis). Movement toward

a stimulus is considered a positive response, while movement away from a stimulus is considered a negative response.



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

CONCEPT IN ACTION

Watch this video to see how the sensitive plant responds to a touch stimulus.

Reproduction

Single-celled organisms reproduce by first duplicating their DNA, which is the genetic material, and then dividing it equally as the cell prepares to divide to form two new cells. Many multicellular organisms (those made up of more than one cell) produce specialized reproductive cells that will form new individuals. When reproduction occurs, DNA containing genes is passed along to an organism's offspring. These genes are the reason that the offspring will belong to the same species and will have characteristics similar to the parent, such as fur color and blood type.

Adaptation

All living organisms exhibit a "fit" to their environment. Biologists refer to this fit as adaptation and it is a consequence of evolution by natural selection, which operates in every lineage of reproducing organisms. Examples of adaptations are diverse and unique, from heat-resistant Archaea that live in boiling hot springs to the tongue length of a nectar-feeding moth that matches the size of the flower from which it feeds. All adaptations enhance the reproductive potential of the individual exhibiting them, including their ability to survive to reproduce. Adaptations are not constant. As an environment changes, natural selection causes the characteristics of the individuals in a population to track those changes.

Growth and Development

Organisms grow and develop according to specific instructions coded for by their genes. These genes provide instructions that will direct cellular growth and development, ensuring that a species' young (Figure 3.1.3) will grow up to exhibit many of the same characteristics as its parents.



Figure 3.1.3**:** Although no two look alike, these kittens have inherited genes from both parents and share many of the same characteristics. (credit: Pieter & Renée Lanser)

Regulation

Even the smallest organisms are complex and require multiple regulatory mechanisms to coordinate internal functions, such as the transport of nutrients, response to stimuli, and coping with environmental stresses. For example, organ systems such as the digestive or circulatory systems perform specific functions like carrying oxygen throughout the body, removing wastes, delivering nutrients to every cell, and cooling the body.

Homeostasis

To function properly, cells require appropriate conditions such as proper temperature, pH, and concentrations of diverse chemicals. These conditions may, however, change from one moment to the next. Organisms are able to maintain internal conditions within a narrow range almost constantly, despite environmental changes, through a process called homeostasis or "steady state"—the ability of an organism to maintain constant internal conditions. For example, many organisms regulate their body temperature in a process known as thermoregulation. Organisms that live in cold climates, such as the polar bear (Figure 3.1.4), have body structures that help them withstand low temperatures and conserve body heat. In hot climates, organisms have methods (such as perspiration in humans or panting in dogs) that help them to shed excess body heat.



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

Energy Processing

All organisms (such as the California condor shown in Figure 3.1.5) use a source of energy for their metabolic activities. Some organisms capture energy from the Sun and convert it into chemical energy in food; others use chemical energy from molecules they take in.



Figure 3.1.5**:** A lot of energy is required for a California condor to fly. Chemical energy derived from food is used to power flight. California condors are an endangered species; scientists have strived to place a wing tag on each bird to help them identify and locate each individual bird. (credit: Pacific Southwest Region U.S. Fish and Wildlife)

Levels of Organization of Living Things

Living things are highly organized and structured, following a hierarchy on a scale from small to large. The atom is the smallest and most fundamental unit of matter. It consists of a nucleus surrounded by electrons. Atoms form molecules. A molecule is a chemical structure consisting of at least two atoms held together by a chemical bond. Many molecules that are biologically important are macromolecules, large molecules that are typically formed by combining smaller units called monomers. An

example of a macromolecule is deoxyribonucleic acid (DNA) (Figure 3.1.6), which contains the instructions for the functioning of the organism that contains it.

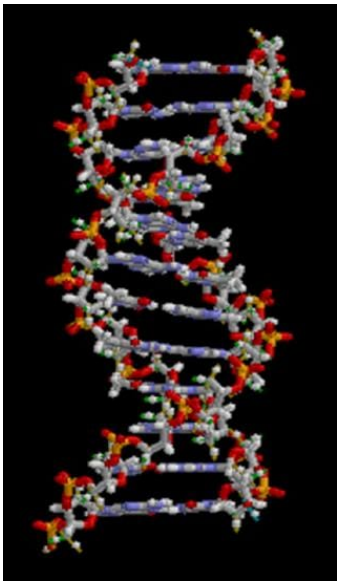


Figure 3.1.6: A molecule, like this large DNA molecule, is composed of atoms. (credit: "Brian0918"/Wikimedia Commons)

CONCEPT IN ACTION

To see an animation of this DNA molecule, click here.

Some cells contain aggregates of macromolecules surrounded by membranes; these are called organelles. Organelles are small structures that exist within cells and perform specialized functions. All living things are made of cells; the cellitself is the smallest fundamental unit of structure and function in living organisms. (This requirement is why viruses are not considered living: they are not made of cells. To make new viruses, they have to invade and hijack a living cell; only then can they obtain the materials they need to reproduce.) Some organisms consist of a single cell and others are multicellular. Cells are classified as prokaryotic or eukaryotic. Prokaryotes are single-celled organisms that lack organelles surrounded by a membrane and do not have nuclei surrounded by nuclear membranes; in contrast, the cells of eukaryotes do have membrane-bound organelles and nuclei.

In most multicellular organisms, cells combine to make tissues, which are groups of similar cells carrying out the same function. Organs are collections of tissues grouped together based on a common function. Organs are present not only in animals but also

in plants. An organ system is a higher level of organization that consists of functionally related organs. For example vertebrate animals have many organ systems, such as the circulatory system that transports blood throughout the body and to and from the lungs; it includes organs such as the heart and blood vessels. Organisms are individual living entities. For example, each tree in a forest is an organism. Single-celled prokaryotes and single-celled eukaryotes are also considered organisms and are typically referred to as microorganisms.

ART CONNECTION

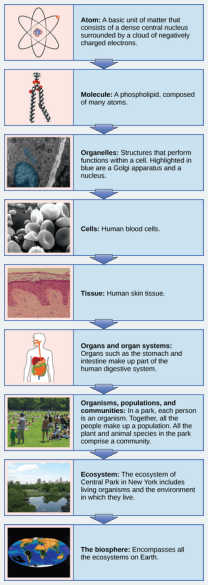


Figure 3.1.7**:** From an atom to the entire Earth, biology examines all aspects of life. (credit "molecule": modification of work by Jane Whitney; credit "organelles": modification of work by Louisa Howard; credit "cells": modification of work by Bruce Wetzel, Harry Schaefer, National Cancer Institute; credit "tissue": modification of work by "Kilbad"/Wikimedia Commons; credit "organs": modification of work by Mariana Ruiz Villareal, Joaquim Alves Gaspar; credit "organisms": modification of work by Peter Dutton; credit "ecosystem": modification of work by "gigi4791"/Flickr; credit "biosphere": modification of work by NASA)

Which of the following statements is false?

A. Tissues exist within organs which exist within organ systems.
B. Communities exist within populations which exist within ecosystems.
C. Organelles exist within cells which exist within tissues.
D. Communities exist within ecosystems which exist in the biosphere.

Answer available at the end of this section.

All the individuals of a species living within a specific area are collectively called a population. For example, a forest may include many white pine trees. All of these pine trees represent the population of white pine trees in this forest. Different populations may live in the same specific area. For example, the forest with the pine trees includes populations of flowering plants and also insects and microbial populations. A community is the set of populations inhabiting a particular area. For instance, all of the trees, flowers, insects, and other populations in a forest form the forest's community. The forest itself is an ecosystem. An ecosystem consists of all the living things in a particular area together with the abiotic, or non-living, parts of that environment such as nitrogen in the soil or rainwater. At the highest level of organization (Figure 3.1.7), the biosphere is the collection of all ecosystems, and it represents the zones of life on Earth. It includes land, water, and portions of the atmosphere.

The Diversity of Life

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

In the 18th century, a scientist named Carl Linnaeus first proposed organizing the known species of organisms into a hierarchical taxonomy. In this system, species that are most similar to each other are put together within a grouping known as a genus. Furthermore, similar genera (the plural of genus) are put together within a family. This grouping continues until all organisms are collected together into groups at the highest level. The current taxonomic system now has eight levels in its hierarchy, from lowest to highest, they are: species, genus, family, order, class, phylum, kingdom, domain. Thus species are grouped within genera, genera are grouped within families, families are grouped within orders, and so on (Figure 3.1.8).

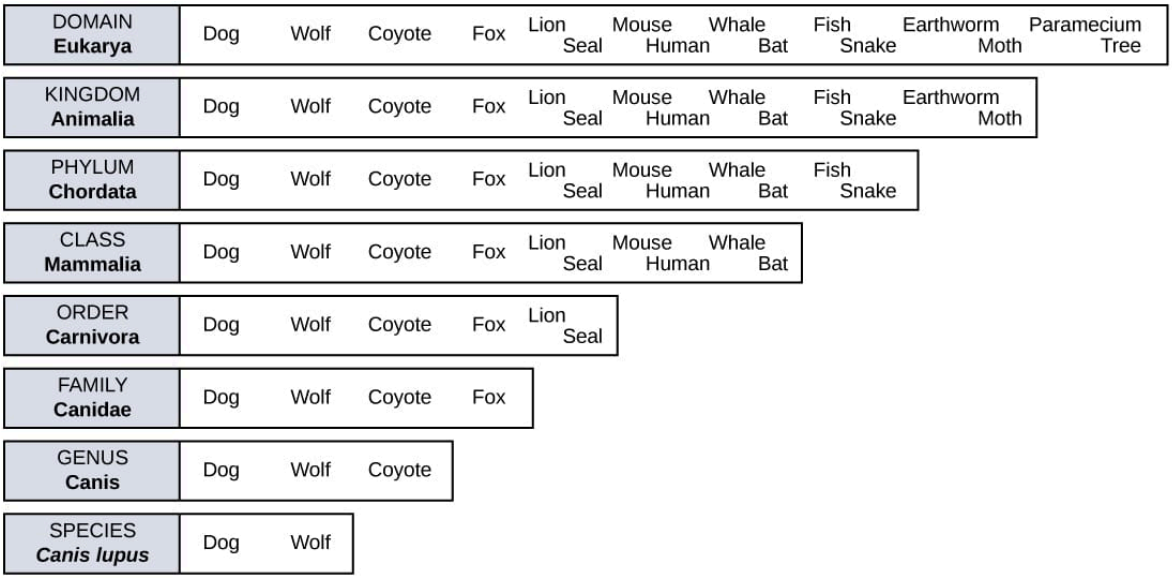


Figure 3.1.8**:** This diagram shows the levels of taxonomic hierarchy for a dog, from the broadest category—domain—to the most specific—species.

The highest level, domain, is a relatively new addition to the system since the 1990s. Scientists now recognize three domains of life, the Eukarya, the Archaea, and the Bacteria. The domain Eukarya contains organisms that have cells with nuclei. It includes the kingdoms of fungi, plants, animals, and several kingdoms of protists. The Archaea, are single-celled organisms without nuclei and include many extremophiles that live in harsh environments like hot springs. The Bacteria are another quite different group of single-celled organisms without nuclei (Figure 3.1.9). Both the Archaea and the Bacteria are prokaryotes, an informal name for cells without nuclei. The recognition in the 1990s that certain "bacteria," now known as the Archaea, were as different genetically and biochemically from other bacterial cells as they were from eukaryotes, motivated the recommendation to divide life into three domains. This dramatic change in our knowledge of the tree of life demonstrates that classifications are not permanent and will change when new information becomes available.

In addition to the hierarchical taxonomic system, Linnaeus was the first to name organisms using two unique names, now called the binomial naming system. Before Linnaeus, the use of common names to refer to organisms caused confusion because there were regional differences in these common names. Binomial names consist of the genus name (which is capitalized) and the species name (all lower-case). Both names are set in italics when they are printed. Every species is given a unique binomial which is recognized the world over, so that a scientist in any location can know which organism is being referred to. For example, the North American blue jay is known uniquely as *Cyanocitta cristata*. Our own species is *Homo sapiens*.



Figure 3.1.9**:** These images represent different domains. The scanning electron micrograph shows (a) bacterial cells belong to the domain Bacteria, while the (b) extremophiles, seen all together as colored mats in this hot spring, belong to domain Archaea. Both the (c) sunflower and (d) lion are part of domain Eukarya. (credit a: modification of work by Rocky Mountain Laboratories, NIAID, NIH; credit b: modification of work by Steve Jurvetson; credit c: modification of work by Michael Arrighi; credit d: modification of work by Frank Vassen)

EVOLUTION IN ACTION: Carl Woese and the Phylogenetic Tree

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

In the past, biologists grouped living organisms into five kingdoms: animals, plants, fungi, protists, and bacteria. The pioneering work of American microbiologist Carl Woese in the early 1970s has shown, however, that life on Earth has evolved along three lineages, now called domains—Bacteria, Archaea, and Eukarya. Woese proposed the domain as a new taxonomic level and Archaea as a new domain, to reflect the new phylogenetic tree (Figure 3.1.10). Many organisms belonging to the Archaea domain live under extreme conditions and are called extremophiles. To construct his tree, Woese used genetic relationships rather than similarities based on morphology (shape). Various genes were used in phylogenetic studies. Woese's tree was constructed from comparative sequencing of the genes that are universally distributed, found in some slightly altered form in every organism, conserved (meaning that these genes have remained only slightly changed throughout evolution), and of an appropriate length.

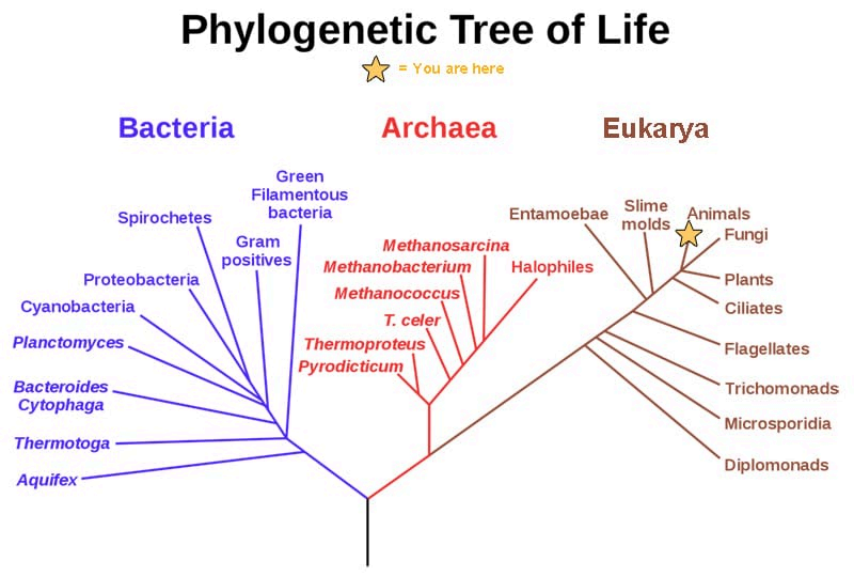


Figure 3.1.10**:** This phylogenetic tree was constructed by microbiologist Carl Woese using genetic relationships. The tree shows the separation of living organisms into three domains: Bacteria, Archaea, and Eukarya. Bacteria and Archaea are organisms without a nucleus or other organelles surrounded by a membrane and, therefore, are prokaryotes. (credit: modification of work by Eric Gaba)

Branches of Biological Study

The scope of biology is broad and therefore contains many branches and sub disciplines. Biologists may pursue one of those sub disciplines and work in a more focused field. For instance, molecular biology studies biological processes at the molecular level, including interactions among molecules such as DNA, RNA, and proteins, as well as the way they are regulated. Microbiology is the study of the structure and function of microorganisms. It is quite a broad branch itself, and depending on the subject of study, there are also microbial physiologists, ecologists, and geneticists, among others.

Another field of biological study, neurobiology, studies the biology of the nervous system, and although it is considered a branch of biology, it is also recognized as an interdisciplinary field of study known as neuroscience. Because of its interdisciplinary nature, this sub discipline studies different functions of the nervous system using molecular, cellular, developmental, medical, and computational approaches.



Figure 3.1.11**:** Researchers work on excavating dinosaur fossils at a site in Castellón, Spain. (credit: Mario Modesto)

Paleontology, another branch of biology, uses fossils to study life's history (Figure 3.1.11). Zoology and botany are the study of animals and plants, respectively. Biologists can also specialize as biotechnologists, ecologists, or physiologists, to name just a few areas. Biotechnologists apply the knowledge of biology to create useful products. Ecologists study the interactions of organisms in their environments. Physiologists study the workings of cells, tissues and organs. This is just a small sample of the many fields that biologists can pursue. From our own bodies to the world we live in, discoveries in biology can affect us in very direct and important ways. We depend on these discoveries for our health, our food sources, and the benefits provided by our ecosystem. Because of this, knowledge of biology can benefit us in making decisions in our day-to-day lives.

The development of technology in the twentieth century that continues today, particularly the technology to describe and manipulate the genetic material, DNA, has transformed biology. This transformation will allow biologists to continue to understand the history of life in greater detail, how the human body works, our human origins, and how humans can survive as a species on this planet despite the stresses caused by our increasing numbers. Biologists continue to decipher huge mysteries about life suggesting that we have only begun to understand life on the planet, its history, and our relationship to it. For this and other reasons, the knowledge of biology gained through this textbook and other printed and electronic media should be a benefit in whichever field you enter.

CAREERS IN ACTION: Forensic Scientist

Forensic science is the application of science to answer questions related to the law. Biologists as well as chemists and biochemists can be forensic scientists. Forensic scientists provide scientific evidence for use in courts, and their job involves examining trace material associated with crimes. Interest in forensic science has increased in the last few years, possibly because of popular television shows that feature forensic scientists on the job. Also, the development of molecular techniques and the establishment of DNA databases have updated the types of work that forensic scientists can do. Their job activities

are primarily related to crimes against people such as murder, rape, and assault. Their work involves analyzing samples such as hair, blood, and other body fluids and also processing DNA (Figure 3.1.12) found in many different environments and materials. Forensic scientists also analyze other biological evidence left at crime scenes, such as insect parts or pollen grains. Students who want to pursue careers in forensic science will most likely be required to take chemistry and biology courses as well as some intensive math courses.



Figure 3.1.12: This forensic scientist works in a DNA extraction room at the U.S. Army Criminal Investigation Laboratory. (credit: U.S. Army CID Command Public Affairs)

Summary

Biology is the science of life. All living organisms share several key properties such as order, sensitivity or response to stimuli, reproduction, adaptation, growth and development, regulation, homeostasis, and energy processing. Living things are highly organized following a hierarchy that includes atoms, molecules, organelles, cells, tissues, organs, and organ systems. Organisms, in turn, are grouped as populations, communities, ecosystems, and the biosphere. Evolution is the source of the tremendous biological diversity on Earth today. A diagram called a phylogenetic tree can be used to show evolutionary relationships among organisms. Biology is very broad and includes many branches and sub disciplines. Examples include molecular biology, microbiology, neurobiology, zoology, and botany, among others.

Glossary

atom - a basic unit of matter that cannot be broken down by normal chemical reactions

biology - the study of living organisms and their interactions with one another and their environments

biosphere - a collection of all ecosystems on Earth

cell - the smallest fundamental unit of structure and function in living things

community - a set of populations inhabiting a particular area

ecosystem - all living things in a particular area together with the abiotic, nonliving parts of that environment

eukaryote - an organism with cells that have nuclei and membrane-bound organelles

evolution - the process of gradual change in a population that can also lead to new species arising from older species

homeostasis - the ability of an organism to maintain constant internal conditions

macromolecule - a large molecule typically formed by the joining of smaller molecules

molecule - a chemical structure consisting of at least two atoms held together by a chemical bond

organ - a structure formed of tissues operating together to perform a common function

organ system - the higher level of organization that consists of functionally related organs

organelle - a membrane-bound compartment or sac within a cell

organism - an individual living entity

phylogenetic tree - a diagram showing the evolutionary relationships among biological species based on similarities and differences in genetic or physical traits or both

population - all individuals within a species living within a specific area

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

tissue - a group of similar cells carrying out the same function

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

Answers

ART CONNECTION

B

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3.2: Prokaryotic Cells

Learning Objectives

By the end of this section, you will be able to do the following:

- Name examples of prokaryotic and eukaryotic organisms
- Compare and contrast prokaryotic and eukaryotic cells
- Describe the relative sizes of different cells
- Explain why cells must be small

Cells fall into one of two broad categories: prokaryotic and eukaryotic. We classify only the predominantly single-celled organisms Bacteria and Archaea as prokaryotes (pro- = “before”; -kary- = “nucleus”). Animal cells, plants, fungi, and protists are all eukaryotes (eu- = “true”).

Components of Prokaryotic Cells

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

A prokaryote is a simple, mostly single-celled (unicellular) organism that lacks a nucleus, or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is in the cell's central part: the nucleoid (Figure 4.5).

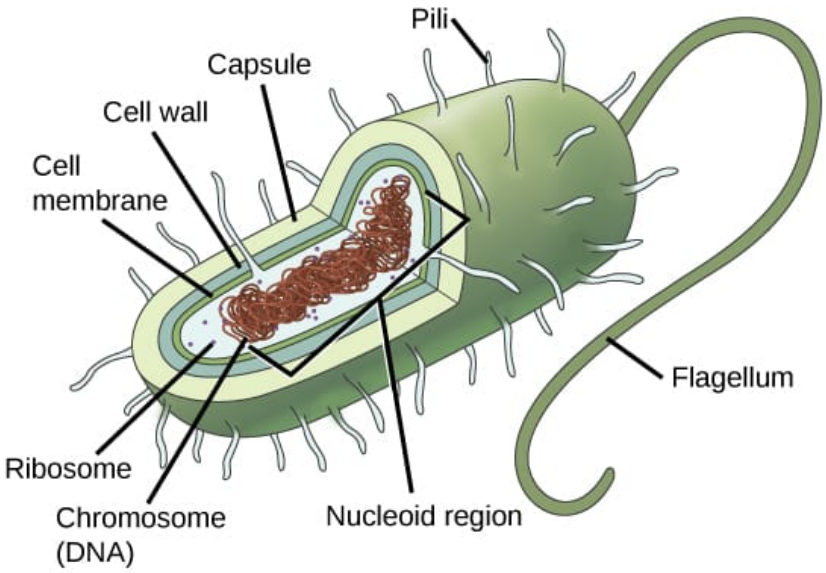


Figure 4.5 This figure shows the generalized structure of a prokaryotic cell. All prokaryotes have chromosomal DNA localized in a nucleoid, ribosomes, a cell membrane, and a cell wall. The other structures shown are present in some, but not all, bacteria.

Most prokaryotes have a peptidoglycan cell wall and many have a polysaccharide capsule (Figure 4.5). The cell wall acts as an extra layer of protection, helps the cell maintain its shape, and prevents dehydration. The capsule enables the cell to attach to surfaces in its environment. Some prokaryotes have flagella, pili, or fimbriae. Flagella are used for locomotion. Pili exchange genetic material during conjugation, the process by which one bacterium transfers genetic material to another through direct contact. Bacteria use fimbriae to attach to a host cell.

Career Connection

Career Connection

MicrobiologistThe most effective action anyone can take to prevent the spread of contagious illnesses is to wash their hands. Why? Because microbes (organisms so tiny that they can only be seen with microscopes) are ubiquitous. They live on doorknobs, money, your hands, and many other surfaces. If someone sneezes into his hand and touches a doorknob, and afterwards you touch that same doorknob, the microbes from the sneezer's mucus are now on your hands. If you touch your hands to your mouth, nose, or eyes, those microbes can enter your body and could make you sick.

However, not all microbes (also called microorganisms) cause disease; most are actually beneficial. You have microbes in your gut that make vitamin K. Other microorganisms are used to ferment beer and wine.

Microbiologists are scientists who study microbes. Microbiologists can pursue a number of careers. Not only do they work in the food industry, they are also employed in the veterinary and medical fields. They can work in the pharmaceutical sector, serving key roles in research and development by identifying new antibiotic sources that can treat bacterial infections.

Environmental microbiologists may look for new ways to use specially selected or genetically engineered microbes to remove pollutants from soil or groundwater, as well as hazardous elements from contaminated sites. We call using these microbes bioremediation technologies. Microbiologists can also work in the bioinformatics field, providing specialized knowledge and insight for designing, developing, and specificity of computer models of, for example, bacterial epidemics.

Cell Size

At 0.1 to 5.0 μm in diameter, prokaryotic cells are significantly smaller than eukaryotic cells, which have diameters ranging from 10 to 100 μm (Figure 4.6). The prokaryotes' small size allows ions and organic molecules that enter them to quickly diffuse to other parts of the cell. Similarly, any wastes produced within a prokaryotic cell can quickly diffuse. This is not the case in eukaryotic cells, which have developed different structural adaptations to enhance intracellular transport.

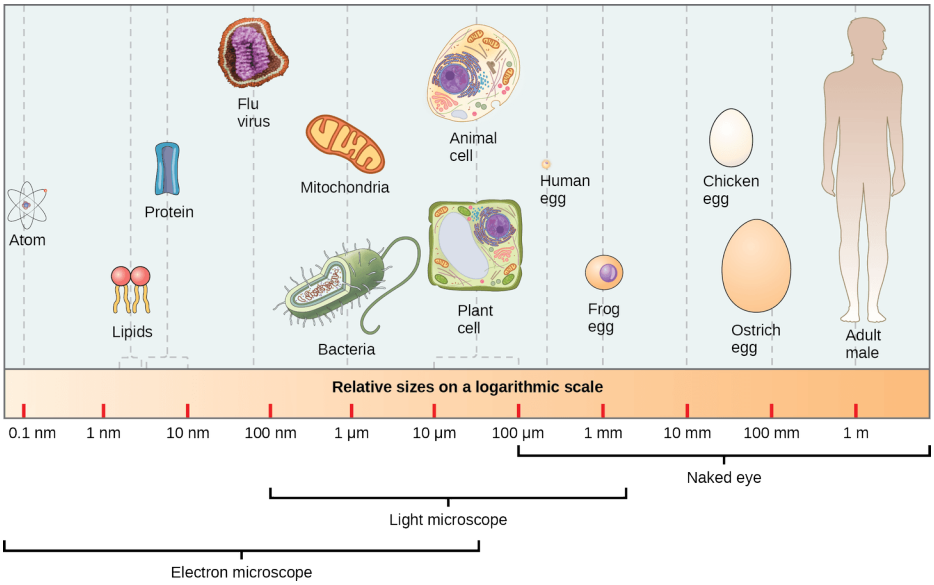


Figure 4.6 This figure shows relative sizes of microbes on a logarithmic scale (recall that each unit of increase in a logarithmic scale represents a 10-fold increase in the quantity measured).

Small size, in general, is necessary for all cells, whether prokaryotic or eukaryotic. Let's examine why that is so. First, we'll consider the area and volume of a typical cell. Not all cells are spherical in shape, but most tend to approximate a sphere. You may remember from your high school geometry course that the formula for the surface area of a sphere is $4\pi r^2$, while the formula for its volume is $4\pi r^3/3$. Thus, as the radius of a cell increases, its surface area increases as the square of its radius, but its volume increases as the cube of its radius (much more rapidly). Therefore, as a cell increases in size, its surface area-to-volume

ratio decreases. This same principle would apply if the cell had a cube shape (Figure 4.7). If the cell grows too large, the plasma membrane will not have sufficient surface area to support the rate of diffusion required for the increased volume. In other words, as a cell grows, it becomes less efficient. One way to become more efficient is to divide. Other ways are to increase surface area by foldings of the cell membrane, become flat or thin and elongated, or develop organelles that perform specific tasks. These adaptations lead to developing more sophisticated cells, which we call eukaryotic cells.

Visual Connection

Visual Connection

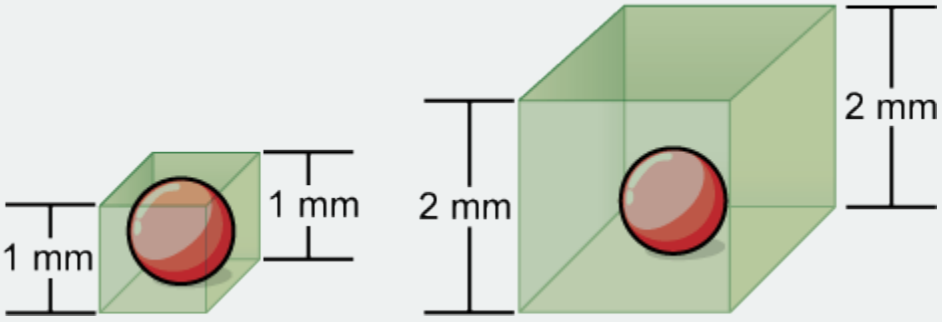


Figure 4.7 Notice that as a cell increases in size, its surface area-to-volume ratio decreases. When there is insufficient surface area to support a cell's increasing volume, a cell will either divide or die. The cell on the left has a volume of 1 mm^3 and a surface area of 6 mm^2, with a surface area-to-volume ratio of 6 to 1; whereas, the cell on the right has a volume of 8 mm^3 and a surface area of 24 mm^2, with a surface area-to-volume ratio of 3 to 1.

Prokaryotic cells are much smaller than eukaryotic cells. What advantages might small cell size confer on a cell? What advantages might large cell size have?

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3.3: Eukaryotic Cells

Learning Objectives

By the end of this section, you will be able to do the following:

- Describe the structure of eukaryotic cells
- Compare animal cells with plant cells
- State the role of the plasma membrane
- Summarize the functions of the major cell organelles

Have you ever heard the phrase “form follows function?” It’s a philosophy that many industries follow. In architecture, this means that buildings should be constructed to support the activities that will be carried out inside them. For example, a skyscraper should include several elevator banks. A hospital should have its emergency room easily accessible.

Our natural world also utilizes the principle of form following function, especially in cell biology, and this will become clear as we explore eukaryotic cells (Figure 4.8). Unlike prokaryotic cells, eukaryotic cells have: 1) a membrane-bound nucleus; 2) numerous membrane-bound organelles such as the endoplasmic reticulum, Golgi apparatus, chloroplasts, mitochondria, and others; and 3) several, rod-shaped chromosomes. Because a membrane surrounds eukaryotic cell’s nucleus, it has a “true nucleus.” The word “organelle” means “little organ,” and, as we already mentioned, organelles have specialized cellular functions, just as your body's organs have specialized functions.

At this point, it should be clear to you that eukaryotic cells have a more complex structure than prokaryotic cells. Organelles allow different functions to be compartmentalized in different areas of the cell. Before turning to organelles, let’s first examine two important components of the cell: the plasma membrane and the cytoplasm.

Visual Connection

Visual Connection

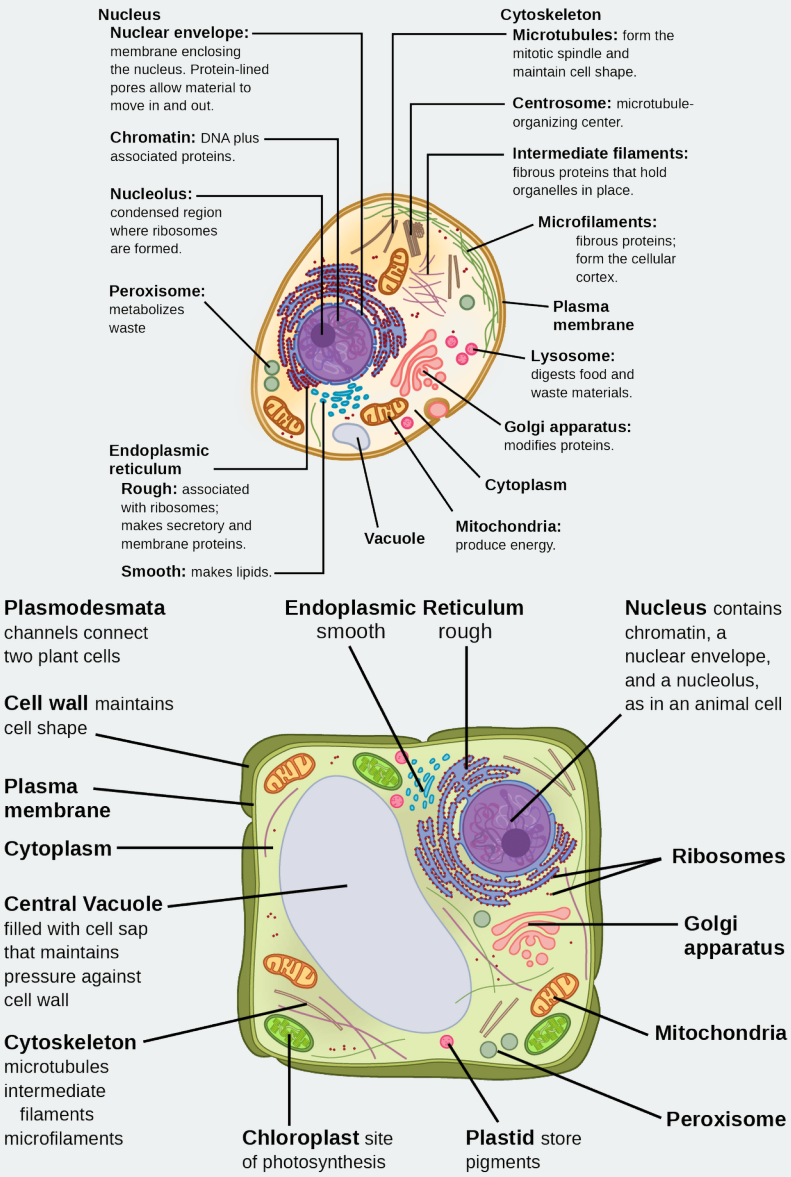


Figure 4.8 These figures show the major organelles and other cell components of (a) a typical animal cell and (b) a typical eukaryotic plant cell. The plant cell has a cell wall, chloroplasts, plastids, and a central vacuole—structures not in animal cells. Most cells do not have lysosomes or centrosomes.

If the nucleolus were not able to carry out its function, what other cellular organelles would be affected?

The Plasma Membrane

Like prokaryotes, eukaryotic cells have a plasma membrane (Figure 4.9), a phospholipid bilayer with embedded proteins that separates the internal contents of the cell from its surrounding environment. A phospholipid is a lipid molecule with two fatty acid chains and a phosphate-containing group. The plasma membrane controls the passage of organic molecules, ions, water, and oxygen into and out of the cell. Wastes (such as carbon dioxide and ammonia) also leave the cell by passing through the plasma membrane.

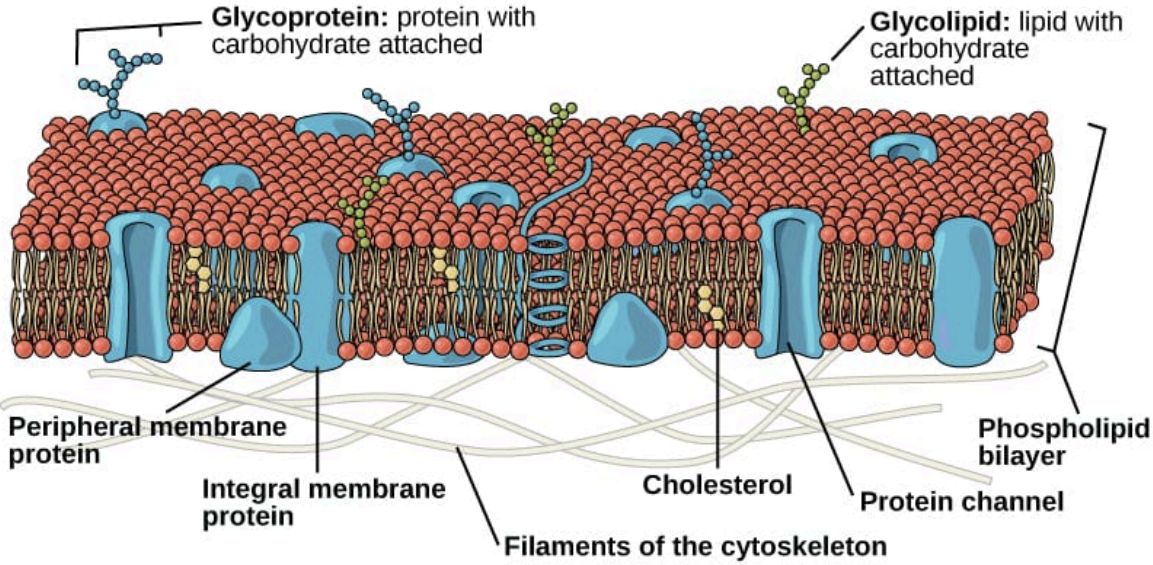


Figure 4.9 The eukaryotic plasma membrane is a phospholipid bilayer with proteins and cholesterol embedded in it.

The plasma membranes of cells that specialize in absorption fold into fingerlike projections that we call microvilli (singular = microvillus); (Figure 4.10). Such cells typically line the small intestine, the organ that absorbs nutrients from digested food. This is an excellent example of form following function. People with celiac disease have an immune response to gluten, which is a protein in wheat, barley, and rye. The immune response damages microvilli, and thus, afflicted individuals cannot absorb nutrients. This leads to malnutrition, cramping, and diarrhea. Patients suffering from celiac disease must follow a gluten-free diet.

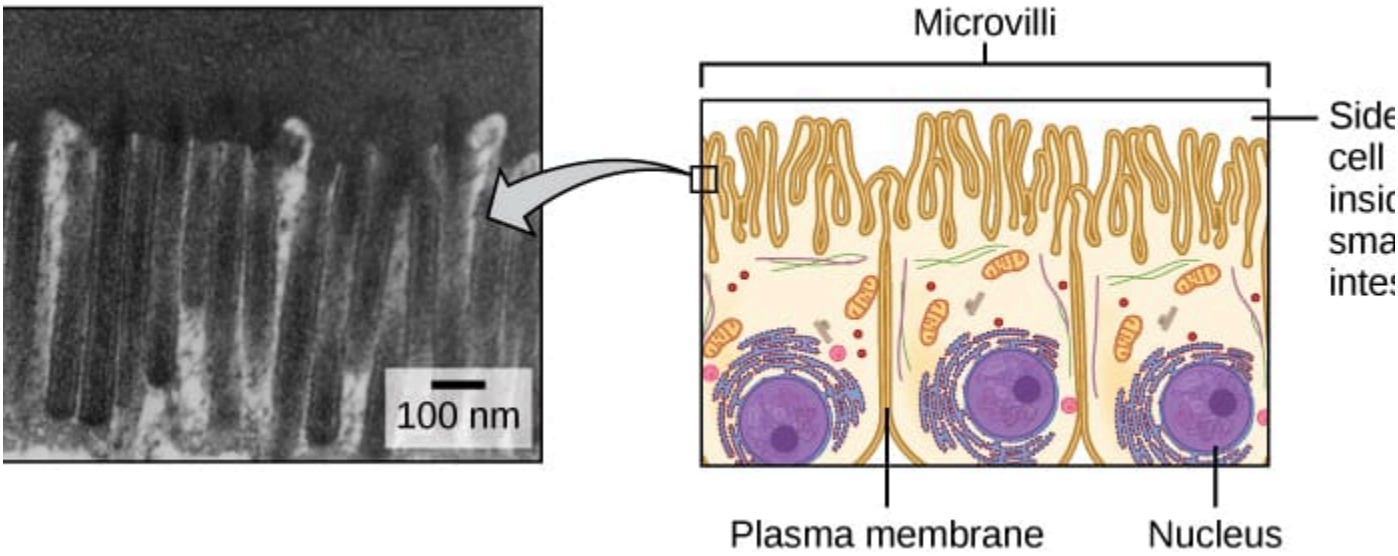


Figure 4.10 Microvilli, as they appear on cells lining the small intestine, increase the surface area available for absorption. These microvilli are only on the area of the plasma membrane that faces the cavity from which substances will be absorbed. (credit "micrograph": modification of work by Louisa Howard)

The Cytoplasm

The cytoplasm is the cell's entire region between the plasma membrane and the nuclear envelope (a structure we will discuss shortly). It is comprised of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals (Figure 4.8). Even though the cytoplasm consists of 70 to 80 percent water, it has a semi-solid consistency, which comes from the proteins within it. However, proteins are not the only organic molecules in the cytoplasm. Glucose and other simple sugars, polysaccharides, amino acids, nucleic acids, fatty acids, and derivatives of glycerol are also there. Ions of sodium, potassium, calcium, and many other elements also dissolve in the cytoplasm. Many metabolic reactions, including protein synthesis, take place in the cytoplasm.

The Nucleus

Typically, the nucleus is the most prominent organelle in a cell (Figure 4.8). The nucleus (plural = nuclei) houses the cell's DNA and directs the synthesis of ribosomes and proteins. Let's look at it in more detail (Figure 4.11).

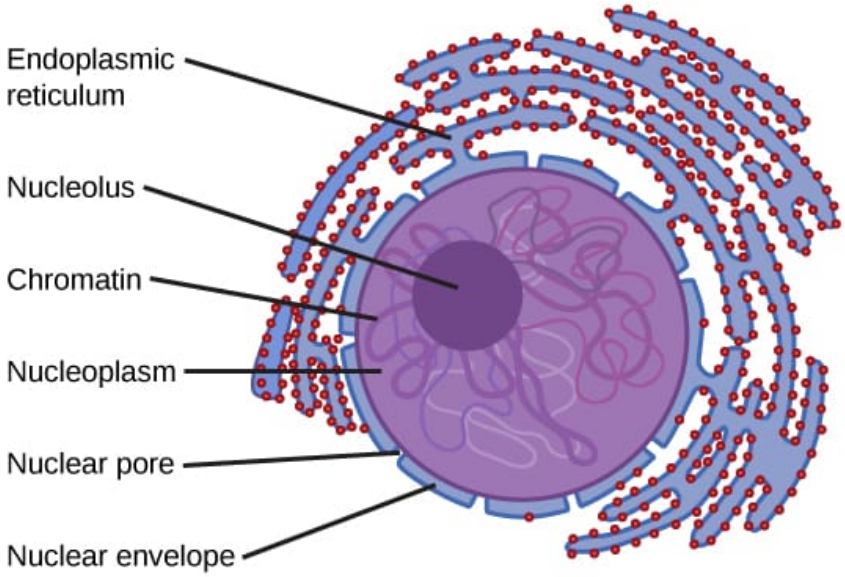


Figure 4.11 The nucleus stores chromatin (DNA plus proteins) in a gel-like substance called the nucleoplasm. The nucleolus is a condensed chromatin region where ribosome synthesis occurs. We call the nucleus' boundary the nuclear envelope. It consists of two phospholipid bilayers: an outer and an inner membrane. The nuclear membrane is continuous with the endoplasmic reticulum. Nuclear pores allow substances to enter and exit the nucleus.

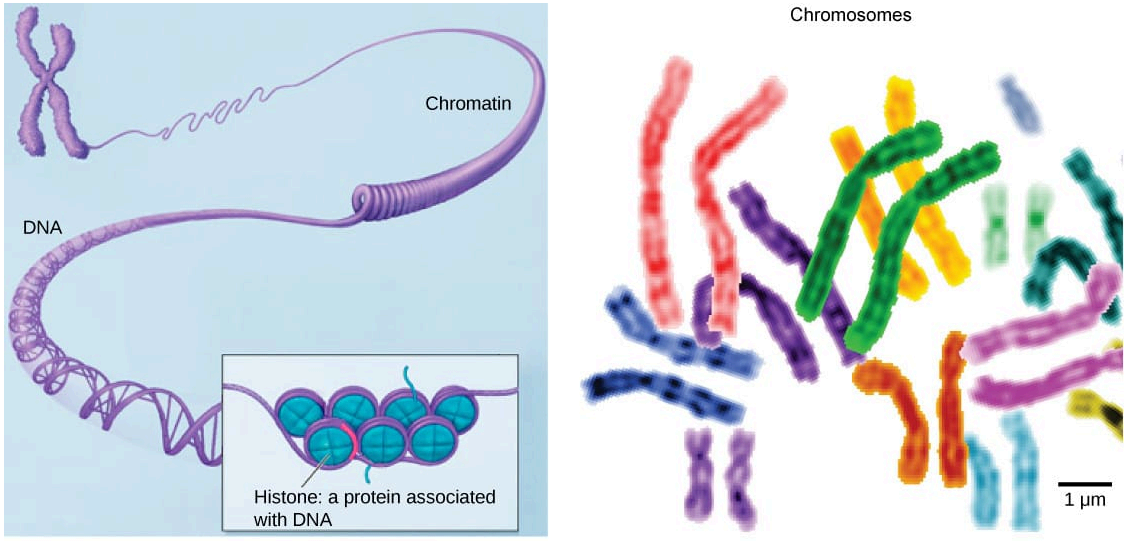
The Nuclear Envelope

The nuclear envelope is a double-membrane structure that constitutes the nucleus' outermost portion (Figure 4.11). Both the nuclear envelope's inner and outer membranes are phospholipid bilayers.

The nuclear envelope is punctuated with pores that control the passage of ions, molecules, and RNA between the nucleoplasm and cytoplasm. The nucleoplasm is the semi-solid fluid inside the nucleus, where we find the chromatin and the nucleolus.

Chromatin and Chromosomes

To understand chromatin, it is helpful to first explore chromosomes, structures within the nucleus that are made up of DNA, the hereditary material. You may remember that in prokaryotes, DNA is organized into a single circular chromosome. In eukaryotes, chromosomes are linear structures. Every eukaryotic species has a specific number of chromosomes in the nucleus of each cell. For example, in humans, the chromosome number is 46, while in fruit flies, it is eight. Chromosomes are only visible and distinguishable from one another when the cell is getting ready to divide. When the cell is in the growth and maintenance phases of its life cycle, proteins attach to chromosomes, and they resemble an unwound, jumbled bunch of threads. We call these unwound protein-chromosome complexes chromatin (Figure 4.12). Chromatin describes the material that makes up the chromosomes both when condensed and decondensed.



(a) (b)

Figure 4.12 (a) This image shows various levels of chromatin's organization (DNA and protein). (b) This image shows paired chromosomes. (credit b: modification of work by NIH; scale-bar data from Matt Russell)

The Nucleolus

We already know that the nucleus directs the synthesis of ribosomes, but how does it do this? Some chromosomes have sections of DNA that encode ribosomal RNA. A darkly staining area within the nucleus called the nucleolus (plural = nucleoli) aggregates the ribosomal RNA with associated proteins to assemble the ribosomal subunits that are then transported out through the pores in the nuclear envelope to the cytoplasm.

Ribosomes

Ribosomes are the cellular structures responsible for protein synthesis. When we view them through an electron microscope, ribosomes appear either as clusters (polyribosomes) or single, tiny dots that float freely in the cytoplasm. They may be attached to the plasma membrane's cytoplasmic side or the endoplasmic reticulum's cytoplasmic side and the nuclear envelope's outer membrane (Figure 4.8). Electron microscopy shows us that ribosomes, which are large protein and RNA complexes, consist of two subunits, large and small (Figure 4.13). Ribosomes receive their “orders” for protein synthesis from the nucleus where the DNA transcribes into messenger RNA (mRNA). The mRNA travels to the ribosomes, which translate the code provided by the sequence of the nitrogenous bases in the mRNA into a specific order of amino acids in a protein. Amino acids are the building blocks of proteins.

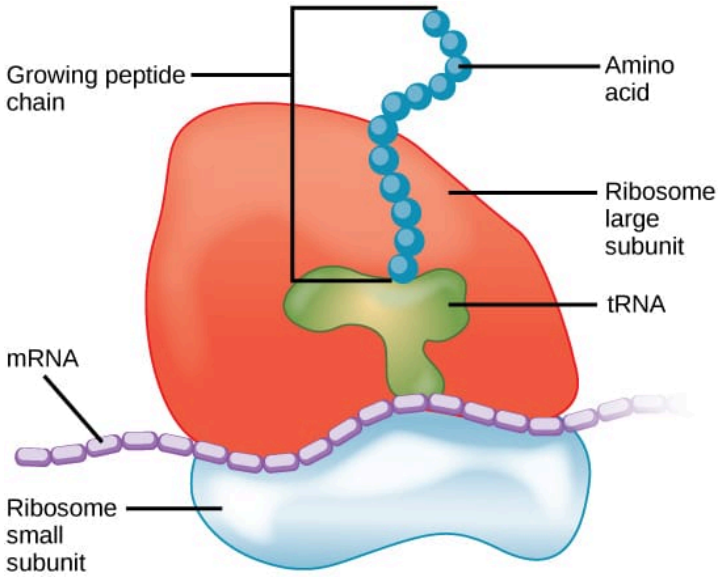


Figure 4.13 A large subunit (top) and a small subunit (bottom) comprise ribosomes. During protein synthesis, ribosomes assemble amino acids into proteins.

Because protein synthesis is an essential function of all cells (including enzymes, hormones, antibodies, pigments, structural components, and surface receptors), there are ribosomes in practically every cell. Ribosomes are particularly abundant in cells that synthesize large amounts of protein. For example, the pancreas is responsible for creating several digestive enzymes and the cells that produce these enzymes contain many ribosomes. Thus, we see another example of form following function.

Mitochondria

Scientists often call mitochondria (singular = mitochondrion) “powerhouses” or “energy factories” of both plant and animal cells because they are responsible for making adenosine triphosphate (ATP), the cell’s main energy-carrying molecule. ATP represents the cell's short-term stored energy. Cellular respiration is the process of making ATP using the chemical energy in glucose and other nutrients. In mitochondria, this process uses oxygen and produces carbon dioxide as a waste product. In fact, the carbon dioxide that you exhale with every breath comes from the cellular reactions that produce carbon dioxide as a byproduct.

In keeping with our theme of form following function, it is important to point out that muscle cells have a very high concentration of mitochondria that produce ATP. Your muscle cells need considerable energy to keep your body moving. When your cells don’t get enough oxygen, they do not make much ATP. Instead, producing lactic acid accompanies the small amount of ATP they make in the absence of oxygen.

Mitochondria are oval-shaped, double membrane organelles (Figure 4.14) that have their own ribosomes and DNA. Each membrane is a phospholipid bilayer embedded with proteins. The inner layer has folds called cristae. We call the area surrounded by the folds the mitochondrial matrix. The cristae and the matrix have different roles in cellular respiration.

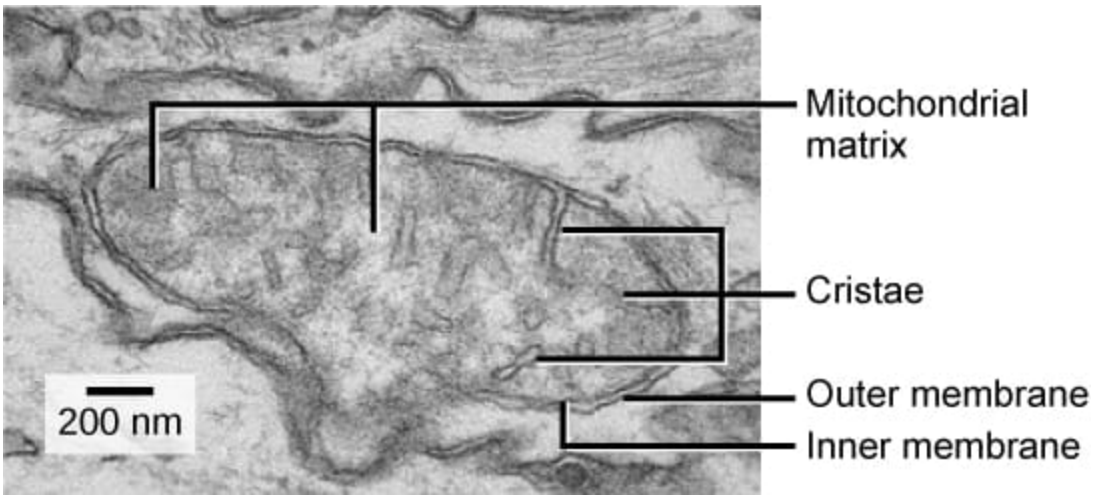


Figure 4.14 This electron micrograph shows a mitochondrion through an electron microscope. This organelle has an outer membrane and an inner membrane. The inner membrane contains folds, called cristae, which increase its surface area. We call the space between the two membranes the intermembrane space, and the space inside the inner membrane the mitochondrial matrix. ATP synthesis takes place on the inner membrane. (credit: modification of work by Matthew Britton; scale-bar data from Matt Russell)

Peroxisomes

Peroxisomes are small, round organelles enclosed by single membranes. They carry out oxidation reactions that break down fatty acids and amino acids. They also detoxify many poisons that may enter the body. (Many of these oxidation reactions release hydrogen peroxide, H_2O_2, which would be damaging to cells; however, when these reactions are confined to peroxisomes, enzymes safely break down the H_2O_2 into oxygen and water.) For example, peroxisomes in liver cells detoxify alcohol. Glyoxysomes, which are specialized peroxisomes in plants, are responsible for converting stored fats into sugars. Plant cells contain many different types of peroxisomes that play a role in metabolism, pathogene defense, and stress response, to mention a few.

Vesicles and Vacuoles

Vesicles and vacuoles are membrane-bound sacs that function in storage and transport. Other than the fact that vacuoles are somewhat larger than vesicles, there is a very subtle distinction between them. Vesicle membranes can fuse with either the plasma membrane or other membrane systems within the cell. Additionally, some agents such as enzymes within plant vacuoles break down macromolecules. The vacuole's membrane does not fuse with the membranes of other cellular components.

Animal Cells versus Plant Cells

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

The Centrosome

The centrosome is a microtubule-organizing center found near the nuclei of animal cells. It contains a pair of centrioles, two structures that lie perpendicular to each other (Figure 4.15). Each centriole is a cylinder of nine triplets of microtubules.

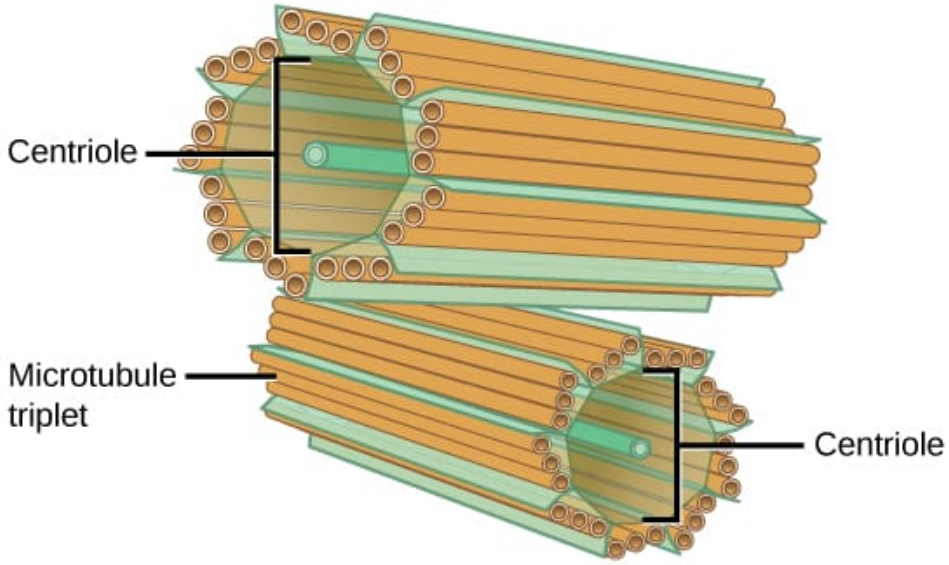


Figure 4.15 The centrosome consists of two centrioles that lie at right angles to each other. Each centriole is a cylinder comprised of nine triplets of microtubules. Nontubulin proteins (indicated by the green lines) hold the microtubule triplets together.

The centrosome (the organelle where all microtubules originate) replicates itself before a cell divides, and the centrioles appear to have some role in pulling the duplicated chromosomes to opposite ends of the dividing cell. However, the centriole's exact function in cell division isn't clear, because cells that have had the centrosome removed can still divide, and plant cells, which lack centrosomes, are capable of cell division.

Lysosomes

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

The Cell Wall

If you examine Figure 4.8, the plant cell diagram, you will see a structure external to the plasma membrane. This is the cell wall, a rigid covering that protects the cell, provides structural support, and gives shape to the cell. Fungal and some protistan cells also have cell walls. While the prokaryotic cell walls' chief component is peptidoglycan, the major organic molecule in the plant (and some protists') cell wall is cellulose (Figure 4.16), a polysaccharide comprised of glucose units. Have you ever noticed that when you bite into a raw vegetable, like celery, it crunches? That's because you are tearing the celery cells' rigid cell walls with your teeth.

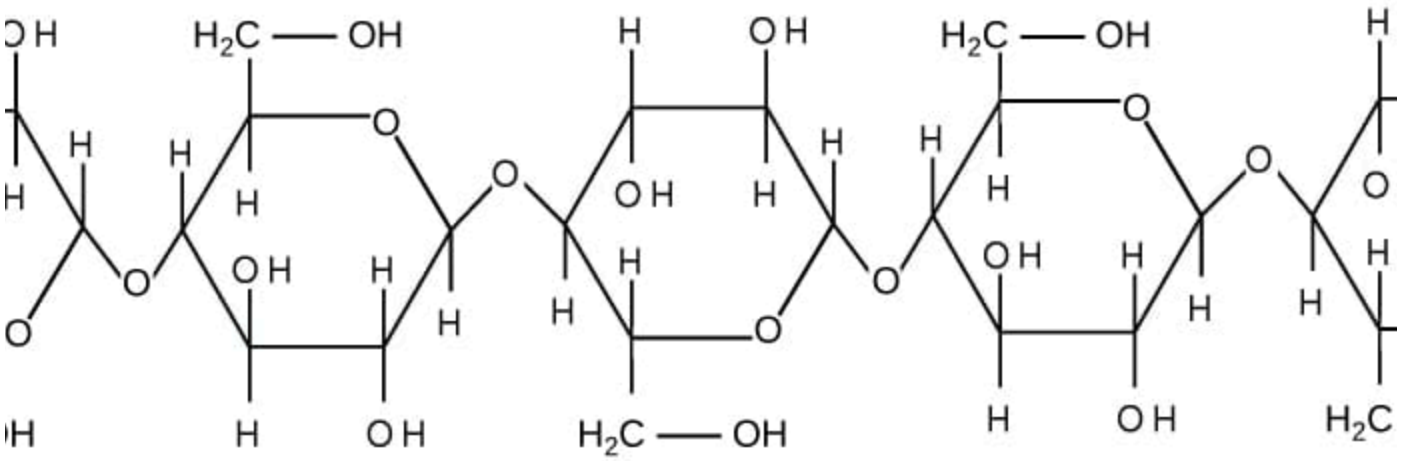


Figure 4.16 Cellulose is a long chain of β-glucose molecules connected by a 1-4 linkage. The dashed lines at each end of the figure indicate a series of many more glucose units. The size of the page makes it impossible to portray an entire cellulose molecule.

Chloroplasts

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

Like mitochondria, chloroplasts have outer and inner membranes, but within the space enclosed by a chloroplast's inner membrane is a set of interconnected and stacked fluid-filled membrane sacs we call thylakoids (Figure 4.17). Each thylakoid stack is a granum (plural = grana). We call the fluid enclosed by the inner membrane that surrounds the grana the stroma.

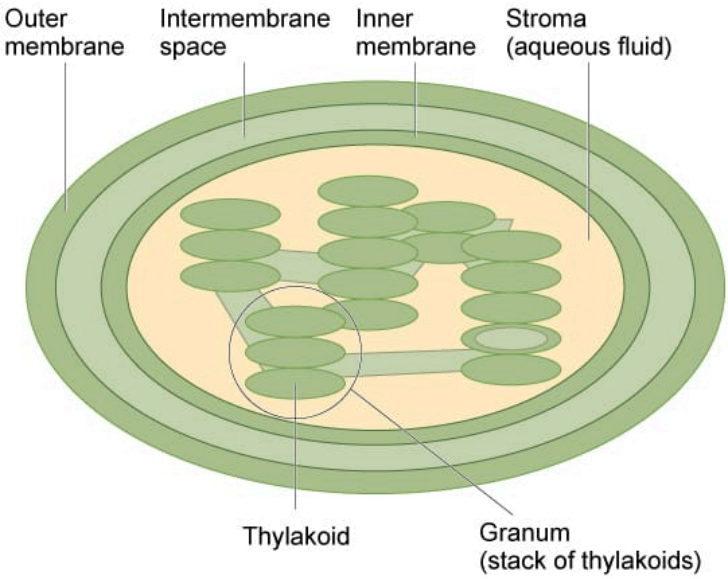


Figure 4.17 The chloroplast has an outer membrane, an inner membrane, and membrane structures - thylakoids that are stacked into grana. We call the space inside the thylakoid membranes the thylakoid space. The light harvesting reactions take place in the thylakoid membranes, and sugar synthesis takes place in the fluid inside the inner membrane, which we call the stroma. Chloroplasts also have their own genome, which is contained on a single circular chromosome.

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

relegated to an organelle.

Evolution Connection

Evolution Connection

EndosymbiosisWe have mentioned that both mitochondria and chloroplasts contain DNA and ribosomes. Have you wondered why? Strong evidence points to endosymbiosis as the explanation.

Symbiosis is a relationship in which organisms from two separate species depend on each other for their survival. Endosymbiosis (endo- = “within”) is a mutually beneficial relationship in which one organism lives inside the other. Endosymbiotic relationships abound in nature. We have already mentioned that microbes that produce vitamin K live inside the human gut. This relationship is beneficial for us because we are unable to synthesize vitamin K. It is also beneficial for the microbes because they are protected from other organisms and from drying out, and they receive abundant food from the environment of the large intestine.

Scientists have long noticed that bacteria, mitochondria, and chloroplasts are similar in size. We also know that bacteria have DNA and ribosomes, just like mitochondria and chloroplasts. Scientists believe that host cells and bacteria formed an endosymbiotic relationship when the host cells ingested both aerobic and autotrophic bacteria (cyanobacteria) but did not destroy them. Through many millions of years of evolution, these ingested bacteria became more specialized in their functions, with the aerobic bacteria becoming mitochondria and the autotrophic bacteria becoming chloroplasts.

The Central Vacuole

Previously, we mentioned vacuoles as essential components of plant cells. If you look at Figure 4.8b, you will see that plant cells each have a large central vacuole that occupies most of the cell's area. The central vacuole plays a key role in regulating the cell’s concentration of water in changing environmental conditions. Have you ever noticed that if you forget to water a plant for a few days, it wilts? That’s because as the water concentration in the soil becomes lower than the water concentration in the plant, water moves out of the central vacuoles and cytoplasm. As the central vacuole shrinks, it leaves the cell wall unsupported. This loss of support to the plant's cell walls results in the wilted appearance.

The central vacuole also supports the cell's expansion. When the central vacuole holds more water, the cell becomes larger without having to invest considerable energy in synthesizing new cytoplasm.

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3.4: VIRUSES

Viruses are acellular, parasitic entities that are not classified within any kingdom. Viruses are not cells and cannot divide. They infect a host cell and use the host's replication processes to produce identical progeny virus particles. Viruses infect organisms as diverse as bacteria, plants, and animals and exist in a netherworld between a living organism and a nonliving entity. Living things grow, metabolize, and reproduce. Viruses replicate, but to do so, they are entirely dependent on their host cells.

3.4.1: Viral Evolution, Morphology, and Classification

3.4.2: Virus Infections and Hosts

3.4.3: Prevention and Treatment of Viral Infections

3.4.4: Key Terms

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3.4.1: Viral Evolution, Morphology, and Classification

Learning Objectives

By the end of this section, you will be able to do the following:

- Describe how viruses were first discovered and how they are detected
- Discuss three hypotheses about how viruses evolved
- Describe the general structure of a virus
- Recognize the basic shapes of viruses
- Understand past and emerging classification systems for viruses
- Describe the basis for the Baltimore classification system

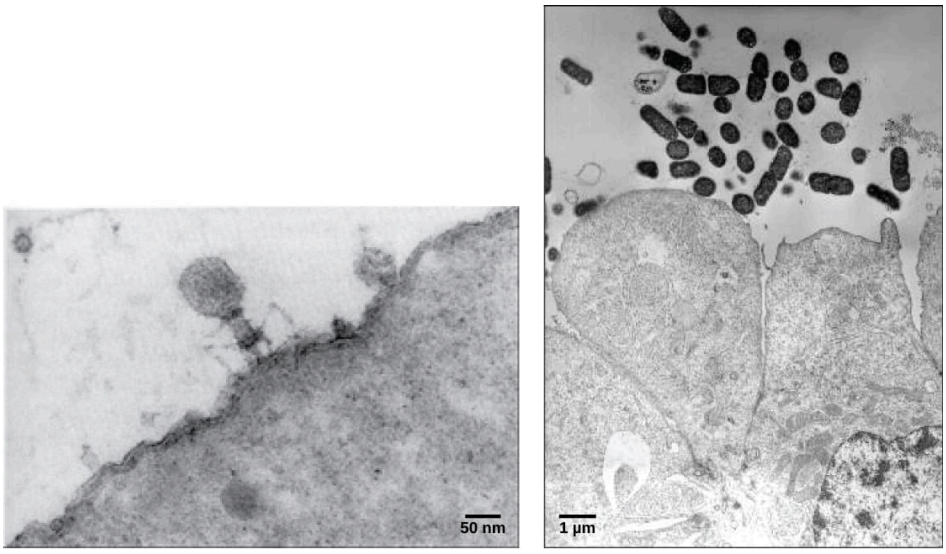
Viruses are noncellular parasitic entities that cannot be classified within any kingdom. They can infect organisms as diverse as bacteria, plants, and animals. In fact, viruses exist in a sort of netherworld between a living organism and a nonliving entity. Living things grow, metabolize, and reproduce. In contrast, viruses are not cellular, do not have a metabolism or grow, and cannot divide by cell division. Viruses *can* copy, or replicate themselves; however, they are entirely dependent on resources derived from their host cells to produce progeny viruses—which are assembled in their mature form. No one knows exactly when or how viruses evolved or from what ancestral source because viruses have not left a fossil record. Some virologists contend that modern viruses are a mosaic of bits and pieces of nucleic acids picked up from various sources along their respective evolutionary paths.

Viruses are diverse entities: They vary in structure, methods of replication, and the hosts they infect. Nearly all forms of life—from prokaryotic bacteria and archaeans, to eukaryotes such as plants, animals, and fungi—have viruses that infect them. While most biological diversity can be understood through evolutionary history (such as how species have adapted to changing environmental conditions and how different species are related to one another through common descent), much about virus origins and evolution remains unknown.

Discovery and Detection

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

Most virions, or single virus particles, are very small, about 20 to 250 nanometers in diameter. However, some recently discovered viruses from amoebae range up to 1000 nm in diameter. With the exception of large virions, like the poxvirus and other large DNA viruses, viruses cannot be seen with a light microscope. It was not until the development of the electron microscope in the late 1930s that scientists got their first good view of the structure of the tobacco mosaic virus (TMV) (Figure 21.1), discussed above, and other viruses (Figure 21.2). The surface structure of virions can be observed by both scanning and transmission electron microscopy, whereas the internal structures of the virus can only be observed in images from a transmission electron microscope. The use of electron microscopy and other technologies has allowed for the discovery of many viruses of all types of living organisms.



(a) (b)

Figure 21.2 Most virus particles are visible only by electron microscopy. In these transmission electron micrographs, (a) a virus is as dwarfed by the bacterial cell it infects, as (b) these *E. coli* cells are dwarfed by cultured colon cells. (credit a: modification of work by U.S. Dept. of Energy, Office of Science, LBL, PBD; credit b: modification of work by J.P. Nataro and S. Sears, unpub. data, CDC; scale-bar data from Matt Russell)

Evolution of Viruses

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

Most scholars agree that viruses don't have a single common ancestor, nor is there a single reasonable hypothesis about virus origins. There are current evolutionary scenarios that may explain the origin of viruses. One such hypothesis, the "devolution" or the *regressive hypothesis*, suggests that viruses evolved from free-living cells, or from intracellular prokaryotic parasites. However, many components of how this process might have occurred remain a mystery. A second hypothesis, the escapist or the *progressive hypothesis*, suggests that viruses originated from RNA and DNA molecules, or self-replicating entities similar to transposons or other mobile genetic elements, that escaped from a host cell with the ability to enter another. A third hypothesis, the *virus first hypothesis,* suggests that viruses may have been the first self-replicating entities before the first cells. In all cases, viruses are probably continuing to evolve along with the cells on which they rely on as hosts.

As technology advances, scientists may develop and refine additional hypotheses to explain the origins of viruses. The emerging field called virus molecular systematics attempts to do just that through comparisons of sequenced genetic material. These researchers hope one day to better understand the origin of viruses—a discovery that could lead to advances in the treatments for the ailments they produce.

Viral Morphology

Viruses are noncellular, meaning they are biological entities that do not have a cellular structure. They therefore lack most of the components of cells, such as organelles, ribosomes, and the plasma membrane. A virion consists of a nucleic acid core, an outer protein coating or capsid, and sometimes an outer envelope made of protein and phospholipid membranes derived from the host cell. Viruses may also contain additional proteins, such as enzymes, within the capsid or attached to the viral genome. The most obvious difference between members of different viral families is the variation in their morphology, which is quite diverse. An interesting feature of viral complexity is that the complexity of the host does not necessarily correlate with the complexity of the virion. In fact, some of the most complex virion structures are found in the bacteriophages—viruses that infect the simplest living organisms, bacteria.

Morphology

Viruses come in many shapes and sizes, but these features are consistent for each viral family. As we have seen, all virions have a nucleic acid genome covered by a protective capsid. The proteins of the capsid are encoded in the viral genome, and are called *capsomeres*. Some viral capsids are simple helices or polyhedral "spheres," whereas others are quite complex in structure (Figure 21.3).

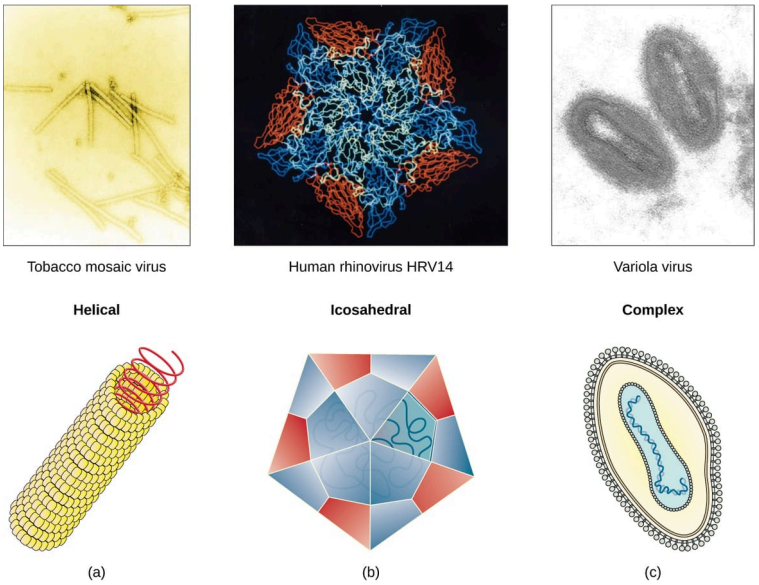


Figure 21.3 Viral capsids can be (a) helical, (b) polyhedral, or (c) have a complex shape. (credit a "micrograph": modification of work by USDA ARS; credit b "micrograph": modification of work by U.S. Department of Energy)

In general, the capsids of viruses are classified into four groups: helical, icosahedral, enveloped, and head-and-tail. *Helical capsids* are long and cylindrical. Many plant viruses are helical, including TMV. *Icosahedral viruses* have shapes that are roughly spherical, such as those of poliovirus or herpesviruses. *Enveloped viruses* have membranes derived from the host cell that surrounds the capsids. Animal viruses, such as HIV, are frequently enveloped. *Head-and-tail viruses* infect bacteria and have a head that is similar to icosahedral viruses and a tail shaped like helical viruses.

Many viruses use some sort of *glycoprotein* to attach to their host cells via molecules on the cell called *viral receptors*. For these viruses, attachment is required for later penetration of the cell membrane; only after penetration takes place can the virus complete its replication inside the cell. The receptors that viruses use are molecules that are normally found on cell surfaces and have their own physiological functions. It appears that viruses have simply evolved to make use of these molecules for their own replication. For example, HIV uses the CD4 molecule on T lymphocytes as one of its receptors (Figure 21.4). CD4 is a type of molecule called a *cell adhesion molecule*, which functions to keep different types of immune cells in close proximity to each other during the generation of a T lymphocyte immune response.

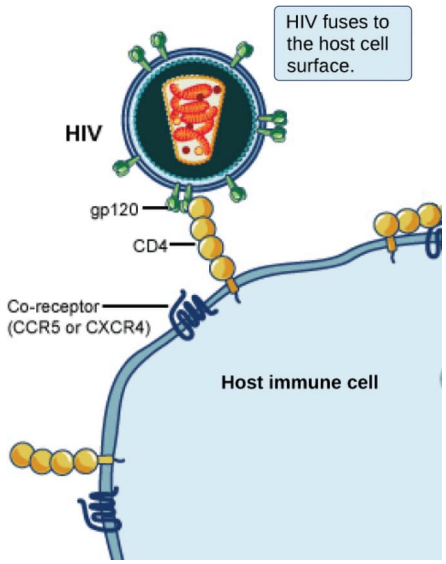


Figure 21.4 A virus and its host receptor protein. The HIV virus binds the CD4 receptor on the surface of human cells. CD4 receptors help white blood cells to communicate with other cells of the immune system when producing an immune response. (credit: modification of work by NIAID, NIH)

One of the most complex virions known, the T4 bacteriophage (which infects the *Escherichia coli*) bacterium, has a tail structure that the virus uses to attach to host cells and a head structure that houses its DNA.

Adenovirus, a non-enveloped animal virus that causes respiratory illnesses in humans, uses glycoprotein spikes protruding from its capsomeres to attach to host cells. Non-enveloped viruses also include those that cause polio (poliovirus), plantar warts (papillomavirus), and hepatitis A (hepatitis A virus).

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

Overall, the shape of the virion and the presence or absence of an envelope tell us little about what disease the virus may cause or what species it might infect, but they are still useful means to begin viral classification (Figure 21.5).

Visual Connection

Visual Connection

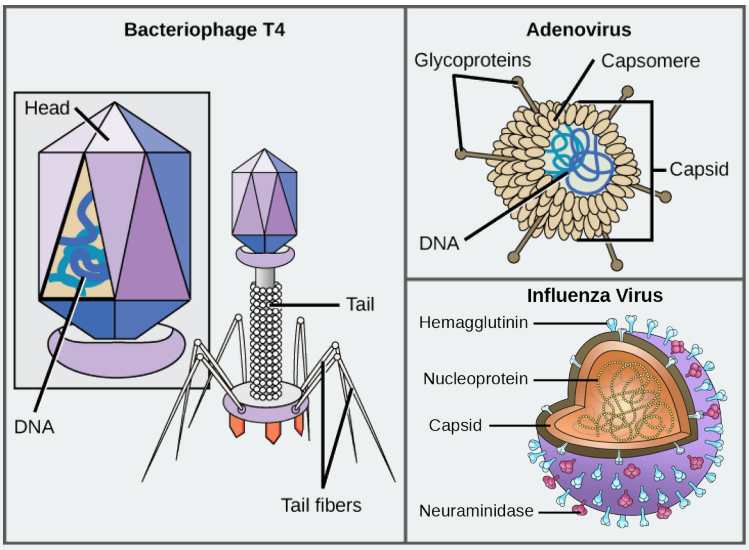


Figure 21.5 Complex Viruses. Viruses can be either complex or relatively simple in shape. This figure shows three relatively complex virions: the bacteriophage T4, with its DNA-containing head group and tail fibers that attach to host cells; adenovirus, which uses spikes from its capsid to bind to host cells; and the influenza virus, which uses glycoproteins embedded in its envelope to bind to host cells. The influenza virus also has matrix proteins, internal to the envelope, which help stabilize the virion's shape. (credit "bacteriophage, adenovirus": modification of work by NCBI, NIH; credit "influenza virus": modification of work by Dan Higgins, Centers for Disease Control and Prevention)

Which of the following statements about virus structure is true?

a. All viruses are encased in a viral membrane.
b. The capsomere is made up of small protein subunits called capsids.
c. DNA is the genetic material in all viruses.
d. Glycoproteins help the virus attach to the host cell.

Types of Nucleic Acid

Unlike nearly all living organisms that use DNA as their genetic material, viruses may use either DNA or RNA. The virus core contains the genome—the total genetic content of the virus. Viral genomes tend to be small, containing only those genes that encode proteins which the virus cannot get from the host cell. This genetic material may be single- or double-stranded. It may also be linear or circular. While most viruses contain a single nucleic acid, others have genomes divided into several segments. The RNA genome of the influenza virus is segmented, which contributes to its variability and continuous evolution, and explains why it is difficult to develop a vaccine against it.

In DNA viruses, the viral DNA directs the host cell's replication proteins to synthesize new copies of the viral genome and to transcribe and translate that genome into viral proteins. Human diseases caused by DNA viruses include chickenpox, hepatitis B, and adenoviruses. Sexually transmitted DNA viruses include the herpes virus and the human papilloma virus (HPV), which has been associated with cervical cancer and genital warts.

RNA viruses contain only RNA as their genetic material. To replicate their genomes in the host cell, the RNA viruses must encode their own enzymes that can replicate RNA into RNA or, in the retroviruses, into DNA. These *RNA polymerase enzymes* are more likely to make copying errors than DNA polymerases, and therefore often make mistakes during transcription. For this reason, mutations in RNA viruses occur more frequently than in DNA viruses. This causes them to change and adapt more rapidly to their host. Human diseases caused by RNA viruses include influenza, hepatitis C, measles, and rabies. The HIV virus, which is sexually transmitted, is an RNA retrovirus.

The Challenge of Virus Classification

Because most viruses probably evolved from different ancestors, the systematic methods that scientists have used to classify

prokaryotic and eukaryotic cells are not very useful. If viruses represent “remnants” of different organisms, then even genomic or protein analysis is not useful. Why?, *Because viruses have no common genomic sequence that they all share*. For example, the 16S rRNA sequence so useful for constructing prokaryote phylogenies is of no use for a creature with no ribosomes! Biologists have used several classification systems in the past. Viruses were initially grouped by shared morphology. Later, groups of viruses were classified by the type of nucleic acid they contained, DNA or RNA, and whether their nucleic acid was single- or double-stranded. However, these earlier classification methods grouped viruses differently, because they were based on different sets of characters of the virus. The most commonly used classification method today is called the Baltimore classification scheme, and is based on how messenger RNA (mRNA) is generated in each particular type of virus.

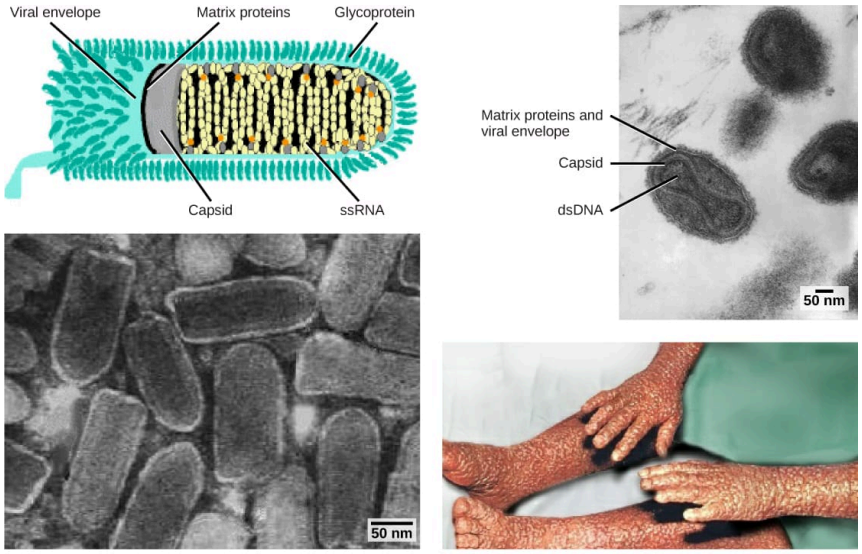
Past Systems of Classification

Viruses contain only a few elements by which they can be classified: the viral genome, the type of capsid, and the envelope structure for the enveloped viruses. All of these elements have been used in the past for viral classification (Table 21.1 and Figure 21.6). Viral genomes may vary in the type of genetic material (DNA or RNA) and its organization (single- or double-stranded, linear or circular, and segmented or non-segmented). In some viruses, additional proteins needed for replication are associated directly with the genome or contained within the viral capsid.

Virus Classification by Genome Structure

Genome Structure	Examples
• RNA • DNA	• Rabies virus, retroviruses • Herpesviruses, smallpox virus
• Single-stranded • Double-stranded	• Rabies virus, retroviruses • Herpesviruses, smallpox virus
• Linear • Circular	• Rabies virus, retroviruses, herpesviruses, smallpox virus • Papillomaviruses, many bacteriophages
• Non-segmented: genome consists of a single segment of genetic material • Segmented: genome is divided into multiple segments	• Parainfluenza viruses • Influenza viruses

Table 21.1



(a) Rabies virus (b) Variola virus

Figure 21.6 Viruses can be classified according to their core genetic material and capsid design. (a) Rabies virus has a single-stranded RNA (ssRNA) core and an enveloped helical capsid, whereas (b) variola virus, the causative agent of smallpox, has a double-stranded DNA (dsDNA) core and a complex capsid. Rabies transmission occurs when saliva from an infected mammal enters a wound. The virus travels through neurons in the peripheral nervous system to the central nervous system, where it impairs brain function, and then travels to other tissues. The virus can infect any mammal, and most die within weeks of infection. Smallpox is a human virus transmitted by inhalation of the variola virus, localized in the skin, mouth, and throat, which causes a characteristic rash. Before its eradication in 1979, infection resulted in a 30 to 35 percent mortality rate. (credit "rabies diagram": modification of work by CDC; "rabies micrograph": modification of work by Dr. Fred Murphy, CDC; credit "small pox micrograph": modification of work by Dr. Fred Murphy, Sylvia Whitfield, CDC; credit "smallpox photo": modification of work by CDC; scale-bar data from Matt Russell)

Viruses can also be classified by the design of their capsids (Table 21.2 and Figure 21.7). Capsids are classified as naked icosahedral, enveloped icosahedral, enveloped helical, naked helical, and complex. The type of genetic material (DNA or RNA) and its structure (single- or double-stranded, linear or circular, and segmented or non-segmented) are used to classify the virus core structures (Table 21.2).

Virus Classification by Capsid Structure

Capsid Classification	Examples
Naked icosahedral	Hepatitis A virus, polioviruses
Enveloped icosahedral	Epstein-Barr virus, herpes simplex virus, rubella virus, yellow fever virus, HIV-1
Enveloped helical	Influenza viruses, mumps virus, measles virus, rabies virus
Naked helical	Tobacco mosaic virus
Complex with many proteins; some have combinations of icosahedral and helical capsid structures	Herpesviruses, smallpox virus, hepatitis B virus, T4 bacteriophage

Table 21.2

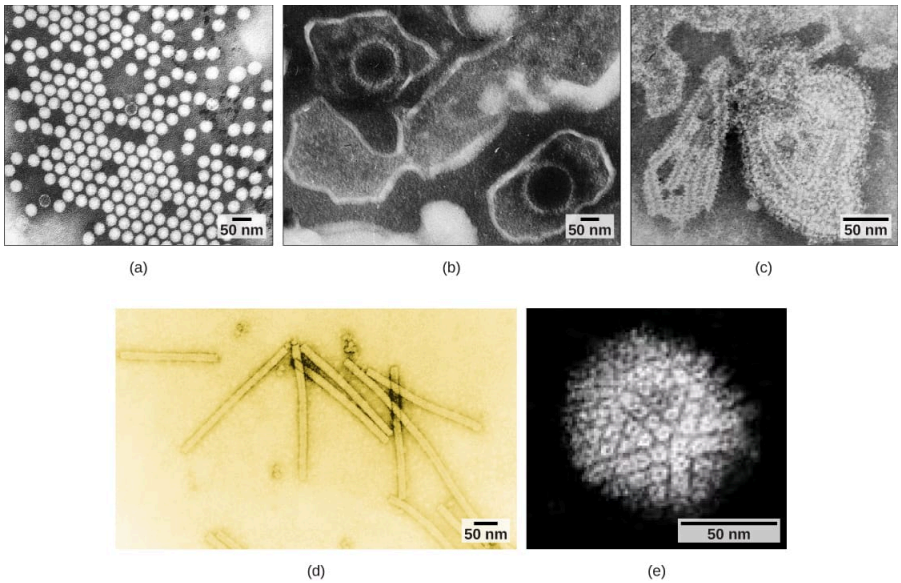


Figure 21.7 Transmission electron micrographs of various viruses show their capsid structures. The capsid of the (a) polio virus is naked icosahedral; (b) the Epstein-Barr virus capsid is enveloped icosahedral; (c) the mumps virus capsid is an enveloped helix; (d) the tobacco mosaic virus capsid is naked helical; and (e) the herpesvirus capsid is complex. (credit a: modification of work by Dr. Fred Murphy, Sylvia Whitfield; credit b: modification of work by Liza Gross; credit c: modification of work by Dr. F. A. Murphy, CDC; credit d: modification of work by USDA ARS; credit e: modification of work by Linda Stannard, Department of Medical Microbiology, University of Cape Town, South Africa, NASA; scale-bar data from Matt Russell)

Baltimore Classification

The most commonly and currently used system of virus classification was first developed by Nobel Prize-winning biologist David Baltimore in the early 1970s. In addition to the differences in morphology and genetics mentioned above, the Baltimore classification scheme groups viruses according to how the mRNA is produced during the replicative cycle of the virus.

Group I viruses contain double-stranded DNA (dsDNA) as their genome. Their mRNA is produced by transcription in much the same way as with cellular DNA, using the enzymes of the host cell.

Group II viruses have single-stranded DNA (ssDNA) as their genome. They convert their single-stranded genomes into a dsDNA intermediate before transcription to mRNA can occur.

Group III viruses use dsRNA as their genome. The strands separate, and one of them is used as a template for the generation of mRNA using the RNA-dependent RNA polymerase encoded by the virus.

Group IV viruses have ssRNA as their genome with a *positive polarity*, which means that the genomic RNA can serve directly as mRNA. Intermediates of dsRNA, called *replicative intermediates*, are made in the process of copying the genomic RNA. Multiple, full-length RNA strands of *negative polarity* (complementary to the positive-stranded genomic RNA) are formed from these intermediates, which may then serve as templates for the production of RNA with positive polarity, including both full-length genomic RNA and shorter viral mRNAs.

Group V viruses contain ssRNA genomes with a negative polarity, meaning that their sequence is complementary to the mRNA. As with Group IV viruses, dsRNA intermediates are used to make copies of the genome and produce mRNA. In this case, the negative-stranded genome can be converted directly to mRNA. Additionally, full-length positive RNA strands are made to serve as templates for the production of the negative-stranded genome.

Group VI viruses have diploid (two copies) ssRNA genomes that must be converted, using the enzyme reverse transcriptase, to dsDNA; the dsDNA is then transported to the nucleus of the host cell and inserted into the host genome. Then, mRNA can be produced by transcription of the viral DNA that was integrated into the host genome.

Group VII viruses have partial dsDNA genomes and make ssRNA intermediates that act as mRNA, but are also converted back into dsDNA genomes by reverse transcriptase, necessary for genome replication.

The characteristics of each group in the Baltimore classification are summarized in Table 21.3 with examples of each group.

Baltimore Classification

Group	Characteristics	Mode of mRNA Production	Example
I	Double-stranded DNA	mRNA is transcribed directly from the DNA template	Herpes simplex (herpesvirus)
II	Single-stranded DNA	DNA is converted to double-stranded form before RNA is transcribed	Canine parvovirus (parvovirus)
III	Double-stranded RNA	mRNA is transcribed from the RNA genome	Childhood gastroenteritis (rotavirus)
IV	Single stranded RNA (+)	Genome functions as mRNA	Common cold (picornavirus)
V	Single stranded RNA (-)	mRNA is transcribed from the RNA genome	Rabies (rhabdovirus)
VI	Single stranded RNA viruses with reverse transcriptase	Reverse transcriptase makes DNA from the RNA genome; DNA is then incorporated in the host genome; mRNA is transcribed from the incorporated DNA	Human immunodeficiency virus (HIV)
VII	Double stranded DNA viruses with reverse transcriptase	The viral genome is double-stranded DNA, but viral DNA is replicated through an RNA intermediate; the RNA may serve directly as mRNA or as a template to make mRNA	Hepatitis B virus (hepadnavirus)

Table 21.3

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3.4.2: Virus Infections and Hosts

Learning Objectives

By the end of this section, you will be able to do the following:

- List the steps of replication and explain what occurs at each step
- Describe the lytic and lysogenic cycles of virus replication
- Explain the transmission of plant and animal viruses
- Discuss some of the diseases caused by plant and animal viruses
- Discuss the economic impact of plant and animal viruses

Viruses are obligate, intracellular parasites. A virus must first recognize and attach to a specific living cell prior to entering it. After penetration, the invading virus must copy its genome and manufacture its own proteins. Finally, the progeny virions must escape the host cell so that they can infect other cells. Viruses can infect only certain species of hosts and only certain cells within that host. Specific host cells that a virus must occupy and use to replicate are called permissive. In most cases, the molecular basis for this specificity is due to a particular surface molecule known as the *viral receptor* on the host cell surface. A specific viral receptor is required for the virus to attach. In addition, differences in metabolism and host-cell immune responses (based on differential gene expression) are a likely factor in determining which cells a virus may target for replication.

Steps of Virus Infections

A virus must use its host-cell processes to replicate. The viral replication cycle can produce dramatic biochemical and structural changes in the host cell, which may cause cell damage. These changes, called cytopathic effects, can change cell functions or even destroy the cell. Some infected cells, such as those infected by the common cold virus known as rhinovirus, die through lysis (bursting) or apoptosis (programmed cell death or “cell suicide”), releasing all progeny virions at once. The symptoms of viral diseases result both from such cell damage caused by the virus and from the immune response to the virus, which attempts to control and eliminate the virus from the body.

Many animal viruses, such as HIV (human immunodeficiency virus), leave the infected cells of the immune system by a process known as *budding*, where virions leave the cell individually. During the budding process, the cell does not undergo lysis and is not immediately killed. However, the damage to the cells that the virus infects may make it impossible for the cells to function normally, even though the cells remain alive for a period of time. Most productive viral infections follow similar steps in the virus replication cycle: *attachment, penetration, uncoating, replication, assembly, and release* (Figure 21.8).

Attachment

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

Link to Learning

Link to Learning

This video explains how influenza attacks the body.

Entry

Viruses may enter a host cell either with or without the viral capsid. The nucleic acid of bacteriophages enters the host cell

"naked," leaving the capsid outside the cell. Plant and animal viruses can enter through *endocytosis* (as you may recall, the cell membrane surrounds and engulfs the entire virus). Some enveloped viruses enter the cell when the viral envelope fuses directly with the cell membrane. Once inside the cell, the viral capsid degrades, and then the viral nucleic acid is released and becomes available for replication and transcription.

Replication and Assembly

The replication mechanism depends on the viral genome. DNA viruses usually use host-cell proteins and enzymes to replicate the viral DNA and to transcribe viral mRNA, which is then used to direct viral protein synthesis. RNA viruses usually use the RNA core as a template for synthesis of viral genomic RNA and mRNA. The viral mRNA directs the host cell to synthesize viral enzymes and capsid proteins, and assemble new virions.

Of course, there are exceptions to this pattern. If a host cell does not provide the enzymes necessary for viral replication, viral genes supply the information to direct synthesis of the missing proteins. Retroviruses, such as HIV (group VI of the Baltimore classification scheme), have an RNA genome that must be reverse transcribed into DNA, which then is incorporated into the host cell genome. To convert RNA into DNA, retroviruses must contain genes that encode the virus-specific enzyme reverse transcriptase that transcribes an RNA template to DNA. Reverse transcription never occurs in uninfected host cells—the enzyme reverse transcriptase is only derived from the expression of viral genes within the infected host cells. The fact that HIV produces some of its own enzymes not found in the host has allowed researchers to develop drugs that inhibit these enzymes without affecting the host's metabolism.

This approach has led to the development of a variety of drugs used to treat HIV and has been effective at reducing the number of infectious virions (copies of viral RNA) in the blood to non-detectable levels in many HIV-infected individuals.

Egress

The last stage of viral replication is the release of the new virions produced in the host organism, where they are able to infect adjacent cells and repeat the replication cycle. As you've learned, some viruses are released when the host cell dies, and other viruses can leave infected cells by budding through the membrane without directly killing the cell.

Visual Connection

Visual Connection

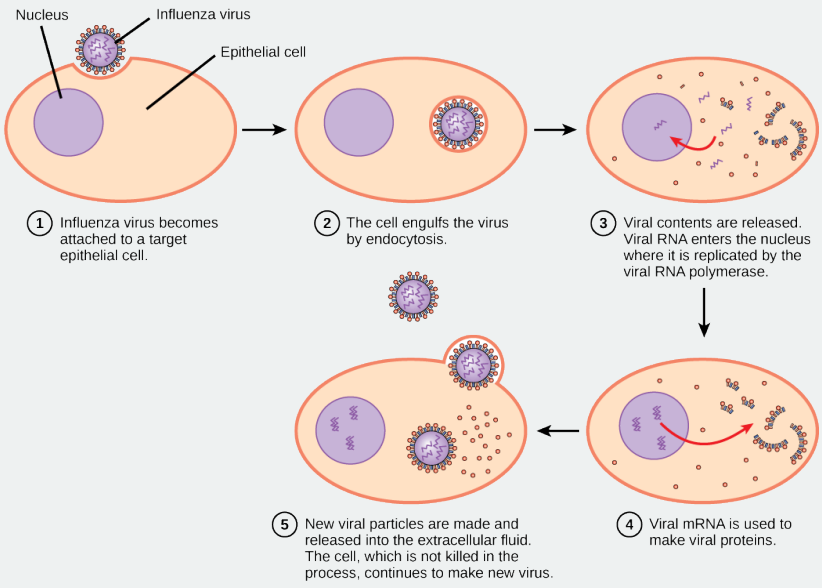


Figure 21.8 The influenza reproductive cycle. In influenza virus infection, glycoproteins on the capsid attach to a host epithelial cell. Following this, the virus is engulfed. RNA and proteins are then made and assembled into new virions.

Influenza virus is packaged in a viral envelope that fuses with the plasma membrane. This way, the virus can exit the host cell

without killing it. What advantage does the virus gain by keeping the host cell alive?

Link to Learning

Link to Learning

Watch a video on viruses, identifying structures, modes of transmission, replication, and more.

Different Hosts and Their Viruses

As you've learned, viruses often infect very specific hosts, as well as specific cells within the host. This feature of a virus makes it specific to one or a few species of life on Earth. On the other hand, so many different types of viruses exist on Earth that nearly every living organism has its own set of *viruses* trying to infect its cells. Even prokaryotes, the smallest and simplest of cells, may be attacked by specific types of viruses. In the following section, we will look at some of the features of viral infection of prokaryotic cells. As we have learned, viruses that infect bacteria are called *bacteriophages* (Figure 21.9). Archaea have their own similar viruses.

Bacteriophages



Figure 21.9 Bacteriophages attached to a host cell (transmission electron micrograph). In bacteriophage with tails, like the one shown here, the tails serve as a passageway for transmission of the phage genome. (credit: modification of work by Dr. Graham Beards; scale-bar data from Matt Russell)

Most bacteriophages are dsDNA viruses, which use host enzymes for DNA replication and RNA transcription. Phage particles must bind to specific surface receptors and actively insert the genome into the host cell. (The complex tail structures seen in many bacteriophages are actively involved in getting the viral genome across the prokaryotic cell wall.) When infection of a cell by a bacteriophage results in the production of new virions, the infection is said to be *productive*. If the virions are released by bursting the cell, the virus replicates by means of a *lytic cycle* (Figure 21.10). An example of a lytic bacteriophage is T4, which infects *Escherichia coli* found in the human intestinal tract. Sometimes, however, a virus can remain within the cell without being released. For example, when a temperate bacteriophage infects a bacterial cell, it replicates by means of a *lysogenic cycle* (Figure 21.10), and the viral genome is incorporated into the genome of the host cell. When the phage DNA is incorporated into the host-cell genome, it is called a prophage. An example of a lysogenic bacteriophage is the λ (lambda) virus, which also infects the *E. coli* bacterium. Viruses that infect plant or animal cells may sometimes undergo infections where they are not producing virions for long periods. An example is the animal *herpesviruses*, including herpes simplex viruses, the cause of oral and genital herpes in humans. In a process called latency, these viruses can exist in nervous tissue for long periods of time without producing new virions, only to leave latency periodically and cause lesions in the skin where the virus replicates. Even though there are similarities between lysogeny and latency, the term lysogenic cycle is usually reserved to describe bacteriophages. Latency will

be described in more detail in the next section.

Visual Connection

Visual Connection

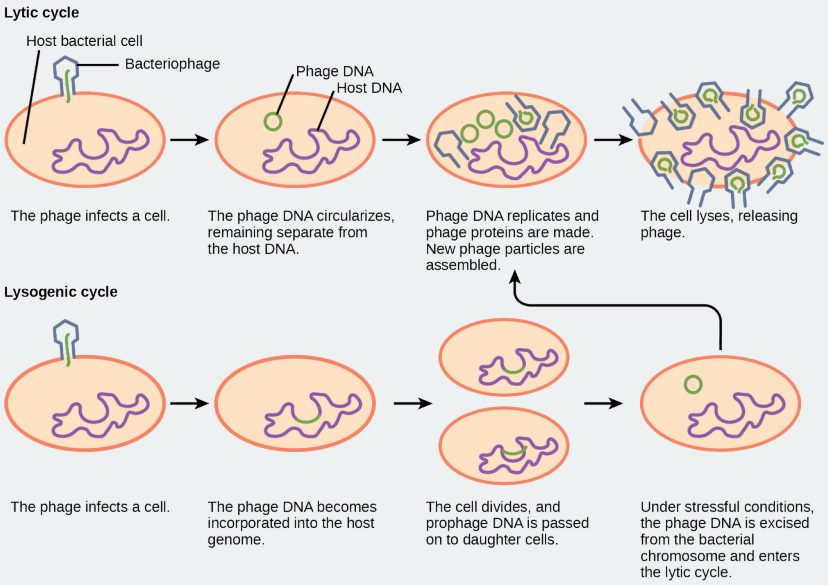


Figure 21.10 A temperate bacteriophage has both lytic and lysogenic cycles. In the lytic cycle, the phage replicates and lyses the host cell. In the lysogenic cycle, phage DNA is incorporated into the host genome, where it is passed on to subsequent generations. Environmental stressors such as starvation or exposure to toxic chemicals may cause the prophage to excise and enter the lytic cycle.

Which of the following statements is false?

a. In the lytic cycle, new phages are produced and released into the environment.
b. In the lysogenic cycle, phage DNA is incorporated into the host genome.
c. An environmental stressor can cause the phage to initiate the lysogenic cycle.
d. Cell lysis only occurs in the lytic cycle.

Plant Viruses

Most plant viruses, like the tobacco mosaic virus, have single-stranded (+) RNA genomes. However, there are also plant viruses in most other virus categories. Unlike bacteriophages, plant viruses do not have active mechanisms for delivering the viral genome across the protective cell wall. For a plant virus to enter a new host plant, some type of mechanical damage must occur. This damage is often caused by weather, insects, animals, fire, or human activities like farming or landscaping. Movement from cell to cell within a plant can be facilitated by viral modification of plasmodesmata (cytoplasmic threads that pass from one plant cell to the next). Additionally, plant offspring may inherit viral diseases from parent plants. Plant viruses can be transmitted by a variety of vectors, through contact with an infected plant's sap, by living organisms such as insects and nematodes, and through pollen. The transfer of a virus from one plant to another is known as horizontal transmission, whereas the inheritance of a virus from a parent is called vertical transmission.

Symptoms of viral diseases vary according to the virus and its host (Table 21.4). One common symptom is *hyperplasia*, the abnormal proliferation of cells that causes the appearance of plant tumors known as galls. Other viruses induce *hypoplasia*, or decreased cell growth, in the leaves of plants, causing thin, yellow areas to appear. Still other viruses affect the plant by directly killing plant cells, a process known as *cell necrosis*. Other symptoms of plant viruses include malformed leaves; black streaks on the stems of the plants; altered growth of stems, leaves, or fruits; and ring spots, which are circular or linear areas of discoloration found in a leaf.

Some Common Symptoms of Plant Viral Diseases

Symptom	Appears as
Hyperplasia	Galls (tumors)
Hypoplasia	Thinned, yellow splotches on leaves
Cell necrosis	Dead, blackened stems, leaves, or fruit
Abnormal growth patterns	Malformed stems, leaves, or fruit
Discoloration	Yellow, red, or black lines, or rings in stems, leaves, or fruit

Table 21.4

Plant viruses can seriously disrupt crop growth and development, significantly affecting our food supply. They are responsible for poor crop quality and quantity globally, and can bring about huge economic losses annually. Others viruses may damage plants used in landscaping. Some viruses that infect agricultural food plants include the name of the plant they infect, such as tomato spotted wilt virus, bean common mosaic virus, and cucumber mosaic virus. In plants used for landscaping, two of the most common viruses are peony ring spot and rose mosaic virus. There are far too many plant viruses to discuss each in detail, but symptoms of bean common mosaic virus result in lowered bean production and stunted, unproductive plants. In the ornamental rose, the rose mosaic disease causes wavy yellow lines and colored splotches on the leaves of the plant.

Animal Viruses

Animal viruses, unlike the viruses of plants and bacteria, do not have to penetrate a cell wall to gain access to the host cell. The virus may even induce the host cell to cooperate in the infection process. Non-enveloped or “naked” animal viruses may enter cells in two different ways. As a protein in the viral capsid binds to its receptor on the host cell, the virus may be taken inside the cell via a vesicle during the normal cell process of *receptor-mediated endocytosis*. An alternative method of cell penetration used by non-enveloped viruses is for capsid proteins to undergo shape changes after binding to the receptor, creating channels in the host cell membrane. The viral genome is then “injected” into the host cell through these channels in a manner analogous to that used by many bacteriophages.

Enveloped viruses also have two ways of entering cells after binding to their receptors: receptor-mediated endocytosis, or fusion. Many enveloped viruses enter the cell by receptor-mediated endocytosis in a fashion similar to that seen in some non-enveloped viruses. On the other hand, fusion only occurs with enveloped virions. These viruses, which include HIV among others, use special fusion proteins in their envelopes to cause the envelope to fuse with the plasma membrane of the cell, thus releasing the genome and capsid of the virus into the cell cytoplasm.

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

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

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

As already discussed, herpes simplex virus can remain in a state of latency in nervous tissue for months, even years. As the virus "hides" in the tissue and makes few if any viral proteins, there is nothing for the immune response to act against, and immunity to the virus slowly declines. Under certain conditions, including various types of physical and psychological stress, the latent herpes simplex virus may be reactivated and undergo a lytic replication cycle in the skin, causing the lesions associated with the disease. Once virions are produced in the skin and viral proteins are synthesized, the immune response is again stimulated and resolves the skin lesions in a few days or weeks by destroying viruses in the skin. As a result of this type of replicative cycle, appearances of cold sores and genital herpes outbreaks only occur intermittently, even though the viruses remain in the nervous tissue for life. Latent infections are common with other herpesviruses as well, including the varicella-zoster virus that causes chickenpox. After having a chickenpox infection in childhood, the varicella-zoster virus can remain latent for many years and reactivate in adults to cause the painful condition known as "shingles" (Figure 21.11).

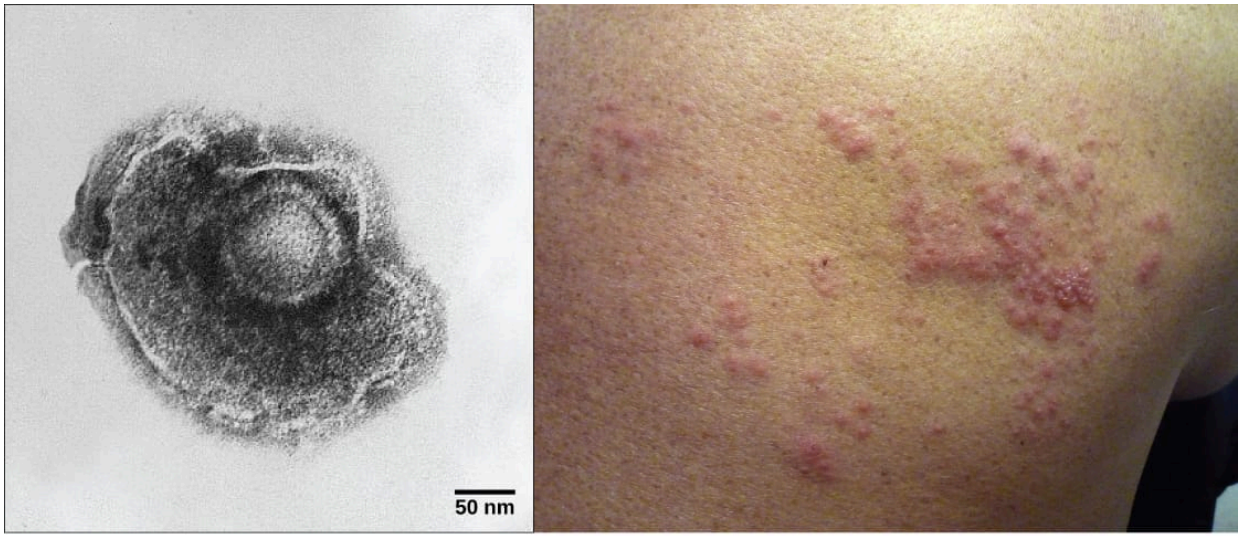


Figure 21.11 A latent virus infection. (a) Varicella-zoster, the virus that causes chickenpox, has an enveloped icosahedral capsid visible in this transmission electron micrograph. Its double-stranded DNA genome becomes incorporated in the host DNA and can reactivate after latency in the form of (b) shingles, often exhibiting a rash. (credit a: modification of work by Dr. Erskine Palmer, B. G. Martin, CDC; credit b: modification of work by "rosmary"/Flickr; scale-bar data from Matt Russell)

Some animal-infecting viruses, including the hepatitis C virus discussed above, are known as oncogenic viruses: They have the ability to cause cancer. These viruses interfere with the normal regulation of the host cell cycle either by introducing genes that stimulate unregulated cell growth (oncogenes) or by interfering with the expression of genes that inhibit cell growth. Oncogenic viruses can be either DNA or RNA viruses. Cancers known to be associated with viral infections include cervical cancer, caused by human papillomavirus (HPV) (Figure 21.12), liver cancer caused by hepatitis B virus, T-cell leukemia, and several types of lymphoma.

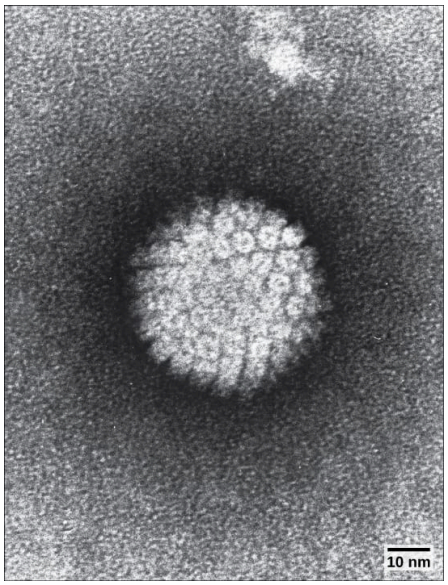


Figure 21.12 HPV, or human papillomavirus, has a naked icosahedral capsid visible in this transmission electron micrograph and a double-stranded DNA genome that is incorporated into the host DNA. The virus, which is sexually transmitted, is oncogenic and can lead to cervical cancer. (credit: modification of work by NCI, NIH; scale-bar data from Matt Russell)

Link to Learning

Link to Learning

This video shows the life cycle of a virus.

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3.4.3: Prevention and Treatment of Viral Infections

Learning Objectives

By the end of this section, you will be able to do the following:

- Identify major viral illnesses that affect humans
- Compare vaccinations and anti-viral drugs as medical approaches to viruses

Viruses cause a variety of diseases in animals, including humans, ranging from the common cold to potentially fatal illnesses like meningitis (Figure 21.13). These diseases can be treated by antiviral drugs or by vaccines; however, some viruses, such as HIV, are capable both of avoiding the immune response and of mutating within the host organism to become resistant to antiviral drugs.

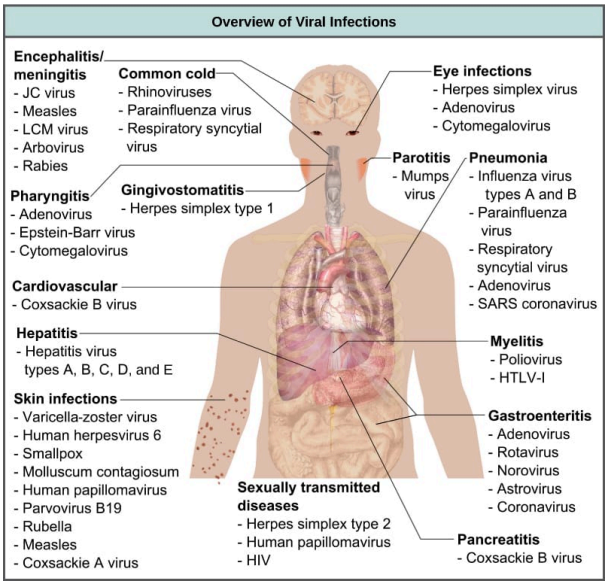


Figure 21.13 A sampling of human viruses. Viruses can cause dozens of ailments in humans, ranging from mild illnesses to serious diseases. (credit: modification of work by Mikael Häggström)

Vaccines for Prevention

The primary method of controlling viral disease is by vaccination, which is intended to prevent outbreaks by building immunity to a virus or virus family (Figure 21.14). Vaccines may be prepared using live viruses, killed viruses, or molecular subunits of the virus. Note that the killed viral vaccines and subunit viruses are both incapable of causing disease, nor is there any valid evidence that vaccinations contribute to autism.

Live viral vaccines are designed in the laboratory to cause few symptoms in recipients while giving them protective immunity against future infections. Polio was one disease that represented a milestone in the use of vaccines. Polio epidemics occurred with increasing frequency and impact as the twentieth century progressed, becoming a terrifying and tragic event each summer. Tens of thousands of people died and many more were paralyzed; children made up a large portion of the victims. Using killed virus tested on HeLa cell line (originally obtained from Henrietta Lacks and then mass produced to meet the need), Jonas Salk developed a successful vaccine. Mass immunization campaigns in the 1950s (killed vaccine) and 1960s (live vaccine) significantly reduced the incidence of the disease. The success of the polio vaccine paved the way for the routine dispensation of childhood vaccines against measles, mumps, rubella, chickenpox, and other diseases.

The issue with using live vaccines (which are usually more effective than killed vaccines), is the low but significant danger that these viruses will revert to their disease-causing form by back mutations. Live vaccines are usually made by attenuating (weakening) the “wild-type” (disease-causing) virus by growing it in the laboratory in tissues or at temperatures different from

what the virus is accustomed to in the host. Adaptations to these new cells or temperatures induce mutations in the genomes of the virus, allowing it to grow better in the laboratory while inhibiting its ability to cause disease when reintroduced into conditions found in the host. These attenuated viruses thus still cause infection, but they do not grow very well, allowing the immune response to develop in time to prevent major disease. Back mutations occur when the vaccine undergoes mutations in the host such that it readapts to the host and can again cause disease, which can then be spread to other humans in an epidemic. This type of scenario happened as recently as 2007 in Nigeria where mutations in a polio vaccine led to an epidemic of polio in that country.

Some vaccines are in continuous development because certain viruses, such as influenza and HIV, have a high mutation rate compared to that of other viruses and normal host cells. With influenza, mutations in the surface molecules of the virus help the organism evade the protective immunity that may have been obtained in a previous influenza season, making it necessary for individuals to get vaccinated every year. Other viruses, such as those that cause the childhood diseases measles, mumps, and rubella, mutate so infrequently that the same vaccine is used year after year.



Figure 21.14 Vaccinations are designed to boost immunity to a virus to prevent infection. (credit: Navy Medicine)

Link to Learning

Link to Learning

Watch this NOVA video to learn how microbiologists are attempting to replicate the deadly 1918 Spanish influenza virus so they can understand more about virology.

Vaccines and Antiviral Drugs for Treatment

In some cases, vaccines can be used to treat an active viral infection. The concept behind this is that by giving the vaccine, immunity is boosted without adding more disease-causing virus. In the case of *rabies*, a fatal neurological disease transmitted via the saliva of rabies virus-infected animals, the progression of the disease from the time of the animal bite to the time it enters the central nervous system may be two weeks or longer. This is enough time to vaccinate individuals who suspect that they have been bitten by a rabid animal, and their boosted immune response is sufficient to prevent the virus from entering nervous tissue. Thus, the potentially fatal neurological consequences of the disease are averted, and the individual only has to recover from the infected bite. This approach is also being used for the treatment of Ebola, one of the fastest and most deadly viruses on Earth. Transmitted by bats and great apes, this disease can cause death in 70 to 90 percent of infected humans within two weeks. Using newly developed vaccines that *boost the immune response* in this way, there is hope that affected individuals will be better able to control the virus, potentially saving a greater percentage of infected persons from a rapid and very painful death.

Another way of treating viral infections is the use of antiviral drugs. Because viruses use the resources of the host cell for replication and the production of new virus proteins, it is difficult to block their activities without damaging the host. For many

years, scientists thought that drugs capable of impacting viruses would be too toxic for the body to endure. To meet this challenge, researcher Gertrude Elion sought to develop drugs that would target only the virus through processes such as inhibiting only viral DNA replication. For example, some of her medicines focused on purines, while others affected DNA polymerase. (Elion has 45 patents ranging from antivirals to immunosuppressants to cancer drugs.) However, we do have some effective antiviral drugs, such as those used to treat HIV and influenza. Some antiviral drugs are specific for a particular virus and others have been used to control and reduce symptoms for a wide variety of viral diseases. For most viruses, antiviral drugs can inhibit the virus by blocking the actions of one or more of its proteins. *It is important to note that the targeted proteins be encoded by viral genes and that these molecules are not present in a healthy host cell.* In this way, viral growth is inhibited without damaging the host. Elion's work with George Hitchens not only led to direct treatments, but, more importantly, changed the entire methodology of drug development. By targeting specific aspects of tumor cells, viruses, and bacteria, they laid the groundwork for many of today's most common and important medicines, used to help millions of people each year. They were awarded the Nobel Prize in 1988.

Antivirals have been developed to treat genital herpes (herpes simplex II) and influenza. For genital herpes, drugs such as acyclovir, developed by Elion, can reduce the number and duration of episodes of active viral disease, during which patients develop viral lesions in their skin cells. As the virus remains latent in nervous tissue of the body for life, this drug is not curative but can make the symptoms of the disease more manageable. For influenza, drugs like Tamiflu (oseltamivir) (Figure 21.15) can reduce the duration of “flu” symptoms by one or two days, but the drug does not prevent symptoms entirely. Tamiflu works by inhibiting an enzyme (viral neuraminidase) that allows new virions to leave their infected cells. Thus, Tamiflu inhibits the spread of virus from infected to uninfected cells. Other antiviral drugs, such as Ribavirin, have been used to treat a variety of viral infections, although its mechanism of action against certain viruses remains unclear.

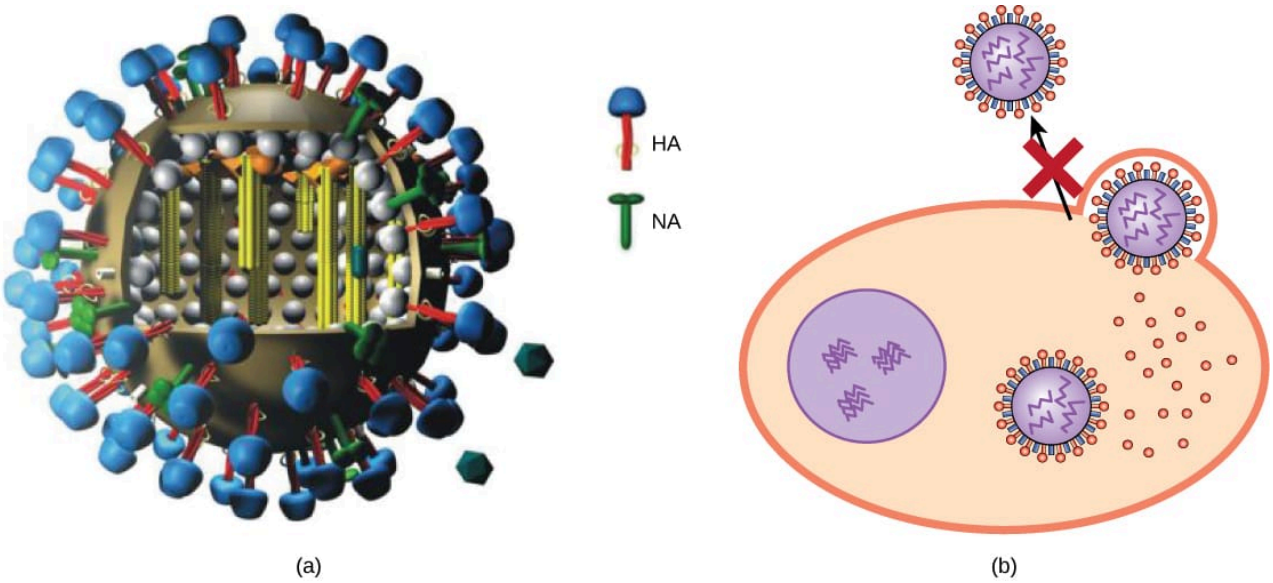


Figure 21.15 Action of an antiviral drug. (a) Tamiflu inhibits a viral enzyme called neuraminidase (NA) found in the influenza viral envelope. (b) Neuraminidase cleaves the connection between viral hemagglutinin (HA), also found in the viral envelope, and glycoproteins on the host cell surface. Inhibition of neuraminidase prevents the virus from detaching from the host cell, thereby blocking further infection. (credit a: modification of work by M. Eickmann)

By far, the most successful use of antivirals has been in the treatment of the retrovirus HIV, which causes a disease that, if untreated, is usually fatal within 10 to 12 years after infection. Anti-HIV drugs have been able to control viral replication to the point that individuals receiving these drugs survive for a significantly longer time than the untreated.

A particular challenge with HIV is its tendency to mutate quickly within the body of an individual patient. This leads to individual drug resistance, and requires a different treatment strategy than many other diseases. David Ho was among the first to propose and develop a method to treat multiple mutations of HIV at the same time. Ho's efforts were a turning point in fighting AIDS. Anti-HIV drugs inhibit viral replication at many different phases of the HIV replicative cycle (Figure 21.16). Drugs have been developed that inhibit the fusion of the HIV viral envelope with the plasma membrane of the host cell (fusion inhibitors), the conversion of its RNA genome into double-stranded DNA (reverse transcriptase inhibitors, like AZT), the integration of the viral DNA into the host genome (integrase inhibitors), and the processing of viral proteins (protease inhibitors).

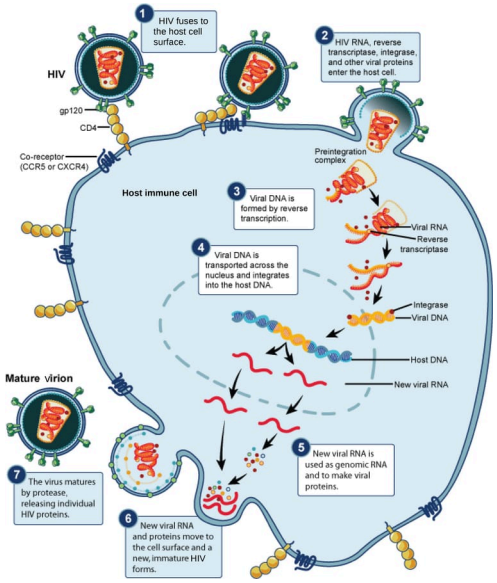


Figure 21.16 Life cycle of HIV. HIV, an enveloped, icosahedral virus, attaches to the CD4 receptor of an immune cell and fuses with the cell membrane. Viral contents are released into the cell, where viral enzymes convert the single-stranded RNA genome into DNA and incorporate it into the host genome. (credit: NIAID, NIH)

Unfortunately, when any of these drugs are used individually, the high mutation rate of the virus allows it to easily and rapidly develop resistance to the drug, limiting the drug's effectiveness. The breakthrough in the treatment of HIV was the development of HAART, *highly active anti-retroviral therapy*, which involves a mixture of different drugs, sometimes called a drug "cocktail." By attacking the virus at different stages of its replicative cycle, it is much more difficult for the virus to develop resistance to multiple drugs at the same time. Still, even with the use of combination HAART therapy, there is concern that, over time, the virus will develop resistance to this therapy. Thus, new anti-HIV drugs are constantly being developed with the hope of continuing the battle against this highly fatal virus.

Everyday Connection

Everyday Connection

Applied Virology:The study of viruses has led to the development of a variety of new ways to treat non-viral diseases. Viruses have been used in gene therapy. Gene therapy is used to treat genetic diseases such as severe combined immunodeficiency (SCID), a heritable, recessive disease in which children are born with severely compromised immune systems. One common type of SCID is due to the lack of an enzyme, adenosine deaminase (ADA), which breaks down purine bases. To treat this disease by gene therapy, bone marrow cells are taken from a SCID patient and the ADA gene is inserted. This is where viruses come in, and their use relies on their ability to penetrate living cells and bring genes in with them. Viruses such as adenovirus, an upper-respiratory human virus, are modified by the addition of the ADA gene, and the virus then transports this gene into the cell. The modified cells, now capable of making ADA, are then given back to the patients in the hope of curing them. Gene therapy using viruses as carriers of genes (viral vectors), although still experimental, holds promise for the treatment of many genetic diseases. Still, many technological problems need to be solved for this approach to be a viable method for treating genetic disease.

Another medical use for viruses relies on their specificity and ability to kill the cells they infect. Oncolytic viruses are engineered in the laboratory specifically to attack and kill cancer cells. A genetically modified adenovirus known as H101 has been used since 2005 in clinical trials in China to treat head and neck cancers. The results have been promising, with a greater short-term response rate to the combination of chemotherapy and viral therapy than to chemotherapy treatment alone. This ongoing research may herald the beginning of a new age of cancer therapy, where viruses are engineered to find and specifically kill cancer cells, regardless of where in the body they may have spread.

A third use of viruses in medicine relies on their specificity and involves using bacteriophages in the treatment of bacterial infections. Bacterial diseases have been treated with antibiotics since the 1940s. However, over time, many bacteria have evolved resistance to antibiotics. A good example is methicillin-resistant *Staphylococcus aureus* (MRSA, pronounced

"mersa"), an infection commonly acquired in hospitals. This bacterium is resistant to a variety of antibiotics, making it difficult to treat. The use of bacteriophages specific for such bacteria would bypass their resistance to antibiotics and specifically kill them. Although phage therapy is in use in the Republic of Georgia to treat antibiotic-resistant bacteria, its use to treat human diseases has not been approved in most countries. However, the safety of the treatment was confirmed in the United States when the U.S. Food and Drug Administration approved spraying meats with bacteriophages to destroy the food pathogen *Listeria*. As more and more antibiotic-resistant strains of bacteria evolve, the use of bacteriophages might be a potential solution to the problem, and the development of phage therapy is of much interest to researchers worldwide.

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3.4.4: Key Terms

acellular - lacking cells

acute disease - disease where the symptoms rise and fall within a short period of time

asymptomatic disease - disease where there are no symptoms and the individual is unaware of being infected unless lab tests are performed

attenuation - weakening of a virus during vaccine development

bacteriophage - virus that infects bacteria

capsid - protein coating of the viral core

cell necrosis - cell death

chronic infection - describes when the virus persists in the body for a long period of time

cytopathic - causing cell damage

envelope - lipid bilayer that encircles some viruses

fusion - method of entry by some enveloped viruses, where the viral envelope fuses with the plasma membrane of the host cell

gall - appearance of a plant tumor

gene therapy - treatment of genetic disease by adding genes, using viruses to carry the new genes inside the cell

horizontal transmission - transmission of a disease between unrelated individuals

hyperplasia - abnormally high cell growth and division

hypoplasia - abnormally low cell growth and division

latency - virus that remains in the body for a long period of time but only causes intermittent symptoms

lysis - bursting of a cell

lysogenic cycle - type of virus replication in which the viral genome is incorporated into the genome of the host cell

lytic cycle - type of virus replication in which virions are released through lysis, or bursting, of the cell

oncogenic virus - virus that has the ability to cause cancer

oncolytic virus - virus engineered to specifically infect and kill cancer cells

pathogen - agent with the ability to cause disease

phage therapy - treatment of bacterial diseases using bacteriophages specific to a particular bacterium

prophage - phage DNA that is incorporated into the host cell genome

vaccine - weakened solution of virus components, viruses, or other agents that produce an immune response

viral receptor - glycoprotein used to attach a virus to host cells via molecules on the cell

virion - individual virus particle outside a host cell

viroid - plant pathogen that produces only a single, specific RNA

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4: CENTRAL DOGMA

4.1: The Cell Cycle
4.2: DNA Structure
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4.1: The Cell Cycle

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 produce two genetically identical cells. The cell cycle has two major phases: interphase and the mitotic phase (Figure 4.1.1). 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.

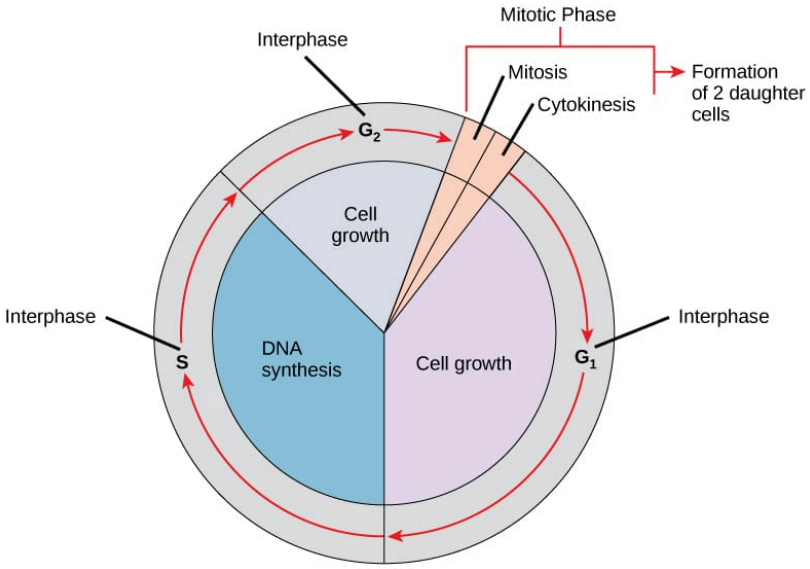


Figure 4.1.1**:** A cell moves through a series of phases in an orderly manner. During interphase, G_1 involves cell growth and protein synthesis, the S phase involves DNA replication and the replication of the centrosome, and G_2 involves further growth and protein synthesis. The mitotic phase follows interphase. Mitosis is nuclear division during which duplicated chromosomes are segregated and distributed into daughter nuclei. Usually the cell will divide after mitosis in a process called cytokinesis in which the cytoplasm is divided and two daughter cells are formed.

Interphase

During interphase, the cell undergoes normal processes while also preparing for cell division. For a cell to move from interphase to the mitotic phase, many internal and external conditions must be met. The three stages of interphase are called G_1, S, and G_2.

G_1 Phase

The first stage of interphase is called the G_1 phase, or first gap, because little change is visible. However, during the G_1 stage, the cell is quite active at the biochemical level. The cell is accumulating the building blocks of chromosomal DNA and the associated proteins, as well as accumulating enough energy reserves to complete the task of replicating each chromosome in the nucleus.

S Phase

Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the S phase (synthesis phase), DNA replication results in the formation of two identical copies of each chromosome—sister chromatids—that are firmly attached at the centromere region. At this stage, each chromosome is made of two sister chromatids and is a duplicated chromosome. The centrosome is duplicated during the S phase. The two centrosomes will give rise to the mitotic spindle, the apparatus that orchestrates the movement of chromosomes during mitosis. The centrosome consists of a pair of rod-like centrioles at right angles to each other. Centrioles help organize cell division. Centrioles are not present in the centrosomes of many eukaryotic species, such as plants and most fungi.

G_2 Phase

In the G_2 phase, or second gap, the cell replenishes its energy stores and synthesizes the proteins necessary for chromosome manipulation. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic spindle. There may be additional cell growth during G_2. The final preparations for the mitotic phase must be completed before the cell is able to enter the first stage of mitosis.

The Mitotic Phase

To make two daughter cells, the contents of the nucleus and the cytoplasm must be divided. The mitotic phase is a multistep process during which the duplicated chromosomes are aligned, separated, and moved to opposite poles of the cell, and then the cell is divided into two new identical daughter cells. The first portion of the mitotic phase, mitosis, is composed of five stages, which accomplish nuclear division. The second portion of the mitotic phase, called cytokinesis, is the physical separation of the cytoplasmic components into two daughter cells.

Mitosis

Mitosis is divided into a series of phases—prophase, prometaphase, metaphase, anaphase, and telophase—that result in the division of the cell nucleus (Figure 4.1.2).

ART CONNECTION

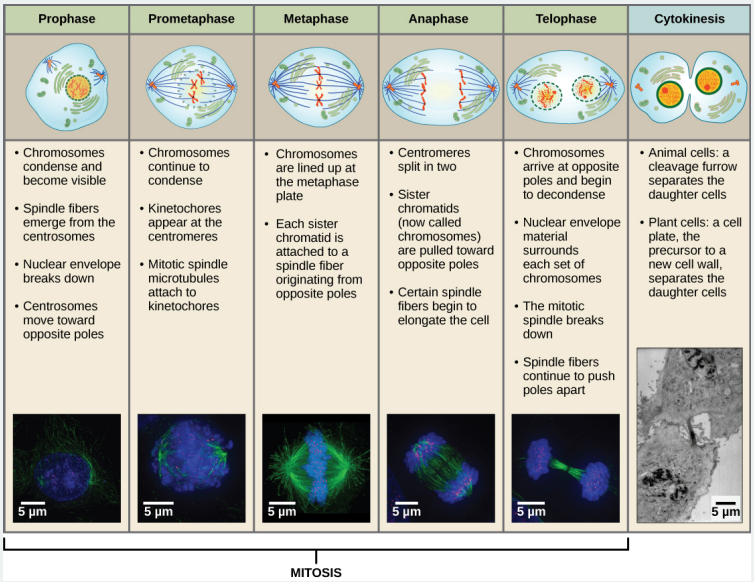


Figure 4.1.2**:** Animal cell mitosis is divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase—visualized here by light microscopy with fluorescence. Mitosis is usually accompanied by cytokinesis, shown here by a transmission electron microscope. (credit "diagrams": modification of work by Mariana Ruiz Villareal; credit "mitosis micrographs": modification of work by Roy van Heesbeen; credit "cytokinesis micrograph": modification of work by the Wadsworth Center, NY State Department of Health; donated to the Wikimedia foundation; scale-bar data from Matt Russell)

Which of the following is the correct order of events in mitosis?

A. Sister chromatids line up at the metaphase plate. The kinetochore becomes attached to the mitotic spindle. The nucleus re-forms and the cell divides. The sister chromatids separate.
B. The kinetochore becomes attached to the mitotic spindle. The sister chromatids separate. Sister chromatids line up at the metaphase plate. The nucleus re-forms and the cell divides.
C. The kinetochore becomes attached to metaphase plate. Sister chromatids line up at the metaphase plate. The kinetochore breaks down and the sister chromatids separate. The nucleus re-forms and the cell divides.

D. The kinetochore becomes attached to the mitotic spindle. Sister chromatids line up at the metaphase plate. The kinetochore breaks apart and the sister chromatids separate. The nucleus re-forms and the cell divides.

During prophase, the “first phase,” several events must occur to provide access to the chromosomes in the nucleus. The nuclear envelope starts to break into small vesicles, and the Golgi apparatus and endoplasmic reticulum fragment and disperse to the periphery of the cell. The nucleolus disappears. The centrosomes begin to move to opposite poles of the cell. The microtubules that form the basis of the mitotic spindle extend between the centrosomes, pushing them farther apart as the microtubule fibers lengthen. The sister chromatids begin to coil more tightly and become visible under a light microscope.

During prometaphase, many processes that were begun in prophase continue to advance and culminate in the formation of a connection between the chromosomes and cytoskeleton. The remnants of the nuclear envelope disappear. The mitotic spindle continues to develop as more microtubules assemble and stretch across the length of the former nuclear area. Chromosomes become more condensed and visually discrete. Each sister chromatid attaches to spindle microtubules at the centromere via a protein complex called the kinetochore.

During metaphase, all of the chromosomes are aligned in a plane called the metaphase plate, or the equatorial plane, midway between the two poles of the cell. The sister chromatids are still tightly attached to each other. At this time, the chromosomes are maximally condensed.

During anaphase, the sister chromatids at the equatorial plane are split apart at the centromere. Each chromatid, now called a chromosome, is pulled rapidly toward the centrosome to which its microtubule was attached. The cell becomes visibly elongated as the non-kinetochore microtubules slide against each other at the metaphase plate where they overlap.

During telophase, all of the events that set up the duplicated chromosomes for mitosis during the first three phases are reversed. The chromosomes reach the opposite poles and begin to decondense (unravel). The mitotic spindles are broken down into monomers that will be used to assemble cytoskeleton components for each daughter cell. Nuclear envelopes form around chromosomes.

CONCEPT IN ACTION

This page of movies illustrates different aspects of mitosis. Watch the movie entitled “DIC microscopy of cell division in a newt lung cell” and identify the phases of mitosis.

Cytokinesis

Cytokinesis is the second part of the mitotic phase during which cell division is completed by the physical separation of the cytoplasmic components into two daughter cells. Although the stages of mitosis are similar for most eukaryotes, the process of cytokinesis is quite different for eukaryotes that have cell walls, such as plant cells.

In cells such as animal cells that lack cell walls, cytokinesis begins following the onset of anaphase. A contractile ring composed of actin filaments forms just inside the plasma membrane at the former metaphase plate. The actin filaments pull the equator of the cell inward, forming a fissure. This fissure, or “crack,” is called the cleavage furrow. The furrow deepens as the actin ring contracts, and eventually the membrane and cell are cleaved in two (Figure 4.1.3).

In plant cells, a cleavage furrow is not possible because of the rigid cell walls surrounding the plasma membrane. A new cell wall must form between the daughter cells. During interphase, the Golgi apparatus accumulates enzymes, structural proteins, and glucose molecules prior to breaking up into vesicles and dispersing throughout the dividing cell. During telophase, these Golgi vesicles move on microtubules to collect at the metaphase plate. There, the vesicles fuse from the center toward the cell walls; this structure is called a cell plate. As more vesicles fuse, the cell plate enlarges until it merges with the cell wall at the periphery of the cell. Enzymes use the glucose that has accumulated between the membrane layers to build a new cell wall of cellulose. The Golgi membranes become the plasma membrane on either side of the new cell wall (Figure 4.1.3).

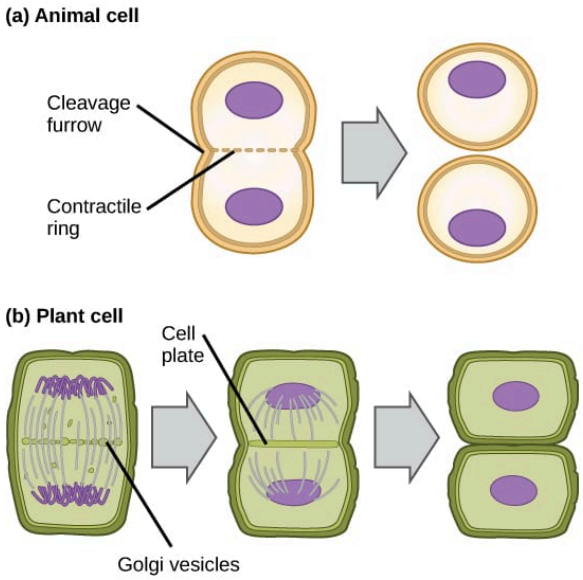


Figure 4.1.3**:** In part (a), a cleavage furrow forms at the former metaphase plate in the animal cell. The plasma membrane is drawn in by a ring of actin fibers contracting just inside the membrane. The cleavage furrow deepens until the cells are pinched in two. In part (b), Golgi vesicles coalesce at the former metaphase plate in a plant cell. The vesicles fuse and form the cell plate. The cell plate grows from the center toward the cell walls. New cell walls are made from the vesicle contents.

G_0 Phase

Not all cells adhere to the classic cell-cycle pattern in which a newly formed daughter cell immediately enters interphase, closely followed by the mitotic phase. Cells in the G_0 phase are not actively preparing to divide. The cell is in a quiescent (inactive) stage, having exited the cell cycle. Some cells enter G_0 temporarily until an external signal triggers the onset of G_1. Other cells that never or rarely divide, such as mature cardiac muscle and nerve cells, remain in G_0 permanently (Figure 4.1.4).

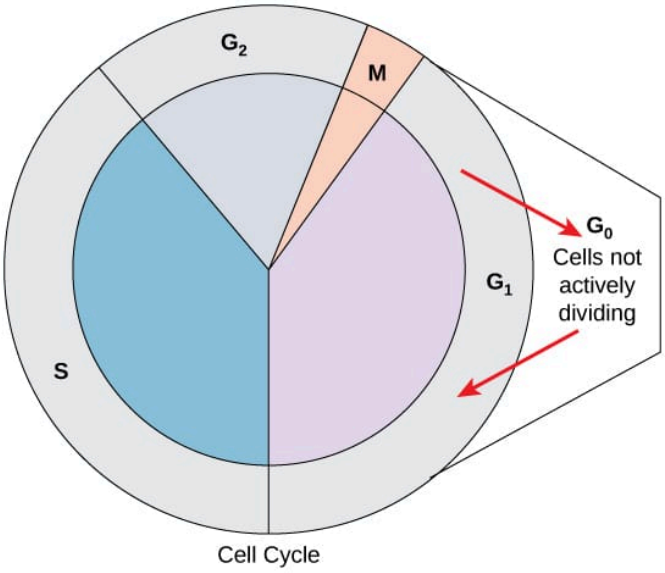


Figure 4.1.4: Cells that are not actively preparing to divide enter an alternate phase called G_0. In some cases, this is a temporary condition until triggered to enter G_1. In other cases, the cell will remain in G_0 permanently.

Control of the Cell Cycle

The length of the cell cycle is highly variable even within the cells of an individual organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development to an average of two to five days for epithelial cells, or to an

entire human lifetime spent in G_0 by specialized cells such as cortical neurons or cardiac muscle cells. There is also variation in the time that a cell spends in each phase of the cell cycle. When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is approximately 24 hours. In rapidly dividing human cells with a 24-hour cell cycle, the G_1 phase lasts approximately 11 hours. The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

Regulation at Internal Checkpoints

It is essential that daughter cells be exact duplicates of the parent cell. Mistakes in the duplication or distribution of the chromosomes lead to mutations that may be passed forward to every new cell produced from the abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell cycle checkpoints at which the cell cycle can be stopped until conditions are favorable. These checkpoints occur near the end of G_1, at the G_2–M transition, and during metaphase (Figure 4.1.5).

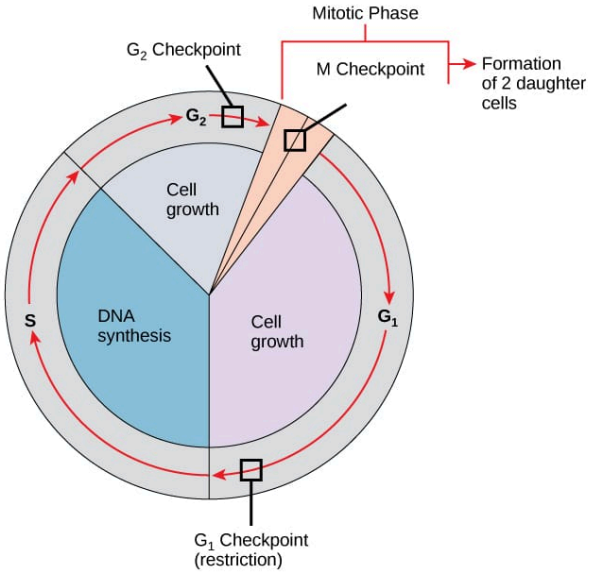


Figure 4.1.5: The cell cycle is controlled at three checkpoints. Integrity of the DNA is assessed at the G_1 checkpoint. Proper chromosome duplication is assessed at the G_2 checkpoint. Attachment of each kinetochore to a spindle fiber is assessed at the M checkpoint.

The G_1 Checkpoint

The G_1 checkpoint determines whether all conditions are favorable for cell division to proceed. The G_1 checkpoint, also called the restriction point, is the point at which the cell irreversibly commits to the cell-division process. In addition to adequate reserves and cell size, there is a check for damage to the genomic DNA at the G_1 checkpoint. A cell that does not meet all the requirements will not be released into the S phase.

The G_2 Checkpoint

The G_2 checkpoint bars the entry to the mitotic phase if certain conditions are not met. As in the G_1 checkpoint, cell size and protein reserves are assessed. However, the most important role of the G_2 checkpoint is to ensure that all of the chromosomes have been replicated and that the replicated DNA is not damaged.

The M Checkpoint

The M checkpoint occurs near the end of the metaphase stage of mitosis. The M checkpoint is also known as the spindle checkpoint because it determines if all the sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until the kinetochores of each pair of sister chromatids are firmly anchored to spindle fibers arising from opposite poles of the cell.

CONCEPT IN ACTION

Watch what occurs at the G_1, G_2, and M checkpoints by visiting this animation of the cell cycle.

Summary

The cell cycle is an orderly sequence of events. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages. In eukaryotes, the cell cycle consists of a long preparatory period, called interphase. Interphase is divided into G_1, S, and G_2 phases. Mitosis consists of five stages: prophase, prometaphase, metaphase, anaphase, and telophase. Mitosis is usually accompanied by cytokinesis, during which the cytoplasmic components of the daughter cells are separated either by an actin ring (animal cells) or by cell plate formation (plant cells).

Each step of the cell cycle is monitored by internal controls called checkpoints. There are three major checkpoints in the cell cycle: one near the end of G_1, a second at the G_2–M transition, and the third during metaphase.

Art Connections

Figure 4.1.2: Which of the following is the correct order of events in mitosis?

A. Sister chromatids line up at the metaphase plate. The kinetochore becomes attached to the mitotic spindle. The nucleus re-forms and the cell divides. The sister chromatids separate.
B. The kinetochore becomes attached to the mitotic spindle. The sister chromatids separate. Sister chromatids line up at the metaphase plate. The nucleus re-forms and the cell divides.
C. The kinetochore becomes attached to metaphase plate. Sister chromatids line up at the metaphase plate. The kinetochore breaks down and the sister chromatids separate. The nucleus re-forms and the cell divides.
D. The kinetochore becomes attached to the mitotic spindle. Sister chromatids line up at the metaphase plate. The kinetochore breaks apart and the sister chromatids separate. The nucleus re-forms and the cell divides.

Answer

D. The kinetochore becomes attached to the mitotic spindle. Sister chromatids line up at the metaphase plate. The kinetochore breaks apart and the sister chromatids separate. The nucleus reforms and the cell divides.

Glossary

anaphase - the stage of mitosis during which sister chromatids are separated from each other

cell cycle - the ordered sequence of events that a cell passes through between one cell division and the next

cell cycle checkpoints - mechanisms that monitor the preparedness of a eukaryotic cell to advance through the various cell cycle stages

cell plate - a structure formed during plant-cell cytokinesis by Golgi vesicles fusing at the metaphase plate; will ultimately lead to formation of a cell wall to separate the two daughter cells

centriole - a paired rod-like structure constructed of microtubules at the center of each animal cell centrosome

cleavage furrow - a constriction formed by the actin ring during animal-cell cytokinesis that leads to cytoplasmic division

cytokinesis - the division of the cytoplasm following mitosis to form two daughter cells

G_0 phase - a cell-cycle phase distinct from the G_1 phase of interphase; a cell in G_0 is not preparing to divide

G_1 phase - (also, first gap) a cell-cycle phase; first phase of interphase centered on cell growth during mitosis

G_2 phase - (also, second gap) a cell-cycle phase; third phase of interphase where the cell undergoes the final preparations for mitosis

interphase - the period of the cell cycle leading up to mitosis; includes G_1, S, and G_2 phases; the interim between two consecutive cell divisions

kinetochore - a protein structure in the centromere of each sister chromatid that attracts and binds spindle microtubules during prometaphase

metaphase plate - the equatorial plane midway between two poles of a cell where the chromosomes align during metaphase

metaphase - the stage of mitosis during which chromosomes are lined up at the metaphase plate

mitosis - the period of the cell cycle at which the duplicated chromosomes are separated into identical nuclei; includes prophase, prometaphase, metaphase, anaphase, and telophase

mitotic phase - the period of the cell cycle when duplicated chromosomes are distributed into two nuclei and the cytoplasmic contents are divided; includes mitosis and cytokinesis

mitotic spindle - the microtubule apparatus that orchestrates the movement of chromosomes during mitosis

prometaphase - the stage of mitosis during which mitotic spindle fibers attach to kinetochores

prophase - the stage of mitosis during which chromosomes condense and the mitotic spindle begins to form

quiescent - describes a cell that is performing normal cell functions and has not initiated preparations for cell division

S phase - the second, or synthesis phase, of interphase during which DNA replication occurs

telophase - the stage of mitosis during which chromosomes arrive at opposite poles, decondense, and are surrounded by new nuclear envelopes

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

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4.2: DNA Structure

Biotech Focus

Viewing the three dimensional structures of molecules, like DNA, remained a challenge in the early part of the twentieth century. Following the discovery of X-rays in 1895, researchers soon proposed that the atomic and molecular structure of a compound, such as a crystal, could be determined by beaming X-rays at them and observing their diffraction patterns. From this work, the field of **X-ray crystallography** was born. X-ray crystallography has been fundamental in numerous scientific breakthroughs, including determining the size of atoms, the lengths and types of chemical bonds, and revealing the structure and function of biological molecules, such as proteins and nucleic acids. Dorothy Crowfoot Hodgkin used X-ray crystallography to determine the structures of cholesterol (1937), penicillin (1946), and vitamin B12 (1956), for which she was awarded the Nobel Prize in Chemistry in 1964. Rosalind Franklin, the British X-ray crystallographer, was one of the first to produce high quality X-ray diffraction images of DNA. From her work, we now know the intricacies of the DNA helix. Today, over 130,000 X-ray crystal structures of proteins, nucleic acids, and other biological molecules have been determined.

Introduction

The first recorded discovery of DNA came in 1869, when an unknown substance found in the pus of wounded soldiers was described by the Swiss physician Friedrich Miescher. As it was found in nuclei of cells, he named this substance "nuclein". In 1878, Albrecht Kossel isolated the nucleic acid component of nuclein. In 1909, Phoebus Levene identified the base, sugar and phosphate components of a nucleotide and would later suggest that DNA consists of a string of four nucleotides (A, C, T, G) linked together through the phosphate groups. In 1910, Albrecht Kossel received the Nobel Prize in Physiology or Medicine for his discovery of the four bases of DNA, as well as of uracil in RNA. In 1928, Frederick Griffith showed that a molecule obtained from pathogenic *Streptococcus pneumonia* strains could be introduced into healthy *S. pneumonia* cells, "transforming" them into pathogenic cells. While we now know that Griffith introduced DNA into these bacteria, he did not know the chemical identity of this transforming molecule. It wasn't until the 1930s, that O. Avery, C. MacLeod, and M. McCarty, using improved purification techniques, showed that the transforming molecule was, in fact, DNA. In spite of these results, DNA was not readily accepted as the genetic material. Confirmation of this would come from the experiments of Alfred Hershey and Martha Chase, who determined that DNA could be passed from bacteriophage to *E.coli* cells. For his contributions to determining DNA as the “stuff of genes”, Hershey shared the 1969 Nobel Prize in Physiology or Medicine with Max Delbruck and Salvador E. Luria.

Learning Objectives

Understanding how DNA is used in biotechnology requires that you understand the structure of this molecule. In this this section, you will be able to:

- Discuss who "discovered the DNA helix"
- Explain the structure of a nucleotide
- Describe how DNA is structured
- Describe the forms of DNA is found in cells: chromatin, chromosomes and plasmids
- Compare and contrast prokaryotic and eukaryotic DNA
- Explain DNA packaging in eukaryotes

The History of the DNA Helix

The history of determining the structure of the DNA helix is rich and long. In 1937, William Astbury used **X-ray crystallography**, a method for investigating molecular structure by observing the patterns formed by X-rays shot through a crystal of a substance, to show that DNA had a regular structure. In 1952, Rosalind Franklin, a researcher in the lab of Maurice Wilkins at King's College, together with her research assistant, Raymond Gosling, produced one of the most well-known X-ray diffraction images of the DNA helix, known as "Photo 51" (Figure 4.2.1). This image helped solidify the theory that DNA was a double helix. At the same time, James Watson and Francis Crick, working together at the University of Cambridge, had started to build a model of the DNA helix.

Using data from other research groups, including that of Rosalind Franklin and Erwin Chargaff, they eventually pieced together the puzzle of the DNA helix and published their seminal work in the journal Nature in 1953. In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine for their work on DNA structure. Because Nobel Prizes are not awarded posthumously, Rosalind Franklin did not receive this award, having died of ovarian cancer in 1958. Several controversies still exist surrounding the discovery of the DNA helix, including the story that James Watson was shown Photo 51 without Rosalind Franklin's permission. Crick and Watson also received a copy of a report containing several of Franklin's crystallographic calculations. How much of this data contributed to their final DNA model will never be known. However, to simply attribute the discovery of the DNA helix to just James Watson and Francis Crick would discount the critical contributions of researchers like William Astbury, Erwin Chargaff, Raymond Gosling, and Rosalind Franklin. Today our knowledge of DNA structure is because of all of them.

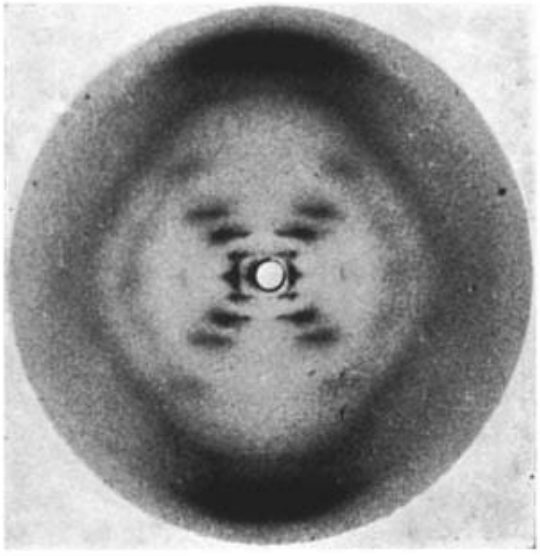


Figure 4.2.1: Photo 51, an X-ray diffraction image of the DNA helix. (Photo 51 by Raymond Gosling, public domain)

Interestingly, in an ironic twist, the Russian biologist Nikolai Koltsov proposed, in 1927, the presence of a "giant hereditary molecule" in cells made up of "two mirror strands that would replicate in a semi-conservative fashion using each strand as a template". A pretty fantastic theory since it was proposed long before Watson, Crick, Gosling, and Franklin worked out the structure of the DNA double-helix.

DNA is Made of Nucleotides and Forms a Double Helix

The DNA molecule is a nucleic acid polymer made of subunits called nucleotides. A **nucleotide** (Figure 4.2.2) consists of three chemical groups:

- a **pentose sugar**
- one to three **phosphate groups**
- a **nitrogenous base**

The sugar component of a nucleotide is a pentose sugar made of five carbons. The carbons are numbered clockwise from the oxygen as 1', 2', 3', 4', and 5' (e.g. 1' is read as “one prime”). In the case of DNA, this pentose sugar is called **deoxyribose** as it lacks an oxygen at the 2' carbon. The nitrogenous bases of DNA are **adenine, guanine, cytosine,** and **thymine** (Figure 4.2.2). Adenine and guanine are known as the **purines**, whereas cytosine and thymine are classified as **pyrimidines**. An introduction to nucleic acids can be found in Chapter 2.7: Nucleotides.

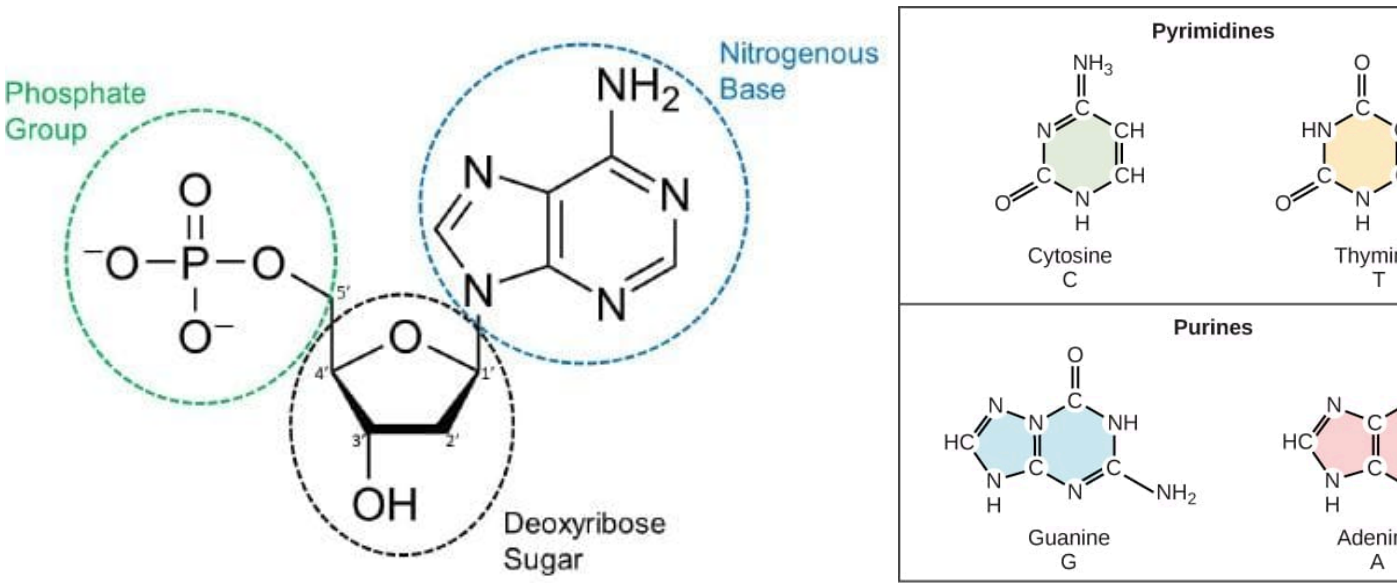


Figure 4.2.2: Nucleotide structure. Left image: The nucleotide consists of three chemical groups: the pentose sugar (black circle), the nitrogenous base attached to the 1' carbon of the sugar (blue circle), and the phosphate group attached to the 5' carbon (green circle); Right image: DNA bases are carbon-based ring structures that are classified as either purines (guanine, adenine) or as pyrimidines (cytosine, thymine). (Nucleotide Structure by Patricia Zuk, CC 4.0; adapted from DAMP chemical structure by Hbf878, CC0 1.0 and OpenStax, CC BY-SA 4.0)

Making a DNA molecule requires linking nucleotides so that they form a long polynucleotide chain. Joining nucleotides is done by forming a **phosphodiester bond** between the phosphate group found attached to the 5' carbon of one nucleotide and the hydroxyl group attached to the 3' carbon of the next nucleotide. The resulting single-strand of DNA has its sugar–phosphate groups lined up as a “backbone” with the nucleotide bases sticking out from this backbone. However, DNA is not a single-strand but two strands that form a coiled **double helix (**Figure 4.2.3). The two DNA strands of the helix have the opposite orientation, or run "anti-parallel" to each other. Hydrogen bonds form between complementary bases (i.e., adenine with thymine; cytosine with guanine), linking the two strands together along their length. Refer back to Chapter 2.7: Nucleotides to read more about the structure of the DNA helix.

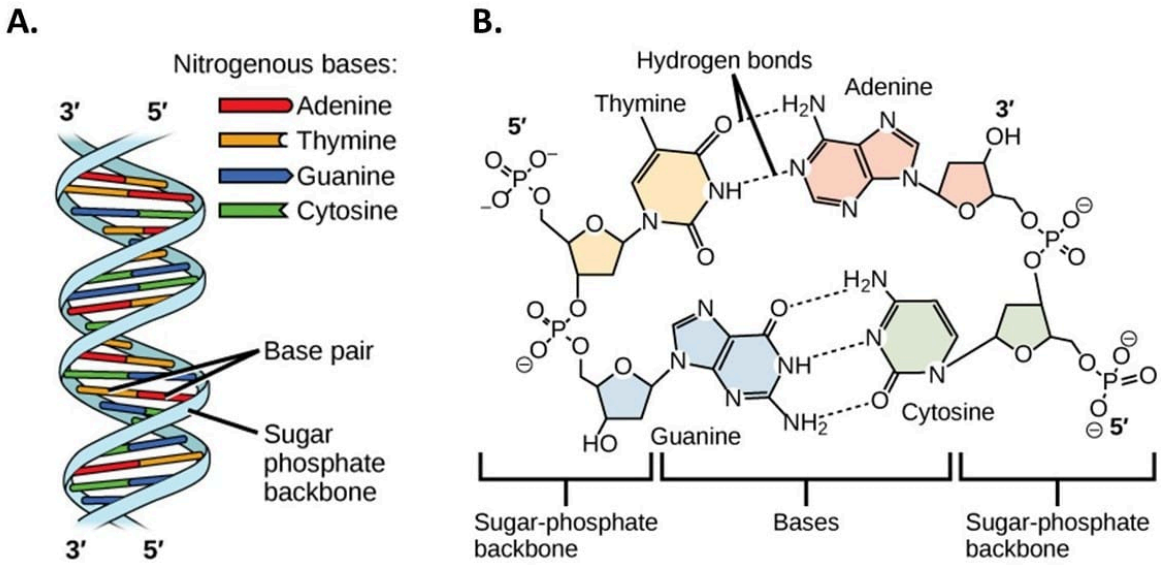


Figure 4.2.3: The DNA Helix. (A) The DNA molecule is made of two single polynucleotide strands joined together to form a helix with the sugar-phosphate backbone (blue curved lines) found on the periphery and the complementary bases (adenine and thymine; guanine and cytosine) connected to each other in the center of the helix. The two DNA strands forming the helix run anti-parallel to each other; (B) Complementary nitrogenous bases connect to each other via hydrogen bonds. (DNA Helix by Jerome Walker and Denis Myts, Concepts of Biology, CC BY-SA 4.0)

DNA Comes in Many Forms: Chromatin, Chromosomes, and Plasmids

Stretched end-to-end, a DNA molecule in a single human cell would come to a length of about 2 meters. The DNA of *E. coli* is about 1000 times as long as the bacterial cell itself. As a result, DNA must be packaged in a very ordered way to fit and function within a cell. The strategy used to package DNA is dependent upon whether the cell is prokaryotic or eukaryotic.

Prokaryotic DNA

Prokaryotic cells, because of the absence of a nucleus, package the majority of their DNA into a region of the cytoplasm known as the **nucleoid**. In most prokaryotic cells, the DNA found within the nucleoid is a large circular piece of double-stranded DNA called a **bacterial chromosome** or **genophore** (Figure 4.2.4). However, other bacteria may have linear chromosomes found within this region.

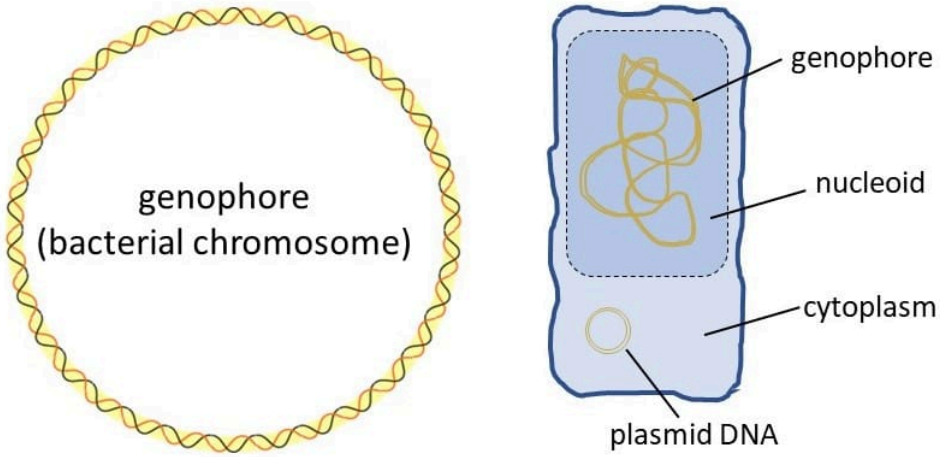


Figure 4.2.4: The nucleoid and genophore. Left image - the bacterial chromosome is a large, circular piece of double-stranded DNA called the genophore. Right image - the genophore is found within the nucleoid region of the cytoplasm. Outside of the nucleoid, bacteria can have smaller pieces of circular DNA, called plasmids. (The Genophore by Patricia Zuk, CC BY 4.0; adapted from Bacterial Transformation by Rosanna Hartline, CC BY-SA 4.0)

To ensure the bacterial chromosome can fit into the nucleoid, it is packaged through **supercoiling.** Supercoiling happens in both prokaryotic and eukaryotic cells when the DNA helix is subject to strain by being either underwound or overwound (Figure 4.2.5). A good analogy for supercoiling is the twists found in a telephone cord. Most DNA found in bacterial cells is underwound, or negatively supercoiled, because it makes the separation of the two DNA strands easier during DNA replication and transcription. Several structural proteins and enzymes within the nucleoid help to maintain the supercoiled nature of the bacterial chromosome. One of these enzymes is known as a **topoisomerase**.

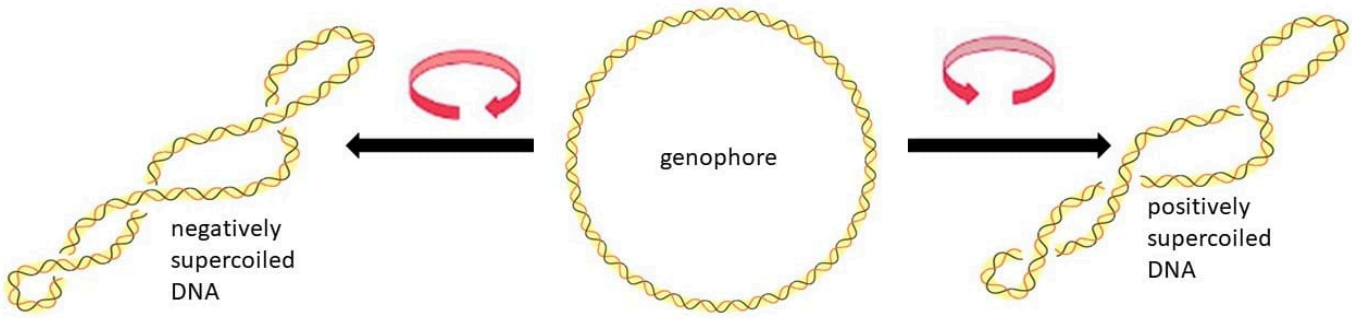


Figure 4.2.5: Supercoiled DNA. The bacterial chromosome, or genophore (center image), found within the nucleoid region, can be underwound or negatively supercoiled (left image) or can be overwound or positively supercoiled (right image). (Supercoiled DNA by Patricia Zuk, CC BY 4.0; adapted from DNA Topoisomers by Fdardel, CC BY-SA 4.0)

The bacterial chromosome contains the genes responsible for the day-to-day functions of the cell. However, in addition to the chromosome, bacteria also have smaller pieces of circular DNA known as plasmids. A **plasmid** is a small, extrachromosomal DNA molecule that is found outside of the nucleoid and can replicate independently of the bacterial chromosome. Naturally occurring plasmids contain genes for specialized functions, such as antibiotic resistance, virulence, nitrogen fixation, or conjugation. They are significantly smaller than the bacterial chromosome, typically several thousand base pairs, although plasmids as large as several hundred thousand base pairs have been found.

In addition, plasmids can be artificially constructed and introduced into bacterial cells. These types of plasmids (Figure 4.2.6) serve as tools in genetics and biotechnology labs, where they are used to clone and amplify genes for commercial applications. Plasmids that are sold commercially contain several regions in common with naturally occurring ones, such as antibiotic resistance genes. In addition, they will have a region for the insertion of genes, referred to as a **multiple cloning site** or **MCS**. This MCS is flanked by a **promoter sequence** and a **polyadenylation signal** so that the inserted gene will be properly transcribed into mRNA by the bacterial cell. More about these flanking regions of DNA can be found in Chapter 3.3 Transcription of RNA.

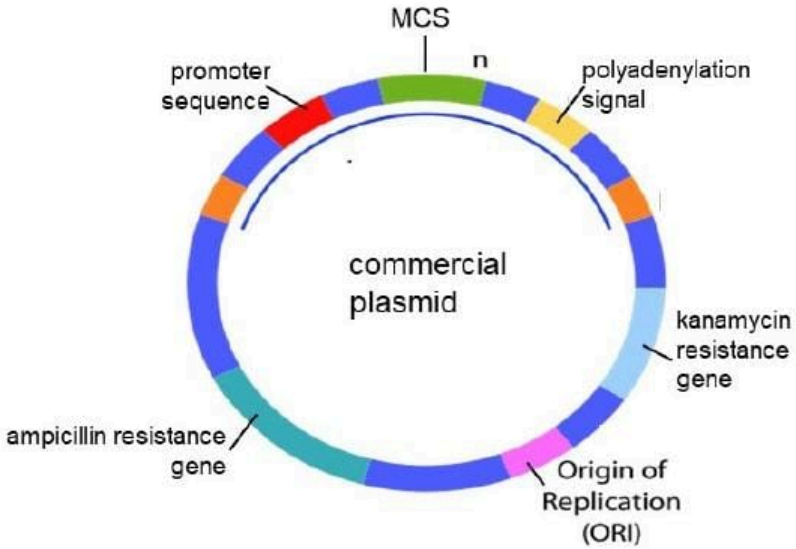


Figure 4.2.6: Plasmid DNA. The elements of a typical commercial plasmid includes sequences such as resistance genes for antibiotics like ampicillin or kanamycin, a multi-cloning site (MCS), a promoter sequence, and an origin of replication. (Ti plasmid by Mouagip, Public Domain)

Eukaryotic DNA

If the DNA of all 46 human chromosomes were stitched together into a single molecule, it would be over 2 meters long. This is a problem that all eukaryotic cell have - the DNA is too long to fit into a nucleus. As such, eukaryotic cells must employ a unique type of packaging strategy to fit their DNA inside the nucleus. Eukaryotes begin to package their DNA by converting it into a

form called **chromatin**. Chromatin is a complex of DNA and proteins. Eukaryotic cells have two types of chromatin: **euchromatin** and **heterochromatin**. In euchromatin, the DNA is found associated with proteins known as **histones**. A histone is often referred to as a **octamer** - a complex of 8 small, positively charged protein subunits (H2A, H2B, H3, and H4) found in the histone as pairs (Figure 4.2.7). To form euchromatin, the DNA helix wraps around a histone octamer almost two times, creating a structure called a **nucleosome**. The DNA is then secured to the histone octamer by the histone protein, H1. The interaction between the DNA and the histone to form a nucleosome is simply the result of the attraction between negatively charged DNA and positively charged histone proteins. The nucleosome is linked to the next one by a short strand of "linker" DNA that is free of histones and is essentially the DNA helix. The length of linker DNA varies from cell type to cell type but is consistent within the cell, producing nucleosomes at regular intervals. Because of its appearance under the electron microscope, euchromatin is frequently referred to as "beads on a string", with the "bead" as the nucleosome and the "string" the linker DNA (Figure 4.2.7). Once formed, certain sections of euchromatin condense further when neighboring nucleosomes stack compactly onto each other to form a 30-nm–wide fiber called **heterochromatin**. Heterochromatin condensing is thought to be the result of interactions between the H1 proteins.

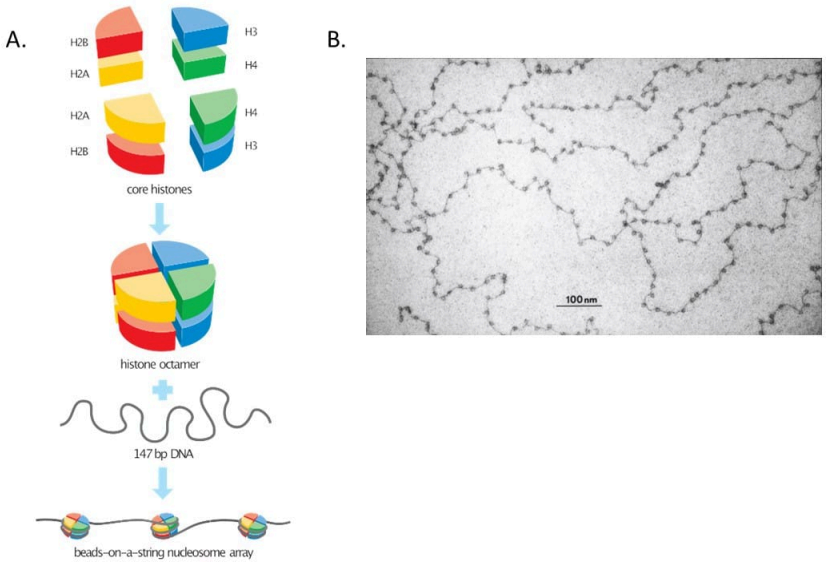


Figure 4.2.7: (A) The histone octamer is made of two subunits each of four core histone proteins (H2A, H2B, H3, and H4). About 147 bp of DNA helix is wrapped around the histone octamer to form the nucleosome. The nucleosomes are arrayed in a loosely packed "beads-on-a-string" form called euchromatin. (Chromatin Structure by David O Morgan, CC0). (B) An electron microscope image of euchromatin showing several nucleosomes. (Nucleosomes by ORNL History, CC BY 2.0)

Eukaryotic cells, in the interphase stage of the cell cycle, will be a mixture of heterochromatin and euchromatin. However, as the cell progresses towards division (i.e. mitosis or meiosis), the DNA is packaged beyond the heterochromatin level to form a **chromosome**. A chromosome ("chromo" = color; "soma" = body) is a highly organized thread-like structure composed of chromatin and associated proteins. The chromosome represents the highest level of DNA organization found within a eukaryotic cell. In dividing cells, condensing into a chromosome begins after the DNA has been replicated and concludes during metaphase (Figure 4.2.8). Euchromatin is condensed into heterochromatin. The heterochromatin forms loops that are 300 nm wide. These loops are condensed further to form the 700 nm wide **chromatid**. During metaphase, the chromosome is made of two chromatids, known as **sister chromatids**, joined at a region of heterochromatin called a **centromere**. Once cell division is complete, the DNA will de-condense back to its euchromatin and heterochromatin forms.

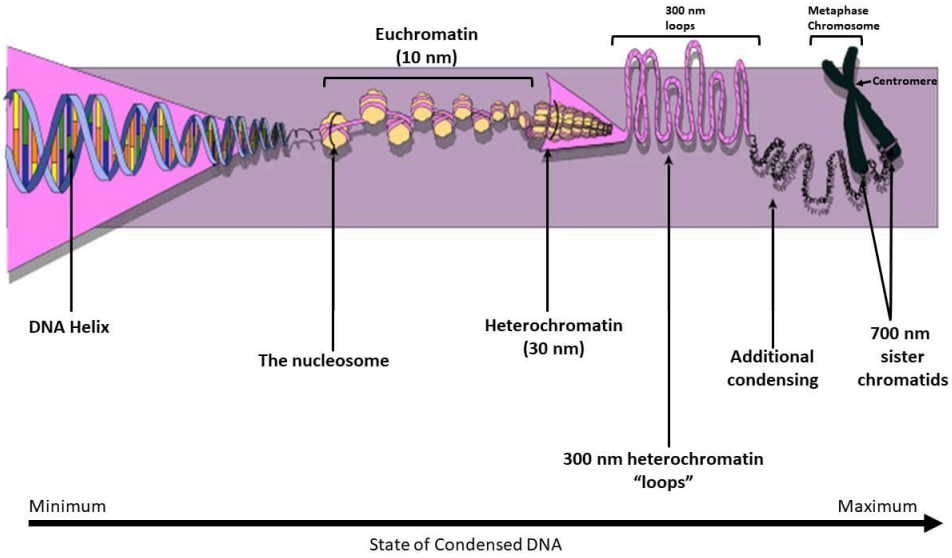


Figure 4.2.8: DNA packing. DNA is packaged to form chromatin and then a chromosome. The DNA helix is wrapped around the histone to form the nucleosome. An array of nucleosomes is known as euchromatin. The euchromatin is condensed into a 30 nm heterochromatin fiber. Heterochromatin is looped and condensed further to form the 700 nm chromatid found in the duplicated chromosome. (DNA packing by Patricia Zuk, CC 4.0; adapted from DNA packaging by ◇◇◇◇◇◇◇, CC BY-SA 4.0)

Concept in Action

Animation: DNA Packaging

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The form of chromatin in the eukaryotic cell has a direct correlation to the function of its DNA. The majority of chromatin in the nucleus is euchromatin and represents the form of DNA where transcription occurs. Stretches of euchromatin are rich in genes and are transcriptionally active in the cell. DNA in the form of heterochromatin has fewer genes and is thought to be inaccessible to the transcription machinery. As such, it represents a more inactive or "silent" form of DNA. For more information about transcription, refer to Chapter 3.3 Transcription of RNA. All eukaryotic cells will have stretches of heterochromatin that do not de-condense to euchromatin but persist as "permanent" heterochromatin. Examples of these regions are the **centromere** and **telomeres** of the chromosome.

Key Concepts

The DNA helix is made of two anti-parallel polynucleotide strands that resemble a "spiral staircase". The sugar-phosphate groups of the nucleotides join to form an outer "backbone" that coils like the rails, with the bases projecting to the inside of the helix, like the steps of the staircase.

The major concepts to remember are:

- the two polynucleotide chain of the DNA helix run in the opposite direction to one another (i.e. are anti-parallel)
- the bases of the DNA helix pair to one another in a complementary fashion - C with G and A with T
- DNA can be found in a prokaryotic cell as a circular chromosome, called a genophore, and as small pieces of circular DNA, known as plasmids
- DNA in eukaryotic cells is found as chromatin or chromosomes
- Eukaryotic cells have two forms of chromatin: euchromatin and heterochromatin

- Euchromatin is made of a DNA helix wrapped around histone protein complexes
- Heterochromatin is formed by condensing euchromatin further into larger coils
- Heterochromatin is condensed further to form the chromatids of a chromosome

Glossary

Adenine (A): a nitrogenous base that pairs with thymine (T) in DNA through two hydrogen bonds.

Base pair: a pair of complementary nitrogenous bases in DNA, consisting of adenine-thymine (A-T) and guanine-cytosine (G-C).

Chromatin: a form of organized DNA found within the nucleus of a cell; found as two types: euchromatin and heterochromatin

Chromosome: the most highly condensed and organized form of DNA; a thread-like structure composed of DNA and associated proteins

Complementary base pairing: the specific pairing of nitrogenous bases in DNA (A pairs with T, G pairs with C) through hydrogen bonding

Cytosine (C): a nitrogenous base that pairs with guanine (G) in DNA through three hydrogen bonds

Deoxyribonucleic Acid (DNA): a molecule that carries genetic instructions for the growth, development, function, and reproduction of living organisms

Deoxyribose: a five-carbon sugar found in DNA nucleotides

Double Helix: a twisted ladder-like structure of DNA, consisting of two strands running in opposite directions

Euchromatin: a type of chromatin; composed of the DNA helix wound around proteins called histones

Gene: a segment of DNA that contains the instructions for synthesizing a specific protein or RNA molecule

Genophore: the chromosome equivalent found in prokaryotes and viruses

Guanine (G): a nitrogenous base that pairs with cytosine (C) in DNA through three hydrogen bonds

Heterochromatin: a type of chromatin; composed of euchromatin wound into a large solenoid structure through its associated with nuclear proteins

Histone: a complex of assembled histone proteins that provides structural support for euchromatin; classified into 5 types: 1, 2A, 2B, 3, 4, and 5

Nucleosome: the section of euchromatin that is composed of a DNA helix wrapped around a histone

Nucleotide: the basic building block of DNA, consisting of one to three phosphate groups, a deoxyribose sugar, and a nitrogenous base

Phosphodiester bond: the bond found between nucleotides in DNA and RNA

Purine: the organic bases adenine and guanine found in nucleic acids

Pyrimidine: the organic bases cytosine, uracil and thymine found in nucleic acids

Supercoiling: the twisting of the DNA helix

Thymine (T): a nitrogenous base that pairs with adenine (A) in DNA

Topoisomerase: the enzyme complex responsible for the elimination of supercoiling

Transcription: the process of copying a gene's DNA sequence into messenger RNA (mRNA)

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4.3: Replication of DNA

Biotech Focus

In 1983, while working at the biotechnology company Cetus Corporation, Dr. Kary Mullis created a laboratory process for DNA amplification that mimicked the *in vivo* process of DNA replication. This process, named **Polymerase Chain Reaction or PCR**, used repeated cycles of heating and cooling to exponentially replicate a targeted DNA segment. Mullis set-up three water baths: a high temperature bath for DNA denaturation (95°C), a lower temperature bath for primer annealing (e.g. 50°C to 60°C), and a third water bath for the extension phase of the polymerase (72°C). The DNA was incubated in each water bath for a defined time period and then repeated over and over again. Initially, the process used a DNA polymerase from *E. coli*, which was not stable enough at the high temperatures required for DNA denaturation and had to be replenished during each cycle. The breakthrough came with the discovery of **Taq polymerase,** an enzyme isolated from the thermophilic bacterium *Thermus aquaticus*, which could withstand the high denaturing temperatures. PCR quickly gained recognition for its versatility and has became a foundational tool in genetics, diagnostics, forensics, and research. Mullis' work earned him the Nobel Prize in Chemistry in 1993.

Introduction to DNA Replication

During cell division, each daughter cell receives an identical copy of the DNA. For this to happen, the DNA must be copied through a process called **DNA replication**. The replication of DNA occurs during the synthesis phase, or S phase, of the cell cycle, before the cell enters mitosis or meiosis. The structure of the double helix provides a hint as to how DNA is copied. During DNA replication, each of the two "parental" DNA strands that make up the double helix will separate and serve as a template from which the new "daughter" DNA strands are copied. The newly made daughter DNA strand is complementary to the parental DNA strand. After replication, each new DNA helix will consist of one parental DNA strand and one new daughter DNA strand. This is known as **semi-conservative replication** because each of the parental DNA strands remains (i.e. is conserved) within the new helix (Figure 4.3.1).

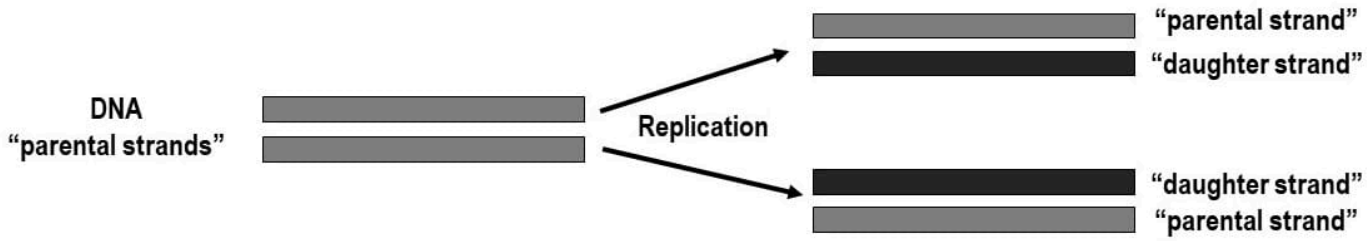


Figure 4.3.1: The semi-conservative replication of DNA. During DNA replication the two original "parental" strands of DNA (gray rectangles) serve as templates for the synthesis of new "daughter" strands (black rectangles). After DNA replication, each DNA helix is made of a parental strand and a daughter strand. (Semi-conservative Replication by Patricia Zuk, CC BY 4.0)

Learning Objectives

DNA is replicated in cells and in the laboratory using a series of enzyme-catalyzed steps. At the end of this section, you will be able to:

- List the major steps of DNA replication
- Explain the process of DNA replication in prokaryotes
- Define the role for each component of DNA replication
- Compare and contrast DNA replication in prokaryotes and eukaryotes
- Explain the steps of the Polymerase Chain Reaction (PCR) workflow
- List and explain the components of a PCR reaction
- Explain the events of one PCR cycle

- Explain how real-time PCR differs from semi-quantitative PCR

The Mechanism of DNA Replication

The process of DNA replication requires many components and is comprised of many stages. However, the process can be simplified into the following steps:

1. The DNA helix is unwound at the origin of replication
2. RNA primers bind to specific sequences of DNA
3. DNA polymerases bind the primers and "read" the parental strands in order to create the new daughter strands. One new strand is made continuously, while the other strand is made in pieces
4. Primers are removed, new DNA nucleotides are put in place of the primers and missing phosphodiester bonds are re-formed

Both the secondary and tertiary structures of DNA make it impossible to replicate. To eliminate the tertiary DNA structure of supercoiling, a **topoisomerase** enzyme complex breaks a phosphodiester bond in one of the two DNA strands, eliminating the supercoiling. The secondary structure of the helix is then eliminated by "unwinding" the helix into its two parental template strands. This step is accomplished by an enzyme called a **DNA helicase (or helicase)** (Figure 4.3.2). The helicase recognizes a specific sequence of DNA known as an **origin of replication**. Two helicase complexes bind at this sequence and move along the DNA in opposite directions. As they move, they unwind or "unzip" the double-stranded DNA into single-strands. As the DNA helix unwinds, Y-shaped structures called **replication forks** are formed. Two replication forks are formed at each origin of replication as two helicase complexes move away from one another. The forks will grow in opposite directions as replication proceeds. Bacterial chromosomes, with their smaller sizes, typically have one origin of replication. The larger eukaryotic chromosomes will have several origins along their lengths so that replication can occur simultaneously from several places along each chromosome.

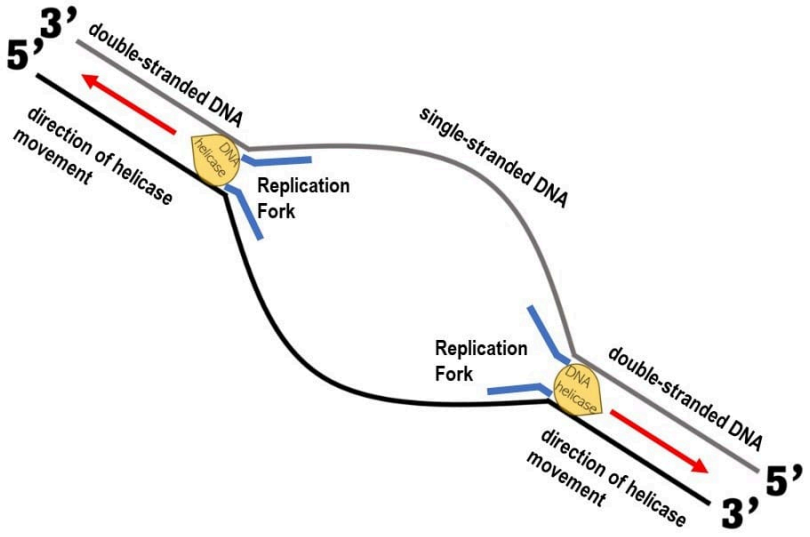


Figure 4.3.2: The DNA Helicase. A DNA helicase (yellow circles) binds to double-stranded DNA and unwinds it. Two DNA helicases move in opposite directions (red arrows), working together to produce single-stranded DNA ready for replication. The region of DNA unwinding is called a replication fork (blue lines). (The DNA Helicase by Patricia Zuk, CC BY 4.0; adapted from DNA Helicase by RosieScienceGal, CC BY-SA 4.0)

The unwinding of the DNA helix produces the two parental strands that serve as templates for daughter strand synthesis. The parental strands are prevented from reforming the DNA helix through the binding of several small **single-stranded binding proteins (SSBs)** to each parental strand. As the helicase unwinds the helix, the parental DNA is "copied" by a **DNA polymerase**, a large enzyme complex made of several subunits, each with specific functions in replication. The DNA polymerase is limited, however, in that it cannot bind single-stranded DNA. To fix this, an enzyme complex called a **primase** creates a short **RNA primer** that is complementary to the parental DNA template. The DNA polymerase clamps itself onto the parental DNA strand at the 3' end of the primer and glides along this strand towards its 5' end. As it moves to the 5' end, it uses the parental strand as a template, adding and linking complementary DNA nucleotides to create the DNA daughter strand. As DNA replication proceeds, RNA primers are removed and the gaps filled with new DNA nucleotides by another type of DNA polymerase. Finally, missing phosphodiester bonds between nucleotides in the daughter strands are formed by a **DNA ligase** enzyme complex, creating a

continuous piece of daughter DNA.

DNA replication (Figure 4.3.3) is a complex process that presents numerous challenges to the cell. Several of these are solved by enzyme complexes like the helicase (which unwinds the DNA helix) and the primase (which creates the primer). But the biggest challenges are: 1) the fact that both parental strands must be replicated at the same time and 2) the requirement that daughter DNA must be made in the 5' to 3' direction. In other words, incoming DNA nucleotides can only be added to a daughter DNA strand at its 3' end. The first challenge is solved easily. Two DNA polymerases, linked to one another, replicate both parental templates at the same time. The second challenge is more difficult. The requirement that daughter DNA "grows" in the 5' to 3' direction is not a problem for the DNA polymerase replicating the parental strand that is in the 3' to 5' orientation. The daughter DNA strand made from this template will be synthesized continuously in a process known as **leading strand synthesis**. The daughter strand made through this process is often called the **leading strand**.

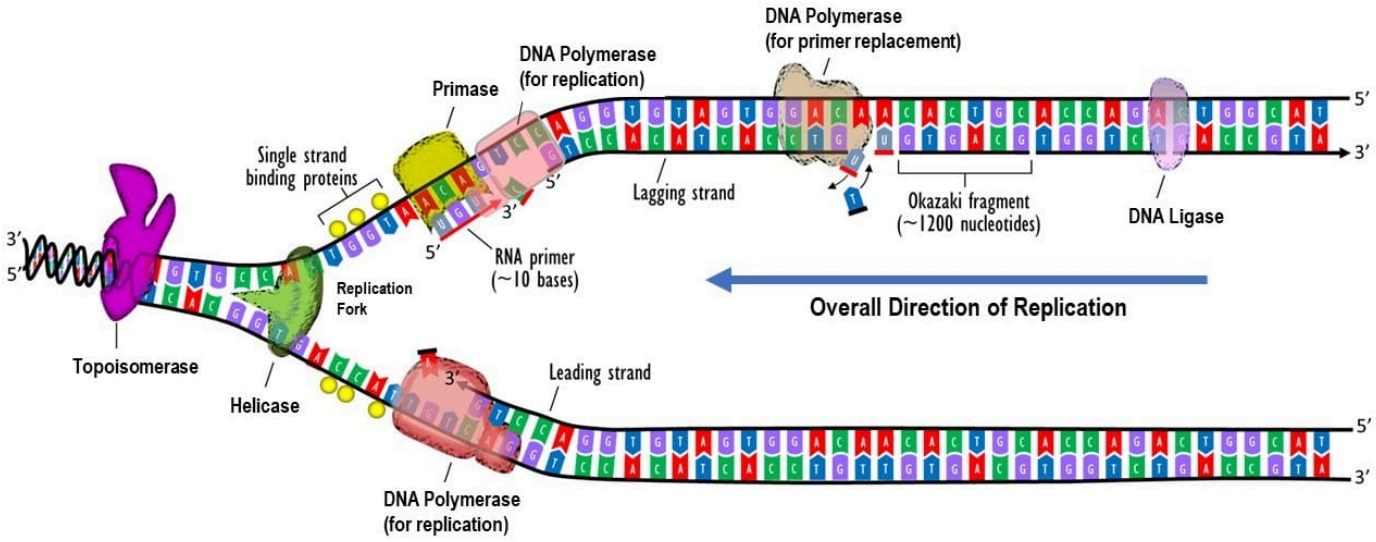


Figure 4.3.3: DNA replication. The replication of DNA involves multiple enzyme complexes, including a Topoisomerase (dark purple enzyme) to remove supercoiling, a Helicase (green enzyme) to unwind the DNA helix into single template strands, a Primase (yellow enzyme) to create RNA primers, and DNA polymerases for replication (orange enzyme) and for RNA primer removal (tan enzyme) and replacement with DNA. The single strands of DNA are prevented from reforming the helix by the binding of several small single-stranded binding proteins (yellow circles). The leading daughter strand (Leading strand) is synthesized continuously. The lagging daughter strand (Lagging strand) is made in pieces known as Okazaki fragments of about 1200 nucleotides. The DNA Ligase (light purple) joins the Okazaki fragments together to complete synthesis of the lagging strand. (DNA Replication by Patricia Zuk, CC BY 4.0; adapted from DNA Replication in Prokaryotes by Eunice Laurent, CC BY-SA 4.0)

However, replication of the other parental template strand that is in the 5' to 3' orientation cannot be done continuously. Instead, this template must be replicated in short pieces called **Okazaki fragments**, in a process called **lagging strand synthesis**. In lagging strand synthesis, a short stretch of parental DNA, beginning with an RNA primer, is "looped" so that it *has its orientation reversed* (i.e. from 5' to 3' to 3' to 5'). This reversal of orientation to 3' to 5' means that the DNA polymerase can synthesize daughter DNA in the 5' to 3' direction and it creates an Okazaki fragment. The DNA polymerase then takes a new section of parental DNA and reverses and replicates it. As these Okazaki fragments are made, a **DNA ligase** will link them together by forming new phosphodiester bonds, thus creating a continuous piece of DNA that is the lagging strand.

Concept in action

Animation: DNA Replication

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DNA Replication in Prokaryotes vs. Eukaryotes

DNA replication was first studied in prokaryotes, primarily because of the small size of the genome. As an example, *E. coli* has 4.6 million base pairs in its single, circular chromosome, and all of it gets replicated in approximately 42 minutes, starting from a single origin of replication and proceeding around the chromosome in both directions. This means that approximately 1000 nucleotides are added per second. The process is much more rapid than in eukaryotes, whose rate of replication ranges from 500 to 5000 nucleotides added per minute at each replication fork.

While there are many similarities in DNA replication between prokaryotes and eukaryotes, there are several important differences (Table 4.3.1. The major difference lies in the composition and function of the DNA polymerases. Specifically, three polymerases (DNA polymerase I, II, and III) are involved in prokaryotic DNA replication, whereas eukaryotes utilize five polymerases (alpha, beta, delta, gamma, and epsilon).

Table 4.3.1: ***Comparison of DNA replication in prokaryotes and eukaryotes.***

Category	Prokaryotes	Eukaryotes
Replication site	nucleoid	nucleus
DNA organization	single, circular chromosome	multiple, linear chromosomes
DNA condensing	supercoiling	chromatin condensed into chromosomes
Origin of replication	one	multiple
Direction of replication	bidirectional	bidirectional
Replication rate	1000 - 2000 nucleotides per second	100 nucleotides per second
Removal of supercoiling	DNA topoisomerase II (DNA gyrase)	DNA topoisomerase II (DNA gyrase)
Unwinding of DNA helix	Helicase	Helicase
RNA primer production	Primase	DNA polymerase a
Synthesis of DNA	DNA polymerase III	DNA polymerase d (lagging strand) DNA polymerase e (leading strand)
Removal of RNA primers	DNA polymerase I	RNAse H???
Replacement of primers with DNA	DNA polymerase I	DNA polymerase e
Joining of DNA fragments	DNA Ligase	DNA Ligase
DNA repair	DNA polymerase II	DNA polymerases, b and d

Polymerase Chain Reaction (PCR): DNA Replication in the Laboratory

Polymerase chain reaction (PCR) is a laboratory protocol that amplifies a small amount of DNA into significant amounts that can be manipulated in a lab. PCR has become an incredibly powerful tool in the lab, widely used in forensics, research and clinical labs. Included in the myriad of uses for PCR is its use in amplifying enough DNA for downstream analysis (e.g. sequencing, restriction digest, DNA fingerprinting) and cloning.

The workflow for PCR is relatively straightforward (Figure 4.3.4). PCR reactions are set-up and placed into a "PCR machine" or **thermocycler**. The thermocycler is programmed to run a repetitive series of **PCR cycles**. Upon the conclusion of the PCR program, the PCR reactions are analyzed through agarose gel electrophoresis, to confirm the presence of the correct PCR product, in addition to other downstream applications, such as sequencing.

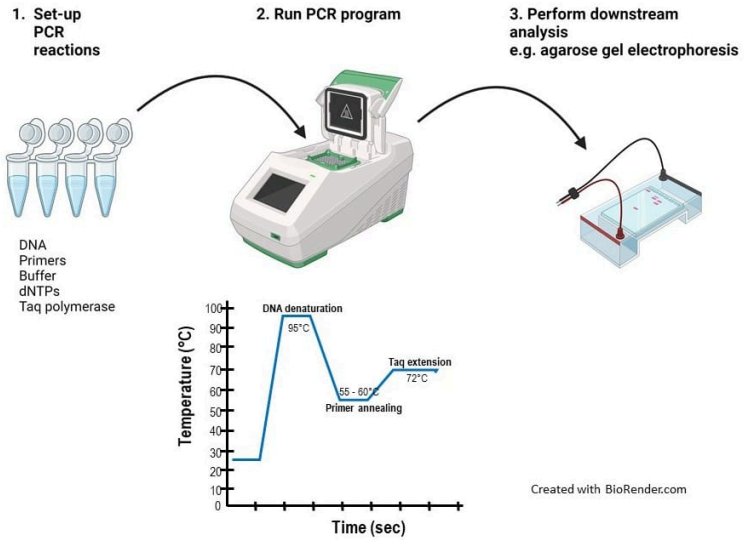


Figure 4.3.4: The PCR workflow. 1) Set up PCR reactions: The PCR reaction is set up in PCR tubes to contain the DNA to be amplified (DNA), together with sequence-specific primers (Primers), a polymerase-specific buffer (Buffer), DNA nucleotides (dNTPs), and aTaq polymerase. 2) Run PCR Program: The PCR tubes are placed into a PCR machine programmed to repeatedly cycle through three specific temperatures (lower figure): DNA denaturation at 95°C, Primer annealing at 55°C to 60°C , and Taq extension at 72°C. 3). Perform downstream analysis: Following PCR, the reactions are analyzed through downstream applications, such as agarose gel electrophoresis. (The PCR Workflow by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2024))

The PCR reaction

In amplifying DNA, PCR mimics DNA replication of the cell. Indeed, the components found in a PCR reaction are the same components, or very similar, to those found in cells undergoing DNA replication (Figure 4.3.5).

The components of a PCR reaction are:

1. **The DNA polymerase:** At the core of a PCR reaction is a DNA polymerase capable of replicating a DNA template. The most common DNA polymerase used in PCR reactions is called **Taq polymerase** – a thermostable bacterial polymerase isolated from *Thermus aquaticus,* a thermostable DNA polymerase isolated from the bacteria Thermus aquaticus in 1988. Taq polymerase maintains activity across a wide range of temperatures that will be encountered during a PCR cycle.
2. **The template DNA:** The DNA added to the PCR reaction will contain the target region of DNA to be amplified.
3. **Two DNA primers:** As with *in vivo* DNA replication, replication by Taq polymerase requires a primer to initiate DNA synthesis. PCR uses two primers, known as the **forward primer** and the **reverse primer**. The forward and reverse primers will flank the DNA region to be amplified and are made of short sequences of DNA complementary to the DNA template. The forward primer sequence is designed to be complementary to the anti-sense template and the reverse primer is designed to be complementary to the sense DNA strand. PCR primers are often referred to as **oligonucleotides** or **"oligos"** because of their short length. Rather than being made of RNA, the two primers are made of DNA. However, they will act as the *in vivo* RNA primer does in replication by base pairing to the template DNA and providing a 3' end from which the Taq polymerase can extend.
4. **The four DNA Nucleotides:** As with *in vivo* DNA replication, the replicated DNA strands will be synthesized using the four DNA nucleotide bases, dATP, dGTP, dCTP, and dGTP.
5. **A reaction buffer:** To optimize Taq polymerase activity, the PCR reaction will be set-up using a reaction buffer that is a mixture of monovalent and divalent cations (e.g. Mg2+) buffered to a specific pH.

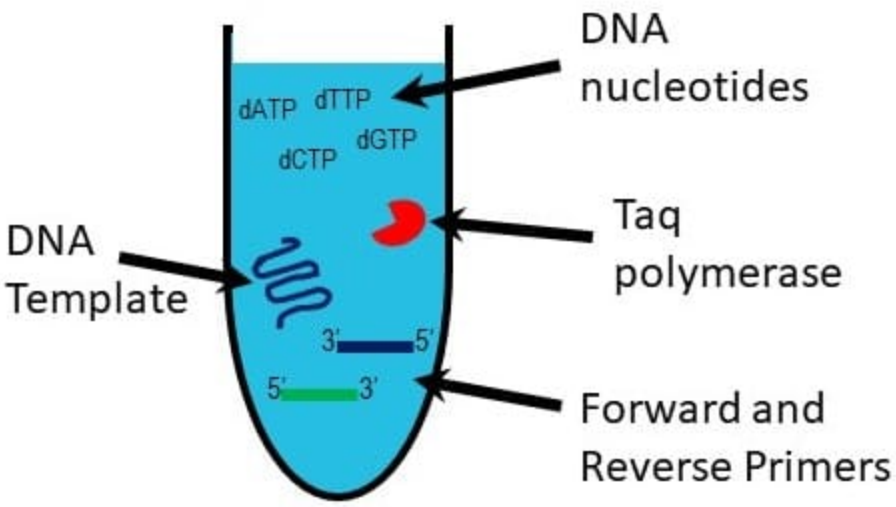


Figure 4.3.5: The PCR reaction. The components of a typical PCR reaction are similar to the components of DNA replication. (The PCR Reaction by Patricia Zuk, CC BY 4.0)

The components listed above are missing some critical components of *in vivo* DNA replication. First and foremost, the helicase of DNA replication is not used in a PCR reaction. The unwinding of the template DNA helix is achieved by incubating the PCR reaction at a very high temperature (i.e. 95°C). This high temperature unwinds or **denatures** the double-stranded DNA into two single strands. There is no need for a topoisomerase because the template DNA used in PCR reactions is typically small enough that supercoiling is not found. Finally, the "reannealing" of the two parental DNA strands back into a helix is prevented by the rapid binding of the forward and reverse primers to their complementary regions. In this way, single-stranded binding proteins (SSBs) are not required.

The PCR "cycle" is made of three temperatures

A PCR reaction is run using a repetitive series of temperatures. Each series is known as a "cycle" and consists of three temperatures (Figure 4.3.6). These temperatures are the:

1. **Denaturing temperature:** A very high temperature (~95°C) that breaks the hydrogen bonds between complementary bases and causes the two parental DNA strands to separate. The denaturing step of PCR is typically very short (~30 to 60 seconds).
2. **Annealing temperature:** A lower temperature designed to promote the base-pairing between the forward and reverse primers to their complementary sequences in the template strands. Because the PCR primers are added to the reaction in excess amounts, when the reaction is cooled, they will anneal to their complementary DNA regions before the two parental strands can re-anneal with each other. Annealing temperatures typically range from 50°C to 60°C and depend on the primer sequences. Annealing times in a PCR reaction usually range from 1 to 2 minutes to ensure sufficient primer annealing.
3. **Extension temperature:** The temperature that allows the Taq polymerase to bind and extend off the annealed primers. For Taq polymerase this temperature is 72°C. Extension steps are usually between 30 and 60 seconds.

A typical PCR program will repeat this three-temp cycle 30 to 40 times. Each cycle increases the amount of target DNA amplified. Only the region of template DNA found between the two annealed primers is replicated At the end of the program, millions of replicated pieces of DNA outlined by the two primers will be created.

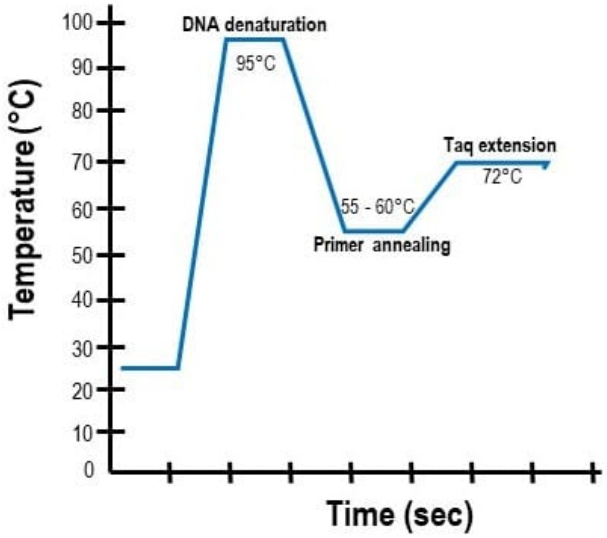


Figure 4.3.6: The PCR cycle. A PCR cycle is made of three temperatures: denaturing, annealing, and extension. The DNA denaturing temperature of 95°C produces single stranded DNA templates. Cooling to the annealing temperature allows for the binding of the primer to its complementary sequence on the template. The extension temperature of 72°C allows extension off the primer leading to template strand replication.
(The PCR Cycle by Patricia Zuk, CC BY 4.0)

The Taq polymerase amplifies the DNA found between the two PCR primers

The strength of PCR lies in its ability to amplify a targeted region of DNA inside a much larger piece. For example, a scientist wanting to study a single human gene (or a specific region inside of it) only needs to amplify that gene (or region) from the enormous human genome of approximately three billion base pairs. “Outlining” the target DNA to be amplified is done using the forward and reverse primers. Therefore, researchers will design these primers to flank the region of DNA they wish to amplify. The animation below illustrates how PCR will amplify the regions between the two primers.

Concept in Action

Animation: PCR amplification of a target region

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Real-time PCR: Quantitative PCR

The PCR described above is known as "semi-quantitative" because it can give a general idea of DNA quantity found in the PCR product. However, this type of PCR can be adapted into a truly quantitative protocol known as **real-time PCR** or **qPCR**. In real-time PCR, a fluorescently-labeled **probe** is used to quantitate the degree of cDNA amplification in the PCR reaction. Two major approaches are used in real-time PCR: **Taqman and SYBR green**.

The "Taqman protocol" was developed by Applied Biosystems (now part of Thermo Fisher). In this protocol, a **20X Gene Expression Mix**, containing custom forward and reverse primers, in addition to a fluorescently-labeled "Taqman probe", is used to amplify a sequence of DNA through a standard PCR reaction. The Taqman probe binds the DNA template somewhere between the forward and reverse primers. The DNA sequence of the probe is bound to a fluorophore and a quencher that prevents fluorescence. Commonly used fluorescent probes include fluorescein (FAM), VIC, HEX, and JOE. When the probe is bound to the

DNA template, the quencher prevents fluorescence. However, as the Taq polymerase amplifies the region between the forward and reverse primers, it displaces the fluorescent probe, which is followed by probe cleavage. As a result, the fluorescent probe is separated from its quencher and is excited. Fluorescent emission is then detected by the thermocycler (Figure 4.3.7). The expression data for each PCR reaction is provided as a value known as a **Ct (threshold cycle) value** - the cycle number at which a detectable fluorescent signal was measured. These Ct values can be analyzed and either converted to absolute values or compared to one another so that changes in gene expression can be determined.

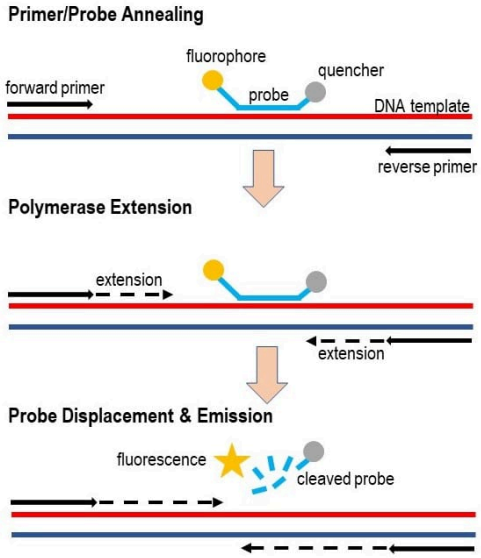


Figure 4.3.7: Real-time PCR quantitation using primers and a Taqman probe. During each cycle of PCR amplification, the forward and reverse primers anneal to their complementary regions on the DNA strand, in addition to a fluorescently-labeled Taqman probe that is comprised of a fluorophore and a quencher. Extension from the primer by the Taq polymerase results in probe displacement from the DNA template. Displacement results in probe cleavage and "release" of the fluorophore from the quencher. The resulting fluorescence is quantified. (Real-time PCR by Taqman by Patricia Zuk, CC BY 4.0)

The "SYBR green protocol" depends on the intercalating dye, **SYBR Green I**. When unbound in the PCR reaction, the SYBR Green dye has negligible fluorescence. However, when the Taq polymerase synthesizes it complementary DNA strand, SYBR Green I is incorporated into the PCR product and emits a strong fluorescent signal. At the start of the next PCR cycle, the fluorescent signal is decreased when the double-stranded DNA (dsDNA) template is denatured but increases again as the PCR product is made. Therefore, the fluorescent signal of the sample is collected at the end of the elongation step of each PCR cycle to determine the relative change in amplified products (Figure 4.3.8). Since SYBR Green I is a non-specific dsDNA binding dye, it cannot distinguish between amplified PCR products and non-specific piece of dsDNA. Therefore, corrective methods must be used in data analysis.

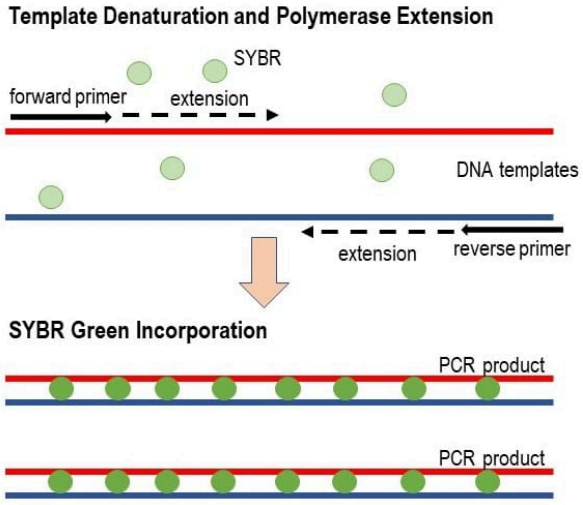


Figure 4.3.8: SYBR green real-time PCR. SYBR green cannot bind single-stranded template DNA and shows negligible fluorescence when dsDNA is denatured. Following polymerase extension from the primers, the SYBR green dye becomes incorporated into the double-stranded PCR product. The fluorescence is then measured. (SYBR Green Real-Time PCR by Patricia Zuk, CC BY 4.0)

Lab Protocols

- Lab Technique: Polymerase Chain Reaction (PCR)
- Lab Technique: Quantitative PCR
- Lab Technique: Agarose Gel Electrophoresis

Key Concepts

DNA replication duplicates the two strands of the DNA helix. Some major concepts to remember are:

- each of the two "parental" DNA strands serve as templates for "daughter" DNA strand synthesis
- the two"parental" DNA strands of the DNA helix are separated by a Helicase enzyme prior to replication
- single-stranded binding proteins hold the two parental DNA strands apart
- DNA replication is performed by a DNA polymerase that reads a parental template strand to create a daughter strand made of complementary DNA nucleotides
- DNA polymerase requires a short primer of RNA to begin replication of a daughter strand. This primer is made by a Primase enzyme
- DNA polymerase makes the daughter DNA strand in the 5' to 3' direction. This means that one daughter strand (i.e. the leading strand) can be synthesized continuously. The other daughter strand (i.e. the lagging strand) is made in short pieces called Okazaki fragments
- the enzyme DNA ligase joins all DNA fragments to finish DNA replication
- after replication, each new DNA helix consists of one parental strand and one new daughter strand (i.e. semi-conservation replication)

Glossary

DNA Ligase: An enzyme that joins Okazaki fragments together by forming phosphodiester bonds.

DNA Polymerase: An enzyme that adds nucleotides to a growing DNA strand during replication.

DNA Replication: The biological process by which a cell makes an identical copy of its DNA before cell division.

Daughter strand: The complementary DNA strand made by DNA polymerase during DNA replication.

Helicase: An enzyme that unwinds the DNA double helix by breaking hydrogen bonds between base pairs.

Leading Strand: The DNA strand that is synthesized continuously in the 5' to 3' direction during replication.

Lagging Strand: The DNA strand that is synthesized discontinuously in short segments known as Okazaki fragments.

Okazaki Fragments: Short DNA fragments synthesized on the lagging strand during replication; joined together by DNA ligase.

Origin of Replication: A specific sequence in the genome where DNA replication begins; the site of binding of the helicase and other replication proteins.

Parental DNA strand: One of the two original strands found in the DNA helix; used as a template for synthesis of the leading and lagging strands.

Primase: An enzyme that synthesizes a short RNA primer to provide a starting point for DNA polymerase.

Replication Fork: The Y-shaped region where the DNA double helix is unwound and new strands are synthesized**.**

RNA Primer: A short RNA sequence synthesized by primase that initiates DNA synthesis.

Topoisomerase: An enzyme that prevents DNA supercoiling and relieves tension ahead of the replication fork.

Single-Strand Binding Proteins (SSBs): Proteins that stabilize the separated DNA strands during replication to prevent them from reannealing.

Semi-Conservative Replication: A method of DNA replication in which each new DNA molecule consists of one original (parental) strand and one newly synthesized strand.

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4.4: Transcription of RNA

Biotech Focus

The process of transcribing DNA into RNA is a complex system involving numerous enzyme complexes all working together in a highly coordinated fashion. In the 1990s, a research group led by **Roger Kornberg** at Stanford University successfully developed an *in vitro* transcription system from baker's yeast that faithfully replicated the *in vivo* process found in cells. The group then used this system to isolate several dozen proteins required for transcription, including the Mediator protein complex required for efficient transcription in eukaryotes. Thanks to this groundbreaking research, we now know that the protein components of transcription are remarkably conserved across the spectrum of eukaryotes, from yeast to human cells. Based on this work, Roger Kornberg won the Nobel Prize in Chemistry in 2006.

Read Roger Kornberg's recollections of his research that culminated in this Nobel Prize.

Introduction

The goal of **transcription** is to make an RNA copy of a gene's DNA sequence. Transcription is the first step in **gene expression**, in which information from a gene is used to produce a functional product, such as RNA or a protein. This flow of genetic information is also known the **central dogma of molecular biology** (Figure 4.4.1), which states that a gene becomes transcribed and then can be translated into a protein.

For a protein-coding gene, the RNA copy, made through transcription, carries the information needed to build the protein. Transcription is relatively straightforward. RNA nucleotides, complementary to the DNA template, are linked together to form an RNA copy known as an **RNA transcript**. The translation of this transcript into a polypeptide sequence is more complex and will require the reading of RNA nucleotides in a specific manner. This process will be covered in Chapter 3.4: Translation.

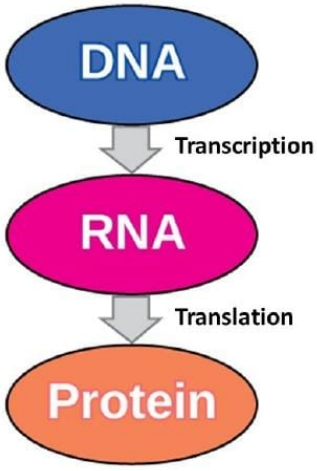


Figure 4.4.1: The Central Dogma of Molecular Biology. DNA is transcribed into RNA which is then translated into protein. (Central Dogma, from LibreText, CC BY-SA 4.0)

Learning Objectives

RNA is a nucleic acid that converts the genetic code found in DNA into the proteins that perform cellular functions.At the end of this section, you will be able to:

- List and explain types of RNA found in cells
- Describe the modifications needed to create mRNA, rRNA, and tRNA
- Explain the structure of a protein-coding gene and its regulatory sequences (i.e., the transcription unit)
- List the types of RNA polymerases involved in transcription
- List and explain the stages of transcription
- Explain the steps involved in mRNA processing
- Explain the spliceosome and its role in spicing
- Explain alternative splicing and its importance

Types of RNA

RNA, like DNA, is a nucleic acid made up of nucleotides. However, there some important differences between RNA and DNA. RNA nucleotides have a pentose sugar, named **ribose,** that has a hydroxyl group (OH), rather than a hydrogen, at the 2' carbon. The bases of RNA are adenine, guanine, cytosine, and **uracil.** Finally, RNA is a single polynucleotide chain. For more information about nucleic acids, refer to Chapter 2.7 Nucleic Acids.

There are several types of RNA found in cells. These RNA types can be broken down into two categories: **coding and non-coding**. There is only one type of RNA that is classified as coding. This RNA is known as **messenger RNA or mRNA** and it is produced following the transcription of protein-coding genes in the nucleus.

There are numerous types of non-coding RNA molecules, including:

- **ribosomal RNA or rRNA**
- **transfer RNA of tRNA**
- **microRNA or miRNA**
- **small nuclear RNA or snRNA**

Non-coding RNA molecules, like coding RNA, are transcribed. However, they are not translated into proteins. More about protein translation can be found in Chapter 3.4: Translation.

mRNA

Messenger RNA, or mRNA, is produced upon transcription of protein-coding genes. This transcription takes place in the **nucleoplasm**, the fluid of the nucleus. Following transcription, this RNA transcript, which is often called **pre-mRNA**, is exported out into the cytoplasm to be modified into mRNA.

Pre-mRNA processing into mRNA involves three events (Figure 4.4.2):

- addition of a modified guanine nucleotide, known as the **5'-methylated cap**, to the 5' end of the pre-mRNA
- addition of a long stretch of adenine nucleotides, known as the **poly-A tail**, to the 3' end of the pre-mRNA
- splicing to remove all **introns** from the pre-mRNA

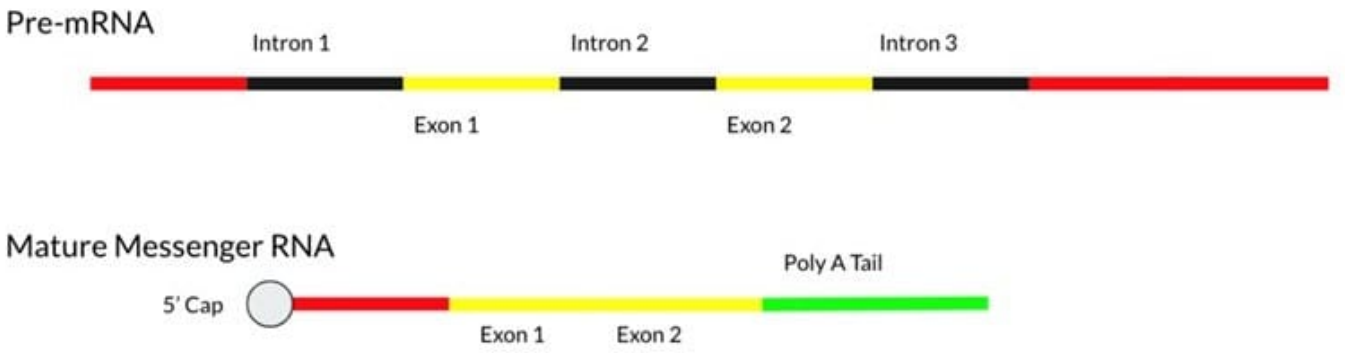


Figure 4.4.2: mRNA processing. Mature mRNA is processed from the pre-mRNA molecule (i.e. RNA transcript) through the addition of a 5'-methylated cap to the 5' end and a polyA tail added to the 3' end. Introns are removed through splicing. (mRNA Maturation by Editorzcj, CC BY-SA 4.0)

rRNA

Ribosomal RNA, or rRNA, makes up the majority of RNA found in cells. rRNA is produced upon transcription of a class of genes called ribosomal genes. This transcription takes place in the **nucleolus** of the nucleus. Following transcription, the rRNA is folded into a series of **stems and loops**, resulting in a characteristic secondary structure (Figure 4.4.3). This mature rRNA will become part of the ribosome, the "machine" for translation of proteins. rRNA molecules are named based on their size. rRNA size is quantified using a technique called **sedimentation**. In sedimentation, molecules are added to columns of sugar and then centrifuged. The rate of sedimentation through the column is denoted with a capital "S". Larger rRNA molecules will have a faster sedimentation rate and a larger "S" value in comparison to smaller rRNA molecules. The types of rRNA molecules made are dependent upon species. For example, humans have the following rRNA molecules: 5S, 5.8S, 18S, and 28S. In contrast, *E.coli* cells produce 5S, 16S, and 23S rRNA molecules. More about rRNA can be found in Chapter 3.4: Translation.

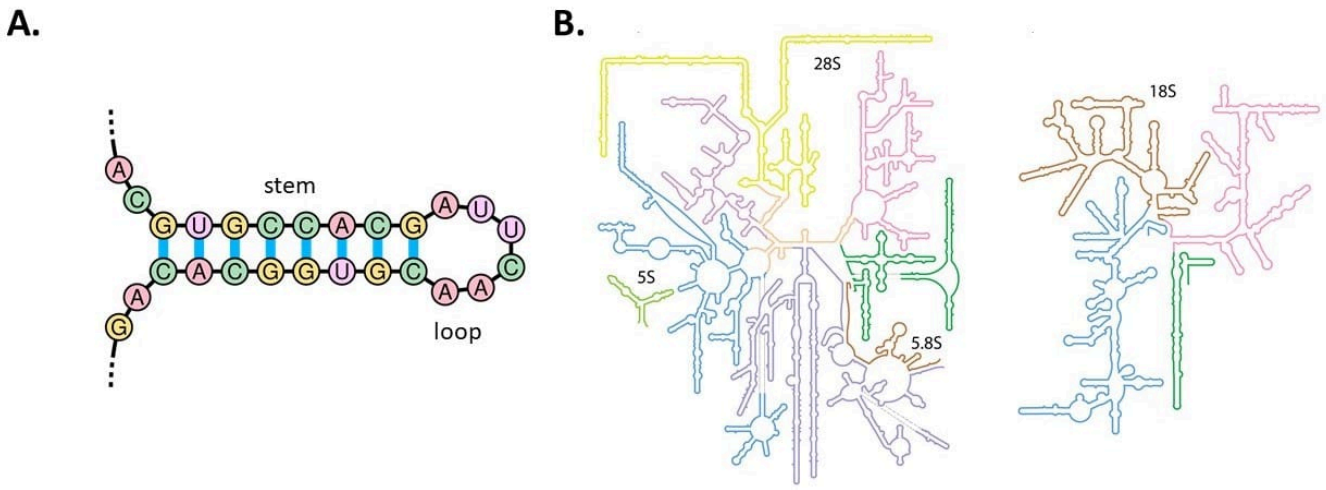


Figure 4.4.3: rRNA molecules (A) The stem-loop configuration of rRNA is produced through complementary base pairing to create a stem, with the non-complementary bases producing a loop. (Stem-loop by Sakurambo, CC BY 2.5)
(B) The 4 types of rRNA molecules found in human cells are 18S and a complex made of 28S, 5.8S, and 5S. (adapted from Petrov et al, CC BY-SA 4.0)

tRNA

Transfer RNA, or tRNA, is transcribed in the nucleoplasm of the nucleus. tRNA is a short, single-stranded RNA molecule approximately 80 nucleotides in length that is then folded into a characteristic secondary structure of stems and loops not unlike rRNA. This secondary structure is then folded further to create a unique tertiary structure (Figure 4.4.4). More about tRNA can be found in Chapter 3.4: Translation.

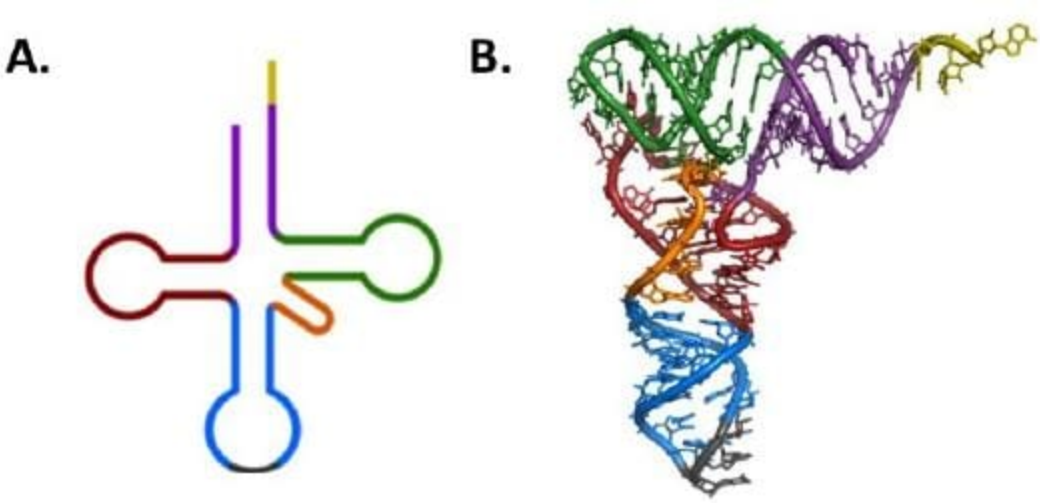


Figure 4.4.4: The tRNA molecule. (A) The secondary structure of the tRNA molecule shows numerous stem and loop regions. (B) The tertiary structure shows the proposed three-dimensional structure of a tRNA molecule. (tRNA-Phe by Yikrazuul, CC BY-SA 3.0)

Genes are Transcribed

Not every stretch of DNA in our genome is transcribed into RNA. A significant portion of our genome is considered to be "junk" DNA, sequences to which a function has not yet been assigned. Those stretches of DNA that are transcribed are known as genes. A **gene** is defined as a sequence of DNA that is transcribed into functional RNA. Protein-coding genes are transcribed into coding RNA (i.e. mRNA). Non-protein coding genes are transcribed into a specific type of non-coding RNA.

The eukaryotic gene is part of a "transcription unit", which is comprised of the stretch of DNA that is transcribed, in addition to several regulatory sequences that will control transcription (Figure 4.4.5).

For a protein-coding gene, the gene itself is made of three regions:

- the **coding sequence (CDS)**, which is the majority of the transcribed DNA. The coding sequence is often called an **Open Reading Frame (ORF)**. The CDS/ORF is made of **exons**, which are DNA sequences that will be translated into amino acids, and **introns**, which are intervening stretches of DNA that are not translated
- the **5' untranslated region (5'UTR)**, a regulatory DNA sequence before (upstream from) the coding sequence
- the **3' untranslated region (3'UTR)**, a regulatory DNA sequence after (downstream from) the coding sequence

Both prokaryotic and eukaryotic protein-coding genes will have UTRs flanking the coding sequence. However, these UTRs are significantly shorter in prokaryotes.

Associated with the gene are regulatory sequences known as the **promotor** and the **enhancer.** The promoter is a DNA sequence located immediately upstream of the gene that "promotes" transcription. The majority of prokaryotic and eukaryotic genes will have some type of promoter. In both prokaryotes and eukaryotes, the promoter region of most genes typically contains a sequence of DNA known as a **core promoter**. The DNA sequence of this core promoter is very similar in many prokaryotic and eukaryotic genes (i.e., is conserved). The most well-known eukaryotic core promoter has the sequence "TATAAA" and is known as the **TATA box.** In prokaryotic cells, this core promoter sequence is "TATAAT" and is called the Pribnow box, after its discoverer. In eukaryotes, additional sequences can be found next to the core promoter. These sequences are called **proximal promoter elements (PPEs)** and collectively they are known as the **proximal promoter**. The sequences making up the proximal promoter will vary from gene to gene. If present, they function as regulators of transcription.

In addition to a promoter region, eukaryotic genes are also associated with a stretch of DNA known as the enhancer. As its name implies, the enhancer "enhances" the efficiency of transcription. The enhancer is usually found at a significant distance upstream of the promoter and gene, although some enhancers can be found downstream of the gene. The enhancer is a sequence that is unique to eukaryotic cells and is not found in prokaryotes.

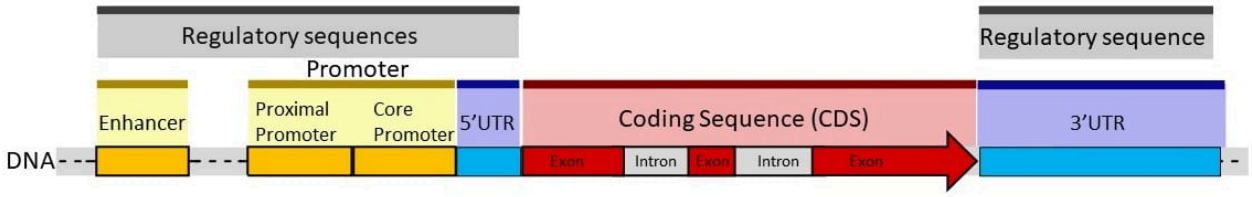


Figure 4.4.5: The transcription unit of a eukaryotic gene. The transcription unit includes a coding sequence/CDS (shown in red) and regulatory sequences known as an enhancer and a promoter (shown in yellow). The promoter region of a transcription unit often contains distinct sequences known as the core promoter and the proximal promoter. The coding sequence contains sequences that will be expressed as amino acids in the polypeptide (exons) and regions that will be spliced out (introns). The coding sequence is also flanked on either side by untranslated regions, or UTRs (shown in blue). (The Transcription Unit by Patricia Zuk, CC BY 4.0; adapted from Gene Structure by Thomas Shafee, CC BY-SA 4.0)

Transcription by the RNA polymerase

The enzyme complex responsible for transcription is the **RNA polymerase**. In prokaryotes, there is a single RNA polymerase that transcribes all genes. In eukaryotes, there are three RNA polymerases: RNA polymerase I, RNA polymerase II, and RNA polymerase III. Each of these are responsible for the transcription of specific RNA molecules in the eukaryotic cell. Table 4.4.1 summarizes these polymerases.

Table 4.4.1: ***Comparison of RNA polymerases***

RNA Polymerase	Transcription Location	RNAs transcribed
RNA polymerase I	nucleolus	5.8S, 18S, and 28S rRNA
RNA polymerase II	nucleoplasm	mRNA
RNA polymerase III	nucleoplasm	5S rRNA, tRNA

Both prokaryotic and eukaryotic RNA polymerases are made of several subunits, with each subunit performing a specific task in transcription. For example, specific subunits in the polymerase act to recognize the promoter sequence, open the DNA helix (i.e., act like a helicase), or hold the two DNA strands apart (i.e. act like SSBs). Additional subunits bind to the DNA template and synthesize the RNA transcript by linking RNA nucleotides together.

Transcription: from DNA to RNA

In both prokaryotes and eukaryotes, transcription occurs in three stages: **initiation, elongation, and termination**.

Initiation

Transcription begins with the binding of a large complex of proteins, called the **Transcription Initiation Complex,** to the promoter of the transcription unit (Figure 4.4.6). Contained with this complex is the RNA polymerase. The RNA polymerase recognizes the sequence of the core promoter and binds to the DNA at that region. This binding places the RNA polymerase in close proximity to the first nucleotide to be transcribed, otherwise known as the **transcription start site (TSS).** In eukaryotes, the TSS is found in the 5' UTR. Polymerase binding in eukaryotes is made more efficient by the proximal promoter elements found next to the core promoter. In eukaryotes, the binding of the RNA polymerase to the promoter region is further enhanced by the presence of DNA-binding proteins called **transcription factors**. Transcription factors called **general transcription factors** will help position the RNA polymerase at the core promoter, while more specialized transcription factors, called **specific transcription factors**, will bind to the proximal promoter and help regulate RNA polymerase activity. Additional transcription factors, known as **activators**, bind the gene's enhancer, significantly enhancing the initiation of transcription by the RNA polymerase. In order to do this, a "DNA bending protein" bends the DNA so that the enhancer sequence with its activators is brought into proximity of the promoter. Additional proteins, forming a **mediator complex,** facilitates the interaction between the activators at the enhancer and the transcription factors at the promoter so that the initiation of transcription occurs efficiently and rapidly. Together with the RNA polymerase, the transcription factors, activators, and the mediator complex make up a significant portion of the Transcription Initiation Complex. Once this complex is finished forming, the RNA polymerase acts to partially

unwind the DNA helix at the TSS creating a "transcription bubble". Transcription then enters the elongation stage.

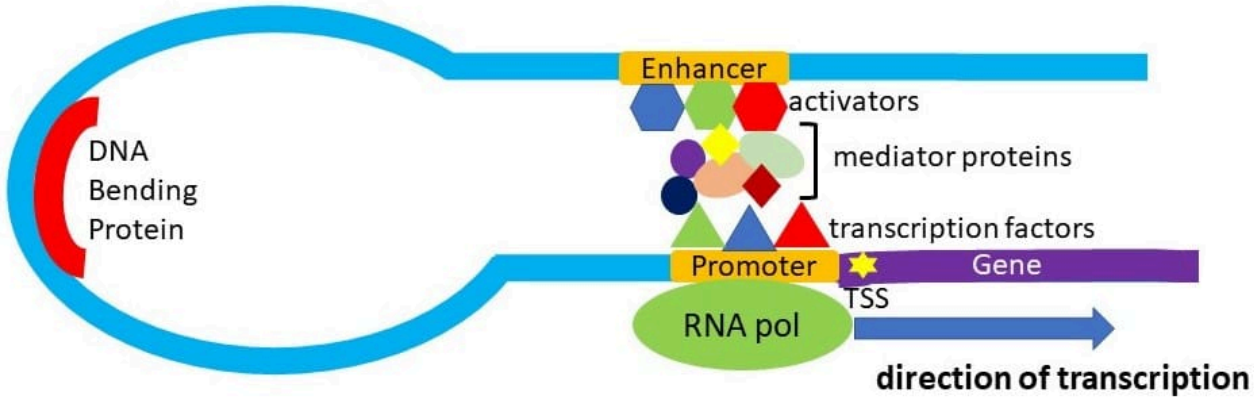


Figure 4.4.6: The transcription initiation complex. The start of transcription in eukaryotes requires a transcription initiation complex containing several proteins, including transcription factors, activators, and a mediator protein complex that facilitates the interactions between these proteins. A DNA bending protein bends the DNA, bringing the enhancer region close to the promoter region. The transcription initiation complex forms to increase the efficiency of RNA polymerase (RNA pol) binding to the promoter and the initiation of transcription at the transcription start site (TSS). (The Transcription Initiation Complex by Patricia Zuk, CC BY 4.0; adapted from Regulation of transcription by Bernstein0275, CC BY-SA 4.0)

Elongation

Elongation in both prokaryotes and eukaryotes occurs when the RNA polymerase reads the DNA sequence and links the incoming RNA nucleotides together to produce the RNA transcript (Figure 4.4.7). The RNA polymerase reads the DNA strand with the 3' to 5' orientation so that it can synthesize the RNA transcript in the 5' to 3' direction. This DNA strand is referred to as the **template strand**. The strand in the 5' to 3' direction is not read by the RNA polymerase and is called the non-template strand. During elongation, the RNA polymerase acts as a helicase to unwind the DNA helix, increasing the size of the transcription bubble. It holds the two DNA strands away from each other, acting as single-stranded binding proteins (SSBs). The RNA polymerase also does not require primers to bind to the DNA template strand. As such, a primase is not required for transcription. The RNA transcript that is made during elongation is complementary to the template strand, with the nucleotide uracil (U) used in place of thymine (T). This means if the template sequence is TAAGTG, the RNA transcript sequence would be UUCAC.

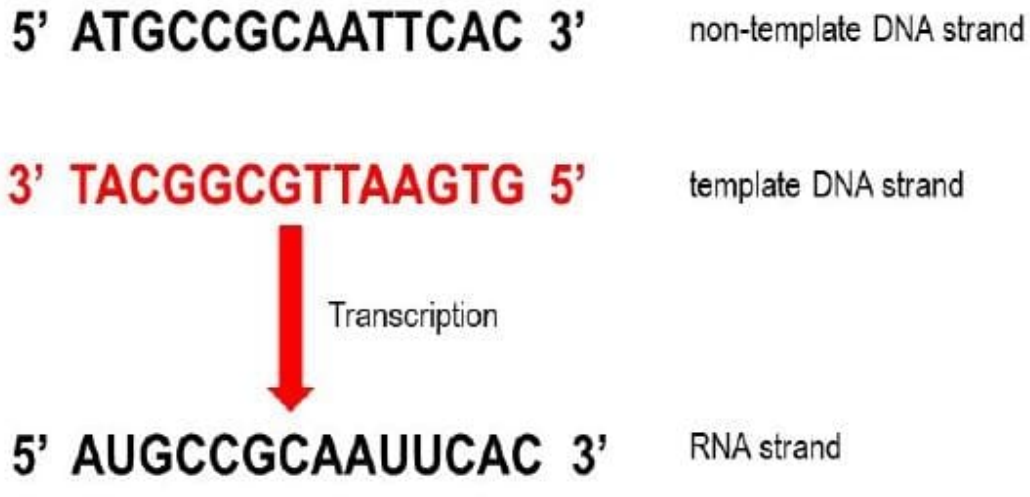


Figure 4.4.7: Transcription elongation. During transcription elongation, the DNA strand with the 3' to 5' orientation is used as the template for transcription (template DNA strand, red letters). The DNA strand with the 5' to 3' orientation (non-template strand, black letters) is not involved in transcription. In this figure, the template DNA sequence of TACGGCGTAAGTG is transcribed into an RNA strand with the sequence AUGCCGCAAUUCAC. (Transcription Elongation by Patricia Zuk, CC BY 4.0)

Termination

Once the gene is transcribed, the RNA polymerase needs to dissociate from the template strand and release the newly made RNA transcript. This is happens at a **termination sequence** located at the 3' end of the gene. The termination sequence can vary depending on the gene and on whether that gene is prokaryotic or eukaryotic. In eukaryotes, the termination sequence is found within the 3'UTR. The sequence is usually comprised of repeated nucleotide sequences that result in the RNA polymerase stalling, detaching from the template, and freeing the RNA transcript.

Concept in Action

Animation: Transcription

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

mRNA Processing in Eukaryotes

Newly transcribed RNA molecules made from eukaryotic protein-coding genes undergo specific processing steps in order to turn them into mature mRNA molecules. As listed in the section on mRNA above, these modifications are: the addition of a "cap" to the 5' end of the RNA, the addition of a "poly-A tail" to the 3' end of the RNA, and the removal of introns within the coding sequence of the gene. These three modifications must be completed before the resulting mRNA can be transferred from the nucleus to the cytoplasm so that it can translated into a protein. These modifications also increase the stability of the mRNA once in the cytoplasm. As a result, eukaryotic mRNAs last for several hours, whereas the typical prokaryotic mRNA, which lack most of these modifications, lasts no more than five seconds before being degraded.

Addition of the "Cap"

As the RNA transcript is being transcribed, a modified guanine nucleotide called 7-methylguanosine, is added as a “cap” to the 5' end of the growing RNA transcript through an unusual 5'-to-5' triphosphate linkage. This nucleotide is referred to as the **5' methylated cap** owing to the presence of a methyl group attached to the guanine base of the nucleotide (Figure 4.4.8). The 5' methylated cap plays a role in the export of the mRNA from the nucleus and protects the 5' end of the mRNA from degradation by RNAses, RNA-degrading enzymes. As will be discussed in Chapter 3.4: Translation, the cap also helps to regulate the start of translation.

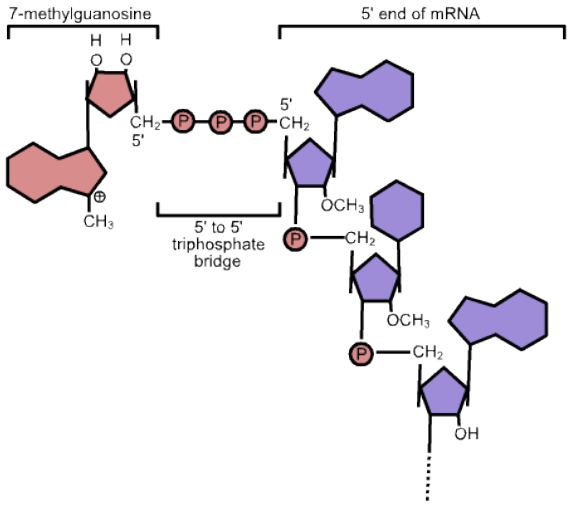


Figure 4.4.8: The 5' methylated cap of mRNA. mRNA is capped with a unique nucleotide called 7-methylguanosine (pink) that is attached to the 5' end of the mRNA (blue) through a unique 5' to 5' triphosphate bridge. (5' cap structure by Zephyris, CC BY-SA 3.0)

Addition of the "Tail"

Following the termination of transcription, a long chain of adenine nucleotides, called the **poly-A tail**, is added to the 3' end of the RNA transcript through a process called **polyadenylation**. A sequence in the 3'UTR region, known as the **polyadenylation signal** (AAUAAA) determines the location of this poly-A tail. During polyadenylation, the majority of the 3'UTR is cleaved off and a specialized RNA polymerase, called polyA polymerase, catalyzes the addition of about 250 adenine nucleotides. Like the cap, the poly-A tail plays a role in nuclear export, protects the RNA transcript from RNAse activity (at the 3' end) and plays a role in regulating translation. Interestingly, the addition of a poly-A tail to prokaryotic transcripts enhances mRNA degradation.

Splicing

The coding sequence of a eukaryotic protein-coding gene will contain regions that will be translated into the amino acids of a polypeptide chain. These regions are called **exons** because they are expressed. In addition, there are regions that will not be translated as amino acids. These intervening sequences are called **introns.** Introns must be removed or "spliced out" of the RNA transcript and the exons ligated or joined together during processing. The process of removing introns and reconnecting exons is called **splicing.** Splicing take place in the nucleus by a complex of RNA and proteins, called a **spliceosome.** The spliceosome is made up of five **snRP subunits**, with each snRP comprised of a stretch of **snRNA (small, nuclear RNA)** and supportive proteins (Figure 4.4.9).

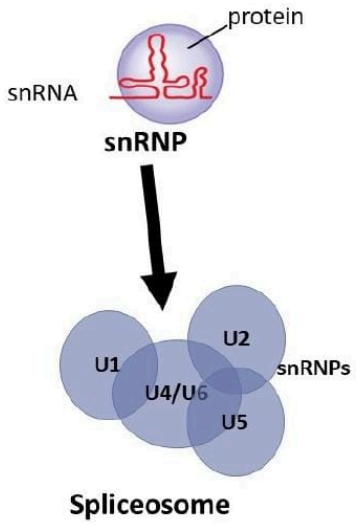


Figure 4.4.9: The spliceosome. Splicing is performed in eukaryotes by a spliceosome. The spliceosome is made of subunits called small nuclear ribonucleoproteins (snRPs). Each snRP is made of protein and a piece of small nuclear RNA (snRNA). Five snRPs (U1, U2, U4, U5, U6) assemble to form the functional spliceosome. (The Spliceosome by Patricia Zuk, CC BY 4.0)

The individual snRPs (U1, U2, U4, U5, and U6) assemble in a specific order on the coding sequence of the RNA transcript. They assemble at sequences flanking the intron called **splice sites.** The sequence at the start of an intron is called the **donor site** and usually has the nucleotides "GU" somewhere within the sequence. The sequence at the end of the intron is called the **acceptor site** and contains the nucleotides "AG" within its sequence. These two sequences are recognized by specific parts of the spliceosome. Once the spliceosome forms, it cuts out the intron and ligates the flanking exons (Figure 4.4.10). Following splicing, translation of the coding sequence produces the polypeptide that will become the functional protein.

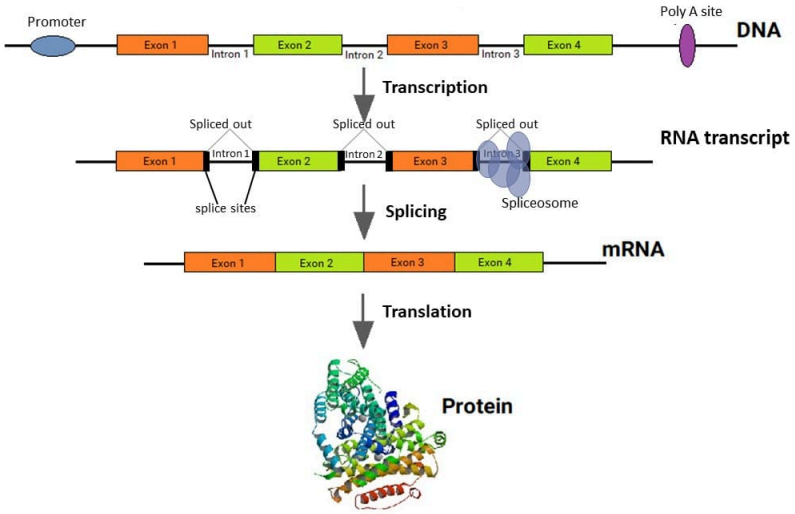


Figure 4.4.10: Intron Splicing in eukaryotes. DNA is transcribed into an RNA transcript containing introns and exons. The spliceosome (blue circles) assembles at an intron using splice site sequences found flanking the intron (shown in black). Each intron is spliced out and exons are ligated together by the spliceosome. Translation of the spliced mRNA produces a polypeptide which will become a functional protein. (Intron Splicing by Patricia Zuk, CC BY 4.0; adapted from Transcript and Splicing by Ganeshmanohar, CC BY-SA 4.0)

The splicing activity of the spliceosome is actually performed by the *snRNA of the spliceosome and not the proteins*. An RNA with enzymatic activity is classified as a **ribozyme**. Therefore, the spliceosome is a ribozyme.

Concept in Action

Animation: Splicing

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While all introns must be spliced from eukaryotic mRNA, exons also can be spliced. The removal of exons by the spliceosome creates novel mRNA transcripts and new "versions" of polypeptides. In this way, multiple polypeptide isoforms can be made from one coding sequence, depending on the exons spliced out. The process of exon removal to create different polypeptide isoforms is known as **alternative splicing** (Figure 4.4.11). Alternative splicing increases genetic diversity as more than one polypeptide can be made from a single mRNA.

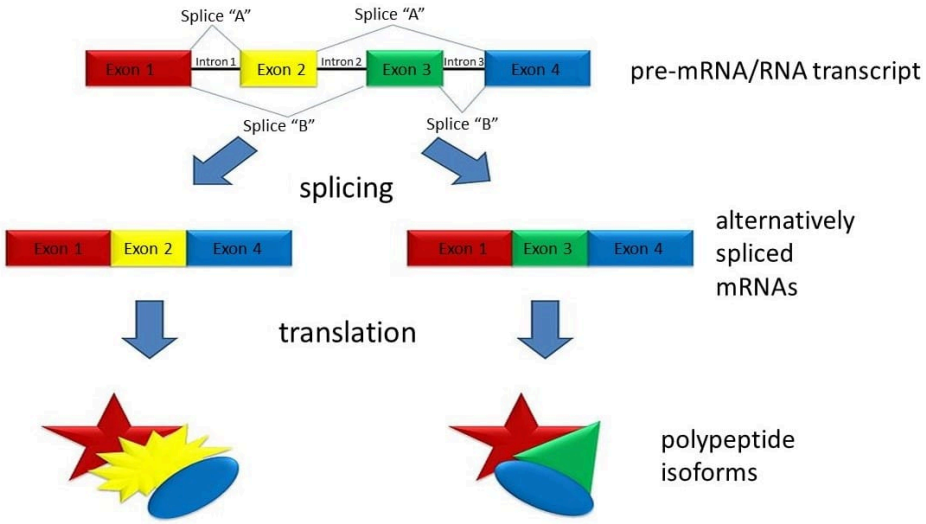


Figure 4.4.11: Alternative splicing of RNA in eukaryotes.The removal of different exons can produce distinct, alternatively spliced mRNA molecules that, when translated, produce different protein isoforms. In splice plan "A", exon 3 is spliced out of the RNA transcription along with all introns; exons 1, 2, and 4 are retained. In splice plan "B", exon 2 is spliced out of the RNA transcription along with all introns; exons 1, 3, and 4 are retained. As each exon produces a region of the polypeptide, different polypeptide isoforms can be produced. (Alternative Splicing by Patricia Zuk, CC BY 4.0; adapted from Splicing Overview by Agathman, CC BY-SA 3.0)

Splicing must be carefully performed by the spliceosome. If the splicing process errs by even a single nucleotide, the nucleotide sequence of the rejoined exons would be shifted, and the resulting protein would be nonfunctional following translation.

Key Concepts

Transcription is the first step in gene expression in which the DNA coding sequence is copied to make an RNA molecule. However, distinctions do exist. Protein-coding genes in both prokaryotes and eukaryotes are transcribed into mRNA molecules. These mRNA molecules will form the starting point for the last step in gene expression: translation. Transcription is performed by an RNA polymerase, which links nucleotides to form an RNA transcript.

The major concepts to remember are:

- Prokaryotes and eukaryotes share many similarities when undergoing transcription
- Only the DNA strand with the 3' to 5' orientation serves as a template for transcription
- Transcription has three stages: initiation, elongation, and termination

- Transcription of DNA into RNA is performed by an RNA polymerase
- Protein-coding genes are transcribed into mRNA molecules
- In eukaryotes, mRNA is made through the addition of a 5' cap, and a 3' poly-A tail to the RNA transcript. All introns are cut out of the mRNA coding sequence through splicing.
- In eukaryotes, most protein-coding genes require the formation of a transcription initiation complex to increase the efficiency of transcription

Glossary

5' methylated cap: a modified guanine (G) nucleotide found at the 5' end of the mRNA strand; prevents mRNA degredation

3' poly-A tail: a long chain of adenine (A) nucleotides attached to the 3' end of the mRNA strand

Activators: DNA binding proteins that bind to the enhancer regions of a DNA strand

Alternative splicing: the process that allows a single gene to code for multiple proteins by varying the combination of exons included in the final mRNA

Coding sequence: the section of the DNA/RNA sequence that is translated into a polypeptide strand; composed of exons and introns; has the same sequence as the transcribed RNA (with thymine replaced with uracil)

Enhancer: a sequence of DNA that binds activators and enhances the process of transcription

Exon: a section of the coding sequence that is translated into amino acids

Intron: a section of the coding sequence that is not translated

Mediator protein: a large complex of proteins that mediates the interaction between activator proteins and transcription factors during transcription initiation

Messenger RNA (mRNA): a sequence of RNA produced through the process of transcription; specifies the sequence of amino acids following translation; comprised of a coding sequence and flanked by a 5' methylated cap and a 3' poly-A tail

Pre-mRNA: unmodified RNA produced through transcription; must be modified through the addition of a 5' cap, a 3' poly-A tail, and splicing; also called an RNA transcript

Polyadenylation: the addition of a long chain of adenine nucleotides (called the poly-A tail) to the 3' end of mRNA; enhances stability of the mRNA and translation

Promoter sequence: a sequence of DNA where the RNA polymerase binds; determines the site of transcription initiation

Ribosomal RNA (rRNA): a type of RNA that is a structural component of ribosomes; aids in protein synthesis

RNA polymerase: an enzyme that reads the DNA template strand and synthesizes an complementary RNA strand using RNA nucleotides

RNA processing: the modifications made to pre-mRNA, including capping, splicing, and polyadenylation, to produce mature mRNA

RNA transcription: the process by which a DNA sequence is copied into a complementary RNA strand

Spliceosome: a large complex of snRPs that splices out introns (and exons) from the coding sequence of mRNA

Small nuclear RNA (snRNA): a small sequence of RNA found as part of a small nuclear ribonucleoprotein (snRNP)

Small nuclear ribonucleoprotein (snRNP): a combination of an snRNA molecule and a protein; also known as a ribonucleoprotein (RNP)

Splicing: the process of removing introns (and exons) from the pre-mRNA transcript; also called RNA splicing

TATA Box: a specific DNA sequence found in the promoter region that helps position RNA polymerase for transcription initiation

Template strand: the DNA strand copied into a complementary RNA strand through the process of transcription; has the 3' to 5' orientation; also called the anti-sense strand

Termination Sequence: a specific DNA sequence that signals the end of transcription

Transcription factors: DNA binding proteins that bind specific sequences of DNA and assist in the initiation and regulation of transcription

Transfer RNA (tRNA): an RNA molecule that helps decode mRNA sequences into proteins by carrying specific amino acids to the ribosome

Untranslated region (UTR): a stretch of RNA that is not translated; contains important regulatory sequences for transcription and translation; found in front of the coding sequence (5' UTR) and behind the coding sequence (3' UTR); found in both DNA and RNA

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4.5: Translation of the polypeptide

Biotech Focus

One of the first experiments to try and determine the nature of the genetic code was performed by Francis Crick, Sydney Brenner, Leslie Barnett, and RJ Watts-Tobin. In this experiment, a mutagen known as **proflavin** was used to induce mutations in the DNA of **T4 bacteriophage**. The researchers knew that proflavin inserts itself between DNA bases, typically resulting in insertion or deletion of a single base pair. Additional treatment of bacteriophage with more proflavin introduces additional insertions or deletions. The researchers treated bacteriophage with proflavin and then used these mutated bacteriophage to infect *E.coli* bacteria. The infected *E.coli* were then grown on plates of agar. They proposed that proflavin-treated bacteriophage (i.e. mutated) would be unable to kill their bacterial hosts. As a result, the bacteria would grow as a sheet on the plate, known as a "lawn" of bacteria. In contrast, *E.coli* infected with non-treated bacteriophage (i.e. non-mutated) would be killed, producing a clear spot, or a "plaque", on the agar plate. Crick and his colleagues discovered that bacteriophage treated once with proflavin to introduce single insertions or deletions could not kill their *E.coli* hosts. With each round of proflavin exposure, additional single insertions or deletions were introduced and depending on the number of proflavin treatments, plaques would be seen. In this way, the Crick and his colleagues were able to determine that the codon consisted of three DNA nucleotides.

Introduction

Once DNA was accepted as the genetic material, the question became how could such a simple molecule with only four bases account for the inheritance of so many different physical traits? The recognition that enzyme activity could be inherited in the same way as morphological characteristics led to the "*one-gene-one enzyme*" hypothesis that earned G. W. Beadle, E. L. Tatum and J. Lederberg the 1958 Nobel Prize for Physiology and Medicine. When enzymes were later shown to be proteins, the hypothesis became "*one-gene-one protein*". When proteins were shown to be composed of one or more polypeptides, the final hypothesis became ***"one-gene-one-polypeptide".*** This hypothesis stands today. In both prokaryotic and eukaryotic cells, protein-coding genes are expressed (i.e. transcribed and translated) as polypeptides. These polypeptides are then processed so that a functional protein can be produced.

Learning Objectives

Translation decodes the genetic code and produces proteins. At the end of this section, you will be able to:

- Describe the structure of the ribosome
- Explain how tRNA molecules are attached to amino acids
- Explain the genetic code used in translation
- Describe the initiation, elongation, and termination steps of translation
- Define the reading frame of translation and its importance in translation
- List and explain the four levels of protein processing

The Ribosome

The “machine” of protein translation is the **ribosome**. A ribosome is a complex macromolecule composed of rRNA and ribosomal proteins. Ribosomes exist in the cytoplasm of prokaryotes and can be found in the cytoplasm and attached to the rough endoplasmic reticulum of eukaryotes. In *E. coli*, there are between 10,000 and 70,000 ribosomes present in each cell at any given time. Both prokaryotic and eukaryotic ribosomes are made of two subunits: **the small subunit** and the **large subunit.** Each subunit is created by combining specific rRNA molecules with polypeptides. In prokaryotes, this assembly takes place in the cytoplasm, with the assembly occurring in the nucleolus in eukaryotes. Table 4.5.1 summarizes the ribosomes of prokaryotes and eukaryotes.

Table 4.5.1: Comparison of Prokaryotic and Eukaryotic Ribosomes

Cell type	Subunit	rRNA	Number of proteins	Location of Synthesis
Prokaryote	Small	16S	21	Cytoplasm
	Large	5S, 23S	34	Cytoplasm
Eukaryote	Small	18S	33	Nucleolus
	Large	5S, 5.8S, 28S	48	Nucleolus

The small subunit of the ribosome has a site that recognizes and binds the mRNA, while the large subunit has three binding sites for tRNA molecules: the **A site (A = aminoacyl)**, the **P site (P = peptidyl)** and the **E site (E = "exit")** (Figure 4.5.1). The tRNA molecule at the P site is attached to the growing peptide chain. The tRNA at the A site is attached to the new, "incoming" amino acid to be added to the peptide chain. The tRNA at the E site has no amino acid and "exits" the ribosome from this site. During translation, these subunits will assemble around the mRNA.

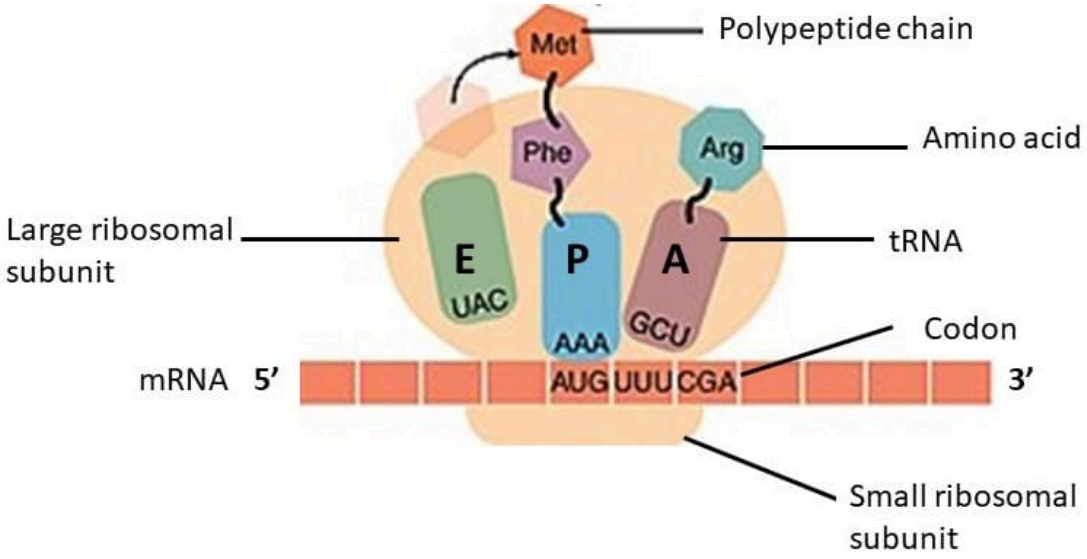


Figure 4.5.1: The ribosome. The assembled ribosome is made of a large and small ribosomal subunit attached to the mRNA molecule. The large ribosomal subunit is shown with three tRNA molecules positioned at the E (left binding site), P (center binding site), and A (right binding site) sites. (The Ribosome by Patricia Zuk, CC BY 4.0; adapted from Translation by OpenStax, CC BY-SA 4.0)

tRNAs

Transfer RNAs (tRNAs) are structural RNA molecules, roughly 70 to 80 nucleotides in length. They are transcribed from genes in the nucleoplasm by RNA polymerase III. Once transcribed, they assume a distinctive secondary and tertiary conformation (Figure 4.5.2). The secondary conformation can be visualized as a series of stems and loops. One loop, the **anti-codon loop**, has a three-nucleotide sequence that is complementary to the codon of the mRNA. At the opposite end of the molecule is the **amino acid attachment site**, the site where the amino acid is covalently bound. This site has a conserved sequence of "CCA". These two loops are also found at the opposite ends of the tRNA molecule when in its tertiary conformation.

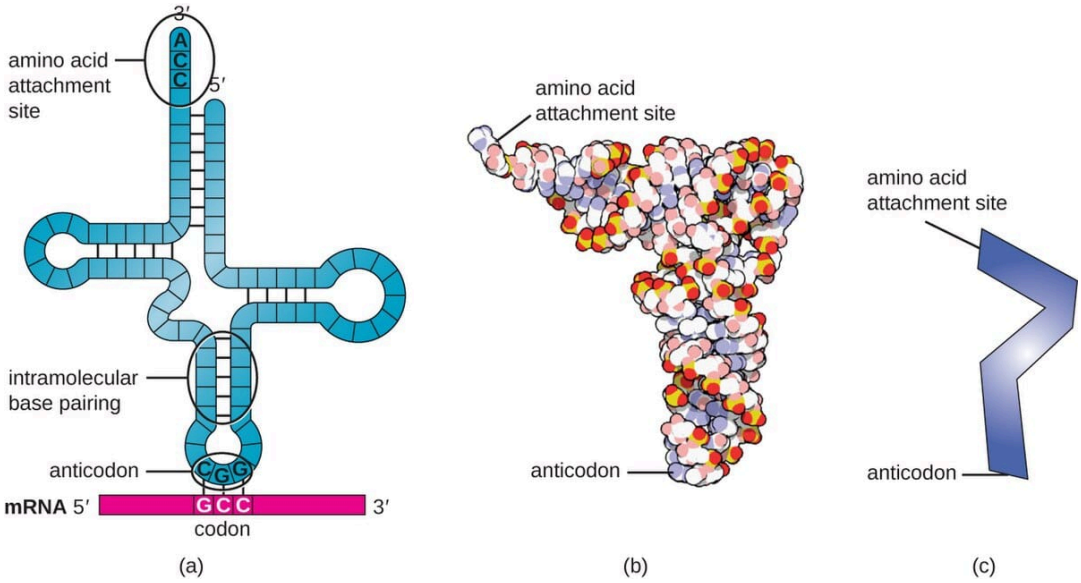


Figure 4.5.2: The tRNA molecule. (a) The secondary structure of a tRNA molecule with its anti-codon loop bound to the mRNA codon (bottom) and its amino acid attachment site (top). (b) Tertiary conformation of the tRNA with these two sites identified. (c) Simplified representation of the tRNA. (attribution provided below)

The attachment of the amino acid to the amino acid site occurs in the cytoplasm by an enzyme complex called an **amino acyl-tRNA synthetase** (Figure 4.5.3). Once attached, the tRNA is said to be "charged". This charging is powered by ATP hydrolysis and is highly specific, requiring that the shape of the enzyme's active site corresponds to the shape of the amino acid and tRNA molecule. Eukaryotic cells have at least 20 synthetases, each responsible for the attachment of a specific amino acid to the correct tRNA molecule. Depending on species and cell type, 40 to 60 types of charged tRNAs are used in eukaryotic translation.

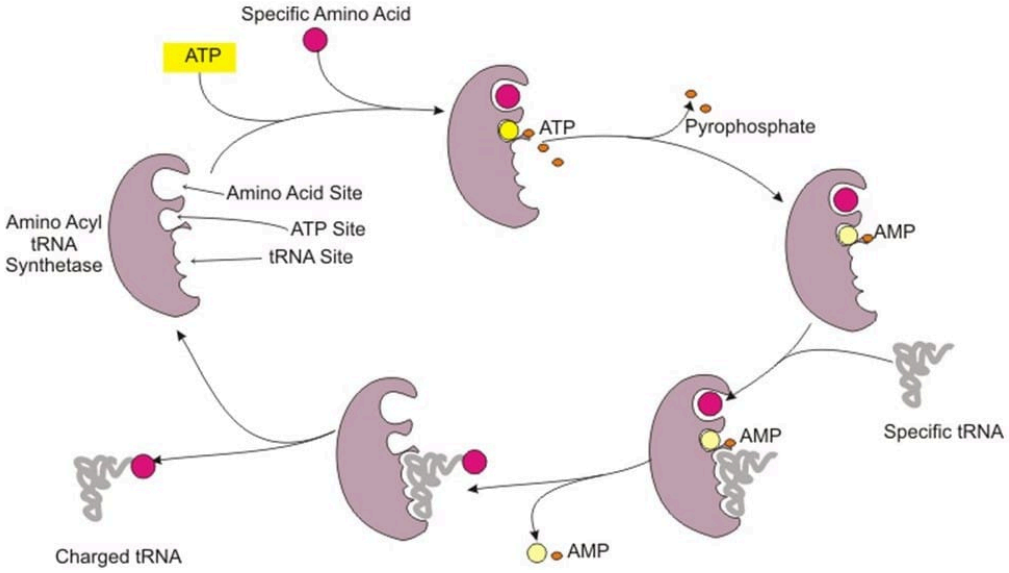


Figure 4.5.3: tRNA "charging". Loading of a tRNA molecule with an amino acid, also known as tRNA "charging", is done using an enzyme called aminoacyl-tRNA synthetase. ATP and the specific amino acid (red circle) bind to their sites on the synthetase, followed by hydrolysis of the ATP to AMP. The corresponding tRNA (gray line) binds the synthetase. The release of the AMP is followed by linking of the amino acid to the tRNA. The "charged" tRNA is released from the synthetase and can be used in translation. (Charge tRNA by Boumphreyfr, CC BY-SA 3.0)

The Genetic Code of Translation

To translate the "message" of the mRNA into a polypeptide strand, the mRNA is read three nucleotides at a time by the ribosome. This three-nucleotide group is called a **codon**. Specific codons correspond to specific amino acids. This relationship between codon sequence and amino acid is known as the **genetic code** (Figure 4.5.4). Of the 64 possible mRNA codons, 61 of them code

for specific amino acids and three specify the termination of translation as **stop codons**. The genetic code is **universal**, meaning that the amino acids coded for are the same in most known organisms. In addition, the genetic code is **degenerate**, meaning a given amino acid can be encoded by more than one codon. For instance, the amino acid leucine is encoded by the codons CUA, CUU, CUC, and CUG. The exception to the degenerate code is the amino acid methionine, coded for by AUG. This is the only amino acid to have a single codon sequence. Similarly, stop codons are encoded by a single codon sequence.

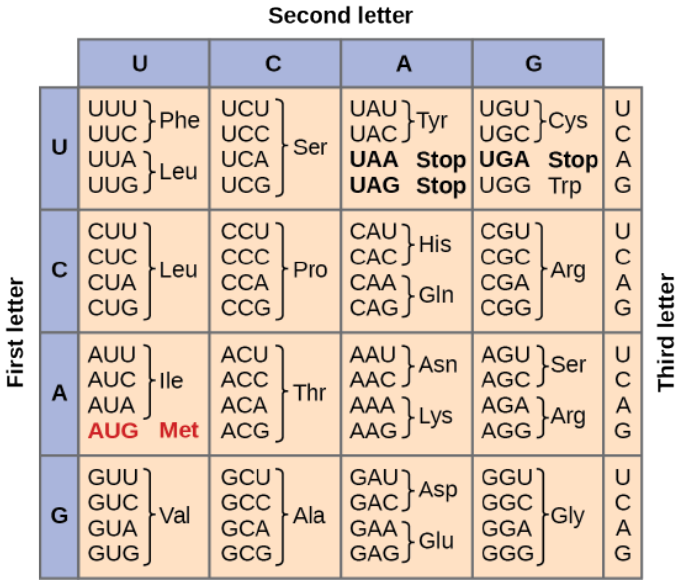


Figure 4.5.4: The genetic code for translation. Specific sequences of 3 nucleotide letters, known as codons, code for specific amino acids or stop codons. (The Codon Table, from OpenStax Biology 2e, CC BY 4.0)

Translation

As with transcription, translation can be broken down into three stages: **initiation, elongation, and termination.** The actual process of translation in prokaryotic and eukaryotic cells is very similar. However, there are some differences in the initiation stage. In addition, because of the lack of a nucleus in prokaryotes, translation can take place at the same time as transcription.

Initiation of Translation

Translation in both prokaryotes and eukaryotes begins with the formation of an **translation initiation complex**, comprised of the small and large ribosomal subunits, the mRNA template, and a special **initiator tRNA (**Figure 4.5.5). In prokaryotes, the initiator tRNA is called **tRNA^{fMet}**. In eukaryotes it is known as **tRNA-Met$_i$**. The assembly of the translation initiation complex requires the assistance of **initiation factors (IFs)** in prokaryotes and **eukaryotic initiation factors (eIFs)** in eukarryotes. To assemble the complex, the 5' end of the mRNA is first recognized by the small subunit. In eukaryotes, the small subunit recognizes the 5' methylated cap. The subunit binds the mRNA and "scans" toward the 3' end of the mRNA looking for the first AUG codon, or the "start codon" . This codon is located in the 5' UTR and is flanked on either side by specific nucleotides. Together, the AUG with its flanking sequences is known as the **Kozak sequence,** named for its discoverer, Marilyn Kozak. Because prokaryotic mRNA molecules do not have a 5' methylated cap, the small subunit recognizes a sequence of mRNA called the **Shine-Dalgarno sequence** (AGGAGG; named for John Shine and Lynn Dalgarno). The prokaryotic small subunit then stops at the AUG located just downstream sequence. Once the small subunit has stopped, the tRNA^{fMet} positions itself at the start codon. In eukaryotes, the tRNA-Met$_i$ accompanies the small subunit to the start codon. The large subunit is the last component to join the translation initiation complex, binding to the small ribosomal subunit so that the initiator tRNA is positioned at the P site.

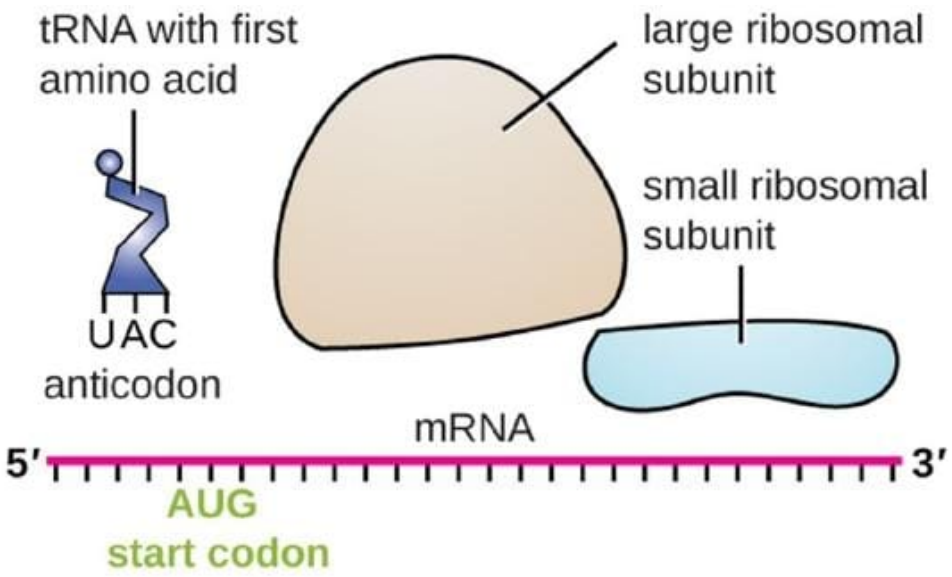


Figure 4.5.5: The translation initiation complex. The components of the translation initiation complex in prokaryotes and eukaryotes include the two ribosomal subunits, the mRNA and the initiator tRNA bound to the first amino acid (i.e., methionine). (attribution provided below)

Elongation of Translation

Once the translation initiation complex forms around the mRNA, a new tRNA molecule, bearing the amino acid that corresponds to the next codon, binds to the ribosome at the A site. This binding represents the start of the **elongation phase**. The rRNA molecules of the ribosome, acting as a ribozyme, catalyze the formation of a peptide bond between the methionine and this new amino acid. The methionine is transferred to the tRNA at the A site, making dipeptide. The ribosome will then shift to the 3' end of the mRNA. A new tRNA/amino acid complex will enter the A site. The tRNA with the two amino acids will shift to the P site and the "empty" tRNA will enter the E site and exit the ribosome. The ribosome will move the dipeptide over to the new amino acid forming a peptide bond and a tripeptide. The ribosome will shift again and the process will repeat itself over and over again. As the ribosome moves to the 3' end of the mRNA, the peptide chain will elongate one amino acid at a time, producing a polypeptide.

Termination of Translation

Eventually, the ribosome's A site will be positioned over one of the three possible stop codons. At this point, a **release factor** will enter the A site, catalyzing the dissociation of the ribosomal subunits from the mRNA and the release of the polypeptide into the cytoplasm. Because the release factor is not bound to an amino acid, *the stop codon does not code for an amino acid*. As long as the mRNA molecule remains intact, the ribosomal subunits can reform around the mRNA to start the process of translation again.

The following summarizes the major events of translation (Figure 4.5.6):

- **Initiation:** A translation initiation complex forms, involving the small ribosomal subunit, the initiator tRNA (with methionine), and the large ribosomal subunit. The small ribosomal subunit binds the mRNA and positions itself at the first AUG (i.e., the start codon), setting the “reading frame” of translation. The initiator tRNA and large subunit bind to complete the complex.
- **Elongation:** A tRNA with a new amino acid binds to the A site of the ribosome. The ribosome catalyzes the formation of a peptide bond between the peptide chain bound to the tRNA at the P site and the new amino acid bound to the tRNA at the A site. The ribosome shifts to the 3’ end of the mRNA so that the tRNA-peptide chain at the A site moves into the P site and the empty tRNA shifts into the E site to exit the ribosome.
- **Termination:** When the A site of the ribosome reaches a stop codon, a release factor binds to the A site, releasing the ribosome and peptide strand. The peptide strand is now considered to be a completed polypeptide

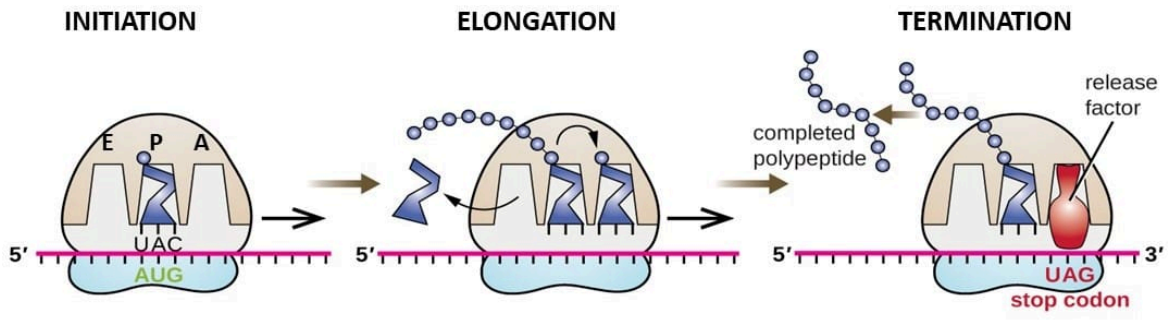


Figure 4.5.6: Translation summary. Initiation: Translation begins when the small and large ribosomal subunits assemble around the mRNA and the first tRNA, bearing a methionine, binds to the AUG start codon that is positioned at the P site. Elongation: Translation continues as new tRNA molecules bind to the mRNA at the A site. The peptide chain at the P site is transferred from its tRNA to the tRNA located at the A site and forms a bond with the new amino acid, growing the peptide chain by one amino acid. Termination: Translation stops when the A site of the ribosome reaches a stop codon and a release factor, releasing the ribosome and peptide strand as a completed polypeptide. (attribution provided below)

Following translation, the polypeptide chain undergoes specific processing steps that drives its folding into a functional protein (see Protein Processing below). In addition, the polypeptide will undergo specific chemical modifications like the attachment of phosphate groups (i.e. phosphorylation) or methyl groups (i.e., methylation) to specific amino acids in the polypeptide sequence. The chemical modifications play important roles in the folding of the polypeptide and its resulting function.

Concept in Action

Animation: Translation

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The Translation Reading Frame

Synthesis of polypeptides in both prokaryotic and eukaryotic cells begins with the amino acid methionine. In the majority of eukaryotic and prokaryotic mRNA sequences, this amino acid is specified by the first AUG codon encountered by the ribosome. Because it is the start of the polypeptide, this codon is called the **start codon**. Three codons, UAA, UAG, UGA, do not specify amino acids, but signal the end of translation. As such, they are called **stop codons**. The sequence of codons found in between the start codon and the stop codon is called the **reading frame**. The reading frame determines the sequence of amino acids in the polypeptide. "Mistakes" found in the mRNA sequence that eliminate or add nucleotides to the reading frame can "shift" the reading frame and alter the sequence of amino acids. These mistakes will be discussed next in Chapter 3.5: Mutations and DNA Repair.

Protein Processing

The translation of mRNA into a polypeptide strand is not the end of protein synthesis. Rather, it is only the beginning. Once translated, the polypeptide strand will undergo additional modifications so that a functional protein is made. These modifications produce distinct levels of **protein conformation or structure**, that directly affect function (Figure 4.5.7). To read more about these levels of structure, go to Chapter 2.6 Proteins.

Primary structure/conformation: The amino acid sequence of the polypeptide produced through translation. The amino acid sequence has a direct effect on the additional conformation levels described below.

Secondary structure/conformation: The folding of the amino acid sequence into alpha helices and beta-pleated sheets. The

secondary level is a result of hydrogen bonding between amino groups and carboxyl groups in neighboring regions of the polypeptide chain.

Tertiary structure/conformation: The unique three dimensional shape of the protein. Protein folding is a spontaneous process driven by interactions between the R groups of amino acids. These interactions include: hydrophobic (van der Waals) interactions, ionic bonding and hydrogen bonds. The final shape that results is the most energetically favorable one and is stabilized by the formation of disulfide bridges that form between the R groups of cysteine amino acids. Protein folding in eukaryotes is monitored by a class of proteins called chaperones.

Quaternary structure/conformation: The shape of a protein complex comprised on more than one protein subunit. Each subunit has its own unique tertiary conformation and interacts with one another through hydrogen bonds and hydrophobic/van der Waals forces. There are many types of proteins complexes, with globular (spherical) and fibrous being the most common. The majority of prokaryotic and eukaryotic proteins are made of multiple protein subunits.

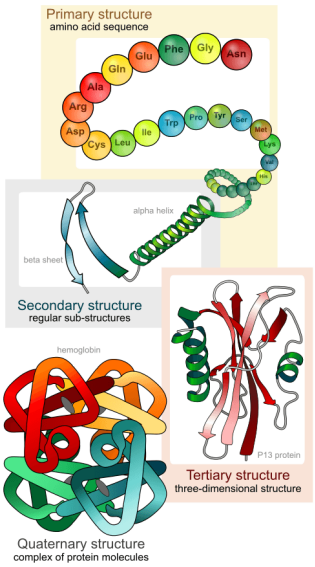


Figure 4.5.7: The four levels of protein conformation. The primary structure of the protein (i.e. the amino acid sequence) is folded into regions of alpha helices and beta-pleated sheets to form the secondary structure of the protein. The folding of the secondary level into a unique three-dimensional shape is the protein's tertiary structure. Complex proteins assembled from more than protein subunit is the protein's quaternary structure. (Main Protein Structure by LadyofHats; public domain)

Key Concepts

Translation is the process that converts the "message" held in the mRNA into the polypeptide made of amino acids. Through specific post-translational modifications the polypeptide becomes a functional protein

Some major concepts to remember are:

- The genetic information in the mRNA "message" is translated into a sequence of amino acids through the process of translation
- Translation is performed by ribosomes that read the mRNA sequence in triplets called codons. Amino acids are brought to the ribosome by tRNA molecules.
- Each codon specifies one amino acid. Most amino acids are encoded for by more than one codon
- The first AUG codon of the mRNA sequence is the start codon and it codes for methionine
- Three codons, UAA, UAG, UGA, are stop codons and do not code for amino acids
- The sequence between the start and stop codon is the reading frame
- Translation is comprised of three stages: initiation, elongation and termination
- Translation initiation involves the assembly of the two ribosomal subunits on the mRNA sequence and the binding of the initiator tRNA to the start codon
- During elongation, the ribosome translates the reading frame into a polypeptide made of amino acids

- In termination, a release factor binds the stop codon and the ribosome dissociates from the mRNA and releases its polypeptide
- Once translated, the polypeptide undergoes additional modification that will produce the secondary, tertiary, and possibly a quaternary level of structure or conformation

Glossary

Anticodon: a sequence of three nucleotides found in tRNA that is complementary to a codon in mRNA; ensures the correct amino acid is added to the growing polypeptide chain

Aminoacyl-tRNA synthetase: an enzyme that attaches the correct amino acid to its corresponding tRNA

Codon: a sequence of three DNA or RNA nucleotides; corresponds to a specific amino acid or a stop signal during translation

Elongation: the process in which amino acids are sequentially added to the growing polypeptide chain

Initiation factor (IF): a protein that helps in the assembly of the ribosome on the mRNA and is required to start the process of translation; known as eukaryotic initiation factors (eIFs) in eukaryotic translation

Initiation: the first stage of translation, where the ribosome assembles around the mRNA and the start codon is recognized

Initiator tRNA: the first tRNA to enter the ribosome; carries methionine; binds the first AUG codon in the mRNA

Kozak sequence: a sequence of mRNA recognized by the small subunit of the eukaryotic ribosome

Messenger RNA (mRNA): a type of RNA that carries genetic instructions from DNA to the ribosome for protein synthesis

Release factor: a protein that binds within the ribosome to stop translation and release the ribosome from the mRNA

Ribosomal RNA (rRNA): a piece of RNA that is a structural and functional component of a ribosome

Ribosome: a large complex comprised of several rRNA molecules and multiple proteins; made in the nucleolus as a small subunit and a large subunit; the engine of translation

Shine-Dalgarno sequence: a sequence of mRNA recognized by the small subunit of the prokaryotic ribosome

Start codon: the first AUG codon of the coding sequence; codes for methionine

Stop codon: one of three codons (UAA, UAG, UGA) that stops translation; does not code for an amino acid

Termination: the final stage of translation, where a stop codon is recognized, and the completed polypeptide is released from the ribosome

Transfer RNA (tRNA): a small stretch of RNA that functions as an amino acid donor during translation

Translation: the process by which ribosomes synthesize proteins using messenger RNA (mRNA) as a template

Attributions

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- Figure 4.5.2
- Figure 4.5.5
- Figure 4.5.6

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4.6: How Genes Are Regulated

For a cell to function properly, necessary proteins must be synthesized at the proper time. All organisms and cells control or regulate the transcription and translation of their DNA into protein. The process of turning on a gene to produce RNA and protein is called gene expression. Whether in a simple unicellular organism or in a complex multicellular organism, each cell controls when and how its genes are expressed. For this to occur, there must be a mechanism to control when a gene is expressed to make RNA and protein, how much of the protein is made, and when it is time to stop making that protein because it is no longer needed.

Cells in multicellular organisms are specialized; cells in different tissues look very different and perform different functions. For example, a muscle cell is very different from a liver cell, which is very different from a skin cell. These differences are a consequence of the expression of different sets of genes in each of these cells. All cells have certain basic functions they must perform for themselves, such as converting the energy in sugar molecules into energy in ATP. Each cell also has many genes that are not expressed, and expresses many that are not expressed by other cells, such that it can carry out its specialized functions. In addition, cells will turn on or off certain genes at different times in response to changes in the environment or at different times during the development of the organism. Unicellular organisms, both eukaryotic and prokaryotic, also turn on and off genes in response to the demands of their environment so that they can respond to special conditions.

The control of gene expression is extremely complex. Malfunctions in this process are detrimental to the cell and can lead to the development of many diseases, including cancer.

Prokaryotic versus Eukaryotic Gene Expression

To understand how gene expression is regulated, we must first understand how a gene becomes a functional protein in a cell. The process occurs in both prokaryotic and eukaryotic cells, just in slightly different fashions.

Because prokaryotic organisms lack a cell nucleus, the processes of transcription and translation occur almost simultaneously. When the protein is no longer needed, transcription stops. As a result, the primary method to control what type and how much protein is expressed in a prokaryotic cell is through the regulation of DNA transcription into RNA. All the subsequent steps happen automatically. When more protein is required, more transcription occurs. Therefore, in prokaryotic cells, the control of gene expression is almost entirely at the transcriptional level.

The first example of such control was discovered using *E. coli* in the 1950s and 1960s by French researchers and is called the *lac* operon. The *lac* operon is a stretch of DNA with three adjacent genes that code for proteins that participate in the absorption and metabolism of lactose, a food source for *E. coli*. When lactose is not present in the bacterium's environment, the *lac* genes are transcribed in small amounts. When lactose is present, the genes are transcribed and the bacterium is able to use the lactose as a food source. The operon also contains a promoter sequence to which the RNA polymerase binds to begin transcription; between the promoter and the three genes is a region called the operator. When there is no lactose present, a protein known as a repressor binds to the operator and prevents RNA polymerase from binding to the promoter, except in rare cases. Thus very little of the protein products of the three genes is made. When lactose is present, an end product of lactose metabolism binds to the repressor protein and prevents it from binding to the operator. This allows RNA polymerase to bind to the promoter and freely transcribe the three genes, allowing the organism to metabolize the lactose.

Eukaryotic cells, in contrast, have intracellular organelles and are much more complex. Recall that in eukaryotic cells, the DNA is contained inside the cell's nucleus and it is transcribed into mRNA there. The newly synthesized mRNA is then transported out of the nucleus into the cytoplasm, where ribosomes translate the mRNA into protein. The processes of transcription and translation are physically separated by the nuclear membrane; transcription occurs only within the nucleus, and translation only occurs outside the nucleus in the cytoplasm. The regulation of gene expression can occur at all stages of the process (Figure $4.6.1$). Regulation may occur when the DNA is uncoiled and loosened from nucleosomes to bind transcription factors (epigenetic level), when the RNA is transcribed (transcriptional level), when RNA is processed and exported to the cytoplasm after it is transcribed (post-transcriptional level), when the RNA is translated into protein (translational level), or after the protein has been made (post-translational level).

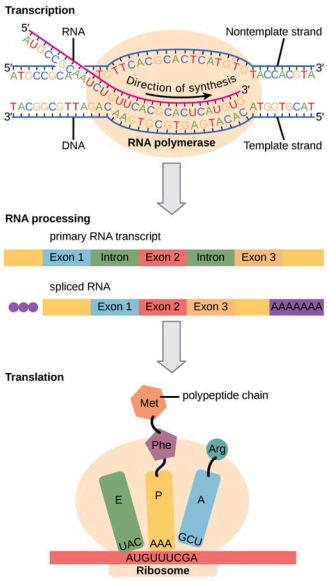


Figure 4.6.1**:** Eukaryotic gene expression is regulated during transcription and RNA processing, which take place in the nucleus, as well as during protein translation, which takes place in the cytoplasm. Further regulation may occur through post-translational modifications of proteins.

The differences in the regulation of gene expression between prokaryotes and eukaryotes are summarized in Table 4.6.1.

Table 4.6.1***:*** *Differences in the Regulation of Gene Expression of Prokaryotic and Eukaryotic Organisms*

Prokaryotic organisms	Eukaryotic organisms
Lack nucleus	Contain nucleus
RNA transcription and protein translation occur almost simultaneously	• RNA transcription occurs prior to protein translation, and it takes place in the nucleus. RNA translation to protein occurs in the cytoplasm. • RNA post-processing includes addition of a 5' cap, poly-A tail, and excision of introns and splicing of exons.
Gene expression is regulated primarily at the transcriptional level	Gene expression is regulated at many levels (epigenetic, transcriptional, post-transcriptional, translational, and post-translational)

EVOLUTION IN ACTION: Alternative RNA Splicing

In the 1970s, genes were first observed that exhibited alternative RNA splicing. Alternative RNA splicing is a mechanism that allows different protein products to be produced from one gene when different combinations of introns (and sometimes exons) are removed from the transcript (Figure 4.6.2). This alternative splicing can be haphazard, but more often it is controlled and acts as a mechanism of gene regulation, with the frequency of different splicing alternatives controlled by the cell as a way to control the production of different protein products in different cells, or at different stages of development. Alternative splicing is now understood to be a common mechanism of gene regulation in eukaryotes; according to one estimate, 70% of genes in humans are expressed as multiple proteins through alternative splicing.

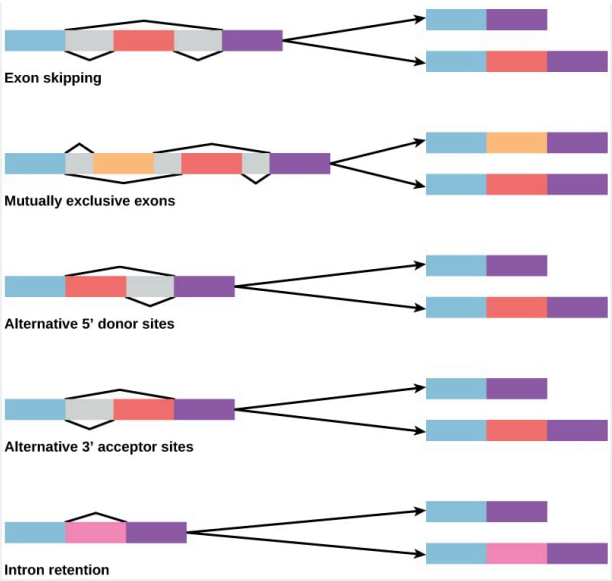


Figure 4.6.2**:** There are five basic modes of alternative splicing. Segments of pre-mRNA with exons shown in blue, red, orange, and pink can be spliced to produce a variety of new mature mRNA segments.

How could alternative splicing evolve? Introns have a beginning and ending recognition sequence, and it is easy to imagine the failure of the splicing mechanism to identify the end of an intron and find the end of the next intron, thus removing two introns and the intervening exon. In fact, there are mechanisms in place to prevent such exon skipping, but mutations are likely to lead to their failure. Such "mistakes" would more than likely produce a nonfunctional protein. Indeed, the cause of many genetic diseases is alternative splicing rather than mutations in a sequence. However, alternative splicing would create a protein variant without the loss of the original protein, opening up possibilities for adaptation of the new variant to new functions. Gene duplication has played an important role in the evolution of new functions in a similar way—by providing genes that may evolve without eliminating the original functional protein.

Summary

While all somatic cells within an organism contain the same DNA, not all cells within that organism express the same proteins. Prokaryotic organisms express the entire DNA they encode in every cell, but not necessarily all at the same time. Proteins are expressed only when they are needed. Eukaryotic organisms express a subset of the DNA that is encoded in any given cell. In each cell type, the type and amount of protein is regulated by controlling gene expression. To express a protein, the DNA is first transcribed into RNA, which is then translated into proteins. In prokaryotic cells, these processes occur almost simultaneously. In eukaryotic cells, transcription occurs in the nucleus and is separate from the translation that occurs in the cytoplasm. Gene expression in prokaryotes is regulated only at the transcriptional level, whereas in eukaryotic cells, gene expression is regulated at the epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels.

Glossary

alternative RNA splicing - a post-transcriptional gene regulation mechanism in eukaryotes in which multiple protein products are produced by a single gene through alternative splicing combinations of the RNA transcript

epigenetic - describing non-genetic regulatory factors, such as changes in modifications to histone proteins and DNA that control accessibility to genes in chromosomes

gene expression - processes that control whether a gene is expressed

post-transcriptional - control of gene expression after the RNA molecule has been created but before it is translated into protein

post-translational - control of gene expression after a protein has been created

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

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4.7: Mutations and DNA Repair

Biotech Focus

In 500 BCE, the Greek physician Hippocrates identified crab-like tumors that he called *karkinos*. From this word, we eventually derived the term **cancer**. Through our study of cancer, researchers began to study **mutagens** and their production of DNA mutations. The association between physical mutagens, like X-rays and ultraviolet (UV) light, and cancer was confirmed in the early part of the 20th century. However, a direct link to DNA mutations and chemical mutagens came later, when, in the 1940s, Charlotte Auerbach and JM Robson found that mustard gas caused mutations in the genomes of fruit flies. Today, through the use of the **Ames test**, a widely employed assay that exposes bacteria to a chemical followed by DNA sequencing, researchers can determine if a chemical will cause DNA mutations and be classified as a mutagen. The Ames test serves as a quick and convenient assay to determine the carcinogenic potential of a compound.

Introduction

The information encoded in the DNA sequence of a protein-coding gene determines the sequence of amino acids that will be found in the polypeptide. Because the amino acid sequence affects the folding of the polypeptide and the function of the protein, the DNA sequence of a protein-coding gene must be free of errors. The correct DNA sequence also determines the secondary structure and function of rRNA and tRNA molecules. Therefore, the correct DNA sequence is essential to the viability and function of an organism. A change in the normal DNA sequence is a **mutation**. Mutations in DNA can be caused by errors in replication, mitosis, meiosis or by exposing DNA to a **mutagen,** an agent that causes a heritable and permanent change in DNA sequence. This agent can be chemical or physical.

Learning Objectives

Errors in DNA sequences are called mutations. At the end of this section, you will be able to:

- Explain how mutations are classified
- Compare small scale vs. large scale mutations
- Explain the types of point mutations (frame-shift, missense, nonsense, and silent)
- Explain the difference between the two substitution mutations (transversion and transition)
- Explain frame-shift mutations (insertions and deletions) and their importance
- List and explain the types of chromosomal mutations
- Explain the major DNA repair mechanisms used in cells

Mutagen Classification

DNA mutations can be classified into two categories: **small-scale and large-scale.** Small-scale mutations affect only one or two nucleotides in a gene, whereas large-scale mutations can affect large stretches of DNA, or even an entire chromosome.

Small-scale mutations include:

- **substitution or point mutations**
- **insertions**
- **deletions**

Large-scale mutations include:

- **duplications**
- **deletions**
- **insertions**

- **inversions**
- **translocations**

Small-Scale Mutations

- **Substitution mutation:** a mutation that exchanges a single nucleotide for another (Figure 4.7.1). An exchange of a purine for a purine (A for G; G for A) or a pyrimidine for a pyrimidine (C for T; T for C) is known as a **transition mutation**. The exchange of a purine for a pyrimidine (C/T for A/G; A/G for C/T) is called a **transversion mutation**. Transition mutations are more common. Substitution mutations can occur as errors made during DNA replication or can also be caused by chemical mutagens. A subsequent round of DNA replication will result in the mutation becoming a change of a base pair. A substitution mutation that occurs in the coding sequence of a protein-coding gene is often called a **point mutation**.

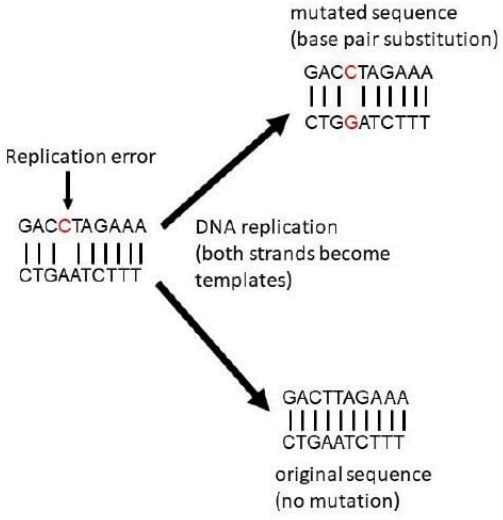


Figure 4.7.1: Substitution Mutations in DNA. Errors in DNA replication result in substitution mutations in the DNA sequence and a change in base pair. The sequence to the left has a replication error in which a T has become an C. Following DNA denaturation, both strands become templates for DNA replication. The top strand with the replication error (red "C") will be used as a template for replication and produce the incorrect base pair of C-G (shown in red). The bottom strand has the correct sequence and the correct base pair of T-A will result following replication. Of the two replicated DNA helices, one will have a change in base pair while the other will be correct. (Substitution Mutations in DNA by Patricia Zuk, CC BY 4.0)

- **Insertion mutation:** an insertion mutation adds one or more nucleotides into a DNA sequence (Figure 4.7.2). They are usually caused by transposable elements, or errors during DNA replication. Insertions in the coding region of a gene may alter splicing of the mRNA (**splice site mutation**), or cause a shift in the reading frame (**frameshift mutation**).
- **Deletion mutation:** a deletion mutation removes one or more nucleotides from the DNA and can occur during DNA replication (Figure 4.7.2). Like insertions, these mutations can alter a splice site or the reading frame of the gene.

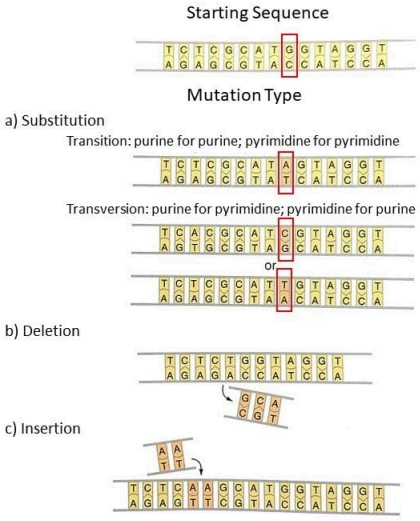


Figure 4.7.2: Point Mutations. Small-scale mutations include substitution, deletion, and insertion mutations. Substitution mutations are classified as transition and transversion mutations. The original sequence is given at the top of the figure. For all sequences the base pair affected by the specific mutation is indicated with a box. (Point Mutations by Patricia Zuk, CC BY 4.0; adapted from Point Mutations and Their Effect by Hullo97; CC BY-SA 4.0)

Point mutations that occur in the coding region of protein-coding genes are more likely to affect the amino acid sequence of the polypeptide. These mutations can be categorized further by their effect on amino acid sequence (Figure 4.7.3) into the following types:

- **Frameshift:** a frameshift is caused by either an insertion or deletion mutation. Due to the triplet nature of the codon, a frameshift results from the insertion/deletion of a number of nucleotides that is not divisible by three. The resulting shift can disrupt the reading frame of the coding sequence.
- **Missense:** a missense mutation changes a nucleotide and results in a different amino acid in the coding sequence
- **Nonsense:** a nonsense mutation results in a premature stop codon
- **Silent:** a silent mutation is a mutation that changes the codon within a coding sequence but does not change the amino acid. This is due to the degenerate nature of the genetic code. Silent mutations can also be found in non-coding regions of a gene, such as an intron or in a UTR, but because they are in the non-coding region of the gene, they are often simply called substitution mutations.

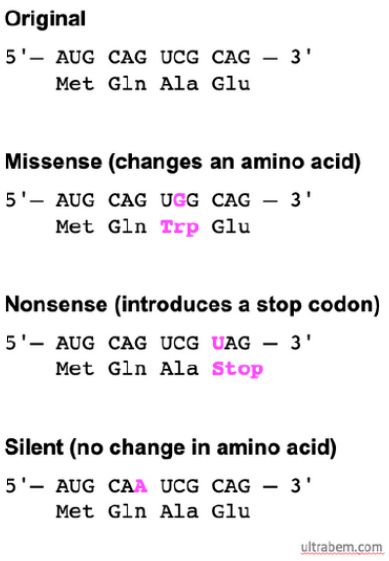


Figure 4.7.3: DNA Point mutations. Small-scale point mutations in a coding region are classified based on their effect on the amino acid sequence. The original sequence is given at the top along with sequences produced by missense, nonsense, and silent mutations. The mutated nucleotide is indicated in pink, along with any change in the amino acid sequence. For clarity, the complementary DNA sequence is not shown. (DNA point mutations by Ultrabem; CCO)

A point mutation can be reversed by another point mutation. This type of point mutation is called a **reversion**. In a "true" reversion, the nucleotide is changed back to its original state and the correct amino restored in the polypeptide sequence. However, reversions can occur elsewhere in the coding sequence. This is known as a second-site reversion and produces a complementary mutation that restores gene function.

Large-Scale Mutations

Large scale mutations involve changes to the structure and composition of chromosomes (Figure 4.7.4). These changes can have significant effects on the number, location, and activity of the genes found on that chromosome.

- **Chromosome Duplication:** also known as an amplification. A chromosomal duplication is a repetition of a specific chromosomal segment so that multiple copies of the genes within that segment are present.
- **Chromosome Translocation:** a mutation that exchanges chromosomal regions between non-homologous (i.e. different) chromosomes. Chromosomal translocations are usually harmless, as they do not result in the gain or loss of genetic information. However, in certain cases, the translocation might result in the fusion of two coding sequences. Such a gene fusion can increase the chances of developing cancer.
- **Chromosome Deletion:** a mutation in which a chromosomal region is deleted, leading to loss of the genes within that region. Chromosomal deletions can have a significant effect on the phenotype of an organism. In humans, several congenital diseases are associated with chromosomal deletions.
- **Chromosome Insertion:** a mutation in which a chromosomal region is inserted into another chromosome, leading to a gain of genes. This type of insertion is the result of a **translocation event**, so the insertion into one chromosome is accompanied by a chromosomal deletion in another chromosome. An alternate type of chromosomal insertion moves a chromosome region within the same chromosome. In both types, because no genetic information is lost, chromosomal insertions are usually harmless.
- **Chromosome Inversion:** a mutation that reverses the orientation of a chromosomal region. Chromosomal inversions may not have a deleterious effect in an organism as long as gene expression is not affected.

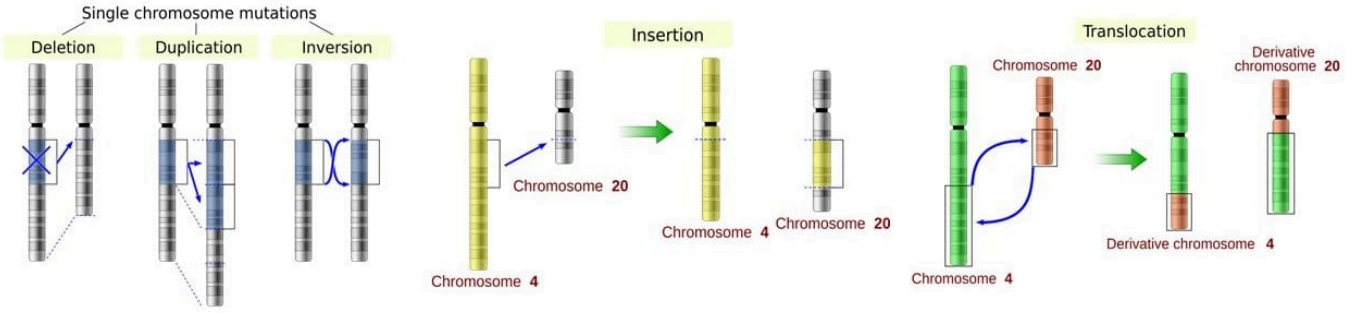


Figure 4.7.4: Large-scale mutations. Large-scale chromosomal mutations involve large chromosomal regions containing many genes. Chromosomal mutations include those involving a single chromosome (deletion, duplication, inversion) or two chromosomes (insertion, translocation). (Five Types of Chromosomal Mutations by YassineMrabet; Public Domain)

DNA Repair

DNA mutations are unavoidable. While some DNA mutations can be beneficial and underlie the evolution of a species, the majority of mutations may be harmful to an organism and must be corrected. Cells have numerous mechanisms for the correction of mutations. These mechanisms are known collectively as DNA repair.

The simplest mechanism of DNA repair is to prevent the mutation in the first place. Both prokaryotic and eukaryotic DNA polymerases have the ability to detect an incorrect nucleotide during DNA replication and replace it with the correct one. This "proofreading" capacity, however, has its limits. The correction must be done immediately. When a DNA polymerase adds the incorrect nucleotide to the 3' end of the daughter DNA strand, it can remove this nucleotide using its 3' to 5' exonuclease activity. An **exonuclease** is an enzyme that removes nucleotides *from the end of a DNA or RNA strand* (Figure 4.7.5). **3' to 5' exonucleases** remove these nucleotides at the 3' end of a nucleic acid, shortening the molecule in the 5' direction. A **5' to 3' exonuclease** would remove nucleotides from the 5' end of the nucleic acid. The DNA polymerase, when correcting a DNA replication error, will pause replication, remove the incorrect nucleotide from the 3' end and then replace it with the correct one. All prokaryotic DNA polymerases have this "proofreading" activity, along with the eukaryotic DNA polymerases delta and epsilon. This type of proofreading must be done immediately as the DNA polymerase cannot correct its mistakes once it continues to replicate the DNA.

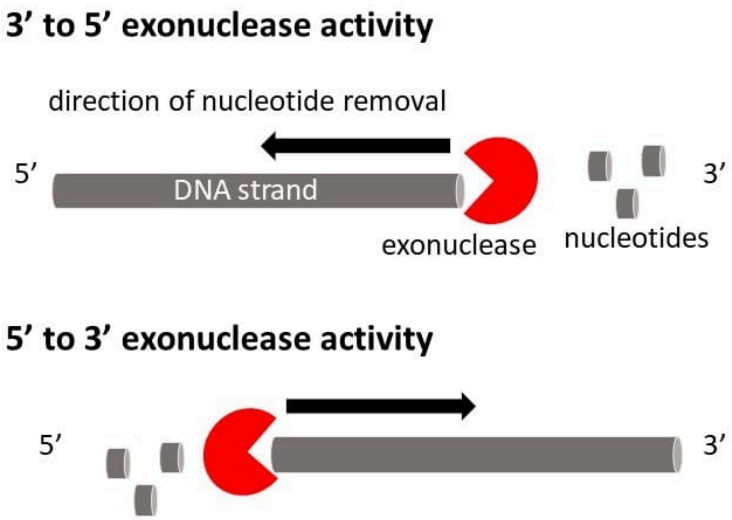


Figure 4.7.5: DNA Exonucleases. Exonucleases are enzymes that remove nucleotides from the end of DNA or RNA. A 3' to 5' exonuclease removes nucleotides at the 3' end in what is called 3' to 5' exonuclease activity. The 5' to 3' exonuclease removed nucleotides at the 5' end in what is known as 5' to 3' exonuclease activity. (DNA Exonucleases by Patricia Zuk, CC BY 4.0)

In addition to proofreading by exonucleases, prokaryotic and eukaryotic cells have additional DNA repair mechanisms. The following are summaries of common repair mechanisms found in human cells (Figure 4.7.6). These mechanisms are similar to

those found in other eukaryotic cells and in prokaryotic cells. Each of these repair mechanisms involve another class of repair enzyme known as an **endonuclease**. An endonuclease is an enzyme that breaks (or "nicks") the phosphodiester bond *within the DNA or RNA strand* so that a nucleotide can be removed and replaced.

- **Base excision repair:** a repair mechanism for the correction of a G-T mismatch in DNA. A **DNA glycosylase** enzyme removes the T base, leaving the remainder of the nucleotide in the DNA strand. An endonuclease "nicks" the DNA strand by breaking the phosphodiester bond upstream of the mutation. Another endonuclease removes the sugar-phosphate remnant. A DNA polymerase replaces the removed nucleotide with the correct one. A DNA ligase replaces the phosphodiester bond.
- **Mismatch repair:** a repair mechanism for the removal of other base pair mismatches. Several protein complexes bind the parental DNA strand. One acts as an endonuclease to nick the mutated daughter DNA strand. Another acts as a helicase unwinds the DNA so that an exonuclease can remove nucleotides, creating a "gap" in the daughter strand that is filled in by a DNA polymerase. A DNA ligase replaces the missing phosphodiester bonds.
- **Nucleotide excision repair:** a repair mechanism for the removal of thymine-thymine dimers. A **thymine-thymine dimer (T-T dimer)** is a type of DNA damage produced by UV light. The UV light causes a bond to form between adjacent thymine bases (i.e. the dimer). This produces a physical distortion in the shape of the DNA helix that can prevent replication. To remove the dimer, a protein complex binds the dimer region. One of the protein complex subunits acts a helicase to unwind the DNA and give access to other components of the protein complex that will act as endonucleases to cut out the region containing the T-T dimer. This gap is filled in by a DNA polymerase and a DNA ligase replaces the missing phosphodiester bonds.

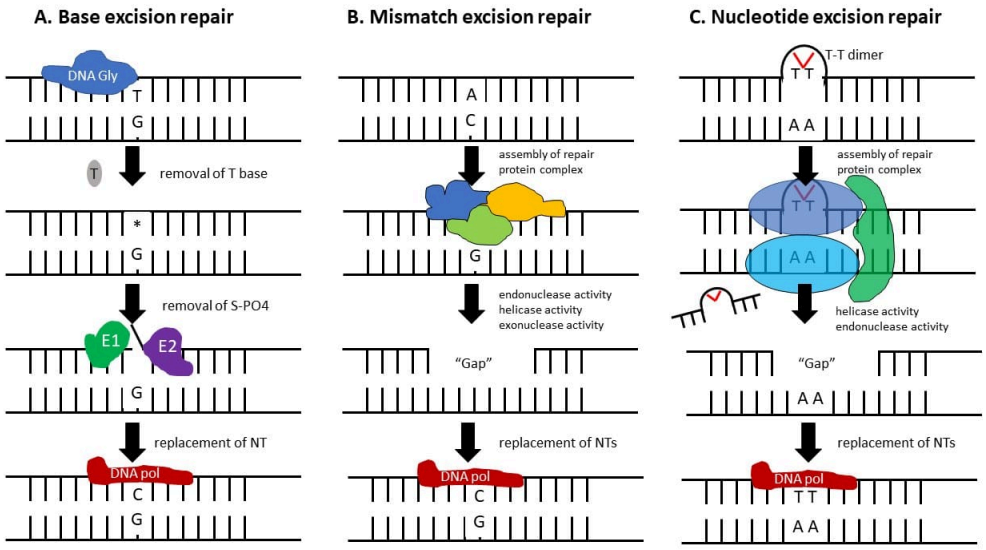


Figure 4.7.6: DNA repair mechanisms in human cells. (A) Base excision repair is performed by the removal of the T base by a DNA glycosylase enzyme, followed by removal of the sugar-phosphate remnant by two endonuclease enzymes (E1, E2). The missing nucleotide is replaced by DNA polymerase (DNA pol) and phosphodiester bonds reformed by DNA ligase (not shown). (B) In mismatch nucleotide repair, a complex made of several proteins binds to the mismatch region. The complex acts as an endonuclease to break a phosphodiester bond in the "incorrect" DNA strand, as a helicase to unwind the DNA helix, and as an exonuclease to remove additional nucleotides to create a "gap". The gap is filled in by DNA polymerase and ligated by DNA ligase (not shown). (C) Thymine-thymine (T-T) dimers are repaired by nucleotide excision repair. A protein complex binds the T-T dimer. The complex unwinds the DNA helix (helicase activity) and removes the dimer and several surrounding nucleotides (endonuclease activity) to create a gap. As with other repair mechanisms, The gap is filled in by DNA polymerase and ligated by DNA ligase (not shown). (DNA Repair Mechanisms by Patricia Zuk, CC BY 4.0)

Key Concepts

DNA mutations can have significant effects on cellular function. Some major concepts to remember are:

- Changes in the DNA sequence, as a result of mutagens or replication errors, result in mutations
- Mutations can be classified as small-scale and large-scale
- Small-scale mutations that affect the coding region of a protein-coding gene are called point mutations
- Point mutations include missense, nonsense, and silent mutations

- Frameshift mutations are caused by insertions or deletions or nucleotides. Frameshift mutations in coding regions can alter the reading frame of translation
- Most errors in DNA replication are repaired by the proofreading capacity of the DNA polymerase
- Other mutations are repaired through DNA repair mechanisms that require multiple proteins acting in a coordinated fashion

Glossary

Deletion - a mutation that removes a nucleotide from a DNA sequence; produces a frame-shift

DNA damage - any alteration or modification to the DNA structure, which can lead to mutations. This includes single- or double-strand breaks, base modifications, and cross-linking

Endonuclease - an enzyme that hydrolyses a phosphodiester bond within a DNA or RNA sequence

Exonuclease - an enzyme that removes a nucleotide from the end of a DNA or RNA sequence; can remove nucleotides from either the 5' or 3' end

Frameshift mutation - a mutation caused by the insertion or deletion of nucleotides in a DNA codon; shifts the reading frame of the codons, potentially leading to a completely altered protein product

Insertion - a mutation that adds a nucleotide from a DNA sequence; produces a frame-shift

Inversion - a type of mutation where a section of DNA is reversed end to end; leads to changes in gene function or regulation

Mismatch repair - a process that fixes base-pairing mismatches, often caused by errors during DNA replication; involves recognition of the mismatch and excision of the incorrect base

Missense mutation - a mutation that changes the codon and the resulting amino acid

Mutagen - any physical or chemical agent that causes changes or mutations in the DNA sequence; includes UV radiation and certain chemicals

Mutagenesis - the process by which mutations are induced or occur naturally within an organism's DNA

Mutation - a change in a DNA sequence

Nonsense mutation - a mutation that changes codon to a stop codon

Point mutation - a mutation that involves a change in a single nucleotide base pair in the DNA sequence; can result in silent, missense, or nonsense mutations

Reading frame - the way of dividing a sequence of nucleotides into a set of consecutive, non-overlapping triplets called codons

Silent mutation - a mutation that does not affect the function of the protein; often produces a codon that codes for the same amino acid

Substitution mutation - a mutation where one nucleotide base is replaced by another; potentially alters the protein if the change affects the codon

Thymine dimers (T-T dimers) - a specific type of pyrimidine dimer, where two adjacent thymine bases bond together, distorting the DNA structure; typically formed by UV light exposure

Translocation - a mutation involving the rearrangement of parts between two non-homologous chromosomes; can lead to the fusion of genes or disruption of regulatory sequences

UV Radiation - a form of electromagnetic radiation that can cause DNA damage, including the formation of thymine dimers and other mutations

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5: GENETIC ENGINEERING AND RECOMBINANT DNA TECHNOLOGY

5.1: Genome Sequencing
5.2: Principles of Genetic Engineering
5.3: Creating Recombinant DNA
5.4: Polymerase Chain Reaction (PCR)

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5.1: Genome Sequencing

iotech Focus

Twenty years ago, the idea of sequencing an entire genome in a day seemed impossible. However in 2005, 454 Life Sciences introduced the **GS20 sequencer** capable of sequencing hundreds of thousands of DNA fragments in a matter of hours. This sequencer was the first of its kind and introduced to the world the concept of "massive parallel sequencing", now known today as **Next Generation Sequencing (NGS)**. In 2007, the GS20 was used to sequence the genome of James Watson and was hailed as a revolution in "personalized medicine". Thanks to NGS, consumers can now submit their DNA and have a copy of their genome, along with an analysis of specific genes, sent to them in weeks. In the research lab, NGS continues to advance fields such as molecular biology, genetics, genomics, evolutionary biology, and medicine.

Check out this podcast on personalized medicine and cancer from the Mayo clinic.

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Introduction

Numerous scientific disciplines, including genomics, rely heavily on the precise determination of DNA sequences. The first method for sequencing DNA was developed in 1975 by Frederick Sanger and Alan Coulson, who used DNA polymerase I from *E. coli* to copy short stretches of a DNA template using radiolabeled nucleotides. These pieces were then fractionated by electrophoresis and the radiograph read to determine the DNA sequence. While successful, the process was very labor intensive as the polymerase could only produce DNA copies of only 80 nucleotides long. Nevertheless, Sanger and Coulson managed to sequence the genome of the bacteriophage phiX174 using this method.

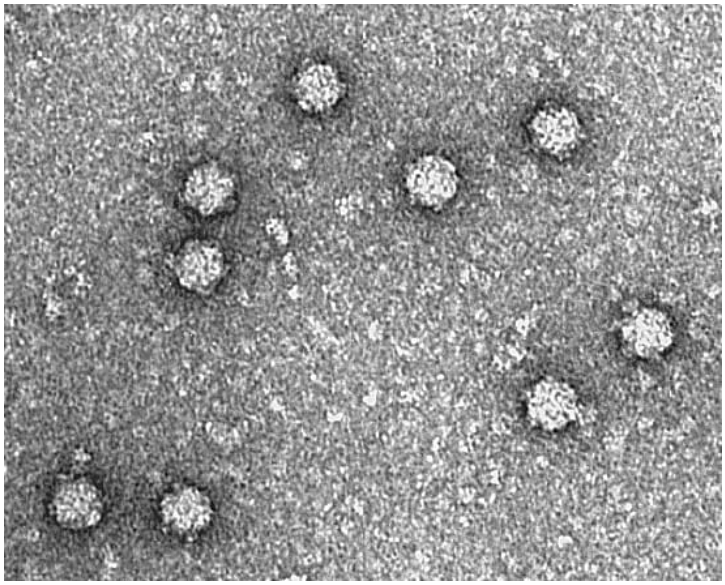


Figure 5.1.1: An electron micrograph of the bacteriophage phiX174. (Bacteriophage Phi X174 by ShiftFn, CC BY-SA 4.0)

In 1977, Sanger and colleagues introduced what is now known as the **dideoxy chain termination method or the "Sanger sequencing method".** Modifications to their original 1975 method now allowed scientists to accurately sequence long stretches

of DNA. Using the Sanger dideoxy chain termination method, Sanger was able to sequence human mitochondrial DNA (16,569 base pairs) and the lambda bacteriophage (48,502 base pairs). For his efforts, Sanger was awarded the Nobel Prize in Chemistry in 1980, along with Walter Gilbert and Paul Berg.

Learning Objectives

DNA sequences gives scientists the exact order of nucleotides in a stretch of DNA. At the end of this section, you will be able to:

- Explain the structure of a dideoxynucleotide (ddNTP)
- Explain the fundamentals of chain termination sequencing using ddNTPs
- Distinguish how shotgun and pair-wise sequencing approaches are used to sequence an entire genome
- Describe how next generation sequencing can sequence an entire genome in a day

Sanger DNA Sequencing

DNA sequencing techniques are based on a cell's DNA replication mechanism. During replication, a primer binds to the parental DNA strand. The DNA polymerase binds to the 3' end of this primer and extends from it, reading the parental DNA template and linking nucleotides to create the daughter DNA strand. The nucleotides are linked together by the DNA polymerase through the creation of phosphodiester bonds. In sequencing, a DNA polymerase binds to a **sequencing primer** that has bound to one of the two DNA strands (e.g. the anti-sense DNA strand). The polymerase extends from this primer and synthesizes a complementary daughter DNA strand. The resulting strand, comprised of the sequencing primer and the daughter DNA strand, is sent to be sequenced.(Figure 5.1.2, left image).

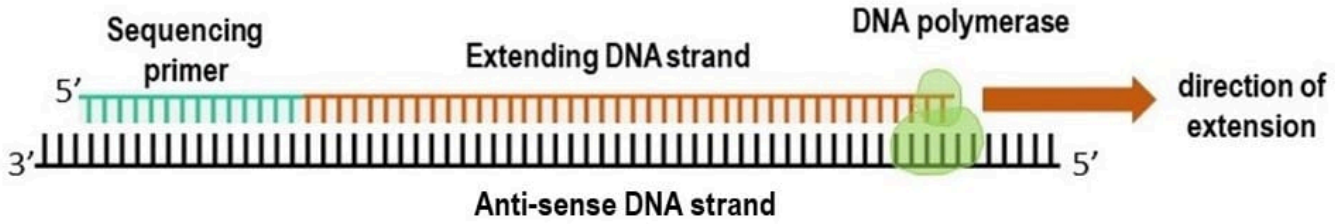


Figure 5.1.2: In DNA sequencing, a DNA polymerase (green circles) extends from a sequencing primer (shown in green), reads a DNA template, such as an anti-sense DNA strand (shown in black), and creates a complementary daughter DNA strand (i.e. the extending DNA strand, shown in orange) that is then sequenced. The direction of polymerase extension is shown with an arrow. (DNA Extension for Sequencing by Patricia Zuk, CC BY 4.0).

As the DNA polymerase creates the daughter strand it incorporates nucleotides (i.e., dNTPs). The Sanger method uses modified nucleotides, called **dideoxynucleotides (ddNTPs),** that lack a hydroxyl (-OH) group at the 3rd carbon in the sugar (Figure 5.1.3).

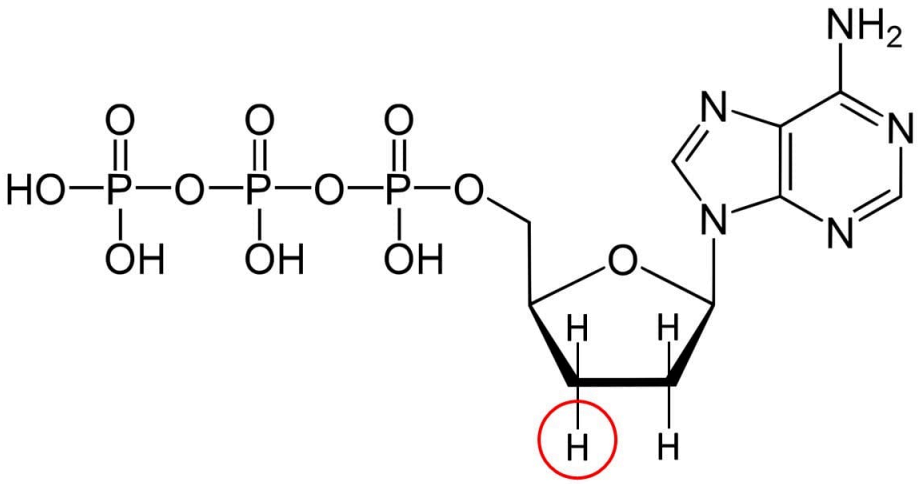


Figure 5.1.3: The dideoxynucleotide (ddNTP). The molecular structure of a ddNTP used in Sanger sequencing shows the absence of a hydroxyl group at the 3' carbon (circled). (The ddNTP by Patricia Zuk, CC BY 4.0; adapted from Molecular Structure of ddATP by Yikrazuul, public domain)

This lack of a 3' OH group prevents formation of a phosphodiester bond with the incoming nucleotide (Figure 5.1.4). As a result, extension of the growing daughter strand is halted when these nucleotides are incorporated (i.e., chain termination).

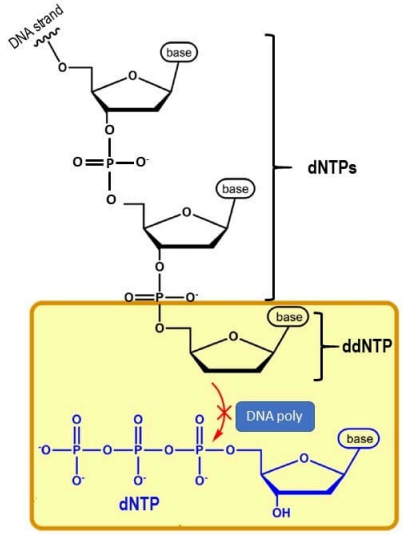


Figure 5.1.4: In Sanger sequencing, a DNA polymerase cannot add an incoming nucleotide (shown at the bottom in blue) because the last nucleotide in the chain (shown at the top in black) is a ddNTP. This ddNTP lacks a hydroxyl group at the 3rd carbon of the sugar and cannot form a phosphodiester bond with the incoming nucleotide. The extension of the DNA chain stops at the ddNTP (i.e., chain termination). (Chain Termination by Patricia Zuk, CC BY 4.0; adapted from Dideoxy Termination of DNA Elongation by Michal Sobkowski, CC BY-SA 3.0)

The steps of Sanger sequencing are relatively straightforward and are shown in Figure 5.1.5. For more information, go to Chapter 13.9: DNA Sequencing.

1. **DNA sequencing reaction set-up**: The DNA sequencing reaction is set up to contain a DNA polymerase, a primer, the DNA template, dNTPs (i.e. dATP, dCTP, dGTP, and dTTP), and fluorescently-labelled ddNTPs. The original labels for these ddNTPs were radioactive, but they are now coupled to fluorescent probes of different colors.
2. **Elongation from primer & chain termination by ddNTPs:** The DNA polymerase extends from the primer and copies the template strand by incorporating nucleotides (dNTPs or ddNTPs). When a ddNTP is added to the daughter DNA strand,

extension is halted at that site (i.e. chain termination). The completed DNA sequencing reaction is a mixture of DNA fragments of increasing sizes, each with a fluorescently-labeled ddNTP located at the end of the fragment.

3. **Capillary gel electrophoresis separation of labeled DNA fragments:** The sample is then analyzed by a **DNA sequencer.** The sequencing reaction is loaded into a capillary gel tube that separates the fluorescently-labeled fragments according to their size and electrical charge. The smaller sequence fragments will migrate through the tube faster in comparison to those sequences that are bigger (i.e., longer). As the fragments migrate down the gel, a laser activates the fluorescent label which is picked up by a detector.
4. **Laser detection of fluorochromes and sequence compilation:** A detector picks up the fluorescent signals and converts them into a chromatograph from which the sequence can be read.

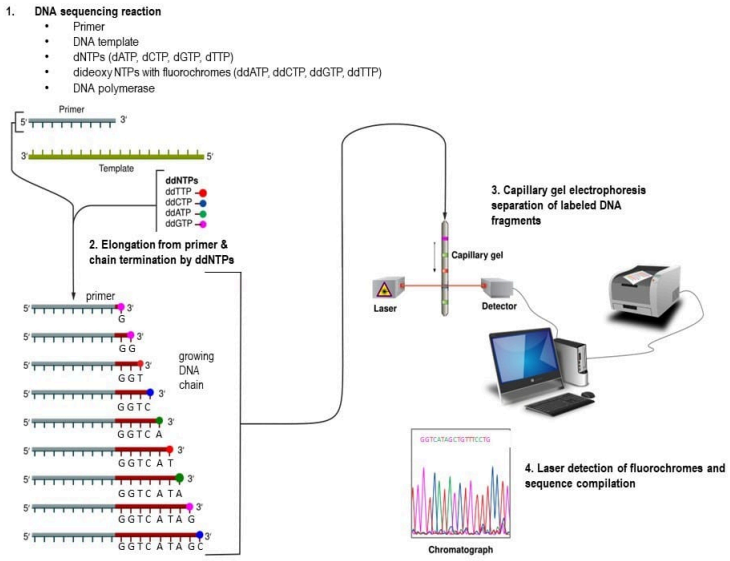


Figure 5.1.5: Sanger sequencing of DNA. The sequencing of DNA is made of the following steps that are described in the text above: (1) DNA sequencing reaction, (2) Elongation from primer & chain termination by ddNTPs, (3) Capillary gel electrophoresis separation of labeled DNA fragments, and (4) Laser detection of fluorochromes and sequence compilation. (Sanger DNA sequencing by Patricia Zuk, CC BY 4.0; adapted from The Sanger Method for DNA Sequencing by Estevezj, CC BY-SA 3.0)

Concept in action

Animation: Sanger Sequencing

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Whole Genome Sequencing

Whole-genome sequencing (WGS) is a process that determines an entire genome's DNA sequence, including nuclear and mitochondrial DNA, in addition to chloroplast DNA (if present). Genomes are cut into smaller fragments and each fragment is sequenced. Computer programs then search for regions of overlap between fragments so that they can be reassembled to create the sequence of the complete genome (Figure 5.1.6).

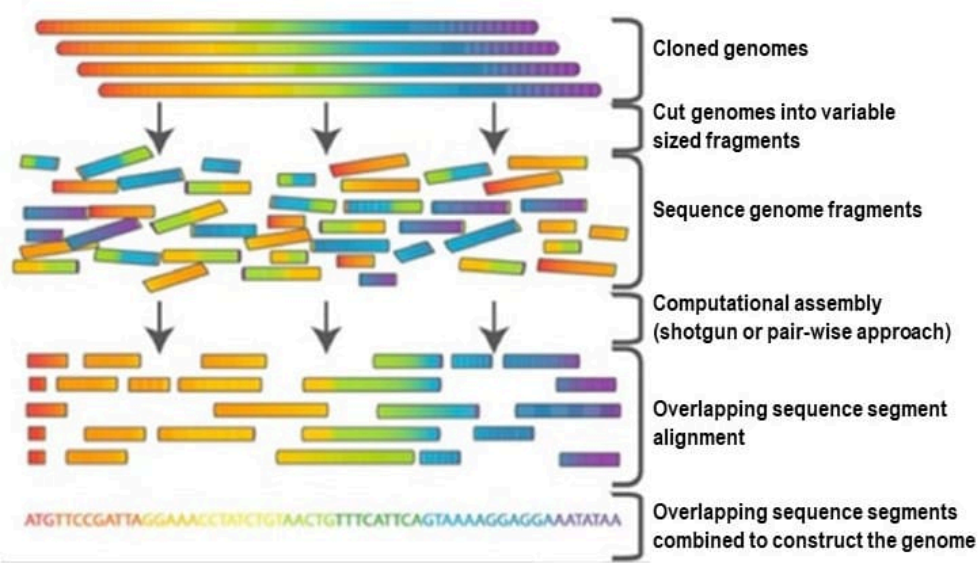


Figure 5.1.6: Whole Genome Sequencing (WGS) approach. WGS is accomplished by cutting the genome into smaller fragments, sequencing the fragments, and then aligning them based on regions of overlap between fragments. "Shotgun" or "pair-wise" approaches are used to align these regions of overlap. With enough fragments and enough overlap, the entire genome sequence can be produced. (Copyright; author via source)

WGS uses either the "shotgun" or "paired end" sequencing methods described below to generate the sequence of entire genome. Because of this, WGS was originally expensive and time consuming. As an alternative to WGS, **exome sequencing** emerged as a lower-cost option. In exome sequencing, only the exons of the DNA's coding region are sequenced. This approach is suitable for diseases in which a protein coding gene is thought to be the underlying cause. However, for determining the sequences of non-protein coding regions, WGS may be the only option. Fortunately, technology has improved the time and cost of WGS. Today, commercial companies offer WGS results in about two days for about $1,000.

Shotgun and Paired End Sequencing

In the **shotgun sequencing** method, DNA fragments are sheared or cut into many smaller pieces. Because this approach is somewhat like when several small pellets are fired from a shotgun, it was named shotgun sequencing. All of the segments are sequenced using the dideoxy chain-sequencing method and powerful computer programs align the sequences by finding regions of overlap amongst the sequences (i.e., computational assembly). With enough sequences the computer can reform the entire DNA sequence. The larger sequence that is assembled from overlapping shorter sequences is called a **contig**.

Table 5.1.1: ***Shotgun Sequencing Alignment***

Strand	Sequence
Original Sequence	AGCATGCTGCAGTCATGCTTAGGCTACGCGC
First Shotgun Sequence	AGCATGCTGCAGTCATG**CT**------- ----------**CT**TAGGCTACGCGC
Second Shotgun Sequence	AGCA**TG**---------- -------**TG**CTGCAGTCATGCTTAGGCTACGCGC
Reassembled Sequence	AGCATGCTGCAGTCATGCTTAGGCTACGCGC

Shotgun sequencing assembles DNA fragments by aligning the end of one fragment with the start of another (Table 5.1.1). Originally, this was sufficient for sequencing small genomes. However, with larger genomes, such as that of a human, modifications to this method was required. In **paired end sequencing,** scientists sequence both ends of a DNA fragment and then analyze both ends for overlap with other fragments (Table 5.1.2). Paired end sequencing is more cumbersome than shotgun sequencing, and requires more sophisticated computer programs, but it produces a more accurate genome sequence because there is more available information.

Table 5.1.2: Paired-end Sequencing Alignment

Strand	Sequence
Original Sequence	-------AGCATGCTGCAGTCATGCTTAGGCTACGCGCAGCATGCTG----------
Aligned Sequences	------**AG**CATGC**TG**---------- ----------**TG**CAGTC**AT** ----------**AT**GCTTAGGC**TA** ----------**TA**CGCGCAGCATGC**TG**----------
Reassembled Sequence	AGCATGCTGCAGTCATGCTTAGGCTACGCGCAGCATGCTG----------

Next Generation Sequencing

While modern day Sanger sequencing instruments are still used by the majority of research facilities to quickly sequence a small DNA construct, they are limited by the relatively small number of sequencing reactions that can be analyzed at one time (e.g. up to 96 samples). **Next-generation sequencing (NGS)** is a powerful platform that enables the sequencing of *thousands to millions of DNA molecules simultaneously*, thus allowing for the sequencing of an entire genome in one day. In NGS, DNA is extracted and broken into small fragments. Short pieces of DNA, called **adaptors**, are attached to each fragment. The adaptors allow for binding of the DNA fragment to the solid support of the sequencer, called a **flow cell**. Each bound DNA fragment is amplified into multiple copies on the flow cell. The amplified fragments are sequenced base by base using different chemical or optical methods, depending on the platform (e.g., Illumina, Ion Torrent, PacBio). The sequences are assembled or aligned to a reference genome to complete the sequence. Bioinformatics tools are used to interpret the results.

NGS permits high throughput and is faster and cheaper per base than Sanger sequencing. It is capable of detecting mutations, insertions, deletions, and can quantitate gene expression levels. NGS is used in whole genome sequencing, exome sequencing, RNA sequencing, and targeted sequencing. NGS has become an integral part of personalized medicine, cancer genomics, infectious disease detection, and other areas of medical biotechnology. However, it requires powerful bioinformatics tools and expertise to interpret the massive amounts of data that the sequencer can generate. In addition, interpretation of the data can be complex. To read more about NGS, go to Chapter 10.2 Diagnostic Biotechnology.

Lab Protocols

Lab Technique: DNA sequencing

Key Concepts

Genomic sequencing can determine the complete DNA sequence of an organism's genome, including all of its genes and non-coding regions. Some important concepts to remember are:

- Sanger sequencing is also known as dideoxy chain termination method
- a dideoxynucleotide (ddNTP) lacks a hydroxyl (OH) group at the 3' carbon in the pentose sugar. This prevents the formation of a phosphodiester bond with an incoming nucleotide
- addition of a ddNTP into a growing DNA strand halts its extension at the site of its incorporation
- DNA sequencing techniques use Sanger sequencing in combination with fluorescently-labeled ddNTPs
- whole genome sequencing techniques sequence smaller fragments of the genome and then assemble the fragments into the complete genome
- Next Generation Sequencing (NGS) can sequence millions of small DNA fragments simultaneously, allowing for the sequencing of a genome in a few hours

Glossary

Adaptors - short, synthetic DNA sequences attached to a DNA fragment; used in Next Generation Sequencing

Alignment - the process of arranging DNA sequences using regions of overlap

Capillary gel - a thin, glass tube filled with a polymeric gel; used to quickly separate molecules, like DNA fragments, according to their size and electrical charge

Chain termination sequencing - a DNA sequencing method that uses dideoxynucleotides (ddNTPs) to terminate the synthesis of the complementary DNA chain; also known as Sanger sequencing

Chromatograph - a graphical representation of the DNA sequence, showing peaks for each nucleotide in the sequence

Computational assembly - the process of aligning DNA fragments into a continuous sequence (i.e., a contig) using computer alignment programs

Contig - a set of DNA sequences that overlap to provide a continuous sequence

Dideoxynucleotide (ddNTP) - a DNA nucleotide used in Sanger sequencing that lacks the 3'-OH (hydroxyl) group necessary for continued 5'-to-3' DNA synthesis; used to terminate the elongation of the complementary DNA strand

DNA polymerase - the enzyme that synthesizes new daughter DNA strands during DNA replication; used in DNA sequencing to create the DNA strand that will be sequenced

Exome sequencing - a sequencing method that sequences all of the protein-coding genes in a genome

Fluorescently-labeled - a compound that has a fluorescent dye attach to it

Genomic sequencing - a sequencing method that sequences the entire genome of an organism

Next Generation Sequencing (NGS) - a high throughput sequencing method that can sequence several genomes in a day; also known as massive parallel sequencing

Paired-end sequencing - a genomic sequencing technique that sequences random DNA fragments followed by alignment of these fragments using the start and end of each fragment

Sanger sequencing - a DNA sequencing method that uses dideoxynucleotides (ddNTPs) to terminate the synthesis of the complementary DNA chain; also known as chain termination sequencing

Shotgun sequencing - a genomic sequencing technique that sequences random DNA fragments followed by alignment of these fragments using the start of each DNA fragment

Whole Genome Sequencing (WGS) - the process of determining the DNA sequence of an organism's entire (or nearly entire) genome at a single time

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5.2: Principles of Genetic Engineering

Biotech Focus

In 1979, researchers at Genentech Inc. and City of Hope Hospital succeeded in cloning the human insulin gene into *E.coli* bacterial cells using the cloning plasmid pBR322. While the expression of this hormone was low, work at Genentech and at Eli Lilly eventually succeeded in increasing the yields of recombinant human insulin made by bacteria. Such work made it possible for a whole host of powerful therapeutic proteins to be made by bacteria. In 1982, recombinant human insulin was approved by the FDA for use by diabetics. In 1983, the drug **Humulin** (i.e., "human insulin") entered the market. Today, genetically modified *E.coli* and yeast strains, like *Saccharomyces cerevisiae,* are the principal sources for the therapeutic production of recombinant human insulin. However, continued genetic engineering research has created transgenic plants for the production of other recombinant proteins. Plants may represent a better option than bacteria and yeast due to their cost effectiveness, ease of production, and absence of human pathogens. For example, recombinant human insulin has been successfully expressed and produced in oilseeds of the plant *Arabidopsis thaliana*. In the future, transgenic seeds may act as "warehouses" for the stockpiling of critical therapeutic compounds like insulin.

Introduction to the Principles of Genetic Engineering

Genetic engineering is the modification and manipulation of an organism's genetic information through the use of laboratory technologies. Genetic engineering alters an organism's genome through the introduction or removal of DNA, or by modifying the sequence of existing genes. Genetic changes may only be a single nucleotide in the DNA sequence, or the replacement of an entire gene. Genetic engineering is a key component of the biotechnology and is used in many settings, including research, medicine, agriculture, and industry. For more information about biotechnology, refer to Chapter 1: Introduction to Biotechnology.

Genetic engineering is often confused with breeding. In **breeding**, an organism (i.e., animal and plants) with a desired characteristic, known as the **phenotype**, is bred to another so that this characteristic is passed on to their offspring. Specific offspring, exhibiting a desired combination of parental characteristics, will be selected and bred again to either perpetuate their phenotype or improve upon the organism. In contrast, genetic engineering takes a gene directly from one organism and delivers it to another. This approach is much faster and can be used to manipulate any gene from any organism.

Concept in Action:

Video: Genetic Engineering

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Learning Objectives

Genetic engineering is at the heart of biotechnology. This section will introduce you to some of the principles and tools of genetic engineering. At the end of this section, you will be able to:

- Explain the difference between genetic engineering and breeding
- List the "tools" used in genetic engineering
- Define DNA cloning and recombinant DNA
- List the major components of a plasmid
- Explain the difference between cloning and expression plasmids

- Define cosmids, and BACs
- Explain restriction enzymes and how they work

The “Tools” of Genetic Engineering

Genetic engineering utilizes several techniques in order to study and manipulate the genome of an organism. Examples of these techniques include:

- DNA cloning (i.e., recombinant DNA technology)
- Restriction digests
- Electrophoresis (DNA and protein)
- Sequencing
- PCR
- Northern, Southern, and Western blotting
- Immunofluorescence
- Transfection
- Transformation
- ELISA assays
- In vitro expression assays

The foundation of genetic engineering is the production of **recombinant DNA (rDNA)**. rDNA is created by combining DNA from different sources - often from different species - into a single piece of DNA that can be inserted into a host organism. rDNA is produced through a lab technique known as **DNA cloning, or cloning**. In a typical cloning protocol, a piece of DNA (usually a gene) is inserted into a bacterial plasmid to produce rDNA (Figure 5.2.1). This rDNA can then be introduced into a bacterial cell so that it can be amplified by the host's replication machinery, or studied for its effect on the cell. The rDNA can also be introduced into a variety of eukaryotic cells (e.g. yeast, insect, mammalian) to study its functional effects. Because this approach is at the heart of genetic engineering, genetic engineering is often called **recombinant DNA (rDNA) technology.**

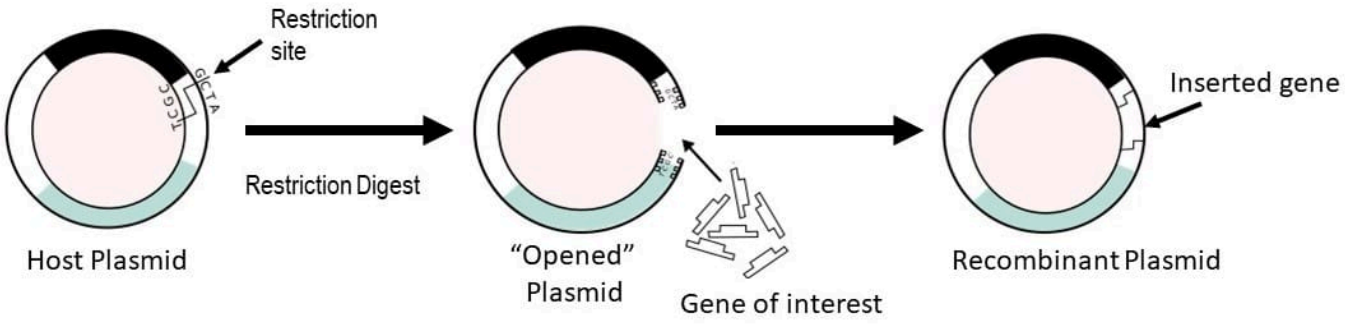


Figure 5.2.1: Creating recombinant DNA (rDNA) through DNA cloning. Left image: A host plasmid can be cut open or "cleaved" at a specific sequence of DNA (i.e., a restriction site) using a technique called a restriction digest. Center image: The restriction digest creates an "opened plasmid" into which a gene of interest can be inserted. Right image: The plasmid containing the inserted gene is known as recombinant DNA. (Creating Recombinant DNA by Patricia Zuk, CC BY 4.0; adapted from Recombinant Formation of Plasmids by Minestrone Soup, CC BY-SA 3.0)

Plasmids, Cosmids, and BACs

DNA cloning requires the use of a DNA construct known as a **vector.** The vectors used in DNA cloning are plasmids, cosmids, artificial chromosomes, and viruses. Choosing which vector to use for DNA cloning will depend on a variety of factors, such as the size of the DNA being cloned.

The most common type of vector used in DNA cloning is the **plasmid**. As introduced in Chapter 3, a plasmid is a small, circular piece of DNA found *outside of the bacterial chromosome*. While plasmids have been identified in yeast, the term plasmid is usually refers to bacterial plasmids. Biotechnology researchers use commercially available plasmids for many uses. These plasmids are derived from naturally occurring ones, but have been genetically engineered themselves so that they can be used in biotechnology protocols.

Plasmids can be classified into two categories:

1. **Cloning plasmids:** also known as cloning vectors; used to amplify the gene product in bacterial cells
2. **Expression plasmids:** used for the transcription and translation of the gene product in a specific cell type (e.g. eukaryotic cells)

Whether the plasmid is to be used for cloning or for expression, it typically has the following major components (Figure 5.2.2):

- **Origin of replication (ori):** allows the vector to replicate within the host bacterial cell and controls the level of replication by the host cell's replication machinery
- **Selection marker(s):** a gene that when expressed helps select for the organism containing the plasmid; usually is a gene that confers antibiotic resistance; plasmids may have more than one selectable marker
- **Multi-cloning site (MCS):** a series of restriction sites that allows the target DNA to be inserted into the plasmid

Cloning plasmids are relatively simple in construction, containing the three components listed above. In contrast, expression plasmids are more complex because they must be constructed to allow for gene expression (i.e. transcription and translation) by the host cell. For example, the MCS in expression plasmids is flanked by a **promoter sequence** positioned upstream of the MCS and a **polyadenylation site** which is positioned downstream (Figure 5.2.2). These sequences allow for continuous transcription of the gene that is inserted into the MCS. However, some expression plasmids contain promoter sequences that will only allow for transcription *if a specific growth condition is employed*, such as a specific temperature or the use of an inducing chemical. These promoters are called **conditional promoters**. Additional regulatory sequences are also found in expression plasmids that allow for protein translation in the host cell. Examples of such sequences would include a transcription initiation site, a ribosome binding site (RBS), and a start codon. Finally, some cloning and expression plasmids may contain "reporter genes" that facilitate the identification of host cell containing the plasmid.

The size of a plasmid can range from a few thousand kilobase pairs (kbps or kb) to 100 kb. Typical cloning plasmids are approximately 3000 to 7000 base pairs (bp) and can accommodate DNA inserts up to approximately 15,000 bp. As such, plasmids are suitable for cloning smaller DNA fragments such as genes or small gene clusters. For larger pieces of DNA, researchers can opt to use cosmids or artificial chromosomes.

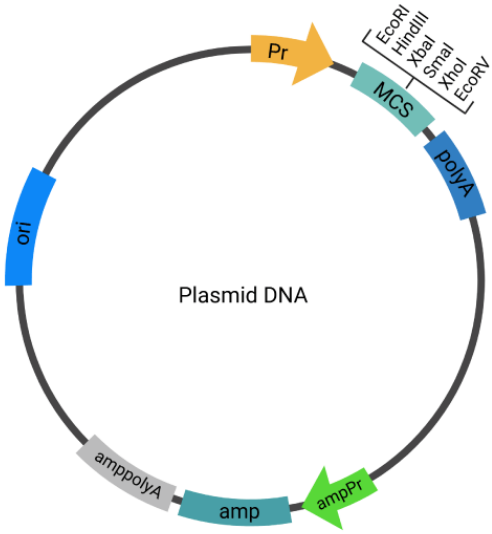


Figure 5.2.2: The plasmid. A plasmid is a small, circular piece of DNA that can be used for the insertion of pieces of DNA. Both cloning and expression plasmids have several basic regions in common. The multi-cloning site (MCS), comprised of multiple restriction sites (e.g. EcoRI, HindIII, XbaI, SmaI, XhoI, EcoRV) is where genes of interest can be inserted. In expression plasmids, the MCS is flanked by a promoter sequences (Pr) and a poly-adenylation sequence (polyA) to help regulate the expression of the inserted gene. An origin of replication (ori) ensures replication of the plasmid by the host bacterial cell. Selection markers, like an antibiotic resistance gene, (amp for ampicillin resistance), are used to select for bacterial cells containing this plasmid. These selection markers can have their own independent promoter and termination regions for expression (ampPr, amppolyA). (Cloning Plasmid, by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2025))

Cosmids are hybrid molecules that combine the features of a plasmid and a bacteriophage. They contain plasmid sequences such as the origin of replication, the MCS, and selection markers, but also contain several regions of bacteriophage DNA. Because

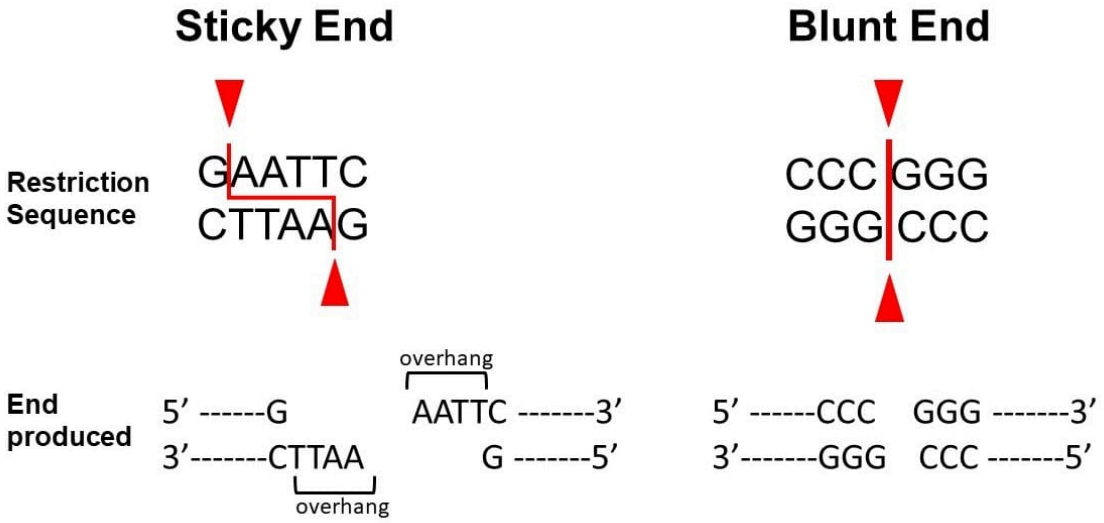
they contain bacteriophage DNA, they can be packaged into bacteriophage particles and replicate via the lytic cycle of the bacteriophage. Cosmids are larger than plasmids and can carry DNA inserts ranging from 30,000 to 45,000 base pairs, so they are used when larger DNA fragments need to be cloned. The presence of bacteriophage DNA helps stabilize larger pieces of DNA, making these vectors more stable than plasmids. Therefore, cosmids are particularly useful for constructing genomic libraries, mapping genes, and isolating large DNA fragments.

A **bacterial artificial chromosome (BAC)** is used to clone extremely large pieces of DNA (e.g. 100,000 to about 300,000 bp). The BACs, with their inserted DNA, can be introduced into bacterial cells. As with cosmids, BACs are used in constructing genomic libraries. Yeast and human versions of the BAC also exist (YAC and HAC, respectively).

Restriction enzymes

The insertion of DNA into a MCS requires the "opening" of the DNA with a **restriction enzyme**. A restriction enzyme (or **restriction endonuclease**) is a bacterial endonuclease that recognizes a specific sequence of DNA and "cuts" the DNA helix at specific points within that sequence. As such, think of a restriction enzyme as a "molecular scissor". The DNA sequence recognized by a restriction enzyme is a **restriction sequence** and its physical location in the DNA is called the **restriction site**. These two terms are often used interchangeably, but they are not the same thing. The restriction sequence is unique to the restriction enzyme. It range from 4 bp to 12 bp in size, and is said to be **palindromic,** meaning it reads the same in one DNA strand going left to right as it does in the other DNA strand going right to left.

All restriction enzymes make two incisions to cut open the DNA helix, one is through a phosphodiester bond on the "sense" DNA strand, and the other is through the a phosphodiester bond on the "anti-sense" strand. Restriction enzymes may cut the restriction sequence in an offset manner to create "overhangs" or "sticky ends" or they may cut the DNA helix in an aligned way to produce "blunt ends" (Figure 5.2.3). The way a restriction enzyme cuts the DNA helix will determine how it is inserted into a vector, like a plasmid.



Figure

: Sticky vs. Blunt ends. Restriction enzymes may cut the DNA of a restriction sequence (red arrows and lines) to produce overhangs known as "sticky ends" or blunted ends known as "blunt ends". The sticky end is a single-stranded DNA overhang (e.g., TTAA and AATT) that forms because the restriction enzyme cuts two DNA strands at different locations. Sticky ends can form hydrogen bonds with complementary DNA sequences (e.g. AATT and TTAA) to reform double-stranded DNA. The blunt end has no overhangs because both DNA strands are cut at the same position (Sticky vs Blunt Ends by Patricia Zuk, CC BY 4.0)

Restriction enzymes are part of a bacterium's defense mechanism. Bacteria infected with bacteriophage DNA use their restriction enzymes to cut the "phage" DNA, rendering it harmless. As such, restriction enzymes are isolated from bacteria. The first restriction enzyme to be studied was isolated from *E.coli* bacteria and was named **EcoRI**. "Eco" indicated the bacteria the enzyme was isolated from (i.e. *E.coli*). "R" stood for the strain of *E.coli* and the Roman numeral "I" indicated it was a type I restriction enzyme. A similar nomenclature scheme is used today to identify all restriction enzymes currently available. To date, over 3,000

restriction enzymes have been isolated and studied, and more than 800 of these are available commercially. A few commonly used restriction enzymes are given in the Table 5.2.1 below.

Table 5.2.1: ***Common Restriction Enzymes and Restriction Sites***

Enzyme	Source	Restriction Site	Cut
BamHI	*Bacillus amyloliquefaciens*	5'GGATCC 3'CCTAGG	5'---G GATCC---3' 3'---CCTAG G---5'
BglII	*Bacillus globigii*	5'AGATCT 3'TCTAGA	5'---A GATCT---3' 3'---TCTAG A---3'
EcoRI	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'
EcoRV	*Escherichia coli*	5'GATATC 3'CTATAG	5'---GAT ATC---3' 3'---CTA TAG--5'
HindIII	*Haemophilus influenzae*	5'AAGCTT 3'TTCGAA	5'---A AGCTT---3' 3'---TTCGA A---5'
KpnI	*Klebsiella pneumoniae*	5'GGTACC 3'CCATGG	5'---GGTAC C---3' 3'---C CATGG---5'
NotI	*Nocardia otitidis*	5'GCGGCCGC 3'CGCCGGCG	5'---GC GGCCGC---3' 3'---CGCCGG CG---5'
PstI	*Providencia stuartii*	5'CTGCAG 3'GACGTC	5'---CTGCA G---3' 3'---G ACGTC---5'
SacI	*Streptomyces achromogenes*	5'GAGCTC 3'CTCGAG	5'---GAGCT C---3' 3'---C TCGAG---5'
SmaI	*Serratia marcescens*	5'CCCGGG 3'GGGCCC	5'---CCC GGG---3' 3'---GGG CCC---5'
StuI	*Streptomyces tubercidicus*	5'AGGCCT 3'TCCGGA	5'---AGG CCT---3' 3'---TCC GGA---5'
XbaI	*Xanthomonas badrii*	5'TCTAGA 3'AGATCT	5'---T CTAGA---3' 3'---AGATC T---5'
XhoI	*Xanthomonas holcicola*	5'CTCGAG 3'GAGCTC	5'---C TCGAG---3' 3'---GAGCT C---5'

Key Concepts

Genetic engineering manipulates and modifies the DNA of an organism using specific "tools" or techniques.

Some major concepts to remember are:

- The genome of a organism can be manipulated in the laboratory through genetic engineering
- Genetic engineering differs from breeding, which selectively crosses specific organisms to produce desired traits in their offspring
- Genetic engineering utilizes many different "tools"
- DNA cloning is at the heart of genetic engineering
- Genetic engineering is also known as recombinant DNA technology
- Plasmids are small, circular pieces of DNA used in cloning
- Cosmids and bacterial artificial chromosomes (BACs) are also used in cloning for large pieces of DNA
- Restriction enzymes are endonucleases that "cut" the DNA helix at specific sequences called restriction sites
- Each restriction enzyme has a unique restriction site
- Restriction enzymes either produce DNA overhangs called "sticky ends" or blunted ends called "blunt ends"

Glossary

Ampicillin resistance gene (ampR) - a selectable marker gene that provides resistance to the antibiotic, ampicillin; commonly found in plasmids

Bacterial Artificial Chromosome (BAC) - a genetically engineered DNA molecule used to clone large sequences of DNA in bacteria

Bacteriophage - a virus that infects bacteria; often used as a vector in recombinant DNA

Base pair (bp) - a unit of DNA length; consist of two complementary nucleotides (A-T or C-G)

Blunt end - produced when a restriction enzyme cuts double-stranded DNA in the middle of a restriction sequence; produces no overhangs

Cloning plasmid - a plasmid used to carry foreign DNA into a host cell for replication

Cosmid - a hybrid plasmid that contains sequences from a bacteriophage and is used to clone large DNA sequences

DNA cloning - the process of making multiple, identical copies of a piece of DNA by inserting the DNA sequence into a plasmid and transforming bacteria with this plasmid

Endonuclease - an enzyme that breaks (i.e. cuts) a phosphodiester bond found within a DNA sequence

Expression plasmid - a plasmid used for expression (i.e. transcription and translation) of an inserted piece of DNA in a host cell

Genetic engineering - the direct modification of an organism's DNA using biotechnology; often done to introduce new traits, to improve resistance to disease, or to produce beneficial products

Kilobase pair (kb) - a unit of DNA length that is 1,000 base pairs long

Plasmid - a small, circular piece of double-stranded DNA found outside of the bacterial chromosome; used in DNA cloning procedures; often used as a vector to introduce foreign DNA into cells

Polyadenylation sequence - a sequence of DNA that regulates the addition of the poly-A tail to mRNA; also called a poly-A sequence

Promoter sequence (promoter) - a sequence of DNA where the RNA polymerase binds; determines the site of transcription initiation

Recombinant DNA (rDNA) - DNA that is created in a laboratory by combining genetic material from different sources

Recombinant plasmid - a type of artificial DNA created by inserting a foreign DNA fragment into a plasmid

Restriction enzyme - an enzyme that cuts double-stranded DNA at a specific sequence; produces either a blunt end or a sticky end; also called a restriction endonuclease

Restriction sequence - the sequence of DNA cut by a restriction enzyme

Restriction site - the physical location of a restriction sequence in a DNA sequence

Selectable marker - a gene included in a plasmid that allows for the identification of successfully transformed cells; often an antibiotic resistance gene

Sticky end - a short, single-stranded overhang on a DNA fragment created by a restriction enzyme that cuts the DNA helix at different locations within the restriction sequence

Transformation - the process of inserting DNA into a bacterial host cell

Vector - a piece of DNA used to transport foreign genetic material into a host cell; includes plasmids, cosmids, BACs, and YACs

Yeast Artificial Chromosome (YAC) - a genetically engineered DNA molecule used to clone large sequences of DNA in yeast

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5.3: Creating Recombinant DNA

Biotech Focus

In 1973 researchers from Stanford and UCSF took a piece of DNA isolated from the African clawed frog, *Xenopus laevis,* containing a ribosomal gene and inserted it into a bacterial plasmid. They introduced the resulting **"recombinant DNA"** into *E. coli* cells and observed the replication of this recombinant DNA along with the bacterial host genome. This groundbreaking experiment was the first time an animal gene was **cloned**.

Listen to *PNAS Science Sessions* (opens in new window) to hear one of those researchers, Stanley Cohen, talk about their original paper on recombinant DNA.

Introduction to Creating Recombinant DNA

Genetic engineering is based upon the creation of **recombinant DNA (rDNA)**. The technology to create rDNA first emerged in the late 1960s, with the discovery of enzymes that could specifically cut and join double-stranded DNA molecules. Today, we know these enzymes as **restriction enzymes** (for cutting) and **ligases** (for joining). rDNA is created through a set of experimental methods that are known, collectively, as **DNA cloning**. DNA cloning (also known as molecular cloning) typically combines 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. The use of the word cloning refers to the replication of cells, containing the rDNA, into a population of cells all with identical recombinant DNA molecules (ie., clones).

Learning Objectives

This section will outline the major steps in DNA cloning. At the end of this section, you will be able to:

- List the basic steps of molecular cloning
- Explain how plasmids and inserts are prepared for cloning
- Explain the differences between directional and blunt-end cloning
- Explain how a plasmid and insert are ligated together to form a piece of recombinant DNA called a recombinant plasmid
- Explain how transformation introduces recombinant plasmids into bacteria

Major Steps of DNA Cloning

DNA cloning is a multi-step process that takes many days in the laboratory to complete. The basic steps of DNA cloning are the same whether a plasmid, cosmid, or artificial chromosome is chosen as the vector. Because plasmids are more commonly used, this page will describe the steps of cloning into a plasmid. These steps are:

1. Isolation and preparation of the insert for cloning
2. Preparation of the plasmid for cloning
3. Combining the plasmid and insert DNA fragments suitably so they form the 'recombinant DNA' molecule (also called the **recombinant plasmid**)
4. Introducing this recombinant plasmid into the host cell (by transformation or transfection)
5. Selecting the host cells that have the correct recombinant plasmid
6. Confirmation of the recombinant plasmid inside the host

Preparation of the insert and plasmid for cloning

Both cloning and expression plasmids contain a specific region made up of multiple restriction sites. This region, the **Multi-Cloning Site, or MCS,** is the site of DNA insertion. In order to insert a piece of DNA into a plasmid (either for cloning or expression) both the DNA and plasmid must be "prepared" or digested with restriction enzymes. While not as important in

a cloning plasmid, insertion of DNA into an expression plasmid must be done in a specific orientation so the DNA can be expressed from the nearby promoter. Thus, in both cloning and expression plasmids, insertion into a MCS is typically done using a "**directional cloning**" strategy where the plasmid and insert are cut with two different restriction enzymes. This type of restriction digest is called a **double digest** because it uses two different restriction enzymes. This digest will produce unique DNA single-stranded overhangs at each end of the insert. DNA overhangs, complementary to the ends of the insert, will also be produced in the plasmid. As a result, there will be only one way that the DNA can be inserted into the plasmid. To learn about the restriction digest, go to Chapter 13.4: Restriction digestion of DNA.

Figure 5.3.2 and Figure 5.3.3 below shows such a directional cloning strategy using the restriction enzymes HindIII and PstI. In the first part of the procedure shown in Figure 5.3.2, both the DNA insert and plasmid are digested with HindIII and PstI. Digestion of the DNA insert with Hind III produces a 5' overhang with the sequence "AGCTT". Digestion of the insert with PstI will produce a 3' overhang of "GGTAC". When the plasmid is digested with the same two enzymes, a HindIII overhang of "TTCGA" and a PstI overhang of "CATGG" is produced (the tiny bit of plasmid DNA found between these two overhangs will be removed).

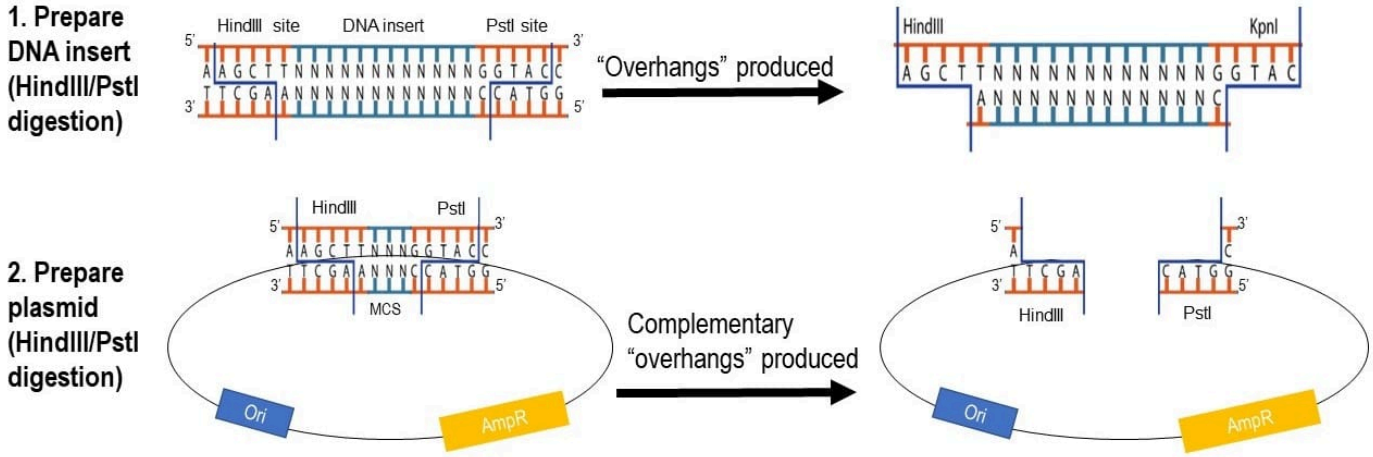


Figure 5.3.2: A directional cloning strategy: Part 1. The first steps of a directional cloning strategy using the restriction enzymes HindIII and PstI are: (1) Prepare the DNA insert (HindIII/PstI digestion) - digestion of insert DNA with HindIII and PstI produces overhangs of AGCT and GTAC, respectively; (2) Prepare the plasmid (HindIII/PstI digestion) - digestion of plasmid DNA with HindIII and PstI produces the complementary overhangs of TCGA and CATG, respectively). (Blue box = Ori; origin of replication; yellow box = AmpR; ampicillin resistance gene) (Directional Cloning Part 1 by Patricia Zuk, CC BY 4.0)

These complementary ends are critical for the correct ligation of the insert and plasmid to form a new piece of recombinant DNA.This is because the DNA insert overhangs of AGCT and GTAC are complementary to the plasmid overhangs of TCGA and CATG (Figure 5.3.3). As a result, there is only one way that the DNA insert can "fit" into the plasmid. Once the insert and plasmid are brought together, an enzyme called a **DNA ligase** will form the phosphodiester bonds required to join these pieces of DNA together. Following ligation, the HindIII and PstI restriction sites are regenerated and can be used to digest out the insert if desired.

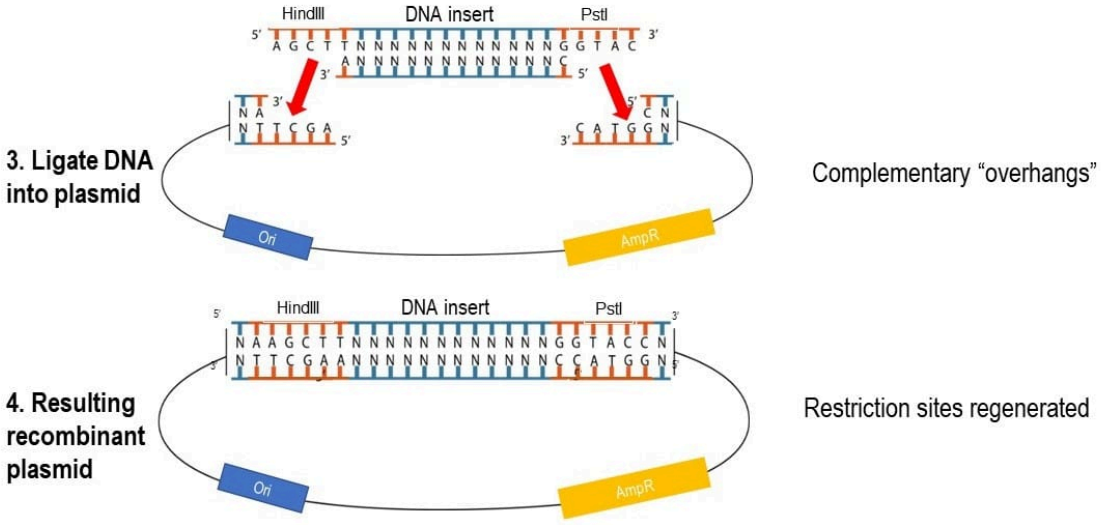


Figure 5.3.3: A directional cloning strategy: Part 2. The remaining steps of the directional cloning strategy are: (3) Ligate DNA into plasmid - ligation of DNA insert and plasmid is accomplished because the overhangs complement one another (AGCT and TCGA; GTAC and CATG). (4) The resulting recombinant plasmid contains the insert DNA and the HindIII and PstI sites are regenerated at either end. (Blue box = Ori; origin of replication; yellow box = AmpR; ampicillin resistance gene) (Directional Cloning Part 2 by Patricia Zuk, CC BY 4.0)

While directional cloning is the preferred strategy, there are times when restriction enzymes that produce a "blunt" end must be used. This technique is called **blunt end cloning**. In blunt end cloning, the restriction enzyme cuts both strands of DNA at the same position within the restriction site. As a result, no overhangs are produced and the ends are said to be "blunt" (Figure 5.3.4).

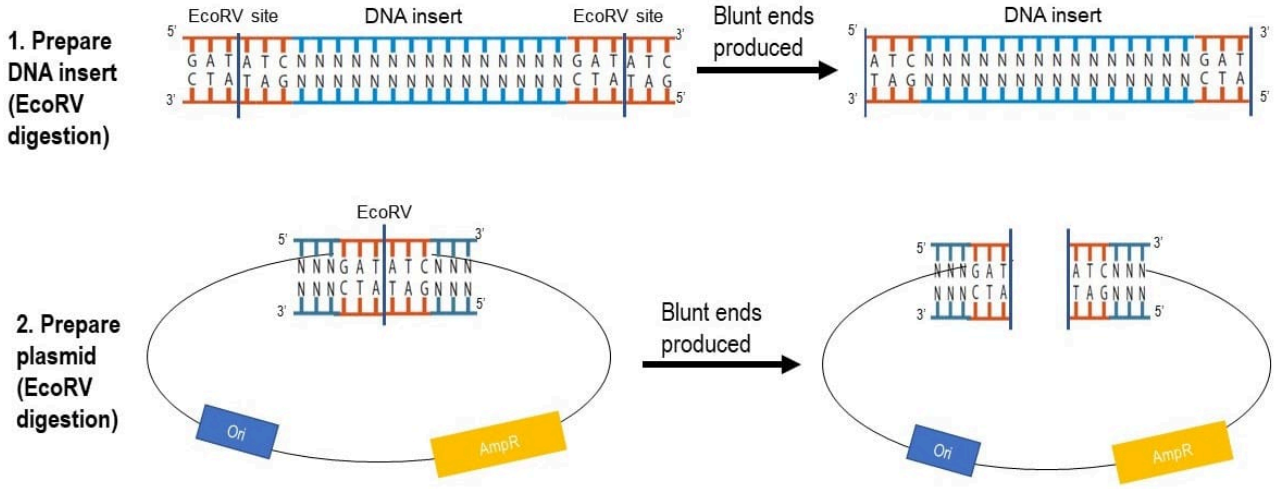


Figure 5.3.4: Blunt end cloning: Part 1. In "blunt end" cloning, both the DNA insert and plasmid are digested with a restriction enzyme that produces blunt ends. The first steps of a blunt end cloning strategy using the enzyme EcoRV are as follows: (1) Prepare the DNA insert (EcoRV digestion) - digestion of insert DNA with EcoRV produces the blunt ends of ATC and GAT at either end of the insert (sense DNA strand sequence given for clarity); (2) Prepare the plasmid (EcoRV digestion) - digestion of plasmid DNA with EcoRV produces the blunt ends of GAT and ATC (sense DNA strand given for clarity). (Blue box = Ori; origin of replication; yellow box = AmpR; ampicillin resistance gene) (Blunt End Cloning Part 1 by Patricia Zuk, CC BY 4.0)

As with directional cloning, once the DNA insert and plasmid are digested with the restriction enzyme, the two are ligated to one another by the DNA ligase. Following ligation, the two EcoRV restriction sites are regenerated and can be used to digest out the insert if desired (Figure 5.3.5).

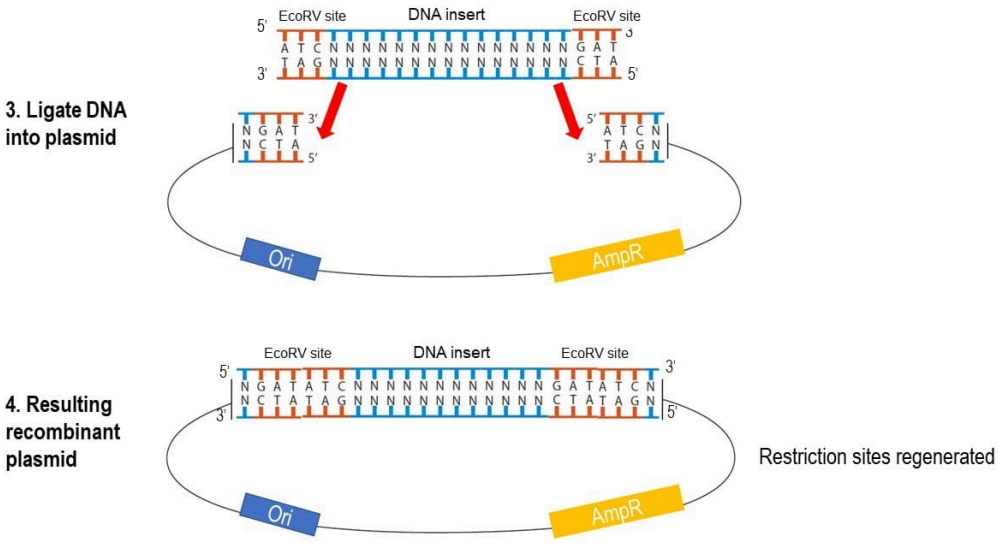


Figure 5.3.5: Blunt end cloning: Part 2. The remaining steps of a blunt end cloning strategy using the restriction enzyme EcoRV are: (3) Ligate DNA into plasmid - ligation of DNA insert and plasmid inserts the DNA into the plasmid. (4) The resulting recombinant plasmid contains the insert DNA and the EcoRV sites are regenerated at either end. (Blue box = Ori; origin of replication; yellow box = AmpR; ampicillin resistance gene) (Blunt End Cloning Part 2 by Patricia Zuk, CC BY 4.0)

Because there are no overhangs produced when using restriction enzymes that produce blunt ends (i.e., blunt end restriction enzymes), it is important that the DNA insert be ligated into the plasmid so that the restriction sites are regenerated. The regeneration of the restriction sites ensures the correct orientation of the insert so that it can be expressed, if desired. Figure 5.3.6 below shows the correct cloning orientation of a DNA insert.

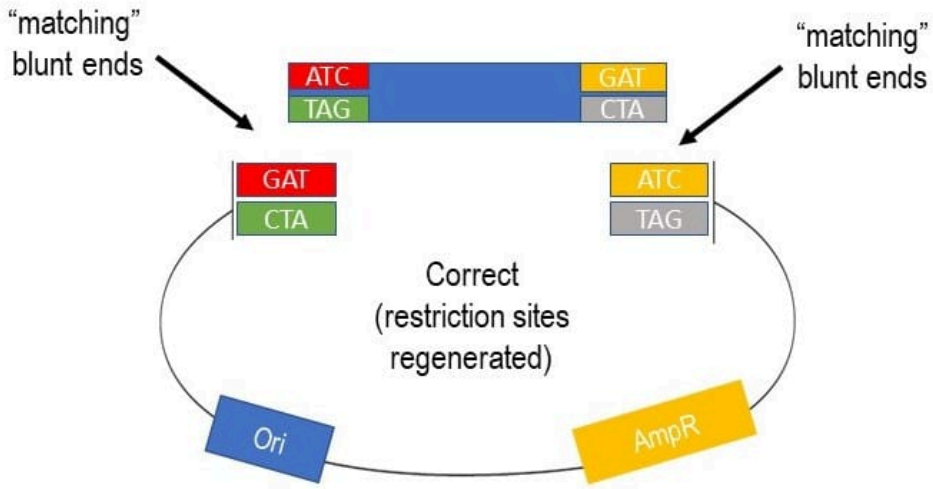


Figure 5.3.6:The correct blunt end cloning direction. The cloning of a DNA insert with blunt ends must be performed so that the restriction sites are regenerated. The sense and anti-sense strands of the DNA insert and plasmid digested with a blunt end restriction enzyme are shown with four different colored rectangles and include their sequences. The correct orientation is shown above with the blunt ends of the DNA insert "matching" up to the blunt ends of the plasmid. This orientation regenerates the EcoRV restriction site sequence of GATATC at both ends of the cloning site. (Correct Blunt End Cloning by Patricia Zuk, CC BY 4.0)

In addition to this orientation, there are three other possibilities for cloning the DNA insert. However, none of these will regenerate the restrictions sites at either end of the DNA insert (Figure 5.3.7). While these other orientations may permit gene expression, the protein made will be incorrect. Therefore, when using a blunt end strategy, it is vital to confirm the orientation of the insert within the plasmid. This is typically done through DNA sequencing.

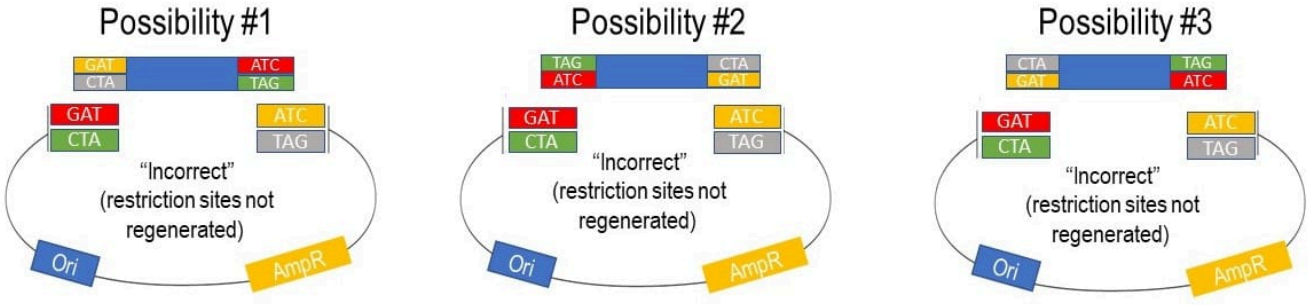


Figure 5.3.7: Incorrect blunt end cloning possibilities. Three possible cloning orientations are shown. Each of these are incorrect because they do not regenerate the restriction site sequence of GATATC at either end of the cloning site. (Blue box = Ori; origin of replication; yellow box = AmpR; ampicillin resistance gene). (Blunt End Cloning Possibilities by Patricia Zuk, CC BY 4.0)

Concept in Action

Animation: Restriction Enzymes

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this Interactive Content online

Ligation of the insert and plasmid to produce the recombinant plasmid

Insertion of the DNA insert into the plasmid requires the formation of new phosphodiester bonds between the two pieces of DNA. This is done by a type of enzyme called a **DNA ligase** (or ligase). Ligation of the insert and plasmid will join these two fragments into one piece, restoring the circular nature of the plasmid and forming a piece of rDNA known as a **recombinant plasmid** (Figure 5.3.8). As described above, ligation will also regenerate the restriction sites used in cloning so that the DNA insert can be cut out again, if desired. It is important to know that DNA ligation is not 100% effective. Some plasmids will resume their circular configuration without incorporating a DNA insert. These "false positives" will need to be identified. To learn about DNA ligation, go to Chapter 13.5: Ligation of insert DNA into a plasmid.

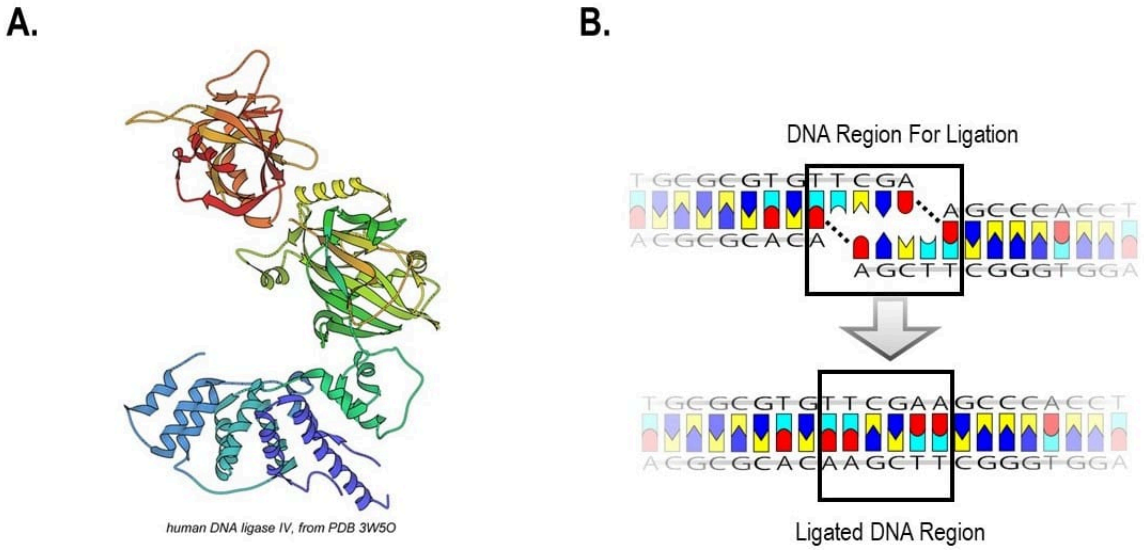


Figure 5.3.8: DNA ligation. (A) The ribbon structure of a human DNA ligase enzyme. (DNA ligase by Dcrjsr, CC BY-SA 4.0) (B) The ligation of DNA by DNA ligase requires the formation of phosphodiester bonds between adjacent nucleotides. Once ligated, the DNA helix is one continuous piece. In the top figure, the region for ligation is shown in the black box with the forming phosphodiester bonds shown as dotted lines. In the bottom figure, the ligated region is shown in the black box. (Ligation by Madprime, CCO 1.0)

Transformation of host cells with the recombinant plasmid

Once the DNA insert has been ligated into the plasmid, the recombinant plasmid is then introduced into bacterial cells so that the bacterial host cell can replicate the recombinant plasmid along with its own DNA. This introduction is done through a technique called **bacterial transformation, or transformation** (Figure 5.3.9). Transformation relies upon the finding that bacteria will take up "naked" DNA from its surroundings if exposed to specific environmental conditions. One common method of transformation is to weaken bacterial membranes through their exposure to ice-cold salt solutions. These weakened bacteria are then mixed with recombinant plasmids and then "heat shocked" at 42°C for a short period of time. In a mechanism not understood, this heat shock allows for the entry of the recombinant plasmids into the bacteria. To learn about bacterial transformation, go to Chapter 13.6: Bacterial transformation.

Transformation is very inefficient. The majority of bacteria will not incorporate recombinant plasmids. As such, a selection process for identifying those bacterial cells containing recombinant plasmids must be used to eliminate those bacterial cells that do not have any recombinant plasmids. This process relies upon the presence of selection markers in the recombinant plasmid. The most common type of selection marker is an **antibiotic resistance gene** - a gene that will confer resistance to specific antibiotics that bacteria are not normally resistant to. Common antibiotic resistance genes will provide resistance to ampicillin, tetracycline, or kanamycin. Bacteria that are transformed with recombinant plasmids are typically grown overnight on agar plates containing a specific antibiotic. Only those bacteria with recombinant plasmids should survive exposure to this antibiotic. These bacteria will propagate on the agar over night and form a **bacterial colony**.

As with many other molecular techniques, antibiotic selection is not foolproof. Some transformed bacteria will contain plasmids that do not contain an insert. Other bacteria may survive exposure to the antibiotic and will have no plasmid at all. As such, confirmation of the recombinant plasmid within the bacteria must be obtained. This confirmation will necessitate the isolation of the recombinant plasmid DNA from bacterial cells (Chapter 13.7: Isolation of DNA from bacteria (i.e., the “mini-prep”), followed by additional tests to confirm the presence of the insert, such as polymerase chain reaction/PCR (Chapter 13.2: Polymerase Chain Reaction (PCR) and agarose gel electrophoresis (Chapter 13.3: Agarose gel electrophoresis).

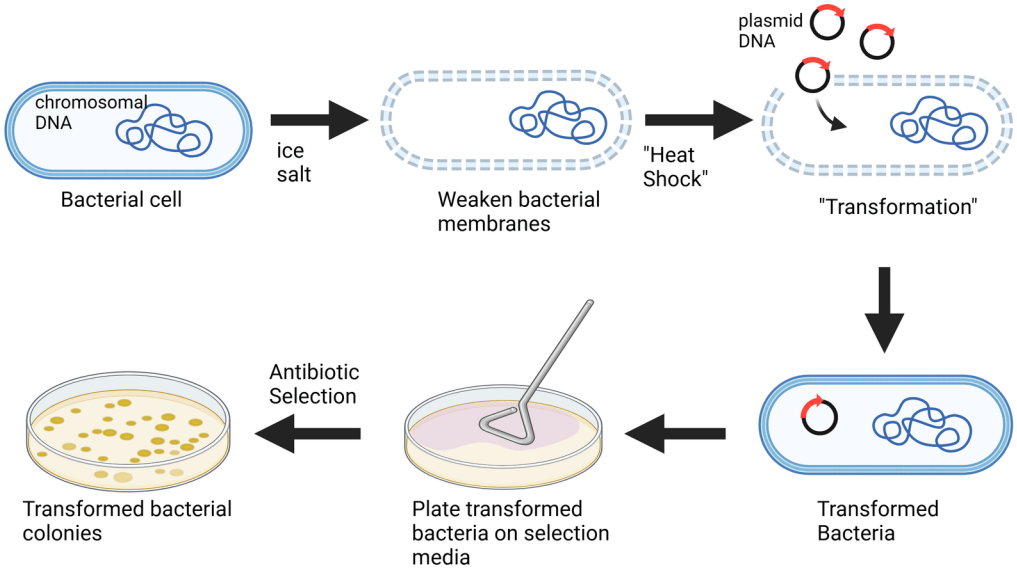


Figure 5.3.9: Transformation of bacterial cells. Bacteria are weakened using ice-cold salt solutions prior to incubation with recombinant plasmids. "Heat Shocking" the weakened bacteria allows for the entry of recombinant plasmids. Plating of transformed bacteria onto agar plates containing an antibiotic should select for those bacteria containing recombinant plasmids. Growth of these bacteria results in the formation of colonies. (Bacteria Transformation by Patricia Zuk, CC BY 4.0; created in BioRender. Zuk, P. (2025))

Recombinant plasmids can also be introduced into mammalian cells through a process known as transfection (Chapter 13.10: Liposomal transfection into mammalian cells). Transfection typically occurs after the recombinant plasmid has been introduced into bacteria, amplified, and the presence of the DNA insert inside the recombinant plasmid has been verified.

Key Concepts

The creation of recombinant DNA is a multi-step process that is known as DNA cloning. Some major concepts to remember are:

- recombinant DNA is created through by inserting DNA into a plasmid
- plasmid DNA is cut open by enzymes called restriction enzymes
- restriction enzymes cut the DNA helix at specific sequences called restriction sites
- many plasmids have a multi-cloning site made up multiple restriction sites
- in directional cloning, the DNA can be inserted into the cloning site of the plasmid in only one direction
- in blunt-end cloning, the DNA can be inserted into the cloning site of the plasmid in multiple directions
- insertion of DNA into a plasmid requires an enzyme called a ligase

Glossary

Antibiotic resistance gene - a gene found within a plasmid that makes the bacteria resistant to a specific antibiotic

Blunt end - produced when a restriction enzyme cuts double stranded DNA in the middle of a restriction site; produces no overhangs

Blunt end cloning - a cloning strategy that uses restriction enzymes that produce blunt ends

Directional cloning - a cloning strategy that uses restriction enzymes that produce sticky ends

DNA cloning - the process of making multiple, identical copies of a piece of DNA by inserting the DNA sequence into a plasmid and transforming bacteria with this plasmid

Ligase - an enzyme that forms a phosphodiester bond between the 3' end of one DNA fragment and the 5' end of another DNA

fragment; also called a DNA ligase

Multi-cloning site (MCS) - several restriction enzyme sites clustered together in a plasmid; the site of insertion of a foreign piece of DNA

Plasmid - a small, circular piece of DNA found outside of the bacterial chromosome; used in DNA cloning procedures

Polylinker - another name for a multi-cloning site (MCS)

Recombinant DNA (rDNA) - DNA that is created in a laboratory by combining genetic material from different sources

Recombinant plasmid - a type of artificial DNA created by inserting a foreign DNA fragment into a plasmid

Restriction enzyme - a protein that cuts double-stranded DNA at specific sequences; produces either a blunt end or a sticky end

Restriction site - the sequence of DNA cut by a restriction enzyme

Sticky end - a short, single-stranded overhang on a DNA fragment created by a restriction enzyme that cuts the DNA at a staggered position

Transformation - the process of inserting DNA into a bacterial host cell

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5.4: Polymerase Chain Reaction (PCR)

Molecular cloning was the first method available to isolate a gene of interest and make many copies of it to obtain sufficient amounts of the DNA to study. Today, there is a faster and easier way to obtain large amounts of a DNA sequence of interest -the polymerase chain reaction (PCR). PCR allows one to use the power of DNA replication to amplify DNA enormously in a short period of time. As you know, cells replicate their DNA before they divide, and in doing so, double the amount of the cell's DNA. PCR essentially mimics cellular DNA replication in the test tube, repeatedly copying the target DNA over and over, to produce large quantities of the desired DNA.

Selective Replication

In contrast to cellular DNA replication, which amplifies all of a cell's DNA during a replication cycle, PCR does targeted amplification to replicate only a segment of DNA bounded by the two primers that determine where DNA polymerase begins replication. Figure 8.34 illustrates the process. Each cycle of PCR involves three steps, denaturing, annealing and extension, each of which occurs at a different temperature.

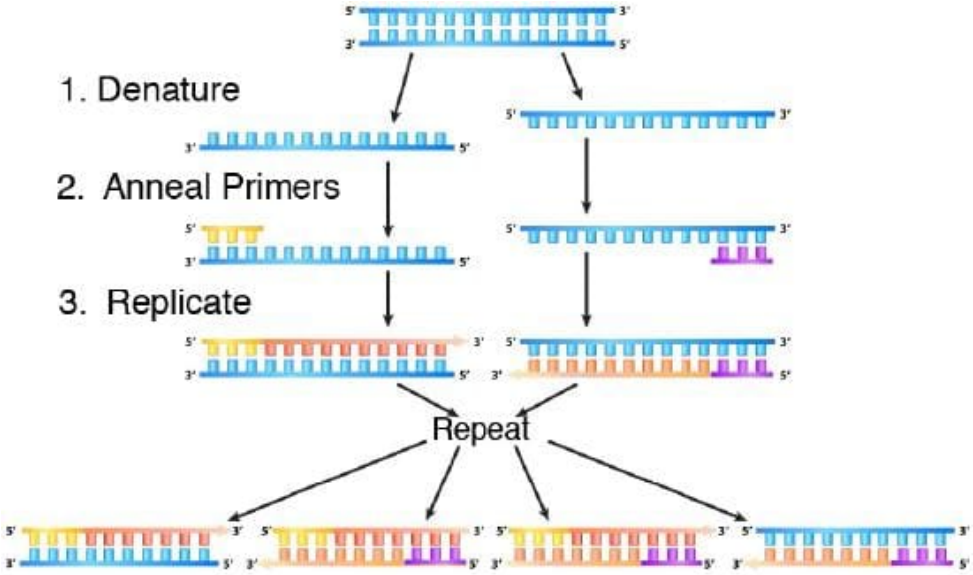


Figure 8.34 - Steps in the polymerase chain reaction. Image by Aleia Kim

The Starting Materials

Since PCR is, basically, replication of DNA in a test-tube, all the usual ingredients needed for DNA replication are required:

- A template (the DNA containing the target sequence that is being copied)
- Primers (to initiate the synthesis of the new DNA strands)
- Thermostable DNA polymerase (to carry out the synthesis). The polymerase needs to be heat stable, because heat is used to separate the template DNA strands in each cycle.
- dNTPs (DNA nucleotides to build the new DNA strands).
- The template is the DNA that contains the target you want to amplify (the "target" is the specific region of the DNA you want to amplify).

The primers are short synthetic single-stranded DNA molecules whose sequence matches a region flanking the target sequence. It is possible to chemically synthesize DNA molecules of any given base sequence, to use as primers. To make primers of the correct sequence that will bind to the template DNA, it is necessary to know a little bit of the template sequence on either side of the region of DNA to be amplified. DNA polymerases and dNTPs are commercially available from biotechnology supply companies.

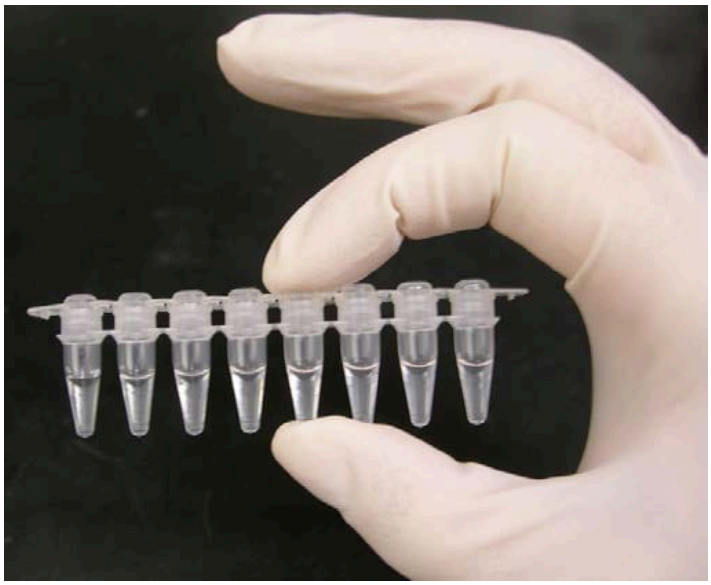


Figure 8.35 - PCR tubes with DNA samples ready for reaction. Wikipedia

First, all of the reagents are mixed together. Primers are present in millions of fold excess over the template. This is important because each newly made DNA strand starts from a primer. The first step of the process involves separating the strands of the target DNA by heating to near boiling.

Next, the solution is cooled to a temperature that favors complementary DNA sequences finding each other and making base pairs, a process called annealing. Since the primers are present in great excess, the complementary sequences they target are readily found and base-paired to the primers. These primers direct the synthesis of DNA. Only where a primer anneals to a DNA strand will replication occur, since DNA polymerases require a primer to begin synthesis of a new strand.



Figure 8.36 - A PCR thermocycler system. Wikipedia

Extension

In the third step in the process, the DNA polymerase replicates DNA by extension from the 3' end of the primer, making a new DNA strand. At the end of the first cycle, there are twice as many DNA molecules, just as in cellular replication. But in PCR, the process is repeated, usually for between 25 and 30 cycles. At the end of the process, there is a theoretical yield of 2^{30} (over 1 billion times) more DNA than there was to start. (This enormous amplification power is the reason that PCR is so useful for forensic investigations, where very tiny amounts of DNA may be available at a crime scene.)

The temperature cycles are controlled in a thermocycler, which repeatedly raises and lowers temperatures according to the set program. Since each cycle can be completed in a couple of minutes, the entire amplification can be completed very rapidly. The resulting DNA is analyzed on a gel to ensure that it is of the expected size, and depending on what it is to be used for, may also be sequenced, to be certain that it is the desired fragment.

Mutagenesis

PCR is frequently used to obtain gene sequences to be cloned into vectors for protein expression, for example. Besides simplicity and speed, PCR also has other advantages. Because primers can be synthesized that differ from the template sequence at any given position, it is possible to use PCR for site-directed mutagenesis. That is, PCR can be used to mutate a gene at a desired position in the sequence. This allows the proteins encoded by the normal and mutant genes to be expressed, purified and compared.

Analysis of gene expression

PCR can also be used to measure gene expression. Where in PCR the amount of amplified product is not determined till the end of all the cycles, a variation called quantitative real-time PCR is used, in which the accumulation of product is measured at each cycle. This is possible because real-time PCR machines have a detector module that can measure the levels of a fluorescent marker in the reaction, with the amount of fluorescence proportional to the amount of amplified product. By following the accumulation of product over the cycles it is possible to calculate the amount of starting template. To measure gene expression, the template used is mRNA reverse-transcribed into cDNA (see below). This coupling of reverse transcription with quantitative real-time PCR is called qRT-PCR.

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6: FORENSIC BIOTECHNOLOGY

6.1: Analysis of STRs

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6.1: Analysis of STRs

Polymorphisms

The difference in nucleotide sequences between humans lies between 0.1-0.4%. This means that people are greater than 99% similar. But when you look at your classmates around the room, you can see that that small difference amounts to quite a bit of variation within our species. The bulk of these differences aren't even within the coding sequences of genes but lie outside in regulatory regions that change the expression of those genes. Imagine if there were mutations to the coding sequences, this could be very deleterious to the well-being of the organism. We say that the coding sequences of genes that ultimately lead to proteins have **selective pressure** to remain the same. The areas outside of the coding sequences have a reduced and sometimes non-existent selection pressure. These areas are allowed to mutate in sequence and even expand or contract. Areas of changes or differences are called **polymorphic** (many forms). If you were to read a repetitive set of sequences and count the repetition, you' would make mistakes and lose count. Likewise, DNA polymerase will make errors or stutter in areas of repetitiveness and produce polymorphic regions.

Tandem Repeats

A type of polymorphism occurs due to these repeats expanding and contracting in non-coding regions. These regions are called variable number tandem repeats (**VNTRs**)or sometimes short tandem repeats (**STRs**). Any region or location on a chromosome is referred to as **locus** (loci for plural). Scientists use polymorphic loci that are known to contain VNTRs/STRs in order to differentiate people based on their DNA. This is often used in forensic science or in maternity/paternity cases. Any variation of a locus is referred to as an **allele**. In standard genetics, we often think of an allele as a variation of a gene that would result in a difference in a physical manifestation of that gene. In the case of STRs, these alleles are simply a difference in the number of repeats. This means the length of DNA within this locus is either longer or shorter and gives rise to many different alleles. VNTRs are referred to as minisatellites while STRs are called **microsatellites**.

CoDIS

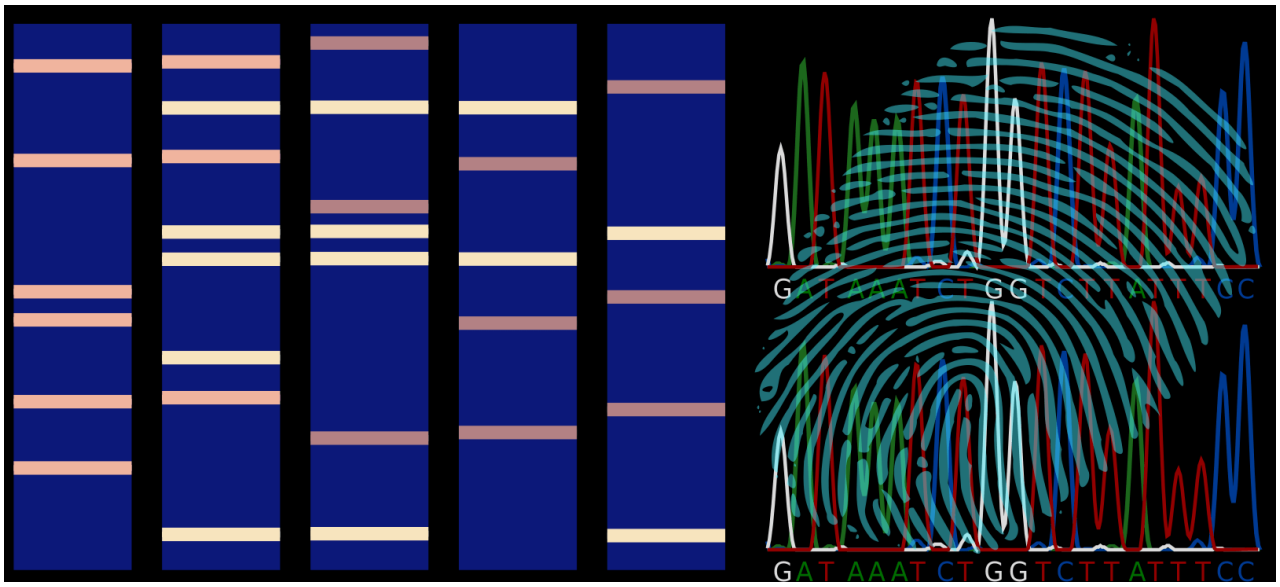


Figure 6.1.1: DNA fingerprinting. Credit: Helixitta, The Photographer and Jeremy Seto (CC-BY-SA 3.0)

The FBI and local law enforcement agencies have developed a database called the Combined DNA Index System (**CoDIS**) that gathers data on a number of STRs. By establishing the number of repeats of a given locus, law enforcement officials can differentiate individuals based on the repeat length of these alleles. CoDIS uses a set of 20 loci that are tested together. As you would imagine, people are bound to have the same alleles of certain loci, especially if they were related. The use of 20 different loci makes it statistically improbable that 2 different people could be confused with each other. Think about this in terms of

physical traits. As you increase the number of physical traits used to describe someone, you are less likely to confuse that person with someone else based on those combinations of traits. Using the CoDIS loci increases the stringency since there are many alleles for each locus. The twentieth locus in CoDIS (called AMEL) discriminates between male and female.

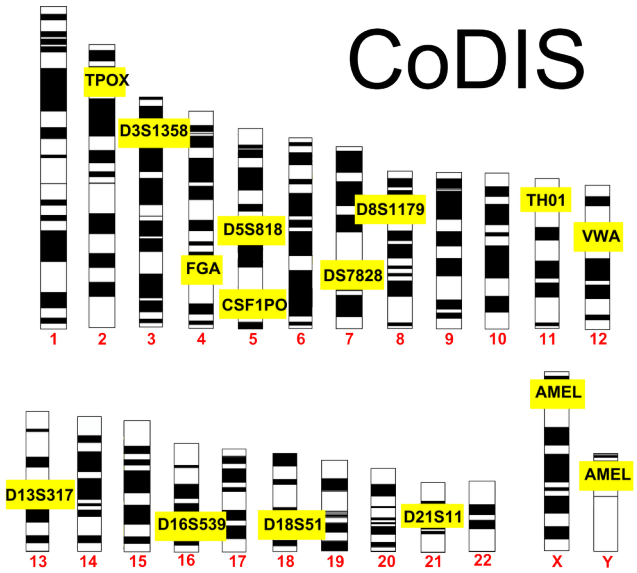


Figure 6.1.2: **CoDIS STRs:** The FBI utilizes 20 different loci to discriminate between people. AMEL discriminates by gender and is located on the X & Y. Credit: Jeremy Seto (CC-BY-NC-SA)

Crime Scene Investigation

This lab uses a CoDIS locus called TH01. TH01 is a locus on chromosome 11 that has a repeating sequence of TCAT. There are reported to be between 3-14 repeats in this locus. With the exception of X and Y in a male, all chromosomes have a homologous partner. Therefore, each individual will have 2 alleles for each CoDIS locus.

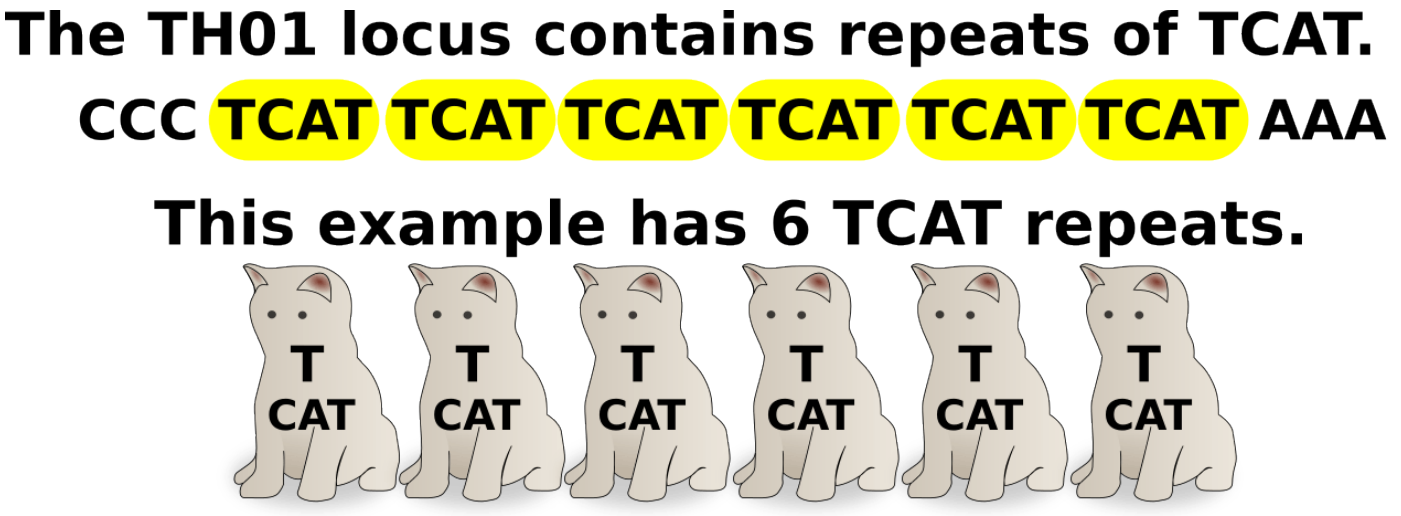


Figure 6.1.2: **TH01 STR:** Outside of the STR, there are flanking areas of known sequence. The primers that amplify TH01 in PCR recognize these flanking sequences to amplify the TCAT repeats. Credit: Jeremy Seto (CC-BY-NC-SA)

At a crime scene, criminals don't often leave massive amounts of tissue behind. Scant evidence in the form of a few cells found within bodily fluids or stray hairs can be enough to use as DNA evidence. DNA is extracted from these few cells and amplified by PCR using the specific primers that flank the STRs used in CoDIS.

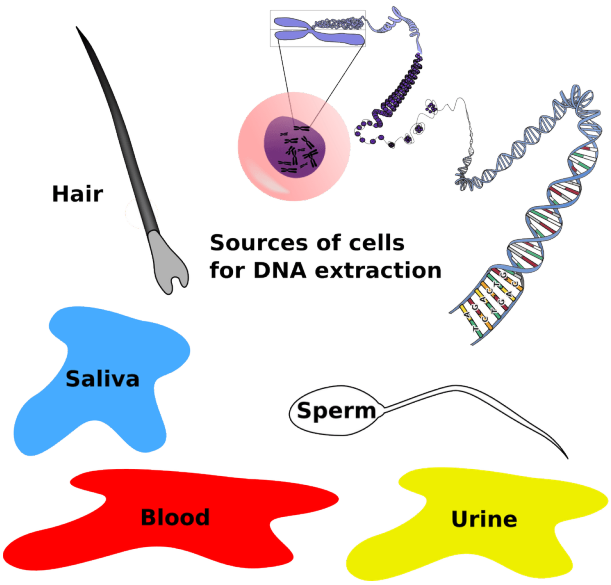


Figure 6.1.4: **DNA evidence from a crime scene:** DNA can be extracted from cells found from various sources at a crime scene. PCR can amplify this small amount of DNA. Credit: Jeremy Seto (CC-BY-NC-SA)

Amplified DNA will be separated by gel electrophoresis and analyzed. Size reference standards and samples from the crime scene and the putative suspects would be analyzed together. In a paternity test, samples from the mother, the child, and the suspected father would be analyzed in the same manner. A simple cheek swab will supply enough cells for this test.

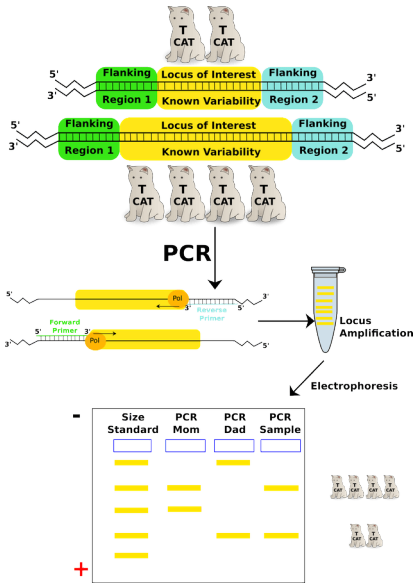


Figure 6.1.5: **TH01 locus used in a Paternity/Maternity test:** Individual PCR reactions are run for each sample (mom, dad, and child). The TH01 primer pair specifically amplifies the locus. Each amplified sample is run on the same gel to resolve the different alleles of TH01 from each individual. From this test, the sample could be the offspring from these 2 parents but the use of more STRs would make it more definitive. Count the TCATs. Credit: Jeremy Seto (CC-BY-NC-SA)

External Resources

- Flash animation walking through what an STR is: http://www.dnalc.org/view/15981-DNA-variations.html

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7: MICROBIAL BIOTECHNOLOGY

7.1: Microbe classification
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7.1: Microbe classification

Biotech Focus

Extremophiles are microorganisms that thrive in extreme conditions where most other life forms would perish. Traditionally, these hardy microbes are found in environments like deep-sea hydrothermal vents, Antarctic ice, or highly acidic hot springs. However, recent studies have revealed a surprising habitat for certain extremophiles: common household appliances. Household appliances such as dishwashers, washing machines, and even coffee makers create unique microenvironments that can mimic some extreme conditions. Dishwashers, for instance, generate high temperatures, rapid temperature shifts, and fluctuating pH levels from detergents—all challenging environments that some extremophiles can endure. These extremophiles, adapted to the challenging conditions of household appliances, hold significant potential for biotechnological innovation. They could be used in many domains, for example, to produce enzymes that could withstand extreme conditions, or to develop methods for sustainable waste management, demonstrating how even the most commonplace settings can contribute to groundbreaking science.

To know more about the study, read The Microbiome of Things: Appliances, Machines, and Devices Hosting Artificial Niche-Adapted Microbial Communities

Introduction

Microbes are microscopic organisms (i.e. microbes) that are too small to be seen by the naked eye. They include bacteria, archaea, fungi, but also viruses. Such a broad definition means that many different types of microbes exist, with different structures, sizes and metabolism. Despite their small size, microbes play critical roles in natural ecosystems. They are also invaluable tools in biotechnology.

Learning Objectives

Microbes are an incredibly important part of biotechnology. At the end of this section, you will be able to:

- Distinguish between prokaryotic and eukaryotic cells, including listing their common structures and how they differ
- List the major microbes used in biotechnology
- Describe the basic morphology of bacterial cells and some of their major structures, including the cell wall
- Explain the "extremophile" nature of archaea
- Describe some basic viral structures

Prokaryotes vs Eukaryotes

All living things are made of one or more cells. Two general types of cells can be found: **prokaryotic (lacking a nucleus)** and **eukaryotic (possessing a nucleus**). Prokaryotic organisms are unicellular, meaning they are made of one cell only (Figure 7.1.1). In contrast, eukaryotic organisms can be unicellular, such as yeast, or multicellular, such as humans.

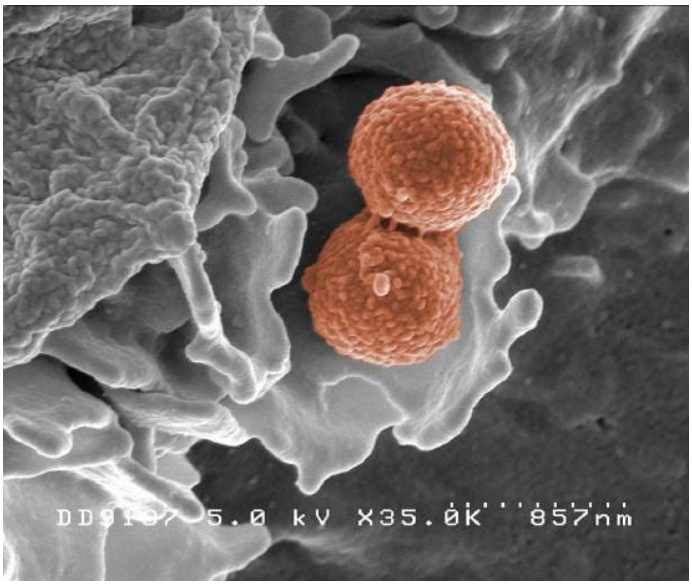


Figure 7.1.1: Methicillin-resistant *Staphylococcus aureus*. A scanning electron micrograph shows two methicillin-resistant *Staphylococcus aureus* (MRSA) bacterial cells (colorized orange) interacting with a human neutrophi (gray). Note the difference in size between the smaller two bacterial cells and the much larger neutrophil (eukaryotic). (Methicillin-Resistance Staphylococcus aureus, by NIAID, CC BY 2.0)

Both prokaryotes and eukaryotes share the same basic elements:

1. a **cytoplasm:** a gel-like substance mainly composed of water, proteins and salts
2. a **cell membrane:** a phospholipid bilayer that surrounds the cytoplasm
3. one or more **chromosomes:** an organized form of DNA; the number is specific to the species
4. **ribosomes:** a non-membranous organelle used for protein synthesis

However, they also differ in many ways. Table 7.1.1 below lists some of these differences.

Table 7.1.1: Prokaryotes vs. Eukaryotes

Feature	Prokaryotes	Eukaryotes
Nucleus	No	Yes
DNA organization	DNA in the form of a circular chromosome known as a genophore	DNA in the form of circular or linear chromosomes; multiple chromosomes found within the nucleus
Membrane-bound organelles	No	Yes (mitochondria, ER, Golgi, lysosomes, peroxisomes, vacuoles, chloroplasts etc....)
Cell division mechanism(s)	Binary fission, budding (asexual reproduction)	Binary fission, budding, mitosis, and meiosis
Cell wall composition	Peptidoglycans (bacteria), pseudopeptidoglycans (archaea)	Cellulose (plant cells), chitin (fungal cells), no cell wall in animals cells
Ribosome composition	70S: 30S and 50S subunits	80S: 40S and 60S subunits
Examples	bacteria, archaea	fungi, plants, animals

Microbial Biotechnology: Cell Types

Microbial biotechnology is the use of microbes for industrial, environmental, and pharmaceutical applications. Over the years, advances in genetic engineering, molecular biology, and bioinformatics have expanded the scope of microbial biotechnology, allowing us to harness these organisms for innovations in a wide range of areas such as agriculture, medicine, bioenergy, and environmental management. What microbes are used in biotechnology is dependent upon the application. For example, bacteria are widely used in biotechnology due to their rapid growth and the ability to manipulate their genetic material easily.

The microbes used in biotechnology are:

1. **Bacteria:** single-celled prokaryotic organisms that lack a nucleus and membrane-bound organelles
2. **Yeast:** single-celled eukaryotic organisms that are classified as fungi
3. **Archaea:** single-celled prokaryotic organisms that are genetically distinct from bacteria
4. **Viruses:** small, infectious organisms that can only replicate inside of a host cell

Bacteria

Bacteria are the oldest and most diverse form of life and comprise the domain Bacteria. They can be found in many shapes and sizes depending on the bacterial species (Figure 7.1.2).

The most common bacterial shapes are:

1. **coccus:** spherical shape (plural = cocci), e.g., *Streptococcus mutans*
2. **bacillus:** rod shape (plural = bacilli), e.g., *Esherichia coli (E.coli)*

While these two are the most common shapes seen, there are additional bacterial shapes including spiral shaped (or **spirillum**), stalked forms, filamentous shapes, and a comma-shaped cell called **vibrio**.

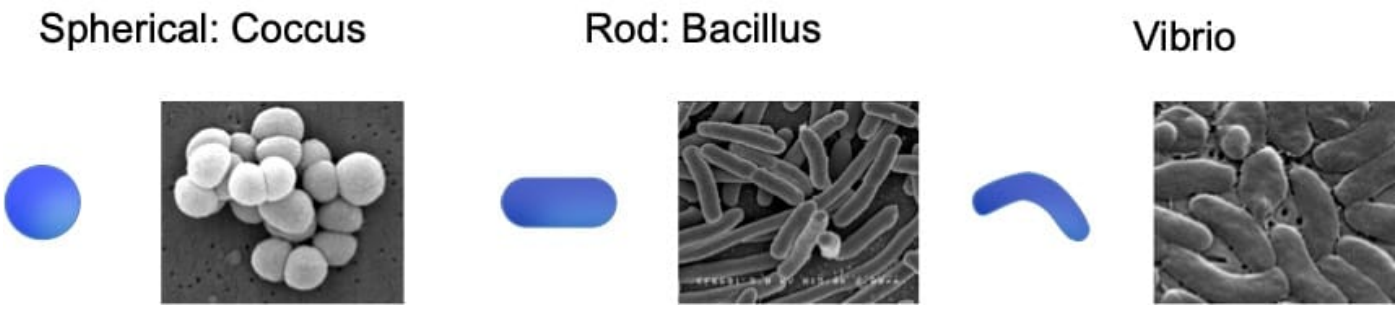


Figure 7.1.2: Common bacterial shapes. Left: the spherical bacterial cell is called coccus (plural: cocci). Center: the rod shaped cell is called bacillus (plural: bacilli). Right: the bent rod shaped cell is called vibrio. (Electron microscopy image credits: Left: Betsy Crane; Center: NIAID; Right: Janice Haney Carr; all images are public domain)

Bacterial species can also be characterized by the arrangement of their cells (Figure 7.1.3). For example, they can found as single cells, in pairs (diplo), in chains (strepto) or in clusters (staphylo). The names of bacteria often reflects their shapes and arrangement, such as the genus *Staphylococcus* (spherical cells arranged in clusters).

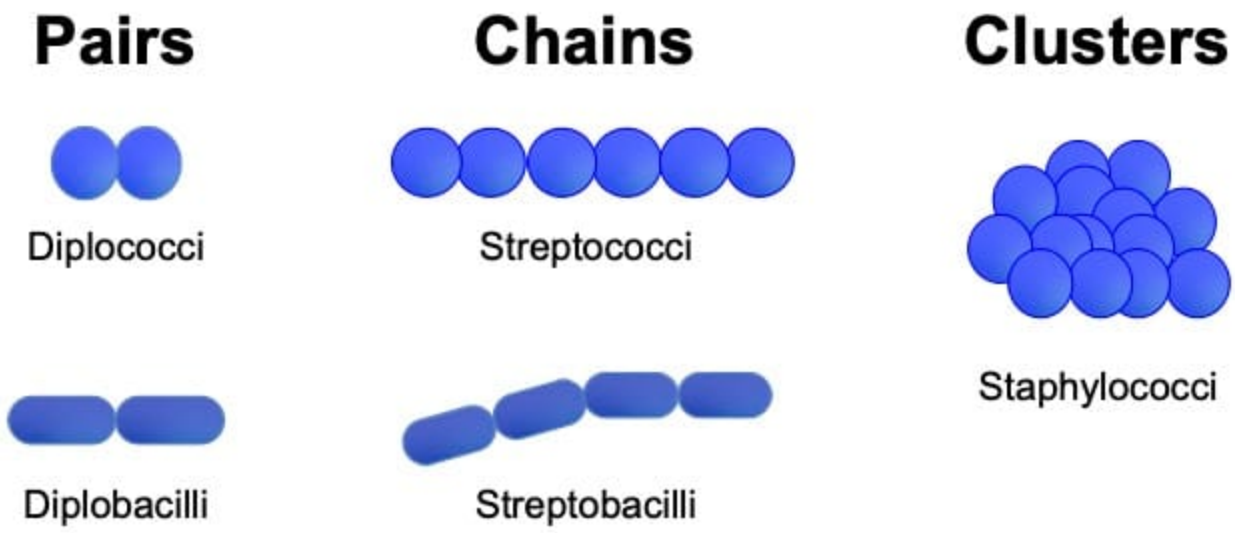


Figure 7.1.3: Bacterial cell arrangements. Left: cells can be arranged in pairs. Cocci found in pairs are called diplococci (top), bacilli in pairs are called diplobacilli (bottom). Center: cells can be arranged in chains. Cocci grouped as a chain are called streptococci, bacilli in chains are called streptobacilli. Right: only cocci can be arranged in clusters and are called staphylococci. (Bacterial Cell Arrangements by Kareen Martin, CC BY 4.0)

The genome of most bacteria is a circular chromosome, called a **genophore**. Because bacteria lack a nucleus, the bacterial chromosome is found in a region of the cytoplasm called the **nucleoid**. In addition to the bacterial chromosome, some bacteria have pieces of extra-chromosomal DNA called plasmids. A **plasmid** is a small, circular double-stranded piece of DNA that replicates independently from the bacterial chromosome and can be passed on from one bacterium to another bacterium. While the genes found in a plasmid are not necessary for the normal metabolism of the bacteria, they confer advantageous traits, such as antibiotic resistance. Plasmids are a valuable tool in molecular biology and genetics, they can be easily manipulated and used as vector to transfer and express genes into a host organism. They can be used to study the effect of the product of a gene or to create genetically modified organism. For more about the bacterial chromosome and plasmids, go to Chapter 3.1 DNA Structure. The bacterial genome can easily be altered either by exposing them to physical or chemical mutagens. They also have the ability to acquire DNA from their environment. Because of these properties, bacteria constitute an invaluable tool in biotechnology.

Although both bacteria and eukaryotic cells possess ribosomes that are responsible for the synthesis of proteins, the bacterial ribosome is smaller and simpler than their eukaryotic counterpart. Prokaryotic ribosomes are called **70S ribosomes**, whereas eukaryotic ribosomes are called 80S ribosomes. The "S" is short for **Svedberg unit** and it is a measure of how fast a particle settles when being centrifuged. The sedimentation rate of a particle depends on a combination of characteristics, such as size, shape, and density. The 70S ribosome indicates that this particle sediments slower that the eukaryotic 80S ribosome, which is due to its smaller size. Prokaryotic ribosomes not only differ from eukaryotic ones in size, but also in their overall structure and composition. These differences make prokaryotic ribosomes sensitive to a wide range of antibiotics and allow for the treatment of bacterial infections without damaging eukaryotic cells.

Most bacteria are surrounded by a **cell wall** composed of a complex polymer called **peptidoglycan** (Figure 7.1.4). Peptidoglycan is composed of repeating disaccharide units. The two sugars of this unit are called **N-acetyl glucosamine (NAG)** and **N-acetyl muramic acid (NAM)**. The NAM monomer within the disaccharide is modified by the addition of 5 to 6 amino acids (i.e., "peptido"). The NAG/NAM disaccharides link together to form long chains of sugars (i.e. "glycan") that crosslink to one another through the peptides. The peptidoglycan-rich cell wall forms a rigid layer that protects and maintains the shape of the bacterium. Numerous antibiotics are effective against bacteria through their ability to affect the assembly or integrity of the peptidoglycan cell wall.

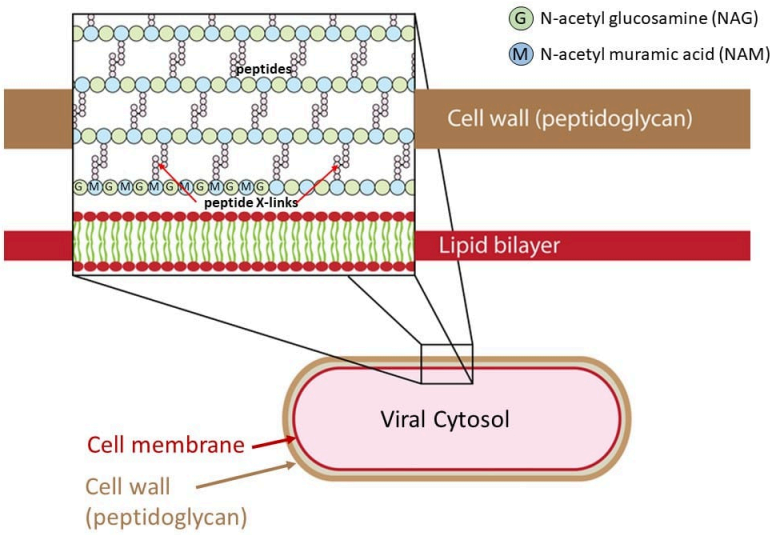


Figure 7.1.4: Peptidoglycan structure. The cell wall of a bacterium is made of peptidoglycan, an alternating series of sugars called N-acetyl glucosamine or NAG (G, green circles) and N-acetyl muramic acid or NAM (M, blue circles). The NAM sugar is modified through the attachment of several peptides (small circles). Cross-linking between the peptides (peptide X-links, shown with red arrows) produces a rigid mesh-like structure that protects the bacteria. (Peptidoglycan Structure by Patricia Zuk, CC BY 4.0; adapted from Peptidoglycan membrane by Bradleyhintze, CC0 1.0)

The bacterial cell wall can be used to classify bacteria as either **gram-positive** or **gram-negative**, a classification technique that is named after the Gram staining method. Gram-positive cell walls consist of a thick peptidoglycan layer that is readily stained through Gram staining. Gram-positive bacteria will be dark purple in color (Figure 7.1.5). In contrast, the gram-negative cell wall consists of a thin peptidoglycan layer sandwiched between the plasma membrane of the bacterial cell and an **outer membrane**. This outer membrane prevents the staining of the cell wall and gram-negative bacteria are pink in color (the color of the Safranin counterstain) (Figure 7.1.5). The outer membrane is a phospholipid bilayer studded with fat and sugar components, called **lipopolysaccharides (LPS) (**Figure 7.1.5). The lipopolysaccharides can be released from the cell wall and contribute to the pathogenicity of the bacteria.

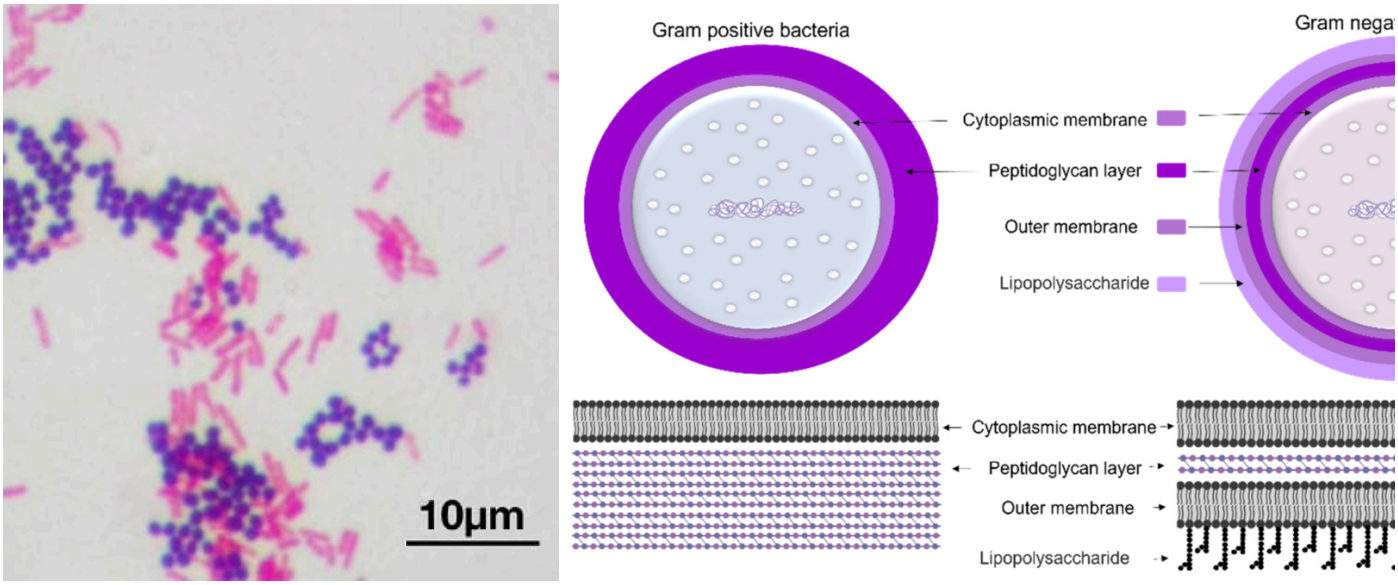


Figure 7.1.5: Gram-positive and gram-negative bacteria. Left image: A micrograph of gram-positive cocci (purple circles) and gram-negative bacilli (pink rods). (Gram stain by Y tambe, CC BY-SA 3.0)
Right image: The cell wall composition of gram-positive bacteria (left) is a thick peptidoglycan layer over the plasma membrane (i.e. cytoplasmic membrane). Gram-negative bacteria (right) have a thinner peptidoglycan layer found in between the cytoplasmic membrane and an outer membrane. The outer membrane is a phospholipid bilayer studded with lipopolysaccharides. (Cell wall composition by Malihe Mehdizadeh Allaf and Hassan Peerhossaini, CC BY-SA 4.0)

Bacteria divide rapidly, mainly through binary fission. In binary fission, the bacteria will duplicate its genetic material and partition it into two daughter cells. Many bacteria divide every 20 minutes. Hence, with the population doubling every 20 minutes, millions of identical cells can be produced in a short period of time. Compare this with the 24 hours doubling time of eukaryotic cells, and it is obvious why bacteria are a favorite organisms in biotechnology.

Archaea

Archaea are a group of single-celled microorganisms that make up the domain, Archaea. They are similar in appearance to bacteria but are genetically and biochemically distinct, with unique evolutionary histories. Archaea thrive in a wide range of environments, including some of the most extreme on Earth, such as hot springs, deep-sea hydrothermal vents, and highly acidic or saline habitats. This ability to survive in extreme conditions is due to their specialized cell membranes and enzymes, which allow them to maintain stability and function. Unlike bacteria, archaea have distinct metabolic pathways and can utilize a variety of energy sources, including methane, sulfur, and hydrogen, making them key players in global nutrient cycles.

Despite their microscopic size, archaea are critical to many ecological processes and have potential applications in biotechnology due to their resilience and unique biochemistry. Their enzymes, known as **extremozymes**, are particularly valuable in industrial processes that require extreme conditions, such as high temperatures, high salinity, or extreme pH levels. For instance, thermostable DNA polymerases from archaea are widely used in PCR (polymerase chain reaction) which requires using cycles with very high temperatures. Archaea are also used in bioremediation, where they help break down pollutants in harsh environments, and in biofuel production, where their metabolic pathways can be harnessed to produce methane or hydrogen. The resilience and adaptability of archaea make them promising candidates for innovations in biotechnology, including drug development, waste management, and sustainable energy solutions.

Yeast

Yeast are single-celled eukaryotic organisms belonging to Domain Eukarya, Kingdom Fungi. Yeast thrive in a variety of environments, including those that are moist and warm. They grow rapidly in aqueous environments and have been extensively studied for their metabolic processes. Yeast is widely used in biotechnology due to its versatility and ease of manipulation. Dating back several millennia., yeast has been employed in the production of bread, beer, and wine through **fermentation** - a biological process that use microbes, like yeast, to convert sugars (e.g. glucose) into energy (i.e. ATP). Nowadays, yeast is also used in many different applications. They are used to produce renewable fuels, like bioethanol, through fermentation. Yeast are used to produce enzymes for industrial applications. The ability of yeast to efficiently express foreign genes and its well-characterized genetics make it a powerful tool in the development of pharmaceuticals, like insulin and vaccines.

Virus

Viruses are microscopic infectious agents that can infect all life forms; bacteria, archaea, animals, plants, and fungi. Even though a virus has both genetic material and protein components, *it is not a living organism*. It is unable to self-replicate and relies on the cellular biochemistry of the host cell it has infected. This makes a virus an **obligate parasite,** or an organism that is required to infect a living cell.

The key features of viruses are (Figure 7.1.6):

1. **Genetic material:** The genetic information of a virus is either DNA or RNA, in either single stranded or double stranded forms. The nucleic acid content of a virus determines how they replication and can be used to classify them. The viruses that carry DNA are called **DNA viruses** (e.g. Herpesvirus) whereas those carrying RNA are called **RNA viruses** (Coronavirus, HIV). The size of viral genome depends on the type of virus. Some viruses contain enough genetic material to make only a few proteins; others code up to 100 proteins. The smaller the genome, the more viral copies can be made by a host cell. In order to replicate, a virus needs to infect a cell and so its genome can be copied. Once in the host cell, it will "hijack" the host's cellular machinery. What aspects of the host cell is used depends on whether the genome is single-stranded or doubled-stranded DNA or RNA. For example, a DNA virus will use the host's DNA polymerase to replicate its genome, followed by host cell transcription and translation. In contrast, RNA viruses have no need of DNA polymerases but will use host cell ribosomes for protein translation.
2. **Capsid:** The capsid is a protein shell that surround the viral genome. The capsid protects the genome from the environment, plays a role in binding the host cell, permits its entry into the host cell, and assists in viral assembly following replication. The protein subunits of the capsid are known as **capsomeres**. The way these capsomeres assemble determines the shape

of the virus. For example, many viruses are **icosahedral**, a shape made of 20 identical triangular faces. Others viruses have a helical capsid, such as the Ebola virus.

3. **Envelope**: The envelope is not found in all viruses. When it is present, the envelope is composed of a phospholipid bilayer that is derived from the host cell. The envelope forms around the virus, when it exists the host cell. As a result of this process, some of the proteins of the host cell membrane will be found embedded within the envelope. These proteins aid in recognition by host cells and facilitate their entry. Viruses with an envelope are called "enveloped" viruses; those lacking an envelope are often called "naked" viruses.
4. **Surface proteins:** Surface proteins can be found embedded in either the capsid or the envelope (if present). Those embedded in the envelope are **glycoproteins** - proteins that have been modified through the addition of short sugar monomers. Most of these proteins are spike-like in shape and are called **spike proteins**. Spike proteins help in viral attachment and entry by binding to receptors at the surface of a cell. They also play a role in evading the host's immune system. Surface proteins found embedded in the capsid are known as **capsid proteins**. Capsid proteins serve as attachment proteins that interact directly with receptors on the host cell's surface. Viral surface proteins are critical for the specificity of the virus, allowing certain viruses to bind and enter only a particular type of cell found in a certain species. While most viruses are highly specific, some viruses may cross the barrier species in a process called "spillover". These viruses are called **zoonotic viruses**. Zoonotic viruses originate in other animals (e.g. bats, birds, pigs) but adapt to infect humans, sometimes leading to endemics or pandemics. The SARS outbreak of 2002-2003 is an example of a zoonotic virus that originated in bats and cats and made the jump to humans.

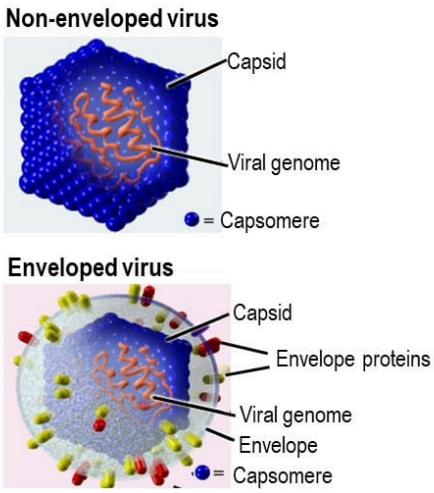


Figure 7.1.6: Enveloped and non-enveloped viruses. Top image: the non-enveloped virus is surrounded by a capsid made of individual capsomeres (blue spheres) that assemble to give the capsid its shape. The capsid surrounds the viral genome (shown in orange). Surface proteins can be found embedded in the capsid (not shown). Bottom image: the enveloped virus has a viral genome and a capsid like the non-enveloped virus. However, the capsid is surrounded by an phospholipid bilayer called the envelope. Envelope proteins (shown in red and yellow) can be found embedded in the envelope. (Enveloped and Non-enveloped Viruses by Patricia Zuk, CC BY 4.0)

Viruses have made major contributions to biotechnology. Viruses are critical to the development of vaccines that train the immune system to recognize and fight infections. They can be used as cloning vectors to introduce foreign genes into host organisms, like bacteria. They have been used in gene therapy to introduce functional genes to specific cell types in order to treat diseases, such as sickle-cell anemia. Viruses called oncolytic viruses have been engineered to infect and destroy cancer cells, while leaving normal cells unaffected. Modified plant viruses can be used as vectors to introduce foreign genes into plant cells in order to create genetically-engineered plants with beneficial traits, such as increased resistance to pests, diseases, or environmental stresses. The future of viral biotechnology is likely to include personalized medicine with custom-designed viruses or vaccines, synthetic viruses to target specific diseases, and improved gene editing tools using viral systems.

Key Concepts

Microbial biotechnology is the use of microbes for industrial, environmental, and pharmaceutical applications. Some important concepts to remember are:

- all living things are made of cells
- there are two types of cells: prokaryotic and eukaryotic
- prokaryotic and eukaryotic cells have DNA in the form of chromosomes, a cytoplasm, ribosomes, and a plasma membrane
- additional features like the presence of a nucleus distinguish eukaryotic cells from prokaryotic cells
- the microbes used in microbial biotechnology include prokaryotic cells (e.g. bacteria and archaea), eukaryotic cells (e.g. yeast), and viruses
- bacteria and archaea are single-celled prokaryotic cells found in Domain Bacteria and Domain Archaea, respectively
- bacteria can be classified using several approaches, including cell shape, cell arrangement and gram staining
- the cell wall of bacteria is made of peptidoglycan, long chains of NAG and NAM sugars that crosslink for rigidity and strength
- gram-positive bacteria have a thick layer of peptidoglycan in their cell wall; the peptidoglycan is stained by the gram stain
- gram-negative bacteria have a thin peptidoglycan layer found in between the cell membrane and an outer membrane; the outer membrane prevents staining by the gram stain
- archaea are found in extreme environments
- yeast are single-celled eukaryotic cells found in Domain Eukarya
- yeast have several uses in biotechnology, including fermentation and genetic engineering
- viruses are non-living, infectious agents that require host cells for their replication
- viruses are made of a nucleic acid genome (DNA or RNA), a capsid, and surface proteins; some viruses may also have a envelope

Glossary

Archaea – a domain of single-celled microorganisms that are genetically distinct from bacteria and eukaryotes, often found in extreme environments

Asexual reproduction – a mode of reproduction in microbes where offspring arise from a single parent, without genetic recombination

Bacillus – a rod-shaped bacterium

Bacteria – single-celled prokaryotic microbes that can be found in diverse environments and classified on parameters like cell shape and gram stain

Binary fission – a common asexual reproduction method in bacteria where a single "parent" cell splits into two identical "daughter" cells

Budding - an asexual reproduction strategy where a small "bud" detaches itself from the "parent" cell, followed by maturation

Capsid – a structure that surrounds and protect the viral genome

Coccus – a spherical-shaped bacterium

Domain - the largest group used to classify organisms; all life can be classified into three domains (Bacteria, Archaea, Eukarya)

Diplo - a pair of bacterial cells joined together; e.g. "diplococcus" - a pair of spherical bacteria joined together

Eukaryote – an organism whose cells contain a nucleus and membrane-bound organelles, including fungi, plants and animals

Extremophile – a microorganism that thrives in extreme conditions, such as high temperatures, acidity, or salinity

Fungi – a kingdom of eukaryotic microorganisms, including yeasts and molds, that obtain nutrients through decomposition

Genophore - the bacterial chromosome

Gram staining – a differential staining technique used to classify bacteria as Gram-positive (purple) or Gram-negative (pink) based on cell wall composition

Host – an organism that provides a habitat or nutrients for a microbe, including pathogens and symbionts

Lipopolysaccharide (LPS) - a component of the outer membrane of gram-negative bacteria; plays a role in immune response and toxicity

Outer membrane - a structure made of phospholipids and lipopolysacchardies found on the outside of the cell wall of Gram-negative bacteria

Peptidoglycan – a polymer found in bacterial cell walls, providing structural strength; used to distinguish gram-positive from gram-negative bacteria

Plasmid – a small, circular DNA molecule found in bacteria that can carry antibiotic resistance genes and other traits

Spirillum – a spiral-shaped bacterium

Staphylo - a cluster of bacterial cells joined together; "staphylococcus" - a chain of spherical bacteria joined together

Strepto - a chain of bacterial cells joined together; "streptobacilli" - a chain of rod-shaped bacteria joined together

Vaccine – a biological preparation that stimulates an immune response against a microbe

Virus – a non-living infectious agent composed of nucleic acids contained in a protein coat; requires a host cell to replicate

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7.2: Industrial Applications of Microbial Biotechnology

Biotech Focus

Modern Meadows Lab looks more like a brewery than a biotech company. But rather than making beer, this biotech company is using microbes to make a material that looks, feels, and performs like leather. Through a process called **biofabrication**, the company uses a specially designed DNA sequence inserted into yeast cells to produce collagen that becomes incorporated into their biofabricated leather. These yeast cells are grown in large fermenters that are no different then those found in beer and wine distilleries and the collagen extracted and processed. Because the product is biofabricated, the properties of the "leather" can be modified to be less flexible or more breathable. At the end of the process, Modern Meadow's "leather" can be used just like leather but without the environmental impact.

To read about Modern Meadow's biofabricated leather, check out The Future of Leather is Growing in a New Jersey Lab.

Introduction

The ability to cultivate and manipulate microbes, like bacteria and yeast, has wide-ranging implications across various industries, including pharmaceuticals, food, agriculture, and biofuels. This chapter explores the diverse applications of microbial biotechnology and highlights some of its key uses.

Learning Objectives

Microbial biotechnology can be applied to industry in diverse ways. At the end of this section, you will be able to:

- Define biofuel and list the common types
- Describe the four generations of biofuels being made today
- Explain the impact of microbial biotechnology on the food industry and the environment

Production of Biofuels by Microbes

As the global population increases, so does the importance of identifying renewable energy sources. Microbes can potentially provide a solution by producing biofuels. A **biofuel** is a type of fuel derived from *biological materials*. The two most common types are **bioethanol** and **biodiesel.** Bioethanol is ethanol produced through the fermentation of carbohydrates and it can be blended with gasoline to reduce carbon emissions and enhance fuel efficiency. For example, the bioethanol, E10, is 10% ethanol, 90% gasoline. Biodiesel is an alternative to petroleum-based diesel and is produced through a chemical process called **transesterification**, where fats or oils react with an alcohol (usually methanol or ethanol) in the presence of a catalyst (such as sodium hydroxide or potassium hydroxide). Both of these biofuels are considered to be renewable energy because they can be replenished. Many microbial species, such as microalgae (i.e. microscopic algae cells), yeast, and bacteria can convert organic matter (i.e. **biomass**) into bioethanol and biodiesel through natural metabolic pathways, resulting in environmentally friendly, sustainable, and cleaner alternatives to fossil fuels. The biomass used by these organisms include plant (e.g. wood, crops, and agricultural residues), animal (manure, fats, and livestock waste), microbial (e.g. bacteria, fungi, or their bioproducts), and wastes (e.g. food scraps, municipal solid waste, and industrial byproducts).

Biofuels can be classified into generations:

1. **First generation (food-based)** - made from food crops
    - source: edible crops (corn, sugarcane, soybean, and vegetable oils)
    - produce: bioethanol (from corn and sugarcane fermentation) and biodiesel (from vegetable oils and other fats)
    - example: the yeast *Saccharomyces cerevisiae* production of bioethanol
    - limitations: competes with the food industry
2. **Second generation (non-food biomass**) - made from non-food biomass

- source: lignocellulosic biomass (i.e. wood, agricultural residues, grasses)
- produce: bioethanol and synthetic biofuels
- example: the yeast *Saccharomyces cerevisiae* production of bioethanol
- advantages: no competition with food industry
- limitations: requires complex processing to break down cellulose and lignin

3. **Third generation (algae and microbial-based)** - made using microbes like algae and bacteria
   - source: algae and genetically-engineering microbes
   - produce: lipids, hydrocarbons, bioethanol, algal biodiesel
   - example: transesterification of lipids- and fermentation by microalgae
   - advantages: fast growth rate, ability to grow on water sources unsuitable for irrigation, and efficient use of carbon dioxide
   - limitations: high production costs and difficulty scaling up
4. **Fourth generation (advanced)** - made using synthetic biology and carbon capture
   - source: genetically-modified organisms in combination with carbon capture technologies
   - produce: carbon-neutral or carbon-negative biofuels; electrofuels (produced from CO2), solar biofuels (convert sunlight into fuel)
   - advantages: highly efficient, potentially carbon-negative
   - limitations: still in the development phase

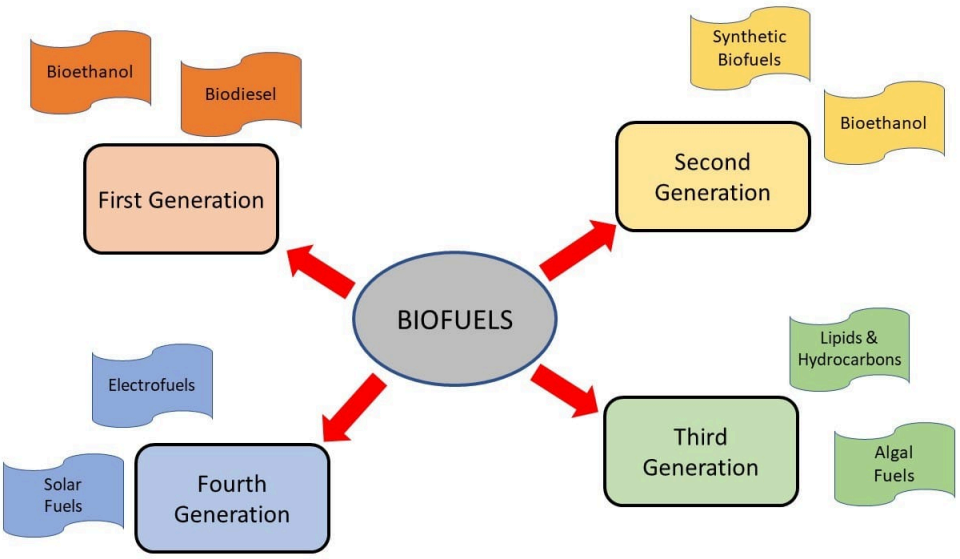


Figure 7.2.1: Biofuel generations. The development of biofuels has undergone several generations. First generation biofuels include bioethanol and biodiesel. Second generation biofuels also include bioethanol, but more advanced biofuels like synthetic biofuels. Third generation biofuels are biological in nature and include algal fuels and lipids and hydrocarbons. The fourth generation are the more advanced and include electrofuels and solar fuels. (Biofuel Generations by Patricia Zuk, CC BY 4.0)

Biotechnology can improve biofuel production by optimizing crop yields, engineering algae for higher lipid content, and developing enzymes that accelerate biomass breakdown. The use of microbes in biofuel production not only reduces dependence on non-renewable energy sources but also helps minimize carbon emissions, making it a key player in the transition toward cleaner energy solutions. However, while promising, many of these types of biofuels still need to be improved to meet the needs of the consumer.

Food Industry

The brewing and food industries rely heavily on fermentation to produce alcohol, bread, yogurt, cheese and various fermented foods. Bacteria like Lactobacillus and Streptococcus are used in the production of yogurt, cheese, pickles, sauerkraut, and kimchi. Yeast (e.g., Saccharomyces cerevisiae) is essential to bread making, beer, and wine production. Finally, molds like Penicillium and Aspergillus and used in blue cheese, soy sauce, and miso. Certain microbes, like Lactobacillus and Acetobacter produce acids, alcohols, and bacteriocins that inhibit spoilage and pathogenic bacteria, helping preserve food. As a result, the food industry has been greatly impacted by advancements in microbial biotechnology. Microbial engineering is being used in industrial molecule **bioproduction** - the use of biological systems, like those found in microbes, in large-scale production of value-added compounds as an alternative to isolating these compounds from natural sources. For example, genetically-modified bacteria (e.g. *Escherichia*

coli, *Corynebacterium glutamicum*) and microalgae species are now being used to produce omega-3s at a commercial scale. Industries are using microbes' biochemical capabilities to sustainably bioconvert industrial by-products into aromas, colorants, flavoring agents, and food additives to meet consumer demands for "natural" products. Addressing food poisoning, particularly from *Staphylococcus aureus*, is also a major focus in the food industry. Recent research explores the molecular characteristics, genotypes, resistance profiles, and enterotoxigenic status of *Staphylococcus aureus* in order understand its prevalence and transmission in food-borne infections.

Environmental Applications

Microbial biotechnology holds immense potential for fostering sustainable environmental and agricultural practices. Microbes are utilized in pollution control and toxic waste treatment, with bioengineered microbes facilitating the biodegradation of waste and oil spills. Genetically-modified microbes can degrade toxic compounds and convert hazardous substances into non-toxic forms. The discovery of new enzymes and molecules can further enhance these processes. In wastewater treatment, biotechnology improves efficiency by increasing plant capacity, replacing synthetic chemical additives, and removing harmful materials. Microalgae, for instance, absorb excess nutrients and organic pollutants from wastewater, improving water quality. The resulting biomass can be re-purposed for biofuel production, biofertilizers, and animal feed. In mining, advances in biotechnology can enhance the extraction of metals from ores by microbes like *Thiobacillus ferrooxidans* by increasing their tolerance to acidic and saline conditions and improving resistance to metals. In sustainable agriculture, microbial biotechnology offers innovative solutions such as biofertilizers that supplement chemical fertilizers, enhance crop yields, and improve soil fertility. Biopesticides present an environmentally friendly alternative to chemical pesticides, posing minimal risks to human health and ecosystems. Additionally, bioherbicides target the defense mechanisms of weeds, reducing farming costs while minimizing environmental impact. Fungal and virus-based insecticides further contribute to sustainable pest management. Despite ongoing challenges in research, development, regulation, and public acceptance, the benefits of microbial biotechnology warrant continued investment and exploration. Future research should focus on understanding microbial communities, optimizing biotechnological applications, and overcoming practical and regulatory hurdles to facilitate widespread implementation.

Key Concepts

Applications of microbial biotechnology has wide ranging impacts on many industries, including agriculture, wastewater treatment, the food industry, and the energy sector.

Some important concepts to remember are:

- Microbes are used in a wide variety of industries, including the generation of biofuels, the creation of food products, the treatment of wastewater, and other industrial applications
- A biofuel is a type of fuel derived from biological materials
- Biofuels, like bioethanol and biodiesel, are renewable because they can be replenished
- There are four generations of biofuels being made today, each with unique sources, advantages, and limitations
- Many types of biofuels still need to be improved to meet the needs of the consumer

Glossary

Biodiesel - a renewable and biodegradable fuel made from the transesterification of vegetable oils, animal fats, and microbial lipids

Bioethanol - ethanol produced through the fermentation of carbohydrates

Biofertilizer - a substance containing living microorganisms that, when applied to soil or plants, enhances nutrient availability—especially nitrogen and phosphorus—promoting healthy plant growth

Bioherbicide - a type of herbicide derived from bacteria, fungi, or viruses or their byproducts; help control unwanted weeds in agriculture

Biomass - organic matter derived from living or recently living organisms such as plants, agricultural waste, and microorganisms; used to produce renewable energy

Biopesticide - a type of pesticide derived from natural materials such as animals, plants, bacteria, or certain minerals, used to control pests in an environmentally friendly way

Bioproduct - any commercial or industrial product derived from a biological source

Bioproduction - the use of biological systems in the large-scale production of compounds

Fermentation - a biological process where microbes (like bacteria and yeast) convert sugars into other products like alcohol, gases, or acids

Lignocellulosic - biomass composed of lignin, cellulose, and hemicellulose; one of the most abundant natural resources; used in biofuel production

Microalgae - microscopic, photosynthetic organisms found in freshwater and marine environments

Transesterification - a chemical process in which fats or oils react with an alcohol in the presence of a catalyst to produce biodiesel

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7.3: Medical Applications of Microbial Biotechnology

Biotech Focus

In 1980, the World Health Organization (WHO) declared the disease **smallpox** officially eradicated. Smallpox is a highly contagious, deadly disease caused by the **variola virus**. Infected individuals become covered with pus-filled lesions, with the majority found around the head (Figure 7.3.1). Smallpox has no known cure, killing 1 in 3 infected people. Those surviving are left with deep scars across their body, with some people blinded by the disease. The first evidence of smallpox were found in Egyptian mummies dating back from 1350 BCE, whose skin was covered with lesions that resembled those of the smallpox rash. To combat this disease, early physicians in Asia and Africa used the practice of **variolation**, which consisted of transferring pus collected from smallpox sores to healthy individuals to induce a milder illness. Although variolation was mostly successful, it presented many risks, such as introducing an opportunistic infection. In the 18th century, **Edward Jenner** created the first smallpox vaccine using the cowpox virus, a closely relative to smallpox. Jenner collected pus from a cowpox blister from the hand of an infected milkmaid and use it to inoculate a cut in a boy's arm. When exposed to smallpox, the boy did not develop the disease. Subsequent advances in research and production has resulted in a safer and more stable vaccine. The current smallpox vaccine is made from a "live" virus related to smallpox. It does not contain the virus and cannot cause smallpox but provides immunity.

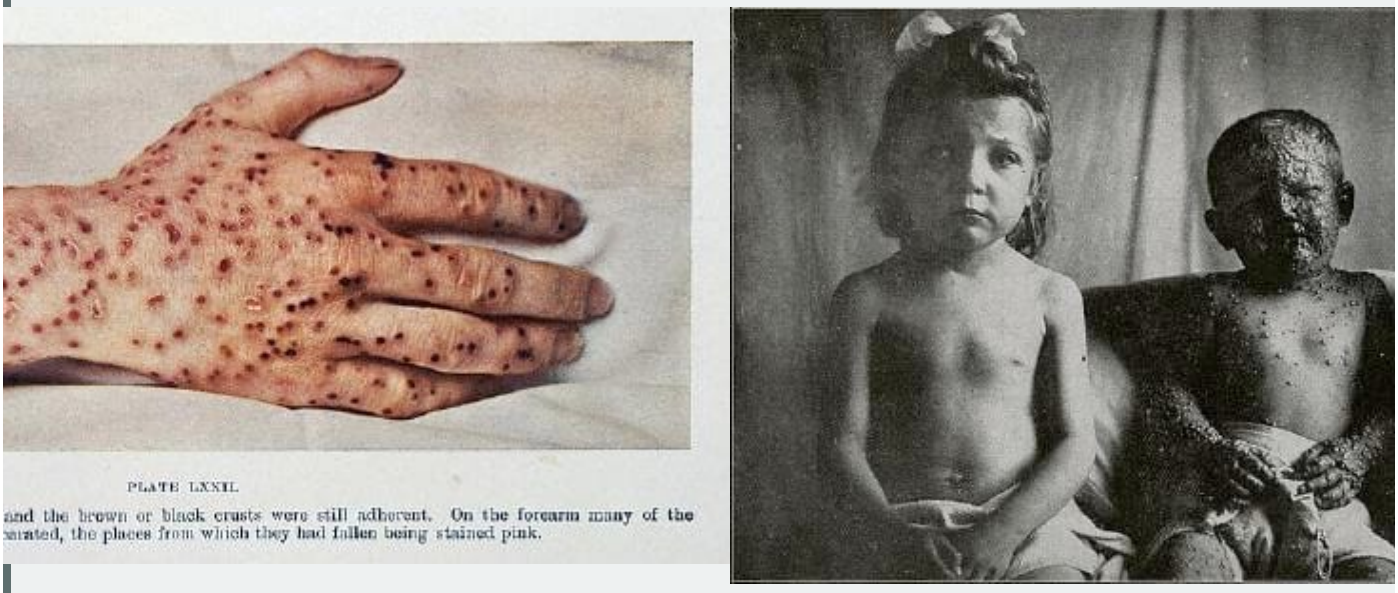


Figure 7.3.1: Left image: Smallpox lesions. (Obsolescent variolous lesions by Wellcome Collection Gallery, CC BY-SA 4.0) Right image: An unvaccinated boy surrounded by his two siblings who were vaccinated for smallpox. Note the absence of lesions in the unvaccinated siblings. (Smallpox in siblings by National Museum of Health and Medicine, CC BY 2.0).

Introduction

For centuries, natural products have been the cornerstones of traditional medicine. Today, microbial biotechnology is revolutionizing medicine by changing how we approach drug discovery and treatment, and offering new solutions to combat diseases, like infections and cancer. Microbes, like bacteria and fungi, are proving to be invaluable in the pharmaceutical industry. Bacteria and fungi are being used as a rich source of **bioactive compounds** with therapeutic potential. Bioactive compounds are naturally occurring chemicals found in plants, animals, microbes, or other biological sources, that have an effect on a living organism. These compounds include antibiotics, anti-fungals, and anti-cancer agents. Genetic engineering of these microbes, together with **synthetic biology** are being used to enhance microbial production of these compounds, discover new drug leads, and develop innovative therapies such as vaccines and targeted drug delivery systems.

Learning Objectives

Microbial biotechnology is a key part of the pharmaceutical industry, revolutionizing the development of drugs and the treatment of diseases. At the end of this page, you will be able to:

- Define key microbial biotechnology terms in relations to medicine
- Describe the three basic types of antibiotics
- Describe how microbial biotechnology is used in the production of antibiotics
- Describe the major types of vaccines that use microbial technology in their production

Microbes as drug factories

Antibiotic Production

An **antibiotic** is a type of antimicrobial agent specifically designed to fight bacterial infections by either killing the bacteria (i.e., bactericidal antibiotics) or by inhibiting their growth (i.e., bacteriostatic antibiotics). Antibiotics are considered to be **secondary metabolites** - compounds not essential for the growth of the organism. Many antibiotics, such as penicillin from *Penicillium* fungi or streptomycin from *Streptomyces* bacteria, are naturally synthesized by microbes to inhibit the growth of bacteria. These antibiotics are called **natural antibiotics.** In addition, there are two other types of antibiotics: semisynthetic, and synthetic (Figure 7.3.2). **Semi-synthetic antibiotics** are modified versions of natural antibiotics, with chemical alterations to enhance their effectiveness, stability, or resistance to bacterial degradation. One well-known example is amoxicillin, an antibiotic derived from penicillin G, and enzymatically modified to remove a side chain from the natural version. **Synthetic antibiotics**, on the other hand, are artificially designed and chemically synthesized compounds that are designed to target critical bacterial processes. Synthetic antibiotics are man-made and are often based on knowledge obtained from natural antibiotics. Synthetic antibiotics include novel compounds, such as the fluoroquinolones, which are engineered to inhibit bacterial DNA replication.

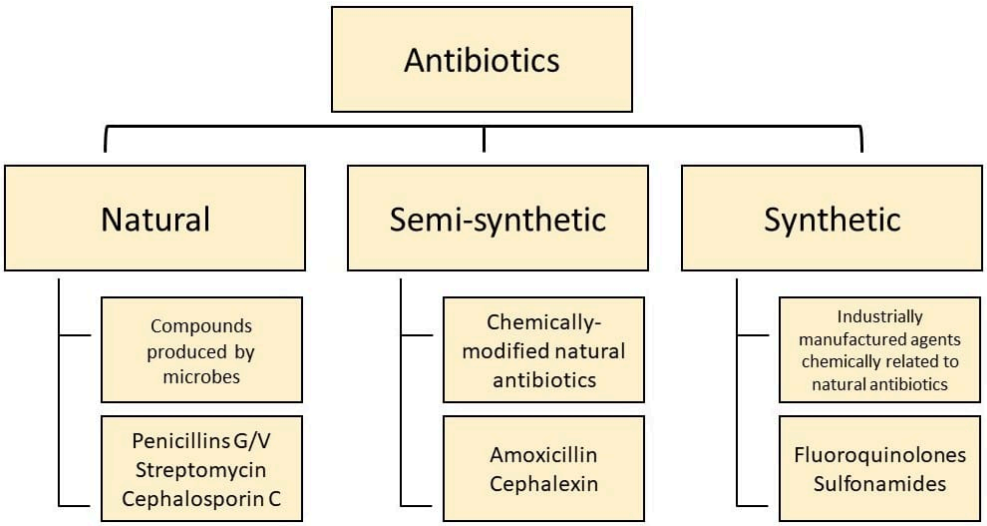


Figure 7.3.2: Types of antibiotics. Antibiotics are classified as natural, semi-synthetic, and synthetic. Natural antibiotics are compounds produced by microbes and include agents such as penicillins G/V, streptomycin, and cephalosporin C. Semi-synthetic antibiotics are chemically-modified natural antibiotics and include compounds like amoxicillin and cephalexin. Synthetic antibiotics are industrially manufactured agents chemically related to natural antibiotics. Synthetic antibiotics include the fluoroquinolone and sulfonamide families of agents. (Types of Antibiotics by Patricia Zuk, CC BY 4.0)

The production of natural and semi-synthetic antibiotics at an industrial scale, together with many synthetic versions, involves a **fermentation step** at some point in their production (Figure 7.3.3). In industrial fermentation, the microbe is grown in large bioreactors (100,000 – 150,000 liters or more), called **fermenters**, containing a liquid growth medium. The size of the microbe population must be controlled very carefully to ensure that maximum yield of the antibiotic is obtained before the cells die. Therefore, oxygen concentration, temperature, pH, and nutrient levels are optimized, closely monitored, and adjusted if necessary.

For more information about fermenters, go to Chapter 7.3 Microbial Cultivation and Manipulation. Once the fermentation process is complete, the antibiotic is extracted and purified. For semisynthetic and synthetic antibiotics, continued chemical processing is required.

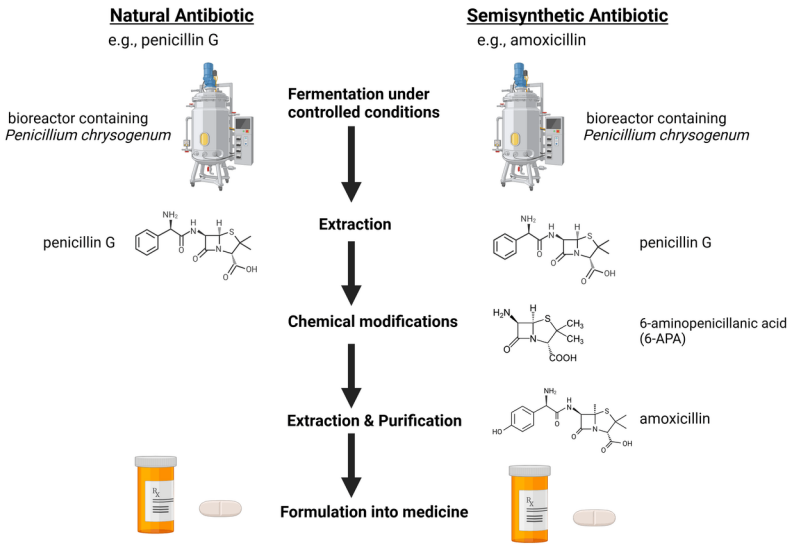


Figure 7.3.2: Natural and synthetic antibiotic production. The steps involved in the production of the natural antibiotic, penicillin G, and the semisynthetic version, amoxicillin are shown in this figure, together with the chemical structures of the critical compounds. Both antibiotics begin with large-scale fermentation of the fungus, *Penicillium chrysogenum* under controlled conditions in a bioreactor. Penicillin G is extracted from the bioreactor. In amoxicillin production, penicillin is enzymatically converted to 6-APA, which is then chemically modified into amoxicillin. At the end of this process, both penicillin G and amoxicillin are formulated into medicines. (Antibiotic production by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2025))

Despite their variety, approximately 99% of antimicrobial agents, such as antibiotics, have little medical or commercial value. Many of these "low-value" antibiotics simply lack a significant advantage over those already in use or have no practical application. This, together with the rise of antibiotic resistance, requires continued exploration and optimization of antibiotic development and production. To promote this, pharmaceutical companies use microbial biotechnology to enhance antibiotic production, develop new antibiotics, or modify existing ones to make them more efficacious. The introduction of mutations (i.e. **mutagenesis**) into the microbial genome to boost antibiotic production is a common microbial biotechnology method. Mutagenesis is produced by exposing microbial cells to **mutagens** such as ultraviolet radiation, x-rays, or certain chemicals. Selection of microbial strains for high-yield antibiotic production is followed by propagation in bioreactors and purification (see Figure 7.3.2). Mutated strains with antibiotic yields as high as 20-fold or more have been produced using this technique.

Biotechnologists can also boost antibiotic production through **gene amplification,** where copies of genes coding for enzymes involved in antibiotic production can be inserted back into the microbial cell, via vectors such as plasmids. Genetic engineering can create new antibiotics by modifying microbial pathways. Altering microbial DNA can be used to produce entirely new synthetic antibiotics. Genes from different bacteria can be combined to generate novel antibiotic structures. Gene editing through techniques like CRISPR are also being explored.

Vaccine Production

A **vaccine** is a biological preparation that stimulates the host's immune system to recognize and fight a specific pathogen (i.e., a virus, bacterium, or other microbe) without causing the actual disease. Vaccines help the body develop immunity by preventing future infections or reducing their severity. There are a wide variety of vaccines technologies used today, with some technologies produced for a specific pathogen (Table 7.3.1). The two most common types of vaccines used today are the **live-attenuated vaccine**, which uses a weakened version of a microbe, and the **inactivated (killed) vaccine**, which uses a killed version. These two vaccines are relatively inexpensive to produce and generate a robust immune response.

Table 7.3.1: Vaccine Types

Vaccine Type	Antigen used	Pathogen	First introduced
Live attenuated (weakened of inactivated)	• uses a weakened (attenuated) form of a virus or bacterium • the pathogen is modified so that it cannot cause disease in healthy individuals but still stimulates a strong and long-lasting immune response.	• measles • mumps • rubella • yellow fever • influenza • varicella (chicken pox) • typhoid	• 1798 (smallpox)
Killed	• uses a killed virus or bacterium	• pertussis • polio • influenza • hepatitis A • rabies	• 1896 (typhoid)
Toxoid	• uses an inactivated toxin produced by bacteria • the toxin is chemically treated to render it harmless but is still capable of stimulating an immune response • the immune response doesn't target the bacteria but creates antibodies that neutralize the toxin during an infection	• tetanus • diptheria	• 1923 (diptheria)
Subunit	• made from a fragment, like sugars and proteins, isolated from a pathogen • the fragments can be produced in large quantities by genetically-engineered bacteria (e.g., *E.coli*) or yeast (e.g., *Saccharomyces cerevisiae, Pichia pastoris*)	• hepatitis A & B • influenza • pertussis • meningococcal • pneumococcal	• 1970 (anthrax)
Viral vector	• uses harmless viruses or bacteria (i.e., the vector) to deliver genetic material into cells • the bacteria or virus is genetically modified to deliver pathogenic genes into host cell • protein production by host cells generates a strong immune responses • viral vector vaccines currently in use; bacteria vector vaccines are experimental	• Ebola	• 2019 (Ebola)
Outer membrane vesicle (OMV)	• uses membrane fragments, called vesicles, that are released from the outer membrane of certain gram-negative bacteria • the vesicles contain various components from the bacterium's outer membrane which are crucial for the immune reaction	• Group B meningococcal	• 1987 (meningococcal group B)

Vaccine Type	Antigen used	Pathogen	First introduced
Protein-polysaccharide conjugate	• uses sugars from the outer capsule of a bacteria • the sugar is coupled (conjugated) to a viral protein to boost the immune response	• *Hemophilus influenzae* type B • pneumococcal • meningococcal • typhoid	• 1987 (*Hemophilus influenzae* type B)

In addition to these two types of vaccines, there are additional types of vaccines that use microbial biotechnology for their development. These more advanced vaccine technologies are capable of decreasing the cost of vaccine production and increasing production efficiency through their use of microbes to produce the antigens and adjuvants that are key to vaccine production, in addition to using microbes that act as vectors to stimulate the immune response.

Key Concepts

Microbial biotechnology is revolutionizing medicine by changing drug discovery and treatment, and offering new solutions to combat diseases, like infections and cancer.

Some important concepts to remember are:

- a bioactive compound is a naturally occurring chemical found in plants, animals, microbes, or other biological sources, that is used to affect an organism
- an antibiotic is a compound that is used to treat bacterial infections
- a natural antibiotic is naturally synthesized by microbes to inhibit the growth of bacteria
- a semisynthetic antibiotic is produced by chemically modifying a natural antibiotic
- a synthetic antibiotic is a man-made antibiotic
- antibiotic production involves a fermentation step
- mutagenesis and gene amplification in microbes are techniques used to boost antibiotic production
- vaccines stimulate the host's immune response to fight a specific pathogen
- microbial biotechnology is used in the production of several kinds of vaccines, such as subunit vaccines and vector-based vaccines

Glossary

Antibiotic - any substance that can destroy or inhibit the growth of bacteria and similar microbes

Antimicrobial agent - a substance that kills or inhibits the growth of microbes such as bacteria, fungi, viruses, and parasites

Bioactive molecule - a substance that interacts with living cells or tissues to influence their behavior, such as promoting growth, differentiation, or healing; examples include growth factors, cytokines, or peptides; also called a bioactive compound

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

Gene amplification - results from the insertion of multiple copies of a gene into the genome of an organism

Metabolite - any substance produced by, or taking part in, a metabolic reaction

Mutagen - any physical or chemical agent that causes changes or mutations in the DNA sequence; includes UV radiation and certain chemicals

Mutagenesis - the process by which mutations are induced or occur naturally within an organism's DNA

Plasmid - a small, circular piece of double-stranded DNA found outside of the bacterial chromosome; used in DNA cloning procedures; often used as a vector to introduce foreign DNA into cells

Secondary metabolite - a substance produced by an organisms that are not directly involved in growth, development and reproduction (these basic functions are carried out by primary metabolites)

Vaccine - a biological preparation that stimulates the immune system to protect against a specific disease

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7.4: Vaccines

Learning Objectives

- Compare the various kinds of artificial immunity
- Differentiate between variolation and vaccination
- Describe different types of vaccines and explain their respective advantages and disadvantages

For many diseases, prevention is the best form of treatment, and few strategies for disease prevention are as effective as vaccination. Vaccination is a form of artificial immunity. By artificially stimulating the adaptive immune defenses, a vaccine triggers memory cell production similar to that which would occur during a primary response. In so doing, the patient is able to mount a strong secondary response upon exposure to the pathogen—but without having to first suffer through an initial infection. In this section, we will explore several different kinds of artificial immunity along with various types of vaccines and the mechanisms by which they induce artificial immunity.

Classifications of Adaptive Immunity

All forms of adaptive immunity can be described as either active or passive. Active immunity refers to the activation of an individual's own adaptive immune defenses, whereas passive immunity refers to the transfer of adaptive immune defenses from another individual or animal. Active and passive immunity can be further subdivided based on whether the protection is acquired naturally or artificially.

Natural active immunity is adaptive immunity that develops after natural exposure to a pathogen (Figure 7.4.1). Examples would include the lifelong immunity that develops after recovery from a chickenpox or measles infection (although an acute infection is not always necessary to activate adaptive immunity). The length of time that an individual is protected can vary substantially depending upon the pathogen and antigens involved. For example, activation of adaptive immunity by protein spike structures during an intracellular viral infection can activate lifelong immunity, whereas activation by carbohydrate capsule antigens during an extracellular bacterial infection may activate shorter-term immunity.

Natural passive immunity involves the natural passage of antibodies from a mother to her child before and after birth. IgG is the only antibody class that can cross the placenta from mother's blood to the fetal blood supply. Placental transfer of IgG is an important passive immune defense for the infant, lasting up to six months after birth. Secretory IgA can also be transferred from mother to infant through breast milk.

Artificial passive immunity refers to the transfer of antibodies produced by a donor (human or animal) to another individual. This transfer of antibodies may be done as a prophylactic measure (i.e., to prevent disease after exposure to a pathogen) or as a strategy for treating an active infection. For example, artificial passive immunity is commonly used for post-exposure prophylaxis against rabies, hepatitis A, hepatitis B, and chickenpox (in high risk individuals). Active infections treated by artificial passive immunity include cytomegalovirus infections in immunocompromised patients and Ebola virus infections. In 1995, eight patients in the Democratic Republic of the Congo with active Ebola infections were treated with blood transfusions from patients who were recovering from Ebola. Only one of the eight patients died (a 12.5% mortality rate), which was much lower than the expected 80% mortality rate for Ebola in untreated patients.[1] Artificial passive immunity is also used for the treatment of diseases caused by bacterial toxins, including tetanus, botulism, and diphtheria.

Artificial active immunity is the foundation for vaccination. It involves the activation of adaptive immunity through the deliberate exposure of an individual to weakened or inactivated pathogens, or preparations consisting of key pathogen antigens.

Mechanisms of Acquisition of Immunity		
	Natural acquired	**Artificial acquired**
Passive	Immunity acquired from antibodies passed in breast milk or through placenta 	Immunity gained through antibodies harvested from another person or an animal 
Active	Immunity gained through illness and recovery 	Immunity acquired through a vaccine 

Figure 7.4.1: The four classifications of immunity. (credit top left photo: modification of work by USDA; credit top right photo: modification of work by "Michaelberry"/Wikimedia; credit bottom left photo: modification of work by Centers for Disease Control and Prevention; credit bottom right photo: modification of work by Friskila Silitonga, Indonesia, Centers for Disease Control and Prevention)

Exercise 7.4.1

1. What is the difference between active and passive immunity?
2. What kind of immunity is conferred by a vaccine?

Herd Immunity

The four kinds of immunity just described result from an individual's adaptive immune system. For any given disease, an individual may be considered immune or susceptible depending on his or her ability to mount an effective immune response upon exposure. Thus, any given population is likely to have some individuals who are immune and other individuals who are susceptible. If a population has very few susceptible individuals, even those susceptible individuals will be protected by a phenomenon called herd immunity. Herd immunity has nothing to do with an individual's ability to mount an effective immune response; rather, it occurs because there are too few susceptible individuals in a population for the disease to spread effectively.

Vaccination programs create herd immunity by greatly reducing the number of susceptible individuals in a population. Even if some individuals in the population are not vaccinated, as long as a certain percentage is immune (either naturally or artificially), the few susceptible individuals are unlikely to be exposed to the pathogen. However, because new individuals are constantly entering populations (for example, through birth or relocation), vaccination programs are necessary to maintain herd immunity.

Vaccination: Obligation or Choice

A growing number of parents are choosing not to vaccinate their children. They are dubbed "antivaxxers," and the majority of them believe that vaccines are a cause of autism (or other disease conditions), a link that has now been thoroughly disproven. Others object to vaccines on religious or moral grounds (e.g., the argument that Gardasil vaccination against HPV may promote sexual promiscuity), on personal ethical grounds (e.g., a conscientious objection to any medical intervention), or on political grounds (e.g., the notion that mandatory vaccinations are a violation of individual liberties).[2]

It is believed that this growing number of unvaccinated individuals has led to new outbreaks of whooping cough and measles. We would expect that herd immunity would protect those unvaccinated in our population, but herd immunity can only be maintained if enough individuals are being vaccinated.

Vaccination is clearly beneficial for public health. But from the individual parent's perspective the view can be murkier. Vaccines, like all medical interventions, have associated risks, and while the risks of vaccination may be extremely low compared to the risks of infection, parents may not always understand or accept the consensus of the medical community. Do such parents have a right to withhold vaccination from their children? Should they be allowed to put their children—and society at large—at risk?

Many governments insist on childhood vaccinations as a condition for entering public school, but it has become easy in most states to opt out of the requirement or to keep children out of the public system. Since the 1970s, West Virginia and Mississippi have had in place a stringent requirement for childhood vaccination, without exceptions, and neither state has had a case of measles since the early 1990s. California lawmakers recently passed a similar law in response to a measles outbreak in 2015, making it much more difficult for parents to opt out of vaccines if their children are attending public schools. Given this track record and renewed legislative efforts, should other states adopt similarly strict requirements?

What role should health-care providers play in promoting or enforcing universal vaccination? Studies have shown that many parents' minds can be changed in response to information delivered by health-care workers, but is it the place of health-care workers to try to persuade parents to have their children vaccinated? Some health-care providers are understandably reluctant to treat unvaccinated patients. Do they have the right to refuse service to patients who decline vaccines? Do insurance companies have the right to deny coverage to antivaxxers? These are all ethical questions that policymakers may be forced to address as more parents skirt vaccination norms.

Variolation and Vaccination

Thousands of years ago, it was first recognized that individuals who survived a smallpox infection were immune to subsequent infections. The practice of inoculating individuals to actively protect them from smallpox appears to have originated in the 10th century in China, when the practice of variolation was described (Figure 7.4.2). Variolation refers to the deliberate inoculation of individuals with infectious material from scabs or pustules of smallpox victims. Infectious materials were either injected into the skin or introduced through the nasal route. The infection that developed was usually milder than naturally acquired smallpox, and recovery from the milder infection provided protection against the more serious disease.

Although the majority of individuals treated by variolation developed only mild infections, the practice was not without risks. More serious and sometimes fatal infections did occur, and because smallpox was contagious, infections resulting from variolation could lead to epidemics. Even so, the practice of variolation for smallpox prevention spread to other regions, including India, Africa, and Europe.



Figure 7.4.2: Variolation for smallpox originated in the Far East and the practice later spread to Europe and Africa. This Japanese relief depicts a patient receiving a smallpox variolation from the physician Ogata Shunsaku (1748–1810).

Although variolation had been practiced for centuries, the English physician Edward Jenner (1749–1823) is generally credited

with developing the modern process of vaccination. Jenner observed that milkmaids who developed cowpox, a disease similar to smallpox but milder, were immune to the more serious smallpox. This led Jenner to hypothesize that exposure to a less virulent pathogen could provide immune protection against a more virulent pathogen, providing a safer alternative to variolation. In 1796, Jenner tested his hypothesis by obtaining infectious samples from a milkmaid's active cowpox lesion and injecting the materials into a young boy (Figure 7.4.3). The boy developed a mild infection that included a low-grade fever, discomfort in his axillae (armpit) and loss of appetite. When the boy was later infected with infectious samples from smallpox lesions, he did not contract smallpox.[3] This new approach was termed vaccination, a name deriving from the use of cowpox (Latin *vacca* meaning "cow") to protect against smallpox. Today, we know that Jenner's vaccine worked because the cowpox virus is genetically and antigenically related to the *Variola* viruses that caused smallpox. Exposure to cowpox antigens resulted in a primary response and the production of memory cells that identical or related epitopes of Variola virus upon a later exposure to smallpox.

The success of Jenner's smallpox vaccination led other scientists to develop vaccines for other diseases. Perhaps the most notable was Louis Pasteur, who developed vaccines for rabies, cholera, and anthrax. During the 20th and 21stcenturies, effective vaccines were developed to prevent a wide range of diseases caused by viruses (e.g., chickenpox and shingles, hepatitis, measles, mumps, polio, and yellow fever) and bacteria (e.g., diphtheria, pneumococcal pneumonia, tetanus, and whooping cough,).



Figure 7.4.3: (a) A painting of Edward Jenner depicts a cow and a milkmaid in the background. (b) Lesions on a patient infected with cowpox, a zoonotic disease caused by a virus closely related to the one that causes smallpox. (credit b: modification of work by the Centers for Disease Control and Prevention)

Exercise 7.4.2

1. What is the difference between variolation and vaccination for smallpox?
2. Explain why vaccination is less risky than variolation.

Classes of Vaccines

For a vaccine to provide protection against a disease, it must expose an individual to pathogen-specific antigens that will stimulate a protective adaptive immune response. By its very nature, this entails some risk. As with any pharmaceutical drug, vaccines have the potential to cause adverse effects. However, the ideal vaccine causes no severe adverse effects and poses no risk of contracting the disease that it is intended to prevent. Various types of vaccines have been developed with these goals in mind. These different classes of vaccines are described in the next section and summarized in Table 7.4.1.

Live Attenuated Vaccines

Live attenuated vaccines expose an individual to a weakened strain of a pathogen with the goal of establishing a subclinical

infection that will activate the adaptive immune defenses. Pathogens are attenuated to decrease their virulence using methods such as genetic manipulation (to eliminate key virulence factors) or long-term culturing in an unnatural host or environment (to promote mutations and decrease virulence).

By establishing an active infection, live attenuated vaccines stimulate a more comprehensive immune response than some other types of vaccines. Live attenuated vaccines activate both cellular and humoral immunity and stimulate the development of memory for long-lasting immunity. In some cases, vaccination of one individual with a live attenuated pathogen can even lead to natural transmission of the attenuated pathogen to other individuals. This can cause the other individuals to also develop an active, subclinical infection that activates their adaptive immune defenses.

Disadvantages associated with live attenuated vaccines include the challenges associated with long-term storage and transport as well as the potential for a patient to develop signs and symptoms of disease during the active infection (particularly in immunocompromised patients). There is also a risk of the attenuated pathogen reverting back to full virulence. Table 7.4.1 lists examples live attenuated vaccines.

Inactivated Vaccines

Inactivated vaccines contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of key antigens on the pathogen.

Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. Typically the response involves only humoral immunity, and the pathogen cannot be transmitted to other individuals. In addition, inactivated vaccines usually require higher doses and multiple boosters, possibly causing inflammatory reactions at the site of injection.

Despite these disadvantages, inactivated vaccines do have the advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. However, inactivated vaccines are not without their side effects. Table 7.4.1 lists examples of inactivated vaccines.

Subunit Vaccines

Whereas live attenuated and inactive vaccines expose an individual to a weakened or dead pathogen, subunit vaccinesonly expose the patient to the key antigens of a pathogen—not whole cells or viruses. Subunit vaccines can be produced either by chemically degrading a pathogen and isolating its key antigens or by producing the antigens through genetic engineering. Because these vaccines contain only the essential antigens of a pathogen, the risk of side effects is relatively low. Table 7.4.1 lists examples of subunit vaccines.

Toxoid Vaccines

Like subunit vaccines, toxoid vaccines do not introduce a whole pathogen to the patient; they contain inactivated bacterial toxins, called toxoids. Toxoid vaccines are used to prevent diseases in which bacterial toxins play an important role in pathogenesis. These vaccines activate humoral immunity that neutralizes the toxins. Table 7.4.1lists examples of toxoid vaccines.

Conjugate Vaccines

A conjugate vaccine is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. Conjugate vaccines have been developed to enhance the efficacy of subunit vaccines against pathogens that have protective polysaccharide capsules that help them evade phagocytosis, causing invasive infections that can lead to meningitis and other serious conditions. The subunit vaccines against these pathogens introduce T-independent capsular polysaccharide antigens that result in the production of antibodies that can opsonize the capsule and thus combat the infection; however, children under the age of two years do not respond effectively to these vaccines. Children do respond effectively when vaccinated with the conjugate vaccine, in which a protein with T-dependent antigens is conjugated to the capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide. Table 7.4.1 lists examples of conjugate vaccines.

Table 7.4.1***:*** *Classes of Vaccines*

Class	Description	Advantages	Disadvantages	Examples
Live attenuated	Weakened strain of whole pathogen	Cellular and humoral immunity	Difficult to store and transport	Chickenpox, German measles, measles, mumps, tuberculosis, typhoid fever, yellow fever
		Long-lasting immunity	Risk of infection in immunocompromised patients	
		Transmission to contacts	Risk of reversion	
Inactivated	Whole pathogen killed or inactivated with heat, chemicals, or radiation	Ease of storage and transport	Weaker immunity (humoral only)	Cholera, hepatitis A, influenza, plague, rabies
		No risk of severe active infection	Higher doses and more boosters required	
Subunit	Immunogenic antigens	Lower risk of side effects	Limited longevity	Anthrax, hepatitis B, influenza, meningitis, papillomavirus, pneumococcal pneumonia, whooping cough
			Multiple doses required	
			No protection against antigenic variation	
Toxoid	Inactivated bacterial toxin	Humoral immunity to neutralize toxin	Does not prevent infection	Botulism, diphtheria, pertussis, tetanus
Conjugate	Capsule polysaccharide conjugated to protein	T-dependent response to capsule	Costly to produce	Meningitis (*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitides*)
			No protection against antigenic variation	
		Better response in young children	May interfere with other vaccines	

Exercise 7.4.3

1. What is the risk associated with a live attenuated vaccine?
2. Why is a conjugated vaccine necessary in some cases?

DNA Vaccines

DNA vaccines represent a relatively new and promising approach to vaccination. A DNA vaccine is produced by incorporating genes for antigens into a recombinant plasmid vaccine. Introduction of the DNA vaccine into a patient leads to uptake of the recombinant plasmid by some of the patient's cells, followed by transcription and translation of antigens and presentation of these antigens with MHC I to activate adaptive immunity. This results in the stimulation of both humoral and cellular immunity without the risk of active disease associated with live attenuated vaccines.

Although most DNA vaccines for humans are still in development, it is likely that they will become more prevalent in the near future as researchers are working on engineering DNA vaccines that will activate adaptive immunity against several different pathogens at once. First-generation DNA vaccines tested in the 1990s looked promising in animal models but were disappointing when tested in human subjects. Poor cellular uptake of the DNA plasmids was one of the major problems

impacting their efficacy. Trials of second-generation DNA vaccines have been more promising thanks to new techniques for enhancing cellular uptake and optimizing antigens. DNA vaccines for various cancers and viral pathogens such as HIV, HPV, and hepatitis B and C are currently in development.

Some DNA vaccines are already in use. In 2005, a DNA vaccine against West Nile virus was approved for use in horses in the United States. Canada has also approved a DNA vaccine to protect fish from infectious hematopoietic necrosis virus.[4] A DNA vaccine against Japanese encephalitis virus was approved for use in humans in 2010 in Australia.

Clinical Focus: Resolution

Based on Olivia's symptoms, her physician made a preliminary diagnosis of bacterial meningitis without waiting for positive identification from the blood and CSF samples sent to the lab. Olivia was admitted to the hospital and treated with intravenous broad-spectrum antibiotics and rehydration therapy. Over the next several days, her condition began to improve, and new blood samples and lumbar puncture samples showed an absence of microbes in the blood and CSF with levels of white blood cells returning to normal. During this time, the lab produced a positive identification of *Neisseria meningitidis*, the causative agent of meningococcal meningitis, in her original CSF sample.

N. meningitidis produces a polysaccharide capsule that serves as a virulence factor. *N. meningitidis* tends to affect infants after they begin to lose the natural passive immunity provided by maternal antibodies. At one year of age, Olivia's maternal IgG antibodies would have disappeared, and she would not have developed memory cells capable of recognizing antigens associated with the polysaccharide capsule of the *N. meningitidis*. As a result, her adaptive immune system was unable to produce protective antibodies to combat the infection, and without antibiotics she may not have survived. Olivia's infection likely would have been avoided altogether had she been vaccinated. A conjugate vaccine to prevent meningococcal meningitis is available and approved for infants as young as two months of age. However, current vaccination schedules in the United States recommend that the vaccine be administered at age 11–12 with a booster at age 16.

In countries with developed public health systems, many vaccines are routinely administered to children and adults. Vaccine schedules are changed periodically, based on new information and research results gathered by public health agencies. In the United States, the CDC publishes schedules and other updated information about vaccines.

Key Concepts and Summary

- Adaptive immunity can be divided into four distinct classifications: **natural active immunity, natural passive immunity, artificial passive immunity,** and **artificial active immunity.**
- Artificial active immunity is the foundation for **vaccination** and vaccine development. Vaccination programs not only confer artificial immunity on individuals, but also foster **herd immunity** in populations.
- **Variolation** against smallpox originated in the 10th century in China, but the procedure was risky because it could cause the disease it was intended to prevent. Modern vaccination was developed by Edward Jenner, who developed the practice of inoculating patients with infectious materials from cowpox lesions to prevent smallpox.
- **Live attenuated vaccines** and **inactivated vaccines** contain whole pathogens that are weak, killed, or inactivated. **Subunit vaccines, toxoid vaccines,** and **conjugate vaccines** contain acellular components with antigens that stimulate an immune response.

Footnotes

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Contributors and Attributions

- Nina Parker, (Shenandoah University), Mark Schneegurt (Wichita State University), Anh-Hue Thi Tu (Georgia Southwestern State University), Philip Lister (Central New Mexico Community College), and Brian M. Forster (Saint Joseph’s University) with many contributing authors. Original content via Openstax (CC BY 4.0; Access for free at https://openstax.org/books/microbiology/pages/1-introduction)

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7.5: How Does SARS-Cov-2 Infect Humans?

In order to develop effective strategies to diagnose, treat, and manage this disease, it is vital to understand exactly how SARS-CoV-2 enters human cells. The virus' surface spike protein mediates SARS-CoV-2 entry into cells by binding to the ACE2 (Angiotensin-converting enzyme 2) receptor in humans through its receptor-binding domain and is proteolytically activated by human proteases. Cell entry of SARS-CoV-2 is preactivated by proprotein convertase furin, reducing its dependence on target cell proteases for entry, thus making it more efficient in cell entry and infection.

In humans, the ACE2 receptor protein is present in many cell types (especially epithelial cells) and tissues including the nose, mouth, lungs, heart, blood vessels, kidneys, liver, and gastrointestinal tract. ACE2 assists in modulating the activities of a protein called angiotensin II (ANG II) which increases blood pressure and inflammation, thereby increasing damage to blood vessel linings and promotes various types of tissue injury. ACE2 converts ANG II to other molecules which effectively counteract the effects of ANG II, such as inflammation and cell death. When the SARS-CoV-2 virus binds to the ACE2 receptor, it prevents ACE2 from performing its normal function to regulate ANG II signaling. As such, ACE2 action is inhibited, removing the protective mechanism from ANG II signaling through increased availability of ANG II to injure tissues, especially in the lungs and heart. **Figure 13.2.1** summarizes the transmission and life-cycle of SARS-CoV-2 causing COVID-19.

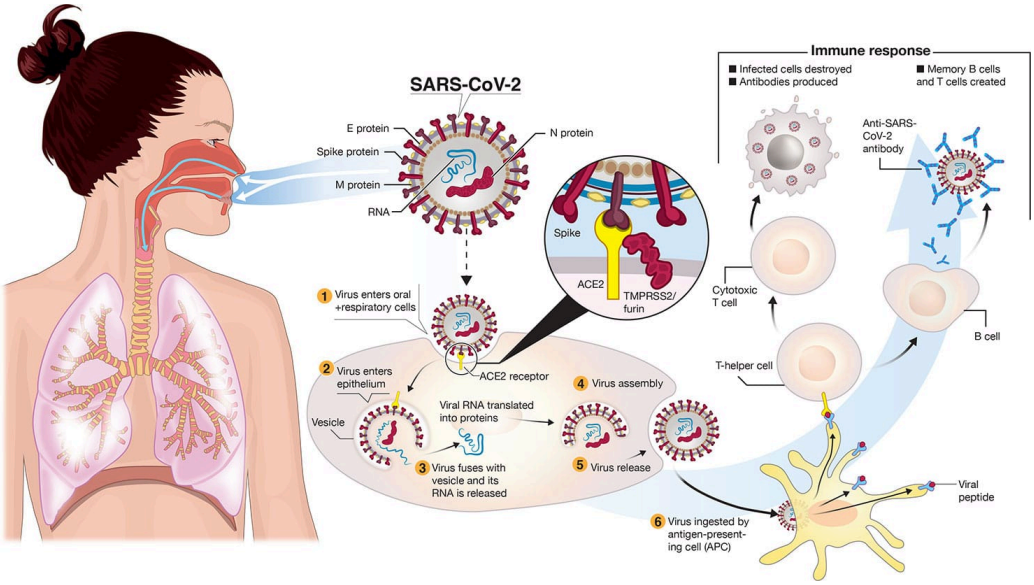


Figure 13.2.1 Transmission and Life-Cycle of SARS-CoV-2 Causing COVID-19. SARS-CoV-2 is transmitted via respiratory droplets of infected cases to oral and respiratory mucosal cells. The virus, possessing a single-stranded RNA genome wrapped in nucleocapsid (N) protein and three major surface proteins: membrane (M), envelope (E) and Spike, replicates and passes to the lower airways potentially leading to severe pneumonia. The gateway to host cell entry (magnified view) is via Spike-converting enzyme 2 (ACE2) interaction with cleavage of Spike in the prefusion state by proteases TMPRSS-2/furin. A simplified depiction of the life cycle of the virus is shown along with potential immune responses elicited. [Long description]

Take a look at the video below, *How the Novel Coronavirus Infects a Cell: Science, Simplified*, by Scripps Research (2020) on YouTube, describing how the novel coronavirus that causes COVID-19 enters the body and infects cells. Illustrated by a Scripps Research scientist, this installment of *Science, Simplified* gives an overview of the entire infection process.

YouTube Video

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- **Figure 13.2.1**Fphar-11-00937-g001 by Colin D. Funk, Craig Laferrière, and Ali Ardakani (2020), CC BY 4.0, via Wikimedia Commons

Reference

Funk, C. D., Laferrière, C., Ardakani, A. (2020, June 19). A snapshot of the global race for vaccines targeting SARS-CoV-2 and the COVID-19 pandemic. *Frontiers in Pharmacology11*. https://doi.org/10.3389/fphar.2020.00937

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Long Description

- **Figure 13.2.1** Transmission and Life-Cycle of SARS-CoV-2 Causing COVID-19. SARS-CoV-2 is transmitted via respiratory droplets of infected cases to oral and respiratory mucosal cells. The virus, possessing a single-stranded RNA genome wrapped in nucleocapsid (N) protein and three major surface proteins: membrane (M), envelope (E) and Spike, replicates and passes to the lower airways potentially leading to severe pneumonia. The gateway to host cell entry (magnified view) is via Spike-converting enzyme 2 (ACE2) interaction with cleavage of Spike in the prefusion state by proteases TMPRSS-2/furin. A simplified depiction of the life cycle of the virus is shown along with potential immune responses elicited. [Back to Figure 13.2.1]

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7.6: Genes Implicated in Severe COVID-19 Infection in Humans

Overview

The information in this section *(Overview)* was extracted from Elhabyan, A. et al. (2020), The role of host genetics in susceptibility to severe viral infections in humans and insights into host genetics of severe COVID-19: A systematic review.

Susceptibility to severe viral infections was reported to be associated with genetic variants in immune response genes. This article systematically reviewed the genes related to viral susceptibility that were reported in human genetic studies (case-reports and genome wide association studies) to understand the role of host viral interactions and to provide insights into the pathogenesis of severe COVID-19. Approximately 15% of cases are severe and some of them are accompanied by a dysregulated immune system and cytokine storm. There is increasing evidence that severe manifestations of COVID-19 might be attributed to human genetic variants in genes related to immune deficiency and/or inflammasome activation (cytokine storm). Forty genes were found to be associated with viral susceptibility and 21 of them were associated with severe SARSCoV disease and severe COVID-19. Some of those genes were implicated in toll-like receptor pathways, others in C-lectin pathways, and others were related to inflammasome activation (cytokine storm).

Table 13.1 Summary of Review (Elhabyan et al., 2020) – Showing Genes Associated with Clinical Manifestation of SARS-CoV/2

Clinical Manifestation	Genes Associated
Susceptibility to SARS-CoV infection	CD14, HLA-B, FCGR2A, CCL2, CCL5, MxA, ABO, MBL, OAS-1, ICAM3, DC-SIGN
Susceptibility to SARS-CoV2 infection	ALOXE3, TMEM181, BRF2, ERAP2, LC6A20, LZTFL1, CCR9, FYCO1, CXCR6, XCR1, ABO, ApoE

A Closer Look at Popular Theories

As researchers work tirelessly to uncover the genetic basis of COVID-19 severity and susceptibility, the following outlines some popular opinions based upon the science, as it stands when this was written. As the science advances, so will our theories and understanding.

- Emily Willingham's (2020) article, "Genes May Influence COVID-19 Risk, New Studies Hint" at *Scientific American*, and Jocelyn Kaiser's (2020) article, "Found: genes that sway the course of the coronavirus" at *Science* from American Association for the Advancement of Science. speak to early theories concerning**Chromosome 3 and ABO blood groups.**
- Another article from from Ann Gibbons (2020) at *Science*, "Neanderthal gene found in many people may open cells to coronavirus and increase COVID-19 severity" discusses the theory related to **Chromosome 2, specifically the DPP4 gene known as the Neanderthal gene** found in many people which may open cells to coronavirus and increase COVID-19 severity.
- An article from the National Institutes of Health (NIH, 2020), "Scientists discover genetic and immunologic underpinnings of some cases of severe COVID-19" speaks to a molecular explanation for **why more men than women die from COVID-19**.
- An article from John Hewitt (2020), *Medical Xpress*, "Unique susceptibility to unique Sars-CoV-2 variants and vaccines" gives a brief overview of how**individuals with different genetic variants in their immune system** components often have very different immune responses to SARS-CoV-2, and explains that they also will have different responses to vaccines.

Take a look at the video below by Dr. Alex Hoischen, Radboud University (Bionano Genomics, 2021), where he discusses his published results on genomic variants found in families with severe COVID-19. In two families with severely ill brothers, mutations were found in the Toll-Like Receptor 7 gene (TLR7), which affects the production of interferons, signaling molecules used to control the immune response. Several other studies have since made similar findings in other genes of the TLR family. Dr. Hoischen discussed how individual patients each may carry individually rare variants, that are collectively common and point to important pathways involved in the disease. His interest in the consortium is based on his understanding that larger SVs have a greater chance to be rare and disruptive, and genome-wide studies have lacked so far in their assessment.

The video below, *Rare Genetic Variants May Predispose to Severe COVID-19,* by Bionano Genomics (2021) on YouTube, discusses

the links between genes and incidence of severe COVID-19 infection.

YouTube Video

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Bionano Genomics. (2021, January 22). Rare genetic variants may predispose to severe COVID-19 (video file). YouTube. https://www.youtube.com/watch?v=RaustWij4yg

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7.7: Approaches for Vaccine Development Against SARS-Cov-2

The elucidation of the genome organization and functional domains of S protein for SARS-CoV **(Figure 13.4.1),** achieved through the work of scientists and geneticists all over the globe, has facilitated a deep understanding of the mode of action of this virus which has led to the development of a myriad of vaccine and drug candidates in an effort to mitigate the spread of this virus, as well as to assist in the diagnosis and management of infected patients.

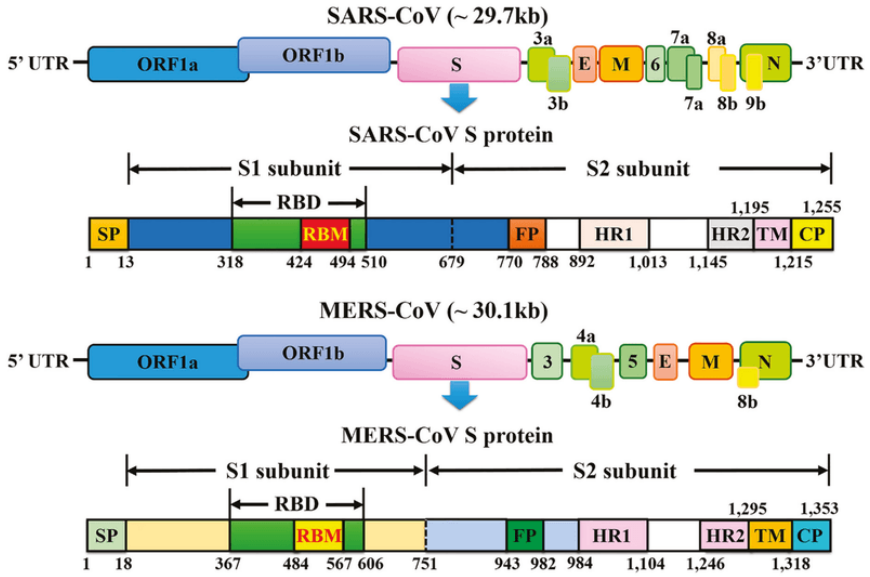


Figure 13.4.1 Schematic Representation of the Genome Organization and Functional Domains of S Protein for SARS-CoV and MERS-CoV. [Long description]

Figure 13.4.1 is a schematic representation of the genome organization and functional domains of S protein for SARS-CoV and MERS-CoV. The single-stranded RNA genomes of SARS-CoV and MERS-CoV encode two large genes, the ORF1a and ORF1b genes, which encode 16 non-structural proteins (nsp1–nsp16) that are highly conserved throughout coronaviruses. The structural genes encode the structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N), which are common features to all coronaviruses. The accessory genes (shades of green) are unique to different coronaviruses in terms of number, genomic organization, sequence, and function. The structure of each S protein is shown beneath the genome organization. The S protein mainly contains the S1 and S2 subunits. The residue numbers in each region represent their positions in the S protein of SARS and MERS, respectively. The S1/S2 cleavage sites are highlighted by dotted lines. SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; CP, cytoplasm domain; FP, fusion peptide; HR, heptad repeat; RBD, receptor-binding domain; RBM, receptor-binding motif; SP, signal peptide; TM, transmembrane domain.

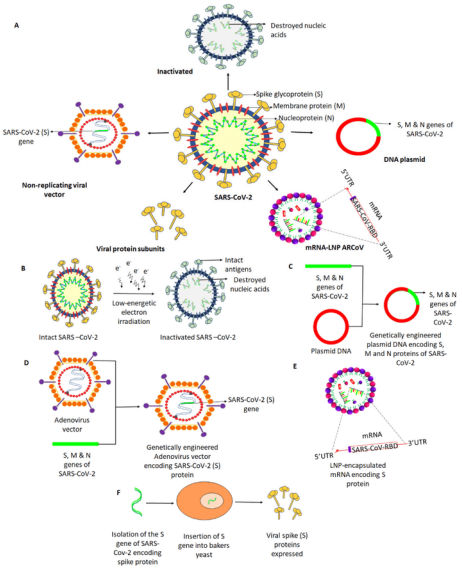


Figure 13.4.2 Different Approaches for the Development of Vaccine Candidates Against SARS-Cov-2 [Long description]

Different approaches for the development of vaccine candidates against SARS-Cov-2 have, and are being, developed and utilized. **Figure 13.4.2** summarizes these methods:

A. Potential vaccines under development, involve five leading platforms (inactivated virus, protein subunit, DNA, RNA, and non-replicating viral vector), as depicted.
B. Intact SARS-CoV-2 is neutralized by treatment with radiation to cease its ability to infect and replicate, while preserving the induction of an immune response.
C. A plasmid DNA is genetically engineered with the S, M, and N genes of SARS-CoV-2 encoding the respective proteins that may facilitate an immune response.
D. A replication-defective Adenovirus (Ad) vector is genetically engineered to express SARS-Cov-2 spike (S) protein.
E. An mRNA (replication-defective) that encodes the S protein of SARS-CoV-2 is encapsulated in a lipid nanoparticle (LNP), which, when injected, induces the body cells to produce the spike protein and direct the immune response.
F. Spike protein-encoding (S) gene of SARS-CoV-2 was isolated and genetically engineered into a baker's yeast, producing the spike protein antigens when grown. The produced S antigens can then be collected and purified.

Watch the video below called, *There are four types of COVID-19 vaccines: here's how they work*, by Gavi, the Vaccine Alliance(2020) on YouTube, which gives a brief overview of the main types of COVID-19 vaccines and the mechanism by which they bring about immunity in a patient.

YouTube Video

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- **Figure 13.4.1** SARS-CoV MERS-CoV genome organization and S-protein domains by Song, et al. (2019), CC BY 4.0, via Wikimedia Commons
- **Figure 13.4.2**Different approaches for the development of vaccine candidates against SARS-Cov-2 by Faheem et al. (2021), CC BY 4.0, via Wikimedia Commons

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Long Descriptions

- **Figure 13.4.1** Schematic representation of the genome organization and functional domains of S protein for SARS-CoV and MERS-CoV. The single-stranded RNA genomes of SARS-CoV and MERS-CoV encode two large genes, the ORF1a and ORF1b genes, which encode 16 non-structural proteins (nsp1–nsp16) that are highly conserved throughout coronaviruses. The structural genes encode the structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N), which are common features to all coronaviruses. [Back to Figure 13.4.1]
- **Figure 13.4.2** Different Approaches for the Development of Vaccine Candidates Against SARS-Cov-2. (A) Potential vaccines under development involve five leading platforms (inactivated virus, protein subunit, DNA, RNA, and non-replicating viral vector), as depicted. (B) Intact SARS-CoV-2 is neutralized by treatment with radiation to cease its ability to infect and replicate, while preserving the induction of an immune response. (C) A plasmid DNA is genetically engineered with the S, M, and N genes of SARS-CoV-2 encoding the respective proteins that may facilitate an immune response. (D) A replication-defective Adenovirus (Ad) vector is genetically engineered to express SARS-Cov-2 spike (S) protein. (E) An mRNA (replication-defective) that encodes the S protein of SARS-CoV-2 is encapsulated in a lipid nanoparticle (LNP), which, when injected, induces the body cells to produce the spike protein and direct the immune response. (F) Spike protein-encoding (S) gene of SARS-CoV-2 was isolated and genetically engineered into a baker's yeast, producing the spike protein antigens when grown. The produced S antigens can then be collected and purified. [Back to Figure 13.4.2]

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8: ENVIRONMENTAL, AGRICULTURAL, AND AQUATIC BIOTECHNOLOGY

8.1: Applications of Environmental Biotechnology
8.2: Applications of Agricultural Biotechnology
8.3: Aquaculture

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8.1: Applications of Environmental Biotechnology

Biotech Focus

The textile industry produces copious amount of wastewater that is full of toxic dyes. These dyes are resistant to breakdown by "normal" bacteria. In response, scientists genetically-modified bacteria to enhance their degradative capacity. To do this, genes to enzymatically process textile dyes, like **azoreductase**, were inserted intro bacterial strains like *Pseudomonas putida*. Introduction of these bacteria into the wastewater effluent resulted in the breakdown of these dyes into harmless byproducts. The modified bacteria cleaned the wastewater much faster than "traditional" methods. They worked even in low-oxygen environments, which is important for deep water treatment.

Introduction

"Gray", or **environmental biotechnology**, uses science, technology, and engineering to study the natural world and solve a variety of environmental problems. Because environmental biotechnology could also imply the harnessing of biological processes for commercial use, the International Society for Environmental Biotechnology now defines environmental biotechnology as "the development, use and regulation of biological systems for remediation of contaminated environments (land, air, water), and for environment-friendly processes (green manufacturing technologies and sustainable development)".

Some of the key applications of gray biotechnology include:

- **Bioremediation:** Using bacteria to clean up oil spills or toxic waste
- **Phytoremediation:** Using plants to clean up polluted soil, water, or air
- **Waste management:** Using engineered bacteria to break down plastics and other difficult to degrade materials
- **Air pollution control:** Using bacteria to trap and break down pollutants from industrial gases
- **Environmental monitoring:** Using bacteria in the development of biosensors that can detect pollution early on in the process

Learning Objectives

"Gray" biotechnology restores ecosystems, reduces pollution, and improves waste management practices to make them eco-friendly. At the end of this page, you will be able to:

- Define gray biotechnology
- Discuss some applications of gray biotechnology, such as phytoremediation and bioremediation

Applications of Gray Biotechnology

The use of living organisms in the reduction of environmental waste and pollutants has become a very popular area of biotechnology. As such, environmental biotechnology integrates extensively with industrial biotechnology. Because agriculture practices can produce significant amounts of waste, environmental biotechnology also intersects with agricultural biotechnology. This means that the applications described below could also be considered to be part of other biotechnology fields.

Phytoremediation

Phytoremediation is a natural and eco-friendly method of cleaning up environmental pollutants using plants and their associated microbes. Phytoremediation is environmentally friendly, aesthetically pleasing, cost-effective compared to conventional cleanup methods and can improve soil health and biodiversity. However, this natural process is slower than conventional methods and is only effect to the depth of the plant roots. In addition, phytoremediation may not work well in areas with high contamination levels.

Types of phytoremediation include:

1. **Phytoextraction:** the plants absorb contaminants, like heavy metals from soil or water through their roots and store them in the tissues of their stems or leaves; the contaminated plants can then be harvested and safely disposed of
   - e.g. sunflowers planted at Chernobyl (Ukraine) absorb cesium-137 and strontium-90
   - e.g. Indian mustard planted at mining sites for the absorption of heavy metals like lead, mercury, selenium, and cadmium.
2. **Phytostabilization:** the plants reduce the movement of heavy metal contaminants by immobilizing them in the soil through root systems, preventing them from spreading to water sources
   - e.g. Buffalo grass, Vetivar grass, willow and poplar trees
3. **Phytodegradation:** the plants use enzymes to break down organic pollutants, like pesticides or petroleum products, into less harmful substances
   - e.g. use of poplar trees at landfills and hazardous waste sites
   - e.g. Vetivar grass planted in oil-contaminated soils
4. **Phytovolatilization:** the plants absorb contaminants, like heavy metals and solvents, and release them into the atmosphere in a less toxic form through transpiration from the leaves
   - e.g. ferns and wetland species
5. **Rhizofiltration:** plant roots, especially those in water, absorb or filter pollutants from contaminated water
   - e.g. water hyacinths use in ponds and canals
6. **Phytodesalination:** uses halophytes (e.g., plants adapted to saline soil) to extract salt from the soil to improve its fertility

Genetic engineering has become a powerful method for enhancing natural phytoremediation capabilities, or for introducing new capabilities into plants. Genes from metal-tolerant organisms may be introduced into other plants to turn them into "hyperaccumulators" capable of absorbing significant amounts of toxic metals and store them safely in their tissues. Plants can be engineered to express degradative enzymes like cytochrome P450 enzymes. Genetic engineering can also be used to turn the native plants that are better adapted to the environmental conditions at a specific cleanup site into plants capable of accelerated phytoremediation.

Bioremediation

The natural ability of organisms to adsorb, accumulate, and degrade pollutants has attracted the use of biotechnology in the treatment of contaminated environments. **Bioremediation** refers to any process that uses a biological system (e.g., bacteria, algae, fungi, and plants), or its products (e.g., enzymes), for the removal of environmental pollutants from air, water, soil, and industrial effluents (Figure 8.1.1. New bioremediation approaches involve the use of genetically-modified bacteria but with strict controls to ensure the bacteria will not harm other ecosystems. Genetically-modified bacteria can produce new enzymes to digest pollutants, can be modified to improve the efficiency of natural pathways, or to make bacteria more resistant to the environmental stresses at the clean up location. *Pseudomonas putida* has been engineered to degrade hydrocarbons and resist toxic metals at clean-up sites. *Deinococcus radiodurans* has been modified to survive elevated radiation levels and clean up nuclear wastes. Engineering bacteria to treat multiple types of pollution at once (e.g., oil and heavy metals) is also being explored. Whatever the approach, the end goal of bioremediation is to remove harmful compounds to improve soil and water quality.

Bioremediation techniques can be classified as *in situ* techniques, which treat polluted sites directly or *ex situ* techniques which are applied to materials that have been removed from a contaminated site.

In situ bioremediation can involve the addition of microbes for the treatment of the pollutant or the addition of specific nutrients to encourage the growth of naturally-occurring microbes at the site. Examples of *in situ* techniques include:

1. **Bioventing:** a method where oxygen is pumped into the soil to help natural bacteria break down pollutants
   - used for the clean up of oil, gasoline, and solvent spills in soil
2. **Biostimulation:** a method where nutrients, like nitrogen or phosphorus, are added to "feed" microbes and speed up their breakdown of the pollutant
   - used for soil, groundwater, or oil spill cleanup
   - does not involve the use of genetically-modified microbes
3. **Bioattenuation:** a method of letting natural processes slowly break down pollutants without the addition of microbes or nutrients
   - scientists monitor the process rather than direct the process



Figure 8.1.1: Biostimulation at the Snake River Plain Aquifer in Idaho. Whey powder is added to the site to promote the utilization of naturally present bacteria. The bacteria at this sitebreak down the carcinogenic compound trichloroethylene (TCE). (Biostimulation by bomazi; Public domain)

Examples of *ex situ* techniques include:

1. **Biopiles:** soil is removed and piled into mounds with an aeration system to encourage bacterial growth
   - microbe use is similar to bioventing
   - used to remove petroleum pollutants
2. **Windrows:** contaminated soil is laid out in long, narrow rows and then regularly turned over to mix in air
   - also used in composting
3. **Land farming:** contaminated soil is spread over a large, flat field and then tilled to give microbes access to air and sunlight
   - treats soils contaminated with petroleum or pesticides

Wastewater Treatment

Wastewater is any water that has been used and is now dirty because it picked up pollutants like chemicals, food scraps, oil and grease, human waste, and industrial toxins. There are two types of wastewater: domestic (toilets, showers, kitchens) and industrial (factories and businesses). Wastewater needs to be cleaned (i.e.,treated) before it can safely go back into the environment or be reused. The process of cleaning wastewater is known as **wastewater treatment**.

Wastewater is treated is 4 steps:

1. **Preliminary treatment:** the filtering of water of particulate matter
2. **Primary treatment:** the separation of wastewater into phases using large tanks
   - heavy solids sink to the bottom of the tank; lighter materials (oil and grease) rise to the top
   - both phases can be removed
3. **Secondary treatment:** the addition of bacteria to the water phase
   - aeration is added to improve bacterial efficiency
   - the water is transferred to a tank to allow for the settling and removal of bacteria
4. **Advanced (tertiary) treatment:** special filters, chemicals, or UV light is used to kill any remaining pathogens and chemicals
   - artificial "swamps" can be constructed to filter water and eliminate the need for harsh chemicals or UV light

Scientists can engineer bacteria to improve the secondary treatment phase of wastewater by making them break down pollutants faster, remove heavy metals, dyes, oils, and pharmaceuticals, and work in harsh environments. Strains of *Pseudomonas* bacteria are one example of a bacterial strain used in wastewater treatment. These bacteria can be engineered to break down oil, heavy metals, or organic pollutants in wastewater. *E. coli* strains are also engineered for wastewater treatment. Some *E. coli* strains can be even be engineered to glow when pollutants are present, turning them into biosensors. As with bioremediation, the introduction of genetically-modified bacteria to wastewater treatment plants are heavily regulated and must be carefully monitored to ensure they do not escape into natural water systems.

Pollution Control

Biotechnology can control pollution in many ways, including phytoremediation and bioremediation. In addition, scientists are now creating **biofilters** that use microbial films to clean dirty air (and water). A biofilter is a moist bed of soil, compost, or wood chips that contains beneficial microbes, such as natural bacteria or genetically-modified bacteria. The dirty air is pushed through the biofilter, giving the bacteria access to the pollutants. Wastewater treatment plants use such biofilters to control the bad odors and harmful gases that result from the treatment process. Bacteria or fungi designed to break down specific pollutants can also be added to a system in a process called **bioaugmentation**. As with biofilters, these microbes are chosen for their degradative ability or they can genetically engineered. Bioaugmentation is also a critical part of bioremediation and wastewater treatment.

Environmental Monitoring

Environmental monitoring is the process of regularly checking air, soil, and water to measure pollution levels and track changes in the environment. When monitoring, scientists collect samples and analyze them. By monitoring, scientists can detect problems early in order to protect ecosystems and human health. One technique being developed for use in environmental monitoring is the creation of biosensors. A **biosensor** is a special device that can detect pollution using biological components, like bacteria, enzymes, or antibodies. The typical biosensor produces some type of "signal" like a color change, light, or an electrical signal. By measuring the signal, scientists can determine how much pollution is present.

A basic biosensor contains the following parts:

- **Bioreceptor:** what detects the pollutant
  - can be an enzyme, antibody, gene, or an entire organism (e.g. microbe)
- **Transducer:** converts the detection into a measurable signal that can be measured
  - light (i.e., fluorescence, luminescence), color change, electrical signal
- **Display:** shows the signal
  - the signal could be displayed used a computer screen, a camera, or something simple as film
  - the display can be coupled to devices that measure the signal

One example is the use of genetically-engineered bacteria like *E.coli, Bacillis subtilis*, or strains of *Pseudomonas* in the detection of arsenic in water. For this biosensor, a gene for a **reporter protein** is inserted into the bacterial genome. This reporter protein is under the control of a promoter capable of being bound by arsenic. The amount of reporter protein expressed, as a result of arsenic binding, can then be detected and measured through a simple bioassay, indicating the presence and concentration of arsenic (Figure 8.1.2).

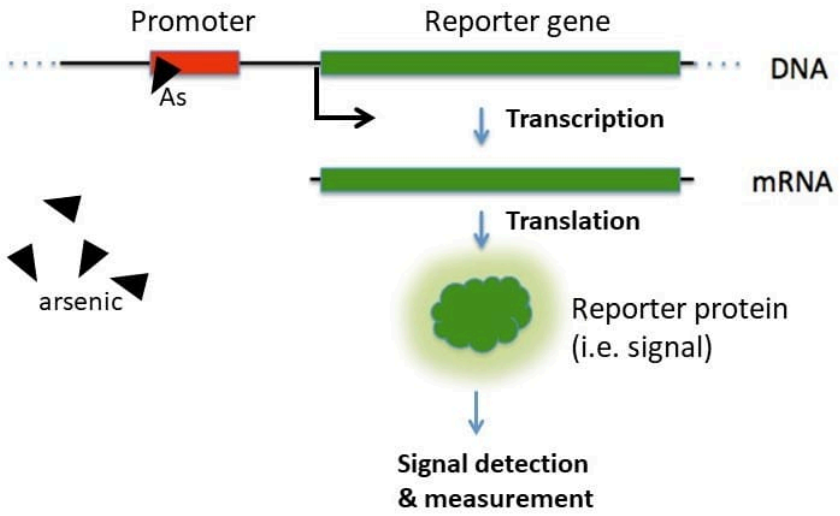


Figure 8.1.2: Biosensors can be built using reporter systems. A typical reporter system has a promoter (red rectangle) capable of being bound by the compound to be detected and measured (e.g., arsenic, As). Binding of this compound results in transcription of the reporter gene into mRNA and then translation into the reporter protein, also called the "signal". This signal could be fluorescent, luminescent, or colorimetric. The signal is detected and then measured as an indication of the amount of compound. (Reporter Systems by Patricia Zuk, CC BY 4.0; modified from Reporter Gene by Transcontrol, CC BY-SA 3.0)

Several types of reporter protein systems can be used in biosensors, including fluorescent (e.g. Green Fluorescent Protein or GFP), luminescent (e.g. firefly luciferase), or colorimetric systems. Each have their own advantages and disadvantages. For example, the fluorescent signal produced by reporter systems using GFP can be measured directly, but the signal can be weak. Reporter systems using luminescent or colorimetric proteins require additional steps to detect the signal but this signal can be amplified, making these systems more sensitive. Ultimately, this type of biosensor is inexpensive to create, and robust. It can be highly sensitive. Moreover, this biosensor is portable and can be used easily for field testing.

Key Concepts

Applications of gray biotechnology range from the use of plants to trap heavy metals in soils and the engineering of bacteria to breakdown hydrocarbons in oil. Some important concepts to remember are:

- plants, and their associated microbes, can be used to clean up environmental pollutants through a process called phytoremediation
- bioremediation uses biological systems, or its products, to remove environmental pollutants
- environmental monitoring can involve the use of biosensors which utilize reporter systems for the detection of a pollutant
- the binding of an environmental pollutant, like a heavy metal, to a reporter system promoter leads to the expression of the reporter protein
- quantitation of a reporter protein can be used to measure the amount of environmental pollutant
- bioremediation, wastewater treatment, and pollution control can use "natural" bacteria or those that have been genetically-engineered
- genetically-engineered bacteria use in environmental biotechnology must be carefully used to prevent their introduction into natural ecosystems

Glossary

Bioassay - a scientific test that uses living organisms to measure the effect or concentration of a chemical substance, such as pollutants or toxins

Bioaugmentation - the process of adding specially selected or genetically-modified microbes to a contaminated environment in order to help break down pollutants faster and more efficiently

Biofilter - a system that uses living materials, like microorganisms on a filter medium (like soil, compost, or synthetic materials), to remove contaminants from air, water, or industrial waste streams

Biosensor - a device that uses a biological component, like an enzyme, antibody, cell, or DNA sequence) to detect and measure specific chemicals, substances, or conditions and convert them into a signal (like light, electricity, or color change)

Effluent - a liquid waste or sewage that flows out of a factory, treatment plant, or other facility; often requires treatment before it can be safely released into the environment

Ex situ - Latin for "off site," referring to treatments that are performed away from the original location of a process or problem

In situ - Latin for "on site"; referring to performing treatments directly at the location where the problem or process naturally occurs

Phytoremediation - the use of plants to clean up contaminants from soil, water, or air by absorbing, containing, degrading, or volatilizing pollutants

Promoter sequence (promoter) - a DNA sequence where the RNA polymerase binds; determines the site of transcription initiation

Remediation - the process of cleaning up, removing, or neutralizing pollutants from soil, water, or air to restore the environment to a healthier state

Reporter system - a tool used to visually or easily measure the activity of a gene, promoter, or cellular process

Wastewater - used or contaminated water that comes from homes, businesses, or industries, often containing pollutants, and requiring treatment before it can be reused or released into natural water bodies

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8.2: Applications of Agricultural Biotechnology

Biotech Focus

Most crops depend on nitrogen fertilizers to grow well. However, producing and applying those fertilizers can be expensive and harmful to the environment. With the advent of the "green" biotechnology field, researchers have become interested on whether crop production can be improved without the use of synthetic fertilizers.

Enter the humble legume.

Legumes, including peas, beans, lentils, and soybeans, can naturally remove nitrogen (N_2) from the atmosphere and convert it into nitrogenous compounds through a process called **nitrogen fixation**. These nitrogenous compounds are used by the legumes to make proteins and nucleotides. This results in enhanced growth even in poor nutrient soils. However, it isn't the legume doing the nitrogen fixation. Rather, the nitrogen fixation is being performed thanks to a symbiotic relationship with certain bacteria. Scientists in the green biotechnology field are now exploring whether this capability can be transferred to other crops like corn, wheat, and rice. One way may be to genetically engineer crops to form similar symbiotic relationships with nitrogen-fixing bacteria. Another way is to engineer the crops themselves to express the necessary bacterial genes from nitrogen fixation.

Whatever method is used, giving plants the ability to fix nitrogen will reduce their reliance on synthetic fertilizers, lower farming costs, and minimize environmental damage. Turning plants into self-feeding powerhouses will certainly have a dramatic impact on the agricultural field.

Introduction

Green biotechnology, often called **agricultural biotechnology**, is the use of science and engineering on agriculture and the environment. It combines biology with technology to solve problems related to food production, sustainability, and environmental challenges. To do this, green biotechnology uses many of the same techniques and tools as other areas of biotechnology. These techniques include genetic engineering, genetically-modified organisms (GMOs), and genome editing (e.g., CRISPR-Cas9). In agriculture, these tools are used to:

- Increase crop yields
- Enhance resistance to pests, diseases, and harsh climates
- Improve nutritional content
- Reduce the need for chemical fertilizers and pesticides

In botany, green biotechnology reduces the need for chemical fertilizers and pesticides, helps crops survive climate stress like drought or extreme temperatures, improves soil and water quality, and increases food security while being more eco-friendly. Some of the key applications of this field of biotechnology include:

- **Genetically Modified (GM) Crops:** Crops engineered to resist pests, tolerate drought, or grow in poor soil
- **Biofertilizers:** Natural microbes used to boost plant growth by fixing nitrogen or breaking down nutrients in the soil
- **Biopesticides:** Natural organisms or compounds that control pests without harmful chemicals

Learning Objectives

"Green" biotechnology improves farming practices to increase crop yields, enhance resistance, improve nutritional content, and reduce the need for chemical treatments. At the end of this page, you will be able to:

- Define green biotechnology
- List some key features of green biotechnology
- Discuss some applications of green biotechnology, such as the development of genetically-modified crops

Applications of Green Biotechnology

Throughout history, farmers have selectively bred plants and animals to achieve desirable traits, such as sweeter apples or larger corn cobs. However, this traditional method can be time-consuming and may inadvertently introduce unwanted characteristics. Biotechnology offers a more precise approach to developing desirable traits. These enhancements can include improved drought resistance, increased tolerance to pests and diseases, enhanced nutritional content in food, or the production of pharmaceuticals and industrial products. The applications described below are only a few examples of where "green" biotechnology can be applied.

Genetically Modified Crops & Foods

Genetically-modified (GM) crops are classified as **Genetically-Modified Organisms (GMOs)** - organisms whose genetic material has been altered using biotechnology. In botany, genetically-modified crops are used for multiple purposes, including to resist herbicides (e.g., "Roundup Ready" soybeans), prevent spoilage (e.g., Flavr Savr® tomatoes), and enhance nutrition (e.g., Golden Rice with added vitamin A).

When making a GMO, the "host" organism whose genome will be altered can range from something as simple as unicellular bacteria and yeast, to multicellular, complex organisms such as animals and plants. To read about how genetically-modified animals are created and used, go to Chapter 8: Animal Biotechnology. For plants, the introduction of a new gene is often done using a bacteria called *Agrobacterium tumefaciens,* a rod-shaped Gram-negative soil bacterium. In plants, *A. tumefaciens* causes "crown gall" disease, in which tumors, called "galls" form within the infected plant. These galls, often at the base of the stem or roots, can disrupt nutrient and water transport, weakening the plant and potentially leading to its death. As such, *A. tumefaciens* is a serious pathogen of many commercial crops like walnuts, grape vines, stone fruits, nut trees, sugar beets, and rhubarb. The persistent nature of the tumors caused by the disease make it particularly harmful for these crops.

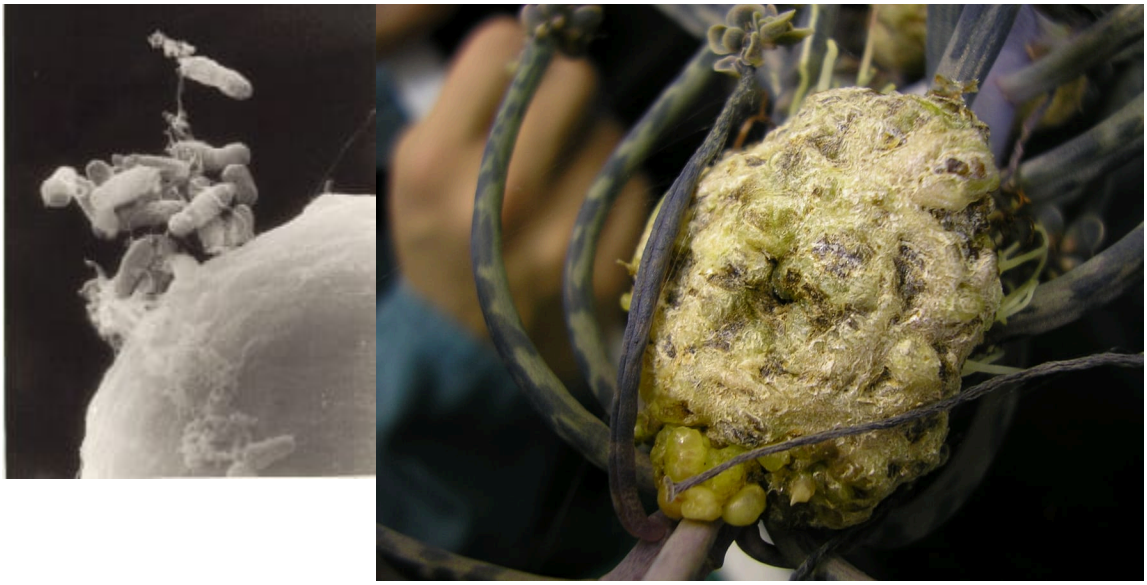


Figure 8.2.1: *Agrobacterium tumefaciens* and crown gall disease. Left image: *Agrobacterium tumefaciens* is a rod-shaped, Gram-negative bacteria that infects plant cells. (*Agrobacterium tumefaciens* by A. G. Matthysse, K. V. Holmes, R. H. G. Gurlitz; Public domain) Right image: Infection with *Agrobacterium tumefaciens* leads to "crown gall" disease - the production of several small tumors (called "galls") on the plant. (Crown Gall Disease by author unknown; CC BY-SA 3.0)

The tumors caused by *A. tumefaciens* are due to the presence of a tumor-inducing plasmid, known as the **Ti plasmid (or T-plasmid).** This plasmid contains all of the genes required for the bacteria to be virulent and efficiently infect plants cells. Once within the cell, the Ti plasmid incorporates a segment of its DNA, called **T-DNA**, into the genome of the host cell. This makes T-DNA an efficient gene delivery method that can be exploited in the laboratory. Today, Ti-based plasmids are used by researchers for the transfer and insertion of their desired gene into a plant genome (Figure 8.2.2). *A. tumefaciens*-infected cells with the Ti plasmid can be selected and used to regenerate new plant tissue. Because plant cells are "totipotent", a single plant cells can be used to recreate an entirely new plant. This means that the genetically-modified cells created using *Agrobacterium tumefaciens* can easily be grown into genetically-modified plants that are clones of the originating cell.

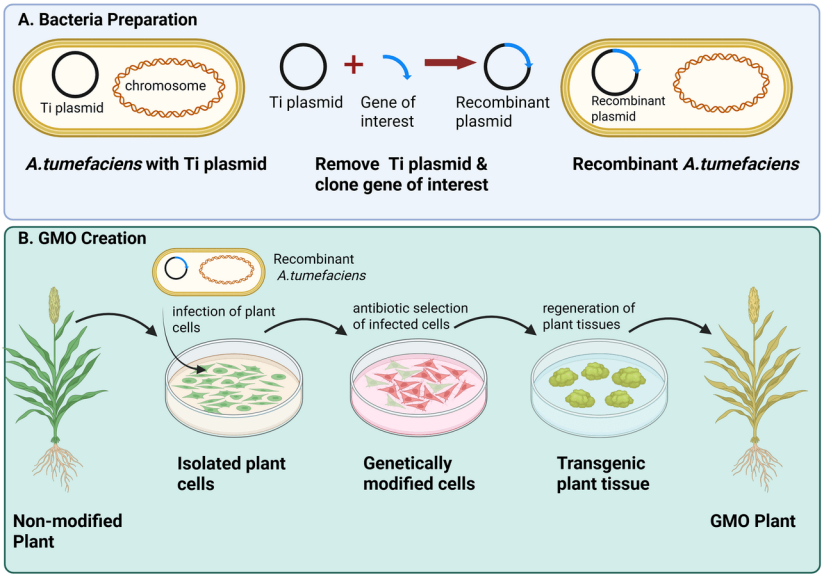


Figure 8.2.2: Production of a genetically-modified plant. (A) Bacteria Preparation (blue box at top of figure): *Agrobacterium tumefaciens* bacteria contain a plasmid called the Ti plasmid. The Ti plasmid is removed from *A.tumefaciens* bacterial cells (black circle) and the gene of interest (blue arrow) is inserted to make a recombinant Ti plasmid (black and blue circle). *A. tumefaciens* bacteria are transformed with this plasmid to produce recombinant *A. tumefaciens* cells. (B) GMO Creation (green box at bottom of figure): Non-modified plants are used for the isolation of plant cells. These plant cells are infected with recombinant *A. tumefaciens* cells. Antibiotic selection of infected cells selects for those genetically-modified cells in the culture (red cells). Expansion of these cells in culture produces transgenic plant tissue that can be developed into the GMO plant. (GMO Plant Production by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2024))

Certain plants, like wheat and corn, do not respond well to Agrobacterium-mediated transformation. As an alternative, botanists can use a **"gene gun"** to introduce their gene of interest into these plants. A gene gun, also known as a biolistic particle delivery system, can be used to deliver genetic material (like DNA or RNA) into prokaryotic or eukaryotic cells, but is typically used on plant cells (Figure 8.2.3). For this, the plant cells are plated onto a surface and placed into the gene gun. Tiny metal particles (usually gold or tungsten) are coated with DNA, containing the desired gene. The particles are loaded onto a plastic membrane or cartridge and placed inside the gene gun. The gene gun is aimed at the cells. A burst of compressed gas (like helium) or a high-voltage electric discharge is applied to the membrane, propelling the particles at high speed into the cells. The particles shoot through the cell walls and membranes, carrying the DNA into the cell interior. Some of the cells will then integrate the DNA into their genome for gene expression. Antibiotic resistance is used to identify those cells containing the integrated DNA. Following development under highly controlled conditions and genetic testing, the organism may be considered a GMO.

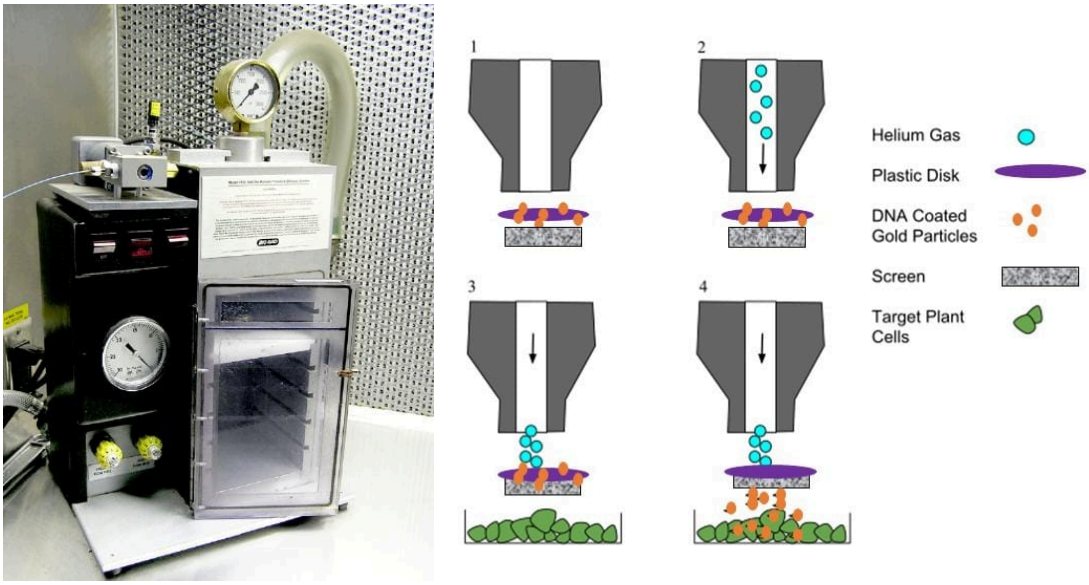


Figure 8.2.3: Gene delivery using a "gene gun". Left image: A picture of a gene gun with the chamber open to receive the cell sample. (Genegun by xmort; Public domain) Right image: 1. DNA-coated gold particles are loaded onto a plastic disk and placed in the gene gun on top of a screen. Cells are plated onto a surface and placed into the gun (not shown). 2. Helium gas fills the chamber and pressure builds against the gold-coated disk. 3. The pressure pushes the disk into the screen. 4. The DNA-coated gold particles are propelled through the screen and into the targeted plant cells. (How the Gene Gun Works by Patricia Zuk CC BY-SA 4.0; adapted from Gene Gun by RachelBrooks15, CC BY-SA 4.0)

While the introduction of genetic material into cells is typically done using **liposomal-mediation transfection** (see Lab Protocol 17.9 Liposomal transfection into mammalian cells), plant cells, with their rigid cell walls made of cellulose, make transfection with liposomes difficult. As such, botanists will use a gene gun to improve their transformation efficiency. This is because the gene gun is capable of "firing" genetic information through the cell wall and plasma membrane. In addition, the gene gun can introduce genetic material into a few plant cells, a single plant tissue or a specific plant organ (e.g. a leaf) in order to observe the effects of genetic manipulation without creating an entire genetically-modified plant.

GMOs like genetically-modified crops have helped address food security and resource sustainability. However, they also raise ethical and environmental concerns. Some have expressed concerns about the potential impacts of GMOs on biodiversity, long-term human health effects, and the monopolization of food resources by biotech companies, making GMOs a complex and often controversial area of modern science.

Biofertilizers and Biopesticides

Once of the focuses of green biotechnology is in the creation and use of biofertilizers and biopesticides that are eco-friendly and sustainable. A **biofertilizer** is a natural fertilizer that contains living microbes that help plants grow by increasing the supply or availability of nutrients in the soil. The types of microbes found will depend on the biofertilizer but can contain beneficial bacteria, fungi, or algae. One important function of biofertilizers is in **nitrogen fixation** - the process by which atmospheric nitrogen gas (N_2) is converted into ammonia (NH_3) or other nitrogenous compounds (Figure 8.2.4). These nitrogenous compounds are used by the plants to synthesize proteins.

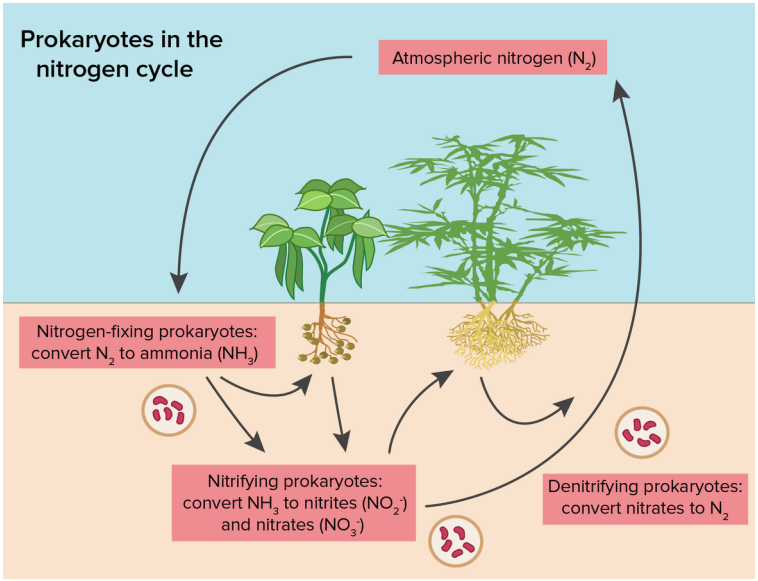


Figure 8.2.4: The nitrogen cycle. Nitrogen-fixing prokaryotes that live in soil and convert atmospheric N_2 intro ammonia (NH_3). In addition, nitrifying prokaryotics convert NH_3 into nitrites (NO_2^-) and nitrates (NO_3^-). Each of these compounds are used by plants. Denitrifying prokaryotes can convert nitrates back into N_2 which returns to the atmosphere. (Prokaryotes in the nitrogen cycle modified from Nitrogen cycle by Johann Dréo; CC BY-SA 3.0)

Biofertilizers are easily applied to seeds, soil, or to plant roots. The seeds can be coated with the biofertilizer prior to planting. This is common for legumes like beans and peas. Alternatively, the biofertilizer can be mixed with compost materials or carrier material, like peat, and the spread into the soil. Other techniques include root "dipping" in which the roots of the plants are dipped into a solution containing the biofertilizer and the spraying of a biofertilizer solution onto leaves. Table 8.2.1 lists some commonly used biofertilizers.

Table 8.2.1: Biofertilizers

Microbe	What They Do	Commonly Used In
Rhizobium (Gram-negative bacteria)	fix nitrogen into the nodules of roots	legumes (beans, peas, lentils)
Azospirillum (Gram-negative bacteria)	fix nitrogen into the soil around the root zone	corn, wheat, rice
Azotobacter (bacteria)	fix nitrogen into soil	vegetable, cereal crops
Cyanobacteria: *Anabaena sp., Nostoc sp.*	fix nitrogen into waterlogged soils	rice fields
Phosphate-solubilizing bacteria (PSB): *Bacillus* and *Pseudomonas* species	make soil phosphorus more available	many commercial crops
Mycorrhizal (fungi)	improve phosphate uptake and water absorption by plant roots	trees, vegetables, flowers

Biopesticides are biologically-based substances that control pests, such as insects, weeds, fungi, and bacteria. Biopesticides tend to be safer for humans, animals, and the environment. They don't pollute the soil or water and break down quickly in the environment. Biopesticides are target-specific as they usually affect only the pest and not beneficial organisms. Moreover, the targeted pests are less likely to develop resistance to the biopesticide. However, biopesticides can be slower-acting than chemical pesticides and may need specific conditions in order to work effectively.

There are several types of biopesticides, including:

- **Microbial biopesticides:** contain living organisms, like bacteria, fungi, viruses
  - e.g. *Bacillus thuringiensis (Bt)*
  - Bt produces toxins that kill insect larvae
- **Botanical pesticides:** pesticides extracted from plants

    - e.g. neem oil
    - disrupts insect hormones and feeding
- **Biochemical pesticides:** natural substances that affect pest behavior
    - e.g. insect sex pheromones
    - interfere with mating to reduce pest populations
- **Entomopathogenic fungi:** fungi that infect and kill insects
    - e.g. Beauveria spores infect and grow inside insect hosts

One of the most widely used biopesticides in both organic and conventional farming is the soil bacteria, *Bacillus thuringiensis* (Bt). Bt bacteria are used to control the populations of several moth and butterfly species, in addition to beetles, flies and mosquitoes. During sporulation, Bt cells produces **crystal (Cry) proteins,** which are also known as δ-endotoxins (Figure 8.2.5). When ingested by certain insects, the alkaline environment of the gut processes and activates the toxin. The activated form of the toxin binds to specific receptors expressed by the epithelial cells lining the mid-gut portion of the digestive system. Receptor activation results in the formation of pores in the epithelial lining. The insect stops feeding and dies within days to a few weeks. Because the receptors to the activated Cry proteins are found only in certain insect species, Bt is safe to beneficial insects, plant cells, and animals. In organic farming, Bt spores and toxins can be directly sprayed onto crops. In addition, genetically-modified crops, such as corn, cotton, and soybeans, have been engineered to produce the Bt toxins themselves. This genetic engineering involved use of a gene gun to introduce the relevant Bt genes.

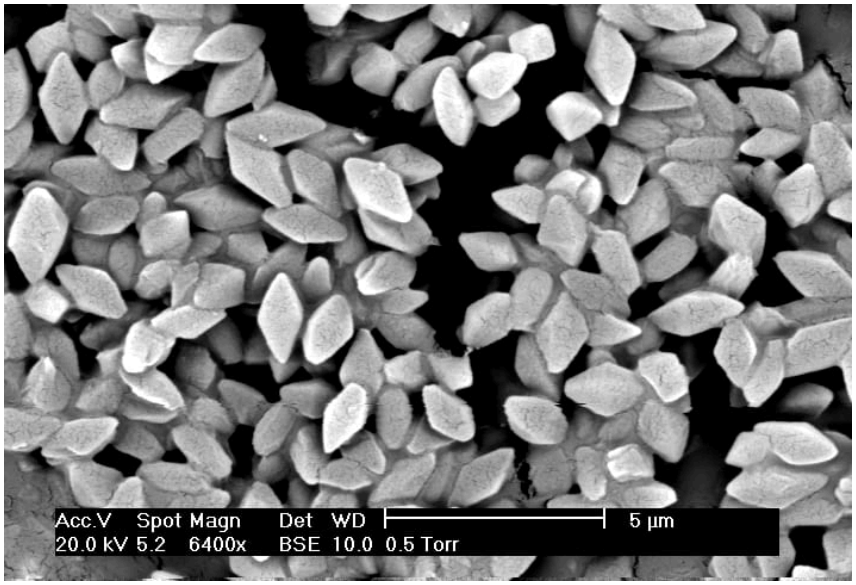


Figure 8.2.5: Bt-produced cry proteins are insecticidal towards certain insect pests. (Crystals of Bt Toxin by Jim Buckman; public domain)

Key Concepts

Applications of green and gray biotechnology can range from the creation of genetically-modified crops to the use of plants to trap heavy metals in soils. Some important concepts to remember are:

- some of the key applications of green biotechnology include genetically-modified crops, biopesticides, biofertilizers, and phytoremediation
- genetic modification of plant cells can be accomplished using a gene gun or transformation using the Ti plasmid of the soil bacteria, *Bacillus thuringiensis*
- biofertilizers contains living microbes that help plants grow by increasing the supply or availability of nutrients in the soil
- biofertilizers can be easily applied to seeds, soil, or to plant roots
- one important role of biofertilizers is nitrogen fixation
- biopesticides are biologically-based substances that control pests, such as insects, weeds, fungi, and bacteria
- there are several types of biopesticides including microbial, botanical, and biochemical
- the bacteria, *Bacillus thuringiensis,* is one of the most commonly used biopesticides in farming
- plants, and their associated microbes, can be used to clean up environmental pollutants through a process called phytoremediation

Glossary

Agriculture - the science and practice of growing crops and raising animals for food, fiber, fuel, and other products used to sustain and enhance human life

Agricultural biotechnology - a branch of biotechnology focused on using scientific tools and techniques, like genetic engineering and molecular markers, to improve plants, animals, and microorganisms used in farming; known as "green" biotechnology

Biofertilizer - a substance containing living microorganisms that, when applied to soil or plants, enhances nutrient availability—especially nitrogen and phosphorus—promoting healthy plant growth

Biopesticide - a type of pesticide derived from natural materials such as animals, plants, bacteria, or certain minerals, used to control pests in an environmentally friendly way

Botany - the scientific study of plants, including their structure, growth, reproduction, metabolism, development, diseases, and interactions with their environment

Crop - a plant that is cultivated and harvested for food, fiber, fuel, or other economic purposes

Genetically-modified (GM) crop - a crop whose DNA has been altered using genetic engineering techniques to introduce traits like pest resistance, herbicide tolerance, or improved nutritional content

Genetically-modified organism (GMO) - any organism whose DNA has been altered using genetic engineering techniques

Pest - any organism that damages or interferes with crops, livestock, food, or property—commonly includes insects, weeds, fungi, and rodents

Pesticide - a chemical or biological agent used to kill or control pests, including insects (insecticides), weeds (herbicides), fungi (fungicides), and others

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8.3: Aquaculture

Aquaculture is the practice of raising aquatic plant and animal species. Much like a terrestrial farm, aquaculture usually includes breeding, rearing, and sometimes harvesting the organisms being raised. Aquaculture typically has two main purposes:

- The first is for the commercial production of organisms. This can be for food, ornamental fish for aquaria, or products like pearls from oysters.
- The other main purpose of aquaculture is to captively breed and raise organisms to bolster wild populations that may be threatened or in decline. For example, there are many salmon hatcheries in California that are intended to supplement populations for commercial fisheries as well as for sport fishing.

Marine aquaculture is the raising marine organisms either in enclosed cages and nets in the ocean or in man-made tanks on land. The most common species produced in U.S. aquaculture operations are mainly oysters, clams, mussels, shrimp, and salmon. There is also production of cod, moi, yellowtail, barramundi, seabass, and seabream, but these are in much smaller quantities. The potential for aquaculture to provide protein sources to the rapidly increasing global population is very high, and research into this technology has been heavily invested in around the world. (Source: NOAA Fisheries - Office of Aquaculture)

There are some issues with aquaculture, however, and marine aquaculture in particular. When fish are raised in a protected and controlled environment, they become adapted to an artificial environment and lack much of the selection pressures that would normally influence genetic traits in wild populations. This often means that fish produced in hatcheries have a lower fitness when released than wild populations. When the hatchery raised fish are released into the wild to build up population sizes, they can compete and breed with wild populations. This has the possibility of reducing the overall fitness of wild populations. (Source: U.S. Fish and Wildlife Service)

- Aquaculture, otherwise known as fish farming refers to breeding, nurturing and harvesting plants and animals from all types of environments; Ex. Ponds, lakes, rivers, and oceans. It includes the production of shellfish and hatchery fish, which are raised to market size in tanks, nets, cages, raceways, or ponds. They are raised for a variety of uses; some will be used for selling in the market for food, however, some are used for stock restoration in which they are raised and then released back into the wild to restore wild populations. Sometimes the fish and plants are traded to corporations/businesses, such as aquariums. They are also grown for pharmaceutical, neurological, or biotechnical purposes. Aquaculture is used in both marine and freshwater environments. The most popular marine species farmed are muscles, clams, shrimp, oysters, and salmon. In freshwater it is dominated by catfish farming, however, tilapia, trout, and bass also have a fair amount of farming. The countries that farm seafood the most are: China at 62%, 26% for all Asian countries besides China, 4.5% from Europe, and 4.5% from the Americas (According to The State of World Fisheries and Aquaculture- 2010). World aquaculture also has a production worth of nearly $100 billion.

The benefits of aquaculture are far and large. They help with sustainability because it lessens the strain on natural stocks. Aquaculture stocks are consistent as well, meaning they are available year round and do not have limitations on how many individuals can be caught. They are also an integral part of the economy, providing thousands of jobs in operations and secondary services. Lastly, and probably the biggest benefit is that aquaculture can be good for the environment; recent studies by NOAA illustrate that aquaculture poses a low risk to the environment. One reason is because there is no bycatch and in some cases, such as shellfish, they feed of the native zooplankton and phytoplankton. Although aquaculture has many benefits for wild populations of the species being farmed, there are also drawbacks because of poor farming habits. One major issue is that farming puts pressure on wild populations that are used to create the fish pellets. It can also intensify the spread of disease to wild populations and compromise the gene pool of native populations if farmed and wild populations breed. Lastly, it can pollute the environment with excess nutrients, such as with excess food and wastes. While there are negative effects to aquaculture, they are often restricted to small areas and for short periods of time.

Link to Resource: columbianewsservice.com/wp-co...quaculture.jpg

Sources:

1. www.nmfs.noaa.gov/aquaculture...01.html#12what

2. www.nmfs.noaa.gov/aquaculture...uaculture.html

3. fishery.about.com/od/Benefits...reBenefits.htm

4. http://www-tc.pbs.org/emptyoceans/ed...s-and-Cons.pdf

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9: STEM CELLS, CLONING, AND GENE THERAPY

9.1: Stem Cells
9.2: Reproductive Cloning
9.3: Therapeutic Biotechnology
9.4: Regenerative Medicine
9.5: Principles of Medical Biotechnology
9.6: Diagnostic Biotechnology

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9.1: Stem Cells

Stem cells are cells that divide by mitosis to form either two stem cells, thus increasing the size of the stem cell "pool", or one daughter that goes on to differentiate, and one daughter that retains its stem-cell properties. How the choice is made is still unknown. However, several genes have been found whose activity prevents a daughter cell from differentiating.

Types of Stem Cells

Several adjectives are used to describe the developmental potential of stem cells; that is, the number of different kinds of differentiated cell that they can become.

1. **Totipotent cells.** In mammals, totipotent cells have the potential to become any type in the adult body and any cell of the extraembryonic membranes (e.g., placenta). The only totipotent cells are the **fertilized egg** and the first 4 or so cells produced by its cleavage (as shown by the ability of mammals to produce identical twins, triplets, etc.). In mammals, the expression totipotent **stem** cells is a misnomer — totipotent cells cannot make more of themselves.
2. **Pluripotent stem cells.** These are true stem cells, with the potential to make any differentiated cell in the body (but probably not those of the placenta which is derived from the trophoblast).

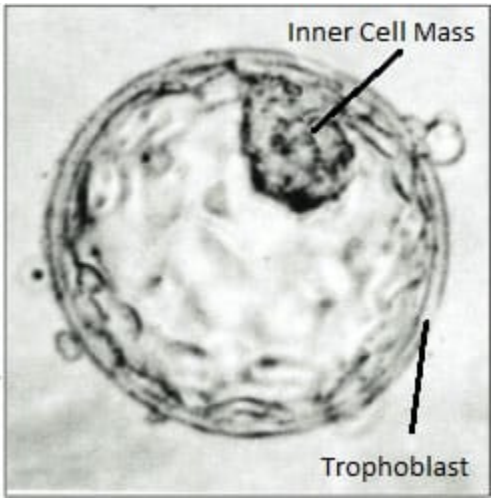


Figure 9.1.1: Human blastocyst showing inner cell mass (top right) and trophoblast. (J. Conaghan).

Three types of pluripotent stem cells occur naturally:

- **Embryonic Stem (ES) Cells.** These can be isolated from the **inner cell mass** (ICM) of the blastocyst — the stage of embryonic development when implantation occurs. For humans, excess embryos produced during in vitro fertilization (IVF) procedures are used. Harvesting ES cells from human blastocysts is controversial because it destroys the embryo, which could have been implanted to produce another baby (but often was simply going to be discarded).
- **Embryonic Germ (EG) Cells.** These can be isolated from the precursor to the gonads in aborted fetuses.
- **Embryonic Carcinoma (EC) Cells.** These can be isolated from teratocarcinomas, a tumor that occasionally occurs in a gonad of a fetus. Unlike the other two, they are usually aneuploid.

All three of these types of pluripotent stem cells can only be isolated from embryonic or fetal tissue. They can be grown in culture, but only with special methods to prevent them from differentiating.

In mice and rats, embryonic stem cells can also:

- contribute to the formation of a healthy chimeric adult when injected into a blastocyst which is then implanted in a surrogate mother;
- enter the germline of these animals; that is, contribute to their pool of gametes;
- develop into teratomas when injected into immunodeficient (SCID) mice. These tumors produce a wide variety of cell types

representing all three germ layers (ectoderm, mesoderm, and endoderm).

Using genetic manipulation in the laboratory, pluripotent stem cells can now be generated from differentiated cells. These **induced pluripotent stem cells** (iPSCs) are described below.

3. **Multipotent stem cells.** These are true stem cells but can only differentiate into a limited number of types. For example, the bone marrow contains multipotent stem cells that give rise to all the cells of the blood but not to other types of cells. Multipotent stem cells are found in adult animals; perhaps most organs in the body (e.g., brain, liver, lungs) contain them where they can replace dead or damaged cells. These **adult stem cells** may also be the cells that — when one accumulates sufficient mutations — produce a clone of cancer cells.

Stem Cells for Human Therapy

The Dream

Many medical problems arise from damage to differentiated cells. Examples:

- Type 1 diabetes mellitus where the beta cells of the pancreas have been destroyed by an autoimmune attack
- **Parkinson's disease**; where dopamine-secreting cells of the brain have been destroyed
- Spinal cord injuries leading to paralysis of the skeletal muscles
- Ischemic stroke where a blood clot in the brain has caused neurons to die from oxygen starvation
- **Multiple sclerosis** with its loss of myelin sheaths around axons
- Blindness caused by damage to the cornea

The great developmental potential of stem cells has created intense research into enlisting them to aid in replacing the lost cells of such disorders. While progress has been slow, some procedures already show promise. Using multipotent "adult" stem cells.

- culturing human epithelial stem cells and using their differentiated progeny to replace a damaged cornea. This works best when the stem cells are from the patient (e.g. from the other eye). Corneal cells from another person (an allograft) are always at risk of rejection by the recipient's immune system.
- the successful repair of a damaged left bronchus using a section of a donated trachea that was first cleansed of all donor cells and then seeded with the recipient's epithelial cells and cartilage-forming cells grown from stem cells in her bone marrow. So far the patient is doing well and needs no drugs to suppress her immune system.

Using differentiated cells derived from embryonic stem (ES) cells. Phase I clinical trials are underway to assess the safety of

- injecting retinal cells derived from ES cells
  - into the eyes of young people with an inherited form of juvenile blindness;
  - into the eyes of adults with age-related macular degeneration.
- injecting glial cells derived from ES cells into patients paralyzed by spinal cord injuries.

The Immunological Problems

One major problem that must be solved before human stem cell therapy becomes a reality is the threat of rejection of the transplanted cells by the host's immune system (if the stem cells are allografts; that is, come from a genetically-different individual).

A Possible Solution

One way to avoid the problem of rejection is to use stem cells that are genetically identical to the host. This is already possible in the rare situations when the patient has healthy stem cells in an undamaged part of the body (like the stem cells being used to replace damaged corneas). But even where no "autologous" stems cells are available, there may be a solution: using **somatic-cell nuclear transfer** .

In this technique,

1. An egg has its own nucleus removed and replaced by
2. a nucleus taken from a somatic (e.g., skin) cell of the donor.
3. The now-diploid egg is allowed to develop in culture to the blastocyst stage when
4. embryonic stem cells can be harvested and grown up in culture.
5. When they have acquired the desired properties, they can be implanted in the donor with no fear of rejection.

Using this procedure it possible to not only grow blastocysts but even have these go on to develop into adult animals — **cloning** — with a nuclear genome identical to that of the donor of the nucleus. The first successful cloning by SCNT was with amphibians. Later, mammals such as sheep (Dolly), cows, mice and others were successfully cloned. And in the 11 November 2007 issue of **Science**, researchers in Oregon reported success with steps 1–4 in rhesus monkeys (primates like us).

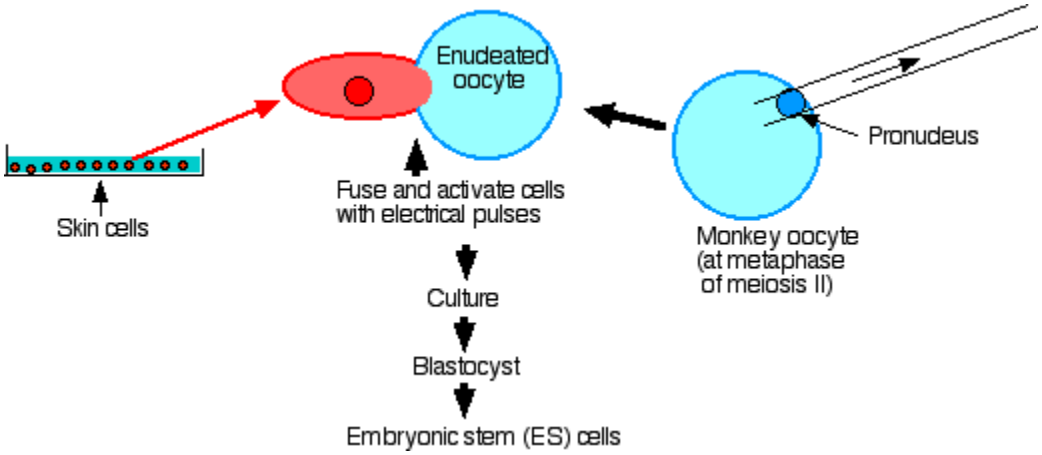


Figure 9.1.2: Somatic cell transfer in rhesus monkeys

Their procedure:

- Remove the spindle and thus all nuclear material from secondary oocytes at metaphase of meiosis II.
- Fuse each enucleated egg with a skin cell taken from a male monkey.
- Culture until the blastocyst stage is reached.
- Extract embryonic stem cells from the inner cell mass.
- Establish that they have the nuclear genome of the male (but mostly the mitochondrial genome of the female).
- Culture with factors to encourage differentiation: they grew cardiac muscle cells (which contracted), and even neuron-like cells.
- Inject into SCID mice and examine the tumors that formed. These contained cells of all three germ layers: ectoderm, mesoderm, and endoderm.
- However, even after more than 100 attempts, they have not been able to implant their monkey blastocysts in the uterus of a surrogate mother to produce a cloned monkey.

This should reassure people who view with alarm the report in May 2013 by the same workers that they have finally succeeded in producing embryonic stem cells (ESCs) using SCNT from differentiated human tissue. The workers assure us that they will not attempt to implant these blastocysts in a surrogate mother to produce a cloned human. And their failure with monkeys suggests that they would fail even if they did try.

While cloning humans still seems impossible, patient-specific ESCs

- could be used in cell-replacement therapy or, failing that,
- provide the material for laboratory study of the basis of — and perhaps treatment of — genetic diseases.

Whether they will be more efficient and more useful than induced pluripotent stem cells remains to be seen.

Questions that Remain to be Answered

- **Imprinted Genes.** Sperm and eggs each contain certain genes that carry an "imprint" identifying them later in the fertilized egg as being derived from the father or mother respectively. Creating an egg with a nucleus taken from an adult cell may not

allow a proper pattern of imprinting to be established. When the diploid adult nucleus is inserted into the enucleated egg (at least those of sheep and mice), the new nucleus becomes "reprogrammed". What reprogramming actually means still must be learned, but perhaps it involves the proper methylation and demethylation of imprinted genes. For example, the inactive X chromosome in adult female cells must be reactivated in the egg, and this actually seems to happen.

- **Aneuploidy.** In primates (in contrast to sheep, cattle, and mice), the process of removing the resident nucleus causes molecules associated with the centrosome to be lost as well. Although injecting a donor nucleus allows mitosis to begin, spindle formation may be disrupted, and the resulting cells fail to get the correct complement of chromosomes (aneuploidy).
- **Somatic Mutations.** This procedure also raises the spectre of amplifying the effect(s) of somatic mutations. In other words, mutations that might be well-tolerated in a single somatic cell of the adult (used to provide the nucleus) might well turn out to be quite harmful when they become replicated in a clone of cells injected later into the patient.
- **Political Controversy.** The goal of this procedure (which is often called **therapeutic cloning** even though no new individual is produced) is to culture a blastocyst that can serve as a source of **ES cells**. But that same blastocyst could theoretically be implanted in a human uterus and develop into a baby that was genetically identical to the donor of the nucleus. In this way, a human would be cloned. And in fact, Dolly and other animals are now routinely cloned this way. The spectre of this is so abhorrent to many that they would like to see the procedure banned despite its promise for helping humans. In fact, many are so strongly opposed to using human blastocysts — even when produced by nuclear transfer — that they would like to limit stem cell research to adult stem cells (even though these are only multipotent).

Possible Solutions to the Ethical Controversy

Induced pluripotent stem cells (iPSCs)

A promising alternative to the use of embryonic stem cells in human therapy are recently-developed methods of genetically reprogramming the nuclei of differentiated **adult** cells so that they regain the pluripotency of embryonic stem (ES) cells.

In June 2007, three laboratories reported that introducing extra copies of only 4 genes into adult mouse skin cells (fibroblasts) enables them to regain the properties of ES cells. When these cells, named **induced pluripotent stem cells** (iPSCs for short), were placed in mouse blastocysts, they participated in building all the tissues of the chimeric mice that resulted. (When placed in tetraploid (**4n**) blastocysts — unable by themselves to develop normally — embryos were formed that thus were clones of the skin cell donor.) The four genes: *c-Myc*, *Sox2*, *Oct3/4*, *Klf4*.

By 2009, several labs had succeeded in producing fertile adult mice from iPSCs derived from mouse embryonic fibroblasts. This shows that iPSCs are just a capable of driving complete development (pluripotency) as embryonic stem cells.

Reprogramming works in humans, too! Using the same four genes, the Yamanaka lab in Japan reported on 20 November 2007, that they now had reprogrammed human skin cells to become induced pluripotent stem cells (**iPSCs**). And the Thomson lab in Wisconsin accomplished the same thing using *SOX2*, *OCT4*, *NANOG*, and *LIN28*.

Further evidence of the remarkable role played by these few genes is the finding that during normal embryonic development of the zebrafish, the same or similar genes (SoxB1, Oct4, Nanog) are responsible for turning on the genes of the zygote. Earlier in development of the blastula, all the genes being expressed (including these) are the mother's — mRNAs and proteins that the mother deposited in the unfertilized egg. It makes sense that the same proteins that can reprogram a differentiated cell into a pluripotent state (iPSCs) are those that produce the pluripotent cells of the early embryo.

These achievements open the possibility of

- creating cells for laboratory study of the basis of genetic diseases.
  Examples: researchers have succeeded in deriving iPSCs from
  - patients with amyotrophic lateral sclerosis (ALS, "Lou Gehrig's disease"), and then causing them to differentiate into motor neurons (the cells affected in the disease) for study of their properties;
  - the skin cells of a patient with an inherited heart disease (long QT syndrome) and causing these to differentiate into beating heart cells for study in the laboratory.
  - The Jaenisch lab reported in the 6 March 2009 issue of **Cell** that they have succeeded in making iPSCs (they call them hiPSCs) from fibroblasts taken from patients with Parkinson's disease. The cells were then differentiated into dopamine-releasing cells — the cells lacking in this disease. What is particularly exciting is that they accomplished this after using the Cre-lox system to remove all the genes (e.g., *SOX2*, *OCT4*, *KLF4*) needed for reprogramming the fibroblasts to an embryonic-stem-cell-like condition.

    - Since that report, other laboratories — using other methods — have also created iPSCs from which all foreign DNA (vector and transgenes) has been removed. Not only should such cells be safer to use in therapy, but these results show that the stimulus to reprogram a differentiated cell into a pluripotent state need only be transitory.
- creating patient-specific cell transplants — avoiding the threat of immunological rejection — that could be used for human therapy.

  Therapy with iPSCs has already been demonstrated in mice. Three examples:

  1. The Jaenisch lab in Cambridge, MA reported (in **Science**, 21 December 2007) that they had successfully treated knock-in mice that make sickle-cell hemoglobin with the human β^S genes (and show many of the signs of sickle-cell disease in humans) by
    - harvesting some fully-differentiated fibroblasts from a sickle-cell mouse;
    - reprogramming these to become iPSCs by infecting them with *Oct4*, *Sox2*, *Klf4*, and *c-Myc*;
    - then removing (using the Cre-lox system) the *c-Myc* to avoid the danger of this oncogene later causing cancer in the recipient mice;
    - replacing the β^S genes in the iPSCs with normal human β^A genes;
    - coaxing, with a cocktail of cytokines, these iPSCs to differentiate in vitro into hematopoietic (blood cell) precursors;
    - injecting these into sickle-cell mice that had been irradiated to destroy their own bone marrow (as is done with human bone marrow transplants). (Although the recipient mice were different animals from the fibroblast donor, they were of the same inbred strain and thus genetically the same — like identical human twins. So the procedure fully qualifies as "**patient-specific**", i.e., with no danger of the injected cells being rejected by the recipient's immune system.)

  The result: all the signs of sickle-cell disease (e.g., anemia) in the treated animals showed marked improvement.

  2. In the 25 July 2013 issue of *Nature*, a team of Japanese scientists report that they were able to manufacture three-dimensional buds of human liver cells. Their process:
    - create human iPSCs from human fibroblasts using the techniques described above;
    - treat these with the substances needed for them to differentiate in liver cell precursors;
    - culture these with a mixture of human endothelial cells and mesenchymal stem cells (to mimic the conditions that occur in normal embryonic development of the liver);
    - implant the resulting solid masses (buds) of liver-like cells into immunodeficient mice.

  The result: the implanted buds developed a blood supply and the mice began to secrete human albumin, human alpha-1-antitrypsin, and to to detoxify injected chemicals just as human livers do.

  3. Workers in the Melton lab at Harvard University reported in the 9 October 2014 issue of **Cell** that they had succeeded in differentiating large numbers of human beta cells from human iPSCs (as well as from human ES cells). When transplanted into diabetic mice, these cells brought their elevated blood sugar levels back down.

Let us hope that what works in mice can someday be developed into a safe therapy that will work in humans. (In the case of Type 1 diabetes mellitus, however, even patient-derived beta cells will still be at risk of the same autoimmune rejection that caused the disease in the first place.)

Despite these successes, iPSCs may not be able to completely replace the need for embryonic stem cells and may even be dangerous to use in human therapy. Several groups have found that human iPSCs contain mutations as well as epigenetic patterns (e.g., methylation of their DNA) that are not found in embryonic stem cells. Some of the mutations are also commonly found in cancer cells.

Other approaches being explored

- ES cells can be derived from a **single cell** removed from an 8-cell **morula**. The success of preimplantation genetic diagnosis (PGD) in humans shows that removing a single cell from the morula does not destroy it — the remaining cells can develop into a blastocyst, implant, and develop into a healthy baby. Furthermore, the single cell removed for PGD can first be allowed to divide with one daughter used for PGD and the other a potential source of an ES cell line.

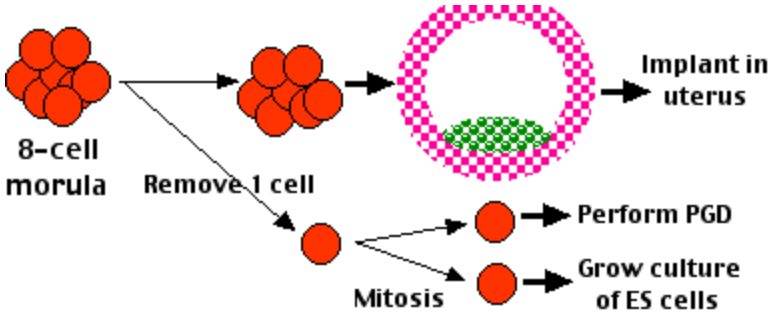


Figure 9.1.1: Preimplantation using ES cell

- In altered nuclear transfer (**ANT**) — a modified version of SCNT (somatic-cell nuclear transfer) — a gene necessary for later implantation (*Cdx2* — encoding a homeobox transcription factor) is turned off (by RNA interference) in the donor nucleus before the nucleus is inserted into the egg. The blastocyst that develops
  - has a defective trophoblast that cannot implant in a uterus
  - but the cells of the inner cell mass are still capable of developing into cultures of ES cells. (The gene encoding the interfering RNA can then be removed using the Cre/*loxP* technique.)
- Jose Cibelli and his team at Advanced Cell Technology reported in the 1 February 2002 issue of **Science** that they had succeeded inIf this form of cloning by parthenogenesis works in humans [It does! — success with unfertilized human eggs was reported in June 2007.], it would have
  - stimulating monkey oocytes to begin dividing without completing meiosis II (therefore still **2n**)
  - growing these until the blastocyst stage, from which they were able to harvest
  - ES cells.
  - the **advantage** that no babies could be produced if the blastocyst should be implanted (two identical genomes cannot produce a viable mammal — probably because of incorrect imprinting);
  - the **disadvantage** that it will only help females (because only they can provide an oocyte!) (But men may have a procedure that works for them next.)
- On 24 March 2006, **Nature** published an online report that a group of German scientists had been able to derive pluripotent stem cells from the stem cells that make spermatogonia in the mouse. Both in vitro and when injected into mouse blastocysts, these cells differentiated in a variety of ways including representatives of all three germ layers. If this could work in humans, it would
  - provide a source of stem cells whose descendants would be "patient-specific"; that is, could be transplanted back into the donor (men only!) without fear of immune rejection.
  - avoid the controversy surrounding the need to destroy human blastocysts to provide embryonic stem cells.
- The 7 January 2007 issue of **Nature Biotechnology** reports the successful production of amniotic fluid-derived stem cells ("AFS"). These are present in the amniotic fluid removed during amniocentesis. With the proper culture conditions, they have been shown to be able to differentiate into a variety of cell types includingSo these cells are pluripotent. Although perhaps not as versatile as embryonic stem cells, they are more versatile than adult stem cells.
  - ectoderm (neural tissue)
  - mesoderm (e.g., bone, muscle)
  - endoderm (e.g., liver)

Applied to humans, none of the above procedures would involve the destruction of a potential human life.

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9.2: Reproductive Cloning

Learning Objectives

- Differentiate reproductive cloning from cellular and molecular cloning

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.

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.

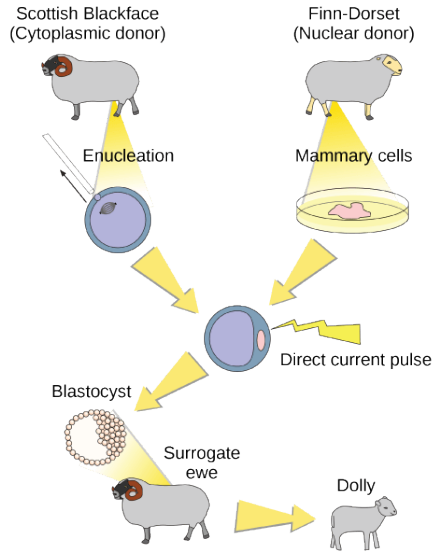


Figure 9.2.1: Reproductive Cloning of Dolly, the Sheep: Dolly the sheep was the first mammal to be cloned. To create Dolly, the nucleus was removed from a donor egg cell. The nucleus from a second sheep was then introduced into the cell, which was allowed to divide to the blastocyst stage before being implanted in a surrogate mother.

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.

Glossary

- **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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9.3: Therapeutic Biotechnology

Biotech Focus

The discovery of insulin in 1922, by Frederick Banting and Charles Best, marked a major breakthrough in medicine and therapy for patients with Type I diabetes. However until the 1970s, Type I diabetics had to rely upon insulin isolated from the pancreas of either cattle or pigs. This necessitated the slaughter of hundreds of thousands of animals each year and could also produce significant immune reactions in certain diabetics. In response, in 1978, David Goeddel and his colleagues at Genentech, successfully inserted the gene for human insulin into *E.coli* cells and confirmed its production by these genetically-modified bacteria. In 1982, recombinant human insulin was approved by the FDA and introduced to the market as **Humulin®** - the first genetically-engineered drug to be approved for human use.

Introduction

Therapeutic biotechnology uses biological systems, organisms, or processes for the development of medical treatments that diagnose, prevent, or cure diseases and disorders. This area of biotechnology relies on the use of tools like recombinant DNA (rDNA) technology, genetic engineering, and gene therapy to create targeted therapies for conditions such as cancer, genetic disorders, autoimmune diseases, and infections. Using these tools, researchers have developed specialized therapies, including monoclonal antibodies, recombinant proteins, and vaccines. Through therapeutic biotechnology, researchers have transformed medicine and significantly improved human health.

Learning Objectives

Through therapeutic biotechnology, researchers can create medical treatments that diagnose, prevent, or cure diseases and disorders. At the end of this page, you will be able to:

- Define therapeutic biotechnology
- Explain gene therapy and some of it approaches
- List some of the pros and cons of viral vectors used in gene therapy
- Explain CAR-T gene therapy and how it can be used to cure cancer
- Explain the potential role of CRISPR in gene therapy
- Define what a therapeutic protein is and explain some major examples
- Explain the difference between a traditional and therapeutic vaccine
- Explain how an mRNA vaccine is made

Gene Therapy

Gene therapy is a therapeutic technique that aims to correct an underlying genetic problem by modifying a person's genes. Through gene therapy a disease can be cured rather than treated. The first step of gene therapy is to identify the faulty gene. Often, the gene at the heart of the issue has been mutated in some way, preventing it from being made or functioning properly. The mutated gene (or genes) can be identified through DNA sequencing and genomic analysis. Once the gene (or genes) has been identified, researchers can begin to design a therapeutic strategy using a gene therapy approach.

There are several approaches used in gene therapy, including:

- Replacing the faulty or missing gene with a normal copy (i.e., "traditional" gene therapy)
- Inactivating or "silencing" the malfunctioning gene using miRNA or siRNA technologies
- Introduction of a new or modified gene to counteract the disease or improve cell function
- Correction of the gene mutation *in situ* through gene editing like CRISPR technology

Traditional gene therapy involves the replacement of a faulty or missing gene through the introduction of "normal" one. This

approach has been used in the treatment of diseases, like cystic fibrosis. Cystic fibrosis can be caused by one of several mutations in the **Cystic Fibrosis Transmembrane conductance Regulato**r (CFTR) gene, a gene that codes for a chloride channel found in the plasma membrane of epithelial cells. This chloride channel regulates the movement of chloride and sodium ions across the plasma membrane and controls the balance of salt and water across the surfaces of epithelial tissues lining the respiratory and digestive systems. When this channel functions properly, it helps keep the mucus coating these epithelial linings thin and slippery. Mutations in the CFTR gene lead to a faulty or absent CFTR protein, resulting in thick and sticky mucus that clogs airways, traps bacteria, and blocks pancreatic enzymes (Figure 9.3.1). Patients suffering from cystic fibrosis have significant breathing problems, lung infections, and digestive issues. Most cystic fibrosis patients will die between the ages of 35 and 50. The introduction of a functional CFTR gene through gene therapy could potentially cure this disease.

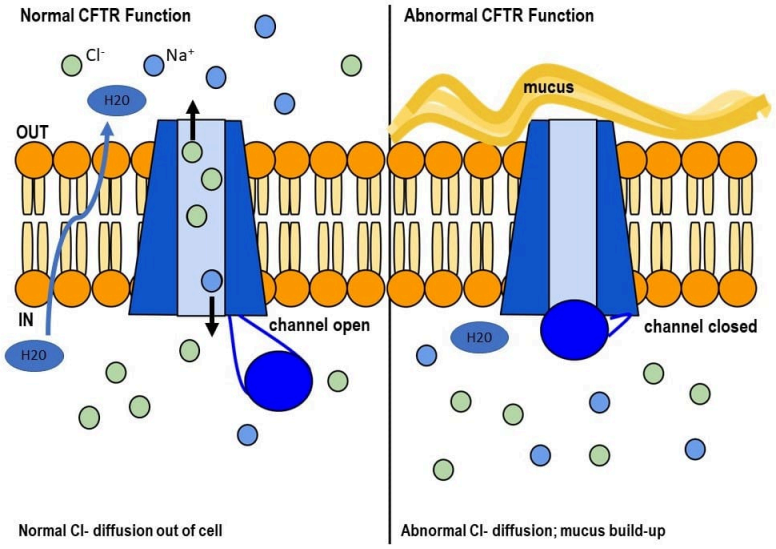


Figure 9.3.1: Cystic fibrosis (CF) and gene therapy. CF is caused by an abnormal CFTR channel protein. The CFTR controls the flow of sodium and chloride ions in and out of the epithelial cells lining the respiratory and digestive systems. In normal CFTR channel function (left panel), the channel is open and chloride ions (Cl-) and sodium ions (Na+) diffuse down their concentration gradients (black arrows). As a result, water (H_2O) moves out of the cell through osmosis, minimizing the thickness of the mucus layer covering the cell. In abnormal CFTR function (right panel), the channel is closed to ion diffusion. Abnormal ion diffusion prevents the osmotic movement of water out of the cell and a thick build-up of mucus outside of the cell results. (CFTR Function in CF by Patricia Zuk, CC BY 4.0; adapted from CFTR Protein Panels by Lbudd14, CC BY-SA 3.0)

Traditional gene therapy to treat diseases like cystic fibrosis was first introduced over thirty years ago to limited success. Challenges associated with the limitations in the size of genes that could be corrected, coupled to restrictions in the diseases that could be targeted, prompted researchers to explore other approaches. One growing research is the use of gene therapy to deliver **microRNA (miRNA)** or **small interfering RNA (siRNA**). As described in Chapter 5.5:Functional Genomics, miRNAs are small non-coding RNA molecules (about 22 nucleotides long) that regulate gene expression by binding to messenger RNAs (mRNAs) and either blocking their translation or causing their degradation. In gene therapy, therapeutic miRNAs could be designed and delivered to down-regulate disease-causing genes, such as oncogenes in cancer. In some diseases, certain miRNAs are underexpressed. By replacing them, normal gene regulation and cell function could be restored. Finally, diseases caused by overactive miRNAs that silence beneficial genes could be inhibited by gene therapy-mediated delivery of anti-miRs or "miRNA sponges", a synthetic or naturally occurring RNA molecule designed to bind (i.e."soak up") microRNAs.

No matter the approach, there are some common steps used in gene therapy. The therapeutic gene is inserted into a delivery system which is usually a viral vector that has been engineered to be safe and non-replicating. The genetic material is then introduced either directly into a patient through intravenous or intramuscular injection (i.e., *in vivo* introduction), or a patient's cells can be removed for genetic manipulation and then reintroduced (i.e., *ex vivo* introduction). Once inside the cells, the new gene will be read by the cell machinery, allowing it to produce a functional protein that restores normal function or fights the disease.

While alternatives to the use of viral vectors are being explored in gene therapy, including the packaging of the corrective genetic material into lipid nanoparticles or direct electroporation into cells, the most common delivery system still remains the viral vector. The choice of viral vector will depend on the size of the gene being cloned, the targeted cell, and the possible immune reaction of the patient.

Common viral vectors include:

1. **Adeno-Associated Virus (AAV)**
   - Pros: Low immune response, doesn't integrate into the genome and reduces risk of genetic mutations, long-lasting expression in non-dividing cells
   - Cons: Small gene capacity (~4.7 kb), requires repeated dosing in dividing cells
   - Currently used for: Eye disorders (e.g., Luxturna for retinal disease), spinal muscular atrophy
2. **Adenovirus**
   - Pros: High gene capacity, efficient delivery to many cell types, fast expression
   - Cons: Strong immune response, usually doesn't integrate into DNA (i.e., temporary expression)
   - Currently used for: Cancer gene therapy, vaccines (e.g., some COVID-19 vaccines)
3. **Lentivirus**
   - Pros: Integrates into the genome for long-term expression, works well in dividing and non-dividing cells
   - Cons: Risk of insertional mutagenesis due to genomic integration, more complex production
   - Currently used for: Blood disorders (e.g., sickle cell disease, β-thalassemia), HIV-related therapies

CAR-T Gene Therapy

***Ex vivo* gene therapy** methods (also called cell-based gene therapy) involve the modification of a patient's cells outside of their body, followed by their re-introduction. The most successful method being used currently is **CAR-T gene therapy** for the treatment of a variety of blood cancers, including leukemia, lymphoma, and multiple myeloma. In CAR-T therapy, a patient's T cells are extracted from their blood and genetically modified in a lab through the introduction of a gene for the **chimeric antigen receptor/CAR.** Expression of this gene results in the expression of the CAR on the surface of the T cell. The modified T cells are expanded in a cell culture facility and infused into the patient's bloodstream (Figure 9.3.2). Through the CAR, the T cells can bind antigens specific to cancer cells and destroy them. While showing promise, significant side effects of CAR-T therapy have been observed including neurological issues and "cytokine release syndrome" which results in fever, shortness of breath, low blood pressure, and in rare cases, heart or lung issues. The FDA is current investigating instances of secondary T-cell lymphomas in people who have been treated with CAR T-cell therapy.

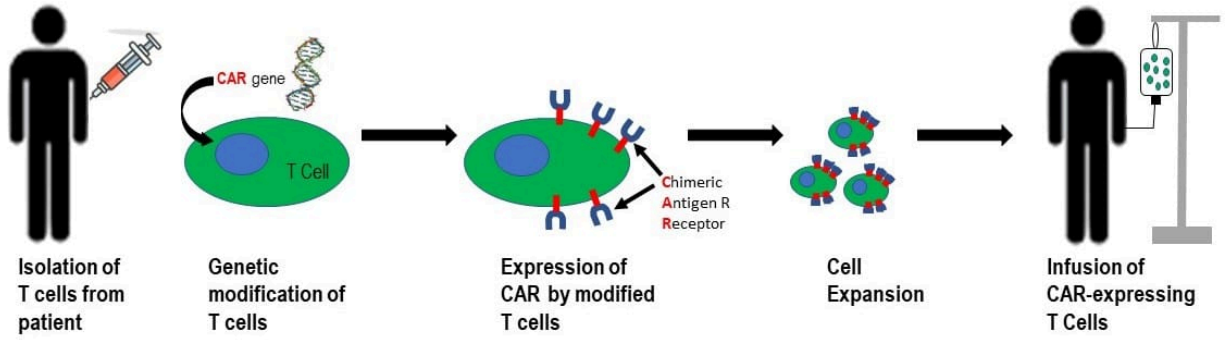


Figure 9.3.2: CAR-T gene therapy. T cells are isolated from a patient and genetically modified by introducing the gene for the Chimeric Antigen Receptor (CAR gene). Gene expression of this gene leads to the expression of the CAR on the surface of T cells. The modified T cells are expanded in number and then infused back into the patient for the targeted of cancerous cells. (CAR-T Therapy Overview by Patricia Zuk, CC BY 4.0)

Gene Therapy and CRISPR

Early trials in gene therapy approaches showed promise but demonstrated limited effectiveness due to challenges like immune responses and short-lived expression. Newer approaches include CRISPR-based gene editing, which aims to correct the mutation directly in the patient's DNA without the need to introduce additional genetic information. As outlined in Chapter 8.1: Applications of Animal Biotechnology, **CRISPR**, or Clustered Regularly Interspaced Short Palindromic Repeats, is derived from a bacterial defense mechanism that protects bacteria from the integration of viral DNA into the bacterial genome. CRISPR uses a **guide RNA**

and the **Cas9 enzyme** to precisely cut DNA at specific locations in the genome. Researchers are exploring its use in correcting, deleting, or replacing genes, making it a powerful tool in combination with gene therapy.

Current gene therapy applications using CRISPR include:

- **Sickle Cell Disease:** used *ex vivo* to edit a patient's blood stem cells in order to "reactivate" fetal hemoglobin and compensate for the defective hemoglobin gene. The edited cells are reinfused into the patient.
- **Leber Congenital Amaurosis (LCA):** uses CRISPR to edit retinal cells *in vivo* to restore vision in patients with this inherited form of blindness
- **Beta-Thalassemia:** similar to sickle cell, CRISPR is being used to edit the genome of bone marrow stem cells to boost healthy hemoglobin production

While the results are exciting, there are limitations to gene therapy and CRISPR. Chief among them is a concern about "off-target" effects. CRISPR may cut unintended parts of the genome and could introduce unintended mutations. Delivery challenges also exist. Getting CRISPR components safely and efficiently into the right cells using viral vectors or nanoparticles is still an obstacle. Finally, an immune response to Cas9 may result in a significant decline in the effectiveness of this technology.

Concept in Action

CRISPR Gene Editing of Sickle-Cell Anemia

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

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Therapeutic Proteins

Therapeutic biotechnology is critical for the development of therapeutic proteins. A **therapeutic protein** is a biologically-engineered protein that is used to treat disease by replacing, enhancing, or modifying a specific physiological function or functions. Therapeutic proteins are typically produced by genetically-modified bacteria, yeast, or mammalian cells that have been modified using rDNA technology. However, therapeutic proteins can also be produced by genetically-modified organisms (i.e., transgenic organisms), such as plants and animals.

There are numerous types of therapeutic proteins with specific functions, including:

- **Enzyme Replacement Therapy (ERT) proteins**: replace missing or defective enzymes in patients with genetic disorders; examples include:
  - lactase: produced to treat lactose intolerance
  - alpha-glucosidase (i.e., Myozyme™); used to treat for Pompe disease
- **Hormones**: used to regulate biological processes like metabolism, growth, and reproduction; examples include:
  - Insulin: produced by either genetically-modified bacteria or yeast; used to treat Type I diabetes without the need for animal donors
  - Human Growth Hormone (HGH): produced using genetically-engineered bacteria; used to treat growth hormone deficiencies in children and adults.
  - Erythropoietin (EPO): produced by genetically-modified mammalian cells; used to stimulate red blood cell production in patients with anemia; also used/abused by endurance athletes such as marathon runners, triathletes, and long-distance cyclers
- **Clotting Factors:** used in the treatment of blood clotting disorders; examples include:
  - Factor VIII and IX: produced using genetically-engineered mammalian cells; used to treat hemophilia and ensure safety and supply without relying on human donors.
- **Monoclonal Antibodies**: target specific antigens for treating cancer, autoimmune diseases, and infections; examples include:
  - Trastuzumab™ (anti-HER2 receptor antibody; Herceptin): produced by genetically-modified mammalian cells; used to

treat certain types of breast and gastric cancers by binding the Human Epidermal Growth Factor Receptor Type 2 (HER2), blocking its function and triggering its destruction by the immune system
    - Adalimumab™ (anti-TNF alpha antibody; Humira): produced by genetically-modified mammalian cells; used to treat certain autoimmune disorders by blocking the function of Tumor Necrosis Factor-alpha (TNF-alpha)
- **Immune modulators:** produced to stimulate or regulate the immune system; examples include
    - Interferons: produced by genetically-modified bacteria or yeast; used to treat certain cancers, multiple sclerosis, and viral infections
    - Interleukin-2: produced by genetically-modified bacteria; used to boost the immune response during chemotherapy

Most therapeutic proteins are produced by genetically-modified bacteria, yeast, or mammalian cells. The production of the protein starts with the selection of the desired gene. This gene is then cloned into a suitable plasmid and the resulting recombinant plasmid is then introduced into the genome of the "host cell" (e.g bacteria or mammalian cell). The modified cells are then grown in the laboratory and the protein isolated. Following extensive confirmation that the therapeutic protein is made properly and functions effectively, the protein is then introduced into the commercial market and made through precise manufacturing processes. To learn more about this DNA cloning process, go to Chapter 4.2: Creating Recombinant DNA.

While the approach for genetically-modifying cells for the production of therapeutic proteins is generally the same, the technique used to introduce the genetic information into a cell depends on the cell type (Figure 9.3.3). For bacteria, this technique is called **transformation**. For mammalian cells, genetic information is introduced through liposomal **transfection**. In transformation, bacteria are treated with ice-cold solutions to weaken their outer wall and plasma membrane, making them "competent" for transformation. They are then mixed with the recombinant plasmid and "heat shocked" to induce internalization of the plasmid. For transfection, the recombinant plasmid is coupled to microscopic **liposomes** that bind to the mammalian cells and are internalized through endocytosis. To learn about transformation, go to Chapter 13.6 Lab Technique: Bacterial Transformation. To learn about transfection, go to Chapter 13.10 Lab Technique: Liposomal Transfection into Mammalian Cells.

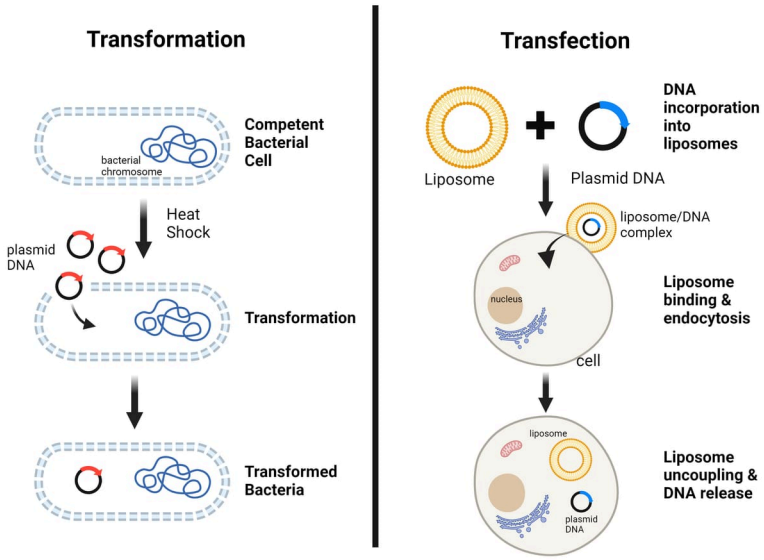


Figure 9.3.3: Transformation vs. Transfection. In transformation, competent bacterial cells undergo a "heat shock" to internalize DNA such as plasmid DNA. The transformed bacteria with its incorporated plasmid DNA begin expression of the new gene product. In transfection, DNA (e.g. plasmid DNA) is added to liposomes to form a liposome/DNA complex. The complex binds eukaryotic cells and is internalized into the cell through endocytosis. Once inside the cell, the complex undergoes uncoupling and the DNA is released into the cytoplasm. (Transformation vs Transfection by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2024))

Vaccines

There are two basic kinds of vaccines: traditional and therapeutic. The **traditional vaccine** stimulates the immune system in order to prevent an infection, whereas a **therapeutic vaccine** is designed to treat cancer, an existing disease, or chronic infection (like HIV or hepatitis) rather than prevent it. rDNA technology is used in the creation of both types of vaccines. To produce a vaccine using rDNA technology, highly specific viral or bacterial proteins that trigger an immune response without causing disease are produced in the lab. The process for this is very similar to recombinant protein production. A gene crucial for the pathogen's ability to infect a host cell or illicit an immune response is cloned into a plasmid and introduced into a host cell for expression. The

expressed protein is purified extensively to ensure that the vaccine contains only the desired protein and no harmful components from the host organism. The purified protein is then used to formulate a vaccine.

While the typical vaccine is made using expressed proteins, the process can be time-consuming. However, the development of **mRNA vaccines** has enabled scientists to dramatically cut down the generation time for a vaccine. Making an mRNA vaccine uses a few steps in common with the traditional vaccine described above (Figure 9.3.4). A gene for a target protein is identified and cloned into a plasmid. However, rather than expressing this gene as a protein in a cell, the mRNA transcript is produced by a cell-free system and isolated. The mRNA is then coupled to lipid nanoparticles that protect the delicate mRNA. In the final step, the mRNA-nanoparticle mixture is injected into muscle tissue whereupon the mRNA enters into the muscle cells and is expressed as a protein. The expressed protein is displayed on the surface of the muscle cell and triggers a immune reaction.

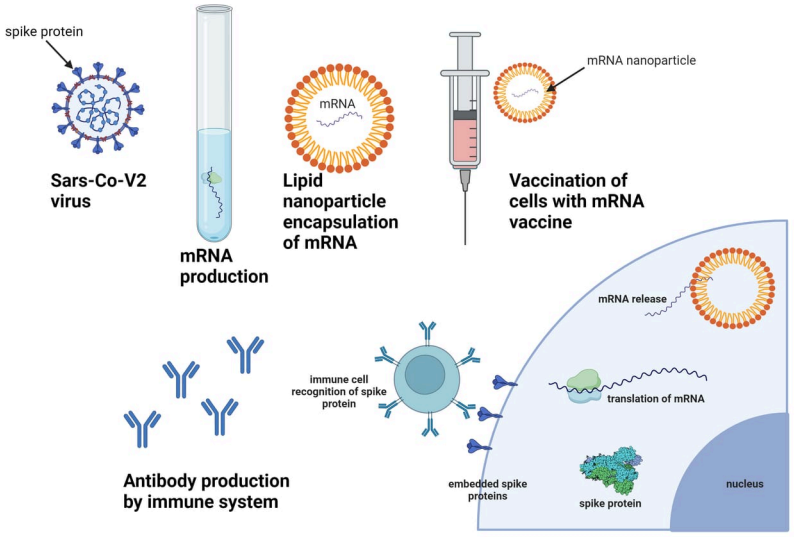


Figure 9.3.4: mRNA vaccine production. Vaccines against the spike protein of the Sars-CoV-2 virus can be made using mRNA. mRNA that codes for the spike protein is synthesized using a cell-free system (mRNA production). The mRNA particles are encapsulated into lipid nanoparticles (lipid nanoparticle encapsulation of mRNA). The mRNA-lipid nanoparticles are used to vaccinate host cells (vaccination of cells with mRNA vaccine). Once inside the cell, the mRNA is released from the nanoparticle and translated by the host cell's ribosomes. The resulting spike proteins are embedded within the cell membrane and recognized by the host's immune cells. Antibodies to the spike protein are made by the host (antibody production by immune system). (mRNA vaccine production by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2024))

Molecular Protocols

- Lab Technique: Bacterial Transformation
- Lab Technique: Transfection of Mammalian Cells

Key Concepts

Therapeutic biotechnology can be used to treat a wide range of diseases and disorders. Some major concepts to remember are:

- therapeutic biotechnology uses biotechnology tools to diagnose, treat, cure, or prevent diseases or disorders
- gene therapy can potentially treat or cure diseases through the introduction of a gene (or genes) that either replaces a "faulty" one or counteracts its harmful effects
- genes can be introduced for gene therapy using viral vectors like adenovirus, adeno-associated virus, or lentivirus
- new methods for the introduction of genes for gene therapy include the use of nanoparticles
- gene therapy can either be performed by introducing corrective genetic information into an organism directly (*in vivo* approach) or by removing the affected cells, genetically manipulating them, and then re-introducing them back into the patient (*ex vivo* approach)
- CAR-T therapy is a type of gene therapy that genetically-manipulates a patients T cells in order to treat cancer

- CRISPR technologies are currently being explored for use in gene therapy
- most therapeutic proteins are made using bacteria, yeast, or mammalian cells that have been modified with recombinant DNA
- some therapeutic proteins are made through genetically-modifying the entire organism with recombinant DNA
- examples of therapeutic proteins are monoclonal antibodies, hormones, an immune modulators
- therapeutic vaccines use recombinant DNA to produce specific antigens for vaccine production

Glossary

CAR (Chimeric Antigen Receptor) - a synthetic receptor engineered into immune cells (usually T cells) that allows them to recognize and bind to specific proteins on the surface of cancer cells

CAR-T therapy (Chimeric Antigen Receptor T-cell Therapy) - a type of immunotherapy where a patient's T cells are collected, genetically modified in the lab to express CARs, and then re-infused for targeted destruction of a cancer cell

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) - a gene-editing tool that uses a guide RNA and a Cas9 enzyme to find and cut specific DNA sequences

Ex vivo - refers to a process or event that happens outside of the body

Gene therapy - a technique that treats or prevents diseases by modifying the genetic make-up of a person using viral or non-viral vectors

In vivo - refers to a process or event that happens inside of a body

Liposome - a spherical vesicle made of phospholipids that mimics the structure of a cell membrane; used in transfection of mammalian cells

mRNA (messenger RNA) - a sequence of RNA produced through the process of transcription; specifies the sequence of amino acids following translation; comprised of a coding sequence and flanked by a 5' methylated cap and a 3' poly-A tail

mRNA vaccine - a vaccine containing mRNA molecules surrounded by lipid nanoparticles

Nanoparticle - a tiny particle that ranges in size from 1 to 100 nanometers (nm) in at least one dimension; can be made of materials such as biodegradable polymers, metals, and lipids

Recombinant DNA (rDNA) - DNA that is created in a laboratory by combining genetic material from different sources

Therapeutic protein - a protein-based compound used to treat, prevent, or cure diseases and medical conditions; often produced using recombinant DNA technologies

Therapeutic vaccine - a vaccine used to prevent diseases; often produced using recombinant DNA technologies

Transformation - the process of inserting DNA into a bacterial host cell

Transfection - the process of introducing DNA into a mammalian cell using liposomes

Vaccine - a biological preparation that stimulates the immune system to protect against a specific disease

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9.4: Regenerative Medicine

Biotech Focus

In 1956, several workers performing an experiment involving uranium fuel rods at the Vinča Nuclear Institute in Yugoslavia were exposed to high doses of radiation. Soon after, these men began experiencing the symptoms of acute radiation syndrome and were immediately transferred to the Curie Institute in Paris, where they underwent pioneering **bone marrow transplants** by the French oncologist Georges Mathé. All but one of the workers survived. This transplant represented the first use of stem cells as a medical treatment. Five years later, Canadian scientists Dr. James Till and Dr. Ernest McCulloch at the Ontario Cancer Institute in Toronto published their discovery of the **hematopoietic stem cell (HSC)**, the stem cell that gives rise to all blood cells. Till and McCulloch injected bone marrow into irradiated mice and observed the formation of colonies in the spleen. Each colony originated from a single HSC and exhibited both self-renewal and differentiation - the two traits of a stem cell. Thanks to their pioneering work, the stem cell field, and related fields like tissue engineering, were made possible.

Introduction

Regenerative medicine is a branch of medicine that focuses on the repair, replacement, or regeneration of human cells, tissues, or organs in order to restore normal function. It takes advantage of the the body's natural healing processes and often uses advanced technologies to accelerate or enhance those processes.

Key components of regenerative medicine include:

1. **Stem Cell Technology**: uses stem cells to develop into specific cell types and repair damaged tissues
2. **Tissue Engineering:** creates biological tissues in the lab using a combination of scaffolds, cells, and biologically active molecules
3. **Biomaterials and scaffolds:** uses synthetic or natural materials to support cell growth and tissue formation

Regenerative medicine has a wide range of applications, including the treatment of bone defects, damaged heart muscle, and spinal cord injuries, to potentially curing diabetes, Parkinson's, and even organ failure without the need for transplantation. One of the most successful early applications of regenerative medicine was in the treatment of skin defects and burns. Early approaches involved the use of tissue grafts taken from the patient's own skin (i.e., autografts), cadavers (i.e., allografts), or animals like pigs (i.e., xenografts). However, each of these came with their own problems, like the secondary defect associated with an autograft or immune rejection associated with a xenograft. These problems led to the need for alternatives that regenerative medicine could provide. Today, physicians can use synthetic skin grafts made of bioengineered scaffolds, seeded with human epidermal and dermal cells, to treat non-healing wounds and partial-thickness burns (e.g., Apligraft, Stratagraft) or "cultured autografts" made of a patient's own keratinocytes grown in the laboratory (e.g. Epicell). With each advancement, regenerative medicine continues to expand its applications in medicine and offers hope for those living with disease, defect, or injury.

Learning Objectives

Regenerative medicine is a branch of medicine focused on repairing, replacing, or regenerating human cells, tissues, or organs to restore normal function. At the end of this page, you will be able to:

- Define key terms associated with regenerative medicine, like stem cell, differentiation, and potency
- List and explain the three key components of regenerative medicine
- List and explain the three major types of potency associated with a stem cell
- Compare and contrast the three major categories of stem cells
- Describe some commonly used stem cells
- List the components used in tissue engineering
- Explain the different types materials used in making a scaffold
- Explain some methods commonly used to make a scaffold

- Explain what a bioactive molecule is and list some types used in tissue engineering

Stem Cell Technology

A **stem cell** is an undifferentiated (i.e., unspecialized) cell that can differentiate (i.e., specialize) into a specific cell type like a muscle cell or a blood cell (Figure 9.4.1). Stem cells also exhibit the property of **self-renewal**, in that they divide to produce a daughter cell that continues on as a stem cell and another daughter cell that is destined to differentiate. **Differentiation** is the biological process in which a stem cell develops into a specialized cell type with a distinct structure and function. This differentiation is critical for the determination of a cell's **lineage**, the developmental history of a cell as it divides and differentiates into other cell types. In order for a lineage to be determined, stem cells "choose" a cell fate that directs the type of cell they will become. The tissue continues its development and the lineage is set. For example, a stem cell population whose cell fate is restricted to the osteogenic lineage will differentiate into osteoblasts, followed by direct bone tissue formation.

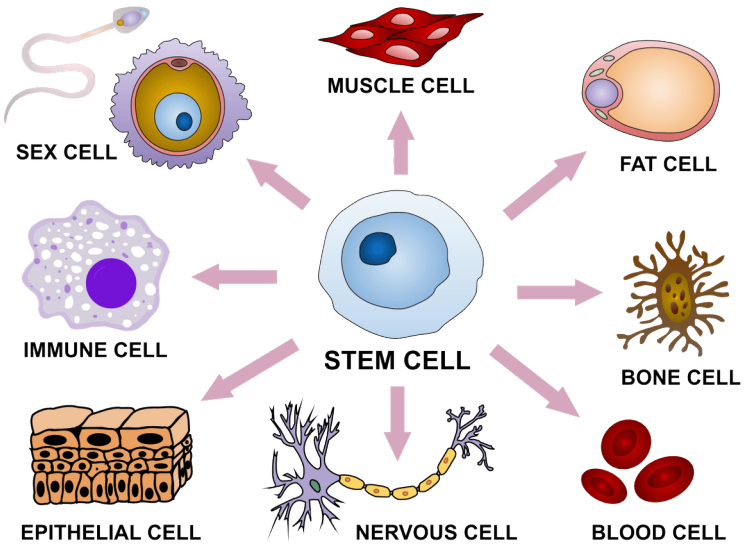


Figure 9.4.1: Stem cell differentiation. The stem cell differentiates in specific cell types such as epithelial, immune, sex, muscle, fat, bone, blood, and nervous cells (i.e., neurons) (Stem Cell by Haileyfournier, CC BY-SA 4.0)

Differentiation of a stem cell is directed by the expression of specific genes that restrict the stem cell's potency and result in its morphological and metabolic transition to a new cell type. Even today, researchers continue to study the cellular and molecular mechanisms that underlie stem cell differentiation in the hopes of more effectively using stem cells in regenerative medicine. The ability of a stem cell to differentiate is determined by their **potency**. Stem cell potency refers to a stem cell's ability to differentiate into different types of cells. Most stem cells can be described as having one of three types of potency (Figure 9.4.2):

- **Totipotent**: stem cells can develop into any cell type in the body and the extraembryonic tissues.
  - e.g., early-stage embryonic stem cells (i.e. up to the 16-cell stage)
- **Pluripotent**: stem cells can become any cell type in the body (i.e., all three germ layers: ectoderm, mesoderm, endoderm), but not extraembryonic tissues
  - e.g., embryonic stem cells and induced pluripotent stem cells
  - Widely used in regenerative medicine
- **Multipotent**: stem cells have limited potency and can differentiate into a limited number of cell types within a germ lineage (e.g., mesoderm)
  - e.g., hematopoietic stem cell (HSC), mesenchymal stem cell (MSC), and the adipose-derived stem cell (ASC)
  - Limited use in regenerative medicine that is restricted to the corresponding cell lineage - e.g. HSCs for generation of blood cells; MSCs for the generation of mesenchymal tissues (i.e., fat, bone, cartilage, muscle)

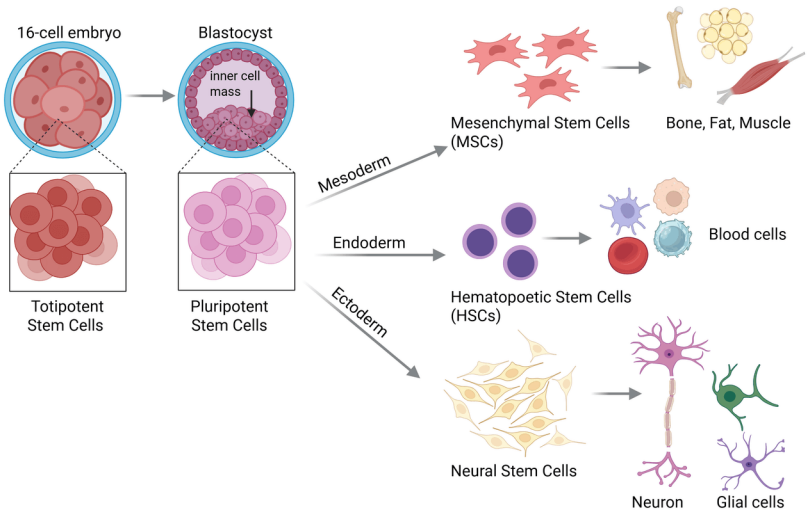


Figure 9.4.2: Stem cells have specific potency. The 16-cell embryo is made of cells called totipotent stem cells. Over time the embryo develops into a blastocyst. The inner cell mass of the blastocyst is a population of pluripotent stem cells. These stem cells develop into the mesoderm, endoderm, and ectoderm germ lines. Germ line development results in the formation of several kinds of multipotent stem cells like mesenchymal stem cells (MSCs) that can develop into bone, fat, and muscle cells, hematopoietic stem cells (HSCs) that form the blood cells, and neural stem cells that develop into neurons and the glial cells of the nervous system. (Stem Cells by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. 2025)

Embryonic development results in the loss of stem cell potency as the totipotent stem cell population of the 16-cell zygote is replaced with the pluripotent stem cells of the inner cell mass. These pluripotent stem cells will become distributed throughout the embryo and will restrict their potency to become the various populations of multipotent stem cells. The loss potency during embryonic development is directed, in part, by environmental signals found in the developing tissue. In humans, the majority of tissues contain multipotent stem cells that direct tissue repair following damage.

With each passing year, there seems to be a new population of stem cells being discovered and used in regenerative medicine. However, there are only three recognized categories of stem cells: embryonic, fetal, and adult. Each stem cell population is classified into one of these three categories. **Embryonic stem cells (ES) cells, fetal stem cells,** and **adult stem cells** differ in their origin, potency, and applications (Table 9.4.1).

Table 9.4.1: Embryonic vs. Adult Stem Cells

Feature	Embryonic Stem Cells (ES cells)	Fetal Stem Cells	Adult Stem Cells
Origin	isolated from the **inner cell mass** of a blastomere (animal) or blastocyst (human)	isolated from fetal tissues after 8 weeks of gestation	isolated from tissues such as bone marrow, adipose tissue, and skin
Potency	totipotent or pluripotent	mostly multipotent	mostly multipotent
Self-renewal ability	high	lower in comparison to ES cells	relatively low; self-renewal depends on the type of adult stem cell
Potential for tumor formation	high - can form teratomas (tumors) due to uncontrolled growth	lower in comparison to ES cells	low
Potential for immune rejection	high - ES cells may be rejected if not genetically matched to the recipient	lower in comparison to ES cells; similar to adult stem cells	low; often isolated from autologous tissues

Feature	Embryonic Stem Cells (ES cells)	Fetal Stem Cells	Adult Stem Cells
Applications	• regenerative medicine - organ regeneration and tissue repair (e.g. Parkinson's, diabetes, spinal cord injuries) • disease modeling • drug testing	• regenerative medicine - organ regeneration and tissue repair (e.g. Parkinson's, diabetes, spinal cord injuries) • disease modeling • drug testing	• regenerative medicine - organ regeneration and tissue repair (e.g. Parkinson's, diabetes, spinal cord injuries) • disease modeling • drug testing
Ethical considerations	high - use of human ES cells requires destruction of human blastocysts	high - used of fetal stem cells requires the use of fetal tissues	low - human adult stem cells are isolated from consenting donors

In practice, a stem cell is identified by its ability to repair or regenerate tissue. Since the discovery of hematopoietic stem cells (HSCs) in the 1960s, stem cells have been utilized in a wide variety of regenerative medicine applications, such as:

1. **Hematopoietic Stem Cells (HSCs):** for the treatment of blood cancers (like leukemia, lymphoma), aplastic anemia, and some immune disorders.
    - uses several sources of HSCs, including bone marrow, peripheral blood, or umbilical cord blood
    - the most widely used and established stem cell therapy worldwide
2. **Epidermal stem cells:** for the treatment of large-area burns using autologous skin grafts
    - use of autologous epidermal stem cells donated by the patient
    - products like Epicel (cultured epidermal autografts) are FDA-approved
3. **Mesodermally-derived stem cells:** for the regeneration of a variety of mesodermally-derived tissues
    - use of autologous stem cells from bone marrow (i.e., MSCs) or adipose tissue (e.g., ASCs)
    - regeneration of cartilage in patients with joint injuries or early osteoarthritis (e.g., Carticel)
    - treatment of spinal cord injuries (e.g., Stemirac)
    - treatment of graft vs. host disease in young children(e.g., Ryanocil)
4. **Adipose-derived Stem Cells (ASCs):** for the repair of complex perianal fistulas that do not respond to conventional treatments
    - use of allogeneic ASCs
    - approved in the EU (e.g., Alofisel),with strong efficacy and safety data

The majority of regenerative medicine applications using stem cells utilize adult stem cells due to their ease of isolation and lack of ethical concerns. This is in direct contrast to the use of ES cells. The ethical considerations that continue to surround the isolation and use of human ES cells has led researchers to develop the **induced Pluripotent Stem Cell (iPSC)**. iPSCs have been artificially reprogrammed to behave like ES cells through the introduction of four specific genes (Oct4, Sox2, Klf4, c-myc) into fully differentiated cells, such as skin or blood cells. This genetic reprogramming appears to return the somatic cell to a more pluripotent state. Because of their high level of potency and their derivation from adult cells, iPSCs have been suggested as a replacement for ES cells. As such, iPSCs are used in disease modeling, drug testing, personalized medicine and regenerative medicine. However, their use in personalized and regenerative medicine has not been as widely explored as ES cells and adult stem cells. Furthermore, the significant degree of genetic changes required to create an iPSC may eliminate them from many applications in regenerative medicine. Despite this, stem cell therapy continues to be promising due to their potential therapeutic application in replacing defective or damaged cells resulting from a variety of disorders and injuries, such as Parkinson disease, heart disease, and diabetes.

Tissue Engineering

Tissue engineering is a multidisciplinary field that combines biology, engineering, and medicine in order to focus on the development of biological substitutes to restore, maintain, or improve tissue function. Tissue engineering combines components:

1. **Cells:** the living units responsible for forming functional tissue
    - can be precursor cells or stem cells
    - can be either **autologous** (harvested from the patient in order to minimize immune rejection) or **allogeneic** (donated cells)
2. **Biological scaffolds:** 3D structures that provide a biocompatible, physical structure for the attachment, proliferation, and

differentiation of cells
    - made of natural, synthetic, or composite materials
3. **Bioactive molecules:** growth factors and other signaling cues that guide cell behavior and tissue development within the scaffold

In the typical tissue engineering approach, these three components interact with one another inside a **bioreactor**, a controlled environment designed to mimic physiological conditions and support tissue formation.

By integrating cells, scaffolds, and signaling molecules, tissue engineering aims to repair or regenerate damaged tissues and organs. Examples of tissues generated through tissue engineering include:

- **Skin:** bioengineered skin substitutes consisting of keratinocytes and fibroblasts layered on a scaffold (e.g., Apligraf, Dermagraft) used for chronic wounds and burns
- **Cartilage:** autologous chondrocyte implantation (ACI) or stem-cell seeded scaffolds to repair joint damage
- **Bone:** hydroxyapatite or tricalcium phosphate scaffolds combined with pre-osteoblast precursors of adult stem cells
- **Blood vessels:** vascular grafts engineered from endothelial and smooth muscle cells for the replacement of damaged vessels; e.g. coronary bypass vessels

Tissue engineering is also being used in the generation of whole organs such as liver, kidney, and heart tissues. Miniature versions of organs, called organoids, are being developed in lab settings, although clinical applications remain limited.

The use of tissue engineering principles in tissue repair is promising. However, there are several obstacles to overcome. Vascularization is one of them. Supplying nutrients to thick tissue layers remains a significant challenge. While autologous cells are often used to decrease the chances of an immune response, such as response can occur due to scaffold materials or altered cell behavior. Regulatory hurdles must be cleared in order to ensure the safety and efficacy of engineered tissues. These hurdles can delay the transition of the clinic. Lastly, manufacturing scale-up remains an issue as generating tissues at a large scale both reproducibly and cost-effectively is a significant challenge.

Biomaterials and Bioscaffolds

A **biomaterial** is any natural or synthetic material that is designed to interact with a biological system (e.g. cells, tissues) for a medical purpose, such as the therapeutic delivery of drugs or cells for tissue repair. Biomaterials must be **biocompatible** and not provoke an immune reaction. They should **bioactive** to promote the attachment, proliferation, and/or the differentiation of cells. They should match the mechanical demands of the target tissue (e.g, load bearing vs. non-load bearing). Finally, many biomaterials are designed to be **biodegradable** so that they degrade safely in the body.

A **bioscaffold (i.e., scaffold)** is a three-dimensional structure made of a biomaterial. The composition of a bioscaffold is chosen to be compatible with the cells being used and the tissue being regenerated. The scaffold must promote the viability of the cells seeded onto the scaffold. In addition, it should also integrate into the surrounding tissue so that the tissue being regenerated is compatible. Scaffold compositions used in tissue engineering are **natural polymers**, **synthetic polymers**, or **composite materials** that combine both natural and synthetic biomaterials. Each of these compositions have advantages and disadvantages that must be considered before being used in a tissue engineering application. For example, synthetic polymers have the advantages of consistent composition, higher mechanical strength, and controlled degradation *in situ*. However, their degradation may create toxic materials. The composition and design of the scaffold ultimately used must consider porosity, mechanical strength, biodegradability, and surface chemistry so as to support cellular function and integration.

Natural polymers include collagen, fibrin, alginate, and chitosan (Table 9.4.2). These materials have the advantages of being bioactive and biocompatible. However, they can be variable in composition and have lower mechanical strength.

Table 9.4.2: ***Natural Scaffold Materials***

Material	Source	Tissue Engineering Application	Key Features
Collagen	animal connective tissues	skin, bone, cartilage	biodegradable, cell-friendly
Fibrin	blood plasma	wound healing, cardiovascular patches	promotes angiogenesis
Alginate	brown seaweed	soft tissue engineering	mild gelation, controlled stiffness
Chitosan	crustacean shells	cartilage, wound healing	antimicrobial, highly biodegradable

Material	Source	Tissue Engineering Application	Key Features
Hyaluronic acid	connective tissue matrix	cartilage repair, cell delivery system	hydrating, similar to cartilage matrix

Common synthetic materials used in biological scaffolds include **polylactic acid (PLA)** and **polyglycolic acid (PGA),** in addition to calcium phosphate-based scaffolds like **hydroxyapatite** (Figure 9.4.3) and **tricalcium phosphate** (Table 9.4.3).

Table: 9.4.3: ***Synthetic Scaffold Materials***

Material	Type	Tissue Engineering Applications	Key Features
PLA (Polylactic acid)	thermoplastic polyester	bone, skin, guidance of nerves	biodegradable, FDA-approved for load-bearing tissues like bone
PGA (Polyglycolic acid)	synthetic polymer	cartilage, nerve, skin	fast degradation
PLGA (Poly(lactic-co-glycolic acid))	co-polymer	bioactive factor delivery, tissue patches	controlled degradation
PCL (Polycaprolactone)	synthetic polymer	ligaments and tendons, cartilage, vascular tissue	slow degradation, flexible
PEG (Polyethylene glycol)	hydrophilic polymer	cartilage, nerve, vascular tissue, cornea	mimics soft tissue
Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$)	calcium phosphate-based material	bone	slow degradation, good for load-bearing tissues, mimics ECM of bone
Tricalcium phosphate (TCP)	calcium phosphate-based material	bone	fast degradation

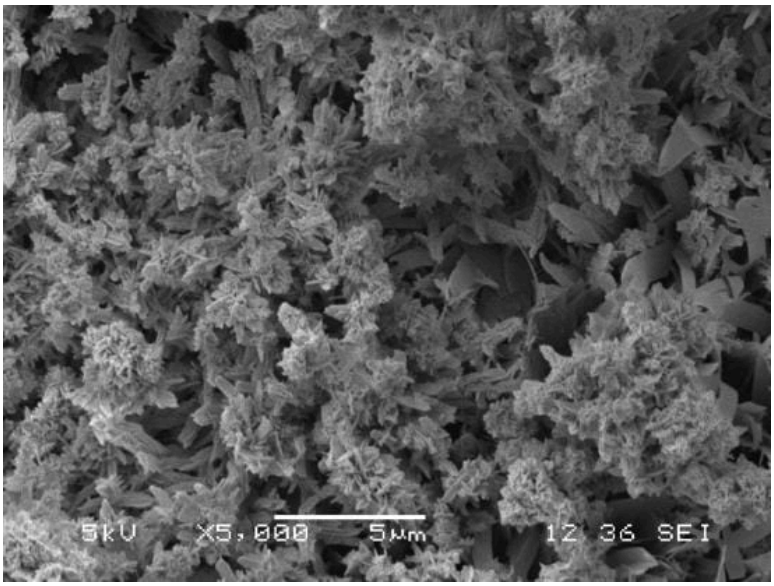


Figure 9.4.3: Hydroxyapatite crystals. This electron micrograph shows an extensive network of hydroxyapatite crystals that closely mimics the matrix of bone tissue. (Crystals of Hydroxylapatite by Nabilah, CC BY-SA 4.0)

Composite scaffolds combine natural and synthetic materials for optimized performance, balancing biocompatibility and mechanical integrity. Examples include collagen-PLGA and hydroxyapatite–PCL for bone and cartilage repair.

Bioscaffolds can be produced using several methods including:

1. **Solvent Casting and Particulate Leaching (SCPL):** used for PLGA, PGA, PCL, hydroxyapatite, tricalcium phosphate scaffolds
    - Production method: a polymer is dissolved in a solvent and mixed with salt or sugar particles (i.e. **porogens**)

    - the mixture is cast into a mold
    - the solvent is evaporated and immersed in water to dissolve the porogen
  - Pros: simple and inexpensive to make; pore sizes controlled by porogen; good for cell infiltration, can be coated with natural materials like collagen
  - Cons: limited control over pore interconnectivity; residual solvent or porogen may be toxic if not fully removed
  - Used for: bone and cartilage
2. **Electrospinning:** used for PLA or PCL synthetic scaffolds and numerous natural materials (e.g., collagen)
  - Production method: a polymer solution is fed through a needle with a high-voltage electric field to create fine fibers that collect on a grounded surface
  - Pros: excellent surface area for cell attachment; mimics native ECM structure well
  - Cons: typically made as a 2D or thin 3D scaffold; poor cell infiltration unless the scaffold is layered
  - Used for: skin, nerve, vascular, and soft tissue
3. **3D printing:** most natural and synthetic scaffold materials
  - Production method: layer-by-layer printing of scaffold material using a 3D printer and its software
  - Pros: Can be customized to print a variety of scaffold shapes
  - Cons: Cost; requires a 3D printer and software
  - Used for: vascular grafts, cartilage, bone scaffolds

Concept in Action

Electrospinning Technology

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Bioactive Molecules

A **bioactive molecule** is any compound that affects tissues or cells by producing a specific physiological response. Common bioactive molecules include growth factors, peptides, and hormones. In order to be a bioactive molecule, the compound must bind to receptors on the cell surface and trigger signaling pathways that influence cell fate and behavior (e.g., migration, growth, differentiation). In tissue engineering, bioactive molecules promote the adhesion, proliferation, and differentiation of cells (including stem cells), promote angiogenesis, nerve regeneration and remodeling of tissues, and modulate the immune response to implants. Table: 9.4.4 below outlines some common bioactive molecules used in tissue engineering.

Table: 9.4.4: ***Bioactive Molecules***

Type of Bioactive Molecule	Function	Example
Growth Factors	Stimulate cell growth, migration, or differentiation	BMPs, VEGF, FGF, EGF, TGF-β
Cytokines	Modulate immune response or inflammation	IL-1, IL-6, TNF-α, IFN-γ
Peptides	Mimic protein domains for cell signaling	RGD peptide (cell adhesion), IKVAV
Hormones	Regulate cell metabolism and function	Insulin, estrogen, parathyroid hormone
Extracellular Matrix (ECM) Proteins	Provide structural and signaling support	Collagen, fibronectin, laminin
Small Molecules	Modulate cell signaling	Dexamethasone, retinoic acid, valproate
Antibodies	Target or block specific receptors or proteins on cells	Anti-VEGF, anti-TNF antibodies
Nucleic Acids	Regulate gene expression	siRNA, miRNA

Bioactive molecules used in tissue engineering can be incorporated into scaffolds for the slow release of the molecule, attached to the surface of a biomaterial to affect surrounding cells, and encapsulated in microspheres/nanoparticles. Today, "smart biomaterials" are being designed to release bioactive factors in response to stimuli like pH, temperature, or electric fields.

Emerging Breakthroughs

Today, there are several promising approaches being proposed for tissue engineering. One such approach is called **"bioprinting"**. Bioprinting is based on 3D printing technology but uses **"bioinks"** which are combinations of biomaterials, living cells, and bioactive materials. Like 3D printing, bioprinted scaffolds are layer by layer in a 3D printer. However, these printers have been modified to use bioinks. Bioprinting is highly precise and can be customized to create complex structures seeded with living cells and embedded with bioactive compounds. However, bioprinting remains expensive, as the bioprinter is more advanced then the standard 3D printer used in labs today. In addition, the bioinks must finely tuned to balance printability with biocompatibility. Bioprinting is currently being used for the creation of soft tissue scaffolds like vascular grafts and tubes. Researchers are using bioprinting to create miniature versions of organs (i.e., **organoids**) in order to advance bioprinting to the point where life-sized organs can be made.

Concept in Action

Bioprinting Human Tissues

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Another approach is the use of **decellularized tissues** (i.e. decellularized ECM or dECM). Tissue-specific scaffolds can be created by removing cells from a donor tissue while preserving the overall 3D structure of the tissue and its matrix proteins. Examples include the experimental use of decellularized trachea, tendons, ligaments, and heart matrix, in addition to the commercial use of decellularized skin grafts (e.g., Alloderm and DermACELL) and small intestine submucosa (e.g., SurgiSIS and Oasis). Decellularized tissues have the advantage of being perfectly suited to the tissue they are repairing. However, the possibility of an immune reaction upon implantation cannot be completely discounted.

Concept in Action

The Science of Decellularization

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The progress made in tissue engineering over the last two decades has been astounding. Of course, obstacles still remain. Organ regeneration is complicated by the sheer size and thickness of the organ. As such, the formation of a functional vasculature and innervation remains elusive in large tissue and organ constructs. Scaling up manufacturing without compromising the bioactivity of the engineered construct is challenging. Ensuring long-term integration and safety in humans and navigating the ethical and regulatory hurdles, especially in organ engineering, will need be to addressed. Despite these challenges, the future remains bright for tissue engineering.

Key Concepts

Regenerative medicine is a branch of medicine that focuses on the repair, replacement, or regeneration of human cells, tissues, or organs in order to restore normal function.

Some important concepts to remember are:

- regenerative medicine uses tissue engineering techniques to repair or regenerate tissues
- tissue engineering uses cells, biomaterials, and bioactive molecules to regenerate and repair tissues
- the cells used in tissue engineering can be precursor cells or stem cells
- stem cells are unspecialized cells that can divide and differentiate
- stem cells can be totipotent, pluripotent, or multipotent
- each stem cell population has its own unique set of advantages and disadvantages
- biomaterials are any natural or synthetic materials that are designed to interact with a biological system
- biomaterials include scaffolds
- scaffolds can be made of natural or synthetic biomaterials or a combination of both
- scaffolds can be made through techniques like particulate leaching, electrospinning, or 3D printing
- bioactive molecules are compounds that affect tissues or cells by producing a physiological response
- bioactive molecules include growth factors, peptides, hormones, or matrix proteins
- emerging technologies in tissue engineering include the use of decellularized matrices or bioprinting
- bioprinting uses "bioinks" that are a combination of living cells, bioactive molecules, and biomaterials

Glossary

Bioactive molecule - a substance that interacts with living cells or tissues to influence their behavior, such as promoting growth, differentiation, or healing; examples include growth factors, cytokines, or peptides

Biocompatible - a material or substance that can be safely introduced into the body to supports normal cellular function without causing an immune response or toxicity

Biodegradable - a material that can be broken down naturally by biological processes in the body into harmless byproducts over time

Bioink - a printable biomaterial, containing cells and biocompatible hydrogels, that is used in 3D printing to fabricate tissue-like structures layer-by-layer

Biomaterial - any natural or synthetic material used to interact with or replace a biological tissue

Bioprinting - a form of 3D printing that uses bioinks to create complex biological structures, such as tissues or organs

Bioscaffold - a 3D framework used in tissue engineering that is made of biomaterials designed to support cell attachment, proliferation, and differentiation

Decellularized - a term that describes a tissue or organ that has had all cellular material removed, leaving behind the extracellular matrix to be used as a scaffold

Differentiation - a biological process by which a less specialized stem cell becomes a more specialized cell type

Extracellular matrix (ECM) - a complex network of proteins and molecules surrounding cells in tissues; provides structural support and biochemical signals for cell behavior

Lineage - the development "history" of a cell

Multipotent - a term that describes stem cells that can differentiate into multiple, but limited, cell types within a specific cell lineage; e.g., mesenchymal stem cells into bone, fat, or cartilage

Organoid - a miniature, simplified version of an organ grown in vitro from stem cells, that mimics key structural and functional

properties of the real organ

Pluripotent - a term that describes stem cells that can give rise to all cell types in the body; found in the inner cell mass of the embryo

Polymer - a large molecule made up of repeating units called monomers

Potency - the measure of a stem cell's ability to differentiate into different cell types; ranges from totipotent to unipotent

Precursor cell - a cell that is restricted in its lineage to only one type of cell; e.g., a pre-osteoblast precursor becomes an osteoblast

Regenerative medicine - a field of medicine that aims to restore or replace damaged tissues and organs using techniques like stem cell therapy, tissue engineering, and gene editing

Scaffold - a natural or synthetic 3D structure that provides physical support for cells used in tissue engineering; also called a bioscaffold

Stem cell - an undifferentiated cell capable of self-renewal and differentiation depending on its potency

Totipotent - a term that describes stem cells that are able to give rise to all cell types of the body and the extra-embryonic tissues; found in early stage embryos (i.e. up to 16-cell)

Unipotent - a term that describes a cell that has the capacity to differentiate into only one specific cell type, but they still retain the ability to self-renew

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9.5: Principles of Medical Biotechnology

Biotech Focus

A brief history of medical biotechnology:

- ~6000 BCE – 1000 BCE: early humans use fermentation to make bread, beer, and wine
- ~500 BCE: traditional Chinese and Indian medicine begins using plant-based remedies
- 1796: Edward Jenner develops the first vaccine to protect against smallpox
- 1865: Gregor Mendel discovers the basic laws of heredity
- 1885: Louis Pasteur develops the rabies vaccine
- 1919: Károly Ereky coins the term “biotechnology”
- 1953: Watson and Crick discover the double-helix structure of DNA
- 1973: Cohen and Boyer develop recombinant DNA technology by cloning a gene into a bacterial plasmid
- 1978: first synthetic human insulin (Humulin) produced using genetically-engineered bacteria
- 1982: FDA approves Humulin
- 1986–1990s: development of vaccines (e.g., Hepatitis B) and monoclonal antibodies using biotechnology techniques
- 1990: launch of the Human Genome Project to map all human genes
- 1999–2000: first gene therapy trials for genetic disorders begin
- 2003: completion of the Human Genome Project
- 2012: introduction of CRISPR-Cas9 for gene editing
- 2020: mRNA vaccines approved for COVID-19

Introduction

Medical biotechnology is the branch of biotechnology that focuses on using living cells, cell materials, and biological processes in order to develop products and technologies that help prevent, diagnose, and treat diseases. It's an area where biology, medicine, and technology come together to improve human health.

As scientists began to understand biological systems, they began to use them to develop new diagnostic tools and therapeutic strategies. With time, the field of medical biotechnology began to emerge. Key aspects of medical biotechnology include:

1. **Production of medicines and vaccines:** uses genetically-engineered organisms, such as bacteria or yeast to produce therapeutic compounds like drugs, hormones, antibodies and vaccines
2. **Gene therapy:** treats genetic diseases by repairing, replacing, or modifying faulty genes inside a patient’s cells
3. **Tissue engineering and regenerative medicine:** grows artificial tissues and organs in the lab to replace damaged ones
4. **Diagnostic tools:** develops advanced biotech-based tests to quickly and accurately detect diseases
5. **Personalized medicine:** tailors medical treatments based on an individual’s genetic profile to maximize effectiveness and minimize side effects

In many cases, medical biotechnology can lead to more cost-effective treatments and diagnostics compared to traditional methods. However, medical biotechnology raises numerous ethical concerns. For example, ethical debates surround the long-term effects of gene therapy, as well as the potential for modifying the human germline. Concerns about the use of genetic information and patient privacy also have been raised. There are many people who are worried about ensuring equitable access to new biotechnology-based treatments and diagnostics, particularly in underserved populations. How can someone who is economically disadvantaged afford the high costs of tech treatments created using medical biotechnology? Despite these concerns, it is hard to deny that medical biotechnology has revolutionized the medical field and improved human health.

Concept in Action

Red Biotechnology: The Future of Medical Science

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Learning Objectives

Medical biotechnology blends biology, medicine, and technology to create solutions for diagnosing, treating, and preventing diseases. At the end of this page, you should be able to:

- Define medical biotechnology
- List and explain the major principles of medical biotechnology

Principles of Medical Biotechnology

Medical biotechnology relies on the manipulation of biological systems at both the molecular and cellular level so that the resulting treatments are effective, safe, and ethical. To do so, medical biotechnology uses the following (Figure 9.5.1):

1. **Genetic Engineering:** the manipulation of DNA to alter the genetic material of an organism
   - involves the use of recombinant DNA technology
   - often used to insert health genes in order to correct genetic disorders
   - e.g., the production of hormones using genetically modified bacteria
   - to read more about genetic engineering, go to Chapter 4: Genetic Engineering and Recombinant DNA Technology
2. **Cell Culture Technology:** the growth of cells outside of their natural environment in controlled settings, used for research, vaccine production, and regenerative medicine
   - can include the use of stem cells or organ culture
   - to read more about cell culture, go to Chapter 6: Cell Culture Fundamentals
3. **Molecular Diagnostics:** uses molecular biology techniques to detect genetic mutations, pathogens, or diseases at the molecular level
   - includes areas such as next generation sequencing, microarrays, and PCR
   - to learn about PCR, go to Chapter 3.2: Replication of DNA
   - to read more about DNA sequencing, go to Chapter 5.3: Genome Sequencing
4. **Biomanufacturing:** the production of biological products such as vaccines, therapeutic proteins, and antibodies on a large scale using living systems like bacteria, yeast, or mammalian cells
   - to read more about biomanufacturing, go to Chapter 11.2: Bioprocess Engineering
5. **Immunotechnology:** engineers the immune system for the treatment or prevention of diseases
   - e.g., developing monoclonal antibodies for cancer therapy
   - e.g., CAR-T cell therapy
6. **Bioinformatics:** the application of computational tools to understand biological data, such as analyzing gene sequences or modeling biological processes
   - includes areas such as genomics, proteomics
   - to read more about genomics, proteomics, and bioinformatics, go to Chapter 5: Genomics and Proteomics
7. **Ethical and Regulatory Compliance:** ensures that biotechnology applications meet ethical standards and are approved by regulatory authorities
   - includes regulatory organizations like the FDA and compliance procedures, such as GMP

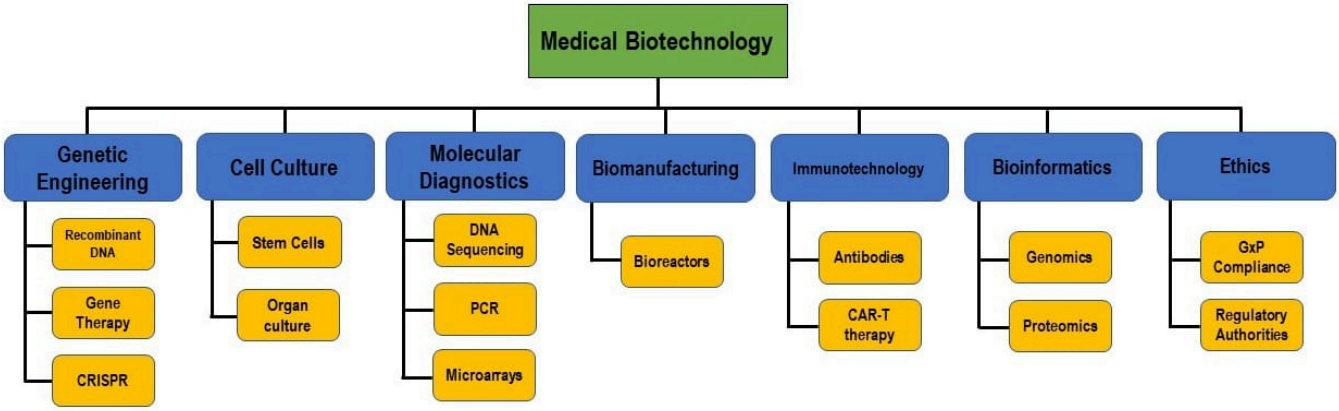


Figure 9.5.1: Medical Biotechnology Principles. (Medical Biotechnology by Patricia Zuk, CC BY 4.0)

Key Concepts

Medical biotechnology uses biological systems in the production of therapeutic compounds, like drugs and vaccines.

Some important concepts to remember are:

- Medical biotechnology manipulates biological systems at the molecular and cellular level to create treatments are effective, safe, and ethical
- Medical biotechnology involves areas such as genetic engineering, cell culture, bioinformatics, and biomanufacturing

Glossary

Bioinformatics - an interdisciplinary field that combines biology, computer science, mathematics, and statistics to analyze and interpret biological data

Biomanufacturing - the production of biological products, such as vaccines, therapeutic proteins, or enzymes using living cells or organisms in industrial settings

Cell Culture - a laboratory technique used to maintain and/or grow eukaryotic cells in vitro; usually refers to mammalian cells

Genetic engineering - the direct modification of an organism's DNA using biotechnology; often done to introduce new traits, to improve resistance to disease, or to produce beneficial products

Immunotechnology - the use of immune system-based tools and techniques for diagnostics, vaccine development, and treatment of diseases

Molecular diagnostics - techniques used to detect and measure specific genetic material or proteins in a sample in order to diagnose diseases at the molecular level

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9.6: Diagnostic Biotechnology

Biotech Focus

Conventional laboratory techniques for diagnosing metastatic breast cancers include a combination of imaging, tissue analysis, and biomarker testing. Tumor biopsies are tested for common breast cancer biomarkers like the ER marker (estrogen receptor) and the HER2 marker (human epidermal growth factor receptor 2; also called HER2/neu) using immunological methods, like FISH, and the BRCA-2 marker using molecular techniques such as DNA sequencing. These methods can be time-consuming and expensive to perform.

However, researchers at Hacettepe University and Abant İzzet Baysal University in Turkey have created a potential means of rapidly diagnosing HER2-positive breast cancer using a unique biosensor called a **quartz crystal microbalance (QCM).** A QCM is a **piezoelectric sensor**, means that it uses a thin quartz crystal that oscillates at a specific frequency. When molecules accumulates on the crystal, the change in mass of the crystal decreases its oscillation frequency. This frequency shift is directly proportional to the mass of the material that has bound to the crystal surface, even at the nanogram or even picogram level. To detect breast cancer, researchers can coat the crystal with nanoparticles bound to transferrin, a iron-binding protein. As a result of their enhanced metabolic activity, aggressively-growing breast cancer cells are known to overexpress the receptor to transferrin (i.e., the transferrin receptor). As a result, breast cancer cells will bind to this sensor via their transferrin receptor and change its oscillation frequency.

To test the QCM biosensor, breast cancer cells were added to a transferrin-coated crystal to allow for binding. The oscillation frequency was measured as an indication of the concentration of this cancer biomarker. Researchers found that the QCM biosensor function correlated well with the number of transferrin receptors on the cells, making this a potential diagnostic tool for identifying the early stages of breast cancer, monitoring its progression, or evaluating a patient's response to therapy.

To learn about recent advances in using a QCM in detecting breast cancer, include coating the crystal's nanoparticles with antibodies to the HER2/neu biomarker, check out this article.

Introduction

Diagnostic biotechnology involves the use of biological tools and techniques to detect and/or measure **biomarkers** associated with diseases, genetic conditions, or infections. The exploitation of these tools and techniques has led to the industrial production of biological products for diagnostic purposes. These products are now used in clinical laboratories around the world. A **biomarker, or biological marker**, is a measurable indicator of some biological state or condition. Biomarkers are often used to indicate a normal or abnormal process, identify a disease, or evaluate a response to treatment. In medicine, biomarkers are usually molecules found in blood, other body fluids, or in tissues.

Biomarkers are classified according to their use and include:

- **Diagnostic biomarkers:** used to diagnose a condition like a disease
  - e.g., estrogen and progesterone receptors used in the diagnosis of breast cancer
- **Prognostic biomarkers:** markers that assess the progression of a diagnosed disease, regardless of treatment
  - e.g., Brain Natriuretic Peptide (BNP) expression used to assess heart failure prognosis
- **Predictive biomarkers:** markers that predict a response to a specific treatment
  - e.g., HER2 receptor used to predict response to chemotherapies
- **Monitoring biomarkers:** markers that track disease progression or treatment response over time
  - e.g., troponin synthesis used to monitor heart disease
  - e.g., creatinine levels used to monitor kidney disease
- **Susceptibility biomarkers:** markers that identify an increased risk of developing a disease
  - e.g., BRCA1/BRCA2 mutations used to assess the risk of breast cancer
  - e.g., LDL levels used to assess atherosclerosis
- **Pharmacodynamic/Response biomarkers:** markers that indicate a biological response to a drug in order to assess whether the drug is working
  - e.g., decrease in hemoglobin A1c (HBA1c) levels in response to diabetes treatment

- **Safety biomarkers:** markers that indicate toxicity of adverse effects of a specific treatment
  - e.g., liver enzymes monitored for drug-induced liver injury

Here are some key areas used in diagnostic biotechnology:

1. **Molecular Diagnostics**
2. **Immunodiagnostics**
3. **Point of Care (POC) Diagnostics**

Learning Objectives

Diagnostic biotechnology uses biological tools and techniques to diagnose diseases, disorders, and conditions. At the end of this page, you will be able to:

- List and define the key areas of diagnostic biotechnology
- Explain next-generation sequencing
- Explain the DNA microarray
- Explain Fluorescent In Situ Hybridization (FISH)
- Describe the structure of an antibody
- Explain the different types of ELISAs
- Explain how the "sandwich" ELISA is performed and how this protocol differs from the direct and indirect ELISA
- Define point-of-case diagnostics (POC)
- Explain how a lateral flow assay (LFA) works
- Define biosensor and explain how one works in POC
- Explain how the glucose monitor works as a biosensor
- Explain the lab-on-a-chip

Molecular Diagnostics

Molecular diagnostics is a field of medical testing that uses molecular biology techniques to detect and measure specific DNA, RNA, or protein markers in cells or tissues. These tests are used to diagnose diseases, predict risk, guide treatment, and monitor health conditions at the molecular level.

Key techniques used in molecular diagnostics are:

1. **Polymerase Chain Reaction (PCR):** amplifies specific DNA or RNA sequences
   - used to detect the presence of specific genetic markers associated with diseases and infections
   - to read more about PCR, go to Chapter 3.2: Replication of DNA
2. **Real-Time PCR (qPCR):** quantifies the amount of genetic material in a sample
   - used to quantitate the expression level of genetic markers
   - to read more about PCR, go to Chapter 3.2: Replication of DNA
3. **Next-Generation Sequencing (NGS):** sequences entire genomes or targeted regions for mutations
4. **Microarrays:** analyzes the expression levels of thousands of genes at once
   - two major types: DNA microarrays and protein microarrays
   - to read about protein microarrays, go to Chapter 5.6: Proteomics
5. **In situ hybridization (e.g., FISH):** detects specific DNA or RNA in cells

Next Generation Sequencing

While modern day Sanger sequencing instruments are still used by the majority of research facilities to quickly sequence a small DNA construct, they are limited by the relatively small number of sequencing reactions that can be analyzed at one time (e.g. 8 to 96). **Next-generation sequencing (NGS)** is a powerful platform that enables the sequencing of thousands to millions of DNA molecules simultaneously, thus allowing for the sequencing of an entire genome in one day. In NGS, large-scale DNA libraries are first created by shearing the genome into smaller fragments, denaturing them into single strands and attaching specialized oligonucleotide sequences, called "adaptors" to each end of the DNA fragment (Figure 9.6.1). These adaptors allow for hybridization of the DNA library fragments to complementary sequences called DNA "linkers". These linkers are found coupled to

a specialized glass surface, called a **flow cell**. The adaptors also contain small stretches of sequence that can serve as an "index" to identify the bound fragment once sequencing is complete.

Once bound to their linker, each DNA library fragment is amplified through a multi-step process called **bridge amplification**. This unique amplification method produces thousands of DNA copies identical to the original bound DNA fragment. Because these copies are located at a specific area of the flow cell, they are called "clusters". The DNA in each cluster becomes the starting point for a subsequent sequencing reaction called **Sequencing by Synthesis or (SBS)**. SBS uses using fluorescently-labeled "terminator" nucleotides that are similar to those used in Sanger sequencing. However, these nucleotides are said to be "reversible". During SBS, a DNA polymerase synthesizes a DNA chain that is complementary to the bound library fragment using these fluorescent nucleotides. The addition of each nucleotide is known as a "round" or "cycle". A camera takes an image after each round in order to detect the fluorescent signal. Following incorporation of the nucleotide, the fluorescent tag and terminator are removed, thus permitting a new round. The process of nucleotide incorporation and terminator/fluorescent tag removal is repeated for 50 to 300 cycles, depending on the fragment size.

At the end of these SBS phase, the fluorescent images from each cluster are converted into a sequence of bases called a "read". The reads from each cluster are analyzed using bioinformatic programs that align the DNA sequences using a reference genome or assemble them de novo into a genomic sequence.

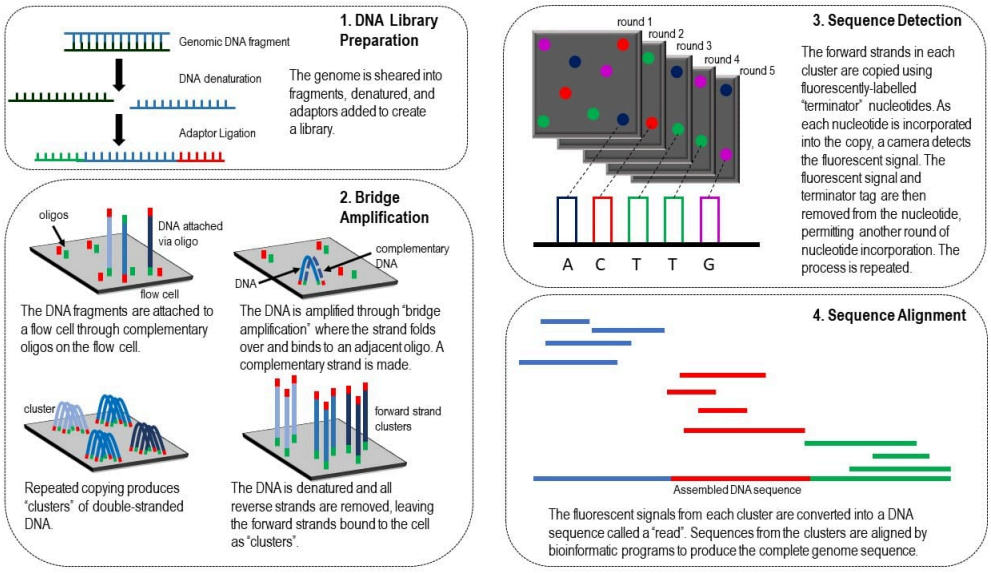


Figure 9.6.1: The Next Generation Sequencing (NGS) workflow. NGS can sequence multiple genomes in a day. (1) DNA Library Production: The genome is sheared into smaller fragments. Each fragment is flanked by two types of adaptors to create a DNA library for sequencing. (2) Bridge Amplification: Through their adaptors, DNA library fragments are bound to complementary DNA oligos located at specific locations on a "flow cell". The bound genomic fragments are then amplified through “bridge amplification” where the DNA strand folds over and binds to an adjacent oligo. A complementary strand is made. Repeated copying produces “clusters” of double-stranded DNA. The DNA is denatured and all reverse strands are removed, leaving the forward strands bound to the cell as “forward strand clusters”. (3) Sequence Detection: The forward strands in each cluster are copied using fluorescently-labeled “terminator” nucleotides. As each nucleotide is incorporated into the copy, a camera detects the fluorescent signal. The fluorescent signal and terminator tag are then removed from the nucleotide, permitting another round of nucleotide incorporation. The process is repeated. (4) Sequence Alignment: The fluorescent signals from each cluster are converted into a DNA sequence called a “read". Sequences from the clusters are aligned by bioinformatic programs to produce the complete genome sequence. (Next Generation Sequencing (NGS) Workflow by Patricia Zuk, CC BY 4.0)

NGS offers researchers a high-throughput option by giving labs the capability to sequence the genomes from multiple individuals at the same time, allowing a genomic comparison between normal individuals and those with a disease or disorder. Through this work, diseases and disorders can be linked to specific DNA mutations.

Concept in Action:

Video: Next Generation Sequencing

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Microarrays

DNA Microarrays can be used to measure the expression of thousands of genes simultaneously and are often used to compare gene expression between different cell types or conditions. The microarray is a solid surface onto which short DNA sequences, called **DNA probes (or probes)**, are affixed in a grid pattern. Each DNA probe corresponds to a specific gene in an organism's genome. To perform a microarray, **total RNA** is extracted from a sample being studied and converted into **complementary DNA (cDNA)**. A fluorescent label is then added to the cDNA. The labeled cDNA sample is added to the microarray, where the cDNA molecules will bind to their complementary probe sequence. The unbound cDNA is washed away, leaving only the labeled cDNA molecules bound to ther DNA probes. The microarray is then scanned with a laser, exciting the fluorescent labels and capturing the fluorescent signal from each spot. Binding of a cDNA molecule to its probe indicates expression of a specific gene. The intensity of fluorescence at each probe spot indicates the expression level of that gene.

Multiple samples can be analyzed using microarrays, ranging from hundreds of samples analyzed by "high-throughput" platforms (e.g., Affymetrix or Illumina) to just two samples (i.e., a dual-color microarray). In a dual-color microarray, the cDNA from each sample is labeled with a different fluorescent label, such as green for the control sample and red for the experimental sample. The samples are applied to the microarray as described above. The fluorescent color and intensity is measured at each DNA probe spot and then used to compare gene expression levels between the two samples (Figure 9.6.2).

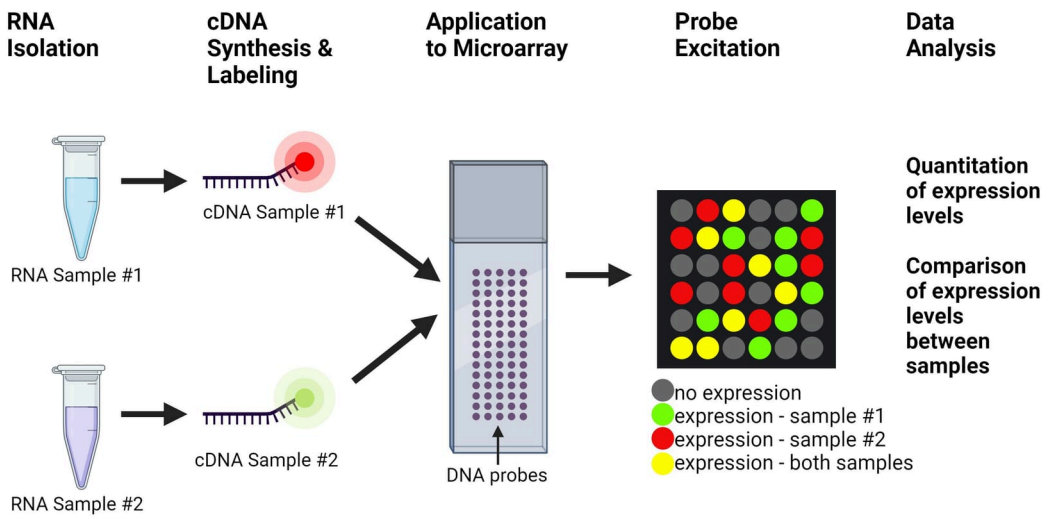


Figure 9.6.2: The steps of dual-color microarray analysis. (1) RNA Isolation: RNA is isolated from two different samples (RNA Sample #1 and RNA Sample #2). (2) cDNA Synthesis & Labeling: cDNA samples are synthesized from these RNA samples and labeled with different fluorescent molecules (cDNA sample #1 with red probe, cDNA sample #2 with green probe). (3) Application to Microarray: the labeled cDNA samples are added to a microarray slide made of thousands of short DNA sequences (DNA probes). Binding of a labeled cDNA to the complementary DNA probe represents expression of a specific gene. (4) Probe Excitation: Excitation of the fluorescent probes produces fluorescent signals that are detected by a fluorescent reader. Spots with red fluorescence represent gene expression in sample #1. Spots with green fluorescence represent expression in sample #2. Spots with yellow fluorescence (i.e. green plus red) represent gene expression by both samples. Empty spots (gray color) indicate no cDNA binding to a probe and no expression of that gene. (5) Data analysis: quantitation of gene expression levels can be determined by measuring fluorescent levels on the slide. Comparison of expression levels between cDNA samples can also be performed. (Microarray Analysis by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. 2025)

Microarrays allow researchers to assess gene expression profiles across thousands of genes simultaneously. Like NGS, data obtained from DNA microarray analysis can be used to compare the genetic "signatures" of a disease or infection to its healthy

counterpart. However, proper data analysis is important since the amount of data generated by a microarray can be significant.

Concept in Action

DNA Microarray Methodology

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Fluorescent *In Situ* Hybridization (FISH)

Fluorescent *In Situ* Hybridization (FISH) is a molecular technique that uses a **fluorescently-labeled probe** to detect specific DNA or RNA sequences in a cell or tissue. Because this hybridization occurs within a cell or tissue, it is considered to be "*in situ*" (i.e., in place). Using a fluorescent microscope, FISH allows scientists to confirm the presence and location of a genetic sequence within the sample. The probes used for FISH can be either DNA or RNA. These DNA or RNA probes are chemically synthesized and are typically designed to be small in length (~20 to 50 nucleotides). The DNA probe is designed to be complementary to a specific DNA sequence in the sample, while an RNA probe is complementary to a specific mRNA or miRNA sequence. Once synthesized, the DNA or RNA probe is then labeled with a fluorescent dye. However, DNA probes can also be labeled with other compounds like fluorescently-bound antibodies. The actual process of FISH will depend on whether the probe is RNA or DNA and how the probes are labeled. To perform FISH, the sample for analysis is prepared by "fixing" the cell or tissue onto a microscope slide (Figure 9.6.3). Chemicals such as paraformaldehyde or formaldehyde are used. Following fixation, the sample is permeabilized with a detergent to allow for penetration of the probe into the sample and the DNA on the slide is denatured. The probe is allowed to bind, or hybridize, to its target sequence in the sample. Excess probes are washed away and the sample is viewed under a fluorescent microscope.

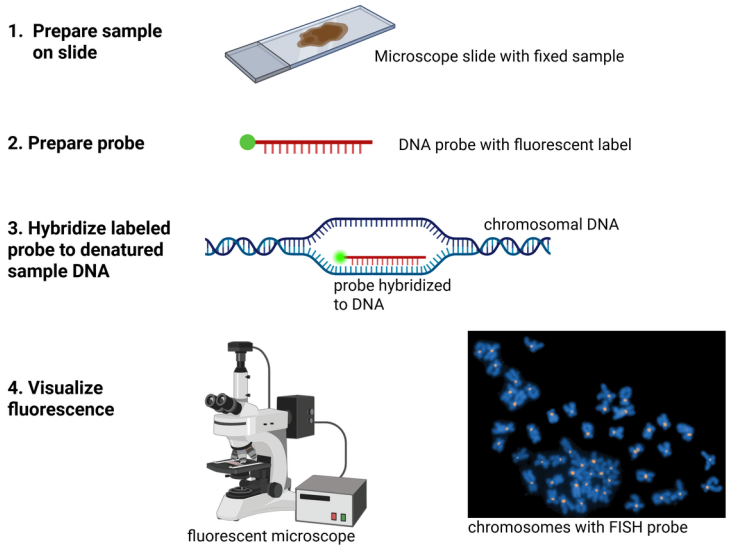


Figure 9.6.3: Fluorescent in situ hybridization (FISH). (1) Prepare sample on slide: samples a fixed to a microscope slide, permeabilized with detergent and the DNA in the sample denatured. (2) Prepare probe: DNA (or RNA) probes are synthesized to be complementary to a target DNA sequence in the sample and then labeled with a fluorescent probe (green circle). (3) Hybridize labeled probe to denature sample DNA: the probe is hybridized to the sample to allow for the binding of the probe sequence to its complementary target on the sample's chromosome. (4) Visualize fluorescence: fluorescence can be viewed using a fluorescent microscope. The physical location of probe binding to chromosomes (shown in blue) is seen as orange fluorescent dots. (FISH by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2025); image credit: Karyotype of Zebrafish by Freeman et al., CC BY-2.0)

FISH is a fast and specific molecular technique that has numerous medical applications, including the detection of chromosomal abnormalities during pre-natal testing (e.g., Down's syndrome), the diagnosis of cancer (e.g. HER2 in breast cancer), the

identification of microbes within a sample, the expression of a gene within a tissue, or the mapping of genes to a specific chromosome. Examples of diseases and syndromes that can be detected using FISH include, acute lymphoblastic leukemia, chronic myelogenous leukemia, Down's syndrome, Cri-du-Chat, and Angelman syndrome. In medicine, FISH is also used to evaluate disease prognosis and remission of a disease like cancer. Treatments can then be tailored to fit the patient.

Immunodiagnostics (Immunoassays)

Immunodiagnostics is a group of diagnostic techniques that use the specificity of **antibodies** to detect and measure substances in biological samples. An **antibody,** also known as an **immunoglobulin**, is a protein produced by the B-cells of the immune system in response to the presence of a foreign substance known as an **antigen**. Antigens are typically sugars or proteins found on the surface of cells (i.e., bacterial, fungal, mammalian) and viruses. The antibody is made of four polypeptide chains, two **heavy chains** and two **light chains**, that link together to form a Y-shape (Figure 9.6.4). The tips of these four chains is known as the **variable region (or antigen-binding site)**. This region changes its shape from antibody to antibody to "match" a specific antigen, giving the antibody the critical property of specificity. The remaining regions of the antibody, called the constant region, can bind to other immune cells or immune components to heighten the immune response. Once bound to the antigen, the antibody can neutralize the antigen, identify the invading cells for destruction by the immune system, and activate the complement system, enhancing the immune response.

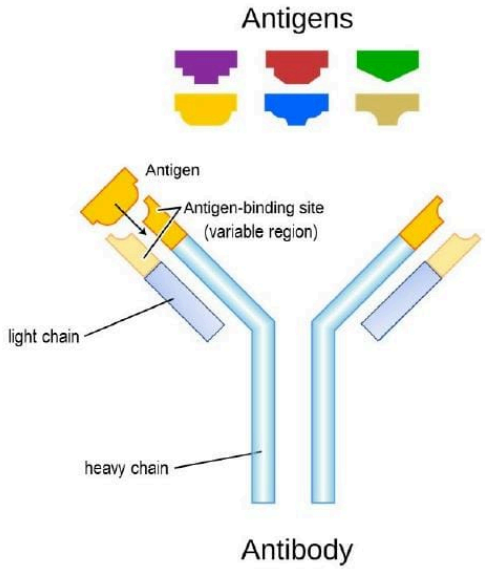


Figure 9.6.4: The structure of an antibody. The antibody is comprised of four polypeptides known as two heavy chains and two light chains. The ends of the light and heavy chains are known as the antigen-binding site or the variable region (shown in yellow). This variable region changes its shape to reflect the specific antigen that binds to this region. (Antibody Structure by Patricia Zuk, CC BY 4.0; modified from Antibody by Fvasconcellos, public domain)

Immunodiagnostic tests are used to identify infectious diseases (e.g., HIV, hepatitis, COVID-19), autoimmune diseases (e.g., rheumatoid arthritis, lupus), and cancer markers (e.g., PSA, CA-125). They are also used in allergy testing and can quantitate substances like hormones (e.g., TSH, insulin) and growth factors (e.g., EGF, VEGF). They are highly specific and sensitive due to the precise interaction between antibodies and antigens. As such, immunodiagnostic tests can detect minute amounts of substances like hormones, proteins, microbes, or antibodies.

Common immunodiagnostic techniques include:

1. **ELISA (Enzyme-Linked Immunosorbent Assay)** – for detecting antigens or antibodies.
2. **Western Blotting** – for detecting specific proteins in a sample.
3. **Immunofluorescence** – uses fluorescent-labeled antibodies to detect targets in cells or tissues.
4. **Lateral Flow Assays** – like pregnancy tests or rapid COVID-19 tests.
5. **Radioimmunoassay (RIA)** – uses radioactive isotopes to detect small amounts of substances.

The Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA stands for **Enzyme-Linked Immunosorbent Assay**. The ELISA is a common laboratory technique that is used to detect and measure an antigen, such as a protein, found in a sample. Samples that are assayed using an ELISA can range from blood and urine (assayed in clinical labs) or cell lysates (analyzed in clinical and research labs). At the end of the ELISA, a "signal" is produced that can be detected and measured. Antigens that can be detected through ELISAs include antibodies, hormones, growth factors, and toxins. ELISAs are used to diagnose diseases (e.g. HIV, hepatitis), detect allergens in food, monitor hormone levels in blood and urine, test for toxins or pathogens in water or food, and measure antibody levels (i.e., titers) in blood following vaccination.

Concept in Action

The ELISA

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There are several types of ELISAs (Figure 9.6.5), including the **direct ELISA** and **indirect ELISA**. In both of these ELISAs, the sample is added to a solid surface in order to bind the antigen to it. The direct ELISA uses an enzyme-bound primary antibody to directly bind the antigen. The direct ELISA is quick and easy to perform but produces a low signal intensity as there is only a single type of antibody used. The antigen in the indirect ELISA is bound by a primary antibody, followed by an enzyme-coupled secondary antibody. Because multiple secondary antibodies can bind a single primary antibody, the indirect ELISA produces an amplified signal. It is also a more flexible ELISA because different types of secondary antibodies can be used. The direct and indirect ELISA use an antigen-coated solid support. In contrast, the **"sandwich" ELISA** starts with a solid support coated with an antibody. In the "sandwich" ELISA, the sample protein to be detected is captured or "sandwiched" in between the antibody-coated surface and the detection antibodies used for quantitation. The sandwich ELISA has high sensitivity and amplification, but non-specific antibody binding can interfere with the results.

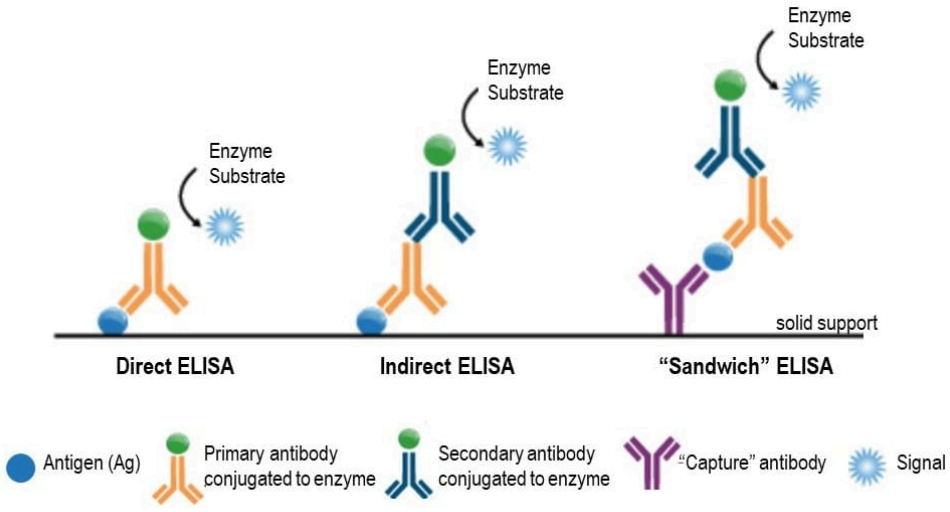


Figure 9.6.5: ELISA types. Some of the major types of ELISAs are direct, indirect, and "sandwich". In the direct ELISA, a primary antibody, coupled to an enzyme binds the antigen bound to the solid support. In the indirect ELISA, a primary antibody binds the antigen, which is then bound by the enzyme-coupled secondary antibody. In the "sandwich" ELISA, the solid surface is coated with a "capture" antibody. This capture antibody binds the antigen. The bound antigen is then detected with a primary antibody and enzyme-coupled secondary antibody. For each ELISA type, the antibody-bound enzyme processes a substrate into a signal that can be detected and quantified. (ELISA Types by Patricia Zuk, CC BY 4.0; modified from ELISA Types by Allan.richard5093, CC BY-SA 4.0)

To perform an "sandwich" ELISA, the following steps are taken:

1. **Coating:** A solid surface, usually a 96-well plate, is coated with an antibody
2. **Blocking:** Non-specific binding sites on the plate are blocked to prevent background signal
3. **Sample addition:** The sample is added and binding of the target to the antibody occurs
4. **Probing:** A target-specific primary antibody is added to the plate to bind and identify the bound target
5. **Washing:** Excess primary antibody is removed
6. **Detection:** A secondary antibody that is linked to an enzyme is added to bind the primary antibody
7. **Washing:** Excess secondary antibody is removed
8. **Substrate Addition:** A substrate specific for the enzyme is added; the enzyme reacts with the substrate to produce a color change or a fluorescent or luminescent signal
9. **Measurement:** The intensity of the signal is measured using a spectrophotometer or luminometer; this measurement is proportional to the amount of the target molecule

The procotols for direct and indirect ELISAs are very similar to the "sandwich" ELISA. Each protocol is summarized below in Table 9.6.1.

Table 9.6.1: ELISA Protocols

ELISA Step	Direct ELISA	Indirect ELISA	"Sandwich" ELISA
1. Coating	Coat with sample	Coat with sample	Coat with "capture" antibody
2. Blocking	Yes	Yes	Yes
3. Sample addition	No	No	Yes
4. Probing	Yes, primary antibody coupled to enzyme	Yes; uncoupled primary antibody	Yes; uncoupled primary antibody
5. Washing	Yes	Yes	Yes
6. Detection	No	Yes, secondary antibody coupled to enzyme	Yes, secondary antibody coupled to enzyme
7. Washing	No	Yes	Yes
8. Substrate Addition	Yes	Yes	Yes
9. Measurement	Yes	Yes	Yes

Lateral flow assays

A **lateral flow assay (LFA)** is a simple, fast, and portable test used to detect the presence of a substance in a liquid sample like water, blood, or saliva. These substances can include proteins, pathogens, or chemicals. The best example of an LFA is a pregnancy test. However, other LFAs include those that will detect viral infections (e.g., the COVID-19 test), bacterial contamination, and the presence of heavy metals in water.

The LFA (Figure 9.6.6) is made of the following parts:

- a **sample pad** where the sample is introduced
- a **conjugate pad** that contains detector molecules, such as antibodies or DNA probes, that mix with the sample
  - the detector molecules are tagged with a visible label
- a **nitrocellulose membrane** as the core of the LFA
  - multiple membranes could be present with each strip performing a specific function
  - the membrane has a **test line** that indicates whether the target is present with a physical line and a **control line** that confirms whether the test worked
- an **absorbant pad (wicking pad)** that pulls the liquid through the strip and soaks up excess fluid

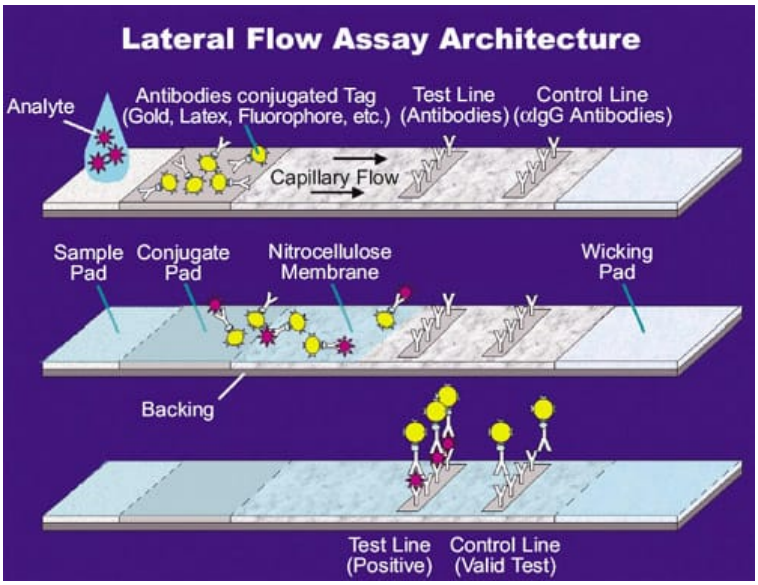


Figure 9.6.6: The lateral flow assay. The lateral flow assay (LFA) test strip is made of a sample pad, a conjugate pad containing antigen-specific antibodies conjugated to a tag like a gold nanoparticle, a nitrocellulose membrane with an antibody-bound test line, and a wicking pad. The sample (i.e. the analyte) is applied to the sample pad and capillary action draws the sample through the remaining parts of the LFA test strip. The antigens found in the sample bind to the conjugated antibodies in the conjugate pad. If the antigen also binds these antibodies found in the test strip, the conjugated tags will form a physical line and indicate a positive result. (Lateral Flow Assay, no author, public domain)

LFAs are easy to use. The sample is added to the sample pad and the liquid flows along the conjugate pad and into the nitrocellulose pad due to capillary action. If the target substance is present it will bind to the tagged detector molecules. The resulting complexes will stick to the test line found in the nitrocellulose membrane and create a visible line to indicate a positive test result.

Point of Care Diagnostics

The LFA describe above is an example of **Point of Care Diagnostics (PCD)**. PCD are medical tests done at or near the place where the patient is located. PCD allows for rapid diagnostics, rather than waiting for a distant lab to provide results. PCD are relatively easy to perform and can be performed by medical personnel and even the patient themselves. The rapid turn around time gives medical personnel the chance to make quick decisions for the health and well-being of the patient.

Examples of PCD include:

- **Lateral Flow Assays (LFAs)**
- **Glucose Monitors**
- **Lab-on-a-Chip**

Each of these PCD examples provide fast results (often within minutes), are portable and easy to use, require minimal training to operate, and can be used at home, in clinics, ambulances, and even at remote locations where clinics are unavailable.

Lab-on-a-Chip

More advanced PCD are being made possible thanks to **lab-on-a-chip (LOC)** technology. An LOC is a device that integrates one or several laboratory functions on a single integrated circuit, known as the "chip" (Figure 9.6.7). This chip is only a few millimeters to a few square centimeters and is designed to achieve automation and high-throughput screening (HTS). LOCs can handle extremely small fluid volumes of blood, urine, or saliva, even down to less than a picoliter (i.e., a trillionth of a liter). The LOC is very similar to an LFA, but can provide more complex analysis.

To use an LOC, a tiny drop of fluid is placed into the chip. **Microfluidic channels** move the liquid within the chip using either capillary action, electric fields, pressure, or vacuum. As the liquid moves through the chip, it passes through reaction chambers where it mixes with reagents that allow for the detection of targets like DNA, proteins, or bacteria. The results can appear as a visual color change, a fluorescent signal, or an electrical signal that is sent to an computer or a phone app.

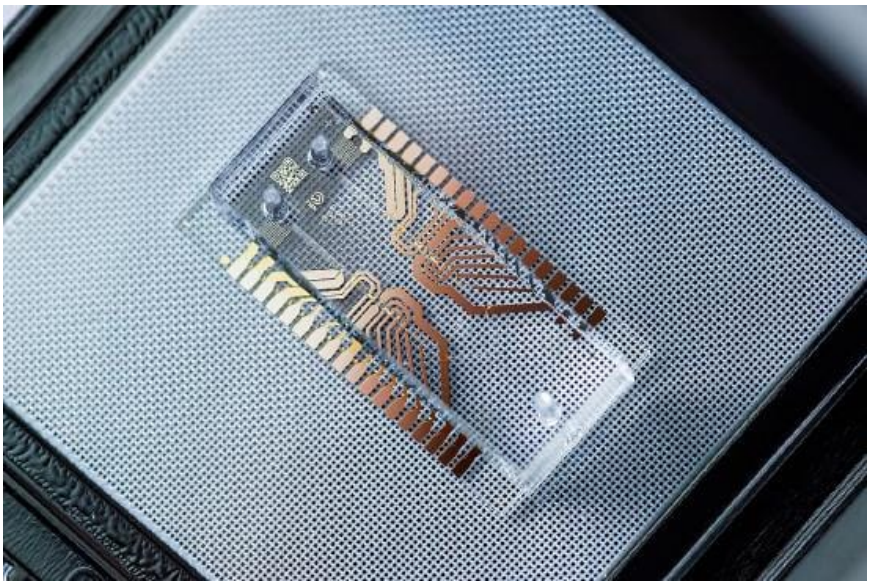


Figure 9.6.7: a Microfluidic chip. The microfluidic chip is a key element of lab-on-a-chip devices that are outside hospitals. The chip consists of multiple microfluidic channels and a detection system of metal electrodes on a glass substrate. (Microfluidic Chip by FMNLab, CC BY-SA 4.0)

Like immunodiagnostic tests, LOC technology can diagnose diseases, measure hormones and growth factors, and detect toxins. However, their more advanced technology also allows for the analysis of DNA or RNA (i.e. portable DNA sequencers), blood and urine. LOC technology may soon become an important part of efforts to improve global health, as a part of PCD. In countries with few healthcare resources, LOCs may be able to provide rapid and accurate diagnosis of things like infectious diseases.

Biosensors

A **biosensor** is a device that uses a biological component, like an enzyme, antibody, or DNA sequence, to detect a specific substance. The biosensor then converts that detection into a readable signal, such as light, color, or an electric signal. The results are usually displayed as a digital reading, a color change or graph.

The main parts of the biosensor (Figure 9.6.8) are the:

1. **Bioreceptor** - the biological part that recognizes and binds the target being detected
2. **Transducer** - converts the biological reaction into a signal
3. **Display** - shows the result

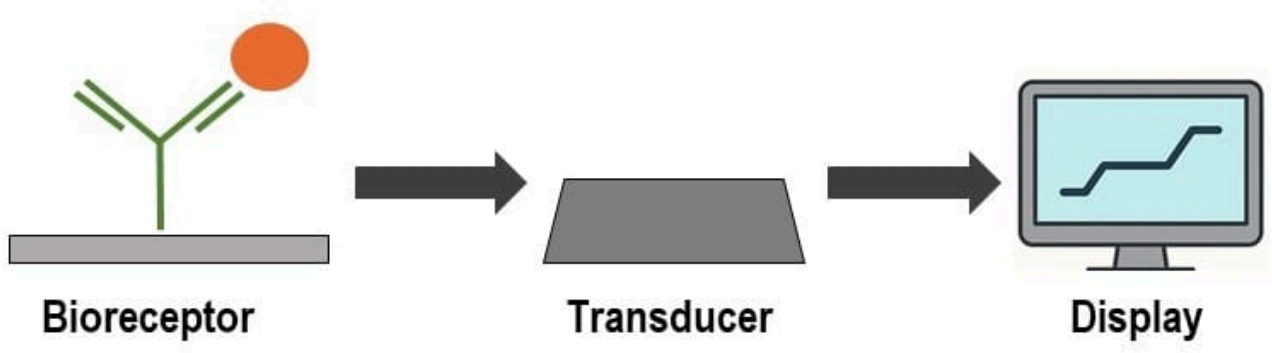


Figure 9.6.8: The biosensor. The biosensor is made of three components: the bioreceptor for detection of the target, the transducer which converts the detection into a measurable signal, and the display which displays the results (The Biosensor by Patricia Zuk, CC BY 4.0)

Perhaps the most well-known biosensor is the **glucose monitor**. The bioreceptor in this sensor is an enzyme called **glucose**

oxidase. Glucose oxidase binds glucose and, through oxidation, converts it to gluconolactone and hydrogen peroxide. This enzyme is physically bound to an electrode. The oxidation reaction releases electrons which flow through the electrode as an electrical current. The amount of current flowing through the electrode is proportional to enzyme activity and glucose concentration in the blood.- The result in then displayed as milligrams per deciliter (mg/dL) or millimoles per liter (mmol/L). In addition to the glucose monitor, biosensors are also used in biotechnology research, drug development, environmental monitoring, and food safety.

Key Concepts

Diagnostic biotechnology involves the use of biological tools and techniques to detect diseases, genetic conditions, or infections. Some important concepts to remember

- molecular diagnostics uses molecular tools like PCR and sequencing to associate DNA sequences with diseases, disorders, and infections
- next-generation sequencing can sequence thousands of DNA fragments at a time and sequence a entire genome in a few hours
- DNA microarrays can analyze sequences from multiple samples at the same time
- NGS and DNA microarrays allow researchers to compare normal genomic sequences to those associated with a specific disease, disorder, or infection
- FISH is used to physically identify the location of a specific DNA or RNA sequence in a genome and can be used to diagnose chromosomal abnormalities, cancer, and microbial contamination
- immunodiagnostics like the ELISA or lateral flow assay (LFA) uses antibodies for diagnosis
- the ELISA is used to measure the amount of a specific antigen or antibody in a sample
- the lateral flow assay (LFA) is a test strip embedded with an antigen-specific antibody
- the LFA is used to quickly identify the presence of an antigen in a sample
- point-of-care diagnostics (PCD) are medical tests performed at local site other than hospital, clinic, or research lab
- PCD include the LFA, biosensors, and the lab-on-a-chip
- a biosensor, like a glucose monitor, uses biological component, like an enzyme, antibody, or DNA sequence, to detect a specific substance in a sample
- advanced biosensors can be combined into a lab-on-a-chip capable of performing multiple chemical steps in a diagnosis

Glossary

Adaptors - short, synthetic DNA sequences attached to a DNA fragment; used in Next Generation Sequencing

Alignment - the process of arranging DNA or amino acid sequences using regions of sequence overlap

Antibody - a Y-shaped protein produced by B cells of the immune system that recognizes and binds to a specific antigen; also called an immunoglobulin

Antigen - a substance (usually a protein or sugar) that triggers an immune response in the body; often used in vaccines

Bioreceptor - a biological molecule or structure that interacts with a target substance

Capillary action - the movement of liquid through a narrow space, like a thin tube or porous material, due to cohesion between water molecules or adhesion between water and the surface

Capillary gel - a thin, glass tube filled with a polymeric gel; used to quickly separate molecules, like DNA fragments, according to their size and electrical charge

Complementary DNA (cDNA) - DNA that is synthesized from an RNA template (usually mRNA) using the enzyme reverse transcriptase

Computational assembly - the process of aligning DNA fragments into a continuous sequence (i.e., a contig) using computer alignment programs

Dideoxynucleotide (ddNTP) - a DNA nucleotide used in Sanger sequencing that lacks the 3'-OH (hydroxyl) group necessary for continued 5'-to-3' DNA synthesis; used to terminate the elongation of the complementary DNA strand

Fluorescently-labeled - a compound that has a fluorescent dye attached to it

High-throughput screening (HTS) - a method for scientific discovery that uses robotics, data processing/control software, liquid handling devices, and sensitive detectors, to allow a researcher to quickly conduct millions of chemical, genetic, or pharmacological tests; used in drug discovery and the fields of biology, materials science and chemistry

Microfluidics - the science and technology of manipulating small volumes of fluids in channels with dimensions of tens to hundreds of micrometers; typically involves volumes of microliters to picoliters

Microfluidic channels - tiny fluid-conducting pathways etched or molded into microfluidic devices; guide fluid flow precisely for chemical reactions, separation, or detection on diagnostics chips or cards

Next Generation Sequencing (NGS) - a high throughput sequencing method that can sequence several genomes in a day; also known as massive parallel sequencing

Oligonucleotide - a short, single-stranded fragment of a nucleic acid, like DNA or RNA, typically containing between 2 and 50 nucleotides; also called an "oligo"

Point of Care Diagnostics (PCD) - medical tests done at or near the place where the patient is located; also called Point of Care Testing (POCT)

Probe - a single strand of DNA or RNA that is complementary to a nucleotide sequence of interest

Reagent - any chemical or biological substance used in a laboratory test or reaction to cause a chemical reaction or detect a target; includes enzymes, antibodies, dyes, and buffers

Sanger sequencing - a DNA sequencing method that uses dideoxynucleotides (ddNTPs) to terminate the synthesis of the complementary DNA chain; also known as chain termination sequencing

Total RNA - the complete set of RNA molecules extracted from a cell or tissue at a given time; includes mRNA, tRNA, and rRNA

Transducer - a cellular component that converts a biological reaction into a signal

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10: ANIMAL BIOTECHNOLOGY

10.1: Applications of Animal Biotechnology
10.2: Genetic Engineering in Animals
10.3: Principles of Mammalian Cell Culture

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10.1: Applications of Animal Biotechnology

Biotech Focus

What comes first, the chicken or the egg?

Well, thanks to biotechnology and **precision fermentation,** turns out its neither one of them. Precision fermentation is an emerging food technology that uses microbes, like yeast, to produce specific functional food ingredients, some of which traditionally require farm animals. One target of this technology is the egg. The "white" of a chicken egg is 88% water, 10% protein (mainly ovalbumin), and trace minerals and carbohydrates. At EVERY Company, scientists use genetically-modified yeast cells to produce the ovalbumin found in chicken egg whites. The yeast are genetically-modified with the gene for chicken ovalbumin and grown in large fermenting tanks. Today, the company uses this technology to produce egg white proteins without the hen. What results is called the EVERY Egg™ - the world's first "henless" egg. So similar is the EVERY Egg™ to the real thing, EVERY Egg™ debuted in 2023 at Eleven Madison Park in New York City, a three-star Michelin restaurant considered among the world's best of the best.

To learn more about precision fermentation, check out the article The Eggs of the Future will be from Precision Fermentation.

Introduction

Animal biotechnology is a branch of biotechnology in which recombinant DNA techniques are used to genetically engineer (i.e. modify the genome of) animals in order to improve their suitability for use in the livestock industry, medicine, or pharmaceuticals. The last decade has seen significant advances in animal biotechnology, with researcher making progress in sequencing animal genomes, gene expression, and the proteomic analysis of animal cells. Through their work, animal biotechnology has lead to increased food production from healthier, faster-growing livestock, increased disease resistance as engineered animals are less vulnerable to infections, improved breeding using assisted reproductive technologies, and medical breakthroughs through transgenic animals producing human medicines.

Learning Objectives

Animal biotechnology genetically engineer animals to improve their use in the livestock industry, medicine, and pharmaceuticals. At the end of this section, you will be able to

- Describe how genetic engineering (gene addition, gene silencing) is used to improve the health of animals and their productivity
- Describe how biopharming can be used to produce compounds beneficial to humans
- Describe how liposomes and nanoparticles are an alternative to viral vectors in gene delivery
- Describe how CRISPR works in gene editing
- Describe the types of cell cloning

Techniques & Applications of Animal Biotechnology

Animal biotechnology uses specific biological techniques and tools. Some of these techniques include **genetic engineering, gene editing,** and **cell cloning**. Their applications in animal biotechnology can be wide and varied.

Genetic Engineering

Genetic engineering uses recombinant DNA techniques to introduce, remove, or modify genes within an organism's genome. Several terms are used to describe genetically engineered animals, including genetically-modified, genetically-altered, genetically-manipulated, and transgenic. For an introduction to genetic engineering and the tools used for this field, see Chapter

4: Genetic Engineering & Recombinant DNA Technology.

The two principle methods in genetic engineering are gene introduction/addition and gene silencing. The goal of **gene addition** is to introduce a functional copy of a desired gene into the genome of an organism. In humans, this gene is usually added in order to replace a defective or missing one. However in animals, gene addition is most often used to introduce a new trait to an animal or improve upon an existing one. The new or modified trait(s) can improve the health of a species, enhance its nutritional profile or its productivity, or enable the use of animal products for use by humans. Examples include the addition of genes that make pigs resistant to Porcine Reproductive and Respiratory Syndrome (PRRS), or genes to cows to protect them from Bovine Spongiform Encephalopathy (BSE) (i.e., mad cow disease). Increasing disease resistance using gene addition in endangered animals is seen as a promising way of improving the health and survival of these animals. Gene addition has also been suggested as a means of re-introducing extinct animals, such as the woolly mammoth, by inserting mammoth genes into the elephant genome. Commercially, the addition of the Pacific Chinook salmon growth hormone gene, in combination with a promoter from ocean pout (an eel-like fish) into Atlantic salmon has resulted in the production of AquAdvantage® salmon, a genetically engineered salmon with a two-fold increase in growth rate when compared to their unmodified counterparts. Regulatory genes have also been added to the genomes of cattle, pigs, and sheep in order to increase their muscle mass, thus decreasing the time it take for these animals to come to market. Outside of its commercial applications, gene addition has be used to insert genes for the production of therapeutic compounds by a wide variety of animals for their use in medicine. For more information, go to Chapter 8.2 Genetic Engineering in Animals.

Viral vectors, such as adeno-associated virus (AAV) or lentivirus, can be modified to introduce genes into animal and human cells without causing disease. Viral vectors have the advantage of being able to deliver the functional gene to a significant number of cells. However, the potential for a deleterious immune reaction can be high with the use of viral vectors. In humans, modified viral vectors are currently being using in the treatment of sickle cell anemia, blindness and spinal muscular atrophy (SMA). While the results are promising, the cost of viral-based gene therapy is very high. As an alternative, genes can be introduced using synthetic delivery methods like **liposomes and nanoparticles**. Liposomes and nanoparticles can be used to deliver genes to cells owing to their ability to encapsulate DNA or RNA and protect them from degradation in the bloodstream. The attachment of specific ligands to nanoparticles so that the delivery of genes can be targeted to a specific cell type has shown promise in enhancing treatment efficacy and minimizing side-effects. Encouraging results have been observed using targeted nanoparticles for the delivery of apoptotic genes to lung cancer cells. However, like viral vectors, the immune system can decrease the effectiveness of nanoparticle delivery. In addition, the percentage of cells receiving these nanoparticles is significantly lower versus viral vectors.

In **gene silencing**, a defective gene is "turned off" inside of a cell. The most common way of doing this is through the use of **RNA interference (RNAi)** technology. RNAi mechanisms within a cell target specific mRNA molecules for degradation or inhibit their expression into proteins. RNAi molecules, including **small interfering RNA (siRNA)** and **microRNA (miRNA)** can be delivered and introduced into cells using nanoparticles, antibodies or modified viruses. RNAi molecules can also be attached to cell surface molecules for internalization. The first commercial RNAi-based therapeutic, approved by the FDA in 2018 is sold as ONPATTRO® for the treatment of hereditary amyloidogenic transthyretin (hATTR) amyloidosis. Today, the gene therapy using RNAi is being explored in the treatment of Huntington's disease and certain cancers. For more about gene silencing and RNAi, read Chapter 5.5: Functional Genomics.

Gene Editing using CRISPR

Gene editing allows for the precise and direct modification of a genome by adding, removing, or altering DNA at a specific location. Like genetic engineering, gene editing modifies an organism's genome to change a specific trait or characteristic. These modifications can include gene addition or gene silencing. However, gene editing typically does not introduce foreign DNA into a host's genome but uses DNA from the same species. One powerful method for gene editing is the technique known as **CRISPR**, or Clustered Regularly Interspaced Short Palindromic Repeats. CRISPR is derived from a bacterial defense mechanism that protects bacteria from the integration of viral DNA (from bacteriophage) into the bacterial genome. For this mechanism, bacteria evolved a location in the genome called a **CRISPR locus.** The CRISPR locus is made of short regions of repetitive bacterial DNA known as **direct repeats** (Figure 10.1.1). In between these repeats, the bacteria inserts short sequences of bacteriophage DNA (approximately 20-50 nucleotides each) known as **"spacers"**. These spacers serve as a "memory" of previous bacteriophage infections and act as future defense against another invasion by the same bacteriophage. Transcription of the CRISPR locus followed by RNA processing produces several pieces of CRISPR RNA or **crRNA**. Each crRNA will contain one of the bacteriophage spacers. The bacteria also produces an additional piece of RNA called **tracrRNA**, which becomes associated with a type of bacterial nuclease called a **CRIPSR-associated nuclease (Cas)**. Bacteria express several Cas isoforms. However, the most commonly studied one is **Cas9.** Upon infection of the bacteria with a bacteriophage, the tracrRNA matches up the crRNA with its corresponding piece of bacteriophage DNA. When this match occurs the Cas9 enzyme cuts the bacteriophage DNA, rendering it inactive.

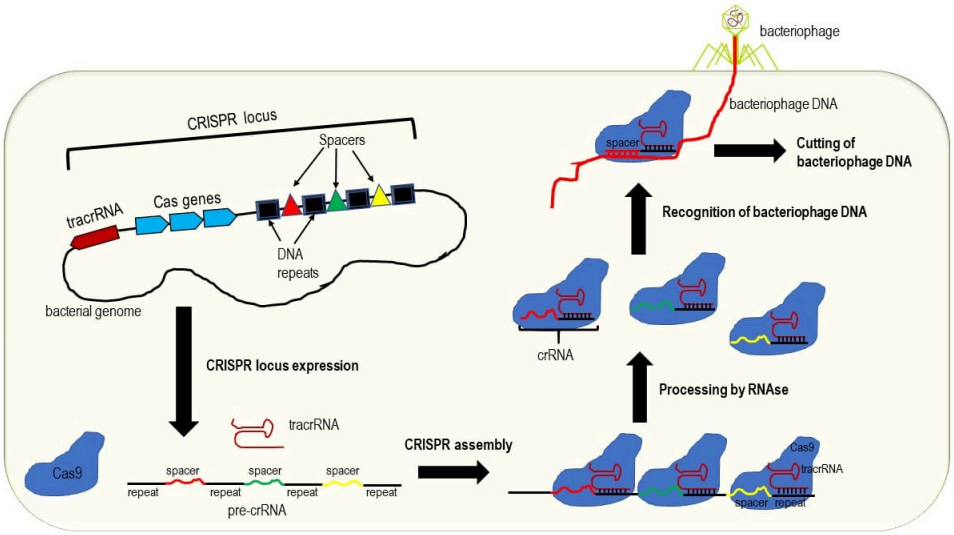


Figure 10.1.1: The CRISPR system of bacterial immunity. Adaptive immunity against bacteriophage infection is achieved in bacteria through the CRISPR system. The CRIPSR locus is comprised of several palindromic repeats of DNA (DNA repeats, black squares) found in between regions of integrated bacteriophage DNA known as spacers (red, green and yellow triangles). The CRISPR locus also contains genes for several isoforms of the Cas enzyme (Cas gene, blue arrows), the gene for tracrRNA (red arrow), and a leader sequence (not shown) that plays an essential role in transcription of the spacers and DNA repeats. CRISPR locus gene expression produces several Cas isoforms, of which Cas9 plays an essential role in immunity. Transcription from the locus also produces tracrRNA (red line), and a piece of "pre-crRNA" comprised of the spacers and DNA repeats. CRISPR assembly results in the hybridization of the tracrRNA with its complementary sequence found in the pre-cRNA and the binding of the Cas9 to the pre-cRNA as well. Processing by an RNAse, cuts the pre-cRNA into several pieces of crRNA (i.e., a spacer and a DNA repeat), each associated with a piece of tracrRNA, and a Cas9 protein. Upon infection by a bacteriophage, the spacer in the crRNA binds to its complementary region in the bacteriophage DNA. The Cas9 enzyme cuts the bacteriophage DNA at that region, rendering it inactive. (CRISPR by Patricia Zuk, CC BY 4.0)

Since its discovery, the CRISPR system has been modified to edit the genome of mammalian cells in the laboratory. For this, researchers combined the tracrRNA and crRNA into a **single guide RNA** (**sgRNA**) that retains the functions of both its crRNA and tracrRNA components. This sgRNA can be designed in the lab to match up with a specific sequence within the mammalian cell's genome where DNA changes are desired. The Cas enzyme is provided to the cell via a DNA plasmid for gene expression or directly as purified protein. The sgRNA directs the Cas9 enzyme to the targeted DNA sequence where it will unwind a short stretch of host DNA. The RNA in the crRNA component can then bind to its complementary DNA sequence. The Cas9 enzyme cuts both strands of the host's DNA helix at that site. Sensing damage to its genome, the cell will repair the cut. However, because mammalian DNA repair is error-prone, the cell will often introduce mutations to the repaired site. If this area of the genome is a gene, the gene with the error will likely be inactivated. As such, CRISPR is often used to "knock-out" gene function.

CRISPR can also be used to excise a mutated section of a gene and replace it with the correct sequence. This can be done if an exogenous piece of DNA is provided to the cell during the repair process. If an entire gene sequence is provided to the cell during repair, the cell can insert this gene. In more advanced applications, Cas9 can be fused to other enzymes for delivery to a DNA sequence. Once there, these enzymes can modify the DNA sequence directly, producing mutations like stop codons to inhibit gene expression or changing the sequence of a mutated gene back to the correct sequence. While a powerful piece of technology, the efficiency of CRISPR gene editing is not 100% so correctly edited cells must be identified. Additionally, scientists are continuing to study the incidence of non-intended edits to the genome when using CRISPR-based systems.

Concept in Action

Animation: CRISPR - Gene editing and beyond

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Cloning and Somatic Cell Nuclear Transfer (SCNT)

Somatic Cell Nuclear Transfer (SCNT), otherwise known as "cell cloning", transfers a diploid (2n) nucleus from a donor cell into a recipient egg in which the nucleus has been removed (i.e., enucleated)(Figure 10.1.2). The genetically-modified egg is programmed by the egg's cytoplasm to begin its development into an embryo that is genetically identical to the donor cell. As such, this early stage embryo is often referred to as a **clone**. In **reproductive cloning**, the clone is implanted, through embryonic transfer, into a surrogate mother for continued development and delivery. Reproductive cloning has been employed to produce genetically identical copies of animals that have exceptional traits, such as enhanced milk production. Despite a high failure rate, numerous animals have been cloned and delivered using this method, including Dolly the sheep in 1996. Reproductive cloning holds promise for the conservation of extremely endangered animals and researchers have also proposed that Reproductive cloning could be used to "resurrect" extinct animals using frozen cells and suitable surrogate mothers (e.g. the woolly mammoth). In **therapeutic cloning**, the clone is cultured and allowed to develop to the blastomere stage (or blastocyst stage for human embryos). Embryonic stem cell (ES) cells are retrieved from this stage. The ES cells are used in regenerative medicine for the development of new tissues and organs.

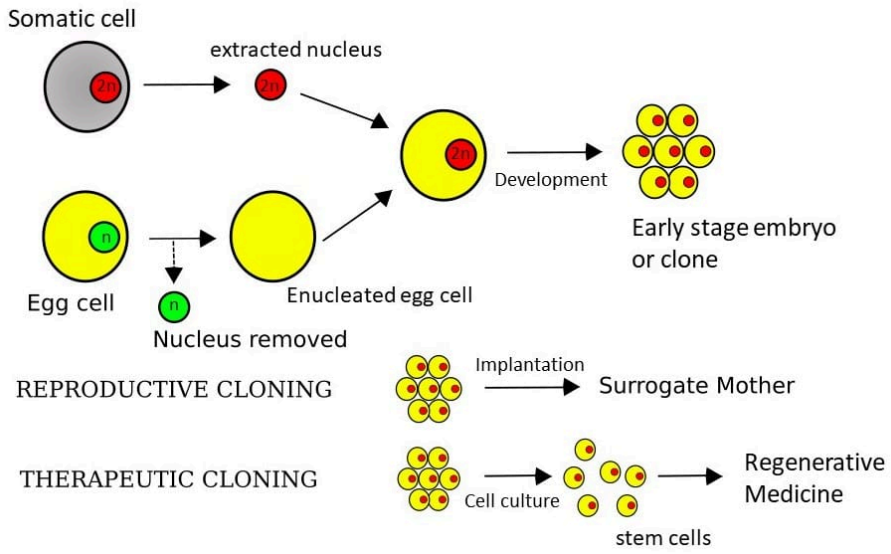


Figure 10.1.2: Somatic Cell Nuclear Transfer (SCNT). The diploid nucleus from a somatic cell with the desired genes is removed and inserted into a recipient egg cell in which the haploid nucleus has been removed. The genetically modified egg cell is then allowed to undergo development to form an early stage embryo that is known as a clone. In reproductive cloning, the clone is implanted into a surrogate mother for continued development and delivery. In therapeutic cloning, the clone is placed into cell culture in order to produce stem cells that can be used in downstream applications, such as regenerative medicine. (SCNT by Patricia Zuk, CC BY 4.0; adapted from Somatic Cell Nuclear Transfer by Wikibob, CC BY-SA 3.0)

Concept in Action

Video: Microinjection of Nuclei for SCNT

This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

Both reproductive and therapeutic cloning have raised concerns about cloning humans and the destruction of human embryos for the isolation of human ES cells. Today, most countries have stringent legal and ethical rules surrounding SCNT and cloning.

Key Concepts

The techniques used in animal biotechnology are wide and varied and include advanced techniques like CRISPR for gene editing and SCNT for cell cloning.

Some important concepts to remember are:

- genetic engineering and gene editing use recombinant DNA techniques to introduce, remove, or modify genes within an organism's genome
- gene editing allows for the precise and direct modification of a genome by adding, removing, or altering DNA at a specific location
- foreign DNA is usually added through genetic engineering; non-foreign DNA is added through gene editing
- genetically engineered organisms are referred to a transgenic organisms
- gene addition introduces a functional copy of a desired gene into the genome of an organism
- gene silencing inhibits the expression of a defective gene inside a cell

Glossary

Animal biotechnology – the application of genetic engineering, cloning, and molecular biology to modify animals for agriculture, medicine, and industry

Biopharming – the use of genetically-engineered plants or animals to produce valuable medical or therapeutic compounds such as drugs, vaccines and antibodies; also called pharmaceutical farming or "pharming"

Cloning – the process of creating a genetically identical copy of an organism, often using somatic cell nuclear transfer (SCNT)

Cell cloning - also known as somatic cell nuclear transfer (SCNT)

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) - a gene-editing tool that uses a guide RNA and a Cas9 enzyme to find and cut specific DNA sequences

Disease-resistant animals – genetically modified or selectively bred animals that are less susceptible to infections

Embryo Transfer (ET) – a reproductive technology where fertilized embryos are transferred into a surrogate mother to improve breeding efficiency

Gene editing – the modification of an cell's DNA to introduce beneficial traits; uses technologies like CRISPR

Genetic engineering - the direct modification of an organism's DNA using biotechnology; often done to introduce new traits, to improve resistance to disease, or to produce beneficial products

Genetically Modified Organism (GMO) - any organism whose DNA has been altered using genetic engineering techniques

Gene silencing - the inhibition of a gene's expression through natural or artificial means; can be achieved in the laboratory using

siRNA or miRNA techniques

Genome - the entirety of the genetic information in a cell

Enucleated - a cell in which the nucleus has been removed; used in cell cloning

Liposome - a spherical vesicle made of phospholipids that mimics the structure of a cell membrane; used in transfection of mammalian cells

Recombinant DNA (rDNA) technology – techniques that combine DNA from different sources, used to create genetically modified animals

Reproductive cloning – cloning that uses SCNT technology to create identical animals for research, agriculture, or conservation

RNA interference - a lab technique used to target specific mRNA molecules for degredation; uses wither siRNA or miRNA molecules to affect mRNA stability and expression

Somatic cell nuclear transfer (SCNT) - a process in which a diploid (2n) nucleus from a donor cell is transferred into an enucleated recipient egg

Transgenic animal – an animal that carries genes from another species, typically used for research, medicine, or agriculture

Therapeutic cloning - cloning that uses SCNT technology for the isolation of embryonic stem cells

Xenotransplantation – the process of transplanting cells, tissues, or organs from one species to another, typically from animals to humans

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10.2: Genetic Engineering in Animals

Biotech Focus

In the late 1990s, researchers at the National University of Singapore added the gene encoding the green fluorescent protein from jellyfish to the genome of wild zebrafish (*Danio rerio*). Under natural white and UV light, these fish glowed a bright green. Recognizing a commercial application, Alan Blake and Richard Crockett founded the **GloFish** brand in 2001 to market and sell these genetically modified fish. After two and a half years of research and consultation with experts, GloFish were introduced to the US market in late 2003 to be sold as tropical aquarium fish. Because aquarium fish are not used as a food source, the US Food and Drug Administration (FDA) determined that GloFish did not pose a greater environmental risk than unmodified zebrafish and opted not to regulate the sale of these fish. However, some environmentalists became concerned about the possibility of GloFish escaping into the wild and thriving. In 2014, a single GloFish was spotted in Florida, and in 2015, groups of GloFish were spotted in Brazil. These fish likely escaped from ornamental fish farms in both locations. How their presence is affecting natural ecosystems remains unknown.

Introduction

Genetic engineering in animals involves modifying an animal's DNA in order to introduce desirable traits that improve the animals health, or enhances its productivity. By using tools like CRISPR-Cas9, recombinant DNA technology, and cloning, scientists can insert, delete, or modify specific genes in animals for various applications. Genetic engineering of animals has revolutionized research, agriculture (i.e., livestock management), the treatment of diseases, medicine and the pharmaceutical industry. Animals have been genetically modified for disease resistance, faster growth, and improved nutritional value. Transgenic animals are now used routinely to produce pharmaceutical proteins, and genetically-engineered pigs are being developed for the transplantation of organs into humans (i.e., **xenotransplantation**). Research on engineering animals also helps scientists study genetic diseases and drug development. Despite its benefits, genetic engineering in animals raises ethical concerns, including animal welfare, ecological risks, and regulatory challenges. As this technology advances, careful evaluation will be needed to balance innovation with ethical responsibility.

Learning Objectives

Genetically-modified animals have found use in a wide variety of industries, including agriculture and medicine. Transgenic animals form the core of "biopharming", disease management, and xenotransplantation. At the end of this page, you will be able to:

- Define appropriate terms like GMO and transgenic
- Outline the basic steps in making a GMO
- Describe how genetically-modified animals can be used a bioreactors to produce pharmaceuticals
- Describe how genetically-modified animals are used to manage diseases
- Describe how genetically-modified animals are used in xenotransplantation

Genetically modified organisms (GMOs)

Genetically-modified organisms (GMOs) are organisms whose genetic material has been altered through genetic engineering techniques. This alteration has been performed to express desirable traits or improve resilience of an organism. When the gene being introduced comes from a different organism, the GMO is often referred to as a **transgenic organism**. This means that all transgenic organisms are GMOs, but not all GMOs are transgenic.

The history of GMOs dates back to the early 1980s, when the human insulin gene was introduced into the genome of the bacteria *E.coli*. These genetically-engineered bacteria were quickly approved for medical use, revolutionizing diabetes treatment. In agriculture, the "Flavr Savr" tomato became the first genetically modified crop to be approved for commercial sale in 1994. Engineered to delay ripening, it sparked the rapid development of other GMO crops, such as pest-resistant corn and

herbicide-tolerant soybeans. Since then, GMO applications have expanded into numerous fields, including medicine, biofuels, and environmental management, becoming an integral part of biotechnology.

- 1982: FDA approves the first consumer GMO product developed through genetic engineering: human insulin to treat diabetes.
- 1986: The federal government establishes the Coordinated Framework for the Regulation of Biotechnology. This policy describes how the U.S. Food and Drug Administration (FDA), U.S. Environmental Protection Agency (EPA), and U.S. Department of Agriculture (USDA) work together to regulate the safety of GMOs.
- 1990s: The first wave of GMO produce created through genetic engineering becomes available to consumers: summer squash, soybeans, cotton, corn, papayas, tomatoes, potatoes, and canola.
- 2003: The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations develop international guidelines and standards to determine the safety of GMO foods.
- 2005: GMO alfalfa and sugar beets are available for sale in the United States.
- 2015: FDA approves an application for the first genetic modification in an animal for use as food, a genetically engineered salmon.
- 2016: Congress passes a law requiring labeling for some foods produced through genetic engineering and uses the term “bioengineered,” which will start to appear on some foods.

To create a GMO, researchers study and identify a gene responsible for giving an organism a desired trait. This in itself can be difficult because the degree to which a single gene affects a trait depends on the desired trait. Once a gene has been identified, there are several steps that are followed in the laboratory:

- the gene is copied from an organism through polymerase chain reaction (PCR)
- the copied gene is ligated into a cloning plasmid to create a recombinant plasmid
- the recombinant plasmid is introduced into another organism (i.e., the "host" organism)
- the host organism is allowed to develop under laboratory conditions
- the resulting GMO is studied extensively in the laboratory to ensure that it exhibits the desired trait and does not pose a threat to the unmodified organism found in nature.

The "host" organism whose genome will be altered can range from something as simple as unicellular bacteria and yeast, to multicellular, complex organisms such as animals and plants. The way in which the gene is introduced into the host organism will depend on the host organism. For animals, recombinant plasmids are typically introduced into cultured embryonic stem (ES) cells through a process called **transfection**. In transfection, the DNA is incorporated into small particles, called **liposomes**, in order to facilitate the entrance of the DNA into the cell. Once expression of the introduced gene is confirmed, the modified ES cells are then microinjected into an early stage embryo (i.e. the blastocyst) for incorporation into its inner cell mass. Alternatively, recombinant plasmids can be directly microinjected into into a fertilized egg (i.e. the zygote), or the gene can be inserted into the zygote's genome using CRISPR. Following implantation of the genetically altered embryo into a surrogate female, the offspring are assessed for their expression of the new gene (Figure 10.2.1). For more information about transfection, go to Chapter 13.10: Liposomal transfection into mammalian cells.

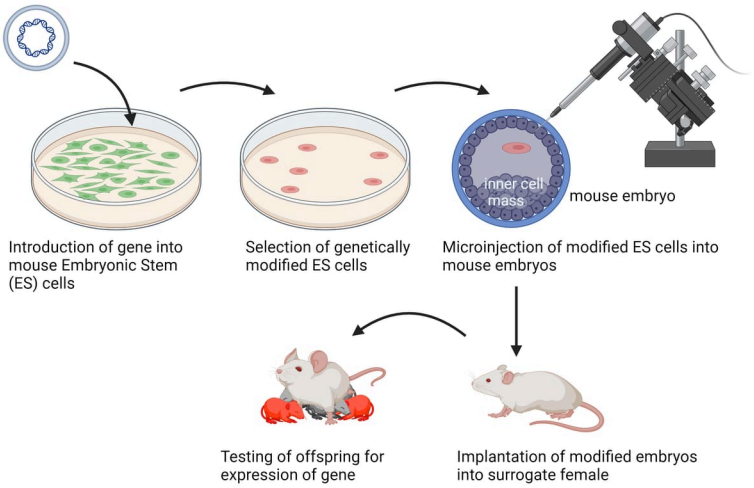


Figure 10.2.1: GMO animal production. One method of producing a genetically-modified organism (GMO) is shown in this figure. The gene is introduced into embryonic stem cells (ES cells) that have been isolated from the host organism, such as a mouse. The selection of genetically modified mouse ES cells are is performed to ensure a purified population. Microinjection of these modified ES cells into a mouse embryo results in the incorporation of the cells into the inner cell mass of the embryo. Incorporation is followed by implantation of the modified embryo into a surrogate female for continued development. Testing of the offspring for expression of the gene (red mice) identifies the GMO. (GMO animal production; Patricia Zuk CC BY 4.0; figure created in BioRender. Zuk, P. (2024))

Genetically-Engineered Animals in Medicine

Animals as Bioreactors

Drug production, regenerative therapies, xenotransplantation, and disease modeling all use genetically-engineered animals. For example, spider silk genes have been inserted into goats, allowing them to produce silk proteins in their milk, which can be used for medical sutures and bulletproof materials. This technology, known as **biopharming** or "pharming", has also been used for the insertion of human proteins into animal genomes, essentially turning these modified animals into "living bioreactors". For example, insertion of the gene encoding anti-thrombin, a natural blood protein that prevents excessive blood-clotting, into the genome of goats has enabled them to produce this therapeutic protein in their milk (Figure 10.2.2). The resulting product, known as ATryn® is currently used to treat conditions like deep vein thrombosis, hereditary anti-thrombin deficiency or for preventing clots during surgery or childbirth.

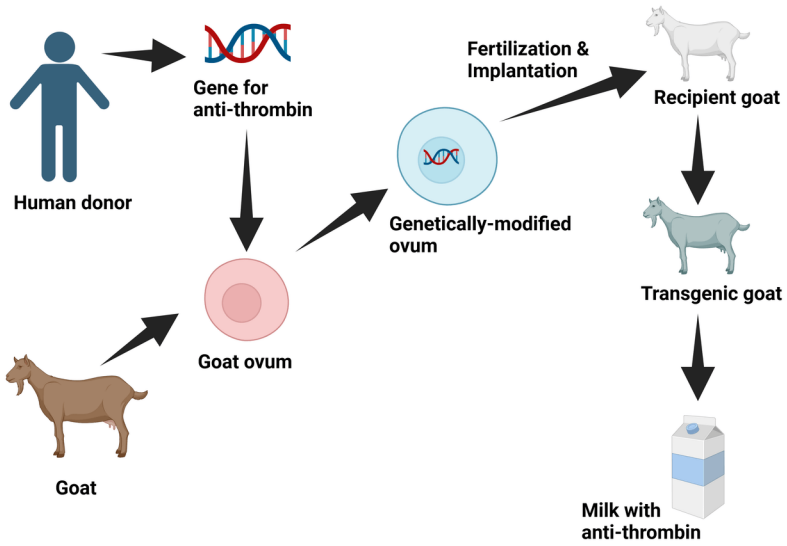


Figure 10.2.2: Biopharming of human anti-thrombin. The human anti-thrombin gene is isolated from a human donor and a goat is used for the donation of an ovum. The gene is microinjected into a goat ovum (pink cell) to created a genetically-modified ovum (blue cell). The ovum is then fertilized and implanted into a recipient goat (shown in white). The resulting transgenic goat (shown in gray) is bred for milk production. The milk, containing the anti-thrombin, can then be processed and the clotting agent extracted and purified. (Biopharming by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2025))

Additional examples of biopharming and the use of animals as bioreactors include:

- Transgenic cows for the production of human insulin and human growth hormone in their milk
- Transgenic cows modified with human immunoglobulin genes and challenged with specific pathogens to produce human antibodies
- Transgenic goats engineered to produce spider silk proteins in their milk for medical sutures and biomaterials
- Transgenic rabbits and the production of the hormone erythropoietin (EPO)
- Transgenic sheep production of alpha-1 anti-trypsin for the treatment of cystic fibrosis and emphysema
- Transgenic sheep and pigs in the production of clotting factor IX

Such applications, while exciting, require the breeding of large amounts of animals and the isolation of specific tissues like blood. As a result, many have called into question the ethics of using transgenic animals.

Disease Management

The use of animals as bioreactors for pharmaceutical production is an established method for the treatment and/or management of a disease. However, there are additional approaches like the production of disease-resistant animals through genetic engineering. For example, the genetic manipulation of cattle is being used to protect herds from deadly infections like foot-and-mouth disease. Genetic engineering of mosquitoes is revolutionizing how the spread of mosquito-borne diseases like malaria, dengue, Zika, and chikungunya is limited in affected populations. Genetically-modified mosquitoes have been created that prevent their reproduction (e.g. Oxitec's Friendly™ Aedes Mosquitoes). The males contain a "self-limiting gene" that, when expressed in their offspring, causes them to die before they reach adulthood. The release of these males into the wild population eventually leads to population collapse. Since their approval and introduction, decreases of over 90% have been measured *Aedes aegypti* mosquito populations in Brazil, Florida, and the Cayman Islands. Disease management through vaccine production has also seen significant advancements using genetic engineering. Chickens can be genetically modified to express a specific pathogenic protein in their eggs. The protein can then be isolated and purified from the egg and used for immunization, offering a faster, more efficient, and cost-effective way to manufacture vaccines for human and animal diseases. Since a single chicken can lay over 300 eggs per year, large amounts of vaccines can be made for a lower cost versus traditional vaccine culture techniques. These vaccines can also be quickly produced for viruses that show a rapid rate of mutation (e.g. influenza, COVID-19).

Xenotransplantation

Xenotransplantation is the process of transplanting cells, tissues, or organs from one species to another, typically from animals

to humans (Figure 10.2.3). This technology is being explored as a solution to the shortage of human organ donors for patients in need of transplants. To increase success rates, animal organs can be genetically-modified through somatic cell nuclear transfer (SCNT) to knock out immunoreactive porcine genes and to express human immune proteins, in the hope of decreasing the risk of immune rejection. Pigs have become the primary choice for xenotransplantation because their organs are similar in size and function to human organs. Prior to use, those pig genes that would illicit an immune reaction are silenced. In addition, the presence of the PERV (Porcine Endogenous Retrovirus) genome within the genome of the pig is removed to prevent infection of human recipients. For this, scientists are exploring the use of CRISPR to silence or edit out these genes. Human genes are then inserted to help prevent immune rejection and blood clotting.

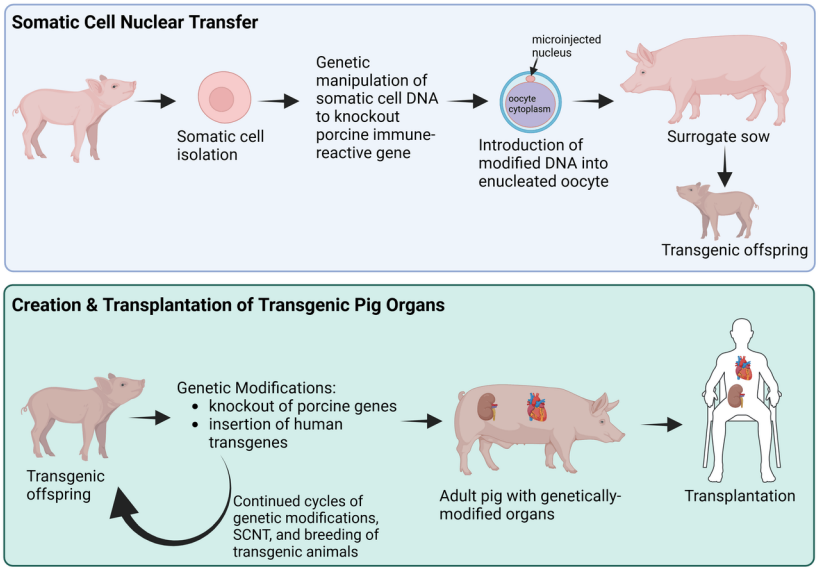


Figure 10.2.3: Xenotransplantation. The modification of pig organs for human transplantation is done in two phases: Somatic Cell Nuclear Transfer (top box in blue) and the creation and transplantation of transgenic pig organs (bottom box in green). In SCNT, a somatic cell (pink) is isolated from a pig. Genetic manipulation of the somatic cell's DNA is performed to knock-out genes that could cause immune rejection in humans. Introduction of the modified DNA into an enucleated oocyte is accomplished by microinjection of the nucleus taken from the modified somatic cell. The oocyte is activated to initiate embryonic development (not shown) and then implanted into a surrogate sow. The resulting transgenic offspring is then used in the creation and transplantation of transgenic pig organs. In this process, the transgenic offspring undergo repeated cycles of additional genetic manipulation (e.g. knock-out of porcine genes, insertion of transgenes), SCNT, and breeding to produce transgenic pigs that express human immune antigens. The adult transgenic pigs are used for the harvesting of genetically-modified organs, such as the heart and kidneys. These organs are used for transplantation into humans. (Xenotransplantation by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2025))

In 2021, the first genetically-modified pig heart was successfully transplanted. In 2024, a modified pig kidney was transplanted and functioned in a brain-dead patient for over a month. In 2024, physicians at Massachusetts General Hospital transplanted a genetically-edited pig kidney into a 62 year old living patient. Genetically-modified pig hearts were first transplanted into living patients in 2022 and 2023. Most recently, in 2025, a genetically-modified pig liver was transplanted into a brain-dead human recipient in order to monitor its function and potential rejection. As the technology improves, pig organs are likely to see increased use in transplant facilities.

While xenotransplantation provides hope for alleviating organ shortages, there are several problems associated with this technology. Even with careful gene editing, rejection of the modified organ is still a risk. The presence of PERV in the pig genome presents concerns about unanticipated cross-species infections. In response, researchers have begun to explore the use of modified primate organs in place of pig organs. However, primate use is likely to increase the opposition people feel about using animals for human organ transplants.

Molecular Lab Protocols

- Lab Technique: Liposomal transfection into mammalian cells

Key Concepts

Genetically-modified animals form the core of areas like "biopharming", disease management, and xenotransplantation, offering advancements in medicine, agriculture, and research.

Some important concepts to remember are:

- Genetically-modified organisms (GMOs) are organisms whose genetic material has been altered through genetic engineering techniques
- The introduction of foreign genes into an organism produces a GMO known as transgenic
- The method used to introduce genetic information into an organism depends on the organism itself
- In medicine, transgenic animals such as goats, cows, and chickens are modified to produce therapeutic proteins in a process known as biopharming
- Transgenic animals can also be used in the production of vaccines, and in the management of diseases
- Transgenic animals can also be engineered for the production of disease-resistant animals
- Genetically-modified pig organs with reduced potential for immune rejection are being developed for xenotransplantation
- Innovations using transgenic animals raise important ethical considerations

Glossary

Biopharming - the use of genetically engineered plants or animals to produce valuable medical or therapeutic compounds such as drugs, vaccines, and antibodies; also called pharmaceutical farming or "pharming"

Blastocyst - an embryonic stage comprised of a hollow, fluid-filled ball of cells; develops about 5–6 days after fertilization in humans and consists of a thin outer layer of cells and an inner cell mass made of embryonic stem cells (ES cells)

ES cells (Embryonic Stem Cells) - totipotent stem cells derived from the inner cell mass of a blastocyst, an early-stage embryo

GMO (Genetically-Modified Organism) - an organism whose genetic material has been altered through genetic engineering techniques

Inner cell mass - a group of cells found within the blastocyst stage of embryonic development; a source of ES cells

Transfection - the process of introducing foreign DNA (or RNA) into eukaryotic cells using chemicals, like liposomes or calcium phosphate

Transgenic organism - an alternate term used for a GMO; often used to refer to genetically-modified animals

Xenotransplantation - the process of transplanting cells, tissues, or organs from one species to another, typically from animals to humans

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10.3: Principles of Mammalian Cell Culture

Biotech Focus

In 1951, **Henrietta Lacks (1920 - 1951)** entered Johns Hopkins hospital for the surgical removal of cervical adenocarcinoma. Cells biopsied from her tumor were given to George Otto Gey (pronounced "Guy") and Gey, together with his wife Margaret, began culturing these cells. Despite having failed to culture other types of cells before, the carcinoma cells from Mrs. Lacks survived and rapidly grew under *in vitro* conditions. They named the cell line **HeLa**, from the letters of Henrietta Lack's name, and began to distribute these cells to other labs around the country. Today, Hela cells are one of the most commonly cultured cell lines in culture facilities. They divide - quickly and indefinitely under standard culture conditions, a hallmark of an immortalized cell line. They have been credited with numerous discoveries, including the development of the polio vaccine, and have been used in drug development, the study of cellular aging, and cancer. However, Mrs. Lacks was not asked for her permission to distribute the cells to Dr. Gey. Patient consent to use their tissues for research was not a common practice back then. Nor did Mrs. Lack's or her family receive any compensation when the cells entered the US marketplace. The commercialization of HeLa cells and subsequent profits has since raised questions about ownership and informed consent of patients.

To learn more about Henrietta Lacks and George Guy, read "*The Immortal Life of Henrietta Lacks*" (2010) by Rebecca Skloot.

Introduction

Cell culture is the growth of cells separate from the donor organism using a liquid, semi-solid or solid growth medium. A more modern definition would be the growth of cells from a multi-cellular organism under controlled conditions. Cell culture gives researchers a method for studying the behavior of cells, under experimental conditions, free from the variations that might arise within a complex, multicellular organism. Such research has elucidated the mechanisms of complex processes such as normal embryologic development, disease progression and how drugs can impact the health and function of an organism. Today, cell culture is an integral part of basic scientific research, pharmaceutical development, bioengineering labs and many other kinds of labs performing research that impacts human health. It is truly one of the most important skills a biologist can have.

The term cell culture is often used interchangeably with **organ culture** and **tissue culture**, but they are not the same thing. Cell culture is the *culture of individual cells* - taken from plant or animal. Organ culture refers to the the *culturing of mammalian tissues* isolated from an organ, or the organ itself. Finally, tissue culture is frequently associated with the *culturing of plant cells or tissues* (Figure 10.3.1).

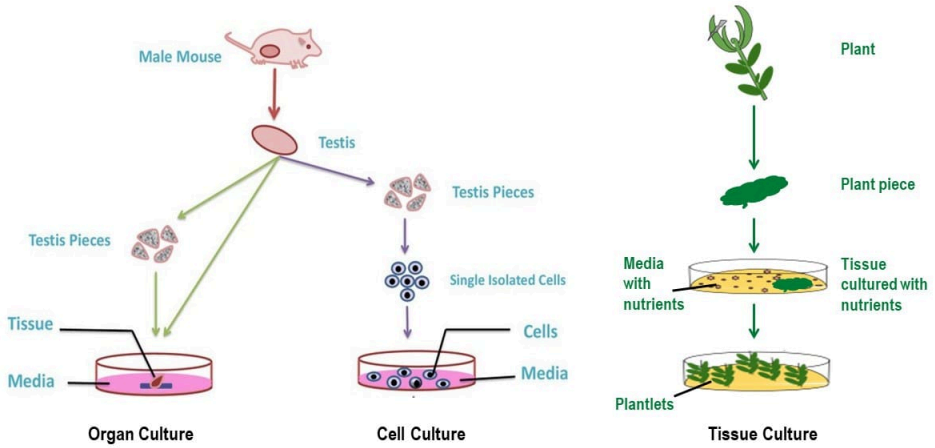


Figure 10.3.1: Cell vs. Organ vs. Tissue Culture. In organ culture, a tissue, like the testis, is isolated from an animal (such as a male mouse) and cut into pieces (testis pieces). The resulting tissue is placed into culture dishes with media for maintenance of the tissue. In cell culture, the tissue pieces are used to isolate cells that are then placed into culture dishes with media. In tissue culture, tissue is isolated from plants (plant piece) and placed into media with nutrients. Over time, the plant cells within the tissue will differentiate and form small "plantlets". (Cell vs. Organ vs. Tissue Culture by Patricia Zuk CC BY 4.0, adapted from Testis Organ Culture by Ibtisham et al. CC BY-SA 3.0 and Simple Micropropagation Technique by MarchaelaM, CC BY-SA 4.0)

Learning Objectives

Cells and tissues can be cultured outside of the organism using *in vitro* culture techniques. At the end of this section, you will be able to:

- Define the types of cell culture: adherent, suspension, organ, and tissue
- Distinguish between primary cultures, secondary cultures, cell lines, and cell strains
- Compare a cell line with a cell strain
- Describe the two types of cell cultures
- List the conditions for cell culture
- List some cell culture reagents
- List and describe the three types of cultured cells
- List and describe the major types of culture media
- Explain biosafety considerations
- List and explain the four BSL levels

Types of Cell Culture

Adherent culture: a type of *in vitro* culture in which cells grow on a surface as a single layer of cells (Figure 10.3.2). Most cells must grow under adherent culture conditions because they require a surface on which to adhere in order to survive and divide. The culture surface is provided in the form of specially-treated and sterilized **cell culture plastic-ware**. It provides the cell with a charged surface that is essential for its adherence. Normal cells grown under adherent conditions will stop growing once they are surrounded on all side by other cells, produced a **monolayer** of cells. This inhibition is called **contact-growth inhibition** and adherent culture can also be known as monolayer culture. Transformed cells will not demonstrate this property. Nearly all normal vertebrate cells grow under adherent conditions.

Suspension culture: a type of *in vitro* culture in which the cells grow suspended within the culture medium; no adherence is required (Figure 10.3.2). While not as common as adherent culture, there are cell types, such as immortalized cells and certain types of immune cells, that will grow suspended in culture medium. This type of culture requires specialized equipment in order to keep the cells suspended so that they receive adequate oxygen and nutrients and do not settle out due to gravity. Most suspension

cultures will either use **roller balls** or **"spinner" flasks**. Roller balls are placed on machines that continuously turn the bottle, while magnetic stirrers within spinner flasks keep the medium and cells suspended.

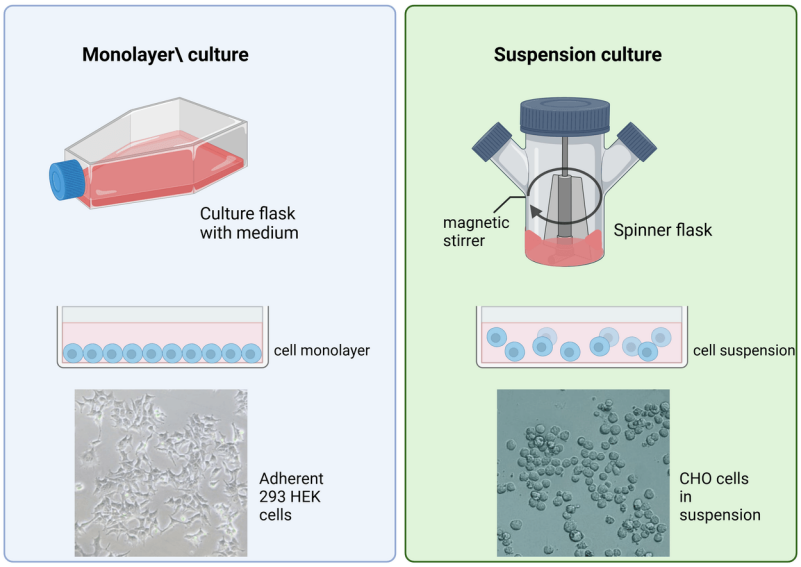


Figure 10.3.2: Adherent vs. Suspension Culture. In adherent culture (blue box on left), cells are grown specially-treated plastic-ware, like a culture flask, whose surfaces promote cell adherence (culture flask with medium). The cells in adherent culture will grow as a cell monolayer. Adherent 293 HEK cells are shown. In suspension culture (green box on right), cells can be grown in vessels known as Spinner flasks, whose magnetic stirrer keeps the cells suspended and exposed to oxygen and nutrients. The cells in suspension culture are non-adherent and "float" within the medium (cell suspension). CHO cells in suspension are shown. (Types of Culture by Patricia Zuk, CC BY 4.0; figure created in BioRender. Zuk, P. (2025); photo credits: HEK 293 cells by Panzer VI-II public domain; CHO cells by Jamiri, CC BY-SA 3.0)

Organ culture: a type of *in vitro* culture that grows either whole organs or parts of organs. One of the major advantages of organ culture is its maintenance of tissue architecture and function within the organ. Organ culture is complex and requires delicate handling *in vitro* as it is essential that the tissue is never disrupted or damaged. The media used for a growing organ culture are generally the same as those used for tissue culture. Organ culture technology has contributed to advances in embryology, inflammation, cancer, drug development, and stem cell research.

Tissue culture: a type of *in vitro* culture that maintains and/or grows plant cells, tissues, or organs. Tissue culture of plants is widely used to create exact clones of a plant. Plant cells, pieces of leaves, stems or roots, given the required nutrients and plant hormone, are often used to generate a new plants through tissue culture.

Cell Culture Types

Cell culture is an integral part of basic scientific research, pharmaceutical development, bioengineering labs, and many other kinds of labs performing research that impacts human health. As a result, the types of cells that are cultured are extremely varied but can be placed into one of four major categories based on their morphology (i.e., their appearance) in culture:

1. **Epithelial-like:** cells attach to a substrate and appear flattened and polygonal in shape
2. **Lymphoblast-like:** cells do not attach but remain in suspension with a spherical shape
3. **Fibroblast-like:** cells attach to an substrate appears elongated and bipolar

Table \({10{.}3{.}\eatSpaces{1} \endSpaces}\ below lists some of the more commonly cells cultured cells.

Table 10.3.1: ***Commonly Culture Cells***

Cell Abbreviation	Cell Name	Description
3T3	“**3**-day **T**ransfer **300,000** inoculum”	mouse fibroblast cell line; derived from Swiss albino mouse embryonic fibroblasts; original culture "transferred" every "3" days at a density of "300,000" cells per culture; used to derive additional 3T3 cell lines

Cell Abbreviation	Cell Name	Description
3T3-L1	**3T3** cell, subclone **L1**	mouse fibroblasts (immortalized pre-adipocyte cell line); derived from 3T3 cells
MC-3T3	"**M**ouse **C**alvarial" from **3T3** cells	mouse fibroblasts (immortalized pre-osteoblast cell line); derived from mouse calvaria (i.e., bone)
CHO	"**C**hinese **H**amster **O**vary"	hamster epithelial cells (immortalized cell line); numerous subclones available
L6		rat myoblasts (immortalized cell line)
PC12	**P**heo**c**hromocytoma-12	rat neuroblastic cells (non-immortal cell line); derived from pheochromocytoma cells in adrenal medulla
COS	"**C**V-1 in **O**rigin, carrying the **S**V40 genetic material"	monkey kidney fibroblasts (immortalized cell line); produced by transforming CV cells with SV40 viral DNA; numerous cell lines available (e.g. COS-1, COS-7)
MDCK	**M**adin **D**arby **C**anine **K**idney	canine epithelial cells (non-immortal cell line)
Saos-2	"**Sa**rcoma **Os**teogenic"	human fibroblasts (pre-osteoblast cell line); derived from osteosarcoma patient's bone
HEK 293	"**H**uman **E**mbryonic **K**idney" experiment number **293**	human epithelial cell line (immortalized); derived from human fetal kidney tissue; produced by transfecting HEK cells with adenoviral DNA
H1, H9		human Embryonic Stem (ES) cell lines; derived from inner cell mass of human blastocysts
MCF-7	**M**ichigan **C**ancer **F**oundation-7	human breast cancer cell line (immortalized); derived from metastatic breast tumor
HeLa*	"**He**nrietta **La**cks"	human epithelial cells (immortalized cell line); derived from metastatic cervical tumor

Primary vs. Secondary Cultures vs. Cell Lines

Many cells used in *in vitro* culture are derived from either animal or plants tissues. The process of isolating these cells is well-defined and will result in a primary culture. From this culture, others can be derived, such as a cell line or cell strain.

A **primary culture** can be prepared from a tissue that is removed from an organism. The tissue can be dissected out and cut into small pieces or treated with enzymes to release cells from the tissue. The resulting cellular mixture is then placed in suitable culture environment. For an adherent primary culture, the non-adherent cells will be removed when the medium is changed and the adherent cells can be expanded. A primary culture is a **heterogeneous** (i.e. mixed) population of many different cell types because of its origin from a multi-cellular tissue. The cells in the primary culture, known as **primary cells**, have a finite life span in culture, unless they are immortalized (see below). Sub-culturing of these primary cells can expand their numbers and lead to either a secondary culture of a cell line. Many primary cell types will not divide i*n vitro* (e.g. macrophages and neurons). As such, these cells can be maintained *in vitro* for a limited amount of time.

A **cell line** is produced by sub-culturing (or passaging) a primary cell culture (see Chapter 13.13: Cell Culture). A cell line may be referred to as a **secondary culture**. Dissociating the primary cells from the culture dish and transferring them to a new one will kill those cells that are not as "hardy" as the others, removing them from the subsequent culture. As a result, a cell line is more **homogenous** (i.e. uniform) than the primary culture. Most cell lines are produced by passaging multiple times in order to increase the removal of contaminating cell types. While the composition of a cell line is relatively homogenous, the cells of a cell line are not all identical to one another. As with primary cultures, cell lines have a finite life span in culture unless immortalized.

A **cell strain** is produced from either a primary culture or a cell line. The cells are diluted over and over again until a single cell is achieved. This single cell is expanded into a cell population known as a cell strain. In most cell strains, a single cell with a specific trait is selected and expanded. The expansion of these single cells in number is called **cloning** as every cell in a strain originates from the same "starting" cell. The cloning process can produce multiple cell strains (or **sub-clones**), with each strain having a

unique set of characteristics. Because the cell strain is derived from a single cell, it is a homogenous population, with each cell in the culture being of the same type. However, the cells of a homogenous cell strain are not identical to one another as genetic changes can occur as they are maintained *in vitro*.

Cell line and cell strain are frequently used interchangeably but they not the same thing. A cell line is produced from a primary culture, while a cell strain is produced from a single cell taken from a primary culture or cell line. Cell strains have a finite life-span in culture and do not change their properties over time. Because they are often isolated from cell lines, cell strains are also more homogenous and specialized then the cell line. Table 10.3.2 below compares a cell line with a cell strain.

Table 10.3.2: Comparison of Cell Line with Cell Strain

Feature	Cell Line	Cell Strain
Definition	A population of cells derived from a primary culture	A population of cells derived from a primary culture or cell line and have been selected for a specific trait (or traits)
Origin	Usually created by subculturing cells from a primary culture to eliminate contaminating cell types	Derived from a single cell through a process called "cloning"
Composition	Can be heterogenous	Homogenous
Lifespan	Can be finite or immortal in culture	Typically finite in culture

Immortalized Cells

Cell lines and cell strains that can only be cultured for a limited time-span before the cells die are known as **finite**. Through a process called **transformation**, the genetic make-up of a cell line or cell strain can be altered and the cells can be maintained indefinitely in culture. These cells are said to be **immortalized.** Because they can be cultured continuously, immortalized cell lines and cell strains are also called **continuous cell lines** or **continuous cell strains**. Immortalized cell lines can be produced from cancerous primary cultures, from cell lines in which genetic mutations have occurred naturally during culture, or following the direct introduction of oncogenes into the cultured cell. The genetic changes of transformation “turn off” the cellular mechanisms that control cell death, permitting continuous growth of the cells in culture. With their infinite life-span in culture, immortalized cell lines and cell strains are used extensively in biotechnology and biomedical applications. Table 10.3.3 below outlines some of the advantages and disadvantages of immortalized cells.

Table 10.3.3: Advantages and Disadvantages of Immortalized Cells

Advantages	Disadvantages
fast growth & unlimited proliferation; decreased need to produce primary cultures	genetic changes during transformation lead to altered gene expression and cellular functions; cells cannot be used for the study of normal physiology
easy to maintain; more robust under suboptimal conditions	transformation produces cells with characteristics of cancer cells (e.g. altered chromosome number, uncontrolled proliferation); limits relevance for certain non-cancerous processes
reduced dependence of serum and substrate - grow more easily in suspension conditions (loss of anchorage dependence)	cells can accumulate additional genetic mutations over time that can compromise research results
ability to grow at higher cell density (loss of contact inhibition)	may exhibit reduced resemblance to tissue of origin, such as loss of tissue-specific gene expression; cells cannot be used for highly specialized studies
homogenous; similar cultures produced with each passage; results in consistent experimental results	cross-contamination with other cells lines common
well-studied and widely used in research; extensive data available on their growth, behavior, genetic profile, and functional characteristics	prone to contamination with mycoplasma

Advantages	Disadvantages
cost-effective; unlimited growth and ease of culture make cells more economical for large-scale experiments or industrial applications	

Cell Culture Conditions & Nutrients

The culturing of cells requires well-defined and precise external conditions that mimic those found in the cell's *in situ* environment. The exact values for the conditions listed below can vary according to the type of cell being cultured.

- Temperature
- CO2 concentration
- pH
- Humidity

In addition to these physical conditions, cells also require essential nutrients. As with the external conditions listed above, the concentrations and types of the nutrients given below will vary based on the cell being cultured.

- Amino acids
- Carbohydrates
- Vitamins
- Minerals
- Growth factors and hormones

Cells are provided these nutrients through their **culture medium**. For animal cell culture, the culture medium is used to change the physical and chemical properties of the environment surrounding the cells. Specifically, the culture medium is critical for the maintenance of pH & bicarbonate levels, salt concentration, and osmolarity.

Culture Medium

The two basic types of culture medium are natural and synthetic.

- **Natural culture medium** is made up of biological substances
  - Concentrated plasma "clots" – plasma separated from blood serum
  - Tissue extracts – extracts from chicken embryos, bone marrow, liver, and spleen
  - Biologic fluids – lymphatic fluid
- **Synthetic culture medium** is made up of a **basal medium** and **supplements**

Basal medium (Figure 10.3.3) provides the cells with a "baseline" of amino acids, salts, glucose, vitamins, and minerals for their viability and growth. Basal medium can be as simple as a balanced salt solution or a more complex formulation, like BME, DMEM, or Ham's medium, that can be purchased from a variety of cell culture companies.



Figure 10.3.3: Basal Medium for Cell Culture. Basal medium contains glucose, plus essential amino acids, salts, vitamins, and minerals for cell viability and growth *in vitro*. (DMEM cell culture medium by Lilly_M, CC BY-SA 3.0)

Table 10.3.4 shows the compositions of four of the more common basal media: Basal Medium Eagle (BME) or Eagle's Medium, Minimal Essential Medium (MEM), alpha-Minimal Essential Medium (a-MEM), and Dulbecco's Modified Eagle's Medium (DMEM).

Table 10.3.4: Composition of Common Basal Media

Component	Basal Medium Eagle (BME) i.e. Eagle's Medium	Minimal Essential Medium (MEM)	alpha-Minimal Essential Medium (a-MEM)	Dulbecco's Modified Eagle's Media (DMEM)
Vitamin B12			1.36	
Thiamine (Vitamin B1)	1	1	1	4
Sodium pyruvate			110	110
Sodium phosphate (NaH_2PO_4-H_2O)	140	140	140	125
Sodium chloride (NaCl)	6800	6800	6800	6400
Sodium bicarbonate ($NaHCO_3$)	2200	2200	2200	3700
Riboflavin (Vitamin B2)	0.1	0.1	0.1	0.4
Pyridoxal hydrochloride	1	1	1	4
Potassium chloride (KCl)	400	400	400	400
Phenol red	10	10	10	15

Component	Basal Medium Eagle (BME) i.e. Eagle's Medium	Minimal Essential Medium (MEM)	alpha-Minimal Essential Medium (a-MEM)	Dulbecco's Modified Eagle's Media (DMEM)
Niacinamide (Vitamin B3)	1	1	1	4
Magnesium sulfate ($MgSO_4$)	97.67	97.67	97.67	97.67
Lipoic acid			0.2	
L-valine	23.5	46	46	94
L-tyrosine	26	52	52	104
L-tryptophan	4	10	10	16
L-threonine	24	48	48	95
L-serine			25	42
L-proline			40	
L-phenylalanine	16.5	32	32	66
L-methionine	7.5	15	15	30
L-lysine	36.47	73	73	146
L-leucine	26	52	52	105
L-isoleucine	26	52	52	105
L-histidine		42	42	
L-histidine	8		31	42
L-glutamine	292	292	292	584
L-glutamic acid			75	
L-cysteine	16	31	31	63
L-cysteine			100	
L-aspartic acid			30	
L-asparagine			50	
L-arginine	21	126	126	84
L-alanine			25	
i-Inositol	2	2	2	7.2
Glycine			50	30
Folic acid (Vitamin B9)	1	1	1	4
Ferric nitrate ($Fe(NO_3)_3{\cdot}9H_2O$)				0.1
D-Glucose (dextrose)	1000	1000	1000	1000

Component	Basal Medium Eagle (BME) i.e. Eagle's Medium	Minimal Essential Medium (MEM)	alpha-Minimal Essential Medium (a-MEM)	Dulbecco's Modified Eagle's Media (DMEM)
D-calcium pantothenate	1	1	1	4
Choline chloride	1	1	1	4
Calcium chloride ($CaCl_2$)	200	200	200	200
Biotin (Vitamin B7)	1		0.1	
Ascorbic acid (Vitamin C)			50	

Culture supplements complete the basal medium by providing additional nutrients, proteins, amino acids and vitamins, and also the appropriate pH. The most commonly added supplements are **serum and antibiotics.** Serum provides the cells with key proteins, minerals, lipids, hormones, growth factors, and adhesion factors. Antibiotic supplementation (i.e. penicillin and streptomycin) decrease the chances of microbial proliferation when combine with proper aseptic technique. Synthetic medium may also contain additional amino acids for growth and proliferation, like L-glutamine, cysteine and tyrosine. Fungal contamination can be decreased through the supplement amphotericin B.

Depending on the type of cells being cultured, there are several types of synthetic culture media that can be used.

- **Serum-containing synthetic medium:**
  - basal medium + serum + antibiotics (optional)
  - serum is usually obtained from animal sources (e.g. horses, fetal cow) that have been raised and slaughtered for this purpose - ethical issues
  - chances of microbial contamination can be high
  - serum properties can vary from batch to batch and company to company - results in inconsistent cultures
  - human serum can be used – expensive and highly regulated due to disease transmission
- **Serum-free synthetic medium:**
  - uses **crude protein fractions** such as bovine serum albumin, or globulins to replace serum
  - provides more consistent results
  - only specific cell types can grow using this kind of medium
  - protein fractions are still obtained from animals
  - cells may require additional growth factors and hormones to optimize growth
- **Chemically-defined synthetic medium:**
  - type of serum-free medium
  - uses highly purified protein fractions - e.g. recombinant proteins
  - concentrations of these fractions are known and optimized for cell growth
- **Protein-free synthetic medium:**
  - type of serum-free medium
  - undefined components such as peptide fractions are used
- **Xeno-free synthetic medium:**
  - no animal components used
  - human sourced components are used as supplements - e.g. human serum albumin used as a source of protein
  - typically used for the maintenance and growth of lymphocytes
  - expensive and highly regulated due to disease transmission

Biosafety Considerations

Biosafety is a concept that refers to the protection of human health and the environment from the possible adverse effects of

pathogenic and/or genetically modified organisms and micro-organisms that are used in research and development (R&D) and biotechnology. Biosafety is important when working with cell cultures because certain cell cultures may contain pathogens that may be harmful to humans or the environment. Therefore, it is the responsibility of all lab personnel to ensure that each lab member is operating safely and that accidents do not occur.

The typical hazard encountered in a cell culture lab is a biohazards. To mitigate exposure, culture labs rely upon two very important aspects: personal protective equipment and biological safety.

Personal Protective Equipment

Personal protective equipment (PPE) protects the user against specific hazards in the laboratory. Safety glasses, lab coats and gloves are examples of PPE that are commonly used in labs. Safety glasses are important pieces of PPE and should be selected based on the specific risks anticipated (e.g. splash risk, shortwave, ultraviolet radiation). Because cell culture is done using a biosafety cabinet, eye protection when culturing cells is not necessary. However, outside of the biosafety cabinet, eye protection may be necessary depending on the lab protocol being performed.

Besides eye protection, it is important to limit exposure of the skin to microbes and chemicals using gloves. The type of glove used is like safety glasses – it is dependent upon the hazard encountered in the culture lab. Most gloves used in culture labs are either **latex, nitrile, or vinyl**. Latex is the simplest material and the most common kind of glove. However, many people may exhibit skin sensitivity to latex. Some disposable latex gloves are also flammable. Nitrile gloves may be used as an alternative in the culture lab. Nitrile is thicker than latex, does not irritate most user's skin and provides a more substantial barrier.

Wearing a lab coat protects clothing and is often worn in a cell culture lab. Many cell culture labs have dedicated lab coats for culture work. In addition to a lab coat, the clothing worn must be appropriate for the lab. Open toed shoes are not allowed in a lab. Long pants are also required. While not necessary, long-sleeved shirts may also be worn. However, short-sleeves are fine if wearing a lab coat.

Biological Safety

The safety of personnel working in a culture lab is described by set of bio-containment precautions known as **Biosafety Levels (BSLs)**. The Centers for Disease Control (CDC) have established four BSLs, classified as *BSL-1 through BSL-4*, that describe the risk level associated with a particular biological agent along with the corresponding microbiological practices, safety equipment, and facility safeguards lab personnel shall use with handling such an agent (Table 10.3.5. Biological labs are classified using these BSLs.

Table 10.3.5: Biosafety Levels

BSL Level	Description	Biologic Agents	Practices & Precautions
BSL-1	Risk level: Minimal Facility requirement: Standard laboratory Special Equipment needed: No	Non-pathogenic, well characterized microbes Agents not known to cause illness or disease is health adults e.g. E.coli	Standard microbiologic practices, PPE (gloves, lab coats)

BSL Level	Description	Biologic Agents	Practices & Precautions
BSL-2	Risk level: Moderate Facility requirement: Limited access lab, biohazard signs Special Equipment needed: Yes	Pathogenic microbes Agents could cause mild to serious illness e.g. Influenza, herpes, common cold	Biosafety cabinet for aerosols, enhanced PPEs, sharps precautions
BSL-3	Risk level: High Facility requirement: Controlled access lab, negative air pressure ventilation Special Equipment needed: Yes	Pathogenic microbes - indigenous or exotic Agents cause disease that could be lethal if untreated e.g. SARS-CoV1, SARS-CoV2, tuberculosis, avian influenza, plague	Biosafety cabinet for all activities, respirators, air is HEPA-filtered upon lab exit, decontamination system, personnel shower out of lab
BSL-4	Risk level: Extremely High Facility requirement: Isolated facility, full-suit positive pressure Special Equipment needed: Yes	Pathogenic microbes - dangerous and exotic Agents cause disease that have no treatment e.g. Ebola virus, Marburg virus, smallpox virus	Biosafety cabinet for all activities, full-body air-supplied suits, air is HEPA-filtered upon lab exit, extensive decontamination of lab personnel and lab materials. chemical and personnel showers out of lab

BSL-1

- the basic level of protection
- appropriate for labs working with well-characterized agents that do not cause disease in healthy humans
- no specialized equipment needed
- labs must have a door to limit access to lab
- precautions: standard microbiological practices (i.e., wash hands, area decontamination with bleach or isopropanol); personal protective equipment (PPE) recommended but not required
- type of lab found in high schools and colleges
- example of agents: E.coli, bacillus, saccharomyces

BSL-2:

- involves use of biological agents that pose a moderate risk to personnel and environment
- biological may cause human disease by ingestion or through percutaneous or mucous membrane exposure; treatment is readily available if exposed

- BSL-2 labs are also able to work with samples contaminated with fluids like blood and can also conduct experiments that involve recombinant DNA
- precautions: BSL-1 precautions plus BSL-2 specific requirements
  - use of personal protective equipment like gloves and lab coats
  - use of a biosafety cabinet for cell culture
  - use of other physical containment equipment to limit aerosol transmission or splashing of infectious materials
  - lab must have a door to limit access to lab
- personnel must undergo specific training in handling pathogenic agents
- the lab classification for most cell culture labs in biotechnology and pharmaceutical companies, national labs, research universities and hospital
- examples of agents: influenza, salmonella, staphylococcus, hepatitis

BSL-3

- involve use of pathogens with significant risk to personnel and environment
- pathogens have a known potential for aerosol transmission that may cause serious or potentially lethal infections in humans
- precautions: BSL-1 and BSL-2 precautions plus BSL-3 specific requirements
  - use of biosafety cabinet for cell culture
  - the lab entrance must be separated from other areas of the building
  - the lab must have two sets of self-closing doors and sealed windows to minimize escape of aerosols
  - the lab must have a specialized ventilation system with filtration to prevent airborne circulation of pathogens
  - lab personnel are monitored medically on a regular basis and offered relevant immunizations
  - lab personnel must wear solid-front protective clothing (e.g. gowns that tie in the back) and face shields or eye protection
- personnel undergo an enhanced level of training in handling pathogenic agents
- examples of agents: SARS-CoV, mycobacterium, West Nile virus, additional viruses

BSL-4

- involve use of pathogens with significant risk to personnel
- pathogens pose a high risk of life-threatening disease by infectious aerosols for which no treatment is available
- precautions: BSL-1 through BSL-3 precautions plus BSL-4 specific requirements
  - the lab entrance must be separated from other areas of the building
  - the lab entrance uses airlocks to prevent escape of aerosols
  - the lab must have a specialized ventilation system with filtration to prevent airborne circulation of pathogens
  - work must be done either in a Class II or Class III biosafety cabinet
  - personnel using the Class II cabinet must wear a full-body, positive-pressure suit with HEPA-filtration
  - personnel leaving the lab must pass through a chemical shower for decontamination of pressure suit, then enter a room to remove the suit, and then take a shower before donning clothes
  - all materials leaving the lab must undergo rigorous decontamination, including lab waster, air, and water
- personnel require exceptional level of training
- examples of agents: Ebola virus, smallpox virus, Marburg virus

Lab Protocols

- Chapter Lab Technique: Cell Culture

Key Concepts

In vitro cell culture is used to grow cells outside of the donor organism by using a liquid, semi-solid or solid growth medium.

Some important concepts to remember are:

- The growth of cultured cells, tissues, or organs requires precisely controlled physical conditions and chemical reagents
- The types of cells that can be cultured are wide and varied and depend on the goal(s) of the experiment

- *In vitro* culture can also be used to maintain tissues and organs outside of the organism, through the process of organ culture
- Primary cultures are established following the removal of cells from a tissue and placement into culture
- Sub-culturing of a primary culture produces a cell line with increased homogeneity when compared to the primary culture
- Isolation of single cells from a cell line, followed by *in vitro* expansion produces cells strains with specific characteristics
- While the most common cell types grown *in vitro* are non-pathogenic, biosafety considerations during culture are essential.

Glossary

Adherent cells - cells that must attach to a surface (e.g. a culture dish) to grow

Basal medium - a liquid mixture of amino acids, salts, glucose, vitamins, and minerals essential for mammalian cell viability and growth

Biosafety - the principles and protocols used to prevent the unintentional exposure of workers to biological agents or toxins; used also to reduce the risk of their accidental release into the environment

Biosafety level (BSL) - a classification system used to define the containment precautions required when working with biological agents that may pose a potential risk to human health and the environment; there are biosafety levels (BSL-1 to BSL-4), each with increasing degrees of containment, protective measures, and operational requirements

BME (Basal Medium Eagle) - a common basal medium used for cell culture; also called Eagle's medium

Cell culture - a laboratory technique used to maintain and/or grow eukaryotic cells *in vitro*

Cell line - a population of cells derived from primary cultures that can grow and multiply *in vitro*

Cell strain - a population of cells derived from a single cultured cell that can grow and multiply *in vitro*

Culture medium - a mixture of basal medium and supplements used to support the viability and growth of eukaryotic cells *in vitro*

DMEM (Dulbecco's Modification of Eagle's Medium) - a common basal medium used for cell culture; a modified version of Eagle's medium

Immortalized cells - eukaryotic cells that grow and multiply indefinitely *in vitro* due to genetic transformation

In vitro - refers to biological or chemical process that occurs outside of an organism and in a controlled laboratory environment like a test tube or culture dish; Latin for "in glass"

Organ culture - a laboratory technique used to maintain and/or grow whole organs or pieces of an organ *in vitro*

Primary culture - a population of eukaryotic cells derived from a tissue and maintained *in vitro*

Personal protective equipment (PPE) - specialized clothing or equipment designed to protect individuals from exposure to hazards that could cause injury or illness

Serum - the fluid portion of blood plasma that remains after the blood has clotted and the clotting factors have been removed; contains various proteins, hormones, electrolytes, and nutrients that can support cells *in vitro;* often added to basal medium and supplements to create culture medium

Supplements - additional factors added to basal medium to support cell growth in vitro and/or prevent contamination; often added to basal medium in combination with serum to create culture medium

Tissue culture - a laboratory technique used to maintain and/or grow tissues *in vitro*; usually refers to the culture of plant tissues *in vitro*

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11: GOVERNMENT OVERSIGHT OF BIOTECHNOLOGY

11.1: Environmental Laws and Regulations
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11.1: Environmental Laws and Regulations

Although environmental laws are generally considered a 20th century phenomenon, attempts have been made to legislate environmental controls throughout history. In 2,700 B.C., the middle-eastern civilization in Ur passed laws protecting the few remaining forests in the region. In 80 A.D., the Roman Senate passed a law to protect water stored for dry periods so it could be used for street and sewer cleaning. During American colonial times, **Benjamin Franklin** argued for "public rights" laws to protect the citizens of Philadelphia against industrial pollution produced by animal hide tanners.

Significant environmental action began at the beginning of the 20th century. In 1906, Congress passed the “Antiquities Act,” which authorizes the president to protect areas of federal lands as national monuments. A few years later, **Alice Hamilton** pushed for government regulations concerning toxic industrial chemicals. She fought, unsuccessfully, to ban the use of lead in gasoline. She also supported the legal actions taken by women who were dying of cancer from their exposure to the radium then used in glow-in-the-dark watch dials. During the early 1960’s, biologist **Rachel Carson** pointed out the need to regulate pesticides such as DDT to protect the health of wildlife and humans.

With the establishment of the **Environmental Protection Agency (EPA)** in 1970, environmental law became a field substantial enough to occupy lawyers on a full-time basis. Since then, federal and state governments have passed numerous laws and created a vast network of complicated rules and regulations regarding environmental issues. Moreover, international organizations and agencies including the **United Nations**, the **World Bank**, and the **World Trade Organization** have also contributed environmental rules and regulations.

Because of the legal and technical complexities of the subjects covered by environmental laws, persons dealing with such laws must be knowledgeable in the areas of law, science and public policy. Environmental laws today encompass a wide range of subjects such as air and water quality, hazardous wastes and biodiversity. The purpose of these environmental laws is to prevent, minimize, remedy and punish actions that threaten or damage the environment and those that live in it. However, some people believe that these laws unreasonably limit the freedom of people, organizations, corporations and government agencies by placing controls on their actions.

Federal Laws

Early attempts by Congress to enact laws affecting the environment included the **Antiquities Act** in 1906, the **National Park Service Act** in 1916, the **Federal Insecticide**, **Fungicide and Rodenticide Act** in 1947 and the **Water Pollution Control Act** in 1956. The **Wilderness Act** of 1964, protected large areas of pristine federal lands from development and ushered in the new age of environmental activism that began in the 1960’s. However, it was the **National Environmental Policy Act (NEPA)** enacted in 1969 and the formation of the Environmental Protection Agency (EPA) in 1970 that started environmental legislation in earnest. The main objective of these two federal enactments was to assure that the environment would be protected from both public and private actions that failed to take into account the costs of damage inflicted on the environment.

Many consider NEPA to be the most far-reaching environmental legislation ever passed by Congress. The basic purpose of NEPA is to force governmental agencies to comprehensively consider the effects of their decisions on the environment. This is effected by requiring agencies to prepare detailed **Environmental Impact Statements** (EIS) for proposed projects. The EPA is the government's environmental watchdog. It is charged with monitoring and analyzing the state of the environment, conducting research, and working closely with state and local governments to devise pollution control policies. The EPA is also empowered to enforce those environmental policies. Unfortunately, the agency is sometimes caught up in conflicts between the public wanting more regulation for environmental reasons and businesses wanting less regulation for economic reasons. Consequently, the development of a new regulation can take many years.

Since 1970, Congress has enacted several important environmental laws, all of which include provisions to protect the environment and natural resources. Some of the more notable laws include:

- The **Federal Clean Air Act** (1970, 1977 & 1990) established national standards for regulating the emission of pollutants from stationary and mobile sources.
- The **Federal Water Pollution Control Act** (1972) amended by the **Clean Water Act** (1977, 1987), established water quality standards; provides for the regulation of the discharge of pollutants into navigable waters and for the protection of wetlands.
- The **Federal Safe Drinking Water Act** (1974, 1977 & 1986) set drinking water standards for levels of pollutants; authorizing

the regulation of the discharge of pollutants into underground drinking water sources.
- The **Toxic Substances Control Act** (1976) provided for the regulation of chemical substances by the EPA and the safety testing of new chemicals.
- The **Resource Conservation and Recovery Act** (1976) established cradle-to-grave regulations for the handling of hazardous wastes.
- The **Comprehensive Environmental Response**, **Compensation and Liability Act** (1980), also known as the **Superfund**program, provided for the cleanup of the worst toxic waste sites.
- The **Food Security Act** (1985, 1990) later amended by the **Federal Agriculture Improvement and Reform Act** (1996), discouraged cultivation of environmentally sensitive lands, especially wetlands, and authorized incentives for farmers to withdraw highly erodible lands from production.

The application, or enforcement, of an environmental law is not always straightforward, and problems can arise. Often, the biggest problem is that Congress fails to allocate the funds necessary for implementing or enforcing the laws. Administrative red tape may make it impossible to enforce a regulation in a timely manner. It also may be unclear as to which agency (or branch of an agency) is responsible for enforcing a particular regulation. Furthermore, agency personnel decline to enforce a regulation for political reasons.

State Laws

Most states, like California, have enacted their own environmental laws and established agencies to enforce them. California faced some of its first environmental challenges in the mid-1800's, with regard to debris from the hydraulic mining of gold. Water quality concerns, dangers of flooding, negative impact on agriculture and hazards to navigation prompted the state to act.

Some of California's environmental regulations preceded similar federal laws. For example, California established the nation's first air quality program in the 1950s. Much of the federal Clean Air Act amendments of 1990 were based upon the **California Clean Air Act of 1988**. California also pioneered advances in vehicle emission controls, control of toxic air pollutants and control of stationary pollution sources before federal efforts in those areas. The **Porter-Cologne Act of 1970**, upon which the state's water quality program is based, also served as the model for the federal Clean Water Act.

International Treaties and Conventions

Conventions, or treaties, generally set forth international environmental regulations. These conventions and treaties often result from efforts by international organizations such as the **United Nations (UN)** or the **World Bank**. However, it is often difficult, if not impossible, to enforce these regulations because of the sovereign rights of countries. In addition rules and regulations set forth in such agreements may be no more than non-binding recommendations, and often countries are exempted from regulations due to economic or cultural reasons. Despite these shortcomings, the international community has achieved some success via its environmental agreements. These include an international convention that placed a moratorium on whaling (1986) and a treaty that banned the ocean dumping of wastes (1991).

The UN often facilitates international environmental efforts. In 1991, the UN enacted an **Antarctica Treaty**, which prohibits mining of the region, limits pollution of the environment and protects its animal species. The United Nations Environment Program (UNEP) is a branch of the UN that specifically deals with worldwide environmental problems. It has helped with several key efforts at global environmental regulations:

- **The 1987 Montreal Protocol on Substances that Deplete the Ozone Layer**. As a result of this global agreement, industrialized countries have ceased or reduced the production and consumption of ozone-depleting substances such as chlorofluorocarbons.
- **The Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade**. This agreement enhances the world's technical knowledge and expertise on hazardous chemicals management.
- **The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)**. This agreement protects over 30,000 of the world's endangered species.
- In 1995 **UNEP and the International Olympic Committee (IOC)** signed a partnership agreement to develop environmental guidelines for sports federations and countries bidding to host the Olympic games.
- **The Rotterdam Convention** (1998) addressed the growing trade in hazardous pesticides and chemicals. Importing countries must now give explicit informed consent before hazardous chemicals can cross their borders.
- **The International Declaration on Cleaner Production** (1998). The signatories commit their countries to implement cleaner industrial production and subsequent monitoring efforts.

In 1992, the UN member nations committed their resources to limiting greenhouse gas (e.g., carbon dioxide) emissions at or below 1990 levels, as put forth by the **UN Framework Convention on Climate Change**. Unfortunately, the agreement was non-binding and by the mid-1990's, it had had no effect on carbon emissions. The 1997 **Kyoto Protocol** was a binding resolution to reduce greenhouse gases. Although the United States initially supported the resolution, the Senate failed to ratify the treaty, and by 2001 the resolution was opposed by President Bush as threatening the United States economy.

California's state environmental regulations are sometimes more stringent than the federal laws (e.g., the California Clean Air Act and vehicle emissions standards). In other program areas, no comparable federal legislation exists. For example, the California **Integrated Waste Management Act** established a comprehensive, statewide system of permitting, inspections, enforcement and maintenance for solid waste facilities and sets minimum standards for solid waste handling and disposal to protect air, water and land from pollution. Also, **Proposition 65** (**Safe Drinking Water and Toxic Enforcement Act**) requires the Governor to publish a list of chemicals that are known to the State of California to cause cancer, birth defects or other reproductive harm.

Despite the state's leadership in environmental programs and laws, the creation of a cabinet-level environmental agency in California lagged more than two decades behind the establishment of the federal EPA. Originally, organization of California's environmental quality programs was highly fragmented. Each separate program handled a specific environmental problem (e.g., the **Air Resources Board**), with enforcement responsibility falling to both state and local governments. It was not until 1991 that a California EPA was finally established and united the separate programs under one agency.

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11.2: The Food and Drug Administration (FDA)

The FDA (www.fda.gov) is an administrative agency created to regulate food and drug supplies in the United States for the safety and health of its citizens. *FDA is an agency within the Department of Health and Human Services*. It should be noted that the FDA has traditionally focused on the US markets, however, with the global market growth (of imports and exports of products and raw materials) *the FDA now manages over 2 trillion dollars of goods manufactured in over 150 nations worldwide (FDA, FDA Global engagement report, 2016).*

International Harmonization Efforts

What happens when a biotechnology company based in the United States or any other country wants to expand to overseas marketplaces? Conflicting regulatory standards made trade difficult until an effort began in the 1990s to harmonize international standards so that important medical products could be bought and sold with less regulatory 'red tape.' *The FDA has been the leader in this effort both in the U.S. and around the globe. The FDA assists both foreign and domestic manufacturers in compliance with CGMP, CGP, GLP, Safe and Sanitary Processing, and other regulations.* (http://www.fda.gov/ForIndustry/default.htm).

Organization

The FDA is vast and complex. A complete organizational chart (2017) is found on their website here.

The FDA, like most organizations, change with changing the economy, world harmonization, and emerging technologies and products. The current hierarchy consists of the Office of the Commissioner overseeing five offices and directorates. Those offices oversee eight centers.

Like the federal government, the FDA possesses the following powers:

1. **Legislative**: The FDA has the authority to create and issue rules.
2. **Executive**: The FDA has the power to conduct investigations.
3. **Judicial**: The FDA has the jurisdiction to review the evidence and make judgments on a product.

The FDA has "product centers" headquartered, largely in the Washington, D.C. area, (the NCTR is in Jefferson, Arkansas). FDA "field offices" are located throughout the United States. The field offices are "the eyes and ears" of the FDA, and it is from these offices that operational personnel enforce the law.

Explore!

Visit the FDA website and select each tab at the top of the page. Each tab corresponds to each of the different types of products the FDA regulates. Briefly describe each.

Product	Description
Drugs	A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease. The site includes drug approval, safety, availability, regulatory information, research and consumer information relating to drugs

FDA Field Offices

Office of the Commissioner: Leadership of the agency's scientific activities, communication, legislative liaison, policy and planning, women's and minority health. (fda.gov). www.fda.gov/about-fda/fdaorganization/office-commissioner

Office of Medical Products and Tobacco: Provides advice and counsel to the Commissioner on all medical product and tobacco-related programs and issues. www.fda.gov/about-fda/office-medicalproducts-and-tobacco/patient-affairs-staff

- Center for Biologics Evaluation and Research
- Center for Devices and Radiological Health
- Center for Drug Evaluation and Research
- Center for Tobacco Products

Office of Foods and Veterinary Medicine: Leads a functionally unified FDA Foods Program that addresses food and feed safety, nutrition, and other critical areas to achieve public health goals.

- Office of Foods and Veterinary Medicine
- Center for Food Safety and Applied Nutrition
- Center for Veterinary Medicine

Office of Global Regulatory Operations and Policy: Provides leadership for FDA's domestic and international product quality and safety efforts. www.fda.gov/about-fda/fda-organization/officeglobal-regulatory-operations-and-policy

Office of Operations: Provides agency-wide services including information technology, financial management, procurement, library services, and freedom of information, FDA history, and facilities. www.fda.gov/about-fda/office-operations/office-ethics

FDA Product Centers

Now, let's examine more closely the functions of several important product-oriented centers: CDER, CBER, CDRH, CVM, CTP, NCTR, and CFSAN.

Center for Drug Evaluation and Research (CDER): The CDER oversees the *regulation of drugs*. The official definition of a drug is "an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; articles designed to affect the structure or any function of the body of man or other animals." (FDA, 2016) CDER regulates over-the-counter and prescription drugs, including biological therapeutics (act as drugs), and generic drugs. This work covers more than just medicines. For example, fluoride toothpaste, antiperspirants, and dandruff shampoos are all considered "drugs." Why? More on this in a later chapter!

Center for Biologics Evaluation and Research (CBER): The CBER oversees the *regulation of biologics*. The official definition of a biological product is 'any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product' (FDA, 2016). More information on a biologic in the following video. Note, therapeutic biologics are biologics that act like drugs and therefore are overseen by CDER instead. More on this in a later chapter!

Center for Devices and Radiological Health (CDRH): Oversees the *regulation of medical devices* and radiation-emitting products, and also includes biotechnology products used in diagnostics, such as HIV or pregnancy tests. You will be surprised to learn about some unusual products that are considered medical devices. https://www.fda.gov/AboutFDA/Centers...dTobacco/CDRH/

Center for Veterinary Medicine, and (CVM): The CVM is a product center, which oversees the *regulation of food, food additives, drugs and biologics for animals*. They also conduct research that helps the FDA ensure the safety of animal drugs, food for animals, and food products made from animals. However, they do not oversee pre-clinical animal studies. Those fall under the purview of the product center of the product the pre-clinical studies are for. Here is a video on the CVM put out by the FDA.

Center for Food Safety and Applied Nutrition (CFSAN): CFSAN oversees *food safety and purity*. It has the power to regulate all domestic and imported food *except for meat, poultry, and eggs* (USDA regulates those). They oversee the safety of food ingredients developed through biotechnology, dietary supplements, food additives, and proper labeling of food. CFSAN is also concerned with food contamination, such as biological pathogens and naturally occurring toxins.

Center for Tobacco Products (CTP): The Center for Tobacco Products (CTP) oversees *the implementation of the Family Smoking Prevention and Tobacco Control Act*. Some of the Agency's responsibilities under the law include setting performance standards, reviewing premarket applications for new and modified-risk tobacco products, requiring new warning labels, and establishing and enforcing advertising and promotion restrictions. (fda.gov)

National Center for Toxicological Research (NCTR): FDA's research center conducts peer-reviewed research and develops

new scientific tools for FDA to improve public health. This research produces innovative tools to assist in solving complex health issues, anticipated toxicological problems, and enhances the science of regulatory decision-making at the FDA. The NCTR publishes an annual report outlining their projects and can be found here.

Test Your Knowledge!

Go to this website for a tutorial about the Food and Drug Administration.

Scroll down and press on the sound icon to hear videos. At the very bottom of the page is a self-quiz. Briefly summarize the mission of the FDA and outline the primary duty of each of the following FDA offices:

Center or Office	Summary of Main Duties
CFSAN	
CDER	
CBER	
CDRH	
CVM	
NCTR	
ORA	
CTP	

Rulemaking, Adjudication, Guidelines, and Points to Consider

The primary administrative activities of the FDA are rulemaking and adjudication.

- **Rulemaking** affects both the public and the drug and device manufacturers. When new rules are proposed, the FDA publishes them electronically in the code of federal regulations (CFR), which is available to the public at www.ecfr.gov.
- **Adjudication** is the term used to describe how the FDA responds to requests for approval to investigate or market a product. The FDA reviews each submission as a separate case even if each submission refers to the same product. Upon conclusion of its review, the FDA responds with its disposition or order.

Federal regulations are first published in the Federal Register (FR) by the executive departments and agencies of the Federal Government published every business day by **the National Archives and Records Administration (NARA)**.

The Federal Register is a legal newspaper where the public is given notice of proposed new rules and intended actions, allowing time for comment. When the FDA finalizes a rule, it publishes its response to public comments in a preamble to the rule.

This bureaucratic process is quite time-consuming and, therefore, the FDA employs two additional methods of transmitting information to the public and industry: Guidelines and Points to Consider, which are published on the FDA website. *Neither the Guidelines nor the "Points to Consider" represent any legal regulation and may be challenged by scientific or other types of evidence*.

- **Guidelines** represent the FDA's formal position on long-standing issues. Guidelines also communicate procedures or standards of general applicability that are acceptable to the FDA.
- **Points to Consider** are intended to disclose the FDA's current position on any area considered new or rapidly changing, such as some parts of the biotechnology industry. In other words, the FDA has not formulated a formal stance.

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11.3: Regulation of Plant GMOs

Food Ingredients

Although the FDA has little regulatory control over food before it goes to the market, what it does have control over is food ingredients, such as food additives. Food additives include intentional components added to food as well as unintentional additives from the manufacturing process.

All food additives are approved before use – a list of approved food additives can be found on the FDA website. Any substance that the FDA considers **'Generally Recognized as Safe (GRAS)'** does not need to be approved before use. https://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm091048.htm

"*GRAS*" *is an acronym for the phrase Generally Recognized As Safe*. Under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (the Act), any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excepted from the definition of a food additive. https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras

Regulation of Agricultural Biotechnology

FDA regulates the safety of food for humans and animals, including foods produced from **genetically engineered (GE) plants**. Foods from GE plants must meet the same food safety requirements as foods derived from traditionally bred plants. While genetic engineering is sometimes referred to as "genetic modification" producing "genetically modified organisms (GMOs)," FDA considers "genetic engineering" to be the more precise term. www.fda.gov/food/food-new-plantvarieties/consumer-info-about-food-geneticallyengineered-plants



Figure 11.3.1: Genetically engineered plums to resist plum pox carried by aphids. Image by Agriculture Research Service, public domain.

What are Genetically Engineered (GE) Organisms?

Crop improvement happens all the time, and genetic engineering is just one form of it. We use the term "genetic engineering" to refer to genetic modification practices that utilize modern biotechnology. In this process, scientists make targeted changes to a plant's genetic makeup to give the plant a new desirable trait. For example, two new apple varieties have been genetically engineered to resist browning associated with cuts and bruises by reducing levels of enzymes that can cause browning.

Humans have been modifying crops for thousands of years through selective breeding. Early farmers developed cross-breeding methods to grow numerous corn varieties with a range of colors, sizes, and uses. For example, the garden strawberries that consumers buy today resulted from a cross between a strawberry species native to North America and a strawberry species native to South America.

Why Genetically Engineer Plants?

Developers genetically engineer plants for many of the same reasons that traditional breeding is used. They may want to create plants with better flavor, higher crop yield (output), greater resistance to insect damage, and immunity to plant diseases. Traditional breeding involves repeatedly cross-pollinating plants until the breeder identifies offspring with the desired combination of traits. The breeding process introduces many genes into the plant. These genes may include the gene responsible for the desired trait, as well as genes responsible for unwanted characteristics. Genetic engineering isolates the gene for the desired trait, adds it to a single plant cell in a laboratory, and generates a new plant from that cell. By narrowing the introduction to only one desired gene from the donor organism, scientists can eliminate unwanted characteristics from the donor's other genes. *Genetic engineering is often used in conjunction with traditional breeding to produce the genetically engineered plant varieties on the market today.*

Am I Eating Food from Genetically Engineered Plants?

The planting of **genetically engineered (GE) crops** was allowed for the first time in the United States in 1995. Today, it is estimated that over 90% of soy, and over 80% of cotton and canola products come from genetically modified crops. Genetically modified crops were created to improve productivity and cut costs by improving insect resistance, virus resistance, herbicide tolerance, and similar issues that affect commercial crops.

The majority of GE plants are used to make ingredients that are used in other food products. Such ingredients include corn starch in soups and sauces, corn syrup used as a sweetener, corn oil, canola oil, and soybean oil in mayonnaise, salad dressings, bread, and snack foods.

Voluntary Pre-Market Engagement for New Plant Varieties

In the 1992 policy on new plant varieties, FDA recommended that developers consult with FDA about bioengineered foods under development; since the issuance of the 1992 policy, developers have routinely done so. To date, FDA's interactions with developers have taken three forms: **biotechnology final consultations**, **new protein consultations**, and rarely, the establishment of a food master file or submission of a **food additive petition**.

In the Federal Register of January 18, 2001 (the premarket notification proposal; 66 FR 4706, available as text and 193 KB PDF), FDA proposed a rule that would *require that developers submit a scientific and regulatory assessment of the bioengineered food 120 days before the bioengineered food is marketed*. In the premarket notification proposal, FDA recommended that developers continue the practice of consulting with the agency before submitting a premarket notice.

A developer who intends to commercialize a bioengineered food may meet with the FDA to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the bioengineered food in an initial consultation. With or without an initial consultation, a developer may submit to FDA a summary of its scientific and regulatory assessment of the food (the final consultation). Submissions to the agency under the 1997 guidance are designated **biotechnology notification files (BNF)**. FDA evaluates the submission and responds to the developer by letter. FDA maintains an inventory of Biotechnology Consultations on Food from GE Plant Varieties, which includes the agency's response to the developer and the text of the agency's final memorandum regarding the submission.

1. Biotechnology Consultations: www.fda.gov/food/submissions...-consultations
2. New Protein Consultations: https://www.fda.gov/food/submissions...-consultations
3. Food Additive Petitions: www.fda.gov/food/submissions-bioengineered-new-plantvarieties/food-master-files-food-additive-petitions

Regulatory Agencies for GE Crops

The FDA regulates human, and animal food from genetically engineered (GE) plants the same way they regulate all food. The existing FDA safety requirements impose a clear legal duty on everyone in the farm to table continuum to market safe foods to consumers, regardless of the process by which such foods are created. It is unlawful to produce, process, store, ship, or sells to consumers unsafe foods.

Three federal agencies evaluate new crop varieties developed using genetic engineering: the FDA, the USDA, and the EPA.

www.fda.gov/food/food-new-plant-varieties/how-fda-regulates-foodgenetically-engineered-plants

1. **FDA**: Evaluates food and feed safety. The FDA's Center for Food Safety and Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM) evaluate new GE crops. They look for increased allergens, toxins, and changes in nutrition or composition. The FDA's main concerns here are threats to human health through food and threats to animal health through feed. What the FDA is comparing these genetically modified products is their unmodified counterpart. The FDA may then issue a statement about the modified food's substantial equivalence but does not approve the food as "safe," per se.
2. **USDA**: Ensures agricultural and environmental safety. The USDA, through the Biotechnology Regulatory Service (**BRS**) office of the Animal and Plant Health Inspection Service (**APHIS**), regulates all GE crops before commercial release. The USDA's primary concern is whether the new plant will harm agriculture or the environment. The USDA's authority over these matters is derived from the Plant Protection Act of 2000.
3. **EPA**: Evaluates food safety and environmental issues associated with new pesticides. The EPA regulates GM crops that have altered pesticide characteristics. Bt GM corn is an example of a product that the EPA would evaluate. Bt corn is genetically modified to contain what's known as a "Plant Incorporated Protectant" (PIP). In other words, the Bt corn produces its insecticide. The EPA evaluates this type of product for its impact on the environment and human health.

Test Your Knowledge!

Visit the EPA website: http://www.epa.gov/ The EPA is currently responsible for regulating GE plants. Why do you think the EPA is concerned about GE plants? What is the *Food Quality Protection Act*?

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11.4: Regulation of Animal GMOs and Medical Foods

FDA, EPA, or USDA: Animals with Intentional Genomic Alterations

On January 19, 2017, the FDA released for public comment draft revised Guidance for Industry (GFI) #187, "**Regulation of Intentionally Altered Genomic DNA in Animals**." This draft revised guidance expands the scope of the existing GFI #187 to address animals with intentionally altered genomic DNA developed through the use of genome editing technologies, as well as techniques such as rDNA in genetic engineering.

AquAdvantage Salmon: First Approved

AquAdvantage Salmon has been genetically engineered to reach a growth marker important to the aquaculture industry more rapidly than its non-GE farm-raised Atlantic salmon counterpart. It does so because it contains an rDNA construct that is composed of the growth hormone gene from Chinook salmon under the control of a promoter (a sequence of DNA that turns on the expression of a gene) from another type of fish called an ocean pout.

Is it Safe?

Based on a comprehensive analysis of the scientific evidence, as required by the Federal Food, Drug, and Cosmetic Act (FD&C Act), the FDA determined that AquAdvantage Salmon meets the statutory requirements for safety and effectiveness under the FD&C Act. As part of its evaluation, the FDA examined data comparing three groups of fish: non-GE farm-raised Atlantic salmon from both the sponsor's farm and from a different commercial farm, and AquAdvantage Salmon. This study compared key hormones (including estradiol, testosterone, 11-ketotestosterone, T3, T4, and insulin-like growth factor 1 (IGF1)) and found no biologically relevant differences. *The salmon are safe to eat and has a comparable nutritional profile to that of the non-GE farm-raised Atlantic salmon. The introduced DNA is safe for the fish itself, and the salmon meets the sponsor's claim about faster growth.*

The FDA also analyzed the potential environmental impact that approval of the original AquAdvantage Salmon application and a supplemental application would have on the quality of the human environment in the United States and issued its Environmental Assessments and Findings of No Significant Impact. https://www.fda.gov/media/93823/download

Containment

Under the approval and subsequent supplements to the original approval, AquAdvantage Salmon are subject to stringent conditions to prevent the possibility of escape into the wild. The salmon cannot be raised in ocean net pens: instead, the approval allows for them to be grown only at specific land-based facilities: one in Canada, where the breeding stock is kept, and Indiana, where the fish for market will be grown out using eggs from the Canada facility. Both the Canada and Indiana facilities have multiple and redundant physical barriers to prevent eggs and fish from escaping, including metal screens on tank bottoms, standpipes, and incubator trays to prevent the escape of eggs and fish during hatching or rearing.

Read more here: www.fda.gov/animal-veterinary/animals-intentional-genomicalterations/aquadvantage-salmon-fact-sheet

FDA, EPA, or USDA: Oxitec Mosquito

Oxitec mosquito is a type of "living pesticide," genetically engineered to carry a gene that prevents female offspring from surviving but allowing male progeny to survive. The male offspring, in turn, half will carry this self-limiting gene. Resulting in the decline of the target mosquito population – the Zika and yellow-fever mosquito vector Aedes aegypti. Learn more about the company and its technology, here, www.oxitec.com/our-technology.



Figure 11.4.1: Zika vector mosquito, Aedes aegypti. Image by CDC, 2006, public domain

On October 5, 2017, FDA issued final Guidance for Industry (GFI) #236 –Clarification of FDA and EPA Jurisdiction over Mosquito-Related Products, which clarifies that *mosquito-related products intended to function as pesticides by preventing, destroying, repelling, or mitigating mosquitoes for population control purposes, and that are not intended to cure, mitigate, treat or prevent a disease are not "drugs" under the FD&C Act, and will be regulated by the EPA under the Federal Insecticide, Fungicide, and Rodenticide Act.*

The FDA will continue to have jurisdiction over mosquito-related products that are intended to prevent, treat, mitigate, or cure a disease. With the issuance of final guidance #236, Oxitec Ltd's genetically engineered mosquito, with its proposed claim to control the population of wild-type Aedes aegypti mosquitoes, now falls under EPA's regulatory authority and all related regulatory questions should be directed to the EPA. www.fda.gov/animal-veterinary/animals-intentional-genomicalterations/oxitec-mosquito.

Findings, no significant impact: https://www.fda.gov/media/99731/download

Labeling and Intentionally Altered Food

National Bioengineered Food Disclosure Standard was announced on December 20, 2018. The National Bioengineered Food Disclosure Law, passed by Congress in July of 2016, directed USDA to establish this national mandatory standard for disclosing foods that are or may be bioengineered. The Standard defines bioengineered foods as those that contain detectable genetic material that has been modified through certain lab techniques and cannot be created through conventional breeding or found



in nature. https://www.ams.usda.gov/rules-regulations/be

The Standard requires food manufacturers, importers, and certain retailers to ensure the disclosure of bioengineered foods. Regulated entities have several disclosure options: text, symbol, electronic or digital link, and text message. Additional options such as a phone number or web address are available to small food manufacturers or for small and very small packages. Watch this video to learn more: youtu.be/08FHkS-pwHw, List of bioengineered foods: https://www.ams.usda.gov/rules-regulations/be/bioengineered-foods-list

Medical Food

Food can be more than meeting basic nutritional and safety standards – it can be used to reduce the risk of disease. *Medical foods are distinguished from other foods in their formulation – specifically to treat a disease or disorder.* They are intended for the specific management of a disease or disorder for which distinctive nutritional requirements exist based on scientific evidence. They are not the same as nutritional supplements. Medical foods are a distinct class of food because they are not classified as food nor medicine. However, this is food that the FDA intends to be used under medical supervision by a patient requiring medical care.

File:Novartis Fibersource HN medical food on IV pole.JPG

Figure 11.4.1: Fibersource HN medical food deployed on an IV pole. Image by BrokenSphere at Wikimedia, CC BY 3.0.

Foods such as these, for "*special dietary uses,*" were first brought up in regulation in 1941, and added to the FD&C Act in 1976. In 1988 Congress amended the Orphan Drug Act to include a statutory definition of "medical food." The term medical food, as defined in section 5(b) of the Orphan Drug Act (21 U.S.C. 360ee (b) (3)) is: "...a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation."

Medical foods are food, and therefore are regulated the same as foods. However, they cannot be marketed for a condition that can be managed or treated solely by a regular diet. That being said, any ingredient added to a medical food must be recognized as safe (GRAS). Medical foods do not need to be registered with the FDA, but the manufacturing facilities do for inspection purposes. Although they are not drugs, they still must meet the requirements for the manufacture and labeling of foods, which include CGMP regulations in manufacturing, packaging, and handling of human food. There is a particular compliance program for medical foods.

Test Your Knowledge!

Go to FDA's Medical Food Guidance's Q&A and answer the following questions

1. Does FDA regulation medical foods as drugs? Why/why not?
2. Do medical foods require clinical studies or premarket review (PMA)?
3. What labeling requirements apply to medical foods?
4. What is the purpose of FDA's compliance program for medical foods?
5. Does medical food require a prescription? Why/Why not?

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11.5: Principles of Quality Assurance (QA) and Quality Control (QC)

Biotech Focus

In the fall of 2004, the US experienced a severe influenza vaccine shortage due to a production issue at Chiron Corporation's Liverpool, England plant. FDA and British inspectors at the plant revealed violations of "good manufacturing practices" leading to contamination of vials of the vaccine with the bacteria *Serratia marcescens*. Inspectors identified potential errors in the filling of the vials, possibly compromising the sterility of the vaccine. The FDA concluded that none of the Chiron vaccine was safe for distribution in the US and a **recall** was issued of over a million doses of the vaccine shipped to the US. This recall effectively halved the expected US supply of influenza vaccine, necessitating a shift in vaccination recommendations and priority groups.

Read about the Fluvirin recall here.

Introduction

Because biotechnology products impact human health, quality is not optional *it is essential*. From therapeutic proteins to gene therapies, the integrity of biotechnology products (and services) must meet the highest standards. To ensure standards are met, biotechnology facilities rely upon **quality assurance (QA)** and **quality control (QC)** (Figure 11.5.1). Through QA, product quality is built into the process from design to delivery, with an emphasis on identifying defects and errors before they happen. If they do, companies can use QC procedures to correct. As such, both QA and QC are integral components of a biotechnology company's production of safe and effective products.

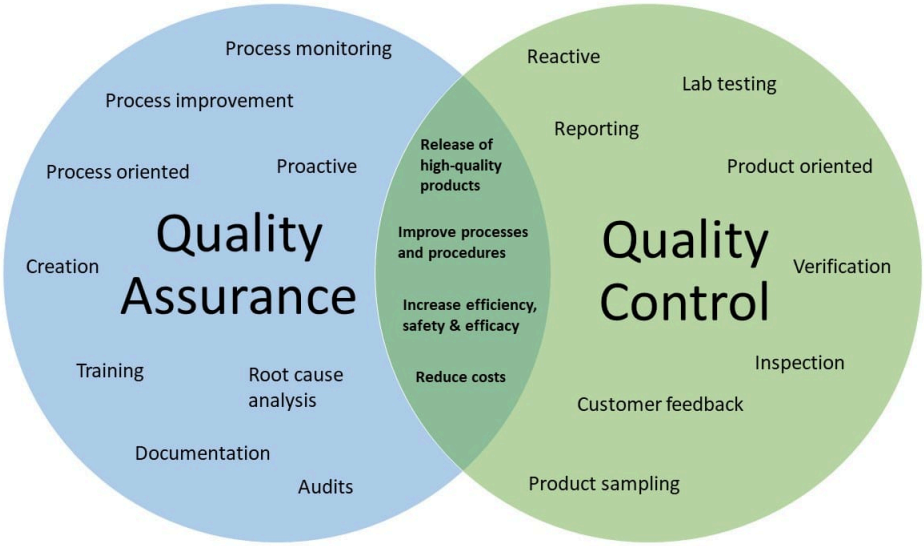


Figure 11.5.1: QA versus QC. Quality assurance (QA) is proactive and process oriented and involves process improvement, monitoring, documentation, audits and training. Quality control (QC) is reactive and product oriented and involves product sampling, lab testing, verification, inspection, and customer feedback. Both emphasize the release of high-quality products, improvement of processes and procedures, increase of efficiency, safety and efficacy, and the reduction of costs. (QA vs. QC by Patricia Zuk, CC BY 4.0)

Learning Objectives

Quality assurance (QA) and quality control (QC) are used by biotechnology companies to identify errors before they happen or to correct them when they do. At the end of this page, you will be able to:

- Define QA and QC and distinguish between them
- Compare and contrast major features of QA and QC
- Explain the major principles of QA and QC
- List the different types of QA and QC
- Explain the role of ISO standards in QA and QC
- Explain the ISO 9000 family of standards
- Explain the purpose of ICH guidelines in pharmaceuticals
- Explain the major categories of ICH guidelines
- Explain the difference between a standard and a specification
- Discuss the roles of QA and QC in biotechnology manufacturing

Quality Assurance (QA) vs. Quality Control (QC)

QA and QC are often confused with one another. While they are related to one another and are both used in product development, they are distinct processes. QA is a systematic approach that ensures all processes involved in the production, testing, and documentation of manufactured products are conducted in accordance with defined standards, specifications, and regulations. QA is process-oriented and pro-active. It focuses on *preventing errors before they occur*. The mantra for QA is "right the first time". QC reviews the quality of all factors involved in production. It is the process of testing and inspection that is used by manufacturers to identify defects or deviations from specifications in raw materials, in-process samples, and final products. QC is product-oriented and reactive. It ensures that outputs meet established quality standards and, when they don't, identifies *where and when errors occurred*. Table 11.5.1: below summarizes the focus, purpose, responsibilities, and activities of QA and QC.

Table 11.5.1: Quality Assurance vs. Quality Control

Feature	Quality Assurance (QA)	Quality Control (QC)
Focus	The process of making a product (from design to delivery)	the product being made
Purpose	Prevention of defects before production (proactive)	Identification and correction of defects during or after production (reactive)
Activities	Documentation, audits, training, SOPs	Testing, sampling, inspection
Responsibility	Quality Assurance department	Quality Control department

Major Principles of Quality Assurance

1. **Compliance with Regulatory Standards:** QA ensures that all products and processes meet regulatory requirements set by regulatory agencies
2. **Document Control:** QA ensures that all procedures are properly documented in **Standard Operating Procedures (SOPs)**, which must be approved, regularly reviewed, and archived
3. **Change Control:** Any proposed changes to materials, methods, or equipment must go through a formal **change control process**, including risk assessment and justification for the change
4. **Training and Qualification:** QA is responsible for ensuring all personnel are properly trained according to internal and external regulations (e.g., Occupational Health and Safety) and their training is documented and regularly updated
5. **Auditing and Self-Inspection:** QA is responsible for conducting internal and external audits that help verify compliance with standards and specifications and uncover opportunities for improvement
6. **Corrective and Preventive Action (CAPA):** QA oversees CAPA systems that investigate the root causes of deviations from standards and specifications and implements actions to prevent recurrence of these deviations

Types of QA

- **Product QA:** focuses on ensuring that the final product meets the specified requirements and standards
- **Process QA:** focuses on ensuring that the processes used to create the product or service are efficient, effective, and consistent
- **Functional QA:** focuses on ensuring that the product functions as intended and meets all functional requirements

- **Supplier QA:** focuses on ensuring that the suppliers of materials or components meet the required quality standard

Major Principles of Quality Control

1. **Raw Material Testing**: All raw materials must be verified for identity, purity, and potency before use in production
2. **In-Process Control (IPC):** QC requires the monitoring of processes while they are being performed (i.e., real-time monitoring) in order to ensure that critical parameters remain within acceptable ranges
    - prevents defects and errors by maintaining process conditions within predefined limits
    - e.g. bioreactor conditions during fermentation processes
3. **Acceptance Sampling:** Inspection of a random sample taken from a production batch to decide whether the whole batch meets quality standards
    - identifies defects in products as they are being made
    - samples (and the entire batch) are either accepted or rejected
    - e.g. extraction of a sample from a bioreactor
4. **Final Product Testing:** QC performs tests for material identity, potency, purity, sterility, and stability
    - goal is to detect and remove defective items before they reach the customer
    - e.g. laboratory analysis of a drug
5. **Analytical Method Validation:** All analytical methods used in QC must be validated to demonstrate accuracy, precision, sensitivity, and reproducibility
    - method validation is mandatory to meet standards set by regulatory authorities like the FDA
    - e.g. validation of quantitative QC methods used to assay drug purity and safety
6. **Stability Testing:** QC must ensure products remain effective and safe throughout their shelf life by conducting short-term and long-term stability studies
7. **Batch Release and Certificate of Analysis (CoA):** QC provides the final evaluation of product quality before a "batch" of product is released
    - includes a signed CoA with key test results

Quality Management Systems

In biotechnology, QA and QC ensure that each product is made in accordance with regulations set by agencies like the FDA, USDA, or the European Medicines Agency (EMA). To ensure this, a biotechnology company employs some type of **quality management system (QMS).** A QMS is a highly structured framework that helps an organization deliver a high-quality product (or service) in a consistent manner. The QMS defines and documents all processes, procedures, and responsibilities involved in manufacturing a product (or providing a service).

Biotechnology facilities will use one (or both) of the following QMS:

- **GMP (Good Manufacturing Practice)**
    - a QMS that applies to the manufacturing, processing, and packaging of regulated products, such as pharmaceuticals, medical devices, and foods
    - ensures that products are produced to consistently meet all quality standards and are safe for use
    - emphasizes comprehensive documentation of all manufacturing processes, batch records, and product testing in order to ensure traceability and regulatory compliance
- **GLP (Good Laboratory Practice)**
    - a QMS used in the research and development labs of biotechnology companies
    - ensures the credibility and reliability of scientific data
    - establishes standards for the conducting of a study and the collection, analysis and reporting of its data
    - emphasizes adherence to protocols, documentation, and quality control measures to ensure that research findings are accurate and reproducible

ISO Standards in QA and QC

Every company promises an unwavering commitment to quality. However, how does a company provide proof of this commitment? Companies can assure their customers of the quality of their practices and processes using standards established by the **International Organization for Standardization (ISO).** Founded in 1947, the ISO has over 170 member countries and publishes new standards and revisions to existing standards on a regular basis. As of 2024, there are over 24,000 ISO standards that

cover virtually every industry and sector. Some of the major categories of ISO standards cover quality management systems, environmental management systems, information security, occupational health and safety, food safety, medical devices, and laboratory competence. In QA, ISO's promote principles such as continual improvement and a commitment to safety and quality. They encourage documentation, monitoring, and evaluation of processes to prevent defects and errors. In QC, ISO standards are used to define calibration requirements for equipment, specific testing methods, and the criteria used to accept or reject a product. They help organizations develop a structured approach to inspection and testing, so that non-conforming products can be detected and dealt with.

The following ISO standards are used frequently in biotechnology QA and QC:

- **ISO 9001** – Used to create and implement a QMS
- **ISO 22000** - Used for Food Safety Management Systems
- **ISO 2859** - Used for QC sampling procedures
- **ISO 17025** – Used for testing and calibration of laboratories
- **ISO 13485** – Used for a QMS for medical devices
- **ISO 19011** – Guidelines for auditing management systems

ISO 9001 is part of the ISO 9000 "family" of standards. The ISO 9000 family includes three main standards: ISO 9000, ISO 9001, and ISO 9004. **ISO 9001** is the standard that helps organizations develop and implement a robust QMS like GMP or GLP. The most recent ISO 9001 version is called ISO 9001:2015. Companies achieving this certification are permitted to display a logo on company materials like letterhead, labels, and their website (Figure 11.5.1). This specific ISO is the only one in the ISO 9000 family that is "certified" through an external **ISO audit** - a systematic, independent, and documented process for evaluating whether an organization's management system, such as their QMS, complies with the requirements of a specific ISO standard. Companies will seek "**ISO 9001 certification"** in order to improve their product and service quality, enhance operational efficiency, and support continuous improvement of their processes. This certification can also boost customer satisfaction and confidence and increase market share because it assures customers that the product they are purchasing has a defined level of quality. In certain instances, companies cannot realistically enter a market without ISO 9001 certification. In contrast to ISO 9001, the ISO 9000 and ISO 9004 standards are not certified through an audit. Rather, companies will use ISO 9000 to learn the basic concepts, principles, and terminology important to ISO 9001 certification. If desired, companies can also follow the standards outlined in ISO 9004 in order to go beyond ISO 9001 compliance to achieve long-term success. Because of this, ISO 9000 and ISO 9004 are applicable to any organization regardless of size, industry, or location, whereas ISO 9001 is used by companies that have established a QMS.



Figure 11.5.2: The ISO 9001:2015 logo. (ISO 9001-2015 by Юкатан, CC BY-SA 4.0)

ICH Guidelines

For pharmaceutical companies, products must be deemed as safe, effective, and of high quality. Moreover, the production, testing, and regulatory processes used in QA and QC must be consistent across major global markets. In short, these processes must be

"harmonized". As a result, pharmaceutical companies also follow **ICH guidelines**. These guidelines are internationally harmonized standards developed by the **International Council for Harmonisation (ICH)** of Technical Requirements for Pharmaceuticals for Human Use. The ICH was founded in 1990 and its members include regulatory authorities (e.g., FDA, EMA, PMDA) and industry groups. The goal of ICH guidelines is to "harmonize" pharmaceutical regulations across the U.S., EU, Japan, and more recently, Canada, China, and Brazil. ICH guidelines focus on reducing duplication in drug development, testing, and approval processes. They are recognizes by regulatory agencies worldwide and are often required for drugs that are entering the global market.

ICH guidelines are divided into five categories:

1. **Q – Quality Guidelines:** focuses on chemistry, manufacturing, and controls
   - examples: Q1 (stability testing), Q8 (pharmaceutical quality system)
2. **S – Safety Guidelines:** focuses on preclinical safety and toxicology studies
   - examples: S1 (carcinogenicity), S2 (genotoxicity), S6 (biotechnology products - i.e., preclinical safety)
3. **E – Efficacy Guidelines:** focuses on clinical studies and pharmacology
   - E6 (Good Clinical Practice), E8 (general considerations for clinical trials), E9 (statistical principles)
4. **M – Multidisciplinary Guidelines:** focuses on topics that cross multiple categories
   - M1 (medical terminology), M4 (common technical document), and M9 (biopharmaceutical classification system)
5. **R – Recently Revised/Implemented Guidelines:** introduces updated versions of older guidelines

Quality Standards and Specifications

The terms standard and specification are used frequently in QA and QC. A standard might require or refer to a specification, while a specification might comply with or be derived from a standard. So, while standard and specification are related, they are not the same thing. A **standard** is a broad, consensus-based document that provides the guidelines or rules for a process and product (or service). A standard covers terminology, general principles, and "best practices" and is often applied across an organization or industry. Because of this, the scope of a standard is broad and general. For example, a standard might stipulate that the base of a light bulb be "made of conductive metal". Another example of a standard is the ISO 9001 standard used in a QMS. This standard defines what a QMS should include, but not how every company must implement it.

A **specification** is a more detailed, *technical* document that defines the exact requirements for a product, material, or process. It is often used in manufacturing and engineering. The specification tells a user exactly what the product must be and must do. The scope of a specification is narrow, detailed, and technical. For example, with regards to the light bulb, the specification might stipulate that the conductive metal contain 30 to 40% copper. Lastly, specifications might differ from company to company, but a standard does not change.

Integration of QA and QC in Biotechnology Manufacturing

In biotech manufacturing, QA and QC are deeply integrated into every stage of the process to ensure that biopharmaceutical products are safe, effective, and comply with regulatory standards (e.g., FDA, EMA, ICH). They are embedded in all stages of product development. For example, during upstream processing, QA oversees process validation, while QC tests cell lines and growth media. During downstream processing, QC ensures the purity and integrity of a product. Regulatory agencies like the FDA (and the European Medicines Agency/EMA) require comprehensive QA and QC documentation as part of new drug applications and the licensing of new biologics. Failures in QA or QC can result in clinical trial delays, product recalls, or regulatory action that can result in large fines. Table 11.5.2 below gives several examples of how QA and QC work together in biotechnology manufacturing.

Table 11.5.2: ***Roles of QA and QC In Biotech***

Phase	QA Role	QC Role
Design and Development	• Implement quality manufacturing systems (QMS) like Good Manufacturing Practices (GMP) or Good Laboratory Practices (GLP) early in development • Develops standard operating procedures (SOPs) • Oversees design controls	• Validates analytical test methods • Performs initial raw material testing • Supports method development with data

Phase	QA Role	QC Role
Raw Material Control	• Approves qualified suppliers • Reviews material handling procedures	• Tests incoming raw materials (e.g., media, buffers) • Ensures material identity, purity, and sterility
Upstream and Downstream Processing	• Audits the compliance of processes to SOPs and batch records • Approves change controls and Corrective and Preventive Actions (CAPAs)	• Performs in-process testing (e.g., cell viability, metabolite levels) • Monitors critical quality attributes (CQAs) • Conducts environmental monitoring in clean rooms
Product Testing and Release	• Reviews and approves batch records and QC data for release • Ensures data integrity and traceability • Manages product disposition decisions	• Conducts release testing of products (e.g., potency, purity, sterility) • Confirms compliance with product specifications • Performs stability testing (short-term)
Validation and Continuous Monitoring	• Oversees the development of strategies for process validation • Implements quality risk management (QRM) principles • Ensures systems remain qualified and calibrated	• Collects and analyzes validation data • Performs trend analysis (e.g., control charts) • Performs and supports long-term stability studies

Key Concepts

Both QA and QC are integral components of a biotechnology company's production of safe and effective products. Some important concepts to remember are:

- QA is proactive; QC is reactive - QA focuses on preventing defects, QC focuses on detecting them
- QA is process-based and focuses on the processes and systems that create the product (or service)
- QC is product-based and tests the final product before it is released
- Biotechnology manufacturers use a quality management system (QMS), like GMP or GLP as part of their QA and QC practices
- QA and QC are conducted using a set of standards established by the International Organization for Standardization (ISO).
- ISO 9001 is the current ISO standard used for the QMS
- Pharmaceutical companies will also use International Council for Harmonisation (ICH) guidelines to ensure that the production, testing, and regulatory processes used in QA and QC are consistent for pharmaceuticals entering the global market

Glossary

Audit - a systematic, independent, and documented process for evaluating whether an organization's management system complies with the requirements of a specific ISO standard

Batch - a specific quantity of material or product produced during a single manufacturing cycle under uniform conditions; identified by a batch number and must meet defined quality criteria

Documentation - the systematic process of creating, organizing, and maintaining written records that describe processes, procedures, decisions, designs, and results

Good Manufacturing Practice (GMP) - a system of regulations and guidelines used in pharmaceuticals, biologics, medical devices, and food, that ensure products are consistently produced and controlled according to quality standards; enforced by regulatory bodies

Good Laboratory Practice (GLP) - a quality system concerned with ensuring the integrity, reliability, and reproducibility of laboratory data

International Organization for Standardization (ISO) - an independent, non-governmental international organization that develops and publishes voluntary global standards

ISO standards - internationally recognized guidelines published by the International Organization for Standardization (ISO) in order to provide best practices, technical specifications, and requirements for quality, safety, and efficiency across industries

ISO 9001 - the standard that helps organizations develop and implement a QMS like GMP or GLP

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) - an international organization that develops guidelines to ensure safe, effective, and high-quality medicines are developed and registered efficiently and properly

Quality assurance (QA) - a set of planned and systematic activities implemented in a quality system to ensure that quality requirements for a product or service are fulfilled

Quality control (QC) - the operational techniques and activities used to fulfill requirements for quality, typically through sampling, measurement, and testing

Quality Management System (QMS) - a highly structured framework that helps organizations deliver a high-quality product (or service) in a consistent manner

Product testing - the process of evaluating a product's performance, quality, safety, and compliance with relevant standards, regulations, or customer requirements before it is released to the market or approved for use

Standard Operating Procedure (SOP) - a detailed, written instruction describing how to perform a routine or repetitive activity in a consistent and controlled way; ensures compliance, training, quality, and safety

Standard - a documented general agreement containing technical specifications, guidelines, or criteria that can be used consistently by an industry to ensure that materials, products, processes, or services are fit for purpose

Specification - a detailed and exact statement of technical requirements, including measurements, tolerances, materials, and methods for a product, service, or process; defines what is acceptable or expected and is used for quality control, purchasing, and regulatory compliance

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10.2: Genetic Engineering in Animals
11: Government Oversight of Biotechnology
11.1: Environmental Laws and Regulations
11.2: The Food and Drug Administration (FDA)
11.3: Regulation of Plant GMOs
11.4: Regulation of Animal GMOs and Medical Foods
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)
2: The Molecules of Life
2.1: The Building Blocks of Molecules
2.2: Water
2.3: Macromolecules
2.4: Carbohydrates
2.5: Lipids
2.6: Nucleic Acids
2.7: Proteins
3: Cells and Viruses
3.1: Themes and Concepts of Biology
3.2: Prokaryotic Cells
3.3: Eukaryotic Cells
3.4: Viruses
3.4.1: Viral Evolution, Morphology, and Classification
3.4.2: Virus Infections and Hosts
3.4.3: Prevention and Treatment of Viral Infections
4: Central Dogma
4.2: DNA Structure
4.3: Replication of DNA
4.4: Transcription of RNA
4.5: Translation of the polypeptide
4.6: How Genes Are Regulated
4.7: Mutations and DNA Repair
5: Genetic Engineering and Recombinant DNA Technology
5.1: Genome Sequencing
5.2: Principles of Genetic Engineering
5.3: Creating Recombinant DNA
5.4: Polymerase Chain Reaction (PCR)
6: Forensic Biotechnology
6.1: Analysis of STRs
7: Microbial Biotechnology
7.1: Microbe classification
7.2: Industrial Applications of Microbial Biotechnology
7.3: Medical Applications of Microbial Biotechnology
7.4: Vaccines
7.5: How Does SARS-Cov-2 Infect Humans?
7.6: Genes Implicated in Severe COVID-19 Infection in Humans
7.7: Approaches for Vaccine Development Against SARS-Cov-2
8: Environmental, Agricultural, and Aquatic Biotechnology
8.1: Applications of Environmental Biotechnology
8.2: Applications of Agricultural Biotechnology
9: Stem Cells, Cloning, and Gene Therapy
9.3: Therapeutic Biotechnology
9.4: Regenerative Medicine
9.5: Principles of Medical Biotechnology
9.6: Diagnostic Biotechnology
Introduction to Biotechnology

AZT
3.4.2: Virus Infections and Hosts

B

Bacillus thuringiensis
8.2: Applications of Agricultural Biotechnology

back mutation
3.4.3: Prevention and Treatment of Viral Infections

bacterial artificial chromosomes (BACs)
5.2: Principles of Genetic Engineering

bacterial cell wall composition
7.1: Microbe classification

bacterial chromosome structure
4.2: DNA Structure

bacterial identification
7.1: Microbe classification

bacterial transformation
5.3: Creating Recombinant DNA

bacteriophage
3.4.2: Virus Infections and Hosts

basal medium for cell culture
10.3: Principles of Mammalian Cell Culture

Base Excision Repair
4.7: Mutations and DNA Repair

bases
2.2: Water

basic science
1.2: The Process of Science

bioactive molecules
9.4: Regenerative Medicine

bioaugmentation
8.1: Applications of Environmental Biotechnology

biodiversity
11.1: Environmental Laws and Regulations

biofabrication
7.2: Industrial Applications of Microbial Biotechnology

biofertilizers
8.2: Applications of Agricultural Biotechnology

biofilters
8.1: Applications of Environmental Biotechnology

biofuels
7.2: Industrial Applications of Microbial Biotechnology

biological safety levels (BSLs)
10.3: Principles of Mammalian Cell Culture

biological scaffolds (bioscaffolds)
9.4: Regenerative Medicine

biology
3.1: Themes and Concepts of Biology

biomarker types
9.6: Diagnostic Biotechnology

biomaterials
9.4: Regenerative Medicine

biopesticides
8.2: Applications of Agricultural Biotechnology

biopharming
10.2: Genetic Engineering in Animals

bioprinting
9.4: Regenerative Medicine

bioproduction
7.2: Industrial Applications of Microbial Biotechnology

bioremediation
8.1: Applications of Environmental Biotechnology

biosafety
10.3: Principles of Mammalian Cell Culture

biosensors
8.1: Applications of Environmental Biotechnology
9.6: Diagnostic Biotechnology

biosphere
3.1: Themes and Concepts of Biology

biotechnological applications
1.1: Definition and a Short History of Biotechnology

biotechnology
1.1: Definition and a Short History of Biotechnology
Introduction to Biotechnology

biotechnology notification files (BNF)
11.3: Regulation of Plant GMOs

black biotechnology
1.1: Definition and a Short History of Biotechnology

blue biotechnology
1.1: Definition and a Short History of Biotechnology

blunt end cloning
5.3: Creating Recombinant DNA

breeding vs genetic engineering
5.2: Principles of Genetic Engineering

brown biotechnology
1.1: Definition and a Short History of Biotechnology

budding
3.4.2: Virus Infections and Hosts

buffers
2.2: Water

C

capsid
3.4: Viruses

capsomere
3.4: Viruses

carbohydrates
2.3: Macromolecules
2.4: Carbohydrates

carbonic acid bicarbonate buffer system
2.2: Water

catalysts
2.7: Proteins

cation
2.1: The Building Blocks of Molecules

cell
3.1: Themes and Concepts of Biology

cell cloning
10.1: Applications of Animal Biotechnology
10.3: Principles of Mammalian Cell Culture

cell culture conditions and reagents
10.3: Principles of Mammalian Cell Culture

cell cycle
4.1: The Cell Cycle

cell cycle checkpoints
4.1: The Cell Cycle

cell line
10.3: Principles of Mammalian Cell Culture

cell necrosis
3.4.2: Virus Infections and Hosts

cell plate
4.1: The Cell Cycle

cell strain
10.3: Principles of Mammalian Cell Culture

cell wall
3.3: Eukaryotic Cells

cellreplacementtherapy
9.1: Stem Cells

cellulose
2.4: Carbohydrates

central dogma of molecular biology
4.4: Transcription of RNA

central vacuole
3.3: Eukaryotic Cells

centriole
4.1: The Cell Cycle

centrosome
3.3: Eukaryotic Cells

chain termination DNA sequencing
5.1: Genome Sequencing

chemical bond
2.1: The Building Blocks of Molecules

chitin
2.4: Carbohydrates

chlorophyll
3.3: Eukaryotic Cells

chloroplast
3.3: Eukaryotic Cells

cholesterol
2.5: Lipids

chromatids
4.2: DNA Structure

chromatin
3.3: Eukaryotic Cells

chromatin structure
4.2: DNA Structure

chromosome
3.3: Eukaryotic Cells

chronic infection
3.4.2: Virus Infections and Hosts

Clean Air Act
11.1: Environmental Laws and Regulations

cleavage furrow
4.1: The Cell Cycle

cloning
10.1: Applications of Animal Biotechnology
5.2: Principles of Genetic Engineering
5.3: Creating Recombinant DNA

cloning plasmids
5.2: Principles of Genetic Engineering

coding sequence
4.4: Transcription of RNA

codon table
4.5: Translation of the polypeptide

common restriction enzymes
5.2: Principles of Genetic Engineering

community
3.1: Themes and Concepts of Biology

complementary base pairing
2.6: Nucleic Acids

conjugate vaccine
7.4: Vaccines

conjugate vaccines
7.4: Vaccines
continuous cell lines
10.3: Principles of Mammalian Cell Culture
control
1.2: The Process of Science
cosmids
5.2: Principles of Genetic Engineering
covalent bond
2.1: The Building Blocks of Molecules
CRISPR
10.1: Applications of Animal Biotechnology
9.3: Therapeutic Biotechnology
culture medium, mammalian culture
10.3: Principles of Mammalian Cell Culture
cultured cell types
10.3: Principles of Mammalian Cell Culture
cystic fibrosis and gene therapy
9.3: Therapeutic Biotechnology
cytokinesis
4.1: The Cell Cycle
cytopathic
3.4.2: Virus Infections and Hosts
cytoplasm
3.3: Eukaryotic Cells
cytosol
3.3: Eukaryotic Cells

D

daughter DNA strand
4.3: Replication of DNA
decellularized scaffolds
9.4: Regenerative Medicine
deductive reasoning
1.2: The Process of Science
dehydration synthesis
2.3: Macromolecules
2.4: Carbohydrates
2.5: Lipids
2.7: Proteins
deletion mutations
4.7: Mutations and DNA Repair
Deoxyribonucleic acid (DNA)
2.6: Nucleic Acids
descriptive science
1.2: The Process of Science
diagnostic biotechnology
9.6: Diagnostic Biotechnology
dideoxy chain termination method of DNA sequencing
5.1: Genome Sequencing
dideoxynucleotides (ddNTPs)
5.1: Genome Sequencing
differentiation of stem cells
9.4: Regenerative Medicine
dipeptides
2.7: Proteins
directional cloning
5.3: Creating Recombinant DNA
disaccharides
2.4: Carbohydrates
DNA cloning
5.2: Principles of Genetic Engineering
5.3: Creating Recombinant DNA
DNA helicase
4.3: Replication of DNA
DNA helix
4.2: DNA Structure
DNA ligase
4.3: Replication of DNA
5.3: Creating Recombinant DNA
DNA microarrays
9.6: Diagnostic Biotechnology
DNA mutations
4.7: Mutations and DNA Repair
DNA packaging
4.2: DNA Structure
DNA polymerase
4.3: Replication of DNA
DNA repair mechanisms
4.7: Mutations and DNA Repair
DNA replication
4.3: Replication of DNA
DNA sequencing
5.1: Genome Sequencing
DNA structure
2.6: Nucleic Acids
4.2: DNA Structure
DNA supercoiling
4.2: DNA Structure
DNA vs RNA
2.6: Nucleic Acids
double digest of DNA
5.3: Creating Recombinant DNA

E

E (exit) site of the ribosome
4.5: Translation of the polypeptide
EcoRI
5.2: Principles of Genetic Engineering
ecosystem
3.1: Themes and Concepts of Biology
electron
2.1: The Building Blocks of Molecules
electron transfer
2.1: The Building Blocks of Molecules
electrospinning of scaffolds
9.4: Regenerative Medicine
element
2.1: The Building Blocks of Molecules
embryonic stem (ES) cells
9.4: Regenerative Medicine
embryonicstemcells
9.1: Stem Cells
endonucleases
4.7: Mutations and DNA Repair
enhancers
4.4: Transcription of RNA
envelope
3.4: Viruses
environmental biotechnology
1.1: Definition and a Short History of Biotechnology
8.1: Applications of Environmental Biotechnology
environmental law
11.1: Environmental Laws and Regulations
environmental monitoring
8.1: Applications of Environmental Biotechnology
environmental policy
11.1: Environmental Laws and Regulations
environmental protection
11.1: Environmental Laws and Regulations
Enzyme Linked Immunosorbent Assays (ELISAs)
9.6: Diagnostic Biotechnology
enzymes
2.7: Proteins
EPA
11.1: Environmental Laws and Regulations
epigenetic
4.6: How Genes Are Regulated
essential fatty acids
2.5: Lipids
ester bond
2.5: Lipids
euchromatin
4.2: DNA Structure
eukaryote
3.1: Themes and Concepts of Biology
eukaryotic cell
3.3: Eukaryotic Cells
eukaryotic chromosome structure
4.2: DNA Structure
evaporation
2.2: Water
evolution
3.1: Themes and Concepts of Biology
ex situ bioremediation
8.1: Applications of Environmental Biotechnology
exonuclease types
4.7: Mutations and DNA Repair
expression plasmids
5.2: Principles of Genetic Engineering
extremophiles
7.1: Microbe classification

F

falsifiable
1.2: The Process of Science
fatty acid structure and classification
2.5: Lipids
FDA
11.2: The Food and Drug Administration (FDA)
first generation biofuels
7.2: Industrial Applications of Microbial Biotechnology
fluorescent in situ hybridization (FISH)
9.6: Diagnostic Biotechnology
food biotechnology
1.1: Definition and a Short History of Biotechnology
fourth generation biofuels
7.2: Industrial Applications of Microbial Biotechnology
frameshift mutations
4.7: Mutations and DNA Repair
fusion
3.4.2: Virus Infections and Hosts

G

G0 phase
4.1: The Cell Cycle

G1 phase
4.1: The Cell Cycle

G2 phase
4.1: The Cell Cycle

gall
3.4.2: Virus Infections and Hosts

gene addition
10.1: Applications of Animal Biotechnology

gene editing
10.1: Applications of Animal Biotechnology
9.3: Therapeutic Biotechnology

gene expression
4.4: Transcription of RNA
4.6: How Genes Are Regulated

gene gun
8.2: Applications of Agricultural Biotechnology

gene silencing
10.1: Applications of Animal Biotechnology

gene structure
4.4: Transcription of RNA

gene therapy
3.4.3: Prevention and Treatment of Viral Infections
9.3: Therapeutic Biotechnology

Generally Recognized As Safe (GRAS)
11.3: Regulation of Plant GMOs

genetic code
4.5: Translation of the polypeptide

genetic engineering
1.1: Definition and a Short History of Biotechnology
10.1: Applications of Animal Biotechnology
10.2: Genetic Engineering in Animals
5.2: Principles of Genetic Engineering

genetically engineered (GE) crops
11.3: Regulation of Plant GMOs

genetically engineered (GE) plants
11.3: Regulation of Plant GMOs

genetically modified crops
8.2: Applications of Agricultural Biotechnology

genetically modified organism (GMO)
1.1: Definition and a Short History of Biotechnology
10.2: Genetic Engineering in Animals
8.2: Applications of Agricultural Biotechnology

geneticmanipulation
9.1: Stem Cells

genome sequencing
5.1: Genome Sequencing

GLOFish
10.2: Genetic Engineering in Animals

glucose monitors
9.6: Diagnostic Biotechnology

glycerol structure
2.5: Lipids

glycogen
2.4: Carbohydrates

glycosidic bond
2.4: Carbohydrates

GMO
1.1: Definition and a Short History of Biotechnology

Good Laboratory Practices (GLPs)
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)

Good Manufacturing Practices (GMPs)
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)

gram staining
7.1: Microbe classification

gray biotechnology
1.1: Definition and a Short History of Biotechnology
8.1: Applications of Environmental Biotechnology

green biotechnology
1.1: Definition and a Short History of Biotechnology
8.2: Applications of Agricultural Biotechnology

group I virus
3.4: Viruses

group II virus
3.4: Viruses

group III virus
3.4: Viruses

group IV virus
3.4: Viruses

group V virus
3.4: Viruses

group VI virus
3.4: Viruses

group VII virus
3.4: Viruses

H

hazardous waste
11.1: Environmental Laws and Regulations

HeLa cells
10.3: Principles of Mammalian Cell Culture

hematopoietic stem cells (HSCs)
9.4: Regenerative Medicine

Henrietta Lacks
10.3: Principles of Mammalian Cell Culture

herd immunity
7.4: Vaccines

heterochromatin
4.2: DNA Structure

histones
4.2: DNA Structure

Homeostasis
3.1: Themes and Concepts of Biology

horizontal transmission
3.4.2: Virus Infections and Hosts

Humulin
5.2: Principles of Genetic Engineering
9.3: Therapeutic Biotechnology

hydrogen bond
2.1: The Building Blocks of Molecules

hydrogen bonding
2.2: Water

hydrolysis
2.3: Macromolecules

hydrophilic and hydrophobic
2.2: Water

hyperplasia
3.4.2: Virus Infections and Hosts

hypoplasia
3.4.2: Virus Infections and Hosts

hypothesis
1.2: The Process of Science

I

ICH guidelines
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)

immortalized cells
10.3: Principles of Mammalian Cell Culture

immunoassays
9.6: Diagnostic Biotechnology

immunodiagnostics
9.6: Diagnostic Biotechnology

in situ bioremediation
8.1: Applications of Environmental Biotechnology

in vitro cell culture
10.3: Principles of Mammalian Cell Culture

in vitro transcription systems
4.4: Transcription of RNA

inactivated vaccines
7.4: Vaccines

induced pluripotent stem cells (iPSCs)
9.4: Regenerative Medicine

inductive reasoning
1.2: The Process of Science

industrial biotechnology
1.1: Definition and a Short History of Biotechnology

initiation factors, translation
4.5: Translation of the polypeptide

initiator tRNA
4.5: Translation of the polypeptide

insertion mutations
4.7: Mutations and DNA Repair

intermittent symptom
3.4.2: Virus Infections and Hosts

international treaties
11.1: Environmental Laws and Regulations

interphase
4.1: The Cell Cycle

intron splicing
4.4: Transcription of RNA

ion
2.1: The Building Blocks of Molecules

ionic bond
2.1: The Building Blocks of Molecules

ISO 9001 certification
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)

ISO standards
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)

isotope
2.1: The Building Blocks of Molecules

K

kinetochore
4.1: The Cell Cycle

Kozak sequence of translation
4.5: Translation of the polypeptide

L

lab on a chip
9.6: Diagnostic Biotechnology

lagging strand synthesis
4.3: Replication of DNA

land management biotechnology
1.1: Definition and a Short History of Biotechnology

large scale mutation types
4.7: Mutations and DNA Repair

latency
3.4.2: Virus Infections and Hosts

lateral flow assays (LFAs)
9.6: Diagnostic Biotechnology

leading strand synthesis
4.3: Replication of DNA

legislation
11.1: Environmental Laws and Regulations

life science
1.2: The Process of Science

lipids
2.3: Macromolecules
2.5: Lipids

liposomes
10.1: Applications of Animal Biotechnology
10.2: Genetic Engineering in Animals
9.3: Therapeutic Biotechnology

Live attenuated vaccines
7.4: Vaccines

lysis
3.4.2: Virus Infections and Hosts

lysogenic cycle
3.4.2: Virus Infections and Hosts

lysosome
3.3: Eukaryotic Cells

lytic cycle
3.4.2: Virus Infections and Hosts

M

macromolecule
3.1: Themes and Concepts of Biology

macromolecules
2.3: Macromolecules

mammalian cell culture
10.3: Principles of Mammalian Cell Culture

manufacturing biotechnology
1.1: Definition and a Short History of Biotechnology

marine biotechnology
1.1: Definition and a Short History of Biotechnology

mass number
2.1: The Building Blocks of Molecules

matrix protein
3.4: Viruses

matter
2.1: The Building Blocks of Molecules

medical biotechnology
1.1: Definition and a Short History of Biotechnology
9.5: Principles of Medical Biotechnology

medicalresearch
9.1: Stem Cells

mesodermally derived stem cells
9.4: Regenerative Medicine

messenger RNA (mRNA) structure
4.4: Transcription of RNA

metaphase
4.1: The Cell Cycle

metaphase plate
4.1: The Cell Cycle

methylated cap
4.4: Transcription of RNA

microbial biotechnology
7.1: Microbe classification
7.2: Industrial Applications of Microbial Biotechnology
7.3: Medical Applications of Microbial Biotechnology

microbial identification and classification
7.1: Microbe classification

microRNA (miRNA)
10.1: Applications of Animal Biotechnology

mismatch repair
4.7: Mutations and DNA Repair

missense mutations
4.7: Mutations and DNA Repair

mitochondria
3.3: Eukaryotic Cells

mitosis
4.1: The Cell Cycle

mitotic phase
4.1: The Cell Cycle

mitotic spindle
4.1: The Cell Cycle

molecular diagnostics
9.6: Diagnostic Biotechnology

molecule
3.1: Themes and Concepts of Biology

monomers
2.3: Macromolecules

monosaccharides
2.4: Carbohydrates

monounsaturated
2.5: Lipids

mRNA processing
4.4: Transcription of RNA

mRNA vaccines
9.3: Therapeutic Biotechnology

multi cloning site (MCS)
4.2: DNA Structure
5.2: Principles of Genetic Engineering
5.3: Creating Recombinant DNA

multipotent stem cells
9.4: Regenerative Medicine

multipotentcells
9.1: Stem Cells

mutagens
4.7: Mutations and DNA Repair

mutations
4.7: Mutations and DNA Repair

N

natural active immunity
7.4: Vaccines

natural passive immunity
7.4: Vaccines

natural science
1.2: The Process of Science

negative polarity
3.4: Viruses

neutron
2.1: The Building Blocks of Molecules

next generation sequencing
5.1: Genome Sequencing
9.6: Diagnostic Biotechnology

nitrogen fixation
8.2: Applications of Agricultural Biotechnology

nitrogenous bases
2.6: Nucleic Acids

non polar bonds
2.2: Water

nonpolar covalent bond
2.1: The Building Blocks of Molecules

nonsense mutations
4.7: Mutations and DNA Repair

nuclear envelope
3.3: Eukaryotic Cells

nucleic acids
2.3: Macromolecules
2.6: Nucleic Acids

nucleoid
3.2: Prokaryotic Cells

nucleolus
3.3: Eukaryotic Cells

nucleoplasm
3.3: Eukaryotic Cells

nucleosomes
4.2: DNA Structure

nucleotide excision repair
4.7: Mutations and DNA Repair

nucleotide nomenclature
2.6: Nucleic Acids

nucleotide structure
2.6: Nucleic Acids
4.2: DNA Structure

nucleus
2.1: The Building Blocks of Molecules
3.3: Eukaryotic Cells

O

octet rule
2.1: The Building Blocks of Molecules

Okazaki fragments
4.3: Replication of DNA

oncogenic virus
3.4.2: Virus Infections and Hosts

oncolytic virus
3.4.3: Prevention and Treatment of Viral Infections

one gene one polypeptide hypothesis
4.5: Translation of the polypeptide

organ
3.1: Themes and Concepts of Biology

organ culture
10.3: Principles of Mammalian Cell Culture

organ system
3.1: Themes and Concepts of Biology

organelle
3.1: Themes and Concepts of Biology
3.3: Eukaryotic Cells

organism
3.1: Themes and Concepts of Biology

origin of replication
4.3: Replication of DNA
5.2: Principles of Genetic Engineering

P

P (peptidyl) site of the ribosome
4.5: Translation of the polypeptide
paired end sequencing
5.1: Genome Sequencing
parental DNA strand
4.3: Replication of DNA
peptide types
2.7: Proteins
periodic table of elements
2.1: The Building Blocks of Molecules
permissive
3.4.2: Virus Infections and Hosts
peroxisome
3.3: Eukaryotic Cells
personal protective equipment (PPE)
10.3: Principles of Mammalian Cell Culture
personalized medicine
5.1: Genome Sequencing
pH scale
2.2: Water
phage therapy
3.4.3: Prevention and Treatment of Viral Infections
phosphodiester bond
2.6: Nucleic Acids
4.2: DNA Structure
phospholipid bilayer
2.5: Lipids
phospholipid structure
2.5: Lipids
phylogenetic tree
3.1: Themes and Concepts of Biology
physical science
1.2: The Process of Science
phytoremediation
8.1: Applications of Environmental Biotechnology
plasma membrane
3.3: Eukaryotic Cells
plasmids
4.2: DNA Structure
5.2: Principles of Genetic Engineering
pluripotent stem cells
9.4: Regenerative Medicine
pluripotentcells
9.1: Stem Cells
point mutations
4.7: Mutations and DNA Repair
point of care diagnostics
9.6: Diagnostic Biotechnology
polar bonds
2.2: Water
polar covalent bond
2.1: The Building Blocks of Molecules
polyA tail
4.4: Transcription of RNA
polyadenylation site
5.2: Principles of Genetic Engineering
polylinker
5.3: Creating Recombinant DNA
polymerase chain reaction (PCR)
4.3: Replication of DNA
5.4: Polymerase Chain Reaction (PCR)
polymers
2.3: Macromolecules
polymorphisms
6.1: Analysis of STRs
polypeptides
2.7: Proteins
polysaccharides
2.4: Carbohydrates
polyunsaturated
2.5: Lipids
population
3.1: Themes and Concepts of Biology
positive polarity
3.4: Viruses
potency of stem cells
9.4: Regenerative Medicine
precision fermentation
10.1: Applications of Animal Biotechnology
primary culture
10.3: Principles of Mammalian Cell Culture
primary protein structure
2.7: Proteins
primase
4.3: Replication of DNA
productive
3.4.2: Virus Infections and Hosts
proflavin and protein translation
4.5: Translation of the polypeptide
prokaryote
3.1: Themes and Concepts of Biology
3.2: Prokaryotic Cells
prokaryotes vs. eukaryotes
7.1: Microbe classification
prokaryotic DNA structure
4.2: DNA Structure
prometaphase
4.1: The Cell Cycle
promoters
4.4: Transcription of RNA
5.2: Principles of Genetic Engineering
prophage
3.4.2: Virus Infections and Hosts
prophase
4.1: The Cell Cycle
protein denaturation
2.7: Proteins
protein folding
2.7: Proteins
protein structure and disease, sickle cell anemia
2.7: Proteins
protein structure levels
2.7: Proteins
4.5: Translation of the polypeptide
protein translation stages
4.5: Translation of the polypeptide
protein types
2.7: Proteins
protein, alpha helix
2.7: Proteins
protein, beta pleated sheet
2.7: Proteins
proteins
2.3: Macromolecules
2.7: Proteins
proton
2.1: The Building Blocks of Molecules
purines
2.6: Nucleic Acids
4.2: DNA Structure
pyrimidines
2.6: Nucleic Acids
4.2: DNA Structure

Q

Quality Assurance (QA) and Quality Control (QC)
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)
Quality Management System (QMS)
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)
quality standards and specifications
11.5: Principles of Quality Assurance (QA) and Quality Control (QC)
quantitative PCR (real time PCR)
4.3: Replication of DNA
quartz crystal microbalances
9.6: Diagnostic Biotechnology
quaternary protein structure
2.7: Proteins
quiescent
4.1: The Cell Cycle

R

radioactive isotope
2.1: The Building Blocks of Molecules
radioimmunoassay (RIA) measurement of proteins
2.7: Proteins
radiolabeling
2.3: Macromolecules
reading frame of translation
4.5: Translation of the polypeptide
4.7: Mutations and DNA Repair
recombinant DNA (rDNA) technology
5.2: Principles of Genetic Engineering
5.3: Creating Recombinant DNA
recombinant plasmids
5.3: Creating Recombinant DNA
red biotechnology
1.1: Definition and a Short History of Biotechnology
9.5: Principles of Medical Biotechnology
regenerative medicine
9.1: Stem Cells
9.4: Regenerative Medicine
release factor in translation termination
4.5: Translation of the polypeptide
replication fork
4.3: Replication of DNA
replicative intermediate
3.4: Viruses
reporter proteins
8.1: Applications of Environmental Biotechnology
reproductive cloning
10.1: Applications of Animal Biotechnology

restriction enzymes
5.2: Principles of Genetic Engineering
5.3: Creating Recombinant DNA
restriction sequences and sites
5.2: Principles of Genetic Engineering
reverse transcriptase
3.4: Viruses
reversion mutations
4.7: Mutations and DNA Repair
Ribonucleic Acid (RNA)
2.6: Nucleic Acids
ribosomal RNA (rRNA) structure
4.4: Transcription of RNA
ribosome
3.3: Eukaryotic Cells
ribosome structure
4.5: Translation of the polypeptide
RNA interference (RNAi)
10.1: Applications of Animal Biotechnology
RNA polymerase
4.4: Transcription of RNA
RNA primers in DNA replication
4.3: Replication of DNA
RNA structure
2.6: Nucleic Acids
RNA transcription, stages
4.4: Transcription of RNA
RNA types
4.4: Transcription of RNA

S

S phase
4.1: The Cell Cycle
Sanger sequencing of DNA
5.1: Genome Sequencing
scaffold materials
9.4: Regenerative Medicine
science
1.2: The Process of Science
scientific law
1.2: The Process of Science
scientific method
1.2: The Process of Science
scientific theory
1.2: The Process of Science
second generation biofuels
7.2: Industrial Applications of Microbial Biotechnology
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SYBR Green PCR
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T

Taq polymerase
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Taqman PCR
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S

Sample Word 1 | Sample Definition 1

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