BIOTECHNOLOGY IN SOCIETY



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Book Introduction

Biology is the study of life, but it is also the study of possibility.

Every day, discoveries in biology shape the world around us. Scientists use bacteria to produce medicines, edit genes to treat inherited diseases, engineer crops to feed growing populations, identify pathogens during disease outbreaks, and develop personalized therapies for cancer. Technologies that once seemed like science fiction are becoming part of everyday life, raising exciting opportunities as well as important ethical and social questions.

To understand these innovations, it isn't enough to simply know *what* happens inside living organisms. We must also understand *why* biological systems function the way they do and *how* scientists use that knowledge to solve real-world problems.

This textbook was written with that goal in mind.

Whether you plan to pursue a career in healthcare, biotechnology, education, business, public policy, or another field entirely, biology has become an essential part of scientific literacy. Decisions about vaccines, genetic testing, artificial intelligence in medicine, sustainable agriculture, conservation, forensic science, and emerging medical treatments increasingly require an understanding of biological principles. The more you understand biology, the better prepared you will be to evaluate new discoveries, interpret scientific claims, and make informed decisions as both a professional and a citizen.

Rather than presenting biology as an endless list of vocabulary words and disconnected facts, this book focuses on helping you develop a framework for understanding how living systems function. Throughout the chapters, you'll encounter recurring themes that connect many areas of biology, including the relationship between structure and function, the flow of biological information from DNA to proteins, the transformation of energy within cells, and the importance of regulation in maintaining healthy cellular processes. Together, these concepts provide the foundation for understanding both the remarkable complexity of living organisms and the biotechnologies that are transforming our world.

One of the central ideas you'll encounter is that life depends on information. DNA stores that information, cells interpret it, proteins carry it out, and complex regulatory systems ensure that everything happens at the right place and the right time. From this perspective, many diseases, including cancer, can be understood as disruptions in the normal flow and regulation of biological information. Likewise, many modern biotechnologies work because scientists have learned how to observe, manipulate, or restore these biological processes.

Throughout the book, you'll also discover that biology rarely offers simple answers. Biological systems are incredibly complex, and many scientific breakthroughs come with important trade-offs. Gene editing holds tremendous promise for treating disease, but it also raises questions about fairness, safety, and the limits of human intervention. Advances in reproductive technologies, synthetic biology, artificial organs, and genetic engineering challenge us to think carefully about not only what science *can* do, but also what it *should* do.

For this reason, you'll occasionally be invited to consider the ethical, social, environmental, and economic implications of the technologies you encounter. These discussions are not intended to tell you what to think. Instead, they are designed to encourage thoughtful, evidence-based decision making and to help you appreciate that science exists within a broader social context.

As you work through the chapters, you'll notice another emphasis: biology is not just about memorizing answers it is about asking better questions. Scientists continually observe patterns, gather evidence, test ideas, revise explanations, and build new understanding. This process of inquiry has transformed our understanding of life and continues to drive innovation in medicine, agriculture, environmental science, and biotechnology.

For that reason, many of the activities, examples, and case studies in this textbook encourage you to think like a scientist. Rather than simply recalling information, you'll practice interpreting evidence, making predictions, solving problems, and applying biological concepts to situations that resemble the kinds of challenges scientists, healthcare professionals, and biotechnology researchers face every day.

Above all, I hope this book helps you develop an appreciation for the remarkable complexity and elegance of living systems. Every organism, from a single bacterial cell to the human body, is the product of billions of years of evolution, operating through countless molecular interactions that occur every second of every day. Understanding even a small part of that complexity provides insight into ourselves, the natural world, and the technologies that are shaping our future.

Whether this is your first biology course or a stepping stone toward a future career, I hope you approach each chapter with curiosity. Ask questions. Make connections. Challenge assumptions. Biology is one of the fastest-moving and most influential scientific disciplines of our time, and the concepts you learn here will help you better understand not only living organisms, but also many of the scientific advances that will define the decades ahead.

Welcome to the study of life and to the exciting world of biotechnology. I hope this journey leaves you with more than knowledge. I hope it leaves you with curiosity, confidence, and a deeper appreciation for the remarkable biology that connects us all.

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1: SCIENCE AND TECHNOLOGY

1.1: Nature of Science
1.2: Scientific Method
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1.4: Scientific Ethics
1.5: Science and Technology

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1.1: Nature of Science

Learning Objectives

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

- Identify the shared characteristics of the natural sciences

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 1.1.1: 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 (**Figure** 1.1.1). 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** 1.1.2). 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.

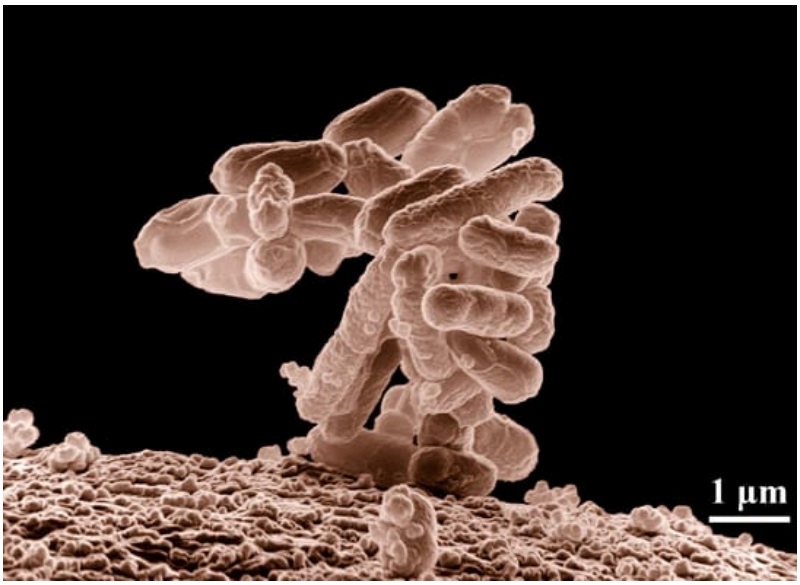


Figure 1.1.2: Biologists may choose to study *Escherichia coli* (*E. coli*), a bacterium that is a normal resident of our digestive tracts but which is also sometimes responsible for disease outbreaks. In this micrograph, the bacterium is visualized using a scanning electron microscope and digital colorization. (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** 1.1.3). 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.

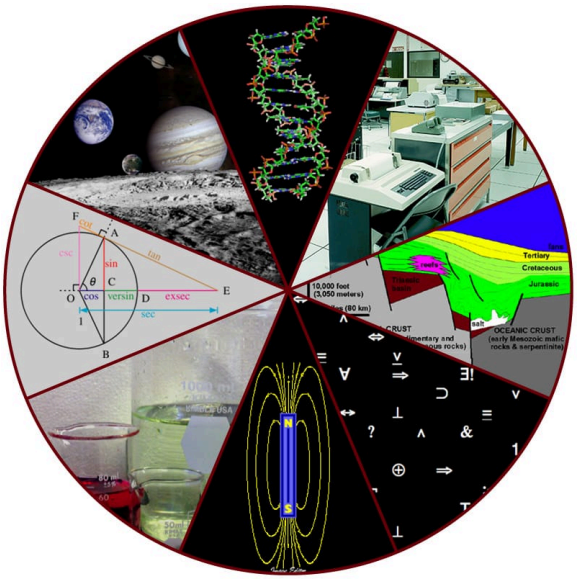


Figure 1.1.3: Some fields of science include astronomy, biology, computer science, geology, physics, chemistry, and mathematics. (credit: "Image Editor"/Flickr)

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.

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.

Key Terms

descriptive science
: a form of science that aims to observe, explore, and find things out

life science
: a field of science, such as biology, that studies living things

natural science
: a field of science that studies the physical world, its phenomena, and processes

physical science
: a field of science, such as astronomy, physics, and chemistry, that studies nonliving matter

science
: knowledge that covers general truths or the operation of general laws, especially when acquired and tested by the scientific method

scientific law
: a description, often in the form of a mathematical formula, for the behavior of some aspect of nature under certain specific conditions

scientific method
: a logical, organized process used to ask questions and find evidence-based answers about the natural world. It typically involves making observations, forming a hypothesis, testing it through experiments, analyzing results, and

drawing conclusions.

scientific theory
a thoroughly tested and confirmed explanation for observations or phenomena

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1.2: Scientific Method

Learning Objectives

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

- Summarize the steps of the scientific method
- Compare inductive reasoning with deductive reasoning

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** 1.2.1), 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 1.2.1: 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** 1.2.2). Using the scientific method, the hypotheses that are inconsistent with experimental data are rejected.

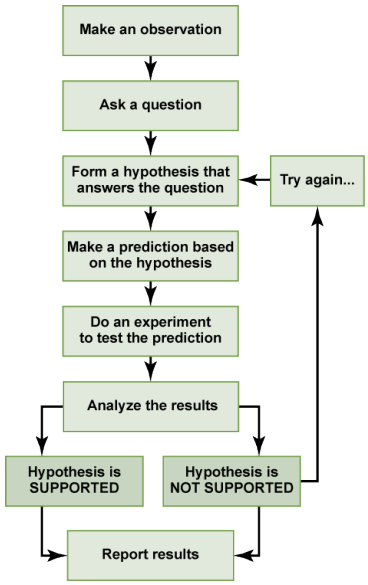


Figure 1.2.2: The scientific method is a series of defined steps that include experiments and careful observation. If a hypothesis is not supported by data, a new hypothesis can be proposed. The flow chart shows the steps in the scientific method. In step 1, an observation is made. In step 2, a question is asked about the observation. In step 3, an answer to the question, called a hypothesis, is proposed. In step 4, a prediction is made based on the hypothesis. In step 5, an experiment is done to test the prediction. In step 6, the results are analyzed to determine whether or not the hypothesis is supported. If the hypothesis is not supported, another hypothesis is made. In either case, the results are reported.

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.

Summary

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.

Key Terms

control
: a part of an experiment that does not change during the experiment

deductive reasoning
: a form of logical thinking that uses a general statement to forecast specific results

falsifiable
: able to be disproved by experimental results

hypothesis
: a suggested explanation for an event, which can be tested

hypothesis-based science
: a form of science that begins with a specific explanation that is then tested

inductive reasoning
: a form of logical thinking that uses related observations to arrive at a general conclusion

prediction
: a statement about what you expect will happen in an experiment based on your hypothesis

scientific method
: a method of research with defined steps that include experiments and careful observation

variable
: a part of an experiment that can vary or change

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1.3: Basic and Applied Science

Learning Objectives

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

- Describe the goals of basic science and applied science
- Evaluate the importance of communication in science
- Analyze ethical and practical responsibilities in scientific work

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** 1.3.1) 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.

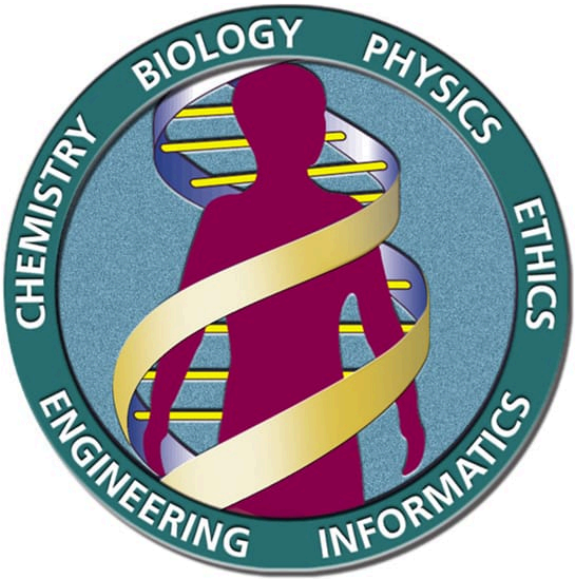


Figure 1.3.1: The Human Genome Project was a 13-year collaborative effort among researchers working in several different fields of science. The project was completed in 2003. (credit: the U.S. Department of Energy Genome Programs)

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

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.

Scientists share their research mainly through peer-reviewed journal articles to ensure accuracy, credibility, and reproducibility. Peer review by experts helps validate the quality of the work. Non-reviewed sources are less reliable and should be used with caution.

Key Terms

applied science
: a form of science that solves real-world problems

basic science
: science that seeks to expand knowledge regardless of the short-term application of that knowledge

peer-reviewed article
: a scientific report that is reviewed by a scientist's colleagues before publication

technology
: tools, methods, or processes created to solve problems or accomplish tasks

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1.4: Scientific Ethics

Learning Objectives

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

- Understand the role of ethics in scientific research
- Evaluate the ethical implications of emerging technologies
- Apply ethical principles to scientific scenarios

Scientific Ethics

Scientists must ensure that their efforts do not cause undue damage to humans, animals, or the environment. They also must ensure that their research and communications are free of bias and that they properly balance financial, legal, safety, replicability, and other considerations. All scientists -- and many people in other fields -- have these ethical obligations, but those in the life sciences have a particular obligation because their research may involve people or other living things. Bioethics is thus an important and continually evolving field, in which researchers collaborate with other thinkers and organizations. They work to define guidelines for current practice, and also continually consider new developments and emerging technologies in order to form answers for the years and decades to come.

For example, bioethicists may examine the implications of gene editing technologies, including the ability to create organisms that may displace others in the environment, as well as the ability to "design" human beings. In that effort, ethicists will likely seek to balance the positive outcomes -- such as improved therapies or prevention of certain illnesses -- with negative outcomes.

Unfortunately, the emergence of bioethics as a field came after a number of clearly unethical practices, where biologists did not treat research subjects with dignity and in some cases did them harm. In the 1932 Tuskegee syphilis study, 399 African American men were diagnosed with syphilis but were never informed that they had the disease, leaving them to live with and pass on the illness to others. Doctors even withheld proven medications because the goal of the study was to understand the impact of untreated syphilis on Black men.

While the decisions made in the Tuskegee study are unjustifiable, some decisions are genuinely difficult to make. Bioethicists work to establish moral and dignifying approaches before such decisions come to pass. For example, doctors rely on artificial intelligence and robotics for medical diagnosis and treatment; in the near future, even more responsibility will lie with machines. Who will be responsible for medical decisions? Who will explain to families if a procedure doesn't go as planned? And, since such treatments will likely be expensive, who will decide who has access to them and who does not? These are all questions bioethicists seek to answer, and are the types of considerations that all scientific researchers take into account when designing and conducting studies.

Bioethics are not simple, and often leave scientists balancing benefits with harm. In this text and course, you will discuss medical discoveries, vaccines, and research that, at their core, have an ethical complexity or, in the view of many, an ethical lapse. In 1951, Henrietta Lacks, a 30-year-old African American woman, was diagnosed with cervical cancer at Johns Hopkins Hospital. Unique characteristics of her illnesses gave her cells the ability to divide continuously, essentially making them "immortal." Without her knowledge or permission, researchers took samples of her cells and with them created the immortal HeLa cell line. These cells have contributed to major medical discoveries, including the polio vaccine. Many researchers mentioned in subsequent sections of the text relied on HeLa cell research as at least a component of their work related to cancer, AIDS, cell aging, and even very recently in COVID-19 research.

Today, harvesting tissue or organs from a dying patient without consent is not only considered unethical but illegal, regardless of whether such an act could save other patients' lives. Is it ethical, then, for scientists to continue to use Lacks's tissues for research, even though they were obtained illegally by today's standards? Should Lacks be mentioned as a contributor to the research based on her cells, and should she be cited in the several Nobel Prizes that have been awarded through such work? Finally, should medical companies be obligated to pay Lacks' family (which had financial difficulties) a portion of the billions of dollars in revenue earned through medicines that benefited from HeLa cell research? How would Henrietta Lacks feel about this? Because she was never asked, we will never know.

To avoid such situations, the role of ethics in scientific research is to ask such questions before, during, and after research or practice takes place, as well as to adhere to established professional principles and consider the dignity and safety of all organisms involved or affected by the work.

Key Terms

bioethics
: the study of ethical questions that arise in biology, medicine, and biotechnology

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1.5: Science and Technology

Learning Objectives

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

- Evaluate the ethical implications of emerging technologies
- Analyze societal and ethical challenges related to adopting new technologies
- Develop informed opinions about how science and technology shape the modern world

Science and Technology

Understanding Technology and Its Impact: A Foundation for Biotechnology

When most people hear the word *technology*, they picture cutting-edge tools like smartphones, drones, artificial intelligence, or CRISPR. These devices dominate headlines and are often seen as symbols of progress. But this narrow view overlooks the broader and more accurate definition of technology: any application of knowledge used to modify or control the natural environment to meet human needs. This includes both high-tech innovations and everyday tools like cooking pots, bandages, or eyeglasses.

Technology spans thousands of years—from ancient agricultural implements to today's robotic surgical systems. It reflects human creativity and our constant efforts to solve problems, improve quality of life, and manage the world around us. This expansive understanding is especially important when studying biotechnology, a field that blends biology with technology to create powerful tools that impact health, agriculture, the environment, and more.

The Relationship Between Science and Technology

Science and technology are deeply interconnected but distinct. Science is a method of inquiry—a systematic approach to understanding the natural world through observation, experimentation, and reasoning. It seeks to answer questions like "How does this work?" and "Why does this happen?" In contrast, technology applies scientific knowledge to solve practical problems and build tools or systems that improve human life.

While modern technology often builds on scientific discoveries, not all technological advances have emerged from a deep understanding of science. Some were developed through trial and error, long before the underlying biological or physical principles were known. For instance, early fermentation techniques used to make products like hard cider or sourdough bread were discovered by observation and passed down through generations. People harnessed microbial activity without knowing what microbes were or how they functioned.

As scientific understanding has advanced, so has our ability to design technologies intentionally. This shift—from accidental discovery to deliberate design—is a hallmark of modern innovation. Today, scientists can manipulate DNA sequences, engineer microbial systems, and develop targeted therapies with precision that was unimaginable in earlier times.

Importantly, the relationship between science and technology is not one-directional. Sometimes, science drives technology. For example, the discovery of DNA's structure paved the way for genetic testing, gene therapy, and biotechnology-based forensics. Other times, technology drives science. Tools like the electron microscope and gene sequencers have opened entirely new fields of inquiry, allowing researchers to study previously invisible biological phenomena.

This reciprocal relationship creates a feedback loop: scientific discoveries enable new technologies, and those technologies, in turn, expand scientific knowledge. Biotechnology exemplifies this dynamic. Technologies such as CRISPR-Cas9, synthetic biology platforms, and high-throughput drug screening are both products of scientific inquiry and tools that accelerate further research.

But the impact of science and technology extends far beyond the lab. Together, they shape society in profound ways. Advances in medicine have increased life expectancy and improved quality of life. Innovations in agriculture have helped feed growing populations. Developments in communication and transportation have reshaped how people connect across the globe.

However, these benefits also bring critical societal questions: Who gets access to these technologies? What risks are acceptable? How can we ensure innovations promote equity, safety, and sustainability?

These aren't abstract concerns. They are central to the field of biotechnology, which touches on everything from public health to environmental management to global ethics. Throughout this course, we will explore biotechnology not only as a set of tools and discoveries but as a force that reflects—and shapes—the values and structures of society.

What Is Biotechnology?

Biotechnology is the use of living systems, organisms, or their components to develop products and technologies that improve human life. It brings together biology and technology to solve problems across medicine, agriculture, environmental science, and industry.

Traditional biotechnology dates back thousands of years. Ancient farmers selectively bred plants and animals for desired traits, and early cultures used fermentation to create bread, cheese, and alcohol. These practices were based on generations of observation and experimentation—often without understanding the biological mechanisms involved.

Modern biotechnology, in contrast, builds on detailed knowledge of molecular and cellular biology. Techniques such as genetic engineering, recombinant DNA, CRISPR-Cas9 gene editing, and synthetic biology allow scientists to make precise changes at the genetic level. This has led to innovations like drought-resistant crops, personalized medicine, lab-grown organs, and even efforts to bring back extinct species.

What sets modern biotechnology apart is not just its sophistication, but the intentional design behind it. Scientists can now engineer biological systems from the ground up or target specific genes for modification—transforming biology from something we observe to something we build.

As we explore this field, we'll see how scientific principles—particularly those from biology, chemistry, and genetics—form the foundation for technological innovation. Throughout this course, we'll examine how deepening scientific knowledge fuels the development of new tools, and how those tools, in turn, open up new avenues of discovery.

The Challenges of Technology Adoption

Even when a technology is developed and proven to work, it doesn't always succeed in becoming widely adopted. There are many factors that can influence whether a technology is embraced by society. One of the classic examples of a technology that failed to live up to its initial promise is the Bell Jet Pack. Demonstrated at fairs in the 1960s, this personal flying device fascinated audiences and sparked the imagination of many. However, despite the excitement, the jet pack was never mass-produced due to various engineering challenges and practical limitations. The technology was simply not ready for widespread use, and the dream of personal flight remains unfulfilled, despite ongoing research.

There are several reasons why technologies may fail to penetrate the market or become mainstream:

- Engineering obstacles, such as technical limitations or safety concerns.
- Economic issues, such as high costs of development or production.
- Ethical concerns, such as fears about safety or unintended consequences.
- Lack of demand, where society may not be ready or willing to embrace a particular innovation.

While the personal jet pack example highlights the gap between innovation and real-world usability, more relevant to today's world—and this course—are the challenges faced by *biotechnologies* attempting to enter mainstream use.

Take *cultured meat*—meat grown from animal cells in a lab. It promises to reduce environmental impact, eliminate animal slaughter, and improve food safety. Yet, despite scientific breakthroughs, it faces hurdles: high production costs, regulatory delays, and public skepticism. As of now, it remains a niche product rather than a mainstream protein source.

Other examples include:

- RNA-based vaccines (like those used for COVID-19) initially faced hesitation from the public, despite years of research backing their safety.
- Gene editing in humans is technically possible, but concerns over ethics, consent, and unintended consequences have

slowed its application.
- Biodegradable plastics and enzyme-based recycling technologies could transform waste management but are struggling with scale-up and market adoption.

These cases show that science alone isn't enough. A technology's success depends on public trust, economic feasibility, cultural acceptance, and effective communication. In this course, we'll explore how these factors intersect, particularly in biotechnology.

Evaluating the Impact of Biotechnology

Every new biotechnology brings both benefits and risks. Vaccines and gene therapies have revolutionized medicine. Genetically modified crops have improved yields and resource efficiency. Biomedical devices like pacemakers and insulin pumps have enhanced millions of lives. At the same time, technologies like GMOs or gene editing prompt debate over safety, ethics, and environmental impact.

Consider the following biotechnologies:

- Vaccines and immunizations – Preventing infectious diseases through engineered immune responses.
- Genetically Modified Organisms (GMOs) – Enhancing crop yield, resistance to pests, and nutrient content.
- Regenerative medicine – Using stem cells to repair or replace damaged tissues and organs.
- Gene therapy and editing – Treating genetic diseases through targeted DNA modification (e.g., CRISPR).
- Lab-grown meat (cultured meat) – Producing animal protein without raising and slaughtering livestock.
- Bioremediation – Using microbes or plants to clean up environmental pollutants.
- Synthetic biology – Designing and constructing new biological systems for applications in health, energy, and industry.
- Reproductive technologies – From in-vitro fertilization (IVF) to potential gene-edited embryos.
- Bio-based plastics and enzyme recycling – Reducing petroleum use and improving waste management.
- Drug discovery and screening – Using biotechnology tools to accelerate pharmaceutical development.

As students, you'll be encouraged to think critically about these technologies. This involves not only weighing their scientific merit but also understanding their broader implications—ethical, economic, and cultural. You'll learn how to evaluate technologies using multiple lenses and draw informed conclusions about their value and risks.

Scientific Literacy and the Pace of Innovation

Scientific and technological change is accelerating. Just in the past few decades, we've seen transformative advances in gene editing, artificial intelligence, renewable energy, and nanotechnology. Keeping up with this pace is a challenge—not just for scientists, but for society as a whole.

That's why scientific literacy is essential. It goes beyond memorizing facts. It's about understanding how science works, how to evaluate evidence, and how to think critically about complex problems. In a world shaped by biotechnology, a scientifically literate public is better equipped to engage in important debates—from vaccine policy to climate change to the ethics of gene editing.

For example, during the COVID-19 pandemic, understanding viral spread, immunity, and vaccine development helped many people make informed health decisions. Similarly, knowledge of genetics empowers individuals to interpret DNA test results, understand their medical risks, or make reproductive choices.

Throughout this course, we'll return to core scientific principles—like the scientific method and molecular biology—to build your capacity for critical analysis. This foundation will help you understand today's technologies and thoughtfully evaluate the choices they present.

Summary

Science and technology are in constant dialogue: science explains how the world works, and technology uses that knowledge to shape the world. Biotechnology embodies this relationship, translating discoveries in biology into practical tools that touch every aspect of our lives.

Yet technological progress alone is not enough. Understanding the science behind these innovations—and their social, ethical, and environmental implications—is key to making responsible decisions in a rapidly changing world.

Scientific literacy empowers us to make informed choices, evaluate innovations critically, and engage in meaningful dialogue about how science and technology should shape our future. This course will prepare you to think critically about biotechnology's potential, its impact, and your role in shaping its future.

Key Terms

biotechnology
: the use of living organisms or their molecules to develop products or improve processes

bioremediation
: using organisms - often bacteria - to clean up pollutants from the environment

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2: CHEMISTRY OF LIFE - CHARACTERISTICS OF LIFE

2.1: Properties of Life

2.2: Levels of Organization of Living Things

2.3: Comparing Prokaryotic and Eukaryotic Cells

2.4: Eukaryotic Cells

2.5: The Building Blocks of Molecules

2.6: Chemical Bonds

2.7: Water

2.8: Chemistry of Life (Exercises)

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2.1: Properties of Life

Learning Objectives

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

- Recognize the core questions biologists ask and explain why defining life is not always straightforward.
- Identify the shared characteristics of living organisms that biologists use to define life.
- Explain how living organisms maintain organization, respond to their environment, and process energy to survive and reproduce.
- Summarize how regulation, homeostasis, growth, and adaptation allow organisms to function across changing environments.

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** 2.1.1 will be discussed later.



Figure 2.1.1: 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** 2.1.2). 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 2.1.2: 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** 2.1.3) will grow up to exhibit many of the same characteristics as its parents.



Figure 2.1.3: Although no two look alike, these kittens have inherited genes from both parents and share many of the same characteristics. One kitten has an orange and white tabby coat, another is entirely black, the third and fourth have a black, white and orange tabby coat but with different patterning. (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** 2.1.4), 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 2.1.4: 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** 2.1.5) 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 2.1.5: 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)

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.

Key Terms

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

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

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

natural selection
: the process by which individuals with traits better suited to their environment are more likely to survive and reproduce

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2.2: Levels of Organization of Living Things

Learning Objectives

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

- Trace the levels of biological organization, from atoms and molecules to organisms, ecosystems, and the biosphere.
- Distinguish among cells, tissues, organs, and organ systems, and explain how increasing complexity supports life.
- Classify living organisms using the taxonomic hierarchy, including domains, kingdoms, and binomial names.
- Describe how evolution and scientific evidence shape our understanding of life's diversity, including the use of phylogenetic trees and modern classification systems.

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** 2.2.1), which contains the instructions for the functioning of the organism that contains it.

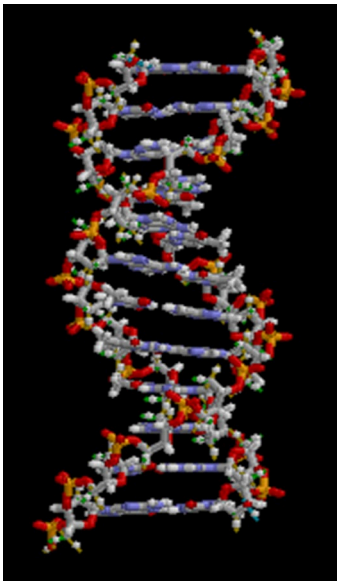


Figure 2.2.1: A molecule, like this large DNA molecule, is composed of atoms. (credit: "Brian0918"/Wikimedia Commons)

CONCEPT IN ACTION



Click to see an animation of this DNA molecule.

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 cell itself 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

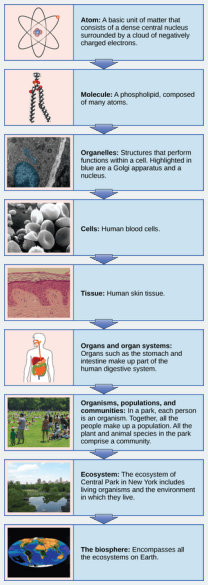


Figure 2.2.2: From an atom to the entire Earth, biology examines all aspects of life. A flow chart shows the hierarchy of living organisms. From smallest to largest, this hierarchy includes: 1 An atom, with protons, neutrons and electrons. 2 Molecules such as the phospholipid shown, made up of atoms. 3 Organelles, such as Golgi apparatus and nuclei, that exist inside cells. 4 Cells, such as a red blood cell. 5 Tissues, such as human skin tissue. 6 Organs such as the stomach and intestine make up the human digestive system, an example of an organ system. 7 Organisms, populations and communities. In a park, each person is an organism. Together, all the people make up a population. All the plant and animal species in the park comprise a community. 8 Ecosystems: The ecosystem of Central Park in New York includes living organisms and the environment in which they live. 9 The biosphere: encompasses all the ecosystems on Earth. (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.

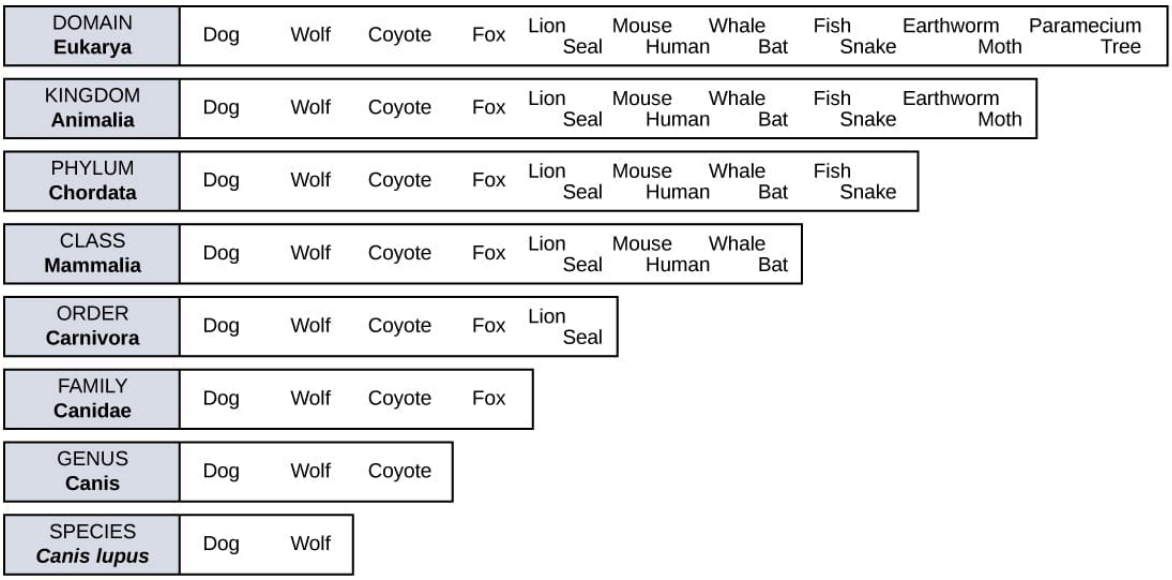
All the individuals of a species living within a specific area are collectively called a population. For example, a forest may include many white pine trees. All of these pine trees represent the population of white pine trees in this forest. Different populations may live in the same specific area. 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** 2.2.2), 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** 2.2.3).



Click to open image description The image is a horizontal, step-like diagram illustrating the taxonomic classification of a dog from the broadest category to the most specific. Each level is shown as a labeled bar stacked vertically, with bars becoming progressively shorter from top to bottom to represent increasing specificity. Within each bar, the names of different organisms are listed to show which ones belong to that classification level. At the top, the widest bar is labeled “DOMAIN: Eukarya.” This bar includes a wide range of organisms: dog, wolf, coyote, fox, lion, seal, mouse, human, whale, bat, fish, snake, earthworm, moth, and paramecium. This level represents the broadest grouping, including all organisms with complex cells. Below it, a slightly narrower bar is labeled “KINGDOM: Animalia.” The list here includes dog, wolf, coyote, fox, lion, seal, mouse, human, whale, bat, fish, snake, earthworm, and moth. The paramecium is no longer included, indicating it does not belong to this kingdom. The next bar down is labeled “PHYLUM: Chordata.” This bar includes dog, wolf, coyote, fox, lion, seal, mouse, human, whale, bat, fish, and snake. The earthworm and moth have been removed at this level. Below that is “CLASS: Mammalia.” This bar is shorter and includes dog, wolf, coyote, fox, lion, seal, mouse, human, whale, and bat. At this level, fish and snake are no longer included. The next level is “ORDER: Carnivora.” This bar narrows further and lists dog, wolf, coyote, fox, lion, and seal. Only meat-eating mammals remain. Below it is “FAMILY: Canidae.” This bar contains dog, wolf, coyote, and fox, indicating these animals are more closely related to each other than to the others listed above. Next is “GENUS: Canis.” This bar is shorter still and includes dog, wolf, and coyote, showing an even closer relationship. Finally, the most specific and narrowest bar at the bottom is labeled “SPECIES: Canis lupus.” This bar includes dog and wolf, indicating that domestic dogs and wolves are classified within the same species in this diagram. Overall, the diagram visually demonstrates how classification becomes more exclusive at each level, with fewer organisms included as the categories move from domain to species.

Figure 2.2.3: 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** 2.2.4). 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*.

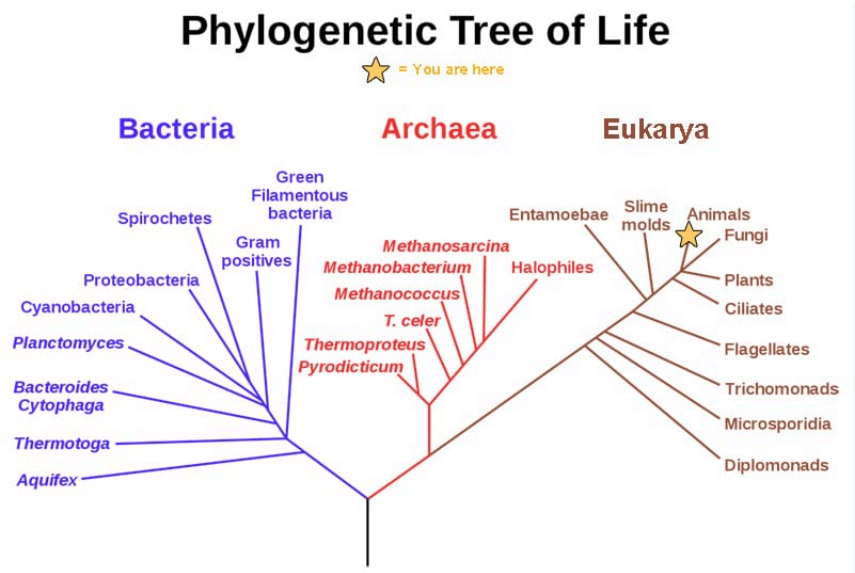


Figure 2.2.4: These images represent different domains. The scanning electron micrograph shows (a) bacterial cells belong to the domain Bacteria, while the (b) extremophiles, seen all together as colored mats in this hot spring, belong to domain Archaea. Both the (c) sunflower and (d) lion are part of 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** 2.2.5). 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.



Click to open image descriptionThe image is a labeled diagram titled “Phylogenetic Tree of Life.” It shows a branching tree structure that represents evolutionary relationships among living organisms based on genetic data. The tree begins at a single point near the bottom center, representing a common ancestor, and splits upward into three major branches corresponding to the domains Bacteria, Archaea, and Eukarya. The left branch, colored blue and labeled “Bacteria,” spreads outward into multiple sub-branches. These branches are labeled with different bacterial groups, including Proteobacteria, Cyanobacteria, Spirochetes, Gram positives, Green filamentous bacteria, Planctomyces, Bacteroides/Cytophaga, Thermotoga, and Aquifex. The branching pattern shows increasing diversity as the lines extend outward from the central trunk. The middle branch, colored red and labeled “Archaea,” has fewer but distinct branches. These include groups such as Methanosarcina, Methanobacterium, Methanococcus, Thermoproteus, Pyrodicticum, T. celer, and Halophiles. These names are clustered on short branching lines that extend from the central archaeal branch. The right branch, colored brown and labeled “Eukarya,” shows a variety of more complex organisms. Branches include Animals, Fungi, Plants, Ciliates, Flagellates, Trichomonads, Microsporidia, Diplomonads, Slime molds, and Entamoebae. These groups are shown diverging from one another along the eukaryotic branch. Two gold star icons labeled “You are here” appear near the top of the diagram: one near the Animals branch within Eukarya and another near the top center, emphasizing the human perspective within this tree. The diagram visually communicates that all life shares a common origin and has diverged into three major domains over time, with Bacteria and Archaea representing prokaryotic life (organisms without a nucleus), and Eukarya representing organisms with more complex cell structures.

Figure 2.2.5: 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 2.2.6: 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** 2.2.6). 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** 2.2.7) 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 2.2.7: 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

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.

Key Terms

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

biosphere
a collection of all ecosystems on Earth

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

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

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Answers

ART CONNECTION

B

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2.3: Comparing Prokaryotic and Eukaryotic Cells

Learning Objectives

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

- Differentiate between prokaryotic and eukaryotic cells based on structure, size, and the presence of a nucleus and organelles.
- Identify the common components found in all cells and describe the key features unique to prokaryotic cells.
- Explain how cell structure supports cell function, including the idea that form follows function.
- Relate cell size to surface area and transport limitations, and explain why eukaryotic cells require internal compartmentalization.

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

Components of Prokaryotic Cells

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

A prokaryotic cell is a simple, single-celled (unicellular) organism that lacks a nucleus, or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is found in the central part of the cell: a darkened region called the nucleoid (**Figure** 2.3.1).

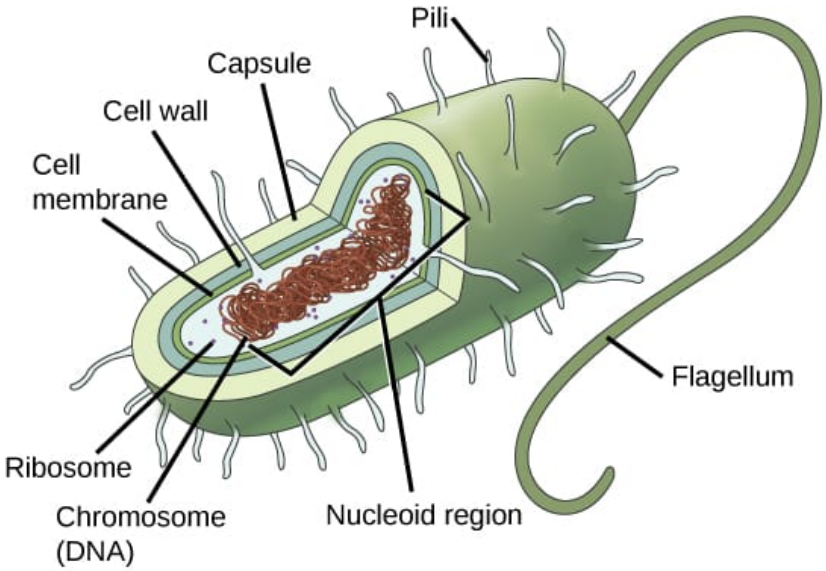


Figure 2.3.1: This figure shows the generalized structure of a prokaryotic cell. The prokaryotic cell has an oval shape. The circular chromosome is concentrated in a region called the nucleoid. The fluid inside the cell is called the cytoplasm. Ribosomes, depicted as small circles, float in the cytoplasm. The cytoplasm is encased in a plasma membrane, which in turn is encased by a cell wall. A capsule surrounds the cell wall. The bacterium depicted has a flagellum protruding from one narrow end. Pili are small protrusions that extend from the capsule in all directions.

Unlike Archaea and eukaryotes, bacteria have a cell wall made of peptidoglycan, comprised of sugars and amino acids, and

many have a polysaccharide capsule (**Figure** 2.3.1). The cell wall acts as an extra layer of protection, helps the cell maintain its shape, and prevents dehydration. The capsule enables the cell to attach to surfaces in its environment. Some prokaryotes have flagella, pili, or fimbriae. Flagella are used for locomotion, while most pili are used to exchange genetic material during a type of reproduction called conjugation.

Eukaryotic Cells

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

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

Cell Size

At 0.1–5.0 µm in diameter, prokaryotic cells are significantly smaller than eukaryotic cells, which have diameters ranging from 10–100 µm (**Figure** 2.3.2). The small size of prokaryotes allows ions and organic molecules that enter them to quickly spread to other parts of the cell. Similarly, any wastes produced within a prokaryotic cell can quickly move out. However, larger eukaryotic cells have evolved different structural adaptations to enhance cellular transport. Indeed, the large size of these cells would not be possible without these adaptations. In general, cell size is limited because volume increases much more quickly than does cell surface area. As a cell becomes larger, it becomes more and more difficult for the cell to acquire sufficient materials to support the processes inside the cell, because the relative size of the surface area across which materials must be transported declines.

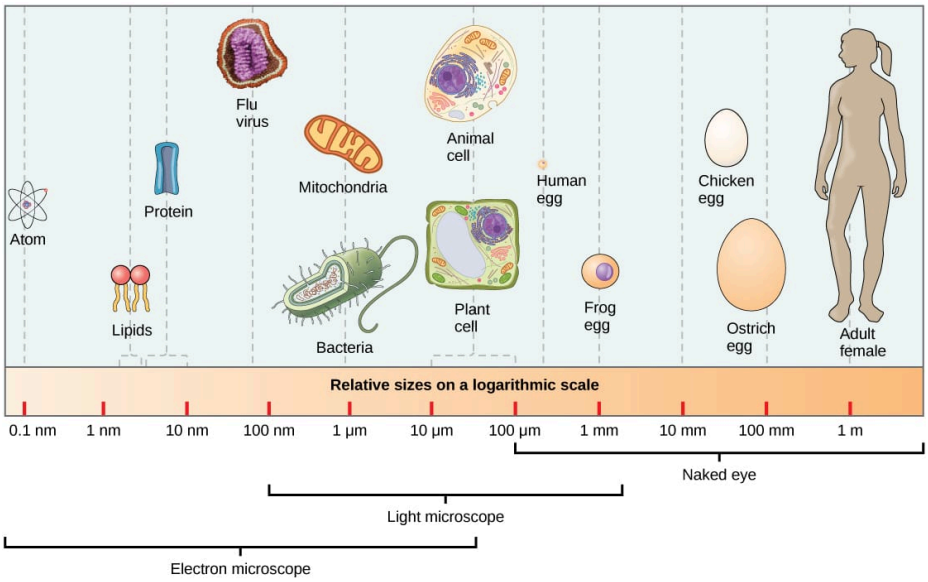


Figure 2.3.2: This figure shows the relative sizes of different kinds of cells and cellular components on a logarithmic scale. Relative sizes on a logarithmic scale, from 0.1 nm to 1 m, are shown. Objects are shown from smallest to largest. The smallest object shown, an atom, is about 1 nm in size. The next largest objects shown are lipids and proteins; these molecules are between 1 and 10 nm. Bacteria are about 100 nm, and mitochondria are about 1 µm. Plant and animal cells are both between 10 and 100 µm. A human egg is between 100 µm and 1 mm. A frog egg is about 1 mm. A chicken egg and an ostrich egg are both between 10 and 100 mm, but an ostrich egg is larger. For comparison, a human is approximately 1 m tall.

Summary

Prokaryotes are predominantly single-celled organisms of the domains Bacteria and Archaea. All prokaryotes have plasma

membranes, cytoplasm, ribosomes, a cell wall, DNA, and lack membrane-bound organelles. Many also have polysaccharide capsules. Prokaryotic cells range in diameter from 0.1–5.0 µm.

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

Key Terms

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

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

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

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2.4: Eukaryotic Cells

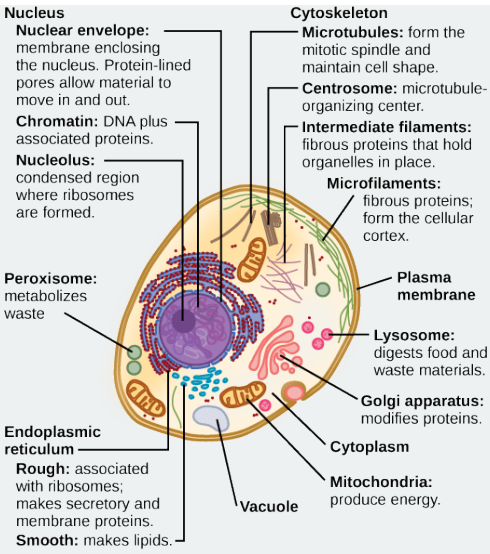
Learning Objectives

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

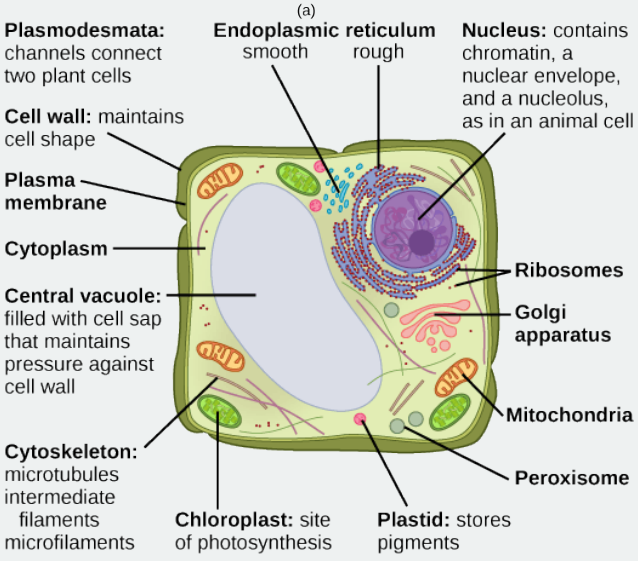
- Explain how eukaryotic cells are organized and why membrane-bound organelles allow many cellular processes to occur at the same time.
- Describe the structure and function of the plasma membrane, cytoplasm, and cytoskeleton, including how form supports function.
- Identify the major organelles of eukaryotic cells and summarize their primary roles in protein production, energy conversion, transport, and waste processing.
- Trace how proteins and lipids move through the endomembrane system, from synthesis to modification, packaging, and delivery.
- Compare animal and plant cells, identifying structures unique to each and explaining how those differences relate to their functions.
- Explain the origins and functions of mitochondria and chloroplasts, including the role of endosymbiosis in eukaryotic evolution.

At this point, it should be clear that eukaryotic cells have a more complex structure than do prokaryotic cells. Organelles allow for various functions to occur in the cell at the same time. Before discussing the functions of organelles within a eukaryotic cell, let us first examine two important components of the cell: the plasma membrane and the cytoplasm.

ART CONNECTION


Nucleus
Nuclear envelope: membrane enclosing the nucleus. Protein-lined pores allow material to move in and out.
Chromatin: DNA plus associated proteins.
Nucleolus: condensed region where ribosomes are formed.
Peroxisome: metabolizes waste
Endoplasmic reticulum
Rough: associated with ribosomes; makes secretory and membrane proteins.
Smooth: makes lipids.
Cytoskeleton
Microtubules: form the mitotic spindle and maintain cell shape.
Centrosome: microtubule-organizing center.
Intermediate filaments: fibrous proteins that hold organelles in place.
Microfilaments: fibrous proteins; form the cellular cortex.
Plasma membrane
Lysosome: digests food and waste materials.
Golgi apparatus: modifies proteins.
Cytoplasm
Mitochondria: produce energy.
Vacuole

(a)


Plasmodesmata: channels connect two plant cells
Endoplasmic reticulum
smooth
rough
Nucleus: contains chromatin, a nuclear envelope, and a nucleolus, as in an animal cell
Cell wall: maintains cell shape
Plasma membrane
Cytoplasm
Central vacuole: filled with cell sap that maintains pressure against cell wall
Ribosomes
Golgi apparatus
Mitochondria
Peroxisome
Cytoskeleton: microtubules intermediate filaments microfilaments
Chloroplast: site of photosynthesis
Plastid: stores pigments

(b)

Figure 2.4.1: This figure shows (a) a typical animal cell and (b) a typical plant cell.
Part a: This illustration shows a typical eukaryotic cell, which is egg shaped. The fluid inside the cell is called the cytoplasm, and the cell is surrounded by a cell membrane. The nucleus takes up about one-half of the width of the cell. Inside the nucleus is the chromatin, which is comprised of DNA and associated proteins. A region of the chromatin is condensed into the nucleolus, a structure in which ribosomes are synthesized. The nucleus is encased in a nuclear envelope, which is perforated by protein-lined pores that allow entry of material into the nucleus. The nucleus is surrounded by the rough and smooth endoplasmic reticulum, or ER. The smooth ER is the site of lipid synthesis. The rough ER has embedded ribosomes that give it a bumpy appearance. It synthesizes membrane and secretory proteins. Besides the ER, many other organelles float inside the cytoplasm. These include the Golgi apparatus, which modifies proteins and lipids synthesized in the ER. The Golgi apparatus is made of layers of flat membranes. Mitochondria, which produce energy for the cell, have an outer membrane and a highly folded inner membrane. Other, smaller organelles include peroxisomes that metabolize waste, lysosomes that digest food, and vacuoles. Ribosomes, responsible for protein synthesis, also float freely in the cytoplasm and are depicted as small dots. The last cellular component shown is the cytoskeleton, which has four different types of components: microfilaments, intermediate filaments, microtubules, and centrosomes. Microfilaments are fibrous proteins that line the cell membrane and make up the cellular cortex. Intermediate filaments are fibrous proteins that hold organelles in place. Microtubules form the mitotic spindle and maintain cell shape. Centrosomes are made of two tubular structures at right angles to one another. They form the microtubule-organizing center.
Part b: This illustration depicts a typical eukaryotic plant cell. The nucleus of a plant cell contains chromatin and a nucleolus, the same as in an animal cell. Other structures that a plant cell has in common with an animal cell include rough and smooth ER, the Golgi apparatus, mitochondria, peroxisomes, and ribosomes. The fluid inside the plant cell is called the cytoplasm, just as in an animal cell. The plant cell has three of the four cytoskeletal components found in animal cells: microtubules, intermediate filaments, and microfilaments. Plant cells do not have centrosomes. Plants have five structures not found in animals cells: plasmodesmata, chloroplasts, plastids, a central vacuole, and a cell wall. Plasmodesmata form channels between adjacent plant cells. Chloroplasts are responsible for photosynthesis; they have an outer membrane, an inner membrane, and stack of membranes inside the inner membrane. The central vacuole is a very large, fluid-filled structure that maintains pressure against the cell wall. Plastids store pigments. The cell wall is localized outside the cell membrane.

What structures does a plant cell have that an animal cell does not have? What structures does an animal cell have that a plant cell does not have?

The Plasma Membrane

Like prokaryotes, eukaryotic cells have a plasma membrane (**Figure** 2.4.2) made up of a phospholipid bilayer with embedded proteins that separates the internal contents of the cell from its surrounding environment. A phospholipid is a lipid molecule composed of two fatty acid chains, a glycerol backbone, and a phosphate group. The plasma membrane regulates the passage of some substances, such as organic molecules, ions, and water, preventing the passage of some to maintain internal conditions, while actively bringing in or removing others. Other compounds move passively across the membrane.

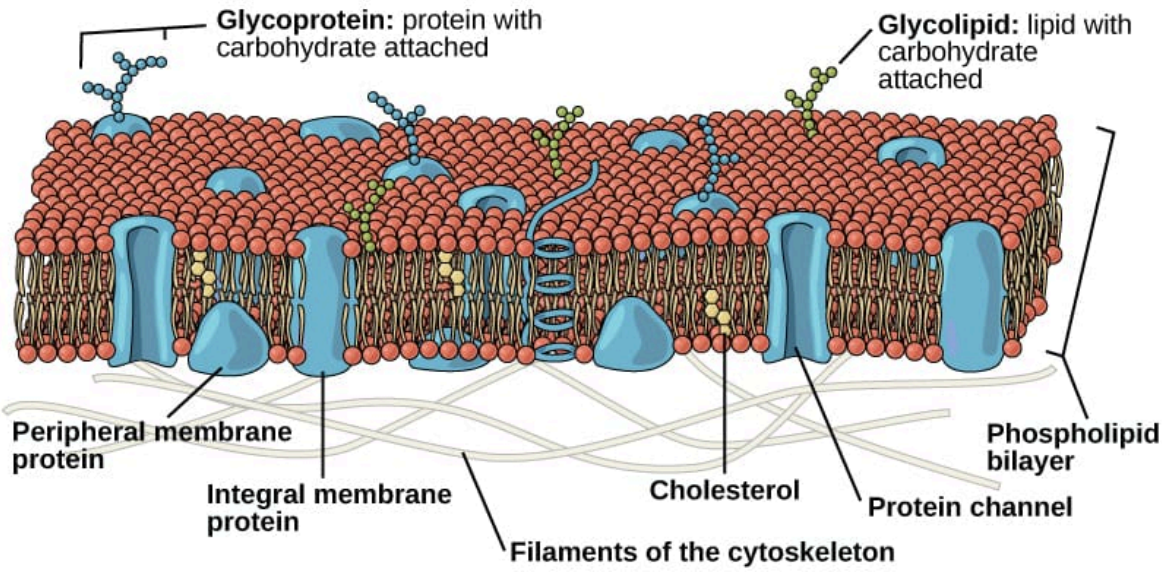


Figure 2.4.2: The plasma membrane is primarily made of a phospholipid bilayer with embedded proteins. Each phospholipid has a hydrophilic (water-attracting) head and two hydrophobic (water-repelling) tails. In the bilayer, the hydrophilic heads face the watery environments inside and outside the cell, while the hydrophobic tails point inward toward each other.
Proteins are found throughout the membrane. Integral membrane proteins extend through the entire bilayer, and some form channels with pores that allow specific substances to pass through. Peripheral membrane proteins are attached to the membrane surface, usually near the phospholipid head groups.
Other membrane components include cholesterol, which helps maintain membrane fluidity, and carbohydrates, which are attached to certain proteins and lipids on the outer surface of the membrane. The cytoskeleton forms a network of protein filaments along the inner surface of the membrane, helping support the cell's shape.

The plasma membranes of cells that specialize in absorption are folded into fingerlike projections called microvilli (singular = microvillus). This folding increases the surface area of the plasma membrane. Such cells are typically found lining the small intestine, the organ that absorbs nutrients from digested food. This is an excellent example of form matching the function of a structure.

People with celiac disease have an immune response to gluten, which is a protein found in wheat, barley, and rye. The immune response damages microvilli, and thus, afflicted individuals cannot absorb nutrients. This leads to malnutrition, cramping, and diarrhea. Patients suffering from celiac disease must follow a gluten-free diet.

The Cytoplasm

The cytoplasm comprises the contents of a cell between the plasma membrane and the nuclear envelope (a structure to be discussed shortly). It is made up of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals (**Figure** 2.4.1). Even though the cytoplasm consists of 70 to 80 percent water, it has a semi-solid consistency, which comes from the proteins within it. However, proteins are not the only organic molecules found in the cytoplasm. Glucose and other simple sugars, polysaccharides, amino acids, nucleic acids, fatty acids, and derivatives of glycerol are found there too. Ions of sodium, potassium, calcium, and many other elements are also dissolved in the cytoplasm. Many metabolic reactions, including protein synthesis, take place in the cytoplasm.

The Cytoskeleton

If you were to remove all the organelles from a cell, would the plasma membrane and the cytoplasm be the only components left? No. Within the cytoplasm, there would still be ions and organic molecules, plus a network of protein fibers that helps to maintain the shape of the cell, secures certain organelles in specific positions, allows cytoplasm and vesicles to move within the cell, and enables unicellular organisms to move independently. Collectively, this network of protein fibers is known as the cytoskeleton. There are three types of fibers within the cytoskeleton: microfilaments, also known as actin filaments, intermediate filaments, and microtubules (**Figure** 2.4.3).

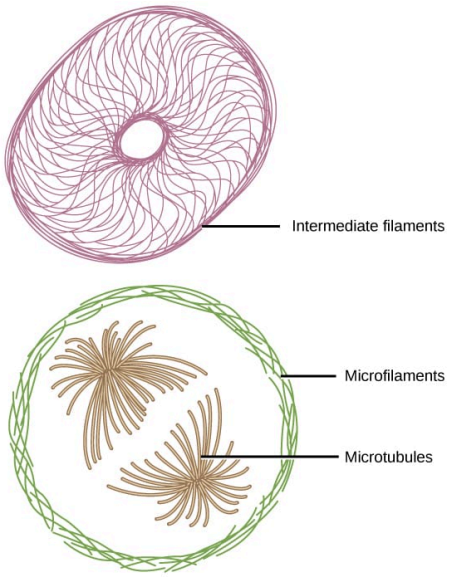


Figure 2.4.3: Microfilaments, intermediate filaments, and microtubules compose a cell's cytoskeleton. Microfilaments line the inside of the plasma membrane, whereas microfilaments radiate out from the center of the cell. Intermediate filaments form a network throughout the cell that holds organelles in place.

Microfilaments are the thinnest of the cytoskeletal fibers and function in moving cellular components, for example, during cell division. They also maintain the structure of microvilli, the extensive folding of the plasma membrane found in cells dedicated to absorption. These components are also common in muscle cells and are responsible for muscle cell contraction. Intermediate filaments are of intermediate diameter and have structural functions, such as maintaining the shape of the cell and anchoring organelles. Keratin, the compound that strengthens hair and nails, forms one type of intermediate filament. Microtubules are the thickest of the cytoskeletal fibers. These are hollow tubes that can dissolve and reform quickly. Microtubules guide organelle movement and are the structures that pull chromosomes to their poles during cell division. They are also the structural components of flagella and cilia. In cilia and flagella, the microtubules are organized as a circle of nine double microtubules on the outside and two microtubules in the center.

The centrosome is a region near the nucleus of animal cells that functions as a microtubule-organizing center. It contains a pair of centrioles, two structures that lie perpendicular to each other. Each centriole is a cylinder of nine triplets of microtubules.

The centrosome replicates itself before a cell divides, and the centrioles play a role in pulling the duplicated chromosomes to opposite ends of the dividing cell. However, the exact function of the centrioles in cell division is not clear, since cells that have the centrioles removed can still divide, and plant cells, which lack centrioles, are capable of cell division.

Flagella and Cilia

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

The Endomembrane System

The endomembrane system (*endo* = within) is a group of membranes and organelles (**Figure** 2.4.3) in eukaryotic cells that work together to modify, package, and transport lipids and proteins. It includes the nuclear envelope, lysosomes, and vesicles, the endoplasmic reticulum and Golgi apparatus, which we will cover shortly. Although not technically *within* the cell, the plasma membrane is included in the endomembrane system because, as you will see, it interacts with the other endomembranous organelles.

The Nucleus

Typically, the nucleus is the most prominent organelle in a cell (**Figure** 2.4.1). The nucleus (plural = nuclei) houses the cell's DNA in the form of chromatin and directs the synthesis of ribosomes and proteins. Let us look at it in more detail (**Figure** 2.4.4).

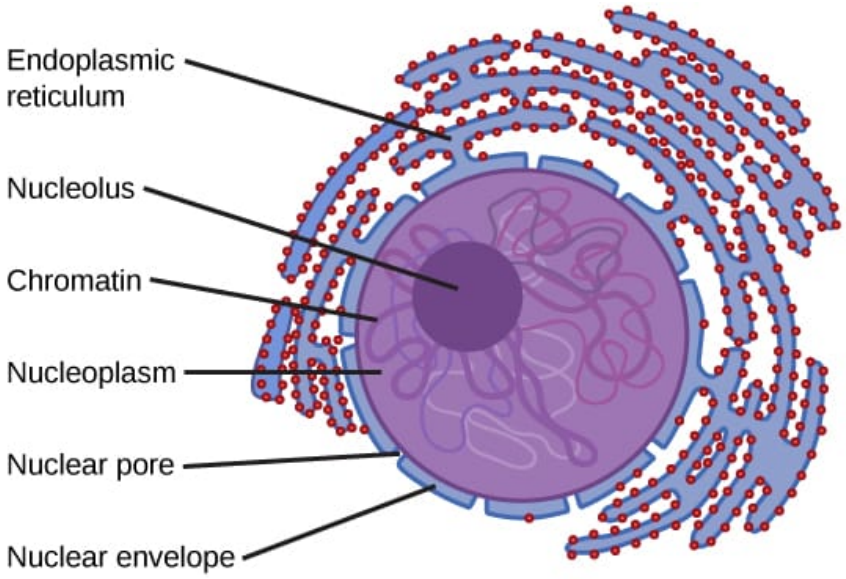


Figure 2.4.4: The outermost boundary of the nucleus is the nuclear envelope.
The nuclear envelope consists of two phospholipid bilayers (membranes)—an outer membrane and an inner membrane—in contrast to the plasma membrane (**Figure** 2.4.2), which consists of only one phospholipid bilayer. In this illustration, chromatin floats in the nucleoplasm. The nucleoid is depicted as a dense, circular region inside the nucleus. The double nuclear membrane is perforated with protein-lined pores. (credit: modification of work by NIGMS, NIH)

The nuclear envelope is a double-membrane structure that constitutes the outermost portion of the nucleus (**Figure** 2.4.4). Both the inner and outer membranes of the nuclear envelope are phospholipid bilayers.

The nuclear envelope is punctuated with pores that control the passage of ions, molecules, and RNA between the nucleoplasm and the cytoplasm.

To understand chromatin, it is helpful to first consider chromosomes. Chromosomes are structures within the nucleus that are made up of DNA, the hereditary material, and proteins. This combination of DNA and proteins is called chromatin. In eukaryotes, chromosomes are linear structures. Every species has a specific number of chromosomes in the nucleus of its body cells. For example, in humans, the chromosome number is 46, whereas in fruit flies, the chromosome number 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, the chromosomes resemble an unwound, jumbled bunch of threads.

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 through the nuclear pores into the cytoplasm.

The Endoplasmic Reticulum

The endoplasmic reticulum (ER) (**Figure** 2.4.7) is a series of interconnected membranous tubules that collectively modify proteins and synthesize lipids. However, these two functions are performed in separate areas of the endoplasmic reticulum: the rough endoplasmic reticulum and the smooth endoplasmic reticulum, respectively.

The hollow portion of the ER tubules is called the lumen or cisternal space. The membrane of the ER, which is a phospholipid bilayer embedded with proteins, is continuous with the nuclear envelope.

The rough endoplasmic reticulum (RER) is so named because the ribosomes attached to its cytoplasmic surface give it a studded appearance when viewed through an electron microscope.

The ribosomes synthesize proteins while attached to the ER, resulting in transfer of their newly synthesized proteins into the lumen of the RER where they undergo modifications such as folding or addition of sugars. The RER also makes phospholipids for cell membranes.

If the phospholipids or modified proteins are not destined to stay in the RER, they will be packaged within vesicles and transported from the RER by budding from the membrane (**Figure** 2.4.7). Since the RER is engaged in modifying proteins that will be secreted from the cell, it is abundant in cells that secrete proteins, such as the liver.

The smooth endoplasmic reticulum (SER) is continuous with the RER but has few or no ribosomes on its cytoplasmic surface (see **Figure** 2.4.1). The SER's functions include synthesis of carbohydrates, lipids (including phospholipids), and steroid hormones; detoxification of medications and poisons; alcohol metabolism; and storage of calcium ions.

The Golgi Apparatus

We have already mentioned that vesicles can bud from the ER, but where do the vesicles go? Before reaching their final destination, the lipids or proteins within the transport vesicles need to be sorted, packaged, and tagged so that they wind up in the right place. The sorting, tagging, packaging, and distribution of lipids and proteins take place in the Golgi apparatus (also called the Golgi body), a series of flattened membranous sacs (**Figure** 2.4.5).

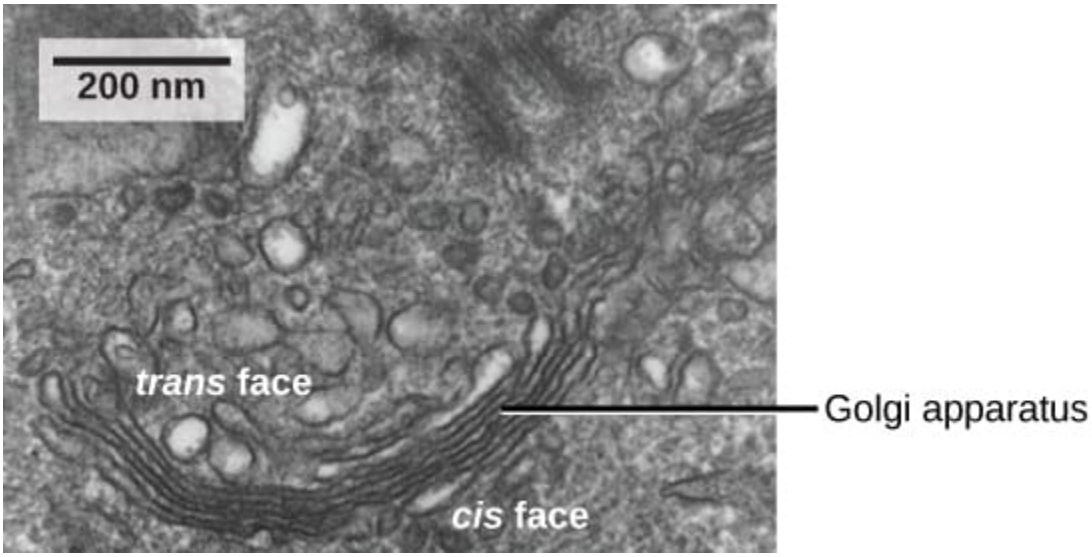


Figure 2.4.5: The Golgi apparatus in this transmission electron micrograph of a white blood cell is visible as a stack of semicircular flattened rings in the lower portion of this image. Several unnamed vesicles can be seen near the Golgi apparatus. (credit: modification of work by Louisa Howard; scale-bar data from Matt Russell)

The Golgi apparatus has a receiving face near the endoplasmic reticulum and a releasing face on the side away from the ER, toward the cell membrane. The transport vesicles that form from the ER travel to the receiving face, fuse with it, and empty their contents into the lumen of the Golgi apparatus. As the proteins and lipids travel through the Golgi, they undergo further modifications. The most frequent modification is the addition of short chains of sugar molecules. The newly modified proteins and lipids are then tagged with small molecular groups to enable them to be routed to their proper destinations.

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

The amount of Golgi in different cell types again illustrates that form follows function within cells. Cells that engage in a great deal of secretory activity (such as cells of the salivary glands that secrete digestive enzymes or cells of the immune system that secrete antibodies) have an abundant number of Golgi.

In plant cells, the Golgi has an additional role of synthesizing polysaccharides, some of which are incorporated into the cell wall

and some of which are used in other parts of the cell.

Lysosomes

In animal cells, the lysosomes are the cell's "garbage disposal." Digestive enzymes within the lysosomes aid the breakdown of proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles. In single-celled eukaryotes, lysosomes are important for digestion of the food they ingest and the recycling of organelles. These enzymes are active at a much lower pH (more acidic) than those located in the cytoplasm. Many reactions that take place in the cytoplasm could not occur at a low pH, thus the advantage of compartmentalizing the eukaryotic cell into organelles is apparent.

Lysosomes also use their hydrolytic enzymes to destroy disease-causing organisms that might enter the cell. A good example of this occurs in a group of white blood cells called macrophages, which are part of your body's immune system. In a process known as phagocytosis, a section of the plasma membrane of the macrophage invaginates (folds in) and engulfs a pathogen. The invaginated section, with the pathogen inside, then pinches itself off from the plasma membrane and becomes a vesicle. The vesicle fuses with a lysosome. The lysosome's hydrolytic enzymes then destroy the pathogen (**Figure** 2.4.6).

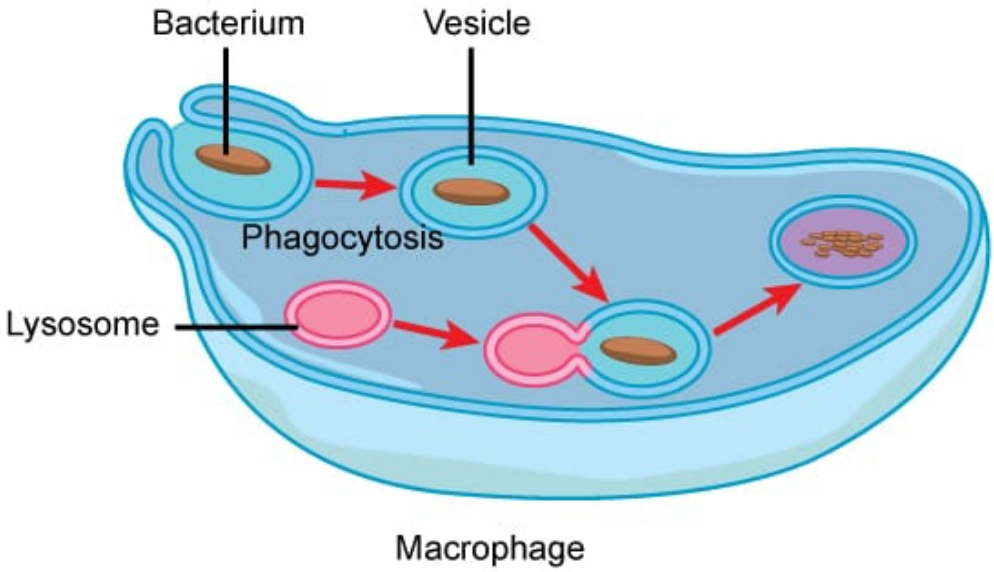


Figure 2.4.6: A macrophage (a type of immune cell) engulfs a bacterium, enclosing it within a membrane-bound vesicle. The vesicle then fuses with a lysosome, whose digestive enzymes break down the bacterium. Other organelles in the cell are omitted for simplicity.

Vesicles and Vacuoles

Vesicles and vacuoles are membrane-bound sacs that function in storage and transport. Vacuoles are somewhat larger than vesicles, and the membrane of a vacuole does not fuse with the membranes of other cellular components. Vesicles can fuse with other membranes within the cell system. Additionally, enzymes within plant vacuoles can break down macromolecules.

ART CONNECTION

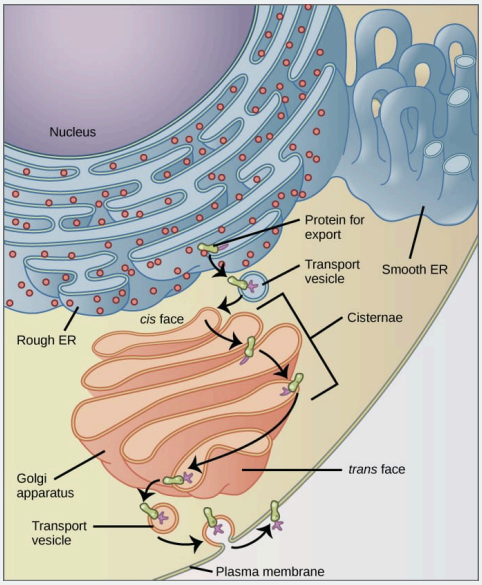


Figure 2.4.7: The endomembrane system works to modify, package, and transport lipids and proteins.
This figure shows the nucleus, rough ER, Golgi apparatus, vesicles, and plasma membrane. The right side of the rough ER is shown with an integral membrane protein embedded in it. The part of the protein facing the inside of the ER has a carbohydrate attached to it. The protein is shown leaving the ER in a vesicle that fuses with the cis face of the Golgi apparatus. The Golgi apparatus consists of several layers of membranes, called cisternae. As the protein passes through the cisternae, it is further modified by the addition of more carbohydrates. Eventually, it leaves the trans face of the Golgi in a vesicle. The vesicle fuses with the cell membrane so that the carbohydrate that was on the inside of the vesicle faces the outside of the membrane. At the same time, the contents of the vesicle are released from the cell. (credit: modification of work by Magnus Manske)

Why does the *cis* face of the Golgi not face the plasma membrane?

Ribosomes

Ribosomes are the cellular structures responsible for protein synthesis. When viewed through an electron microscope, free ribosomes appear as either clusters or single tiny dots floating freely in the cytoplasm. Ribosomes may be attached to either the cytoplasmic side of the plasma membrane or the cytoplasmic side of the endoplasmic reticulum (**Figure** 2.4.7). Electron microscopy has shown that ribosomes consist of large and small subunits. Ribosomes are enzyme complexes that are responsible for protein synthesis.

Because protein synthesis is essential for all cells, ribosomes are found in practically every cell, although they are smaller in prokaryotic cells. They are particularly abundant in immature red blood cells for the synthesis of hemoglobin, which functions in the transport of oxygen throughout the body.

Mitochondria

Mitochondria (singular = mitochondrion) are often called the “powerhouses” or “energy factories” of a cell because they are responsible for making adenosine triphosphate (ATP), the cell’s main energy-carrying molecule. The formation of ATP from the breakdown of glucose is known as cellular respiration. Mitochondria are oval-shaped, double-membrane organelles (**Figure** 2.4.8) that have their own ribosomes and DNA. Each membrane is a phospholipid bilayer embedded with proteins. The inner layer has folds called cristae, which increase the surface area of the inner membrane. The area surrounded by the folds is called the mitochondrial matrix. The cristae and the matrix have different roles in cellular respiration.

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 because muscle cells need a lot of energy to contract.

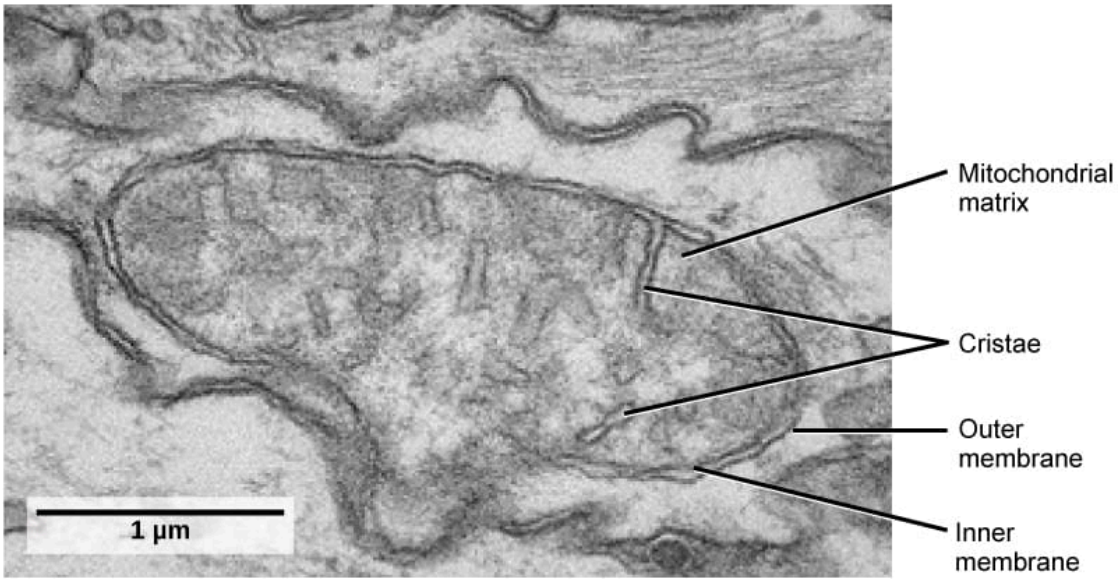


Figure 2.4.8: This transmission electron micrograph shows a mitochondrion as viewed with an electron microscope. Labeled are the inner and outer membranes, the cristae, and the mitochondrial matrix. The mitochondrion has an oval shape, an outer membrane and an inner membrane with many folds called cristae. Inside of the inner membrane is a space called the mitochondrial matrix. (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. Alcohol is detoxified by peroxisomes in liver cells. A byproduct of these oxidation reactions is hydrogen peroxide, H_2O_2, which is contained within the peroxisomes to prevent the chemical from causing damage to cellular components outside of the organelle. Hydrogen peroxide is safely broken down by peroxisomal enzymes into water and oxygen.

Animal Cells versus Plant Cells

Despite their fundamental similarities, there are some striking differences between animal and plant cells (see **Table** 2.4.1). Animal cells have centrioles, centrosomes (discussed under the cytoskeleton), and lysosomes, whereas plant cells do not. Plant cells have a cell wall, chloroplasts, and plastids used for storage, and a large central vacuole, whereas animal cells do not.

The Cell Wall

In **Figure** 2.4.1**b**, the diagram of a plant cell, you see a structure external to the plasma membrane called the cell wall. The cell wall is a rigid covering that protects the cell, provides structural support, and gives shape to the cell. Fungal and protist cells also have cell walls.

While the chief component of prokaryotic cell walls is peptidoglycan, the major organic molecule in the plant cell wall is cellulose, a polysaccharide made up of long, straight chains of glucose units. When nutritional information refers to dietary fiber, it is referring to the cellulose content of food.

Chloroplasts

Like mitochondria, chloroplasts also have their own DNA and ribosomes. Chloroplasts function in photosynthesis and can be found in eukaryotic cells such as plants and algae. In photosynthesis, carbon dioxide, water, and light energy are used to make glucose and oxygen. This is the major difference between plants and animals: Plants (autotrophs) are able to make their own food, like glucose, whereas animals (heterotrophs) must rely on other organisms for their organic compounds or food source.

Like mitochondria, chloroplasts have outer and inner membranes, but within the space enclosed by a chloroplast's inner

membrane is a set of interconnected and stacked, fluid-filled membrane sacs called thylakoids (**Figure** 2.4.9). Each stack of thylakoids is called a granum (plural = grana). The fluid enclosed by the inner membrane and surrounding the grana is called the stroma.

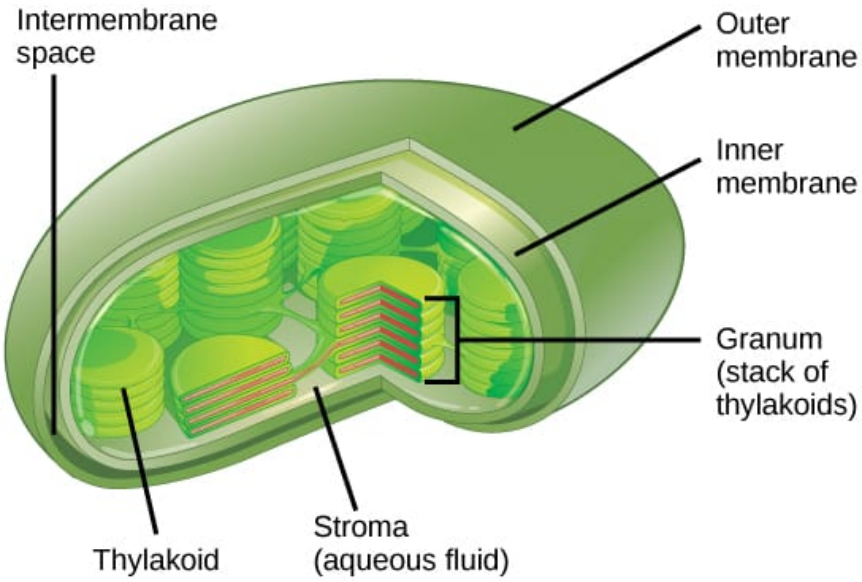


Figure 2.4.9: This illustration shows a chloroplast, which has an outer membrane, an inner membrane and outer membrane, thylakoids, grana, and stroma. The space between the outer and inner membranes is called the intermembrane space. Inside the inner membrane are flat, pancake-like structures called thylakoids. The thylakoids form stacks called grana. The liquid inside the inner membrane is called the stroma, and the space inside the thylakoid is called the thylakoid space.

The chloroplasts contain a green pigment called chlorophyll, which captures the energy of sunlight for photosynthesis. Like plant cells, photosynthetic protists also have chloroplasts. Some bacteria also perform photosynthesis, but they do not have chloroplasts. Their photosynthetic pigments are located in the thylakoid membrane within the cell itself.

EVOLUTION IN ACTION: Endosymbiosis

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

Symbiosis is a relationship in which organisms from two separate species live in close association and typically exhibit specific adaptations to each other. Endosymbiosis (*endo*-= within) is a relationship in which one organism lives inside the other. Endosymbiotic relationships abound in nature. 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 are provided a stable habitat and abundant food by living within the large intestine.

Scientists have long noticed that bacteria, mitochondria, and chloroplasts are similar in size. We also know that mitochondria and chloroplasts have DNA and ribosomes, just as bacteria do. Scientists believe that host cells and bacteria formed a mutually beneficial endosymbiotic relationship when the host cells ingested aerobic bacteria and cyanobacteria but did not destroy them. Through evolution, these ingested bacteria became more specialized in their functions, with the aerobic bacteria becoming mitochondria and the photosynthetic bacteria becoming chloroplasts.

The Central Vacuole

Previously, we mentioned vacuoles as essential components of plant cells. If you look at **Figure** 2.4.1, you will see that plant cells each have a large, central vacuole that occupies most of the cell. The central vacuole plays a key role in regulating the cell's concentration of water in changing environmental conditions. In plant cells, the liquid inside the central vacuole provides turgor pressure, which is the outward pressure caused by the fluid inside the cell. Have you ever noticed that if you forget to water a plant for a few days, it wilts? That is 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 and into the soil. As the central vacuole shrinks, it leaves the cell wall unsupported. This loss of support to the cell walls of a plant results in the wilted appearance. Additionally, this fluid has a very bitter taste, which discourages consumption by insects and animals. The central vacuole also functions to store proteins in developing seed cells.

Extracellular Matrix of Animal Cells

Most animal cells release materials into the extracellular space. The primary components of these materials are glycoproteins and the protein collagen. Collectively, these materials are called the extracellular matrix (**Figure** 2.4.10). Not only does the extracellular matrix hold the cells together to form a tissue, but it also allows the cells within the tissue to communicate with each other.

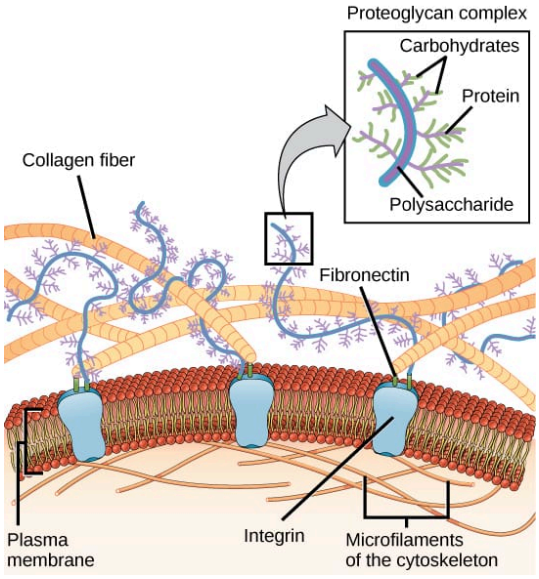


Figure 2.4.10: The extracellular matrix consists of a network of substances secreted by cells.
This illustration shows the plasma membrane embedded with integral membrane proteins called integrins. On the exterior of the cell is a vast network of collagen fibers, which are attached to the integrins via a protein called fibronectin. Proteoglycan complexes also extend from the plasma membrane into the extracellular matrix. A magnified view shows that each proteoglycan complex is composed of a polysaccharide core. Proteins branch from this core, and carbohydrates branch from the proteins. The inside of the cytoplasmic membrane is lined with microfilaments of the cytoskeleton.

Blood clotting provides an example of the role of the extracellular matrix in cell communication. When the cells lining a blood vessel are damaged, they display a protein receptor called tissue factor. When tissue factor binds with another factor in the extracellular matrix, it causes platelets to adhere to the wall of the damaged blood vessel, stimulates adjacent smooth muscle cells in the blood vessel to contract (thus constricting the blood vessel), and initiates a series of steps that stimulate the platelets to produce clotting factors.

Table 2.4.1: This table provides the components of prokaryotic and eukaryotic cells and their respective functions.

Cell Component	Function	Present in Prokaryotes?	Present in Animal Cells?	Present in Plant Cells?
Plasma membrane	Separates cell from external environment; controls passage of organic molecules, ions, water, oxygen, and wastes into and out of the cell	Yes	Yes	Yes
Cytoplasm	Provides structure to cell; site of many metabolic reactions; medium in which organelles are found	Yes	Yes	Yes
Nucleoid	Location of DNA	Yes	No	No

Cell Component	Function	Present in Prokaryotes?	Present in Animal Cells?	Present in Plant Cells?
Nucleus	Cell organelle that houses DNA and directs synthesis of ribosomes and proteins	No	Yes	Yes
Ribosomes	Protein synthesis	Yes	Yes	Yes
Mitochondria	ATP production/cellular respiration	No	Yes	Yes
Peroxisomes	Oxidizes and breaks down fatty acids and amino acids, and detoxifies poisons	No	Yes	Yes
Vesicles and vacuoles	Storage and transport; digestive function in plant cells	No	Yes	Yes
Centrosome	Unspecified role in cell division in animal cells; organizing center of microtubules in animal cells	No	Yes	No
Lysosomes	Digestion of macromolecules; recycling of worn-out organelles	No	Yes	No
Cell wall	Protection, structural support and maintenance of cell shape	Yes, primarily peptidoglycan in bacteria but not Archaea	No	Yes, primarily cellulose
Chloroplasts	Photosynthesis	No	No	Yes
Endoplasmic reticulum	Modifies proteins and synthesizes lipids	No	Yes	Yes
Golgi apparatus	Modifies, sorts, tags, packages, and distributes lipids and proteins	No	Yes	Yes
Cytoskeleton	Maintains cell's shape, secures organelles in specific positions, allows cytoplasm and vesicles to move within the cell, and enables unicellular organisms to move independently	Yes	Yes	Yes
Flagella	Cellular locomotion	Some	Some	No, except for some plant sperm
Cilia	Cellular locomotion, movement of particles along extracellular surface of plasma membrane, and filtration	No	Some	No

Summary

Like a prokaryotic cell, a eukaryotic cell has a plasma membrane, cytoplasm, and ribosomes, but a eukaryotic cell is typically larger than a prokaryotic cell, has a true nucleus (meaning its DNA is surrounded by a membrane), and has other membrane-bound organelles that allow for compartmentalization of functions. The plasma membrane is a phospholipid bilayer embedded with proteins. The nucleolus within the nucleus is the site for ribosome assembly. Ribosomes are found in the cytoplasm or are attached to the cytoplasmic side of the plasma membrane or endoplasmic reticulum. They perform protein synthesis. Mitochondria perform cellular respiration and produce ATP. Peroxisomes break down fatty acids, amino acids, and some toxins. Vesicles and vacuoles are storage and transport compartments. In plant cells, vacuoles also help break down macromolecules.

Animal cells also have a centrosome and lysosomes. The centrosome has two bodies, the centrioles, with an unknown role in cell division. Lysosomes are the digestive organelles of animal cells.

Plant cells have a cell wall, chloroplasts, and a central vacuole. The plant cell wall, whose primary component is cellulose, protects the cell, provides structural support, and gives shape to the cell. Photosynthesis takes place in chloroplasts. The central vacuole expands, enlarging the cell without the need to produce more cytoplasm.

The endomembrane system includes the nuclear envelope, the endoplasmic reticulum, Golgi apparatus, lysosomes, vesicles, as well as the plasma membrane. These cellular components work together to modify, package, tag, and transport membrane lipids and proteins.

The cytoskeleton has three different types of protein elements. Microfilaments provide rigidity and shape to the cell, and facilitate cellular movements. Intermediate filaments bear tension and anchor the nucleus and other organelles in place. Microtubules help the cell resist compression, serve as tracks for motor proteins that move vesicles through the cell, and pull replicated chromosomes to opposite ends of a dividing cell. They are also the structural elements of centrioles, flagella, and cilia.

Animal cells communicate through their extracellular matrices.

Art Connections

Figure 2.4.1: What structures does a plant cell have that an animal cell does not have? What structures does an animal cell have that a plant cell does not have?

Answer

Plant cells have a cell wall, a large central vacuole, chloroplasts, and plastids. Animal cells have lysosomes and centrosomes.

Figure 2.4.7: Why does the *cis* face of the Golgi not face the plasma membrane?

Answer

Because that face receives chemicals from the ER, which is toward the center of the cell.

Key Terms

cell wall
a rigid cell covering made of cellulose in plants, peptidoglycan in bacteria, non-peptidoglycan compounds in Archaea, and chitin in fungi that protects the cell, provides structural support, and gives shape to the cell

central vacuole
a large plant cell organelle that acts as a storage compartment, water reservoir, and site of macromolecule degradation

chloroplast
a plant cell organelle that carries out photosynthesis

cilium
(plural: cilia) a short, hair-like structure that extends from the plasma membrane in large numbers and is used to move an entire cell or move substances along the outer surface of the cell

cytoplasm
the entire region between the plasma membrane and the nuclear envelope, consisting of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals

cytoskeleton
the network of protein fibers that collectively maintains the shape of the cell, secures some organelles in specific positions, allows cytoplasm and vesicles to move within the cell, and enables unicellular organisms to move

cytosol
the gel-like material of the cytoplasm in which cell structures are suspended

endomembrane system
the group of organelles and membranes in eukaryotic cells that work together to modify, package, and transport lipids and proteins

endoplasmic reticulum (ER)
a series of interconnected membranous structures within eukaryotic cells that collectively modify proteins and synthesize lipids

extracellular matrix
the material, primarily collagen, glycoproteins, and proteoglycans, secreted from animal cells that holds cells together as a tissue, allows cells to communicate with each other, and provides mechanical protection and anchoring for cells in the tissue

flagellum
(plural: flagella) the long, hair-like structure that extends from the plasma membrane and is used to move the cell

Golgi apparatus
a eukaryotic organelle made up of a series of stacked membranes that sorts, tags, and packages lipids and proteins for distribution

lysosome
an organelle in an animal cell that functions as the cell's digestive component; it breaks down proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles

microfilament
a thin protein fiber in the cell's cytoskeleton that helps maintain cell shape and enables movement

mitochondria
(singular: mitochondrion) the cellular organelles responsible for carrying out cellular respiration, resulting in the production of ATP, the cell's main energy-carrying molecule

nuclear envelope
the double-membrane structure that constitutes the outermost portion of the nucleus

nucleolus
the darkly staining body within the nucleus that is responsible for assembling ribosomal subunits

nucleus
the cell organelle that houses the cell's DNA and directs the synthesis of ribosomes and proteins

peroxisome
a small, round organelle that contains hydrogen peroxide, oxidizes fatty acids and amino acids, and detoxifies many poisons

plasma membrane
a phospholipid bilayer with embedded (integral) or attached (peripheral) proteins that separates the internal contents of the cell from its surrounding environment

ribosome
a cellular structure that carries out protein synthesis

rough endoplasmic reticulum (RER)
the region of the endoplasmic reticulum that is studded with ribosomes and engages in protein modification

smooth endoplasmic reticulum (SER)
the region of the endoplasmic reticulum that has few or no ribosomes on its cytoplasmic surface and synthesizes carbohydrates, lipids, and steroid hormones; detoxifies chemicals like pesticides, preservatives, medications, and environmental pollutants, and stores calcium ions

vacuole
a membrane-bound sac, somewhat larger than a vesicle, that functions in cellular storage and transport

vesicle
a small, membrane-bound sac that functions in cellular storage and transport; its membrane is capable of fusing with the plasma membrane and the membranes of the endoplasmic reticulum and Golgi apparatus

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2.5: The Building Blocks of Molecules

Learning Objectives

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

- Explain what matter, elements, and atoms are and how they make up living organisms.
- Identify the parts of an atom and describe their charges and roles.
- Use atomic number and mass number to determine the identity of an element and the number of neutrons it contains.
- Recognize how the periodic table organizes elements and why element properties matter for forming 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** 2.5.1). 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.

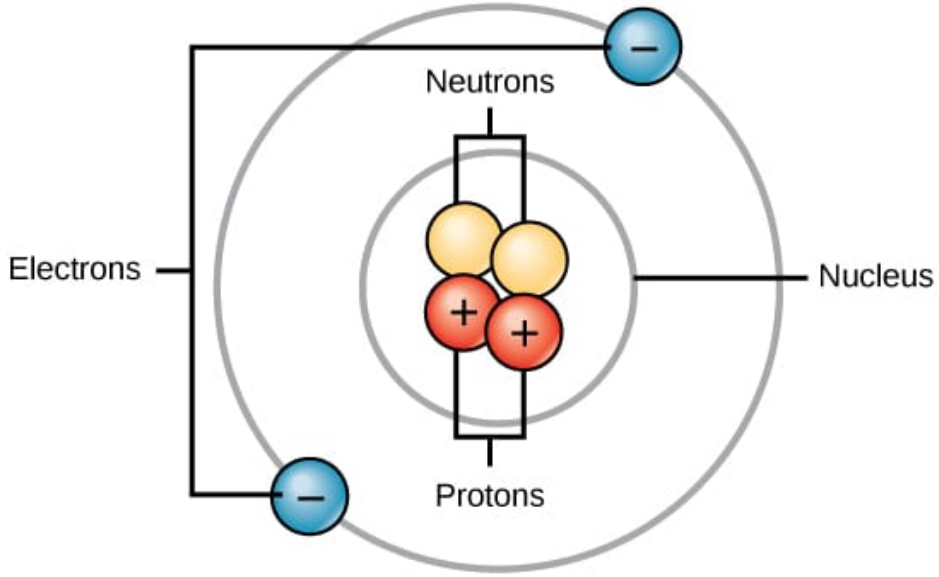


Figure 2.5.1: Atoms are made up of protons and neutrons located within the nucleus, and electrons surrounding the nucleus.
The illustration of this atom shows two neutrons and two protons in the center, with a circle labeled as the nucleus around them. Another circle shows an orbit with two electrons outside of 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.

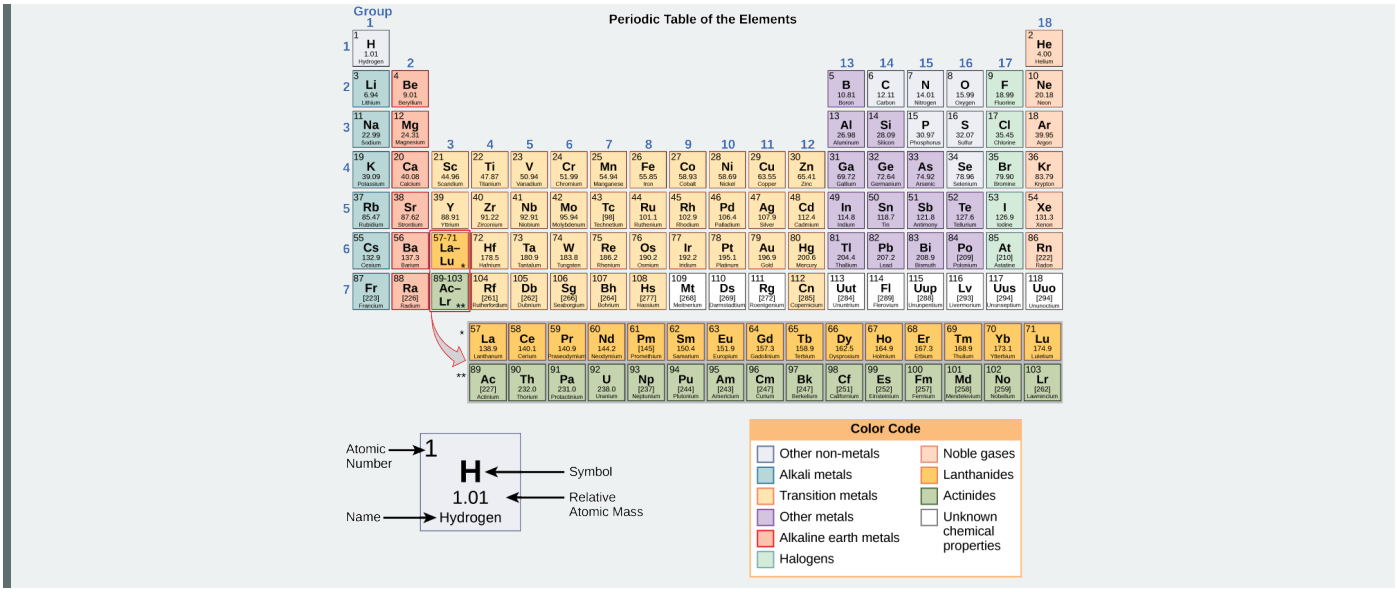
Because protons and neutrons each have a mass of 1, the mass of an atom is equal to the number of protons and neutrons of that atom. The number of electrons does not factor into the overall mass, because their mass is so small.

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** 2.5.2)—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


Periodic Table of the Elements
Group
1
18
2
13
14
15
16
17
3
4
5
6
7
8
9
10
11
12
Atomic Number
1
H
1.01
Hydrogen
Symbol
Relative Atomic Mass
Name
Color Code
Other non-metals
Alkali metals
Transition metals
Other metals
Alkaline earth metals
Halogens
Noble gases
Lanthanides
Actinides
Unknown chemical properties

Click to open image description

The image displays a **color-coded periodic table of the elements** titled *"Periodic Table of the Elements"* at the top center. The table is organized into **horizontal rows (periods)** labeled 1 through 7 on the left side and **vertical columns (groups)** labeled 1 through 18 along the top.

Each element appears in a rectangular box containing:

- **Atomic number** at the top (for example, hydrogen shows "1")
- **Chemical symbol** in large text (for example, "H")
- **Element name** (hydrogen)
- **Relative atomic mass** (for hydrogen, 1.01)

In the **main body of the table**, elements are arranged from left to right in increasing atomic number. The layout forms a wide block with a gap in the middle (in periods 4 and 5) filled by the **transition metals**.

The elements are **color-coded by category**, according to a legend in the lower right corner labeled "Color Code." Categories include:

- Other non-metals (light gray)
- Alkali metals (blue)
- Alkaline earth metals (light red)
- Transition metals (tan)
- Other metals (purple)
- Halogens (light green)
- Noble gases (peach)
- Lanthanides (yellow)
- Actinides (green)
- Unknown chemical properties (gray)

Below the main table are two separated rows:

- The **lanthanides** (elements 57–71), shown as a horizontal strip highlighted in yellow
- The **actinides** (elements 89–103), shown as a strip highlighted in green

Arrows from the main table point down to these two rows, indicating their correct placement between elements **lanthanum (La)** and **hafnium (Hf)**, and between **actinium (Ac)** and **rutherfordium (Rf)**.

In the lower left corner, a **labeled example element box** explains the meaning of each part of an element square, identifying "atomic number," "symbol," "name," and "relative atomic mass."

The overall layout emphasizes patterns:

- Elements in the same **column (group)** share chemical properties
- Properties change across **rows (periods)**
- Metals occupy the left and center, while **nonmetals** are mostly on the right

Figure 2.5.2: The periodic table of the elements. 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** 2.5.3). 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 2.5.3: 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 PhET: Isotopes and Atomic Mass site and run the simulation.

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.

Art Connections

Figure 2.5.2: How many neutrons do (K) potassium-39 and potassium-40 have, respectively?

Answer

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

Key Terms

anion
: a negative ion formed by gaining electrons

atomic number
: the number of protons in an atom

cation
: a positive ion formed by losing electrons

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

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

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

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

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

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

Contributors and Attributions

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2.6: Chemical Bonds

Learning Objectives

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

- Explain how electrons are arranged in atoms and why filled outer shells make atoms more stable.
- Describe how atoms form ions by gaining or losing electrons and how this leads to ionic bonds.
- Compare ionic and covalent bonds, including the difference between polar and nonpolar covalent bonds.
- Recognize weaker interactions, such as hydrogen bonds and van der Waals interactions, and explain why they are important in biological molecules like water, DNA, and proteins.

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 from the previous section, 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** 2.6.1 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.

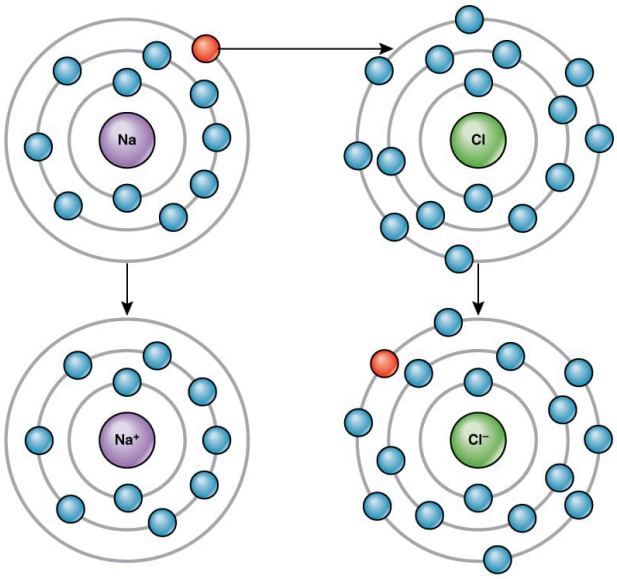


Figure 2.6.1: Elements tend to fill their outermost shells with electrons. To do this, they can either donate or accept electrons from other elements.

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** 2.6.2).

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.

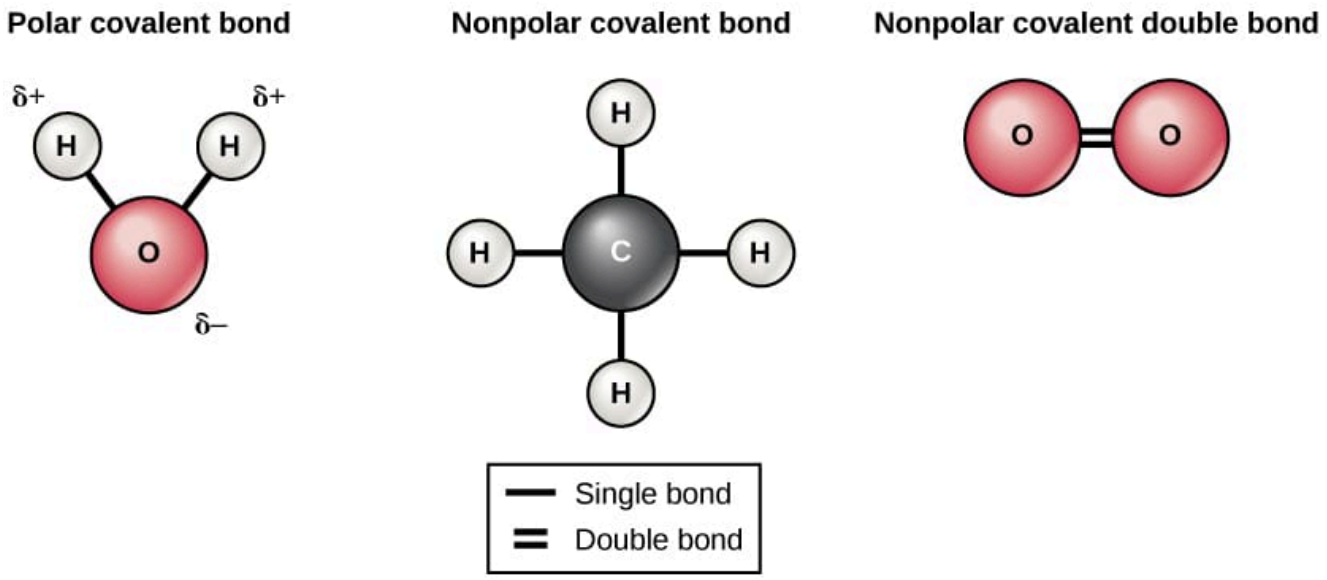


Figure 2.6.2: The water molecule (left) depicts a polar bond with a slightly positive charge on the hydrogen atoms and a slightly negative charge on the oxygen. Examples of nonpolar bonds include methane (middle) and oxygen (right).

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** 2.6.3). 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.

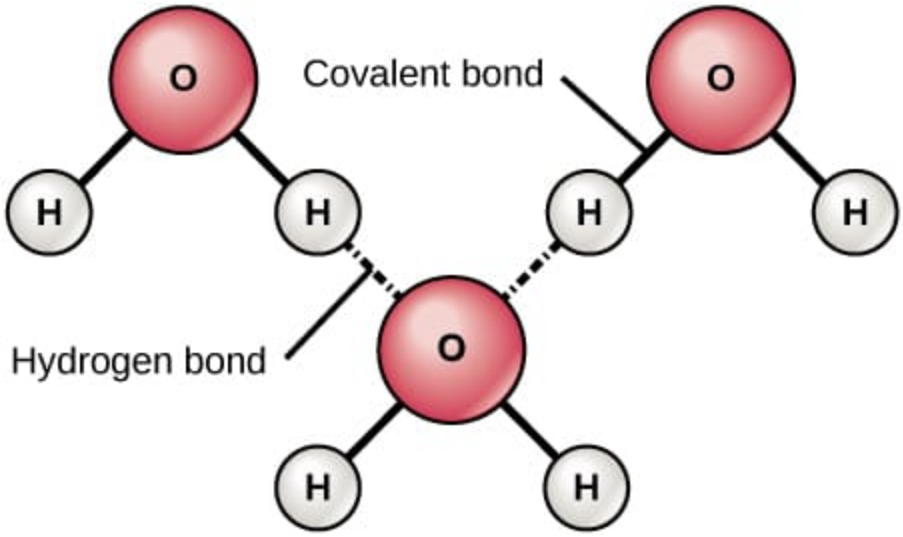


Figure 2.6.3: Hydrogen bonds form between slightly positive (δ+) and slightly negative (δ–) charges of polar covalent molecules, such as water.

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.

CAREERS IN ACTION: Radiography Technician

Have you or anyone you know ever had a magnetic resonance imaging (MRI) scan, a mammogram, or an X-ray? These tests produce images of your soft tissues and organs (as with an MRI or mammogram) or your bones (as happens in an X-ray) by using either radiowaves or special isotopes (radiolabeled or fluorescently labeled) that are ingested or injected into the body. These tests provide data for disease diagnoses by creating images of your organs or skeletal system.

MRI imaging works by subjecting hydrogen nuclei, which are abundant in the water in soft tissues, to fluctuating magnetic fields, which cause them to emit their own magnetic field. This signal is then read by sensors in the machine and interpreted by a computer to form a detailed image.

Some radiography technologists and technicians specialize in computed tomography, MRI, and mammography. They produce films or images of the body that help medical professionals examine and diagnose. Radiologists work directly with patients, explaining machinery, preparing them for exams, and ensuring that their body or body parts are positioned correctly to produce the needed images. Physicians or radiologists then analyze the test results.

Radiography technicians can work in hospitals, doctors' offices, or specialized imaging centers. Training to become a radiography technician happens at hospitals, colleges, and universities that offer certificates, associate's degrees, or bachelor's degrees in radiography.

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

Answer

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

Key Terms

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 shell
a region around an atom where electrons are found

electron transfer
the movement of electrons from one element to another

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

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

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

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

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

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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2.7: Water

Learning Objectives

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

- Explain why water is essential for life and how its polar nature affects how it interacts with other substances.
- Describe how hydrogen bonding gives water its unique properties, including temperature stability, cohesion, and surface tension.
- Understand why water is an effective solvent and how polar and ionic substances dissolve in water.
- Define pH, acids, bases, and buffers, and explain why maintaining a stable pH is critical for living organisms.

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 and the one most critical to life as we know it. Approximately 60–70 percent of your body is made up of water. Without it, life simply would not exist.

Water Is Polar

The hydrogen and oxygen atoms within water molecules form polar covalent bonds. The shared electrons spend more time associated with the oxygen atom than they do with hydrogen atoms. There is no overall charge to a water molecule, but there is 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 seen in Figure 2.6.3. Each water molecule attracts other water molecules because of the positive and negative charges in the different parts of the molecule. Water also attracts other polar molecules (such as sugars), forming hydrogen bonds. When a substance readily forms hydrogen bonds with water, it can dissolve in water and is referred to as hydrophilic (“water-loving”). Hydrogen bonds are not readily formed with nonpolar substances like oils and fats (**Figure** 2.7.1). These nonpolar compounds are hydrophobic (“water-fearing”) and will not dissolve in water.



Figure 2.7.1: As this macroscopic image of oil and water show, oil is a nonpolar compound and, hence, will not dissolve in water. Oil and water do not mix. (credit: Gautam Dogra)

Water Stabilizes Temperature

The hydrogen bonds in water allow it to absorb and release heat energy more slowly than many other substances. Temperature is a measure of the motion (kinetic energy) of molecules. As the motion increases, energy is higher and thus temperature is higher. Water absorbs a great deal of energy before its temperature rises. Increased energy disrupts the hydrogen bonds between water molecules. Because these bonds can be created and disrupted rapidly, water absorbs an increase in energy and temperature changes only minimally. This means that water moderates temperature changes within organisms and in their environments. 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 at the surface of the liquid (such as a body of water, the leaves of a plant, or the skin of an organism) in a process called evaporation. 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.

Conversely, as molecular motion decreases and temperatures drop, less energy is present to break the hydrogen bonds between water molecules. These bonds remain intact and begin to form a rigid, lattice-like structure (e.g., ice) (**Figure** 2.7.2**a**). When frozen, ice is less dense than liquid water (the molecules are farther apart). This means that ice floats on the surface of a body of water (**Figure** 2.7.2**b**). 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.

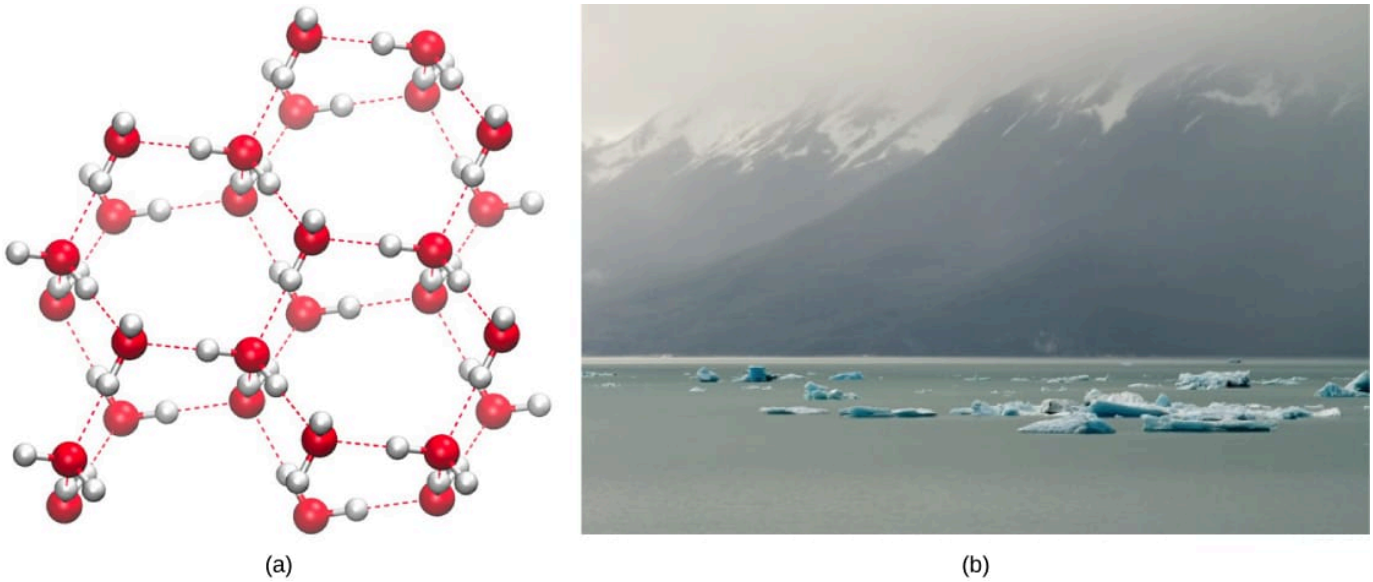


Figure 2.7.2: Figure A shows the lattice-like molecular structure of ice. The lattice structure of ice makes it less dense than the freely flowing molecules of liquid water. Ice's lower density enables it to float on water. Figure B shows a body of water with chunks of ice floating on its surface. There is a foggy backdrop of snow-capped mountains in the distance. (credit a: modification of work by Jane Whitney; credit b: modification of work by Carlos Ponte)

CONCEPTS IN ACTION

Click for a 3-D animation of the structure of an ice lattice. (credit: image created by Jane Whitney using Visual Molecular Dynamics (VMD) software[1])

Water Is an Excellent Solvent

Because water is polar, with slight positive and negative charges, ionic compounds and polar molecules can readily dissolve in it. Water is, therefore, what is referred to as a solvent—a substance capable of dissolving another substance. The charged particles will form hydrogen bonds with a surrounding layer of water molecules. This is referred to as a sphere of hydration and serves to keep the particles separated or dispersed in the water. In the case of table salt (NaCl) mixed in water (**Figure** 2.7.3), the sodium and chloride ions separate, or dissociate, in the water, and spheres of hydration are formed around the ions. A positively charged sodium ion is surrounded by the partially negative charges of oxygen atoms in water molecules. A negatively charged chloride ion is surrounded by the partially positive charges of hydrogen atoms in water molecules. These spheres of hydration are also referred to as hydration shells. The polarity of the water molecule makes it an effective solvent and is important in its many roles in living systems.

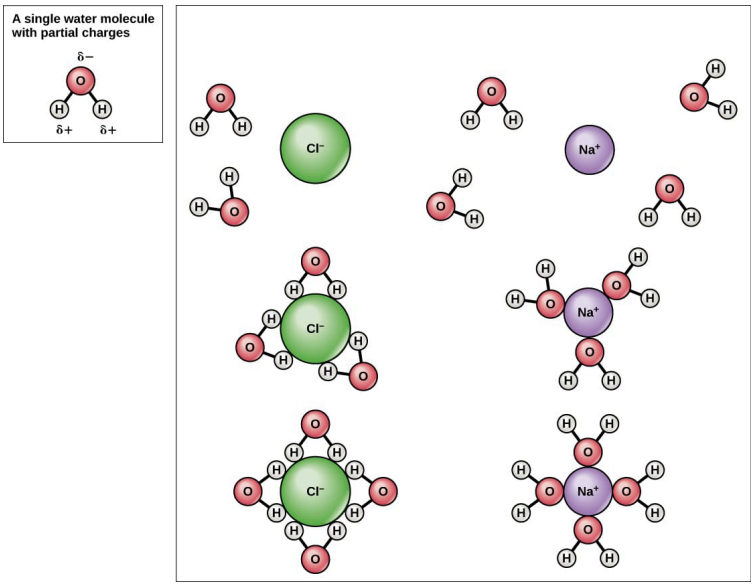


Figure 2.7.3: This illustration depicts spheres of hydration around sodium and chlorine ions. The spheres of hydration are comprised of water molecules. When table salt (NaCl) is mixed in water, spheres of hydration form around the ions.

Water Is Cohesive

Have you ever filled up a glass of water to the very top and then slowly added a few more drops? Before it overflows, the water actually forms a dome-like shape above the rim of the glass. This water can stay above the glass because of the property of cohesion. In cohesion, water molecules are attracted to each other (because of hydrogen bonding), keeping the molecules together at the liquid-air (gas) interface, although there is no more room in the glass. Cohesion gives rise to surface tension, the capacity of a substance to withstand rupture when placed under tension or stress. When you drop a small scrap of paper onto a droplet of water, the paper floats on top of the water droplet, although the object is denser (heavier) than the water. This occurs because of the surface tension that is created by the water molecules. Cohesion and surface tension keep the water molecules intact and the item floating on the top. It is even possible to “float” a steel needle on top of a glass of water if you place it gently, without breaking the surface tension (**Figure** 2.7.4).

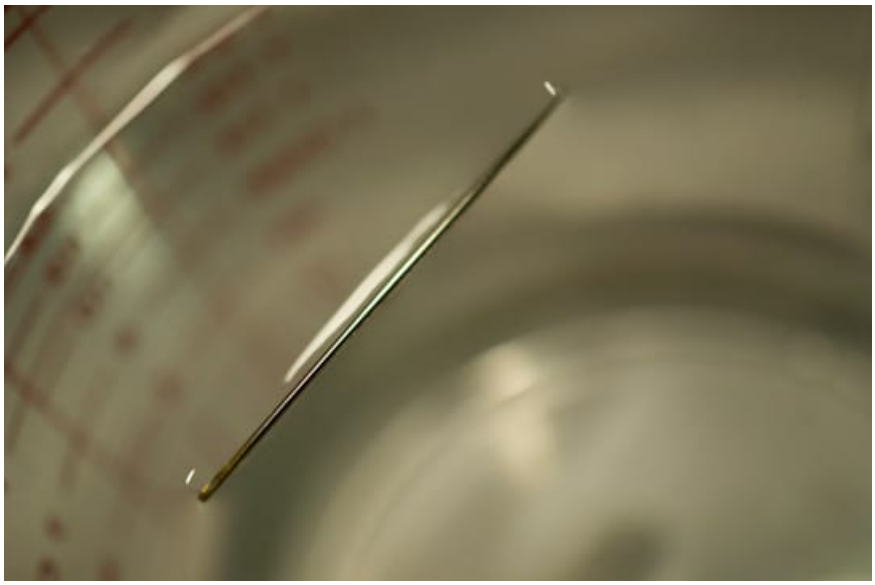


Figure 2.7.4: The weight of a needle on top of water pulls the surface tension downward; at the same time, the surface tension of the water is pulling it up, suspending the needle on the surface of the water and keeping it from sinking. Notice the indentation in the water around the needle. (credit: Cory Zanker)

These cohesive forces are also related to the water's property of adhesion, or the attraction between water molecules and other molecules. This is observed when water "climbs" up a straw placed in a glass of water. You will notice that the water appears to be higher on the sides of the straw than in the middle. This is because the water molecules are attracted to the straw and therefore adhere to it.

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.

CONCEPT IN ACTION

To learn more about water, visit the U.S. Geological Survey Water Science for Schools: All About Water! website.

Buffers, pH, Acids, and Bases

The pH of a solution is a measure of its acidity or alkalinity. You have probably used litmus paper, paper that has been treated with a natural water-soluble dye so it can be used as a pH indicator, to test how much acid or base (alkalinity) exists in a solution. You might have even used some to make sure the water in an outdoor swimming pool is properly treated. In both cases, this pH test measures the amount of hydrogen ions that exists in a given solution. High concentrations of hydrogen ions yield a low pH, whereas low levels of hydrogen ions result in a high pH. The overall concentration of hydrogen ions is inversely related to its pH and can be measured on the pH scale (**Figure** 2.7.5). Therefore, the more hydrogen ions present, the lower the pH; conversely, the fewer hydrogen ions, the higher the pH.

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 concentration of hydrogen ions 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 (ranging from 0.0 to 6.9) is acidic, and anything above 7.0 (from 7.1 to 14.0) is alkaline. The blood in your veins is slightly alkaline (pH = 7.4). The environment in your stomach is highly acidic (pH = 1 to 2). Orange juice is mildly acidic (pH = approximately 3.5), whereas baking soda is basic (pH = 9.0).

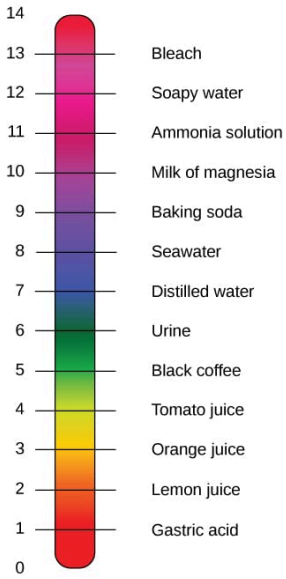


Figure 2.7.5: The pH scale measures the amount of hydrogen ions (H^+) in a substance.
Shown is a rainbow-colored bar represents the pH scale, and an example substance is given at each pH value. Common basic solutions are seawater and bleach. Common acidic solutions are black coffee and lemon juice. (credit: modification of work by Edward Stevens)

Acids are substances that provide hydrogen ions (H^+) and lower pH, whereas bases provide hydroxide ions (OH^-) and raise pH. 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, bases are those substances that readily donate OH^-. The OH^- ions combine with H^+ to produce water, which raises a substance's pH. Sodium hydroxide and many household cleaners are very alkaline and give up OH^- rapidly when placed in water, thereby raising the pH.

Most cells in our bodies operate within a very narrow window of the pH scale, typically ranging only from 7.2 to 7.6. If the pH of the body is outside of this range, the respiratory system malfunctions, as do other organs in the body. Cells no longer function properly, and proteins will break down. Deviation outside of the pH range can induce coma or even cause death.

So how is it that we can ingest or inhale acidic or basic substances and not die? Buffers are the key. Buffers readily absorb excess H^+ or OH^-, keeping the pH of the body carefully maintained in the aforementioned narrow range. Carbon dioxide is part of a prominent buffer system in the human body; it keeps the pH within the proper range. This buffer system involves carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-) anion. If too much H^+ enters the body, bicarbonate will combine with the H^+ to create carbonic acid and limit the decrease in pH. Likewise, if too much OH^- is introduced into the system, carbonic acid will rapidly dissociate into bicarbonate and H^+ ions. The H^+ ions can combine with the OH^- ions, limiting the increase in pH. While carbonic acid is an important product in this reaction, its presence is fleeting because the carbonic acid is released from the body as carbon dioxide gas each time we breathe. Without this buffer system, the pH in our bodies would fluctuate too much and we would fail to survive.

Summary

Water has many properties that are critical to maintaining life. It is polar, allowing for the formation of hydrogen bonds, which allow ions and other polar molecules to dissolve in water. Therefore, water is an excellent solvent. The hydrogen bonds between water molecules give water the ability to hold heat better than many other substances. As the temperature rises, the hydrogen bonds between water continually break and reform, allowing for the overall temperature to remain stable, although increased energy is added to the system. Water's cohesive forces allow for the property of surface tension. All of these unique properties of water are important in the chemistry of living organisms.

The pH of a solution is a measure of the concentration of hydrogen ions in the solution. A solution with a high number of hydrogen ions is acidic and has a low pH value. A solution with a high number of hydroxide ions is basic and has a high pH value. The pH scale ranges from 0 to 14, with a pH of 7 being neutral. Buffers are solutions that moderate pH changes when an acid or base is added to the buffer system. Buffers are important in biological systems because of their ability to maintain constant pH

conditions.

Footnotes

1. 1 Humphrey, W., Dalke, A. and Schulten, K., "VMD—Visual Molecular Dynamics", *J. Molec. Graphics*, 1996, vol. 14, pp. 33-38. http://www.ks.uiuc.edu/Research/vmd/

Key Terms

acid
a substance that donates hydrogen ions and therefore lowers pH

adhesion
the attraction between water molecules and molecules of a different substance

base
a substance that absorbs hydrogen ions and therefore raises pH

buffer
a solution that resists a change in pH by absorbing or releasing hydrogen or hydroxide ions

cohesion
the intermolecular forces between water molecules caused by the polar nature of water; creates surface tension

evaporation
the release of water molecules from liquid water to form water vapor

hydrophilic
describes a substance that dissolves in water; water-loving

hydrophobic
describes a substance that does not dissolve in water; water-fearing

hydroxide ion (OH^-)
a negatively charged ion made of oxygen and hydrogen

kinetic energy
the energy of motion

litmus paper
filter paper that has been treated with a natural water-soluble dye so it can be used as a pH indicator

pH scale
a scale ranging from 0 to 14 that measures the approximate concentration of hydrogen ions of a substance

solvent
a substance capable of dissolving another substance

surface tension
the cohesive force at the surface of a body of liquid that prevents the molecules from separating

temperature
a measure of molecular motion

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2.8: Chemistry of Life (Exercises)

2.5: 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. Each element is designated by its chemical symbol and possesses unique properties. These unique properties allow elements to combine and to bond with each other in specific ways.

Multiple Choice

Magnesium has an atomic number of 12. Which of the following statements is true of a neutral magnesium atom?

A. It has 12 protons, 12 electrons, and 12 neutrons.
B. It has 12 protons, 12 electrons, and six neutrons.
C. It has six protons, six electrons, and no neutrons.
D. It has six protons, six electrons, and six neutrons.

Answer

A

Which type of bond represents a weak chemical bond?

A. hydrogen bond
B. ionic bond
C. covalent bond
D. polar covalent bond

Answer

A

An isotope of sodium (Na) has a mass number of 22. How many neutrons does it have?

A. 11
B. 12
C. 22
D. 44

Answer

A

Free Response

Why are hydrogen bonds and van der Waals interactions necessary for cells?

Answer

Hydrogen bonds and van der Waals interactions form weak associations between different molecules. They provide the structure and shape necessary for proteins and DNA within cells so that they function properly. Hydrogen bonds also give water its unique properties, which are necessary for life.

2.7: Water

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 and the one most critical to life as we know it. Approximately 60–70 percent of your body is made up of water. Without it, life simply would not exist.

Multiple Choice

Which of the following statements is not true?

A. Water is polar.
B. Water stabilizes temperature.
C. Water is essential for life.
D. Water is the most abundant atom in Earth's atmosphere.

Answer

D

Using a pH meter, you find the pH of an unknown solution to be 8.0. How would you describe this solution?

A. weakly acidic
B. strongly acidic
C. weakly basic
D. strongly basic

Answer

C

The pH of lemon juice is about 2.0, whereas tomato juice's pH is about 4.0. Approximately how much of an increase in hydrogen ion concentration is there between tomato juice and lemon juice?

A. 2 times
B. 10 times
C. 100 times
D. 1000 times

Answer

C

Free Response

Why can some insects walk on water?

Answer

Some insects can walk on water, although they are heavier (denser) than water, because of the surface tension of water. Surface tension results from cohesion, or the attraction between water molecules at the surface of the body of water [the liquid-air (gas) interface].

Explain why water is an excellent solvent.

Answer

Water molecules are polar, meaning they have separated partial positive and negative charges. Because of these

charges, water molecules are able to surround charged particles created when a substance dissociates. The surrounding layer of water molecules stabilizes the ion and keeps differently charged ions from reassociating, so the substance stays dissolved.

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3: PROPERTIES OF BIOLOGICAL MOLECULES

3.1: Biological Molecules - Carbon and Carbon Bonding

3.2: Biological Molecules - Carbohydrates

3.3: Biological Molecules - Lipids

3.4: Biological Molecules - Nucleic Acids

3.5: Proteins - Structure

3.6: Proteins - Function

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3.1: Biological Molecules - Carbon and Carbon Bonding

Learning Objectives

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

- Recognize why carbon is central to life and explain how its bonding properties allow complex biological molecules to form.
- Distinguish between monomers and polymers using clear examples from biological and everyday materials.
- Describe how polymerization builds large macromolecules from smaller repeating units.
- Identify the four major classes of biological macromolecules and summarize their general roles in cells.

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

Carbon

It is often said that life is "carbon-based." This means that carbon atoms, bonded to other carbon atoms or other elements, form the fundamental components of many, if not most, of the molecules found uniquely in living things. Other elements play important roles in biological molecules, but carbon certainly qualifies as the "foundation" element for molecules in living things. It is the bonding properties of carbon atoms that are responsible for its important role.

Carbon Bonding

Carbon contains four electrons in its outer shell. Therefore, it can form four covalent bonds with other atoms or molecules. The simplest organic carbon molecule is methane (CH_4), in which four hydrogen atoms bind to a carbon atom (**Figure** 3.1.1).

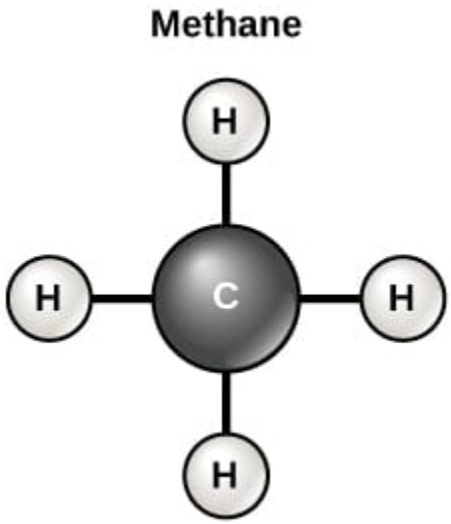


Figure 3.1.1: Carbon can form four covalent bonds to create an organic molecule. The simplest carbon molecule is methane (CH_4), depicted here.

However, structures that are more complex are made using carbon. Any of the hydrogen atoms can be replaced with another carbon atom covalently bonded to the first carbon atom. In this way, long and branching chains of carbon compounds can be made (**Figure** 3.1.2**a**). The carbon atoms may bond with atoms of other elements, such as nitrogen, oxygen, and phosphorus

(**Figure** 3.1.2**b**). The molecules may also form rings, which themselves can link with other rings (**Figure** 3.1.2**c**). This diversity of molecular forms accounts for the diversity of functions of the biological macromolecules and is based to a large degree on the ability of carbon to form multiple bonds with itself and other atoms.

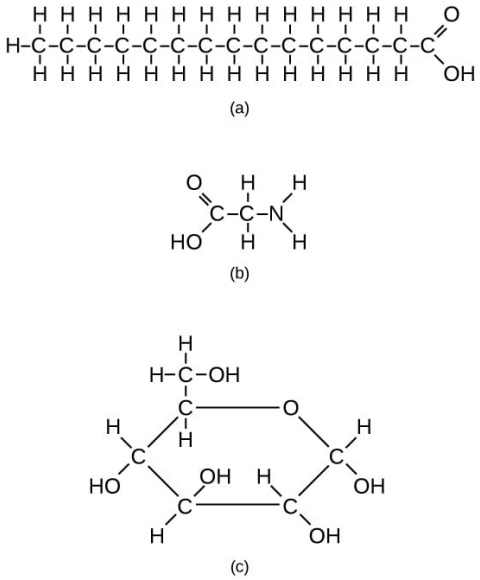


Figure 3.1.2: These examples show three molecules (found in living organisms) that contain carbon atoms bonded in various ways to other carbon atoms and the atoms of other elements. (a) This molecule of stearic acid has a long chain of carbon atoms. (b) Glycine, a component of proteins, contains carbon, nitrogen, oxygen, and hydrogen atoms. (c) Glucose, a sugar, has a ring of carbon atoms and one oxygen atom.

From Carbon Chains to Complex Molecules: Introducing Monomers and Polymers

Now that you've learned about carbon and its unique ability to form strong, flexible bonds with other atoms—including itself—you're ready to explore how those simple carbon-based units can link together to create much larger, more complex molecules.

These larger molecules are central to life—and to biotechnology. Whether it's DNA used in genetic testing, proteins used in lab-grown tissues, or carbohydrates in biofuels, many of the substances you'll encounter in this course are polymers built from smaller subunits called monomers.

What Are Monomers and Polymers?

A monomer is a small, basic molecular unit—often made of carbon, hydrogen, oxygen, and sometimes nitrogen or phosphorus—that can bond chemically with other similar units. When many monomers are linked together, they form a polymer.

You can think of monomers like individual beads on a necklace. A single bead isn't particularly useful on its own, but stringing them together creates a whole piece of jewelry. In the same way, polymer chains can be long, short, straight, branched, or even folded into complex shapes—giving them different functions and properties.

When two monomers are chemically bonded together, the result is called a dimer. If a third monomer is added, it becomes a trimer, and so on. In theory, you could keep naming these small chains based on the number of units, but in practice, we stop keeping track after just a few. Once a molecule is made up of many repeating monomers—typically dozens to thousands—we simply refer to it as a polymer. The key idea is that complex, functional molecules can be built by linking many simple building blocks together in a stepwise fashion.

Polymerization: Building Complexity from Simplicity

Polymerization is the process of joining small repeating units—called monomers—into larger, more complex chains. You can think

of it like snapping together toy bricks or beads on a string: one piece alone doesn't do much, but when you connect many of them, you can build something more functional or useful.

When two monomers are joined together, the result is called a dimer—just a simple pair. If you connect a third, you get a trimer. Add even more, and eventually you stop counting and just call it a polymer, meaning "many parts."

Here's an everyday example:

- A monomer is like a single paperclip.
- A dimer is two paperclips linked together.
- A polymer is a long chain of linked paperclips—something that starts to look like a necklace or a tool you could use to hold things together.

In real life, polymers can be found all around you. Plastic items like water bottles, food containers, or nylon clothing are all made of synthetic polymers—large materials built from repeating chemical units. Similarly, in your body and in nature, important materials like hair, rubber, and some types of glue are also made of natural polymers.

The key takeaway is this: by linking many simple units together, nature (and scientists) can build a huge variety of macromolecules with unique properties and functions.

Summary

Living things are carbon-based because carbon plays such a prominent role in the chemistry of living things. The four covalent bonding positions of the carbon atom can give rise to a wide diversity of compounds with many functions, accounting for the importance of carbon in living things. Monomers are small molecules that serve as building blocks for larger, more complex polymers. Through the process of polymerization, monomers can join to form dimers, trimers, and eventually polymers—many of which are essential to life.

Key Terms

dimer
: a molecule made of two subunits (or two monomers) that are chemically bonded together

macromolecule
: a large molecule, often formed by polymerization of smaller monomers

monomer
: a small, repeating molecular unit that can bond with other monomers to form larger molecules

polymer
: a large molecule made by linking together many monomers in a chain or network

polymerization
: the chemical process that joins monomers together to form a polymer

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3.2: Biological Molecules - Carbohydrates

Learning Objectives

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

- Explain how carbohydrates function as energy sources and structural materials in living organisms.
- Differentiate among monosaccharides, disaccharides, and polysaccharides based on structure and function.
- Compare key polysaccharides (starch, glycogen, cellulose, and chitin) and relate their structures to their biological roles.
- Apply carbohydrate concepts to real-world contexts, including nutrition, digestion, and health.

Carbohydrates

Carbohydrates are macromolecules with which most consumers are somewhat familiar. To lose weight, some individuals adhere to "low-carb" diets. Athletes, in contrast, often "carb-load" before important competitions to ensure that they have sufficient energy to compete at a high level. Carbohydrates are, in fact, an essential part of our diet; grains, fruits, and vegetables are all natural sources of carbohydrates. Carbohydrates provide energy to the body, particularly through glucose, a simple sugar. Carbohydrates also have other important functions in humans, animals, and plants.

Carbohydrates can be represented by the formula $(CH_2O)_n$, where n is the number of carbon atoms in the molecule. In other words, the ratio of carbon to hydrogen to oxygen is 1:2:1 in carbohydrate molecules. Carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides (mono- = "one"; sacchar- = "sweet") are simple sugars, the most common of which is glucose. In monosaccharides, the number of carbon atoms usually ranges from three to six. Most monosaccharide names end with the suffix -ose. Depending on the number of carbon atoms in the sugar, they may be known as trioses (three carbon atoms), pentoses (five carbon atoms), and hexoses (six carbon atoms).

Monosaccharides may exist as a linear chain or as ring-shaped molecules; in aqueous solutions, they are usually found in the ring form.

The chemical formula for glucose is $C_6H_{12}O_6$. In most living species, glucose is an important source of energy. During cellular respiration, energy is released from glucose, and that energy is used to help make adenosine triphosphate (ATP). Plants synthesize glucose using carbon dioxide and water by the process of photosynthesis, and the glucose, in turn, is used for the energy requirements of the plant. The excess synthesized glucose is often stored as starch that is broken down by other organisms that feed on plants.

Galactose (part of lactose, or milk sugar) and fructose (found in fruit) are other common monosaccharides. Although glucose, galactose, and fructose all have the same chemical formula ($C_6H_{12}O_6$), they differ structurally and chemically (and are known as isomers) because of differing arrangements of atoms in the carbon chain (**Figure** 3.2.3).

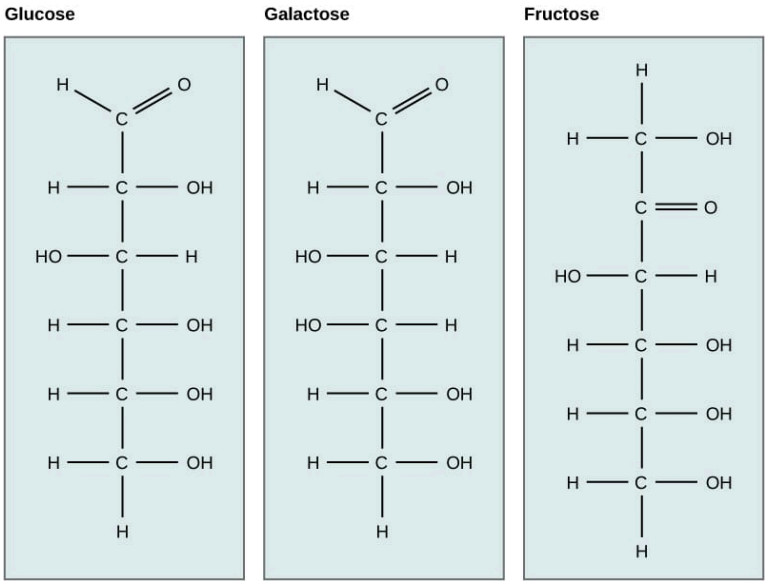


Figure 3.2.3: Glucose, galactose, and fructose are isomeric monosaccharides, meaning that they have the same chemical formula but slightly different structures.

Disaccharides (di- = “two”) form when two monosaccharides undergo a dehydration reaction (a reaction in which the removal of a water molecule occurs). 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.

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

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

Starch is the stored form of sugars in plants and is made up of amylose and amylopectin (both polymers of glucose). Plants are able to synthesize glucose, and the excess glucose is stored as starch in different plant parts, including roots and seeds. The starch that is consumed by animals is broken down into smaller molecules, such as glucose. The cells can then absorb the glucose.

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

Cellulose is one of the most abundant natural biopolymers, a polymer made by a living organism. 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. Cellulose is made up of glucose monomers that are linked by bonds between particular carbon atoms in the glucose molecule.

Every other glucose monomer in cellulose is flipped over and packed tightly as extended long chains. This gives cellulose its rigidity and high tensile strength—which is so important to plant cells. Cellulose passing through our 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 rumen (part of the digestive system of herbivores) and secrete the enzyme cellulase. The appendix also contains bacteria that break down cellulose, giving it an important role in the digestive systems of ruminants. 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. It is made of repeating units of a modified sugar containing nitrogen.

Thus, through differences in molecular structure, carbohydrates are able to serve the very different functions of energy storage (starch and glycogen) and structural support and protection (cellulose and chitin) (**Figure** 3.2.4).

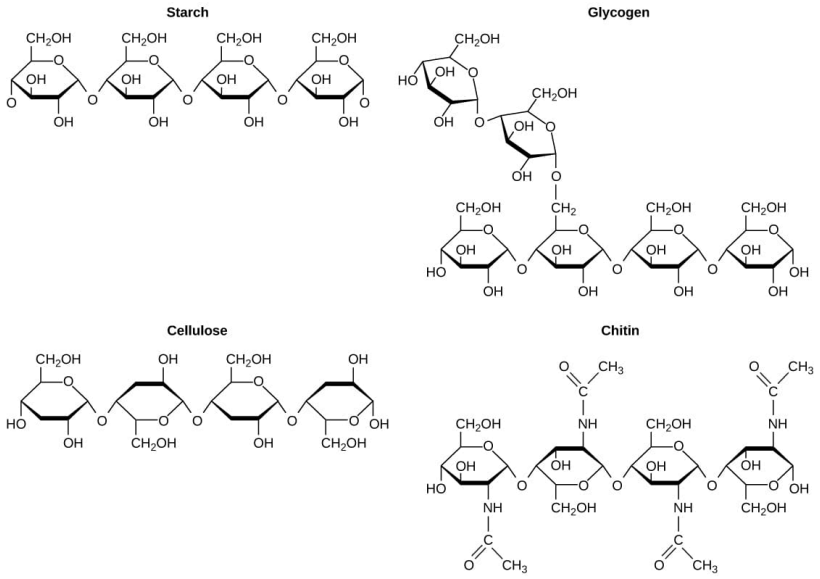


Figure 3.2.4: Although their structures and functions differ, all polysaccharide carbohydrates are made up of monosaccharides and have the chemical formula $(CH_2O)n$.

CAREERS IN ACTION: Registered Dietitian

Obesity is a worldwide health concern, and many diseases, such as diabetes and heart disease, are becoming more prevalent because of obesity. This is one of the reasons why registered dietitians are increasingly sought after for advice. Registered dietitians help plan food and nutrition programs for individuals in various settings. They often work with patients in health-care facilities, designing nutrition plans to prevent and treat diseases. For example, dietitians may teach a patient with diabetes how to manage blood-sugar levels by eating the correct types and amounts of carbohydrates. Dietitians may also work in nursing homes, schools, and private practices.

To become a registered dietitian, one needs to earn at least a bachelor's degree in dietetics, nutrition, food technology, or a related field. In addition, registered dietitians must complete a supervised internship program and pass a national exam. Those who pursue careers in dietetics take courses in nutrition, chemistry, biochemistry, biology, microbiology, and human physiology. Dietitians must become experts in the chemistry and functions of food (proteins, carbohydrates, and fats).

Summary

Carbohydrates are a group of macromolecules that are a vital energy source for the cell, provide structural support to many organisms, and can be found on the surface of the cell as receptors or for cell recognition. Carbohydrates are classified as monosaccharides, disaccharides, and polysaccharides, depending on the number of monomers in the molecule.

Key Terms

biopolymer
: a natural polymer produced by a living organism

carbohydrate
: a biological macromolecule in which the ratio of carbon to hydrogen to oxygen is 1:2:1; carbohydrates serve as energy sources and structural support in cells

cellulose
: a polysaccharide that makes up the cell walls of plants and provides structural support to the cell

chitin
: a type of carbohydrate that forms the outer skeleton of arthropods, such as insects and crustaceans, and the cell walls of fungi

disaccharide
: two sugar monomers that are linked together by a peptide bond

glycogen
: a storage carbohydrate in animals

macromolecule
: a large molecule, often formed by polymerization of smaller monomers

monosaccharide
: a single unit or monomer of carbohydrates

polysaccharide
: a long chain of monosaccharides; may be branched or unbranched

starch
: a storage carbohydrate in plants

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3.3: Biological Molecules - Lipids

Learning Objectives

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

- Describe the defining properties of lipids, including why they are hydrophobic and energy-dense.
- Distinguish among major lipid types (fats, phospholipids, steroids, and waxes) and their functions in cells and organisms.
- Compare saturated, unsaturated, and trans fats and explain how structural differences influence health outcomes.
- Explain how phospholipids and cholesterol contribute to cell membrane structure and function.

Lipids

Lipids include a diverse group of compounds that are united by a common feature. Lipids are hydrophobic ("water-fearing"), or insoluble in water, because they are nonpolar molecules. This is because they are hydrocarbons that include only nonpolar carbon-carbon or carbon-hydrogen bonds. Lipids perform many different functions in a cell. Cells store energy for long-term use in the form of lipids called fats. Lipids also provide insulation from the environment for plants and animals (Figure 3.3.5). For example, they help keep aquatic birds and mammals dry because of their water-repelling nature. Lipids are also the building blocks of many hormones and are an important constituent of the plasma membrane. Lipids include fats, oils, waxes, phospholipids, and steroids.



Figure 3.3.5: Hydrophobic lipids in the fur of aquatic mammals, such as this river otter, protect them from the elements. (credit: Ken Bosma)

A fat molecule, such as a triglyceride, consists of two main components—glycerol and fatty acids. Glycerol is an organic compound with three carbon atoms, five hydrogen atoms, and three hydroxyl (–OH) groups. Fatty acids have a long chain of hydrocarbons to which an acidic 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. In a fat molecule, a fatty acid is attached to each of the three oxygen atoms in the –OH groups of the glycerol molecule with a covalent bond (**Figure** 3.3.6).

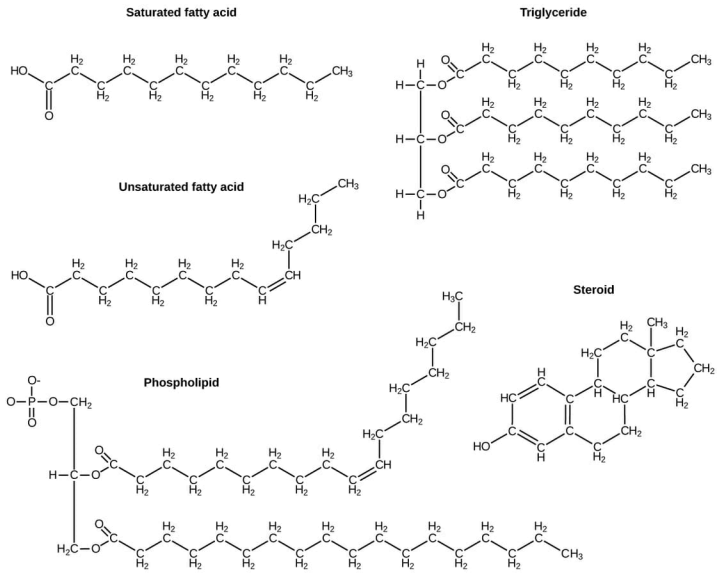


Figure 3.3.6: Lipids include fats, such as triglycerides, which are made up of fatty acids and glycerol, phospholipids, and steroids. Images of the molecular structures of a saturated fatty acid, unsaturated fatty acid, triglyceride, steroid, and phospholipid.

During this covalent bond formation, three water molecules are released. The three fatty acids in the fat may be similar or dissimilar. These fats are also called triglycerides because they have three fatty acids. Some fatty acids have common names that specify their origin. For example, palmitic acid, a saturated fatty acid, is derived from the palm tree. Arachidic acid is derived from *Arachis hypogaea*, the scientific name for peanuts.

Fatty acids may be saturated or unsaturated. In a fatty acid chain, if there are only single bonds between neighboring carbons in the hydrocarbon chain, the fatty acid is saturated. Saturated fatty acids are saturated with hydrogen; in other words, the number of hydrogen atoms attached to the carbon skeleton is maximized.

When the hydrocarbon chain contains a double bond, the fatty acid is an unsaturated fatty acid.

Most unsaturated fats are liquid at room temperature and are called oils. If there is one double bond in the molecule, then it is known as a monounsaturated fat (e.g., olive oil), and if there is more than one double bond, then it is known as a polyunsaturated fat (e.g., canola oil).

Saturated fats tend to get packed tightly and are solid at room temperature. Animal fats with stearic acid and palmitic acid contained in meat, and the fat with butyric acid contained in butter, are examples of saturated fats. Mammals store fats in specialized cells called adipocytes, where globules of fat occupy most of the cell. In plants, fat or oil is stored in seeds and is used as a source of energy during embryonic development.

Unsaturated fats or oils are usually of plant origin and contain unsaturated fatty acids. The double bond causes a bend or a “kink” that prevents the fatty acids from packing tightly, keeping them liquid at room temperature. Olive oil, corn oil, canola oil, and cod liver oil are examples of unsaturated fats. Unsaturated fats help to improve blood cholesterol levels, whereas saturated fats contribute to plaque formation in the arteries, which increases the risk of a heart attack.

In the food industry, oils are artificially hydrogenated to make them semi-solid, leading to less spoilage and increased shelf life. Simply speaking, hydrogen gas is bubbled through oils to solidify them. During this hydrogenation process, double bonds of the *cis*-conformation in the hydrocarbon chain may be converted to double bonds in the *trans*-conformation. This forms a *trans*-fat from a *cis*-fat. The orientation of the double bonds affects the chemical properties of the fat (Figure 3.3.7).

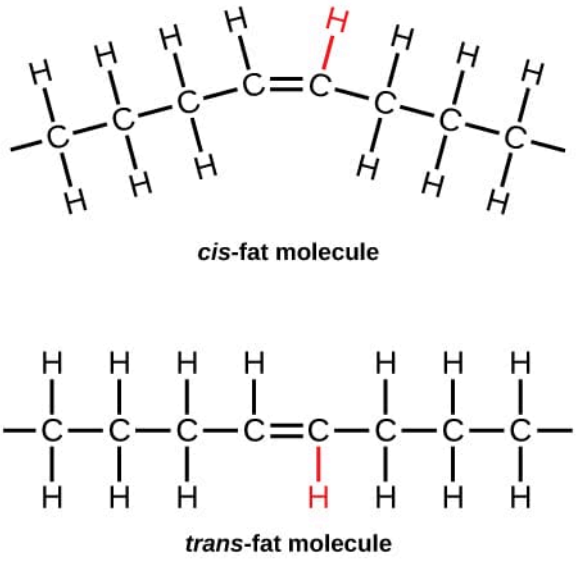


Figure 3.3.7: During the hydrogenation process, the orientation around the double bonds is changed, making a trans-fat from a cis-fat. This changes the chemical properties of the molecule.

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 lipoprotein (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 eliminated the use of *trans*-fats, and U.S. food labels are now required to list their *trans*-fat content.

Essential fatty acids are fatty acids that are required but not synthesized by the human body. Consequently, they must be supplemented through the diet. Omega-3 fatty acids fall into this category and are one of only two known essential fatty acids for humans (the other being omega-6 fatty acids). They are a type of polyunsaturated fat and are called omega-3 fatty acids because the third carbon from the end of the fatty acid participates in a double bond.

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

Like carbohydrates, fats 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. Fats serve as long-term energy storage. They also provide insulation for the body. Therefore, "healthy" unsaturated fats in moderate amounts should be consumed on a regular basis.

Phospholipids

Phospholipids are the major constituent of the plasma membrane. Like fats, they are composed of fatty acid chains attached to a glycerol or similar backbone. Instead of three fatty acids attached, however, there are two fatty acids and the third carbon of the glycerol backbone is bound to a phosphate group. The phosphate group is modified by the addition of an alcohol.

A phospholipid has both hydrophobic and hydrophilic regions. The fatty acid chains are hydrophobic and exclude themselves from water, whereas the phosphate is hydrophilic and interacts with water.

Cells are surrounded by a membrane, which has a bilayer of phospholipids. The fatty acids of phospholipids face inside, away from water, whereas the phosphate group can face either the outside environment or the inside of the cell, which are both aqueous.

Steroids and Waxes

Unlike the phospholipids and fats discussed earlier, steroids have a ring structure. Although they do not resemble other lipids, they are grouped with them because they are also hydrophobic. All steroids have four, linked carbon rings and several of them, like cholesterol, have a short tail.

Cholesterol is a steroid. Cholesterol is mainly synthesized in the liver and is the precursor of many steroid hormones, such as

testosterone and estradiol. It is also the precursor of vitamins E and K. Cholesterol is the precursor of bile salts, which help in the breakdown of fats and their subsequent absorption by cells. Although cholesterol is often spoken of in negative terms, it is necessary for the proper functioning of the body. It is a key component of the plasma membranes of animal cells.

Waxes are made up of a hydrocarbon chain with an alcohol (–OH) group and a fatty acid. Examples of animal waxes include beeswax and lanolin. Plants also have waxes, such as the coating on their leaves, that helps prevent them from drying out.

CONCEPT IN ACTION

For an additional perspective on lipids, explore "Biomolecules: The Lipids" through this Unsaturated vs Saturated vs Trans Fats animation.

Summary

Lipids are a class of macromolecules that are nonpolar and hydrophobic in nature. Major types include fats and oils, waxes, phospholipids, and steroids. Fats and oils are a stored form of energy and can include triglycerides. Fats and oils are usually made up of fatty acids and glycerol.

Key Terms

fat
: a lipid molecule composed of three fatty acids and a glycerol (triglyceride) that typically exists in a solid form at room temperature

hormone
: a chemical signaling molecule, usually a protein or steroid, secreted by an endocrine gland or group of endocrine cells; acts to control or regulate specific physiological processes

lipids
: a class of macromolecules that are nonpolar and insoluble in water

oil
: an unsaturated fat that is a liquid at room temperature

phospholipid
: a major constituent of the membranes of cells; composed of two fatty acids and a phosphate group attached to the glycerol backbone

saturated fatty acid
: a long-chain hydrocarbon with single covalent bonds in the carbon chain; the number of hydrogen atoms attached to the carbon skeleton is maximized

steroid
: a type of lipid composed of four fused hydrocarbon rings

***trans*-fat**
: a form of unsaturated fat with the hydrogen atoms neighboring the double bond across from each other rather than on the same side of the double bond

triglyceride
: a fat molecule; consists of three fatty acids linked to a glycerol molecule

unsaturated fatty acid
: a long-chain hydrocarbon that has one or more than one double bonds in the hydrocarbon chain

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3.4: Biological Molecules - Nucleic Acids

Learning Objectives

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

- Explain the role of nucleic acids in storing and transmitting genetic information.
- Differentiate between DNA and RNA based on structure and cellular function.
- Identify the components of a nucleotide and describe how nucleotides link to form nucleic acids.
- Describe the basic structure of DNA, including the double helix and complementary base pairing.

Nucleic Acids

Nucleic acids are key macromolecules in 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). DNA is the genetic material found in all living organisms, ranging from single-celled bacteria to multicellular mammals.

The other type of nucleic acid, RNA, is mostly involved in protein synthesis. The DNA molecules never leave the nucleus, but instead use an RNA intermediary to communicate with the rest of the cell. Other types of RNA are also involved in protein synthesis and its regulation.

DNA and RNA are made up of monomers known as nucleotides. The nucleotides combine with each other to form a polynucleotide, DNA or RNA. Each nucleotide is made up of three components: a nitrogenous base, a pentose (five-carbon) sugar, and a phosphate group (**Figure** 3.4.1). Each nitrogenous base in a nucleotide is attached to a sugar molecule, which is attached to a phosphate group.

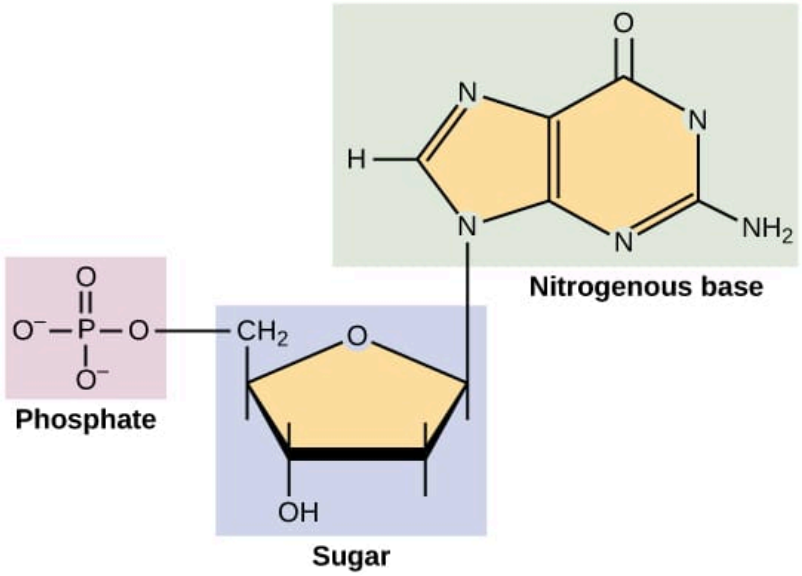


Figure 3.4.1: A nucleotide is made up of three components: a nitrogenous base, a pentose sugar, and a phosphate group.

DNA Double-Helical Structure

DNA has a double-helical structure (**Figure** 3.4.2). It is composed of two strands, or polymers, of nucleotides. The strands are formed with bonds between phosphate and sugar groups of adjacent nucleotides. The strands are bonded to each other at their

bases with hydrogen bonds, and the strands coil about each other along their length, hence the “double helix” description, which means a double spiral.

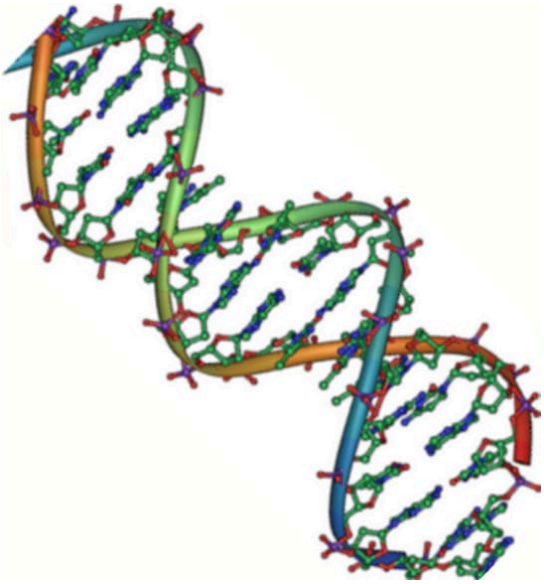


Figure 3.4.2: The double-helix model shows DNA as two parallel strands of intertwining molecules. (credit: Jerome Walker, Dennis Myts)

The alternating sugar and phosphate groups lie on the outside of each strand, forming the backbone of the DNA. The nitrogenous bases are stacked in the interior, like the steps of a staircase, and these bases pair; the pairs are bound to each other by hydrogen bonds. The bases pair in such a way that the distance between the backbones of the two strands is the same all along the molecule.

Summary

Nucleic acids are molecules made up of repeating units of nucleotides that direct cellular activities such as cell division and protein synthesis. Each nucleotide is made up of a pentose sugar, a nitrogenous base, and a phosphate group. There are two types of nucleic acids: DNA and RNA.

Key Terms

deoxyribonucleic acid (DNA)
: a double stranded polymer of nucleotides that stores genetic information in living organisms

nucleic acid
: a biological macromolecule that carries the genetic information of a cell and carries instructions for the functioning of the cell

nucleotide
: a monomer of nucleic acids; contains a pentose sugar, a phosphate group, and a nitrogenous base

ribonucleic acid (RNA)
: a single-stranded polymer of nucleotides that is involved in protein synthesis

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3.5: Proteins - Structure

Learning Objectives

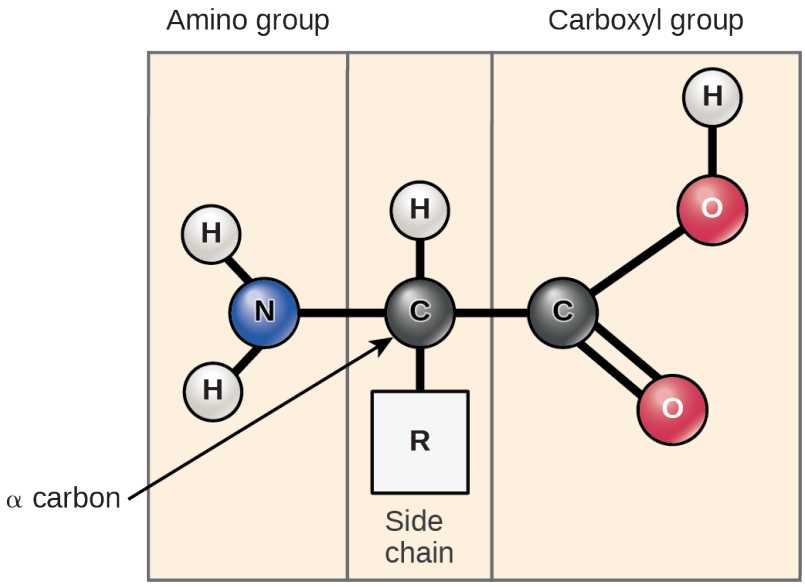
By the end of this section, you will be able to do the following:

- Describe the basic structure of amino acids
- Discuss the relationship between amino acids and proteins
- Evaluate how variations in R groups contribute to protein diversity
- Explain the four levels of protein organization

Proteins are one of the most abundant and functionally diverse macromolecules found in living organisms. To understand why proteins are so versatile, we must first understand their structure. Like all polymers, proteins are composed of smaller building blocks known as monomers. In the case of proteins, these monomers are called amino acids.

Amino Acids

Amino acids are the monomers that comprise proteins. There are 20 different amino acids that are commonly used by cells to build proteins. Each amino acid shares a common core structure: a central carbon atom sometimes referred to as the alpha carbon (α) bonded to four different groups—a hydrogen atom, an amino group (NH_2), a carboxyl group (COOH), and a unique side chain known as the R group (**Figure** 3.5.1). Scientists use the name "amino acid" because these acids contain both amino group and carboxyl-acid-group in their basic structure.

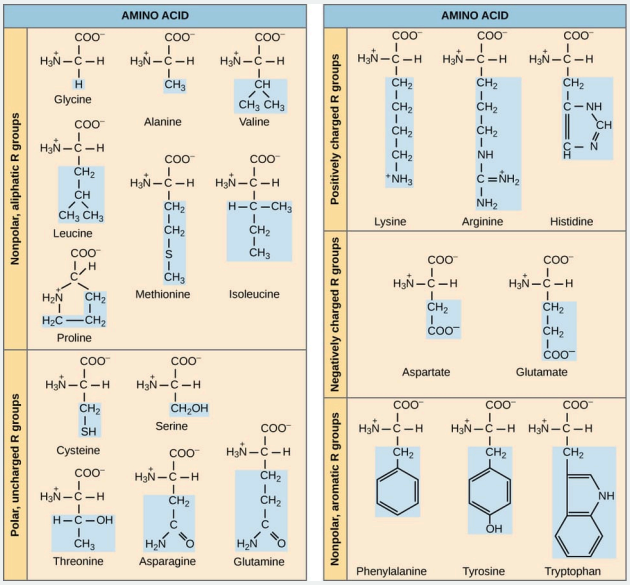


(**Figure** 3.5.1) The molecular structure of an amino acid is shown above. Amino acids have a central asymmetric carbon (alpha carbon), to which an amino group, a carboxyl group, a hydrogen atom, and a side chain are attached. The side chain varies for different amino acids, and is designated as the R - group.

It is the R group that distinguishes one amino acid from another. Some R groups are small and nonpolar (hydrophobic), like those found in alanine, while others are large and polar (hydrophilic), like those found in glutamic acid.

As we mentioned, there are 20 common amino acids present in proteins. Nine of these are essential amino acids in humans because the human body cannot produce them and we obtain them from our diet. For each amino acid, the R group (or side chain) is different (**Figure** 3.5.2). Some R groups are small and nonpolar (hydrophobic), like those found in alanine, while others are large and polar (hydrophilic), like those found in glutamic acid.

Visual Connection

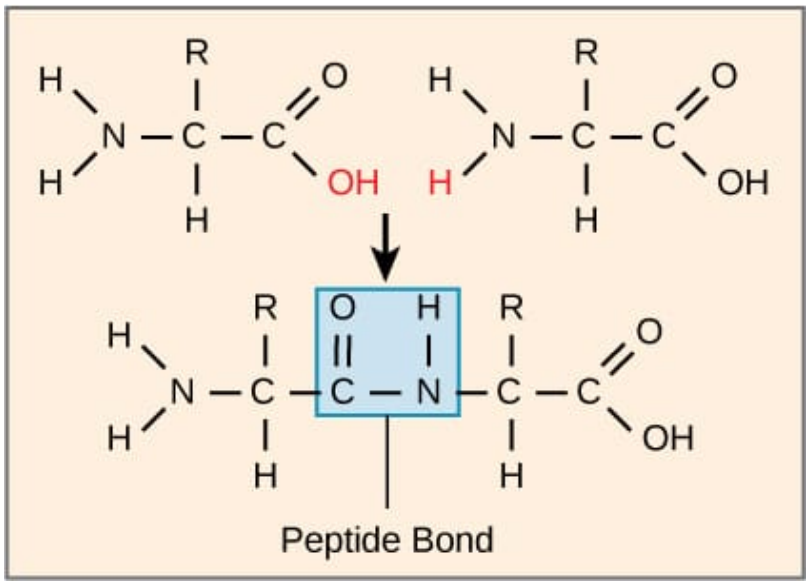


(**Figure** 3.5.2) There are 20 common amino acids commonly found in proteins, each with a different R group that determines its chemical nature.The molecular structures of the twenty amino acids commonly found in proteins are shown above. These are divided into five categories: nonpolar aliphatic, polar uncharged, positively charged, negatively charged, and aromatic. Nonpolar aliphatic amino acids include glycine, alanine, valine, leucine, methionine, isoleucine, and proline. Polar uncharged amino acids include serine, threonine, cysteine, asparagine, and glutamine. Positively charged amino acids include lysine, arginine, and histidine. Negatively charged amino acids include aspartate and glutamate. Aromatic amino acids include phenylalanine, tyrosine, and tryptophan. For example, in the amino acid glycine, the R group is a single hydrogen; but in alanine the R group is upper C upper H subscript 3 baseline.

The chemical nature of the side chain determines the amino acid's nature (that is, whether it is acidic, basic, polar, or nonpolar). For example, the amino acid glycine has a hydrogen atom as the R group. Amino acids such as valine, methionine, and alanine are nonpolar or hydrophobic in nature, while amino acids such as serine, threonine, and cysteine are polar and have hydrophilic side chains. The side chains of lysine and arginine are positively charged, and therefore these amino acids are also basic amino acids. Proline has an R group that is linked to the amino group, forming a ring-like structure. Proline is an exception to the amino acid's standard structure since its amino group is not separate from the side chain (**Figure** 3.5.2) .

The chemical properties of the R groups—whether they are charged, uncharged, polar, or nonpolar—play a critical role in determining how a protein folds and ultimately what shape it takes. Think of the R groups as different colored and shaped puzzle pieces; depending on their properties, some are attracted to each other, some repel each other, and some try to avoid water altogether. This interplay influences the final three-dimensional shape of the protein.

The sequence and the number of amino acids ultimately determine the protein's shape, size, and function. A covalent bond, or peptide bond, attaches to each amino acid, which a dehydration reaction forms. One amino acid's carboxyl group and the incoming amino acid's amino group combine, releasing a water molecule. The resulting bond is the peptide bond (**Figure** 3.5.3) .



(Figure 3.5.3): Peptide bond formation is a dehydration synthesis reaction. The formation of a peptide bond between two amino acids is shown. When the peptide bond forms, the carbon from the carbonyl group becomes attached to the nitrogen from the amino group. The upper O upper H from the carboxyl group and an upper H from the amino group form a molecule of water.

The products that such linkages form are peptides. As more amino acids join to this growing chain, the resulting chain is a polypeptide. Each polypeptide has a free amino group at one end. This end the N terminal, or the amino terminal, and the other end has a free carboxyl group, also the C or carboxyl terminal. 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 combined together, often have bound non-peptide prosthetic groups, have a distinct shape, and have a unique function. After protein synthesis (translation), most proteins are modified. These are known as post-translational modifications. They may undergo cleavage, phosphorylation, or may require adding other chemical groups. Only after these modifications is the protein completely functional.

Link to Learning

Click through this interactive tutorial for the steps of protein synthesis (opens in new window).

Protein Structure

As discussed earlier, the shape of a protein is critical to its function. To understand how the protein gets its final shape or conformation, we need to understand the four levels of protein structure: primary, secondary, tertiary, and quaternary (**Figure** 3.5.4)

The unique sequence and number of amino acids in a polypeptide chain is its primary structure. The unique sequence for every protein is ultimately determined by the gene that encodes the protein. Any change in the gene sequence may lead to a different amino acid being added to the polypeptide chain, causing a change in protein structure and function. In sickle cell anemia, the hemoglobin β chain has a single amino acid substitution, causing a change in both the structure and function of the protein. What is most remarkable to consider is that a hemoglobin molecule is made up of two alpha chains and two beta chains that each consist of about 150 amino acids. The molecule, therefore, has about 600 amino acids. The structural difference between a normal hemoglobin molecule and a sickle cell molecule—that dramatically decreases life expectancy in the affected individuals—is a single amino acid of the 600.

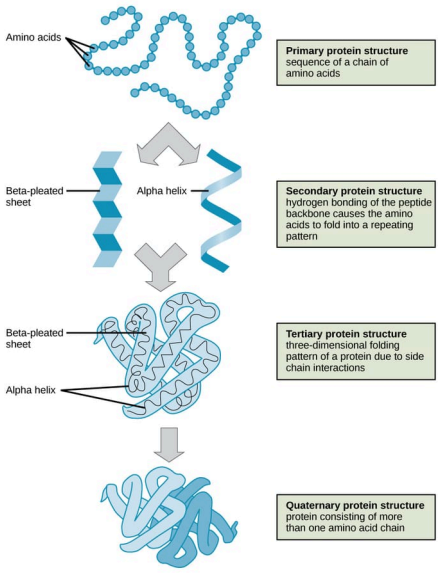
Because of this change of one amino acid in the chain, the normally biconcave, or disc-shaped, red blood cells assume a crescent or “sickle” shape, which clogs arteries. This can lead to a myriad of serious health problems, such as breathlessness, dizziness, headaches, and abdominal pain for those who have this disease.

Folding patterns resulting from interactions between the non-R group portions of amino acids give rise to the secondary structure of the protein. The most common are the alpha (α)-helix and beta (β)-pleated sheet structures. Both structures are held in shape by hydrogen bonds. In the alpha helix, the bonds form between every fourth amino acid and cause a twist in the amino acid chain.

In the β-pleated sheet, the “pleats” are formed by hydrogen bonding between atoms on the backbone of the polypeptide chain. The R groups are attached to the carbons, and extend above and below the folds of the pleat. The pleated segments align parallel to each other, and hydrogen bonds form between the same pairs of atoms on each of the aligned amino acids. The α-helix and β-pleated sheet structures are found in many globular and fibrous proteins.

The unique three-dimensional structure of a polypeptide is known as its tertiary structure. This structure is caused by chemical interactions between various amino acids and regions of the polypeptide. Primarily, the interactions among R groups create the complex three-dimensional tertiary structure of a protein. There may be ionic bonds formed between R groups on different amino acids, or hydrogen bonding beyond that involved in the secondary structure. When protein folding takes place, the hydrophobic R groups of nonpolar amino acids lay in the interior of the protein, whereas the hydrophilic R groups lay on the outside. The former types of interactions are also known as hydrophobic interactions.

In nature, some proteins are formed from several polypeptides, also known as subunits, and the interaction of these subunits forms the quaternary structure. Weak interactions between the subunits help to stabilize the overall structure. Hemoglobin is a good example of a protein with quaternary structure; it consists of four polypeptide subunits that work together to carry oxygen in the blood.



(**Figure** 3.5.4) The four levels of protein structure can be observed in these illustrations. (credit: modification of work by National Human Genome Research Institute)

Open for image description. This diagram illustrates the four levels of protein structure, arranged from top to bottom to show increasing complexity.

At the top, the primary protein structure is depicted as a long chain of connected blue circles representing individual amino acids linked together in a specific sequence. An arrow points to several of the circles and labels them "Amino acids." A text box on the right reads: "Primary protein structure: sequence of a chain of amino acids."

Below this, the diagram shows the secondary protein structure, where portions of the amino acid chain fold into repeating shapes. On the left is a beta-pleated sheet, represented as a stack of folded, zigzag ribbon segments. On the right is an alpha helix, shown as a spiral, ribbon-like coil. A text box explains: "Secondary protein structure: hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern." Arrows indicate the transition from the primary structure to these folded forms.

Further down, the tertiary protein structure is illustrated as a single protein folded into a complex three-dimensional shape. Within the folded structure, alpha helices appear as coiled ribbons and beta-pleated sheets appear as flattened folded regions. Labels identify both structural elements. The protein is shown as a compact, tangled form created by the folding and interaction of secondary structures. A text box states: "Tertiary protein structure: three-dimensional folding pattern of a protein due to side chain interactions."

At the bottom, the quaternary protein structure is represented by a larger assembly of intertwined ribbon-like structures. This level shows multiple folded protein chains associating to form a functional protein complex. A text box reads: "Quaternary protein structure: protein consisting of more than one amino acid chain."

Gray arrows between each level emphasize the progression from a simple linear amino acid sequence to folded structures, then to a fully folded protein, and finally to an assembly of multiple protein subunits. The diagram demonstrates how proteins become increasingly complex as they fold and combine into their functional forms.

Each protein has its own unique sequence and three-dimensional shape, held together by chemical interactions between its parts. This shape is not just a random arrangement—it is critical for the protein's ability to do its job. If the shape changes, the function often changes too.

Proteins can lose their shape when exposed to changes in temperature, pH, or certain chemicals. This process is called denaturation, and it can affect how well—or if—a protein works. Sometimes this shape change is reversible. If the conditions return to normal and the basic sequence of the protein (its primary structure) remains intact, the protein may fold back into its original shape and function again. In other cases, denaturation is permanent, and the protein loses its function completely.

You've likely seen an example of protein denaturation in the kitchen. When you fry or boil an egg, the heat causes proteins in

the egg white, like albumin, to denature. This turns the egg white from clear and liquid to white and solid. Not all proteins are sensitive to high temperatures, however. Some bacteria that live in hot springs produce proteins that are specially adapted to remain stable and functional even under extreme heat.

While we'll explore the different jobs that proteins perform in the next section, it's important to keep in mind that a protein's structure—its precise shape—is key to its function. When that shape changes, so does what the protein can (or can't) do.

CONCEPT IN ACTION

For an additional perspective on proteins, explore this "Biomolecules: The Proteins" interactive animation.

Summary

Proteins are a class of macromolecules that can perform a diverse range of functions for the cell. They help in metabolism by providing structural support and by acting as enzymes, carriers or as hormones. The building blocks of proteins are amino acids. Proteins are organized at four levels: primary, secondary, tertiary, and quaternary. Protein shape and function are intricately linked; any change in shape caused by changes in temperature, pH, or chemical exposure may lead to protein denaturation and a loss of function.

Key Terms

amino ($-NH_2$)
: a functional group found in amino acids and proteins

amino acid
: a monomer of a protein

carboxyl (–COOH)
: functional group that gives molecules acidic properties; found in amino acids and fatty acids

denaturation
: the loss of shape in a protein as a result of changes in temperature, pH, or exposure to chemicals

enzyme
: a catalyst in a biochemical reaction that is usually a complex or conjugated protein

polypeptide
: a long chain of amino acids linked by peptide bonds

protein
: a biological macromolecule composed of one or more chains of amino acids

R-group
: the variable side chain of an amino acid that determines its properties

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3.6: Proteins - Function

Learning Objectives

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

- Identify and describe the major functions of proteins in living systems
- Explain how protein structure determines function
- Describe common types of protein domains and their roles
- Understand how domains contribute to diverse protein functions
- Explain how changes in structure can affect function

Proteins are one of the most abundant and functionally diverse macromolecules found in living organisms. To understand why proteins are so versatile, we must first understand their structure. Like all polymers, proteins are composed of smaller building blocks known as monomers. In the case of proteins, these monomers are called amino acids. The order of amino acids gives each protein its unique structure, and that structure determines its function.

Structure Determines Function

Proteins are folded into three-dimensional shapes that allow them to do specific jobs. A protein's shape is stabilized by a variety of interactions between its amino acids, including hydrogen bonds, ionic bonds, and hydrophobic interactions. When this shape is disrupted—by changes in pH, temperature, or chemicals—the protein may lose its function. This is called denaturation. A familiar example is the egg white turning solid and white when cooked: heat denatures the albumin protein.

Most proteins do not function as a whole uniform shape but instead have separate regions called domains. Each domain typically performs a distinct function, like binding to DNA, RNA, other proteins, or small molecules. These domains can be thought of as modular components—like building blocks—that can be reused in different proteins across evolution. A protein may contain one domain or several, and together these domains give the protein its full function.

Think of a smartphone: it has separate components that each do a specific job—one for the screen, one for the camera, one for the microphone. Similarly, proteins have domains for interacting with other molecules. DNA-binding domains help regulate genes. RNA-binding domains help control gene expression. Protein-protein interaction domains allow proteins to form complexes or signaling chains. Some proteins may even combine two or more of these domains.

Are you wondering whether scientists can combine domains to design new proteins? They can—and they do. In biotechnology, researchers routinely design proteins by assembling domains from different sources. One familiar example is fluorescent fusion proteins used in laboratory research: scientists can take a protein of interest and attach a green fluorescent protein (GFP) domain to it. This allows them to visually track where the protein goes in the cell. Another example with more widespread societal impact is the creation of chimeric antigen receptors (CARs) in CAR T-cell therapy. These engineered proteins combine domains that recognize cancer cells with domains that activate immune responses.

Protein Function

With a solid understanding of how proteins are built and folded, we can now explore the many functions proteins perform in living systems. Because of their diverse shapes and chemical properties, proteins can serve nearly every role required to sustain life.

Structural Support

Some proteins serve as the scaffolding of cells and tissues. These structural proteins provide support, strength, and shape. Collagen, for example, is a fibrous protein in skin, tendons, and ligaments, giving tissues elasticity and durability. Another example is keratin, the protein in hair, nails, and skin. These proteins are composed of amino acid sequences that encourage extended structural formations like alpha-helices, making them tough and resilient. Structural proteins typically contain domains that allow for long, stable interactions between molecules, enabling them to form fibers and sheets.

Communication and Signaling

Proteins also help cells detect and respond to signals. Receptor proteins are embedded in cell membranes and act like molecular antennas. When a signal molecule (ligand) binds to the receptor's extracellular domain, it changes the protein's shape and initiates a response inside the cell. This precise molecular communication is critical for processes like growth, immune responses, and nervous system function. For example, when insulin binds to its receptor on a muscle cell, it sets off a chain reaction that allows glucose to enter the cell. These proteins often contain extracellular ligand-binding domains and intracellular signaling domains. We will discuss this in more detail in Chapter 5 - Response to the Environment.

Transport of Molecules

Proteins are essential for moving substances in and out of cells and around the body. Hemoglobin binds oxygen in the lungs and delivers it to tissues. Membrane transport proteins, such as channels and carriers, control which substances cross cell membranes. These proteins often contain transmembrane domains that create a tunnel or pathway through the membrane. Think of these proteins like subway turnstiles—only the right ticket (molecule) can pass through. They may also have specific binding domains that recognize the molecule to be transported.

Movement

Proteins enable movement, both at the cellular level and in the body. Actin and myosin, proteins found in muscle tissue, interact to contract and relax muscles. Inside cells, proteins help move organelles, vesicles, and other molecules. Flagella, whip-like tails that some bacteria use to swim, are made of protein as well. Movement proteins often have domains that allow them to bind to other cellular components and undergo shape changes that drive motion.

Energy Storage

Some proteins serve as nutrient reserves. Casein in milk supplies amino acids for young mammals. Ovalbumin in egg whites serves a similar function for developing embryos. These storage proteins don't usually need specialized domains for complex interactions. Their value lies in the sheer amount of amino acids they provide, rather than their intricate shape or activity.

Enzymes: Biological Catalysts

One of the most remarkable protein functions is serving as enzymes. Enzymes speed up chemical reactions by lowering the energy needed to get started. Each enzyme has a specific target molecule (substrate) that fits into a region of the enzyme called the active site. This region is usually a carefully shaped domain that allows the enzyme to interact only with certain molecules—much like a lock fits a specific key.

Enzymes are vital for processes like digesting food, copying DNA, and producing energy. For example, the enzyme amylase in your saliva starts breaking down starch the moment you chew.

Proteins in DNA Replication and Protein Synthesis

Although covered in more detail in later chapters, it's worth noting here that proteins are key players in DNA replication and protein synthesis. During DNA replication, proteins with DNA-binding domains help unwind DNA, copy strands, and check for errors. In protein synthesis, RNA-binding proteins play crucial roles in assembling ribosomes and translating the RNA message into proteins. These domains ensure the right molecules are recognized and acted upon at the right time.

Summary

Proteins perform a remarkable range of tasks in the cell—structural support, signaling, transport, movement, storage, catalysis, and information transfer—thanks to their diverse shapes and the presence of specialized domains. Domains function like modular parts that give proteins their unique capabilities. Some proteins have a single domain, while others combine multiple domains to perform more complex tasks. This modularity not only makes proteins versatile in nature but also makes them powerful tools in biotechnology, where scientists combine domains to build proteins with new, targeted functions. Understanding the relationship between protein structure, domains, and function opens the door to appreciating both biology and bioengineering.

Key Terms

domain
: a region on a protein specialized for attaching to specific molecules like DNA, RNA, or other proteins

denaturation
: loss of a protein's shape (and often function) due to environmental changes such as heat or pH

enzyme
: a protein that speeds up chemical reactions without being consumed in the process

ligand
: a molecule that binds specifically to a receptor site of another molecule

modular
: made of interchangeable or combinable parts; used here to describe how protein domains can be rearranged in different proteins

receptor
: a protein that receives signals and triggers a response inside a cell substrate – the molecule an enzyme acts upon

transmembrane domain
: the part of a protein that spans the membrane, usually composed of hydrophobic amino acids

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4: HOW CELLS OBTAIN ENERGY

4.1: Energy and Metabolism

4.2: Enzymes in Action

4.3: Glycolysis

4.4: Citric Acid Cycle and Oxidative Phosphorylation

4.5: Fermentation

4.6: How Cells Obtain Energy (Exercises)

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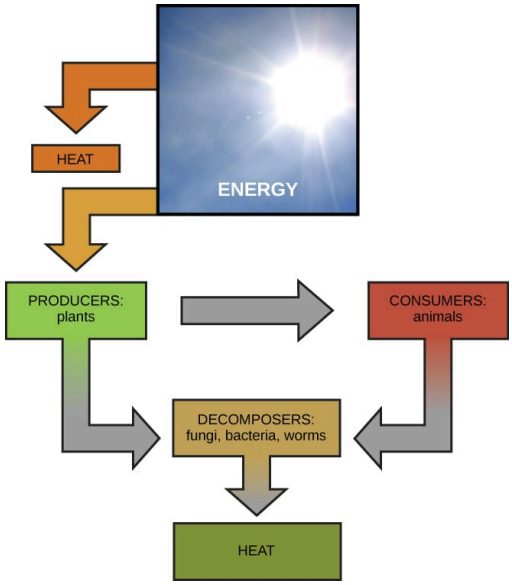
4.1: Energy and Metabolism

Learning Objectives

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

- Explain metabolic pathways and describe the two major types
- Discuss how chemical reactions play a role in energy transfer
- State the first and second laws of thermodynamics
- Explain the difference between kinetic and potential energy
- Describe endergonic and exergonic reactions
- Discuss how enzymes function as molecular catalysts

Scientists use the term bioenergetics to describe the concept of energy flow (**Figure** 4.1.1) through living systems, such as cells. Cellular processes such as the building and breaking down of complex molecules occur through stepwise chemical reactions. Some of these chemical reactions are spontaneous and release energy, whereas others require energy to proceed. Just as living things must continually consume food to replenish their energy supplies, cells must continually produce more energy to replenish that used by the many energy-requiring chemical reactions that constantly take place. Together, all of the chemical reactions that take place inside cells, including those that consume or generate energy, are referred to as the cell's metabolism.



(**Figure** 4.1.1) Ultimately, most life forms get their energy from the sun, as shown above. Producers like plants use photosynthesis to capture sunlight, and consumers like herbivores eat the plants to obtain energy. Carnivores eat the herbivores, and eventual decomposition of plant and animal material contributes to the nutrient pool.

Metabolic Pathways

Consider the metabolism of sugar. This is a classic example of one of the many cellular processes that use and produce energy. Living things consume sugars as a major energy source, because sugar molecules have a great deal of energy stored within their bonds. For the most part, photosynthesizing organisms like plants produce these sugars. During photosynthesis, plants use energy (originally from sunlight) to convert carbon dioxide gas (CO_2) into sugar molecules (like glucose: $C_6H_{12}O_6$). They consume carbon dioxide and produce oxygen as a waste product. This reaction is summarized as:

$$6\,CO_2 + 6\,H_2O \longrightarrow C_6H_{12}O_6 + 6\,O_2$$

Because this process involves synthesizing an energy-storing molecule, it requires energy input to proceed. During the light

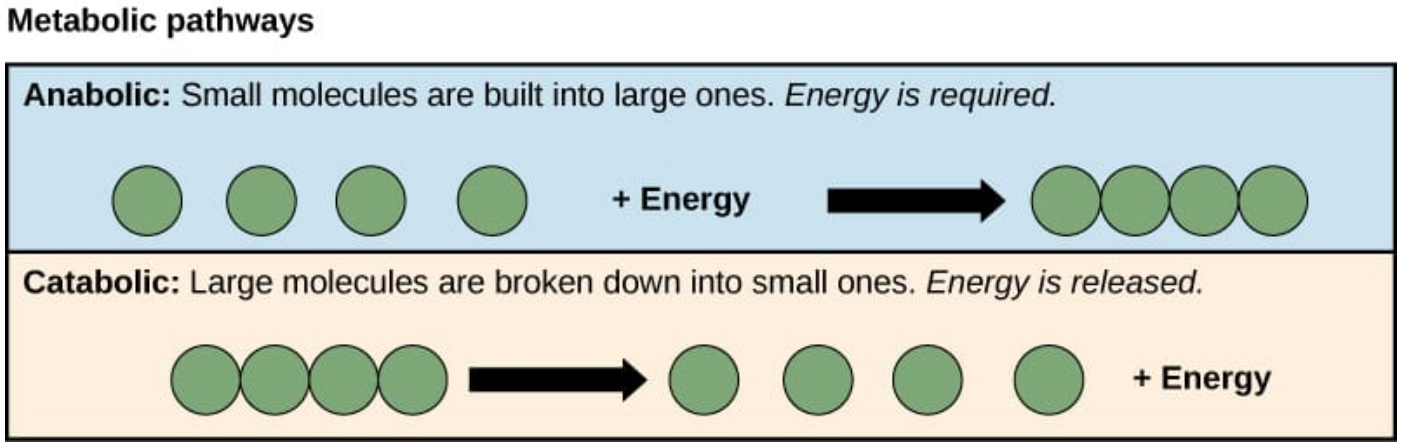
reactions of photosynthesis, energy is provided by a molecule called adenosine triphosphate (ATP), which is the primary energy currency of all cells. Just as the dollar is used as currency to buy goods, cells use molecules of ATP as energy currency to perform immediate work. In contrast, energy-storage molecules such as glucose are consumed only to be broken down to use their energy. The reaction that harvests the energy of a sugar molecule in cells requiring oxygen to survive can be summarized by the reverse reaction to photosynthesis. In this reaction, oxygen is consumed and carbon dioxide is released as a waste product. The reaction is summarized as:

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,H_2O + 6\,CO_2$$

Both of these reactions involve many steps.

The processes of making and breaking down sugar molecules illustrate two examples of metabolic pathways. A metabolic pathway is a series of chemical reactions that takes a starting molecule and modifies it, step-by-step, through a series of metabolic intermediates, eventually yielding a final product. In the example of sugar metabolism, the first metabolic pathway synthesized sugar from smaller molecules, and the other pathway broke sugar down into smaller molecules. These two opposite processes—the first requiring energy and the second producing energy—are referred to as anabolic pathways (building polymers) and catabolic pathways (breaking down polymers into their monomers), respectively. Consequently, metabolism is composed of synthesis (anabolism) and degradation (catabolism) (**Figure** 4.1.2).

It is important to know that the chemical reactions of metabolic pathways do not take place on their own. Each reaction step is facilitated, or catalyzed, by a protein called an enzyme. Enzymes are important for catalyzing all types of biological reactions—those that require energy as well as those that release energy.



(**Figure** 4.1.2) Metabolic pathways are shown above. Anabolic pathways require energy to synthesize larger molecules, four small molecules have energy added to make one large molecule. Catabolic pathways generate energy by breaking down larger molecules, one large molecule is broken down into two components: four small molecules plus energy. Both types of pathways are required for maintaining the cell's energy balance.

Energy

Thermodynamics refers to the study of energy and energy transfer involving physical matter. The matter relevant to a particular case of energy transfer is called a system, and everything outside of that matter is called the surroundings. For instance, when heating a pot of water on the stove, the system includes the stove, the pot, and the water. Energy is transferred within the system (between the stove, pot, and water). There are two types of systems: open and closed. In an open system, energy can be exchanged with its surroundings. The stovetop system is open because heat can be lost to the air. A closed system cannot exchange energy with its surroundings.

Biological organisms are open systems. Energy is exchanged between them and their surroundings as they use energy from the sun to perform photosynthesis or consume energy-storing molecules and release energy to the environment by doing work and releasing heat. Like all things in the physical world, energy is subject to physical laws. The laws of thermodynamics govern the transfer of energy in and among all systems in the universe.

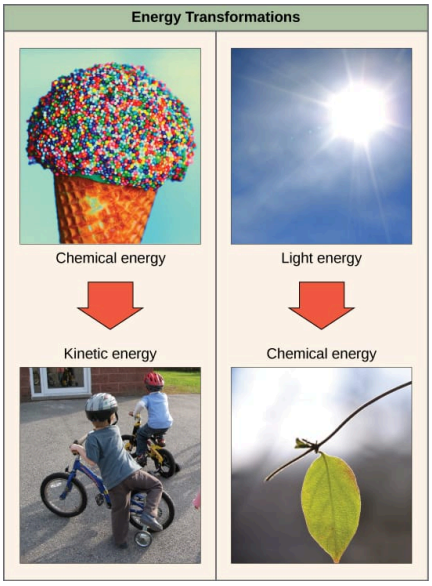
In general, energy is defined as the ability to do work, or to create some kind of change. Energy exists in different forms. For example, electrical energy, light energy, and heat energy are all different types of energy. To appreciate the way energy flows into

and out of biological systems, it is important to understand two of the physical laws that govern energy.

Thermodynamics

The first law of thermodynamics states that the total amount of energy in the universe is constant and conserved. In other words, there has always been, and always will be, exactly the same amount of energy in the universe. Energy exists in many different forms. According to the first law of thermodynamics, energy may be transferred from place to place or transformed into different forms, but it cannot be created or destroyed. The transfers and transformations of energy take place around us all the time. Light bulbs transform electrical energy into light and heat energy. Gas stoves transform chemical energy from natural gas into heat energy. Plants perform one of the most biologically useful energy transformations on earth: that of converting the energy of sunlight to chemical energy stored within organic molecules. Some examples of energy transformations are shown in (**Figure** 4.1.3).

The challenge for all living organisms is to obtain energy from their surroundings in forms that they can transfer or transform into usable energy to do work. Living cells have evolved to meet this challenge. Chemical energy stored within organic molecules such as sugars and fats is transferred and transformed through a series of cellular chemical reactions into energy within molecules of ATP. Energy in ATP molecules is easily accessible to do work. Examples of the types of work that cells need to do include building complex molecules, transporting materials, powering the motion of cilia or flagella, and contracting muscle fibers to create movement.



(**Figure** 4.1.3) Shown are some examples of energy transferred and transformed from one system to another and from one form to another. The food we consume provides our cells with the energy required to carry out bodily functions, just as light energy provides plants with the means to create the chemical energy they need. (credit "ice cream": modification of work by D. Sharon Pruitt; credit "kids": modification of work by Max from Providence; credit "leaf": modification of work by Cory Zanker)

The left side of this diagram depicts energy being transferred from an ice cream cone to two boys riding bikes. The right side depicts a plant converting light energy into chemical energy.

A living cell's primary tasks of obtaining, transforming, and using energy to do work may seem simple. However, the second law of thermodynamics explains why these tasks are harder than they appear. All energy transfers and transformations are never completely efficient. In every energy transfer, some amount of energy is lost in a form that is unusable. In most cases, this form is heat energy. Thermodynamically, heat energy is defined as the energy transferred from one system to another that is not work. For example, when a light bulb is turned on, some of the energy being converted from electrical energy into light energy is lost as heat energy. Likewise, some energy is lost as heat energy during cellular metabolic reactions.

An important concept in physical systems is that of order and disorder. The more energy that is lost by a system to its surroundings, the less ordered and more random the system is. Scientists refer to the measure of randomness or disorder within a system as entropy. High entropy means high disorder and low energy. Molecules and chemical reactions have varying entropy as well. For example, entropy increases as molecules at a high concentration in one place diffuse and spread out. The second law of thermodynamics says that energy will always be lost as heat in energy transfers or transformations.

Living things are highly ordered, requiring constant energy input to be maintained in a state of low entropy.

Potential and Kinetic Energy

When an object is in motion, there is energy associated with that object. Think of a wrecking ball. Even a slow-moving wrecking ball can do a great deal of damage to other objects. Energy associated with objects in motion is called kinetic energy (**Figure** 4.1.4). A speeding bullet, a walking person, and the rapid movement of molecules in the air (which produces heat) all have kinetic energy.

Now what if that same motionless wrecking ball is lifted two stories above ground with a crane? If the suspended wrecking ball is unmoving, is there energy associated with it? The answer is yes. The energy that was required to lift the wrecking ball did not disappear, but is now stored in the wrecking ball by virtue of its position and the force of gravity acting on it. This type of energy is called potential energy (**Figure** 4.1.4). If the ball were to fall, the potential energy would be transformed into kinetic energy until all of the potential energy was exhausted when the ball rested on the ground. Wrecking balls also swing like a pendulum; through the swing, there is a constant change of potential energy (highest at the top of the swing) to kinetic energy (highest at the bottom of the swing). Other examples of potential energy include the energy of water held behind a dam or a person about to skydive out of an airplane.



(**Figure** 4.1.4) The photo on the left shows water behind a dam as potential energy; the photo on the right shows a waterfall as kinetic energy. (credit "dam": modification of work by "Pascal"/Flickr; credit "waterfall": modification of work by Frank Gualtieri)

Potential energy is not only associated with the location of matter, but also with the structure of matter. Even a spring on the ground has potential energy if it is compressed; so does a rubber band that is pulled taut. On a molecular level, the bonds that hold the atoms of molecules together exist in a particular structure that has potential energy. Remember that anabolic cellular pathways require energy to synthesize complex molecules from simpler ones and catabolic pathways release energy when complex molecules are broken down. The fact that energy can be released by the breakdown of certain chemical bonds implies that those bonds have potential energy. In fact, there is potential energy stored within the bonds of all the food molecules we eat, which is eventually harnessed for use. This is because these bonds can release energy when broken. The type of potential energy that exists within chemical bonds, and is released when those bonds are broken, is called chemical energy. Chemical energy is responsible for providing living cells with energy from food. The release of energy occurs when the molecular bonds within food molecules are broken.

CONCEPT IN ACTION

Visit the Phet: Pendulum Lab site and select “Pendulum” from the “Work and Energy” menu to see the shifting kinetic and potential energy of a pendulum in motion.

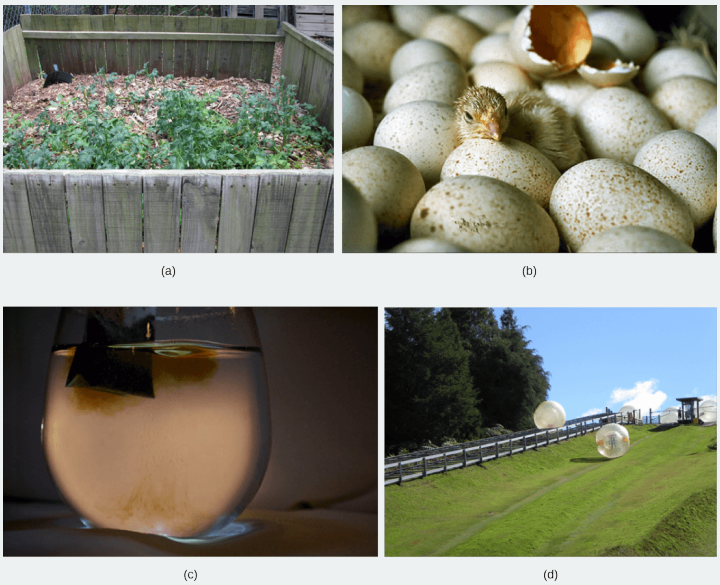
Free and Activation Energy

After learning that chemical reactions release energy when energy-storing bonds are broken, an important next question is the following: How is the energy associated with these chemical reactions quantified and expressed? How can the energy released from one reaction be compared to that of another reaction? A measurement of free energy is used to quantify these energy transfers. Recall that according to the second law of thermodynamics, all energy transfers involve the loss of some amount of energy in an unusable form such as heat. Free energy specifically refers to the energy associated with a chemical reaction that is available after the losses are accounted for. In other words, free energy is usable energy, or energy that is available to do work.

If energy is released during a chemical reaction, then the change in free energy, signified as ΔG (delta G) will be a negative number. A negative change in free energy also means that the products of the reaction have less free energy than the reactants, because they release some free energy during the reaction. Reactions that have a negative change in free energy and consequently release free energy are called exergonic reactions. Think: *ex*ergonic means energy is *ex*iting the system. These reactions are also referred to as spontaneous reactions, and their products have less stored energy than the reactants. An important distinction must be drawn between the term spontaneous and the idea of a chemical reaction occurring immediately. Contrary to the everyday use of the term, a spontaneous reaction is not one that suddenly or quickly occurs. The rusting of iron is an example of a spontaneous reaction that occurs slowly, little by little, over time.

If a chemical reaction absorbs energy rather than releases energy on balance, then the ΔG for that reaction will be a positive value. In this case, the products have more free energy than the reactants. Thus, the products of these reactions can be thought of as energy-storing molecules. These chemical reactions are called endergonic reactions and they are non-spontaneous. An endergonic reaction will not take place on its own without the addition of free energy.

ART CONNECTION



(**Figure** 4.1.5) Shown are some examples of endergonic processes (ones that require energy) and exergonic processes (ones that release energy). From left to right, show a compost pile, a baby chick emerging from a fertilized egg, a teabag's dark-colored contents diffusing into a clear mug of water, and a ball rolling downhill. (credit a: modification of work by Natalie Maynor; credit b: modification of work by USDA; credit c: modification of work by Cory Zanker; credit d: modification of work by Harry Malsch)

Look at each of the processes shown and decide if it is endergonic or exergonic.

There is another important concept that must be considered regarding endergonic and exergonic reactions. Exergonic reactions require a small amount of energy input to get going, before they can proceed with their energy-releasing steps. These reactions have a net release of energy, but still require some energy input in the beginning. This small amount of energy input necessary for all chemical reactions to occur is called the activation energy.

CONCEPT IN ACTION

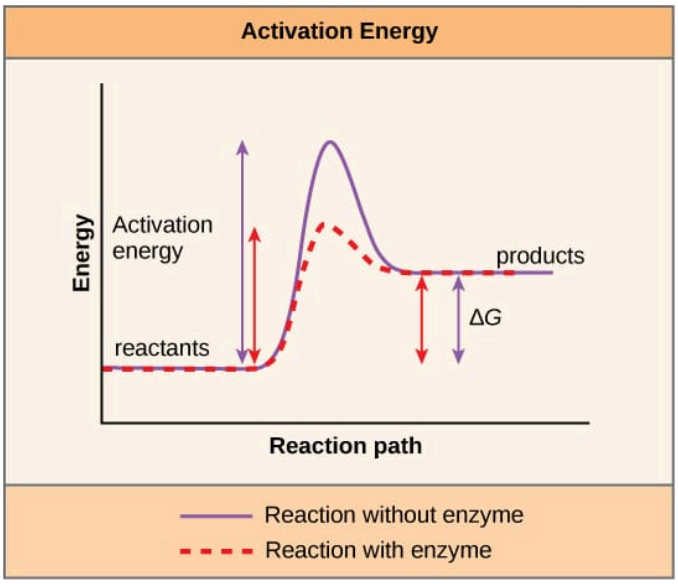
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Watch an Activation energy and Enzymes animation of the move from free energy to transition state of the reaction.

Enzymes

A substance that helps a chemical reaction to occur is called a catalyst, and the molecules that catalyze biochemical reactions are called enzymes. Most enzymes are proteins and perform the critical task of lowering the activation energies of chemical reactions inside the cell. Most of the reactions critical to a living cell happen too slowly at normal temperatures to be of any use to the cell. Without enzymes to speed up these reactions, life could not persist. Enzymes do this by binding to the reactant molecules and holding them in such a way as to make the chemical bond-breaking and -forming processes take place more easily. It is important to remember that enzymes do not change whether a reaction is exergonic (spontaneous) or endergonic. This is because they do not change the free energy of the reactants or products. They only reduce the activation energy required for the reaction to go forward (**Figure** 4.1.6). In addition, an enzyme itself is unchanged by the reaction it catalyzes. Once one reaction has been catalyzed, the enzyme is able to participate in other reactions.



(**Figure** 4.1.6) Enzymes lower the activation energy of the reaction but do not change the free energy of the reaction. A plot of the energy required for a reaction with and without an enzyme is shown above.

The chemical reactants to which an enzyme binds are called the enzyme's substrates. There may be one or more substrates, depending on the particular chemical reaction. In some reactions, a single reactant substrate is broken down into multiple products. In others, two substrates may come together to create one larger molecule. Two reactants might also enter a reaction and both become modified, but they leave the reaction as two products. The location within the enzyme where the substrate binds is called the enzyme's active site. The active site is where the "action" happens. Since enzymes are proteins, there is a unique combination of amino acid side chains within the active site. Each side chain is characterized by different properties. They can be large or small, weakly acidic or basic, hydrophilic or hydrophobic, positively or negatively charged, or neutral. The unique combination of side chains creates a very specific chemical environment within the active site. This specific environment is suited to bind to one specific chemical substrate (or substrates).

Active sites are subject to influences of the local environment. Increasing the environmental temperature generally increases

reaction rates, enzyme-catalyzed or otherwise. However, temperatures outside of an optimal range reduce the rate at which an enzyme catalyzes a reaction. Hot temperatures will eventually cause enzymes to denature, an irreversible change in the three-dimensional shape and therefore the function of the enzyme. Enzymes are also suited to function best within a certain pH and salt concentration range, and, as with temperature, extreme pH, and salt concentrations can cause enzymes to denature.

For many years, scientists thought that enzyme-substrate binding took place in a simple "lock and key" fashion. This model asserted that the enzyme and substrate fit together perfectly in one instantaneous step. However, current research supports a model called induced fit (**Figure** 4.1.7). The induced-fit model expands on the lock-and-key model by describing a more dynamic binding between enzyme and substrate. As the enzyme and substrate come together, their interaction causes a mild shift in the enzyme's structure that forms an ideal binding arrangement between enzyme and substrate.

CONCEPT IN ACTION

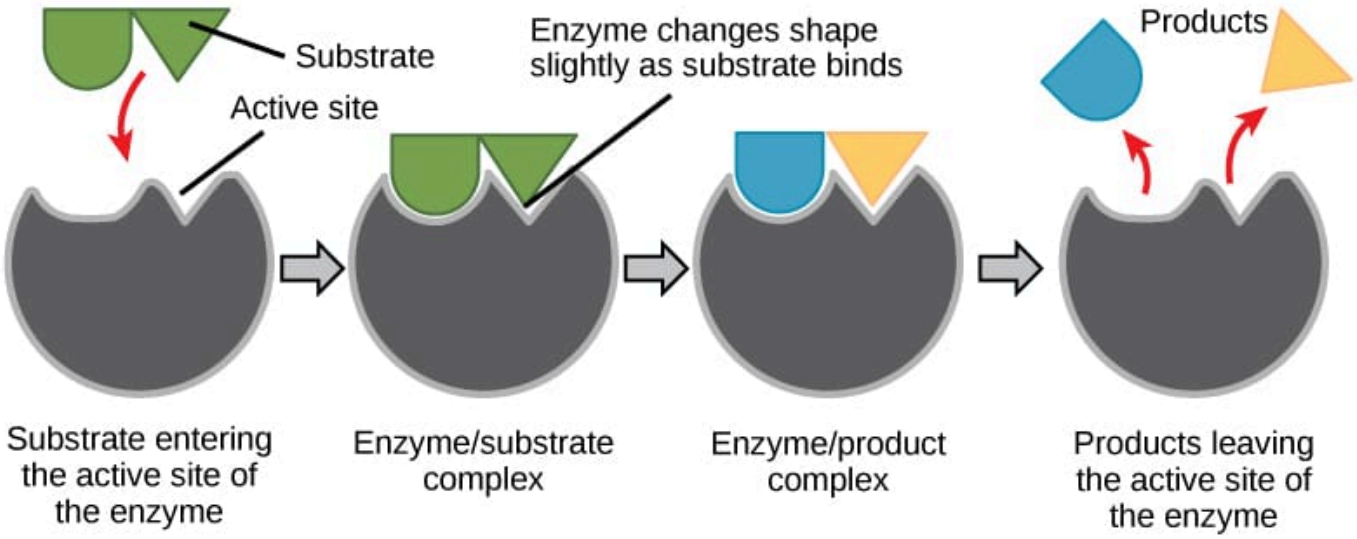
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View this YouTube Video online

View an animation of induced fit.

When an enzyme binds its substrate, an enzyme-substrate complex is formed. This complex lowers the activation energy of the reaction and promotes its rapid progression in one of multiple possible ways. On a basic level, enzymes promote chemical reactions that involve more than one substrate by bringing the substrates together in an optimal orientation for reaction. Another way in which enzymes promote the reaction of their substrates is by creating an optimal environment within the active site for the reaction to occur. The chemical properties that emerge from the particular arrangement of amino acid R groups within an active site create the perfect environment for an enzyme's specific substrates to react.

The enzyme-substrate complex can also lower activation energy by compromising the bond structure so that it is easier to break. Finally, enzymes can also lower activation energies by taking part in the chemical reaction itself. In these cases, it is important to remember that the enzyme will always return to its original state by the completion of the reaction. One of the hallmark properties of enzymes is that they remain ultimately unchanged by the reactions they catalyze. After an enzyme has catalyzed a reaction, it releases its product(s) and can catalyze a new reaction.



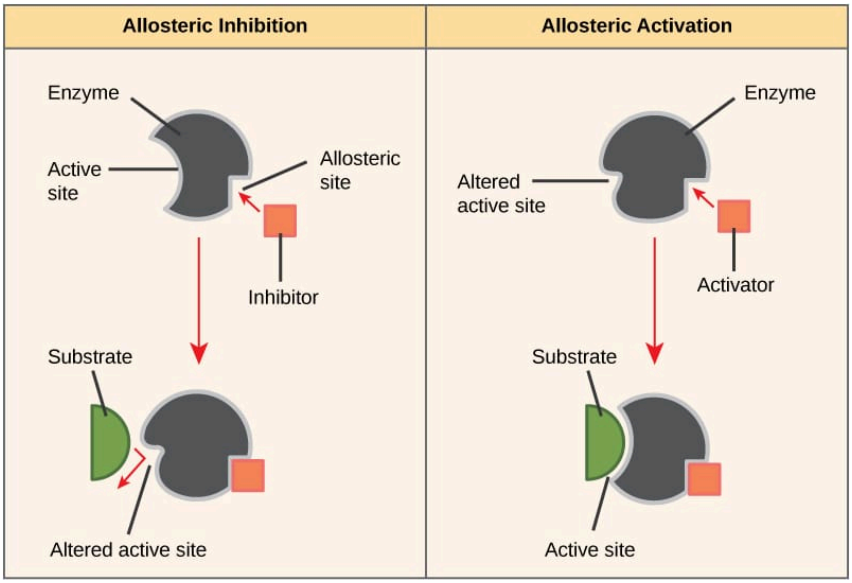
(**Figure** 4.1.7) The induced-fit model is an adjustment to the lock-and-key model and explains how enzymes and substrates undergo dynamic modifications during the transition state to increase the affinity of the substrate for the active site. In the diagram shown above, a substrate binds the active site of an enzyme, and both the shape of the enzyme and the shape of the substrate change. The substrate is converted to product, which leaves the active site.

It would seem ideal to have a scenario in which all of an organism's enzymes existed in abundant supply and functioned optimally under all cellular conditions, in all cells, at all times. However, a variety of mechanisms ensures that this does not happen. Cellular needs and conditions constantly vary from cell to cell, and change within individual cells over time. The required enzymes of stomach cells differ from those of fat storage cells, skin cells, blood cells, and nerve cells. Furthermore, a digestive organ cell works much harder to process and break down nutrients during the time that closely follows a meal compared with many hours after a meal. As these cellular demands and conditions vary, so must the amounts and functionality of different enzymes.

Since the rates of biochemical reactions are controlled by activation energy, and enzymes lower and determine activation energies for chemical reactions, the relative amounts and functioning of the variety of enzymes within a cell ultimately determine which reactions will proceed and at what rates. This determination is tightly controlled in cells. In certain cellular environments, enzyme activity is partly controlled by environmental factors like pH, temperature, salt concentration, and, in some cases, cofactors or coenzymes.

Enzymes can also be regulated in ways that either promote or reduce enzyme activity. There are many kinds of molecules that inhibit or promote enzyme function, and various mechanisms by which they do so. In some cases of enzyme inhibition, an inhibitor molecule is similar enough to a substrate that it can bind to the active site and simply block the substrate from binding. When this happens, the enzyme is inhibited through competitive inhibition, because an inhibitor molecule competes with the substrate for binding to the active site.

On the other hand, in noncompetitive inhibition, an inhibitor molecule binds to the enzyme in a location other than the active site, called an allosteric site, but still manages to block substrate binding to the active site. Some inhibitor molecules bind to enzymes in a location where their binding induces a conformational change that reduces the affinity of the enzyme for its substrate. This type of inhibition is called allosteric inhibition (**Figure** 4.1.8). Most allosterically regulated enzymes are made up of more than one polypeptide, meaning that they have more than one protein subunit. When an allosteric inhibitor binds to a region on an enzyme, all active sites on the protein subunits are changed slightly such that they bind their substrates with less efficiency. There are allosteric activators as well as inhibitors. Allosteric activators bind to locations on an enzyme away from the active site, inducing a conformational change that increases the affinity of the enzyme's active site(s) for its substrate(s) (**Figure** 4.1.8).



(**Figure** 4.1.8) Allosteric inhibition (shown on the left) works by indirectly inducing a conformational change to the active site such that the substrate no longer fits. In contrast, in allosteric activation (shown on the right), the activator molecule modifies the shape of the active site to allow a better fit of the substrate.

CAREERS IN ACTION: Pharmaceutical Drug Developer



(**Figure** 4.1.9) Have you ever wondered how pharmaceutical drugs are developed? Shown above are generic red pills. (credit: Deborah Austin)

Enzymes are key components of metabolic pathways. Understanding how enzymes work and how they can be regulated are key principles behind the development of many of the pharmaceutical drugs on the market today. Biologists working in this field collaborate with other scientists to design drugs (**Figure** 4.1.9).

Consider statins for example—statins is the name given to one class of drugs that can reduce cholesterol levels. These compounds are inhibitors of the enzyme HMG-CoA reductase, which is the enzyme that synthesizes cholesterol from lipids in the body. By inhibiting this enzyme, the level of cholesterol synthesized in the body can be reduced. Similarly, acetaminophen, popularly marketed under the brand name Tylenol, is an inhibitor of the enzyme cyclooxygenase. While it is used to provide relief from fever and inflammation (pain), its mechanism of action is still not completely understood.

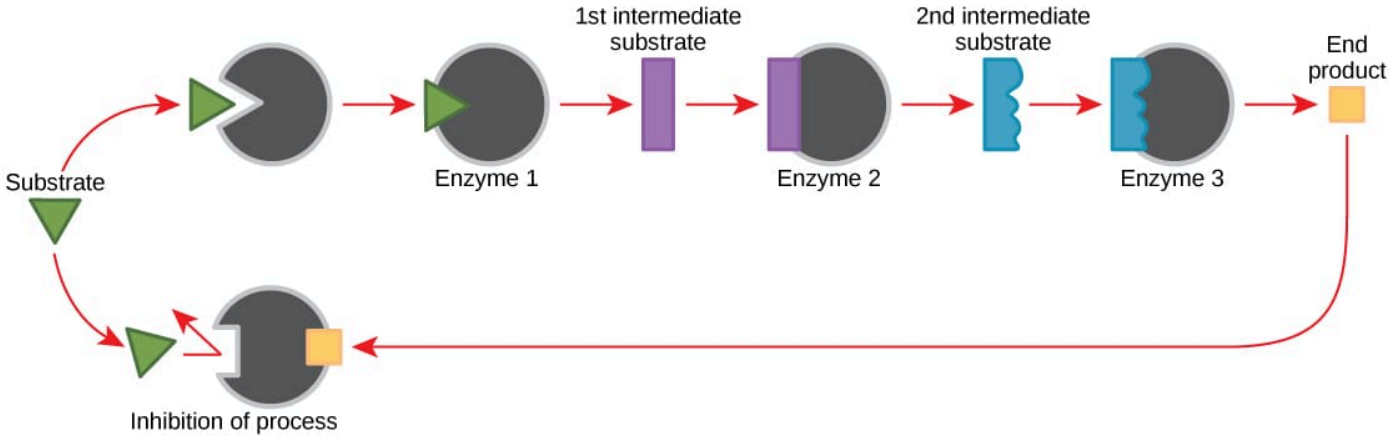
How are drugs discovered? One of the biggest challenges in drug discovery is identifying a drug target. A drug target is a molecule that is literally the target of the drug. In the case of statins, HMG-CoA reductase is the drug target. Drug targets are identified through painstaking research in the laboratory. Identifying the target alone is not enough; scientists also need to know how the target acts inside the cell and which reactions go awry in the case of disease. Once the target and the pathway are identified, then the actual process of drug design begins. In this stage, chemists and biologists work together to design and synthesize molecules that can block or activate a particular reaction. However, this is only the beginning: If and when a drug prototype is successful in performing its function, then it is subjected to many tests from in vitro experiments to clinical trials before it can get approval from the U.S. Food and Drug Administration to be on the market.

Many enzymes do not work optimally, or even at all, unless bound to other specific non-protein helper molecules. They may bond either temporarily through ionic or hydrogen bonds, or permanently through stronger covalent bonds. Binding to these molecules promotes optimal shape and function of their respective enzymes. Two examples of these types of helper molecules are cofactors and coenzymes. Cofactors are inorganic ions such as ions of iron and magnesium. Coenzymes are organic helper molecules, those with a basic atomic structure made up of carbon and hydrogen. Like enzymes, these molecules participate in reactions without being changed themselves and are ultimately recycled and reused. Vitamins are the source of coenzymes. Some vitamins are the precursors of coenzymes and others act directly as coenzymes. Vitamin C is a direct coenzyme for multiple enzymes that take part in building the important connective tissue, collagen. Therefore, enzyme function is, in part, regulated by the abundance of various cofactors and coenzymes, which may be supplied by an organism's diet or, in some cases, produced by the organism.

Feedback Inhibition in Metabolic Pathways

Molecules can regulate enzyme function in many ways. The major question remains, however: What are these molecules and where do they come from? Some are cofactors and coenzymes, as you have learned. What other molecules in the cell provide enzymatic regulation such as allosteric modulation, and competitive and non-competitive inhibition? Perhaps the most relevant

sources of regulatory molecules, with respect to enzymatic cellular metabolism, are the products of the cellular metabolic reactions themselves. In a most efficient and elegant way, cells have evolved to use the products of their own reactions for feedback inhibition of enzyme activity. Feedback inhibition involves the use of a reaction product to regulate its own further production (**Figure** 4.1.10). The cell responds to an abundance of the products by slowing down production during anabolic or catabolic reactions. Such reaction products may inhibit the enzymes that catalyzed their production through the mechanisms described above.



(**Figure** 4.1.10) Metabolic pathways are a series of reactions catalyzed by multiple enzymes. Feedback inhibition, where the end product of the pathway inhibits an upstream process, is an important regulatory mechanism in cells.

The diagram above shows a metabolic pathway in which three enzymes convert a substrate, in three steps, into a final product. The final product inhibits the first enzyme in the pathway by feedback inhibition.

The production of both amino acids and nucleotides is controlled through feedback inhibition. Additionally, ATP is an allosteric regulator of some of the enzymes involved in the catabolic breakdown of sugar, the process that creates ATP. In this way, when ATP is in abundant supply, the cell can prevent the production of ATP. On the other hand, ADP serves as a positive allosteric regulator (an allosteric activator) for some of the same enzymes that are inhibited by ATP. Thus, when relative levels of ADP are high compared to ATP, the cell is triggered to produce more ATP through sugar catabolism.

Summary

Cells perform the functions of life through various chemical reactions. A cell's metabolism refers to the combination of chemical reactions that take place within it. Catabolic reactions break down complex chemicals into simpler ones and are associated with energy release. Anabolic processes build complex molecules out of simpler ones and require energy.

In studying energy, the term system refers to the matter and environment involved in energy transfers. Entropy is a measure of the disorder of a system. The physical laws that describe the transfer of energy are the laws of thermodynamics. The first law states that the total amount of energy in the universe is constant. The second law of thermodynamics states that every energy transfer involves some loss of energy in an unusable form, such as heat energy. Energy comes in different forms: kinetic, potential, and free. The change in free energy of a reaction can be negative (releases energy, exergonic) or positive (consumes energy, endergonic). All reactions require an initial input of energy to proceed, called the activation energy.

Enzymes are chemical catalysts that speed up chemical reactions by lowering their activation energy. Enzymes have an active site with a unique chemical environment that fits particular chemical reactants for that enzyme, called substrates. Enzymes and substrates are thought to bind according to an induced-fit model. Enzyme action is regulated to conserve resources and respond optimally to the environment.

Art Connections

(**Figure** 4.1.5) Look at each of the processes shown and decide if it is endergonic or exergonic.

Answer

A compost pile decomposing is an exergonic process. A baby developing from a fertilized egg is an endergonic

process. Tea dissolving into water is an exergonic process. A ball rolling downhill is an exergonic process.

Key Terms

activation energy
the amount of initial energy necessary for reactions to occur

active site
a specific region on the enzyme where the substrate binds

allosteric inhibition
the mechanism for inhibiting enzyme action in which a regulatory molecule binds to a second site (not the active site) and initiates a conformation change in the active site, preventing binding with the substrate

anabolic
describes the pathway that requires a net energy input to synthesize complex molecules from simpler ones

bioenergetics
the concept of energy flow through living systems

catabolic
describes the pathway in which complex molecules are broken down into simpler ones, yielding energy as an additional product of the reaction

competitive inhibition
a general mechanism of enzyme activity regulation in which a molecule other than the enzyme's substrate is able to bind the active site and prevent the substrate itself from binding, thus inhibiting the overall rate of reaction for the enzyme

endergonic
describes a chemical reaction that results in products that store more chemical potential energy than the reactants

enzyme
a molecule that catalyzes a biochemical reaction

exergonic
describes a chemical reaction that results in products with less chemical potential energy than the reactants, plus the release of free energy

feedback inhibition
a mechanism of enzyme activity regulation in which the product of a reaction or the final product of a series of sequential reactions inhibits an enzyme for an earlier step in the reaction series

heat energy
the energy transferred from one system to another that is not work

kinetic energy
the type of energy associated with objects in motion

metabolism
all the chemical reactions that take place inside cells, including those that use energy and those that release energy

noncompetitive inhibition
a general mechanism of enzyme activity regulation in which a regulatory molecule binds to a site other than the active site and prevents the active site from binding the substrate; thus, the inhibitor molecule does not compete with the substrate for the active site; allosteric inhibition is a form of noncompetitive inhibition

potential energy
the type of energy that refers to the potential to do work

substrate
: a molecule on which the enzyme acts

thermodynamics
: the science of the relationships between heat, energy, and work

Contributors and Attributions

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4.2: Enzymes in Action

Learning Objectives

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

- Describe how enzymes are used in modern biotechnology, medicine, industry, and environmental applications.
- Explain how enzymes contribute to sustainable technologies, including bioremediation, recycling, and biofuel production.
- Discuss how advances in enzyme engineering and synthetic biology are expanding the use of enzymes to solve real-world problems.
- Evaluate the potential benefits of enzyme-based technologies for improving human health, environmental sustainability, and society.

Modern Industrial and Medical Uses of Enzymes

As science advanced, so did our ability to isolate, understand, and apply enzymes. Today, enzymes are used in almost every industry—from food production to medicine to environmental cleanup. Below are several important modern applications.

Lactose-Free Dairy Production

Some people are lactose intolerant, meaning they lack sufficient lactase, the enzyme that breaks down lactose (milk sugar). To help, the dairy industry adds lactase to milk, breaking down lactose before consumption. This makes dairy products more digestible and accessible to millions of people.

Biosensors for Medical Diagnostics

Enzymes are also vital in healthcare technologies. For example, glucose meters used by people with diabetes contain enzymes that specifically react with glucose in a blood sample. This reaction produces an electrical signal proportional to the glucose concentration, allowing for fast and accurate monitoring.

Textile and Fabric Innovation

Enzymes improve the softness, color, and longevity of textiles. Specific enzymes are used to de-pill fabric (remove small fuzz balls), soften materials like cotton and denim, and even help with dye penetration, resulting in less water and chemical usage. These innovations make textile production more sustainable and less polluting.

Biodegradable Plastics and Waste Reduction

Some industrial enzymes help break down plastics, accelerating decomposition and reducing landfill waste. Other enzymes are used in recycling processes to break complex materials down into reusable components more efficiently than heat or chemicals alone.

CONCEPT IN ACTION

Scientists work with microorganisms because these organisms produce special enzymes that enable them to break down harmful substances in the environment. Through a process called bioremediation, these microbial enzymes help convert toxic materials into less harmful compounds like water and carbon dioxide. Because microbes can survive in extreme environments—such as acidic, salty, or cold conditions—they are especially effective at cleaning up pollutants in a wide range of settings. Their natural ability to absorb and degrade pollutants makes them valuable tools for environmental protection. A list of different microbes commonly used in bioremediation is provided below.

Different Microbes Used in Bioremediation

Bacteria

	Species	References
Bacillus licheniformis JUG GS2 (MK106145) and *Bacillus sonorensis*	Naphthalene	(Rabani et al., 2022)
Bacillus sp., *Rhodopirellula* sp., *Rhodovibrio* sp. and *Formosa* sp.	Hydrocarbon	(Machado et al., 2019)
P. cepacia, B. coagulans, B. cereus, and *Serratia ficaria*	Diesel oil	(Miri et al., 2022)
Pseudoalteromonas sp. and *Agarivorans* sp.	Hydrocarbons	(Dell'Anno et al., 2021)
P. aeruginosa and *Aeromonas* sp.	Chromium, uranium, nickel and copper	(Gaur et al., 2022)
E.coli	Hexavalent chromium	(Mohamed et al., 2020)
Oscillatoria laete-virens, Arthrospira platensis, Pseudochlorococcum typicum and *Spirogyra insignis*	Lead	(Song et al., 2019)
Microbacterium sp., *Micrococcus* sp., *Bacillus sp.*, and *Shigella* sp.	Uranium and Arsenic	(Bhakat et al., 2019)
Lysinibacillus sphaericus CBAM5	Lead, cobalt, copper crude oil and chromium	(Kharangate-Lad and D'Souza, 2021)
Pseudomonas aeruginosa	Crude oil	(Mukjang et al., 2022)
Cyclotella cryptica, Pseudochlorococcum typicum, Spirogyra hyaline and *Chlamydomonas reinhardtii*	Mercury	(Shah and Jain, 2020)
Dehalococcoides sp.	Chloroethenes	(Dutta et al., 2022)
Burkholderia sp. and *Myceliophthora thermophila*	N, N-dimethylpphenylenediamine and polycyclic aromatic hydrocarbons	(Mohapatra and Phale, 2021)
Bacillus sp. and *Staphylococcus* sp.	Endosulfans	(Liu et al., 2018)
A. versicolor, Cladosporium sp., *Paecilomyces* sp., *A. fumigatus, Paecilomyces* sp., *Terichoderma* sp. and *Cladosporium* sp.	Cadmium	(Unuofin et al., 2021)
Fusarium sp., *Corynebacterium propinquum, P. aeruginosa* and *Alcaligenes odorans*	Oils	(Pande et al., 2020)
C. reinhardtii, Ulothrix tenuissima and *Spirulina* sp.	Chromium	(Aregbesola et al., 2020)
Ralstonia sp., *Microbacterium* sp., *Pseudomonas* sp. *and Acinetobacter* sp.	Aromatic hydrocarbons	(Basu et al., 2018)
Aerococcus sp., and *Rhodopseudomonas palustris*	Cadmium, lead and chromium	(Sravya and Sangeetha, 2022)
P. aeruginosa, Corynebacterium propinquum, Alcaligenes odorans and *B. subtilis*	Phenol	(Gaur et al., 2018)
K. oxytoca, B. firmus, B. macerans, and Staphylococcus aureus	Vat dyes	(Sangkharak et al., 2020)

	Species	References
Chlorella sp. and *Spirulina* sp.	Lead, nickel and dichromate	(Geetha et al., 2021)
Saccharomyces cerevisiae and *Cunninghamella elegans*	Heavy metals and mercury	(Duc et al., 2021)
Bacillus licheniformis	Dyes	(Mousavi et al., 2021)
Bacillus subtilis and *Pseudomonas fluorescence*	Iron and zinc	(Siric et al., 2022b)
Pseudomonas sp., *Bacillus* sp., *Escherichia* sp., *Shewanella* sp., *Enterobacter* sp. and *Thermus* sp.	Chromium	(Mousavi et al., 2021)

Fungi

Species	Pollutant remediated	References
Phanerochaete chrysosporium	N-heterocyclic explosives, benzene, xylene, ethylbenzene, toluene and organochlorines	(Singh et al., 2020)
Phanerochaete chrysosporium	4,4 dibromodiphenyl ether	(Sen et al., 2019)
Saccharomyces cerevisiae	Arsenic	(Verma et al., 2019)
Aspergillus sp.	Arsenic	(Mohd et al., 2019)
Coprinus comatus	4-Hydroxy-3,5 - dichlorobiphenyl	(Li et al., 2018)
Aspergillus sp. and *Penicillium* sp.	Aliphatic hydrocarbons, polycyclic aromatic hydrocarbons and chlorophenols	(Li et al., 2020)
Aspergillus sp.	N-hexadecane	(Al-Hawash et al., 2018)
Phomopsis liquidambari	Phenanthrene	(Fu et al., 2018)
Ganoderma lucidum	Pyrene	(Agrawal et al., 2018)
Trichoderma sp., *Penicillium* sp. and *Aspergillus* sp.	Cobalt and copper	(Dusengemungu et al., 2020)

Algae

Species	Pollutant remediated	References
Microcystis aeruginosa	Arsenic	(Wang Z. et al., 2018)
Chlamydomonas reinhardti and S. almeriensis and	Arsenic	(Saavedra et al., 2018)
Fucus vesiculosus	Zinc	(Brinza et al., 2020)
Chlorococcum humicola	Iron	(Chugh et al., 2022)
Chlorella sp., *Isochrysis galbana* and *Phaeodactylum tricornutum*	Phenol	(Wu et al., 2022)
F. vesiculosus	Chromium, nickel, cadmium and lead	(Moreira et al., 2019)
Cystoseria indicant	Nickel and cadmium	(Moreira et al., 2019)

Species	Pollutant remediated	References
Chlamydomonas reinhardtii	Chromium and cadmium	(Nowicka et al., 2020)
Microcystis aeruginosa	Cadmium	(Deng et al., 2020)
Scenedesmus accuminatus, *Scenedesmus protuberans* and *Cyclotella* sp.	Cadmium	(Vo et al., 2020)
Chlorococcum humicola	Cobalt	(Chugh et al., 2022)

You can find the table and read the full article here: Bioremediation of environmental wastes: the role of microorganisms

Enzyme-Assisted Drug Delivery and Therapeutics

In pharmaceuticals, enzymes play key roles in targeted drug delivery. Some enzymes activate a drug only once it reaches a specific tissue or cell type, minimizing side effects. Other enzyme-based therapies replace defective enzymes in people with genetic diseases, such as in enzyme replacement therapy for Gaucher's disease.

The Future of Enzymes: Bioinnovation on the Horizon

The future of enzyme use is filled with possibilities, especially in sustainability, medicine, and bioengineering. Scientists are learning how to engineer enzymes—by tweaking their structure at the genetic or protein level—to make them more stable, more efficient, or better suited to specific environments.

Custom Enzymes for Green Energy

One major goal is to create enzymes that help break down plant biomass into fuel. These engineered enzymes could convert agricultural waste into biofuels, offering a renewable alternative to fossil fuels. The idea is to replicate and enhance nature's ability to recycle organic matter for energy production on a large scale.

CONCEPT IN ACTION

Scientists at the National Renewable Energy Laboratory are working on a new way to recycle difficult-to-reuse materials—such as wind turbine blades and plastic bottles—by drawing on nature's enzyme toolkit. They've engineered special enzymes, inspired by those found in plastic-eating bacteria, that can break down durable plastics like PET into building blocks that can be reused to make new materials—even tougher composites like Kevlar. This work highlights how combining enzymes from different organisms with innovative chemistry allows living cells to perform tasks that neither could do alone, paving the way for greener, more sustainable manufacturing processes.

You can read the full Scientific American article here: Breathing new life into renewable energy

Enzymes in Synthetic Biology

In synthetic biology, enzymes are being designed to carry out entirely new functions—such as assembling novel compounds or sensing environmental toxins. Imagine an enzyme that can break down microplastics in the ocean or produce insulin more efficiently than current methods.

CONCEPT IN ACTION

Scientists recently discovered a way to combine tools from chemistry and biology to solve a modern problem: turning plastic waste into something useful. In this research, they used a type of bacteria, *E. coli*, and added enzymes from different species that allowed the bacteria to help break down parts of plastic and turn it into useful materials like medicine. This breakthrough shows how scientists can "borrow" enzymes from nature and put them to work inside cells in new ways—doing things that neither chemistry alone nor natural organisms could do by themselves.

You can read the full Nature Chemistry article here: A biocompatible Lossen rearrangement in *Escherichia coli*

Precision Medicine and Personalized Therapy

The ability to design enzymes for individual patients could one day lead to personalized therapies. Enzymes could be tailored to target unique disease markers or to function in specific tissues or conditions, expanding the possibilities for treating cancer, genetic disorders, and more.

CONCEPT IN ACTION

Scientists have recently created artificial enzymes—called XNAzymes—that are designed to cut up the genetic material of the COVID-19 virus. These lab-made enzymes combine the precision of enzyme-based tools with the clarity of molecular engineering, enabling them to target and break down virus RNA in ways that neither traditional chemistry nor natural enzymes can achieve alone. While this work is still in early stages and mainly a proof of concept, it represents a step in the gradual progression of how new technologies are developed, tested, and eventually applied.

You can read the full Science Daily article here: Synthetic biology meets medicine: 'Programmable molecular scissors' could help fight COVID-19 infection

Summary

Enzymes are ancient, powerful tools—first observed in everyday activities like cheese making or tenderizing meat, and now central to modern life through medicine, industry, and sustainability efforts. From helping ancient cultures process food to enabling precision genetic engineering, enzymes have been and will continue to be essential to human innovation. As we explore ways to improve health, reduce environmental impact, and harness biological systems, enzymes will undoubtedly remain at the forefront of science and society.

Key Terms

Enzyme
A biological catalyst that speeds up chemical reactions without being consumed.

Catalyst
A substance that increases the rate of a chemical reaction.

Lactase
An enzyme that breaks down lactose into glucose and galactose.

Lactose
The primary sugar found in milk and dairy products.

Biotechnology
The use of biological systems or organisms to develop useful products and technologies.

Industrial Enzymes
Enzymes used in manufacturing, food production, medicine, and environmental applications.

Biosensor
A device that uses a biological molecule (often an enzyme) to detect a specific substance.

Bioremediation
The use of microorganisms or their enzymes to remove or neutralize environmental pollutants.

Microorganism (Microbe)
A microscopic organism, such as bacteria, fungi, algae, or yeast.

Biodegradation
The breakdown of materials by living organisms or their enzymes.

Enzyme Replacement Therapy (ERT)
Treatment that replaces a missing or defective enzyme in patients with certain genetic disorders.

Targeted Drug Delivery
Delivering medications specifically to diseased tissues to reduce side effects.

Genetic Disease
A disorder caused by mutations in genes or chromosomes.

Protein Engineering
Modifying enzymes or proteins to improve their function or stability.

Bioengineering
Applying engineering principles to biological systems.

Engineered Enzyme
An enzyme that has been modified to improve or change its activity.

PET (Polyethylene Terephthalate)
A common plastic used in bottles and packaging that some enzymes can degrade.

Artificial Enzyme
A laboratory-designed enzyme with new or enhanced functions.

XNAzyme
A synthetic enzyme made from XNA that can be programmed to cut specific nucleic acids.

RNA (Ribonucleic Acid)
A nucleic acid involved in gene expression and protein synthesis.

Personalized Therapy
Treatments customized for a specific patient.

Environmental Biotechnology
The application of biological systems to solve environmental problems.

Microplastics
Tiny plastic particles that persist in the environment and are difficult to remove.

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4.3: Glycolysis

Learning Objectives

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

- Describe the location of glycolysis in the cell
- Explain how ATP is used by the cell as an energy source
- Describe the overall result in terms of molecules produced of the breakdown of glucose by glycolysis

Even exergonic, energy-releasing reactions require a small amount of activation energy to proceed. However, consider endergonic reactions, which require much more energy input because their products have more free energy than their reactants. Within the cell, where does energy to power such reactions come from? The answer lies with an energy-supplying molecule called adenosine triphosphate, or ATP. ATP is a small, relatively simple molecule, but within its bonds contains the potential for a quick burst of energy that can be harnessed to perform cellular work. This molecule can be thought of as the primary energy currency of cells in the same way that money is the currency that people exchange for things they need. ATP is used to power the majority of energy-requiring cellular reactions.

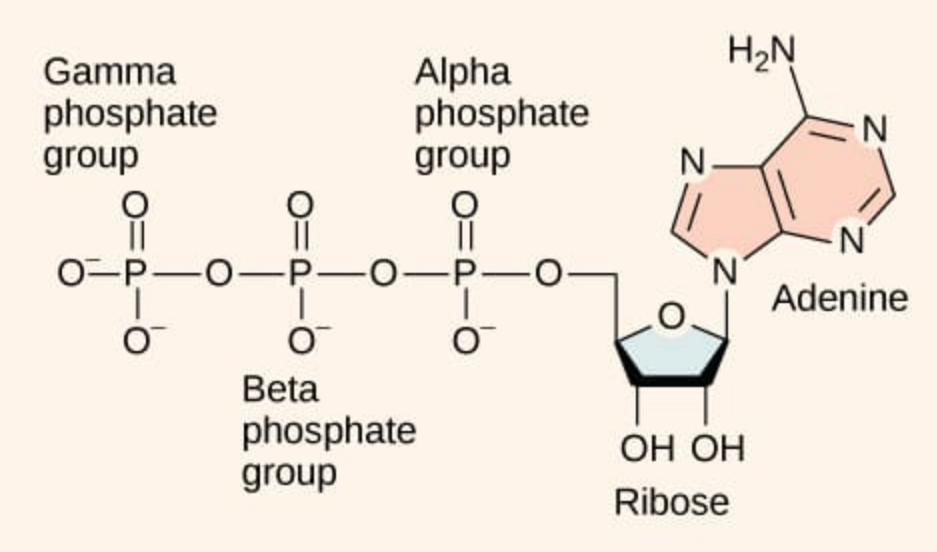
ATP in Living Systems

A living cell cannot store significant amounts of free energy. Excess free energy would result in an increase of heat in the cell, which would denature enzymes and other proteins, and thus destroy the cell. Rather, a cell must be able to store energy safely and release it for use only as needed. Living cells accomplish this using ATP, which can be used to fill any energy need of the cell. How? It functions as a rechargeable battery.

When ATP is broken down, usually by the removal of its terminal phosphate group, energy is released. This energy is used to do work by the cell, usually by the binding of the released phosphate to another molecule, thus activating it. For example, in the mechanical work of muscle contraction, ATP supplies energy to move the contractile muscle proteins.

ATP Structure and Function

At the heart of ATP is a molecule of adenosine monophosphate (AMP), which is composed of an adenine molecule bonded to both a ribose molecule and a single phosphate group (**Figure** 4.3.1). Ribose is a five-carbon sugar found in RNA and AMP is one of the nucleotides in RNA. The addition of a second phosphate group to this core molecule results in adenosine diphosphate (ADP); the addition of a third phosphate group forms adenosine triphosphate (ATP).



(**Figure** 4.3.1) The illustration shows the structure of ATP. This molecule is an adenine nucleotide with a five-carbon ribose, and a string of three phosphate groups. The phosphate groups are named alpha, beta, and gamma in order of increasing distance from the ribose sugar to which they are attached.

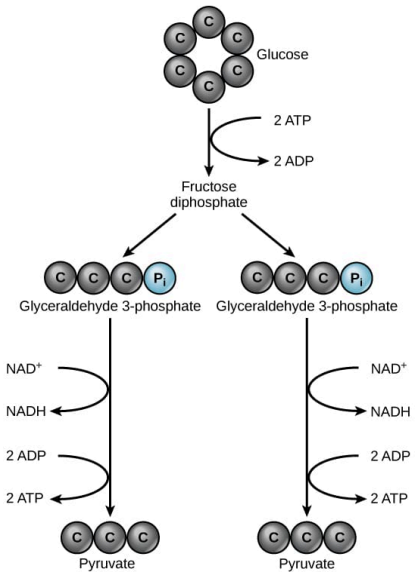
The addition of a phosphate group to a molecule requires a high amount of energy and results in a high-energy bond. Phosphate groups are negatively charged and thus repel one another when they are arranged in series, as they are in ADP and ATP. This repulsion makes the ADP and ATP molecules inherently unstable. The release of one or two phosphate groups from ATP, a process called hydrolysis, releases energy.

Glycolysis

You have read that nearly all of the energy used by living things comes to them in the bonds of the sugar, glucose. Glycolysis is the first step in the breakdown of glucose to extract energy for cell metabolism. Many living organisms carry out glycolysis as part of their metabolism. Glycolysis takes place in the cytoplasm of most prokaryotic and all eukaryotic cells.

Glycolysis begins with the six-carbon, ring-shaped structure of a single glucose molecule and ends with two molecules of a three-carbon sugar called pyruvate. Glycolysis consists of two distinct phases. In the first part of the glycolysis pathway, energy is used to make adjustments so that the six-carbon sugar molecule can be split evenly into two three-carbon pyruvate molecules. In the second part of glycolysis, ATP and nicotinamide-adenine dinucleotide (NADH) are produced (**Figure** 4.3.2).

If the cell cannot catabolize the pyruvate molecules further, it will harvest only two ATP molecules from one molecule of glucose. For example, mature mammalian red blood cells are only capable of glycolysis, which is their sole source of ATP. If glycolysis is interrupted, these cells would eventually die.



(**Figure** 4.3.2) In glycolysis, a glucose molecule is converted into two pyruvate molecules. The diagram above shows glucose at the top with an arrow pointing down to fructose diphosphate, which then splits into two glyceraldehyde 3-phosphate molecules. Each of these forms one NADH and two ATP molecules in the process of each becoming a pyruvate molecule.

Summary

ATP functions as the energy currency for cells. It allows cells to store energy briefly and transport it within itself to support endergonic chemical reactions. The structure of ATP is that of an RNA nucleotide with three phosphate groups attached. As ATP is used for energy, a phosphate group is detached, and ADP is produced. Energy derived from glucose catabolism is used to recharge ADP into ATP.

Glycolysis is the first pathway used in the breakdown of glucose to extract energy. Because it is used by nearly all organisms on earth, it must have evolved early in the history of life. Glycolysis consists of two parts: The first part prepares the six-carbon ring of glucose for separation into two three-carbon sugars. Energy from ATP is invested into the molecule during this step to energize the separation. The second half of glycolysis extracts ATP and high-energy electrons from hydrogen atoms and attaches them to NAD^+. Two ATP molecules are invested in the first half and four ATP molecules are formed during the second half. This produces a net gain of two ATP molecules per molecule of glucose for the cell.

Key Terms

ATP
: (also, adenosine triphosphate) the cell's energy currency

glycolysis
: the process of breaking glucose into two three-carbon molecules with the production of ATP and NADH

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4.4: Citric Acid Cycle and Oxidative Phosphorylation

Learning Objectives

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

- Describe the location of the citric acid cycle and oxidative phosphorylation in the cell
- Describe the overall outcome of the citric acid cycle and oxidative phosphorylation in terms of the products of each
- Describe the relationships of glycolysis, the citric acid cycle, and oxidative phosphorylation in terms of their inputs and outputs.

The Citric Acid Cycle

In eukaryotic cells, the pyruvate molecules produced at the end of glycolysis are transported into mitochondria, which are sites of cellular respiration. If oxygen is available, aerobic respiration will go forward. In mitochondria, pyruvate will be transformed into a two-carbon acetyl group (by removing a molecule of carbon dioxide) that will be picked up by a carrier compound called coenzyme A (CoA), which is made from vitamin B_5. The resulting compound is called acetyl CoA. (**Figure** 4.4.1). Acetyl CoA can be used in a variety of ways by the cell, but its major function is to deliver the acetyl group derived from pyruvate to the next pathway in glucose catabolism.

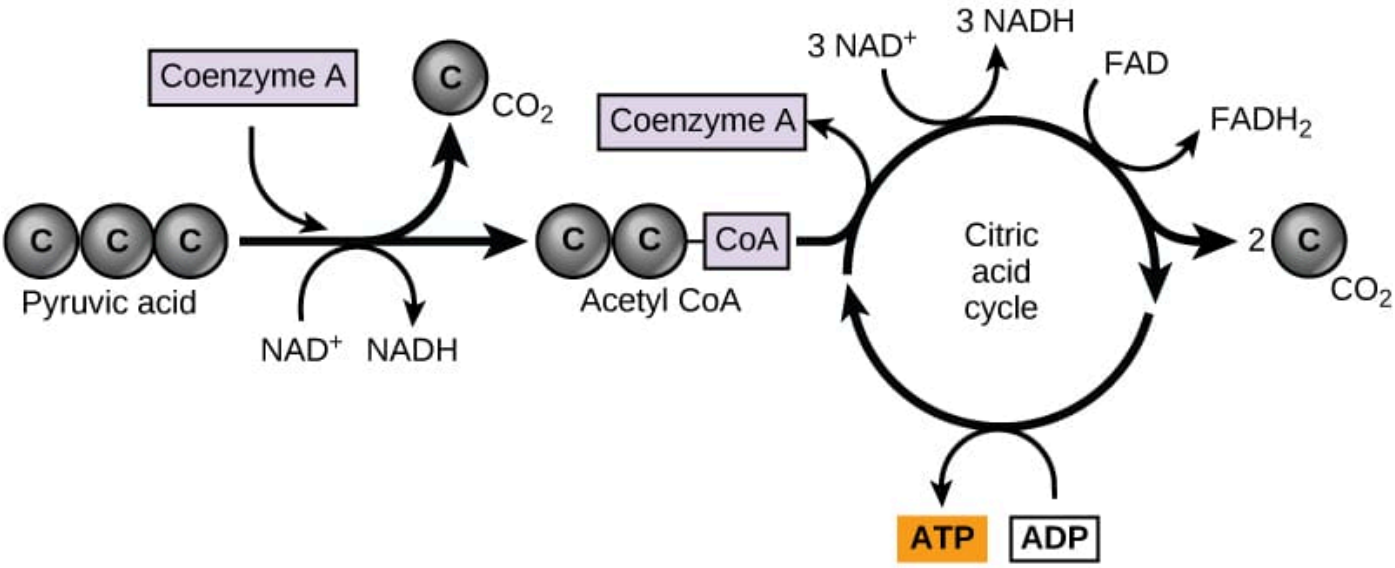


Figure 4.4.1: Pyruvate is converted into acetyl-CoA before entering the citric acid cycle.

The diagram above shows pyruvate becoming a two-carbon acetyl group by removing one molecule of carbon dioxide. The two-carbon acetyl group is picked up by coenzyme A to become acetyl CoA. The acetyl CoA then enters the citric acid cycle. Three NADH, one FADH2, one ATP, and two carbon dioxide molecules are produced during this cycle.

Like the conversion of pyruvate to acetyl CoA, the citric acid cycle in eukaryotic cells takes place in the matrix of the mitochondria. Unlike glycolysis, the citric acid cycle is a closed loop: The last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of chemical reactions that produces two carbon dioxide molecules, one ATP molecule (or an equivalent), and reduced forms (NADH and $FADH_2$) of NAD^+ and FAD^+, important coenzymes in the cell. Part of this is considered an aerobic pathway (oxygen-requiring) because the NADH and $FADH_2$ produced must transfer their electrons to the next pathway in the system, which will use oxygen. If oxygen is not present, this transfer does not occur.

Two carbon atoms come into the citric acid cycle from each acetyl group. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not contain the same carbon atoms contributed by the acetyl group on that turn of the pathway.

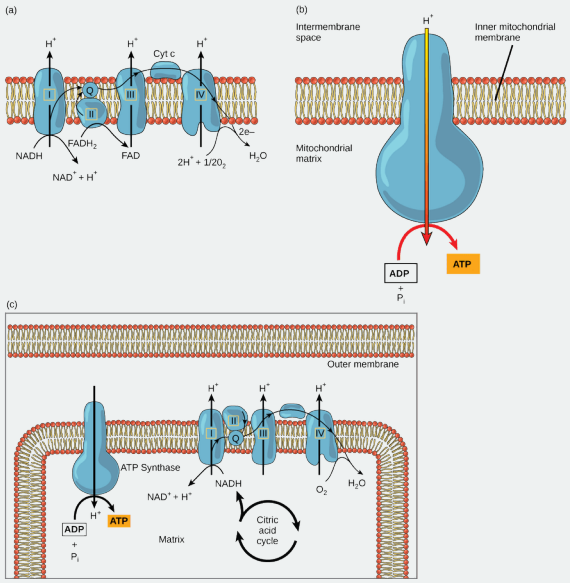
The two acetyl-carbon atoms will eventually be released on later turns of the cycle; in this way, all six carbon atoms from the original glucose molecule will be eventually released as carbon dioxide. It takes two turns of the cycle to process the equivalent of one glucose molecule. Each turn of the cycle forms three high-energy NADH molecules and one high-energy $FADH_2$ molecule. These high-energy carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One ATP (or an equivalent) is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is both anabolic and catabolic.

Oxidative Phosphorylation

You have just read about two pathways in glucose catabolism—glycolysis and the citric acid cycle—that generate ATP. Most of the ATP generated during the aerobic catabolism of glucose, however, is not generated directly from these pathways. Rather, it derives from a process that begins with passing electrons through a series of chemical reactions to a final electron acceptor, oxygen. These reactions take place in specialized protein complexes located in the inner membrane of the mitochondria of eukaryotic organisms and on the inner part of the cell membrane of prokaryotic organisms. The energy of the electrons is harvested and used to generate a electrochemical gradient across the inner mitochondrial membrane. The potential energy of this gradient is used to generate ATP. The entirety of this process is called oxidative phosphorylation.

The electron transport chain (**Figure** $4.4.2$**a**) is the last component of aerobic respiration and is the only part of metabolism that uses atmospheric oxygen. Oxygen continuously diffuses into plants for this purpose. In animals, oxygen enters the body through the respiratory system. Electron transport is a series of chemical reactions that resembles a bucket brigade in that electrons are passed rapidly from one component to the next, to the endpoint of the chain where oxygen is the final electron acceptor and water is produced. There are four complexes composed of proteins, labeled I through IV in **Figure** $4.4.2$**c**, and the aggregation of these four complexes, together with associated mobile, accessory electron carriers, is called the electron transport chain. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and in the plasma membrane of prokaryotes. In each transfer of an electron through the electron transport chain, the electron loses energy, but with some transfers, the energy is stored as potential energy by using it to pump hydrogen ions across the inner mitochondrial membrane into the intermembrane space, creating an electrochemical gradient.

ART CONNECTION



(**Figure** 4.4.2) (**a**) The electron transport chain is a set of molecules that supports a series of oxidation-reduction reactions. **(b)** ATP synthase is a complex, molecular machine that uses an H^+ gradient to regenerate ATP from ADP. (**c**) Chemiosmosis relies on the potential energy provided by the H^+ gradient across the membrane.

Part a: This illustration shows the electron transport chain embedded in the inner mitochondrial membrane. The electron transport chain consists of four electron complexes. Complex I oxidizes NADH to NAD+ and simultaneously pumps a proton across the membrane into the intermembrane space. The two electrons released from NADH are shuttled to coenzyme Q, then to complex III, to cytochrome c, to complex IV, then to molecular oxygen. In the process, two more protons are pumped across the membrane into the intermembrane space, and molecular oxygen is reduced to form water. Complex II removes two electrons from FADH2, thereby forming FAD. The electrons are shuttled to coenzyme Q, then to complex III, cytochrome c, complex I, and molecular oxygen as in the case of NADH oxidation.

Part b: This illustration shows an ATP synthase enzyme embedded in the inner mitochondrial membrane. ATP synthase allows protons to move from an area of high concentration in the intermembrane space to an area of low concentration in the mitochondrial matrix. The energy derived from this exergonic process is used to synthesize ATP from ADP and inorganic phosphate.

Part c: This illustration shows the electron transport chain and ATP synthase enzyme embedded in the inner mitochondrial membrane, and the citric acid cycle in the mitochondrial matrix. The citric acid cycle feeds NADH and FADH2 into the electron transport chain. The electron transport chain oxidizes these substrates and, in the process, pumps protons into the intermembrane space. ATP synthase allows protons to leak back into the matrix and synthesizes ATP.

Cyanide inhibits cytochrome c oxidase, a component of the electron transport chain. If cyanide poisoning occurs, would you expect the pH of the intermembrane space to increase or decrease? What affect would cyanide have on ATP synthesis?

Electrons from NADH and $FADH_2$ are passed to protein complexes in the electron transport chain. As they are passed from one complex to another (there are a total of four), the electrons lose energy, and some of that energy is used to pump hydrogen ions from the mitochondrial matrix into the intermembrane space. In the fourth protein complex, the electrons are accepted by oxygen, the terminal acceptor. The oxygen with its extra electrons then combines with two hydrogen ions, further enhancing the electrochemical gradient, to form water. If there were no oxygen present in the mitochondrion, the electrons could not be removed from the system, and the entire electron transport chain would back up and stop. The mitochondria would be unable to generate new ATP in this way, and the cell would ultimately die from lack of energy. This is the reason we must breathe to draw in new oxygen.

In the electron transport chain, the free energy from the series of reactions just described is used to pump hydrogen ions across the membrane. The uneven distribution of H^+ ions across the membrane establishes an electrochemical gradient, owing to the H^+ ions' positive charge and their higher concentration on one side of the membrane.

Hydrogen ions diffuse through the inner membrane through an integral membrane protein called ATP synthase (**Figure** 4.4.2 **b**). This complex protein acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient from the intermembrane space, where there are many mutually repelling hydrogen ions to the matrix, where there are few. The turning of the parts of this molecular machine regenerate ATP from ADP. This flow of hydrogen ions across the membrane through ATP synthase is called chemiosmosis.

Chemiosmosis (**Figure** 4.4.2**c**) is used to generate 90 percent of the ATP made during aerobic glucose catabolism. The result of the reactions is the production of ATP from the energy of the electrons removed from hydrogen atoms. These atoms were originally part of a glucose molecule. At the end of the electron transport system, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen ions attract hydrogen ions (protons) from the surrounding medium, and water is formed. The electron transport chain and the production of ATP through chemiosmosis are collectively called oxidative phosphorylation.

ATP Yield

The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport chain complexes can pump through the membrane varies between species. Another source of variance stems from the shuttle of electrons across the mitochondrial membrane. The NADH generated from glycolysis cannot easily enter mitochondria. Thus, electrons are picked up on the inside of the mitochondria by either NAD^+ or FAD^+. Fewer ATP molecules are generated when FAD^+ acts as a carrier. NAD^+ is used as the electron transporter in the liver and FAD^+ in the brain, so ATP yield depends on the tissue being considered.

Another factor that affects the yield of ATP molecules generated from glucose is that intermediate compounds in these pathways are used for other purposes. Glucose catabolism connects with the pathways that build or break down all other biochemical compounds in cells, and the result is somewhat messier than the ideal situations described thus far. For example, sugars other than glucose are fed into the glycolytic pathway for energy extraction. Other molecules that would otherwise be used to harvest energy in glycolysis or the citric acid cycle may be removed to form nucleic acids, amino acids, lipids, or other compounds. Overall, in living systems, these pathways of glucose catabolism extract about 34 percent of the energy contained in glucose.

CAREERS IN ACTION: Mitochondrial Disease Physician

What happens when the critical reactions of cellular respiration do not proceed correctly? Mitochondrial diseases are genetic disorders of metabolism. Mitochondrial disorders can arise from mutations in nuclear or mitochondrial DNA, and they result in the production of less energy than is normal in body cells. Symptoms of mitochondrial diseases can include muscle weakness, lack of coordination, stroke-like episodes, and loss of vision and hearing. Most affected people are diagnosed in childhood, although there are some adult-onset diseases. Identifying and treating mitochondrial disorders is a specialized medical field. The educational preparation for this profession requires a college education, followed by medical school with a specialization in medical genetics. Medical geneticists can be board certified by the American Board of Medical Genetics and go on to become associated with professional organizations devoted to the study of mitochondrial disease, such as the Mitochondrial Medicine Society and the Society for Inherited Metabolic Disease.

Summary

The citric acid cycle is a series of chemical reactions that removes high-energy electrons and uses them in the electron transport chain to generate ATP. One molecule of ATP (or an equivalent) is produced per each turn of the cycle.

The electron transport chain is the portion of aerobic respiration that uses free oxygen as the final electron acceptor for electrons removed from the intermediate compounds in glucose catabolism. The electrons are passed through a series of chemical reactions, with a small amount of free energy used at three points to transport hydrogen ions across the membrane. This contributes to the gradient used in chemiosmosis. As the electrons are passed from NADH or $FADH_2$ down the electron transport chain, they lose energy. The products of the electron transport chain are water and ATP. A number of intermediate compounds can be diverted into the anabolism of other biochemical molecules, such as nucleic acids, non-essential amino acids, sugars, and lipids. These same molecules, except nucleic acids, can serve as energy sources for the glucose pathway.

Art Connections

Figure 4.4.2: Cyanide inhibits cytochrome c oxidase, a component of the electron transport chain. If cyanide poisoning occurs, would you expect the pH of the intermembrane space to increase or decrease? What affect would cyanide have on ATP synthesis?

Answer

After cyanide poisoning, the electron transport chain can no longer pump electrons into the intermembrane space. The pH of the intermembrane space would increase, and ATP synthesis would stop.

Key Terms

acetyl CoA
the combination of an acetyl group derived from pyruvic acid and coenzyme A which is made from pantothenic acid (a B-group vitamin)

ATP synthase
a membrane-embedded protein complex that regenerates ATP from ADP with energy from protons diffusing through it

chemiosmosis
the movement of hydrogen ions down their electrochemical gradient across a membrane through ATP synthase to generate ATP

citric acid cycle
a series of enzyme-catalyzed chemical reactions of central importance in all living cells that harvests the energy in carbon-carbon bonds of sugar molecules to generate ATP; the citric acid cycle is an aerobic metabolic pathway because it requires oxygen in later reactions to proceed

electron transport chain
a series of four large, multi-protein complexes embedded in the inner mitochondrial membrane that accepts electrons from donor compounds and harvests energy from a series of chemical reactions to generate a hydrogen ion gradient across the membrane

oxidative phosphorylation
the production of ATP by the transfer of electrons down the electron transport chain to create a proton gradient that is used by ATP synthase to add phosphate groups to ADP molecules

Contributors and Attributions

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4.5: Fermentation

Learning Objectives

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

- Discuss the fundamental difference between anaerobic cellular respiration and fermentation
- Describe the type of fermentation that readily occurs in animal cells and the conditions that initiate that fermentation

In aerobic respiration, the final electron acceptor is an oxygen molecule, O_2. If aerobic respiration occurs, then ATP will be produced using the energy of the high-energy electrons carried by NADH or $FADH_2$ to the electron transport chain. If aerobic respiration does not occur, NADH must be reoxidized to NAD^+ for reuse as an electron carrier for glycolysis to continue. How is this done? Some living systems use an organic molecule as the final electron acceptor. Processes that use an organic molecule to regenerate NAD^+ from NADH are collectively referred to as fermentation. In contrast, some living systems use an inorganic molecule (other than oxygen) as a final electron acceptor to regenerate NAD^+; both methods are anaerobic (do not require oxygen) to achieve NAD^+ regeneration and enable organisms to convert energy for their use in the absence of oxygen.

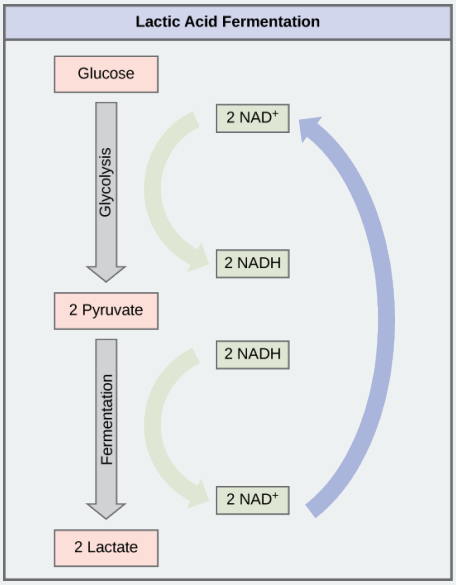
Lactic Acid Fermentation

The fermentation method used by animals and some bacteria like those in yogurt is lactic acid fermentation (**Figure** 4.5.1). This occurs routinely in mammalian red blood cells and in skeletal muscle that has insufficient oxygen supply to allow aerobic respiration to continue (that is, in muscles used to the point of fatigue). In muscles, lactic acid produced by fermentation must be removed by the blood circulation and brought to the liver for further metabolism. The chemical reaction of lactic acid fermentation is the following:

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

The enzyme that catalyzes this reaction is lactate dehydrogenase. The reaction can proceed in either direction, but the left-to-right reaction is inhibited by acidic conditions. This lactic acid build-up causes muscle stiffness and fatigue. Once the lactic acid has been removed from the muscle and is circulated to the liver, it can be converted back to pyruvic acid and further catabolized for energy.

ART CONNECTION

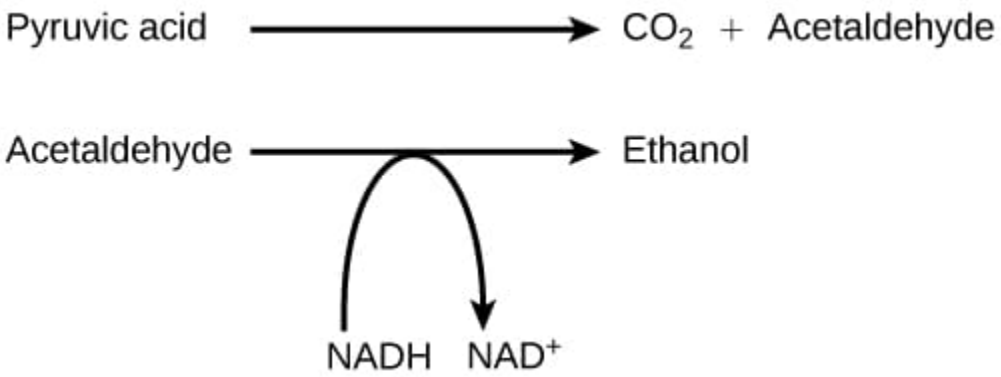


(**Figure** 4.5.1) Lactic acid fermentation is common in muscles that have become exhausted by use. The diagram above shows glucose undergoing glycolysis to become two pyruvate molecules, which then undergo fermentation to become two lactate molecules. During glycolysis, two NAD+ are converted into two high-energy NADH molecules, but during fermentation, these two NADH molecules are reoxidized to become two NAD+ again. NAD+ can then be used in glycolysis.

Tremetol, a metabolic poison found in white snake root plant, prevents the metabolism of lactate. When cows eat this plant, Tremetol is concentrated in the milk. Humans who consume the milk become ill. Symptoms of this disease, which include vomiting, abdominal pain, and tremors, become worse after exercise. Why do you think this is the case?

Alcohol Fermentation

Another familiar fermentation process is alcohol fermentation (**Figure** 4.5.2), which produces ethanol, an alcohol. The alcohol fermentation reaction is the following:



(**Figure** 4.5.2) The reaction resulting in alcohol fermentation is shown.

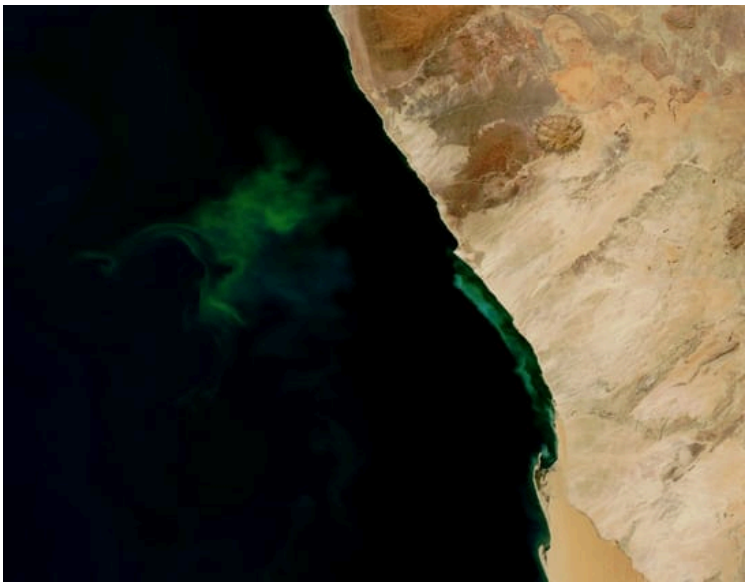
In the first reaction, a carboxyl group is removed from pyruvic acid, releasing carbon dioxide as a gas. The loss of carbon dioxide reduces the molecule by one carbon atom, making acetaldehyde. The second reaction removes an electron from NADH, forming NAD^+ and producing ethanol from the acetaldehyde, which accepts the electron. The fermentation of pyruvic acid by yeast produces the ethanol found in alcoholic beverages (**Figure** 4.5.3). If the carbon dioxide produced by the reaction is not vented from the fermentation chamber, for example in beer and sparkling wines, it remains dissolved in the medium until the pressure is released. Ethanol above 12 percent is toxic to yeast, so natural levels of alcohol in wine occur at a maximum of 12 percent.



(**Figure** 4.5.3) Fermentation of grape juice to make wine produces CO_2 as a byproduct. Fermentation tanks have valves so that pressure inside the tanks can be released.

Anaerobic Cellular Respiration

Certain prokaryotes, including some species of bacteria and Archaea, use anaerobic respiration. For example, the group of Archaea called methanogens reduces carbon dioxide to methane to oxidize NADH. These microorganisms are found in soil and in the digestive tracts of ruminants, such as cows and sheep. Similarly, sulfate-reducing bacteria and Archaea, most of which are anaerobic (**Figure** 4.5.4), reduce sulfate to hydrogen sulfide to regenerate NAD^+ from NADH.



(**Figure** 4.5.4) The green color seen in these coastal waters is from an eruption of hydrogen sulfide. Anaerobic, sulfate-reducing bacteria release hydrogen sulfide gas as they decompose algae in the water. (credit: NASA image courtesy Jeff Schmaltz, MODIS Land Rapid Response Team at NASA GSFC)

CONCEPT IN ACTION

Watch this Yeast Experiment video to see anaerobic cellular respiration in action.

Other fermentation methods occur in bacteria. Many prokaryotes are facultatively anaerobic. This means that they can switch

between aerobic respiration and fermentation, depending on the availability of oxygen. Certain prokaryotes, like *Clostridia* bacteria, are obligate anaerobes. Obligate anaerobes live and grow in the absence of molecular oxygen. Oxygen is a poison to these microorganisms and kills them upon exposure. It should be noted that all forms of fermentation, except lactic acid fermentation, produce gas. The production of particular types of gas is used as an indicator of the fermentation of specific carbohydrates, which plays a role in the laboratory identification of the bacteria. The various methods of fermentation are used by different organisms to ensure an adequate supply of NAD^+ for the sixth step in glycolysis. Without these pathways, that step would not occur, and no ATP would be harvested from the breakdown of glucose.

Summary

If NADH cannot be metabolized through aerobic respiration, another electron acceptor is used. Most organisms will use some form of fermentation to accomplish the regeneration of NAD^+, ensuring the continuation of glycolysis. The regeneration of NAD^+ in fermentation is not accompanied by ATP production; therefore, the potential for NADH to produce ATP using an electron transport chain is not utilized.

Art Connections

Figure 4.5.1: Tremetol, a metabolic poison found in white snake root plant, prevents the metabolism of lactate. When cows eat this plant, Tremetol is concentrated in the milk. Humans who consume the milk become ill. Symptoms of this disease, which include vomiting, abdominal pain, and tremors, become worse after exercise. Why do you think this is the case?

Answer

The illness is caused by lactic acid build-up. Lactic acid levels rise after exercise, making the symptoms worse. Milk sickness is rare today, but was common in the Midwestern United States in the early 1800s.

Key Terms

anaerobic cellular respiration
the use of an electron acceptor other than oxygen to complete metabolism using electron transport-based chemiosmosis

fermentation
the steps that follow the partial oxidation of glucose via glycolysis to regenerate NAD^+; occurs in the absence of oxygen and uses an organic compound as the final electron acceptor

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4.6: How Cells Obtain Energy (Exercises)

4.1: Energy and Metabolism

Cells perform the functions of life through various chemical reactions. A cell's metabolism refers to the combination of chemical reactions that take place within it. Catabolic reactions break down complex chemicals into simpler ones and are associated with energy release. Anabolic processes build complex molecules out of simpler ones and require energy. In studying energy, the term system refers to the matter and environment involved in energy transfers.

Review Questions

Which of the following is not an example of an energy transformation?

A. Heating up dinner in a microwave
B. Solar panels at work
C. Formation of static electricity
D. None of the above

Answer

D

Which of the following is not true about enzymes?

A. They are consumed by the reactions they catalyze.
B. They are usually made of amino acids.
C. They lower the activation energy of chemical reactions.
D. Each one is specific to the particular substrate(s) to which it binds.

Answer

A

Free Response

Does physical exercise to increase muscle mass involve anabolic and/or catabolic processes? Give evidence for your answer.

Answer

Physical exercise involves both anabolic and catabolic processes. Body cells break down sugars to provide ATP to do the work necessary for exercise, such as muscle contractions. This is catabolism. Muscle cells also must repair muscle tissue damaged by exercise by building new muscle. This is anabolism.

Explain in your own terms the difference between a spontaneous reaction and one that occurs instantaneously, and what causes this difference.

Answer

A spontaneous reaction is one that has a negative ΔG and thus releases energy. However, a spontaneous reaction need not occur quickly or suddenly like an instantaneous reaction. It may occur over long periods of time due to a large energy of activation, which prevents the reaction from occurring quickly.

With regard to enzymes, why are vitamins and minerals necessary for good health? Give examples.

Answer

Most vitamins and minerals act as cofactors and coenzymes for enzyme action. Many enzymes require the binding of certain cofactors or coenzymes to be able to catalyze their reactions. Since enzymes catalyze many important reactions, it is critical to obtain sufficient vitamins and minerals from diet and supplements. Vitamin C (ascorbic acid) is a coenzyme necessary for the action of enzymes that build collagen.

4.2: Glycolysis

ATP functions as the energy currency for cells. It allows cells to store energy briefly and transport it within itself to support endergonic chemical reactions. The structure of ATP is that of an RNA nucleotide with three phosphate groups attached. As ATP is used for energy, a phosphate group is detached, and ADP is produced. Energy derived from glucose catabolism is used to recharge ADP into ATP. Glycolysis is the first pathway used in the breakdown of glucose to extract energy.

Multiple Choice

Energy is stored long-term in the bonds of _____ and used short-term to perform work from a(n) _____ molecule.

A. ATP : glucose
B. an anabolic molecule : catabolic molecule
C. glucose : ATP
D. a catabolic molecule : anabolic molecule

Answer

C

The energy currency used by cells is _____.

A. ATP
B. ADP
C. AMP
D. adenosine

Answer

A

The glucose that enters the glycolysis pathway is split into two molecules of _____.

A. ATP
B. phosphate
C. NADH
D. pyruvate

Answer

D

Free Response

Both prokaryotic and eukaryotic organisms carry out some form of glycolysis. How does that fact support or not support the assertion that glycolysis is one of the oldest metabolic pathways?

Answer

If glycolysis evolved relatively late, it likely would not be as universal in organisms as it is. It probably evolved in very primitive organisms and persisted, with the addition of other pathways of carbohydrate metabolism that

evolved later.

4.3: Citric Acid Cycle and Oxidative Phosphorylation

The citric acid cycle is a series of chemical reactions that removes high-energy electrons and uses them in the electron transport chain to generate ATP. One molecule of ATP (or an equivalent) is produced per each turn of the cycle. The electron transport chain is the portion of aerobic respiration that uses free oxygen as the final electron acceptor for electrons removed from the intermediate compounds in glucose catabolism.

Multiple Choice

What do the electrons added to NAD^+ do?

A. They become part of a fermentation pathway.
B. They go to another pathway for ATP production.
C. They energize the entry of the acetyl group into the citric acid cycle.
D. They are converted into NADP.

Answer

B

Chemiosmosis involves

A. the movement of electrons across the cell membrane
B. the movement of hydrogen atoms across a mitochondrial membrane
C. the movement of hydrogen ions across a mitochondrial membrane
D. the movement of glucose through the cell membrane

Answer

C

Free Response

We inhale oxygen when we breathe and exhale carbon dioxide. What is the oxygen used for and where does the carbon dioxide come from?

Answer

The oxygen we inhale is the final electron acceptor in the electron transport chain and allows aerobic respiration to proceed, which is the most efficient pathway for harvesting energy in the form of ATP from food molecules. The carbon dioxide we breathe out is formed during the citric acid cycle when the bonds in carbon compounds are broken.

4.4: Fermentation

If NADH cannot be metabolized through aerobic respiration, another electron acceptor is used. Most organisms will use some form of fermentation to accomplish the regeneration of NAD+, ensuring the continuation of glycolysis. The regeneration of NAD+ in fermentation is not accompanied by ATP production; therefore, the potential for NADH to produce ATP using an electron transport chain is not utilized.

Review Questions

Which of the following fermentation methods can occur in animal skeletal muscles?

A. lactic acid fermentation
B. alcohol fermentation
C. mixed acid fermentation
D. propionic fermentation

Answer

A

Free Response

When muscle cells run out of oxygen, what happens to the potential for energy extraction from sugars and what pathways do the cell use?

Answer

Without oxygen, oxidative phosphorylation and the citric acid cycle stop, so ATP is no longer generated through this mechanism, which extracts the greatest amount of energy from a sugar molecule. In addition, NADH accumulates, preventing glycolysis from going forward because of an absence of NAD^+. Lactic acid fermentation uses the electrons in NADH to generate lactic acid from pyruvate, which allows glycolysis to continue and thus a smaller amount of ATP can be generated by the cell.

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5: HOW CELLS SENSE THEIR ENVIRONMENT

5.1: Components of Plasma Membranes
5.2: Passive Transport
5.3: Facilitated Transport
5.4: Active Transport
5.5: Cell Communication
5.6: Cell Response

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5.1: Components of Plasma Membranes

Learning Objectives

- Describe the function and components of the plasma membrane
- Analyze how the amphiphilic nature of phospholipids influences membrane behavior
- Evaluate the functions of the major components of the plasma membrane and their contributions to membrane structure and cellular function.

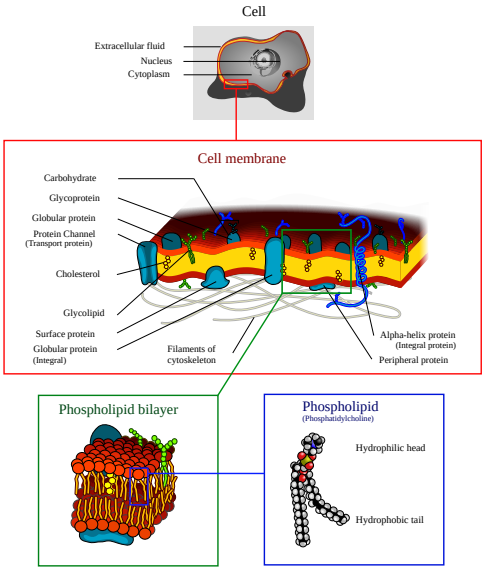
Plasma Membranes

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

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

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

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

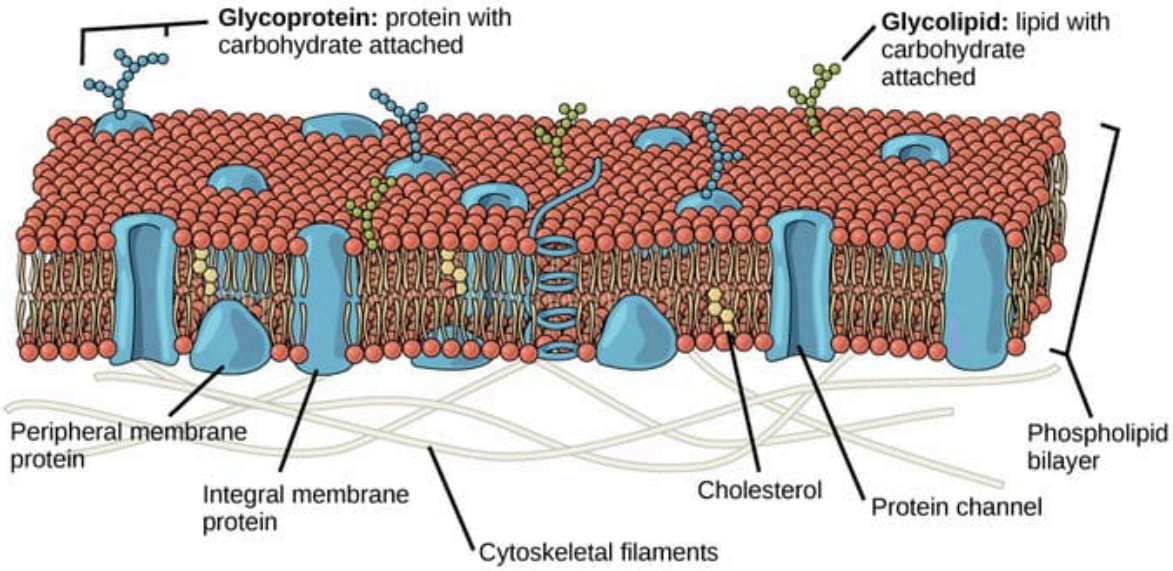


(**Figure** 5.1.1) The plasma membrane is composed of phospholipids and proteins that provide a barrier between the external environment and the cell, regulate the transportation of molecules across the membrane, and communicate with other cells via protein receptors.

Plasma Membrane Structure

Fluid Mosaic Model

To explain the structure of the plasma membrane, S.J. Singer and Garth L. Nicolson proposed the fluid mosaic model in 1972. The model has evolved somewhat over time, but it still best accounts for the structure and functions of the plasma membrane as we now understand them. The fluid mosaic model describes the structure of the plasma membrane as a mosaic of components —including phospholipids, cholesterol, proteins, and carbohydrates—that gives the membrane a fluid character. Plasma membranes range from 5 to 10 nm in thickness. For comparison, human red blood cells, visible via light microscopy, are approximately 8 µm wide, or approximately 1,000 times wider than a plasma membrane. The proportions of proteins, lipids, and carbohydrates in the plasma membrane vary with cell type. For example, myelin contains 18% protein and 76% lipid. The mitochondrial inner membrane contains 76% protein and 24% lipid.



(**Figure** 5.1.2) The fluid mosaic model of the plasma membrane describes the plasma membrane as a fluid combination of phospholipids, cholesterol, and proteins. Carbohydrates attached to lipids (glycolipids) and to proteins (glycoproteins) extend from the outward-facing surface of the membrane.

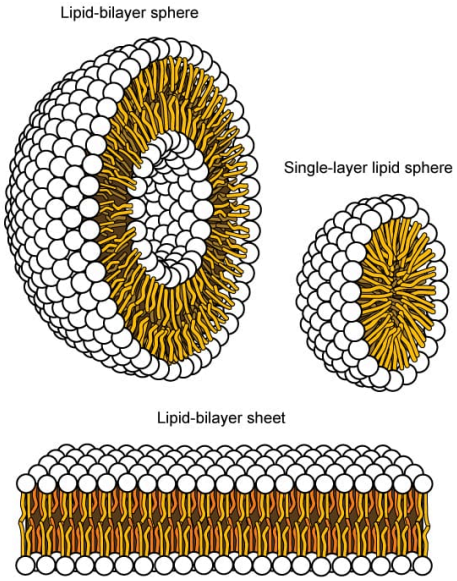
Components of the Plasma Membrane

Table 5.1.1*: The Components and Functions of the Plasma Membrane: The principal components of a plasma membrane are lipids (phospholipids and cholesterol), proteins, and carbohydrates attached to some of the lipids and some of the proteins.*

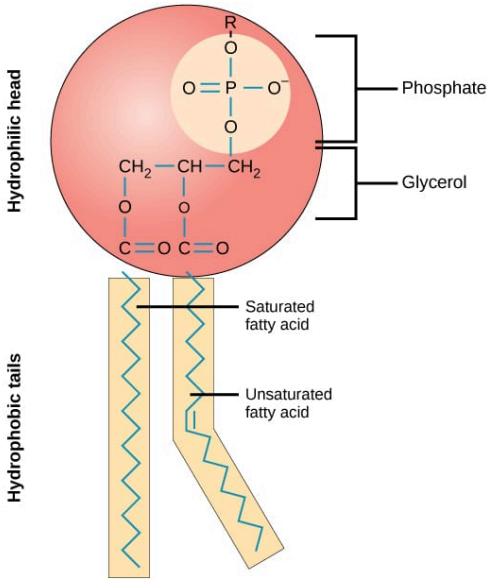
Components	Location
Phosphlipid	Main fabric of the membrane
Cholesterol	Attached between phospholipids and between the two phosppholipid layers
Integral proteins (for example, integrins)	Embedded within the phospholipid layer(s). May or may not penetrate through both layers
Peripheral proteins	On the inner or outer surface of the phospholipid bilayer; not embedded within the phosphlipids
Carbohydrates (components of clycoproteins and glycolipids)	Generally attached to proteins on the outside membrane layer

Phospholipids

The main fabric of the membrane is composed of amphiphilic or dual-loving, phospholipid molecules. The hydrophilic or water-loving areas of these molecules are in contact with the aqueous fluid both inside and outside the cell. Hydrophobic, or water-hating molecules, tend to be non- polar. A phospholipid molecule consists of a three-carbon glycerol backbone with two fatty acid molecules attached to carbons 1 and 2, and a phosphate-containing group attached to the third carbon. This arrangement gives the overall molecule an area described as its head (the phosphate-containing group), which has a polar character or negative charge, and an area called the tail (the fatty acids), which has no charge. They interact with other non-polar molecules in chemical reactions, but generally do not interact with polar molecules. When placed in water, hydrophobic molecules tend to form a ball or cluster. The hydrophilic regions of the phospholipids tend to form hydrogen bonds with water and other polar molecules on both the exterior and interior of the cell. Thus, the membrane surfaces that face the interior and exterior of the cell are hydrophilic. In contrast, the middle of the cell membrane is hydrophobic and will not interact with water. Therefore, phospholipids form an excellent lipid bilayer cell membrane that separates fluid within the cell from the fluid outside of the cell.



(**Figure** 5.1.3) In an aqueous solution, phospholipids tend to arrange themselves with their polar heads facing outward and their hydrophobic tails facing inward.



(**Figure** 5.1.4) This phospholipid molecule is composed of a hydrophilic head and two hydrophobic tails. The hydrophilic head group consists of a phosphate-containing group attached to a glycerol molecule. The hydrophobic tails, each containing either a saturated or an unsaturated fatty acid, are long hydrocarbon chains.

Integral Membrane Proteins

Proteins are a key component of the plasma membrane, working alongside phospholipids to give the membrane its structure and function. Some of the most important proteins found in membranes are integral membrane proteins, which are embedded directly within the phospholipid bilayer. Because they pass into or through the membrane, they must be specially adapted to survive in this unique, partly watery and partly oily environment.

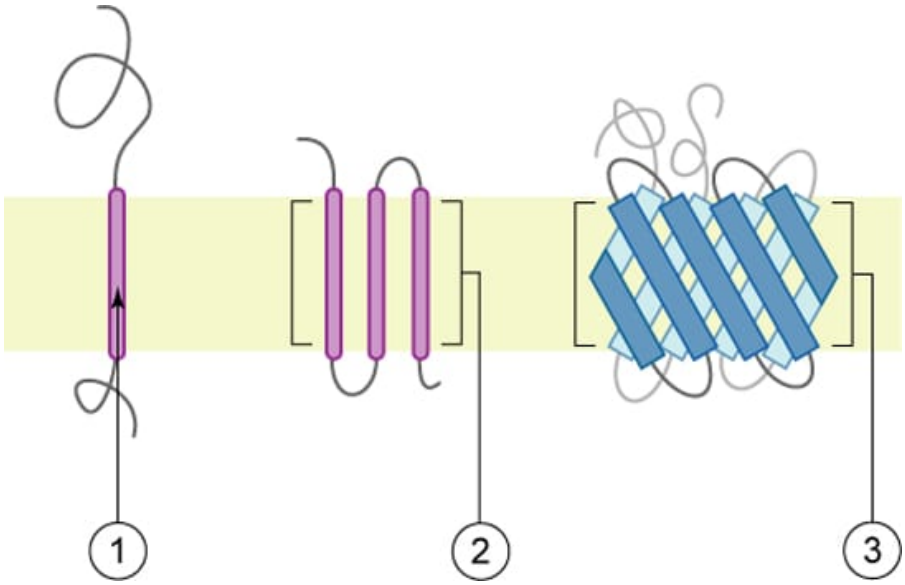
If we zoom in on one of these membrane proteins, as shown in **Figure** 5.1.5, we can see something interesting: different parts of the protein do different jobs. These distinct functional regions are called protein domains, a concept you've already encountered in previous chapters. Protein domains are like specialized "zones" of a protein, each built from a specific stretch of amino acids that folds into a shape that performs a particular task.

In the case of integral membrane proteins, we can usually identify three key domains:

1. Extracellular domain – This part of the protein sticks out into the space outside the cell. It often plays a role in interacting with the external environment—for example, binding to molecules like hormones or signaling compounds. In upcoming sections, you'll see this domain in action in receptors, where the outer domain is the "sensor" that detects signals from the environment.
2. Transmembrane domain – This part spans the hydrophobic core of the membrane. Because the inside of the membrane is oily (hydrophobic), the transmembrane domain is made of nonpolar amino acids that are also hydrophobic. This allows the protein to "sit" comfortably in the membrane without being pushed out. This domain is critical for anchoring the protein in place. In the figure, you can see that some proteins cross the membrane once (example 1), while others snake through it multiple times (example 2), often forming complex channels or transporters.
3. Intracellular domain – This region faces the inside of the cell and often communicates with internal cell structures. It can pass along signals, anchor the protein to internal scaffolding, or interact with other molecules inside the cell. You'll revisit this domain when we explore transport proteins, which help move materials into and out of the cell, and signaling proteins, which respond to messages received at the cell surface.

Some integral proteins are relatively simple, consisting of a single domain that crosses the membrane. Others are much more complex, weaving in and out of the membrane many times and folding into elaborate shapes. Regardless of their complexity, what's important to remember is that each domain of the protein is shaped by its function and the chemical environment it's exposed to. The transmembrane domain, for example, must be made of hydrophobic amino acids so it can live happily in the oily core of the membrane, while the extracellular and intracellular domains are usually hydrophilic so they can interact with water-based fluids outside and inside the cell.

By keeping the idea of protein domains in mind, you can begin to make sense of how a single protein can perform multiple tasks—and how different types of proteins are designed for different roles. This will be especially helpful as you learn more about receptors, transporters, and enzymes, each of which uses its domains in distinct ways to help cells survive, communicate, and respond to their environment.



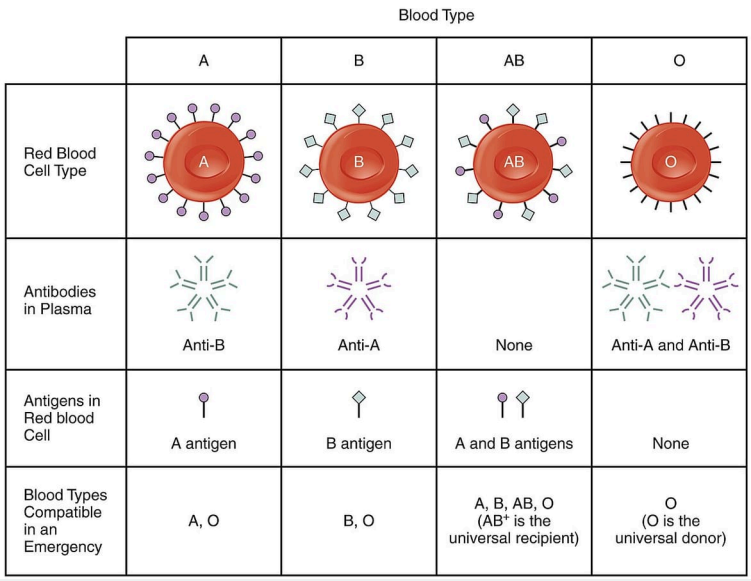
(**Figure** 5.1.5) Integral membrane proteins may have one or more alpha-helices that span the membrane (examples 1 and 2), or they may have beta-sheets that span the membrane (example 3). Each structure includes specialized domains that allow the protein to function in specific environments—within the membrane, and on either side of it.

Carbohydrates

Carbohydrates are the third major component of plasma membranes. They are always found on the exterior surface of cells and are bound either to proteins (forming glycoproteins) or to lipids (forming glycolipids). These carbohydrate chains may consist of 2–60 monosaccharide units and can be either straight or branched. Along with peripheral proteins, carbohydrates form specialized sites on the cell surface that allow cells to recognize each other. This recognition function is very important to cells, as it allows the immune system to differentiate between body cells (called “self”) and foreign cells or tissues (called “non-self”). Similar types of glycoproteins and glycolipids are found on the surfaces of viruses and may change frequently, preventing immune cells from recognizing and attacking them. These carbohydrates on the exterior surface of the cell—the carbohydrate components of both glycoproteins and glycolipids—are collectively referred to as the glycocalyx (meaning “sugar coating”). The glycocalyx is highly hydrophilic and attracts large amounts of water to the surface of the cell. This aids in the interaction of the cell with its watery environment and in the cell’s ability to obtain substances dissolved in the water.

CONCEPT IN ACTION

The carbohydrates attached to glycoproteins on red blood cells determine a person’s A, B, or O blood type. These glycoproteins are recognized by the immune system, which is why mismatched blood types can cause immune reactions.



(**Figure** 5.1.6) ABO Blood types. In this figure, the membrane carbohydrate is represented by the "lollipops". They are termed "antigens". Photo credit: InvictaHOG, 2006. Wikimedia(opens in new window).

Plasma Membrane Functions

Structural and Organizational Roles

The plasma membrane is a dynamic and essential component of all living cells, serving not only as a physical barrier but also as a gateway for communication and exchange with the surrounding environment. It is also structurally asymmetric—the interior surface differs significantly from the exterior in both composition and function. For example, certain proteins on the inner side anchor the membrane to cytoskeletal fibers, while peripheral proteins on the outer surface interact with elements of the extracellular matrix.

Key Functional Roles

Cellular Transport

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

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

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

CONCEPT IN ACTION

We've just explored how integral membrane proteins are anchored within the cell membrane by their transmembrane domains, and how their extracellular and intracellular domains work together to perform various roles. Now let's look at a real-life example that brings these ideas into focus—an integral protein called CFTR, short for *Cystic Fibrosis Transmembrane Conductance Regulator*.

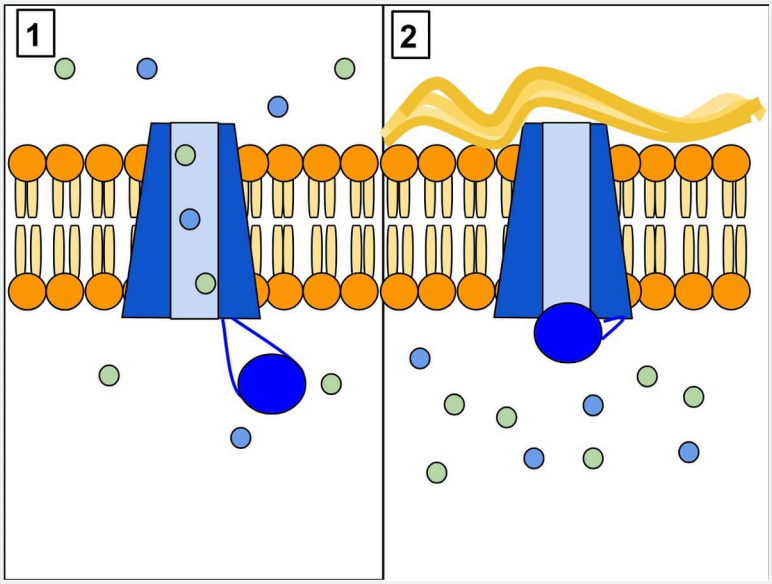
CFTR is a membrane protein found in cells that line parts of the body such as the lungs and digestive system. It plays a key role in helping move chloride ions (Cl^-) across the cell membrane. Though we will explore transport mechanisms in more detail later, it's enough for now to understand that CFTR acts somewhat like a channel or tunnel embedded in the membrane—one that allows ions to pass from one side of the membrane to the other.

As seen in **Figure \(\PageIndex{6}\)**, when CFTR is working properly (Panel 1), chloride ions move through the protein from inside the cell to the outside, helping to maintain proper fluid balance in places like the lungs. This movement of ions draws water with it, which helps keep mucus thin and easier to clear from the airways.

Let's break this down using our knowledge of protein domains:

- The transmembrane domain forms the actual channel through which ions flow. Think of it as the walls of a tunnel that runs through the membrane. For this to work, the protein must have a hollow space—like a pipe—that spans the membrane.
- The extracellular domain is the "entrance" of the tunnel facing the outside of the cell, often into the mucus-lined space in organs like the lungs.
- The intracellular domain acts as the "exit" or interior opening, facing the inside of the cell.

What makes CFTR especially interesting is that it has another specialized intracellular domain that functions like a gate or valve. This gate opens or closes to regulate whether ions can pass through the tunnel. When this domain doesn't work correctly—because of a mutation in the CFTR gene—the gate may stay closed, even when it should be open (Panel 2). As a result, chloride ions can't leave the cell, which also means water doesn't follow. This leads to thick, sticky mucus accumulating in the lungs—a key feature of Cystic Fibrosis.



(**Figure** 5.1.7) The CFTR membrane protein in action. When the CFTR protein is functioning properly (**1**), chloride ions are able to pass through the membrane. When it is not functioning correctly (**2**), ions cannot move through, leading to problems such as thick mucus buildup in the lungs. This condition is known as Cystic Fibrosis. Photo credit: LBudd14(opens in new window), May, 2013. Wikimedia(opens in new window).

Cellular Signaling

Among the most sophisticated functions of the plasma membrane is its ability to transmit signals using complex, membrane-bound proteins. These proteins can function as receptors, which act as receivers of extracellular inputs and as activators of intracellular processes, or as markers, which allow cells to recognize one another and communicate.

Just like transport proteins, membrane receptors and markers are integral proteins embedded within the membrane. But their protein domains are different, and that difference is key to understanding their function. Transport proteins often have transmembrane domains that form channels or passageways, allowing substances like ions or molecules to move across the membrane. In contrast, receptor and marker proteins have domains specialized for communication, not transport.

Membrane receptors, for example, have specific extracellular domains designed to recognize and bind to molecules outside the cell—like hormones, neurotransmitters, or growth factors. When one of these signaling molecules binds to the receptor, the intracellular domain changes shape and triggers a chain reaction inside the cell, often activating a signaling pathway or gene expression. These specialized regions—the binding domain outside the cell and the signaling domain inside the cell—are what allow receptors to function as effective communication hubs.

Some viruses, such as Human Immunodeficiency Virus (HIV), exploit these very domains. HIV binds to the extracellular domain of a specific receptor found on immune cells. Once attached, the virus can gain entry into the cell, hijacking the cell's machinery for its own replication. This highlights how critical domain structure is: a protein's ability to bind or signal depends entirely on having the right shape and surface features in the right location.

Membrane marker proteins also rely on distinct domains. Their extracellular domains often display molecular "tags" or identifiers that act like cellular name badges. This is essential for processes like tissue development, where cells need to recognize and adhere to the right neighbors, and for the immune system, which uses these markers to distinguish between the body's own cells ("self") and foreign invaders ("non-self"). A well-known example is found on red blood cells, where the specific markers on the cell surface determine a person's blood type (A, B, AB, or O). A mismatch in these markers during a transfusion can trigger a dangerous immune response.

In each of these cases, it's the domain structure of the protein—whether it's for binding a signal molecule, triggering an internal response, or displaying a cellular identity—that determines the protein's role. By now, you've seen this pattern again and again: form determines function.

Summary

The plasma membrane is a flexible, protective barrier that surrounds the cell and helps control what enters and leaves. It's made mostly of phospholipids, proteins, and carbohydrates, arranged in a structure known as the fluid mosaic model. This model describes how these components move within the membrane, giving it both structure and flexibility.

The phospholipid bilayer forms the main structure of the membrane, with hydrophilic (water-attracting) heads facing outward and hydrophobic (water-repelling) tails facing inward. Integral and peripheral proteins help with transport, communication, and support. Carbohydrates attached to proteins and lipids play key roles in cell recognition and immune response.

The plasma membrane is selectively permeable, meaning it allows some substances (like oxygen or carbon dioxide) to pass freely while requiring proteins to help move other materials (like glucose or ions). It supports both passive (no energy needed) and active (energy required) transport mechanisms, including processes like endocytosis (bringing in large materials) and exocytosis (removing substances).

Beyond transport, the membrane also helps cells communicate with their environment through receptor proteins, and it anchors cells together into tissues by connecting to the cytoskeleton and the extracellular matrix. These combined roles make the plasma membrane essential for cell survival and function.

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

Key Terms

amphiphilic
: Having one surface consisting of hydrophilic amino acids and the opposite surface consisting of hydrophobic (or lipophilic) ones

diffusion
: the movement of molecules from an area of high concentration to low concentration

endocytosis
: a process in which a cell takes in materials by folding its membrane inward

exocytosis
a process in which a cell releases materials by fusing internal vesicles with the cell membrane

hydrophilic
Having an affinity for water; able to absorb, or be wetted by water, “water-loving."

hydrophobic
Lacking an affinity for water; unable to absorb, or be wetted by water, “water-fearing.”

integral proteins
proteins embedded (completely integrated) in the membrane structure

osmosis
the movement of water across a membrane from lo solute concentrations to high solute concentration

phospholipid
a major component of cell membranes, made of a hydrophilic head and hydrophobic tails

plasma membrane
The semipermeable barrier that surrounds the cytoplasm of a cell.

receptor
A protein on a cell wall that binds with specific molecules so that they can be absorbed into the cell.

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5.2: Passive Transport

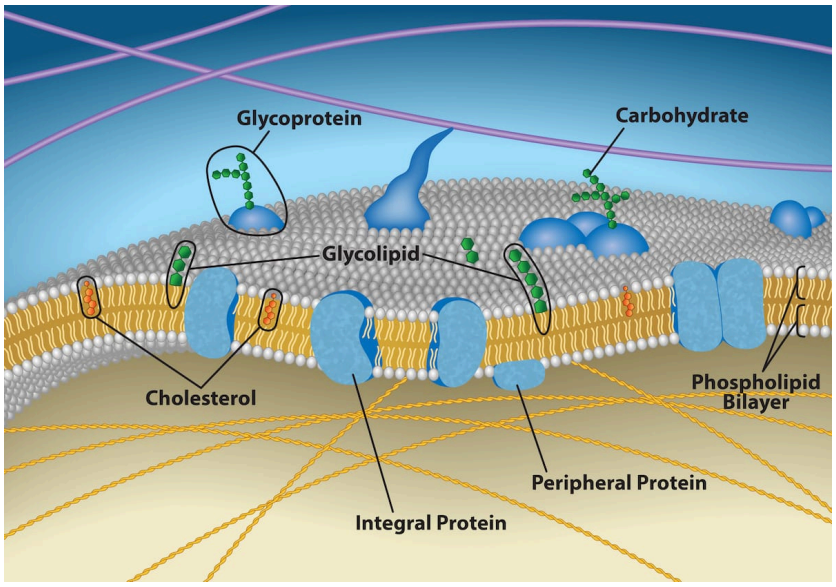
Learning Objectives

- Describe how membrane permeability, concentration gradient, and molecular properties affect biological diffusion rates
- Indicate the manner in which various materials cross the cell membrane
- Describe diffusion and the factors that affect how materials move across the cell membrane
- Explain how membrane proteins assist in facilitated transport.
- Describe the process of osmosis and explain how concentration gradient affects osmosis

Selective Permeability

Plasma membranes are asymmetric, meaning the inside surface of the membrane is different from the outside surface. The two layers (called leaflets) have different types and arrangements of phospholipids and proteins (**Figure** 5.2.1). For example, some proteins on the inner side attach the membrane to the cell's internal support structure, called the cytoskeleton. On the outer side, other proteins help the cell interact with its surroundings. Carbohydrates attached to lipids or proteins are found only on the outside surface, where they help the cell recognize and bind substances from the environment. This structural difference between the two sides of the membrane plays an important role in its ability to selectively control what enters and leaves the cell.

Recall that plasma membranes are amphiphilic; that is, they have both water-attracting (hydrophilic) and water-repelling (hydrophobic) regions. This feature allows some materials to move through the membrane easily, while blocking others. Small, non-polar substances like oxygen, carbon dioxide, and fat-soluble vitamins (A, D, E, and K) can pass directly through the hydrophobic core of the membrane. Fat-soluble drugs and hormones also gain easy entry into cells and are readily transported into the body's tissues and organs. Molecules of oxygen and carbon dioxide have no charge and so pass through membranes by simple diffusion.



(**Figure** 5.2.1) Asymmetry in Plasma Membranes: The exterior surface of the plasma membrane is not identical to the interior surface of the same membrane. Credit: Rao, A., Ryan, K., Fletcher, S., Hawkins, A. and Tag, A. Department of Biology, Texas A&M University.

Polar substances present problems for the membrane. While some polar molecules connect easily with the outside of a cell, they cannot readily pass through the lipid core of the plasma membrane. Additionally, while small ions could easily slip through the spaces in the mosaic of the membrane, their charge prevents them from doing so. Ions such as sodium, potassium, calcium, and chloride must have special means of penetrating plasma membranes. Simple sugars and amino acids also need help with transport across plasma membranes, achieved by various transmembrane proteins (channels), which will be discussed later in this chapter.

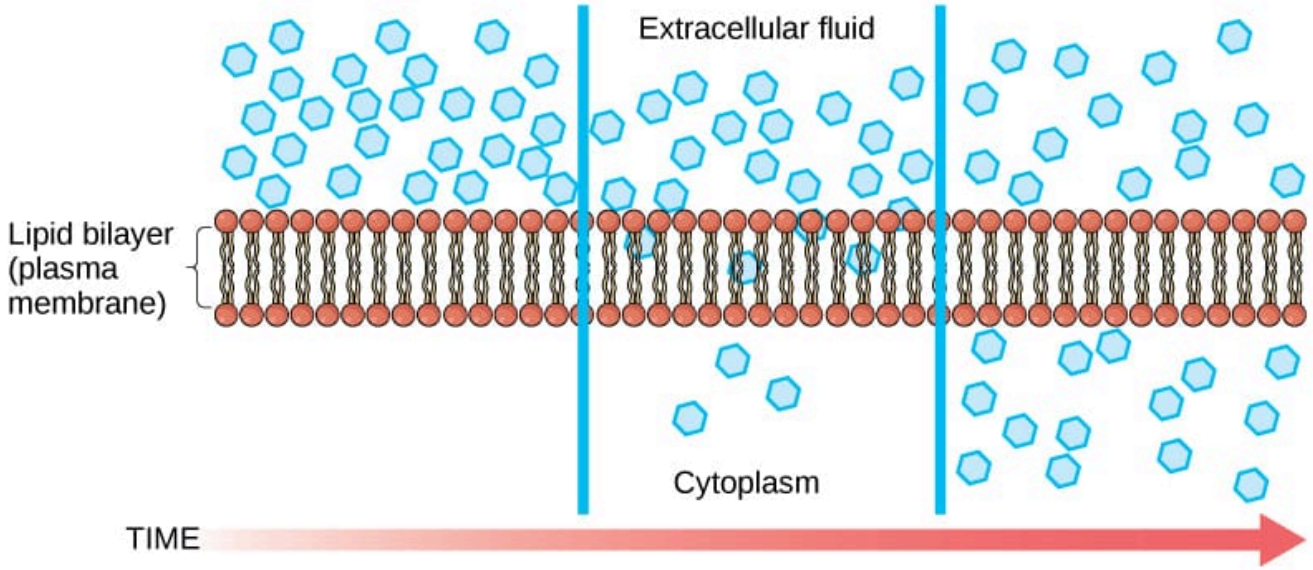
Plasma membranes must allow or prevent certain substances from entering or leaving a cell. In other words, plasma membranes are selectively permeable; they allow some substances to pass through, but not others. If they were to lose this selectivity, the cell would no longer be able to sustain itself, and it would be destroyed. Some cells require larger amounts of specific substances than other cells; they must have a way of obtaining these materials from extracellular fluids. This may happen passively, as certain materials move back and forth, or the cell may have special mechanisms that facilitate transport. Some materials are so important to a cell that it spends some of its energy (hydrolyzing adenosine triphosphate (ATP)) to obtain these materials. Red blood cells use some of their energy to do this. All cells spend the majority of their energy to maintain an imbalance of sodium and potassium ions between the interior and exterior of the cell.

Passive Transport

The most direct forms of membrane transport are passive. Passive transport is a naturally-occurring phenomenon and does not require the cell to exert any of its energy to accomplish the movement. In passive transport, substances move from an area of higher concentration to an area of lower concentration. A physical space in which there is a range of concentrations of a single substance is said to have a concentration gradient.

The passive forms of transport, diffusion and osmosis, move materials of small molecular weight across membranes. Substances diffuse from areas of high concentration to areas of lower concentration; this process continues until the substance is evenly distributed in a system. In solutions containing more than one substance, each type of molecule diffuses according to its own concentration gradient, independent of the diffusion of other substances. Many factors can affect the rate of diffusion, including, but not limited to, concentration gradient, size of the particles that are diffusing, and temperature of the system.

In living systems, diffusion of substances in and out of cells is mediated by the plasma membrane. Over the next few section we will discuss how some materials diffuse readily through the membrane, but others are hindered; their passage is made possible by specialized proteins, such as channels and transporters. The chemistry of living things occurs in aqueous solutions; balancing the concentrations of those solutions is an ongoing problem. In living systems, diffusion of some substances would be slow or difficult without membrane proteins that facilitate transport.



(**Figure** 5.2.2) Diffusion is a type of passive transport. Diffusion through a permeable membrane moves a substance from an area of high concentration (extracellular fluid, in this case) down its concentration gradient (into the cytoplasm). The left part of this illustration shows a substance on one side of a membrane only. The middle part shows that, after some time, some of the substance has diffused across the plasma membrane. The right part shows that, after more time, an equal amount of the substance is on each side of the membrane.

Diffusion

Diffusion is a passive process of transport. A single substance tends to move from an area of high concentration to an area of low concentration until the concentration is equal across a space. You are familiar with diffusion of substances through the air. For example, think about someone opening a bottle of ammonia in a room filled with people. The ammonia gas is at its highest

concentration in the bottle; its lowest concentration is at the edges of the room. The ammonia vapor will diffuse, or spread away, from the bottle; gradually, more and more people will smell the ammonia as it spreads. Materials move within the cell 's cytosol by diffusion, and certain materials move through the plasma membrane by diffusion. Diffusion expends no energy. On the contrary, concentration gradients are a form of potential energy, dissipated as the gradient is eliminated.

Each separate substance in a medium, such as the extracellular fluid, has its own concentration gradient independent of the concentration gradients of other materials. In addition, each substance will diffuse according to that gradient. Within a system, there will be different rates of diffusion of the different substances in the medium.

Factors That Affect Diffusion

Molecules move constantly in a random manner at a rate that depends on their mass, their environment, and the amount of thermal energy they possess, which in turn is a function of temperature. This movement accounts for the diffusion of molecules through whatever medium in which they are localized. A substance will tend to move into any space available to it until it is evenly distributed throughout it. After a substance has diffused completely through a space removing its concentration gradient, molecules will still move around in the space, but there will be no net movement of the number of molecules from one area to another. This lack of a concentration gradient in which there is no net movement of a substance is known as dynamic equilibrium. While diffusion will go forward in the presence of a concentration gradient of a substance, several factors affect the rate of diffusion:

- Extent of the concentration gradient: The greater the difference in concentration, the more rapid the diffusion. The closer the distribution of the material gets to equilibrium, the slower the rate of diffusion becomes.
- Mass of the molecules diffusing: Heavier molecules move more slowly; therefore, they diffuse more slowly. The reverse is true for lighter molecules.
- Temperature: Higher temperatures increase the energy and therefore the movement of the molecules, increasing the rate of diffusion. Lower temperatures decrease the energy of the molecules, thus decreasing the rate of diffusion.
- Solvent density: As the density of a solvent increases, the rate of diffusion decreases. The molecules slow down because they have a more difficult time getting through the denser medium. If the medium is less dense, diffusion increases. Because cells primarily use diffusion to move materials within the cytoplasm, any increase in the cytoplasm's density will inhibit the movement of the materials. An example of this is a person experiencing dehydration. As the body's cells lose water, the rate of diffusion decreases in the cytoplasm, and the cells' functions deteriorate. Neurons tend to be very sensitive to this effect. Dehydration frequently leads to unconsciousness and possibly coma because of the decrease in diffusion rate within the cells.
- Solubility: As discussed earlier, nonpolar or lipid-soluble materials pass through plasma membranes more easily than polar materials, allowing a faster rate of diffusion.
- Surface area and thickness of the plasma membrane: Increased surface area increases the rate of diffusion, whereas a thicker membrane reduces it.
- Distance traveled: The greater the distance that a substance must travel, the slower the rate of diffusion. This places an upper limitation on cell size. A large, spherical cell will die because nutrients or waste cannot reach or leave the center of the cell. Therefore, cells must either be small in size, as in the case of many prokaryotes, or be flattened, as with many single-celled eukaryotes.

CONCEPT IN ACTION

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

Watch this Diffusion of Food Dye In Hot & Cold Water video illustrating the effect of temperature on diffusion of solutions.

A variation of diffusion is the process of filtration. In filtration, material moves according to its concentration gradient through a membrane; sometimes the rate of diffusion is enhanced by pressure, causing the substances to filter more rapidly. This occurs

in the kidney where blood pressure forces large amounts of water and accompanying dissolved substances, or solutes, out of the blood and into the renal tubules. The rate of diffusion in this instance is almost totally dependent on pressure. One of the effects of high blood pressure is the appearance of protein in the urine, which is “squeezed through” by the abnormally high pressure.

Interactive Element

Diffusion across a semipermeable membrane: This interactive shows that smaller molecules have an easier time making it across a semipermeable membrane. Adjust the pore size so the larger molecules can make it through.

Osmosis and Semipermeable Membranes

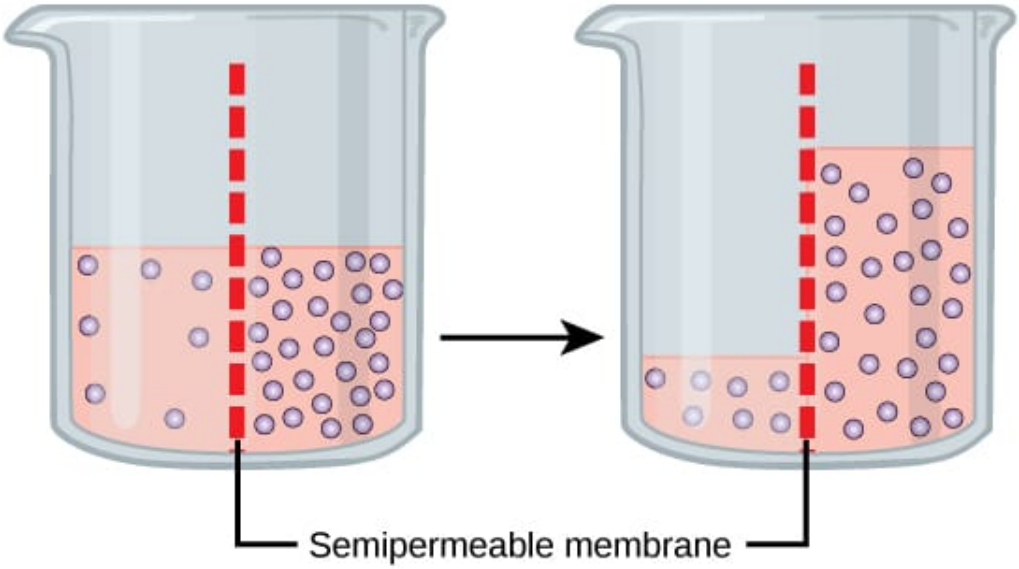
Osmosis is the movement of water through a semipermeable membrane according to the concentration gradient of water across the membrane, which is inversely proportional to the concentration of solutes. Semipermeable membranes, also termed selectively permeable membranes or partially permeable membranes, allow certain molecules or ions to pass through by diffusion.

While diffusion transports materials across membranes and within cells, osmosis transports only water across a membrane. The semipermeable membrane limits the diffusion of solutes in the water. Not surprisingly, the aquaporin proteins that facilitate water movement play a large role in osmosis, most prominently in red blood cells and the membranes of kidney tubules.

Mechanism of Osmosis

Osmosis is a special case of diffusion. Water, like other substances, moves from an area of high concentration to one of low concentration. An obvious question is what makes water move at all? Imagine a beaker with a semipermeable membrane separating the two sides or halves. On both sides of the membrane the water level is the same, but there are different concentrations of a dissolved substance, or solute, that cannot cross the membrane (otherwise the concentrations on each side would be balanced by the solute crossing the membrane). If the volume of the solution on both sides of the membrane is the same but the concentrations of solute are different, then there are different amounts of water, the solvent, on either side of the membrane. If there is more solute in one area, then there is less water; if there is less solute in one area, then there must be more water.

To illustrate this, imagine two full glasses of water. One has a single teaspoon of sugar in it, whereas the second one contains one-quarter cup of sugar. If the total volume of the solutions in both cups is the same, which cup contains more water? Because the large amount of sugar in the second cup takes up much more space than the teaspoon of sugar in the first cup, the first cup has more water in it.



(**Figure** 5.2.3) In osmosis, water always moves from an area of higher water concentration to one of lower concentration. In the diagram shown, the solute cannot pass through the selectively permeable membrane, but the water can.

Returning to the beaker example, recall that it has a mixture of solutes on either side of the membrane. A principle of diffusion is that the molecules move around and will spread evenly throughout the medium if they can. However, only the material capable of passing through the membrane will diffuse through it. In this example, the solute cannot diffuse through the membrane, but the water can. Water has a concentration gradient in this system. Thus, water will diffuse down its concentration gradient, crossing the membrane to the side where it is less concentrated. This diffusion of water through the membrane—osmosis—will continue until the concentration gradient of water goes to zero or until the hydrostatic pressure of the water balances the osmotic pressure. In the beaker example, this means that the level of fluid in the side with a higher solute concentration will go up.

Key Points

The plasma membrane's selective permeability is essential for maintaining the cell's internal stability. It allows certain substances to enter or leave the cell while preventing others from doing so. This selectivity is based on both the structure of the membrane—with its hydrophilic (water-attracting) and hydrophobic (water-repelling) regions—and differences between the membrane's inner and outer surfaces.

Some small, non-polar materials can move easily through the membrane, while polar or charged substances cannot pass through the membrane on their own. These substances must rely on specialized proteins embedded in the membrane to help transport them in or out of the cell. This makes the membrane highly effective at controlling what comes in and goes out.

One of the key ways substances cross the membrane is through passive transport, where materials move naturally from areas of higher concentration to areas of lower concentration, without the use of energy. This movement is guided by a concentration gradient and helps the cell exchange gases, nutrients, and waste efficiently.

The rate of diffusion—a type of passive transport—is influenced by several factors, including the difference in concentration, the size of the molecules, the temperature, and how far the substances have to travel. When molecules spread out evenly across a space, the system reaches what's called dynamic equilibrium, where there is no net movement even though individual molecules keep moving.

Some substances that can't pass through the membrane directly still move by facilitated diffusion, a type of passive transport that uses transport proteins to assist them. These proteins form channels or act as carriers, guiding specific substances across the membrane without energy use.Osmosis is a special case of diffusion that involves the movement of water across a selectively permeable membrane. Water moves from areas where it is more concentrated to areas where it is less concentrated, based on the amount of solutes in the surrounding fluids. This process continues until water levels balance out or pressure stops further movement.

Key Terms

amphiphilic
: having one surface consisting of hydrophilic amino acids and the opposite surface consisting of hydrophobic (or lipophilic) ones

concentration gradient
: a concentration gradient is present when a membrane separates two different concentrations of molecules

diffusion
: the passive movement of a solute across a permeable membrane

osmosis
: the net movement of solvent molecules from a region of high solvent potential to a region of lower solvent potential through a partially permeable membrane

passive transport
: a movement of biochemicals and other atomic or molecular substances across membranes that does not require an input of chemical energy

permeable
: of or relating to substance, substrate, membrane or material that absorbs or allows the passage of fluids

polar
: a separation of electric charge leading to a molecule or its chemical groups having an electric dipole

semipermeable membrane
: a type of biological membrane that will allow certain molecules or ions to pass through it by diffusion and occasionally by specialized facilitated diffusion

solute
: any substance that is dissolved in a liquid solvent to create a solution

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5.3: Facilitated Transport

eeeeeefeLearning Objectives

- Define facilitated diffusion and explain how it differs from simple diffusion.
- Describe the role of integral membrane proteins in transporting polar or charged substances across the plasma membrane.
- Distinguish between channel proteins and carrier proteins in terms of structure and function.
- Explain how carrier proteins undergo conformational change to move substances across the membrane.
- Evaluate how facilitated diffusion supports the selective permeability of the plasma membrane

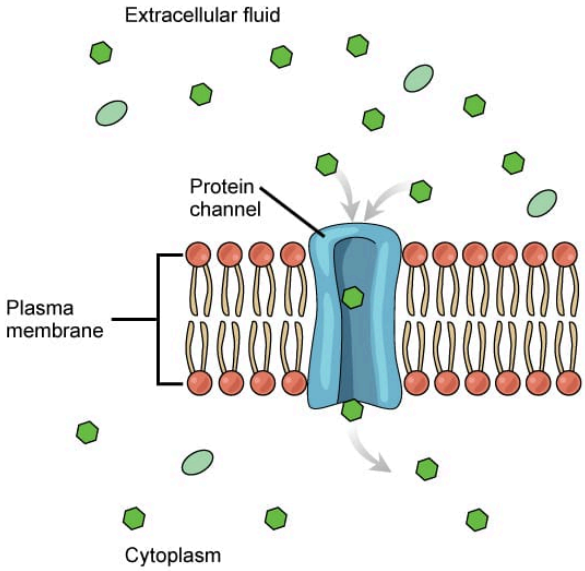
Facilitated Transport

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

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

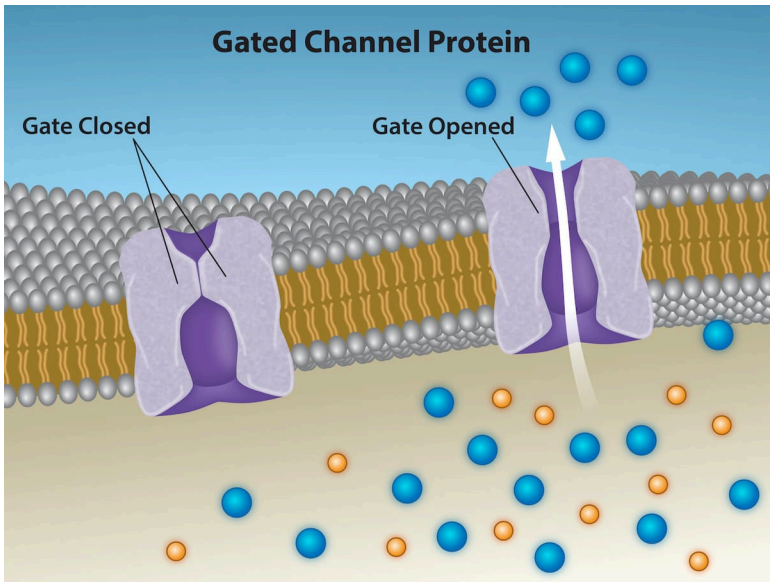
Channels

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



(**Figure** 5.3.1) Facilitated transport moves substances down their concentration gradients. They may cross the plasma membrane with the aid of channel proteins.

Channel proteins help substances cross the cell membrane by forming pores or tunnels that allow specific molecules to pass through. Some channels are always open, enabling a constant flow of materials, while others are gated—opening only in response to certain signals like a change in voltage, the presence of a specific molecule, or mechanical pressure. As with other transport proteins, gated channels have a binding site that is specific for a given molecule or ion.



(**Figure** 5.3.2) When gated channel ion proteins are closed, no ions can pass through them. However, when a channel opens, select (blue) ions diffuse through the channel. Ion channel proteins (like all transport proteins) are highly specific, letting only a specific ion or subset of ions pass. Credit: Rao, A., Ryan, K., Tag, A. and Fletcher, S. Department of Biology, Texas A&M University.

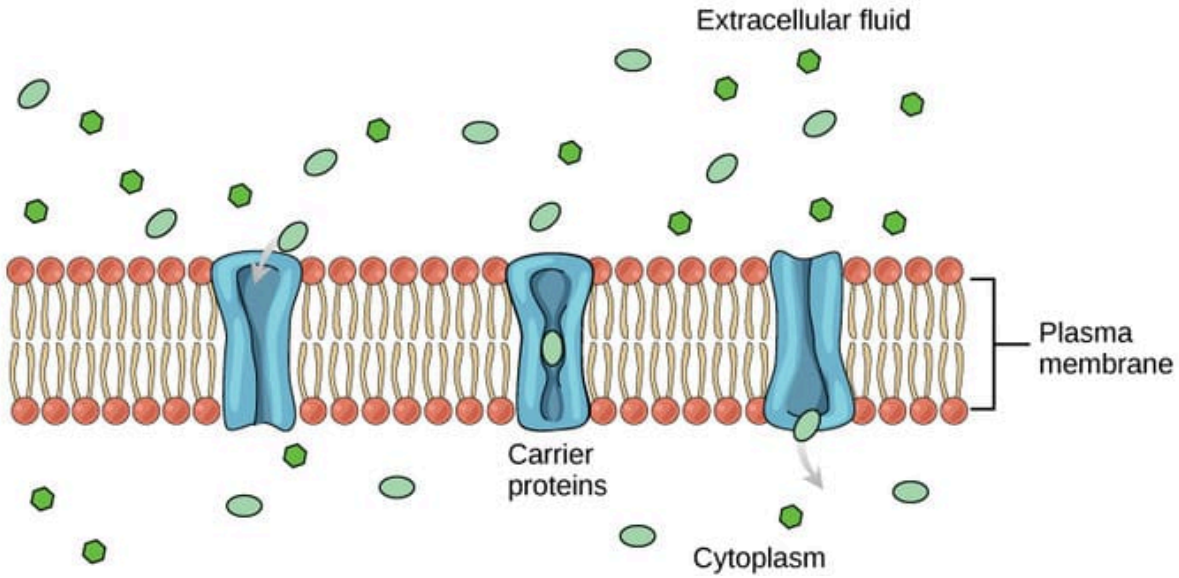
Just as membrane proteins involved in transport are referred to as transport proteins, the subset of transport proteins involved in the transport of ions are referred to as ion channel proteins. A well-known group of channel proteins is the ion channels, which facilitate the movement of charged particles such as sodium (Na^+), potassium (K^+), chloride (Cl^-) or calcium (Ca^{2+}) across the membrane. Ion channels that transport certain ions are especially important in cells involved in electrical signaling, like nerve and muscle cells. Opening and closing ion channels can rapidly change the distribution of charge across the membrane, which plays a key role in nerve impulses and muscle contraction.

Just as not all membrane proteins are involved in the transport of solutes, not all channel proteins transport ions. For instance, aquaporins are specialized channel proteins that allow water molecules to move quickly across the membrane - and are named

accordingly. Aquaporins are particularly important in cells that require rapid water transport, such as those in the kidneys or red blood cells.In general, channel proteins are named and grouped based on the type of substance they transport, whether ions, water, or other small molecules. This classification reflects the diversity of functions these proteins serve in regulating the internal environment of the cell.

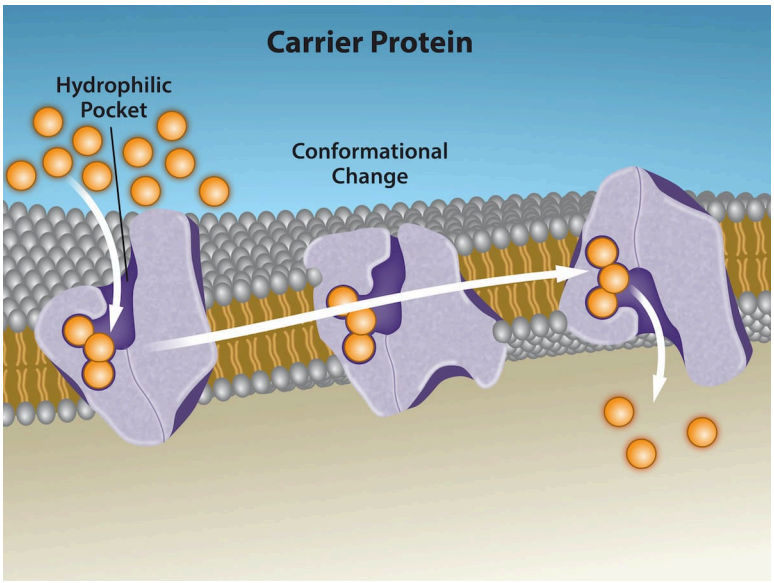
Carrier Proteins

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



(**Figure** 5.3.3) Some substances are able to move down their concentration gradient across the plasma membrane with the aid of carrier proteins. Carrier proteins change shape as they move molecules across the membrane. Note the different structures depicted for the carrier protein in this image.

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



(**Figure** 5.3.4) Carrier proteins change shape (confirmational change) as they move molecules across the membrane. Credit: Rao, A., Tag, A. and Fletcher, S. Department of Biology, Texas A&M University.

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

CONCEPT IN ACTION

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View this YouTube Video online

For an animation of the diffusion process in action, view this short video(opens in new window)on cell membrane transport (4:40).

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View this YouTube Video online

An animation depicting facilitated diffusion (1:36).

Key Points

Facilitated diffusion allows ions and polar molecules to cross the hydrophobic interior of the plasma membrane with the help of integral membrane proteins. These include channel proteins, which form hydrophilic passageways that let substances move down their concentration gradient. Some channel proteins are always open, while gated channel proteins open only in response to specific biological signals—such as a change in voltage or the binding of a molecule—providing the cell with greater control over what enters or exits. Carrier proteins assist by binding to a specific substance and undergoing a conformational change that

moves the substance across the membrane.

Key Terms

channel protein
: a membrane protein that forms an open passage for specific substances to move in or out of the cell without using energy

conformational change
: a shift in the shape of a protein that can affect how it works or interacts with other molecules

facilitated diffusion
: the spontaneous passage of molecules or ions across a biological membrane passing through specific transmembrane integral proteins

gated-channel protein
: a channel that opens or closes in response to signals like chemicals or changes in voltage

ion channel
: a type of channel protein that allows charged particles (ions) to pass through the cell membrane

integral protein
: transmembrane proteins that function as either channels for the material or carriers,, also caled transmembrane proteins

membrane protein
: proteins that are attached to, or associated with the membrane of a cell and are sometimes referred to as integral proteins. Integral proteins are often grouped and named based on their function

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5.4: Active Transport

Learning Objectives

- Describe what active transport is and explain how it differs from passive transport
- Explain the role of energy (ATP) in moving substances across the plasma membrane against their concentration gradients.
- Explain the difference between primary and secondary active transport
- Describe the processes of endocytosis (including phagocytosis, pinocytosis, and receptor-mediated endocytosis) and exocytosis and their functions in the cell
- Give examples of how cells use endocytosis to take in large particles and exocytosis to expel waste
- Explain how defects in endocytosis can affect human health, such as in the case of familial hypercholesterolemia.Describe how cells maintain homeostasis by using active transport mechanisms to control what enters and leaves the cell

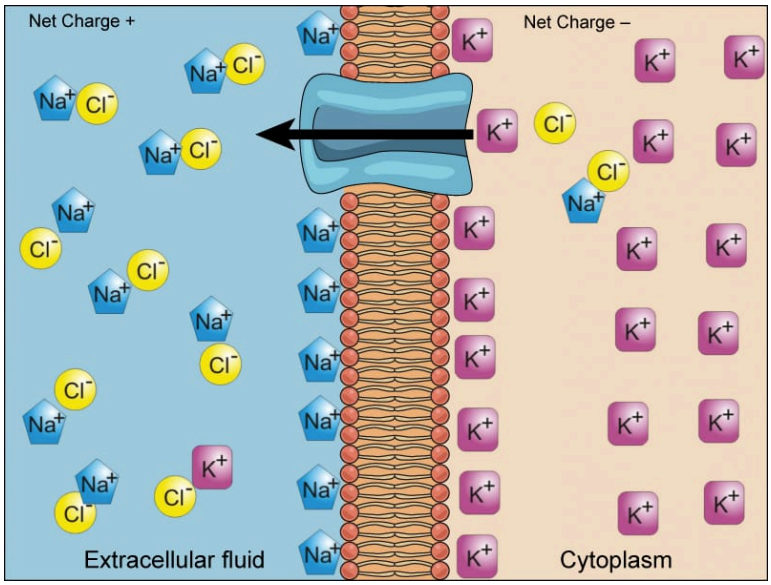
Active Transport

Active transport mechanisms require the use of the cell's energy, usually in the form of adenosine triphosphate (ATP). If a substance must move into the cell against its concentration gradient, that is, if the concentration of the substance inside the cell must be greater than its concentration in the extracellular fluid, the cell must use energy to move the substance. Some active transport mechanisms move small-molecular weight material, such as ions, through the membrane.

In addition to moving small ions and molecules through the membrane, cells also need to remove and take in larger molecules and particles. Some cells are even capable of engulfing entire unicellular microorganisms. You might have correctly hypothesized that the uptake and release of large particles by the cell requires energy. A large particle, however, cannot pass through the membrane, even with energy supplied by the cell.

Electrochemical Gradient

We have discussed simple concentration gradients—differential concentrations of a substance across a space or a membrane—but in living systems, gradients are more complex. Because cells contain proteins, most of which are negatively charged, and because ions move into and out of cells, there is an electrical gradient, a difference of charge, across the plasma membrane. The interior of living cells is electrically negative with respect to the extracellular fluid in which they are bathed; at the same time, cells have higher concentrations of potassium (K^+) and lower concentrations of sodium (Na^+) than does the extracellular fluid. Thus, in a living cell, the concentration gradient and electrical gradient of Na^+ promotes diffusion of the ion into the cell, and the electrical gradient of Na^+ (a positive ion) tends to drive it inward to the negatively charged interior. The situation is more complex, however, for other elements such as potassium. The electrical gradient of K^+ promotes diffusion of the ion *into* the cell, but the concentration gradient of K^+ promotes diffusion *out* of the cell (**Figure** $5.4.1$) . The combined gradient that affects an ion is called its electrochemical gradient, and it is especially important to muscle and nerve cells.

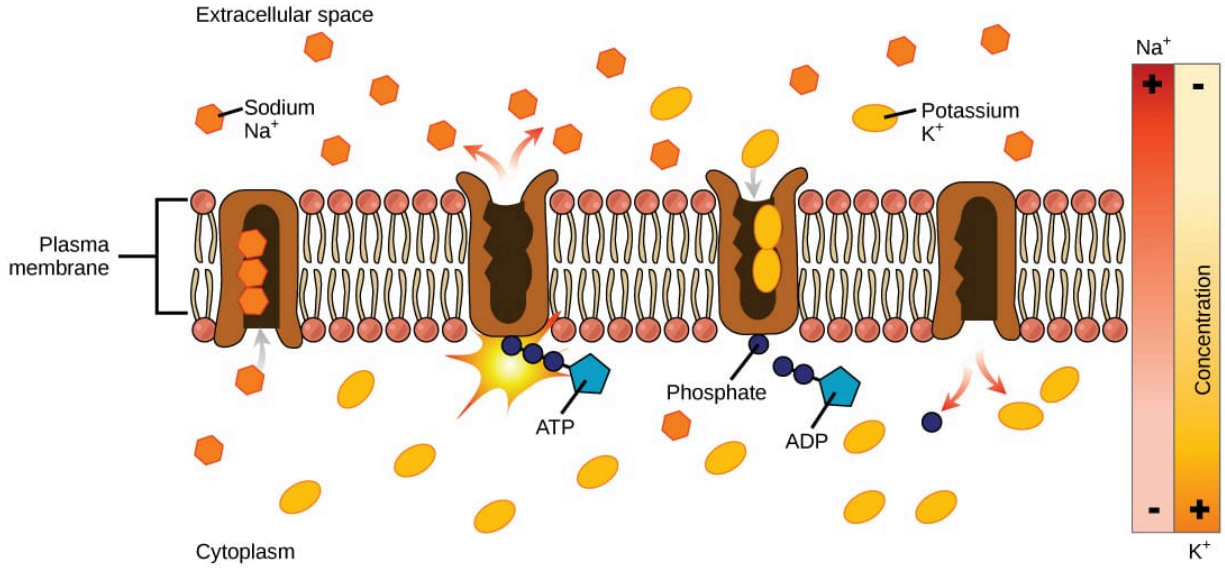


(**Figure** 5.4.1) Electrochemical gradients arise from the combined effects of concentration gradients and electrical gradients. A cell membrane is shown with a protein channel that allows passage of ions into and out of the cell. The cytoplasm has a higher concentration of potassium, and the extracellular fluid has a higher concentration of sodium. An arrow shows movement of a potassium ion out of the cell through the protein channel. (credit: modification of work by "Synaptitude"/Wikimedia Commons)

Moving Against a Gradient

To move substances against a concentration or an electrochemical gradient, the cell must use energy. This energy is harvested from ATP that is generated through cellular metabolism. Active transport mechanisms, collectively called pumps or carrier proteins, work against electrochemical gradients. With the exception of ions, small substances constantly pass through plasma membranes. Active transport maintains concentrations of ions and other substances needed by living cells in the face of these passive changes. Much of a cell's supply of metabolic energy may be spent maintaining these processes. Because active transport mechanisms depend on cellular metabolism for energy, they are sensitive to many metabolic poisons that interfere with the supply of ATP.

Two mechanisms exist for the transport of small-molecular weight material and macromolecules. Primary active transport moves ions across a membrane and creates a difference in charge across that membrane. The primary active transport system uses ATP to move a substance, such as an ion, into the cell, and often at the same time, a second substance is moved out of the cell. The sodium-potassium pump, an important pump in animal cells, expends energy to move potassium ions into the cell and a different number of sodium ions out of the cell (**Figure** 5.4.2). The action of this pump results in a concentration and charge difference across the membrane.



(**Figure** 5.4.2) The sodium-potassium pump moves potassium and sodium ions across the plasma membrane. Initially, the pump's opening faces the cytoplasm, where three sodium ions bind to it. The pump hydrolyzes ATP to ADP and, as a result, undergoes a conformational change. The sodium ions are released into the extracellular space. Two potassium ions from the extracellular space now bind the pump, which changes conformation again, releasing the potassium ions into the cytoplasm. (credit: modification of work by Mariana Ruiz Villarreal)

Secondary active transport describes the movement of material using the energy of the electrochemical gradient established by primary active transport. Using the energy of the electrochemical gradient created by the primary active transport system, other substances such as amino acids and glucose can be brought into the cell through membrane channels. ATP itself is formed through secondary active transport using a hydrogen ion gradient in the mitochondrion.

Endocytosis

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

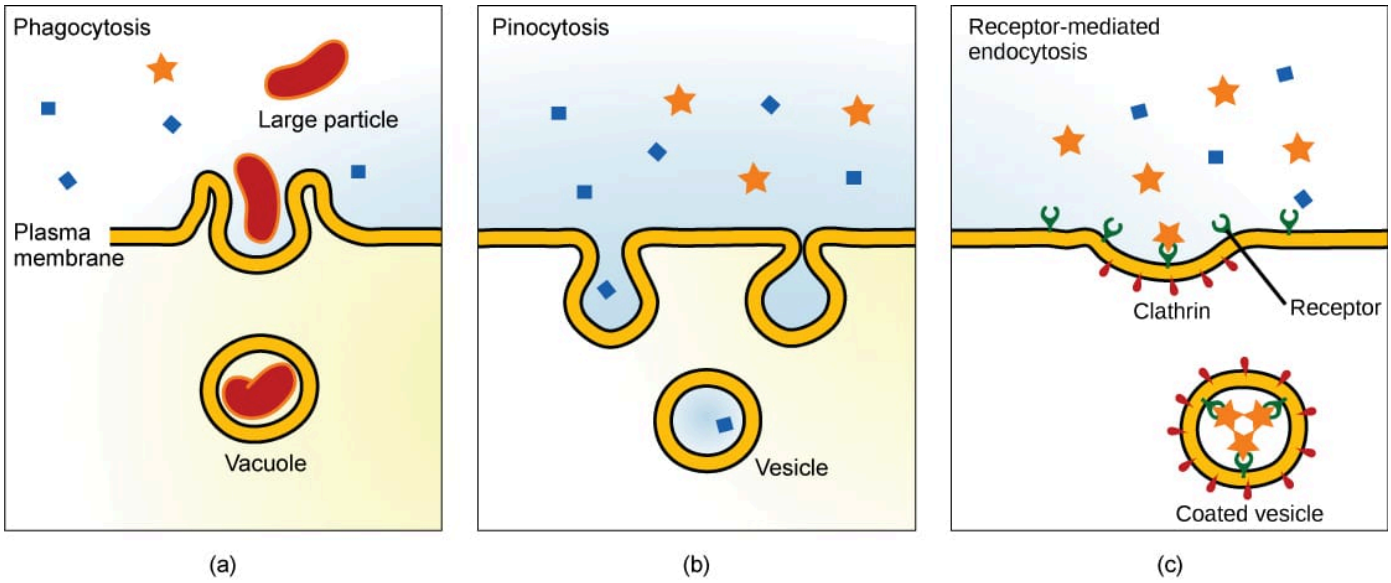


Figure 5.4.3 Three variations of endocytosis are shown. (a) Phagocytosis, the cell membrane surrounds the particle and pinches off to form an intracellular vacuole. (b) Pinocytosis, the cell membrane surrounds a small volume of fluid and pinches off, forming a vesicle. (c) Receptor-mediated endocytosis, uptake of substances by the cell is targeted to a single type of substance that binds at the receptor on the extracellular side. The receptors bind a substance, then pinch off to form a coated vesicle. (credit: modification of work by Mariana Ruiz Villarreal)

Phagocytosis is the process by which large particles, such as cells, are taken in by a cell. For example, when microorganisms invade the human body, a type of white blood cell called a neutrophil removes the invader through this process, surrounding and engulfing the microorganism, which is then destroyed by the neutrophil (**Figure** 5.4.3).

A variation of endocytosis is called pinocytosis. This literally means “cell drinking” and was named at a time when the assumption was that the cell was purposefully taking in extracellular fluid. In reality, this process takes in solutes that the cell needs from the extracellular fluid (**Figure** 5.4.3).

A targeted variation of endocytosis employs binding proteins in the plasma membrane that are specific for certain substances (**Figure** 5.4.3). The particles bind to the proteins and the plasma membrane invaginates, bringing the substance and the proteins into the cell. If passage across the membrane of the target of receptor-mediated endocytosis is ineffective, it will not be removed from the tissue fluids or blood. Instead, it will stay in those fluids and increase in concentration.

Some human diseases are caused by a failure of receptor-mediated endocytosis. For example, the form of cholesterol termed low-density lipoprotein or LDL (also referred to as “bad” cholesterol) is removed from the blood by receptor-mediated endocytosis. In the human genetic disease familial hypercholesterolemia, the LDL receptors are defective or missing entirely. People with this condition have life-threatening levels of cholesterol in their blood, because their cells cannot clear the chemical from their blood.

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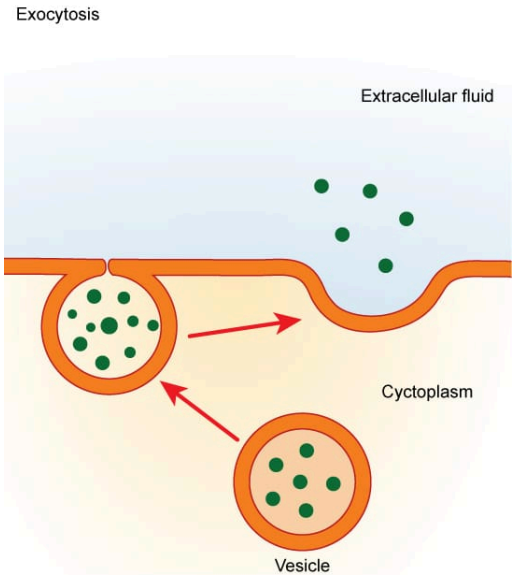
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See receptor-mediated endocytosis animation in action.

Exocytosis

In contrast to these methods of moving material into a cell is the process of exocytosis. Exocytosis is the opposite of the processes discussed above in that its purpose is to expel material from the cell into the extracellular fluid. A particle enveloped

in membrane fuses with the interior of the plasma membrane. This fusion opens the membranous envelope to the exterior of the cell, and the particle is expelled into the extracellular space (**Figure** 5.4.4).



(**Figure** 5.4.4) In exocytosis, a vesicle migrates to the plasma membrane, the membrane of the vesicle fuses with the cell membrane, and releases its contents to the outside of the cell. (credit: modification of work by Mariana Ruiz Villarreal)

Summary

The combined gradient that affects an ion includes its concentration gradient and its electrical gradient. Living cells need certain substances in concentrations greater than they exist in the extracellular space. Moving substances up their electrochemical gradients requires energy from the cell. Active transport uses energy stored in ATP to fuel the transport. Active transport of small molecular-size material uses integral proteins in the cell membrane to move the material—these proteins are analogous to pumps. Some pumps, which carry out primary active transport, couple directly with ATP to drive their action. In secondary transport, energy from primary transport can be used to move another substance into the cell and up its concentration gradient.

Endocytosis methods require the direct use of ATP to fuel the transport of large particles such as macromolecules; parts of cells or whole cells can be engulfed by other cells in a process called phagocytosis. In phagocytosis, a portion of the membrane invaginates and flows around the particle, eventually pinching off and leaving the particle wholly enclosed by an envelope of plasma membrane. Vacuoles are broken down by the cell, with the particles used as food or dispatched in some other way. Pinocytosis is a similar process on a smaller scale. The cell expels waste and other particles through the reverse process, exocytosis. Wastes are moved outside the cell, pushing a membranous vesicle to the plasma membrane, allowing the vesicle to fuse with the membrane and incorporating itself into the membrane structure, releasing its contents to the exterior of the cell.

Key Terms

active transport
: the method of transporting material that requires energy

electrochemical gradient
: a gradient produced by the combined forces of the electrical gradient and the chemical gradient

endocytosis
: a type of active transport that moves substances, including fluids and particles, into a cell

exocytosis
: a process of passing material out of a cell

phagocytosis
: a process that takes macromolecules that the cell needs from the extracellular fluid; a variation of endocytosis

pinocytosis
a process that takes solutes that the cell needs from the extracellular fluid; a variation of endocytosis

receptor-mediated endocytosis
a variant of endocytosis that involves the use of specific binding proteins in the plasma membrane for specific molecules or particles

sodium-potassium pump
a membrane protein that uses energy to move sodium out of the cell and potassium into the cell

Contributors and Attributions

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5.5: Cell Communication

Learning Objectives

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

- Explain why cells need to communicate with one another
- Describe the four basic types of cell signaling
- Understand the role of receptors in receiving messages
- Differentiate between internal and cell-surface receptors
- Identify three main types of cell-surface receptors

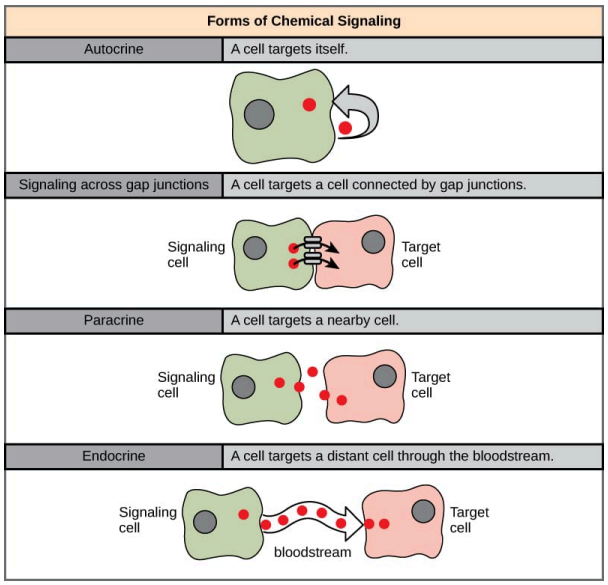
In multicellular organisms, cells must work together to maintain the function and health of the entire organism. Communication between cells (also refered to as cell signaling) allows them to coordinate responses, share information, and make collective decisions. For example, during development, cells communicate to determine their future roles. In the immune system, cells signal one another to launch or stop immune responses. And in everyday maintenance, cells need to monitor and respond to conditions such as nutrient levels or damage. Without reliable communication, cells would act independently, leading to dysfunction or disease.

Cells communicate by sending chemical signals that are detected by other cells. These messages can travel short distances (to nearby cells) or long distances (through the bloodstream or other body fluids). Not all cells are affected by the same signals. Different types of signaling are used for different purposes. The method of communication used depends on the context and the distance the signal must travel (**Figure** 5.5.1).

Types of Cell Signaling

There are four primary types of cell signaling:

- Autocrine Signaling: In autocrine signaling, a cell releases a signal that binds to receptors on its own surface. This type of communication allows a cell to regulate its own activity. For example, a cell might use autocrine signaling to reinforce a signal that tells it to divide or to stop dividing. This can be important in development or in immune responses.
- Paracrine Signaling: Paracrine signaling involves communication between nearby cells. A signaling cell releases molecules into the extracellular space, and nearby cells with the appropriate receptors detect and respond to the signal. This form of signaling is important in many tissues and processes, including inflammation and the development of tissues during embryonic growth.
- Direct Signaling Across Gap Junctions: Some cells are connected by structures called gap junctions (in animals) or plasmodesmata (in plants). These allow small molecules, including signaling molecules, to pass directly from one cell to another. This type of communication is fast and ensures that groups of cells can respond as a unit. For example, heart muscle cells use gap junctions to synchronize contractions.
- Endocrine Signaling: In endocrine signaling, cells release signaling molecules (often called hormones) into the bloodstream or another body fluid. These hormones can travel long distances to reach target cells in different parts of the body. Only cells with the right receptors will respond to the signal. This system is slower than other forms of signaling but can produce widespread and lasting effects, such as regulating metabolism, growth, or reproduction.



(**Figure** 5.5.1) The diagram above shows the different types of chemical signaling. A cell may: target itself (autocrine signaling), a cell connected by gap junctions (signaling across gap junctions), a nearby cell (paracrine signaling), or a distant cell (endocrine signaling). Paracrine signaling acts on nearby cells, endocrine signaling uses the circulatory system to transport ligands, and autocrine signaling acts on the signaling cell. Signaling via gap junctions involves signaling molecules moving directly between adjacent cells.

Receptors and Ligands

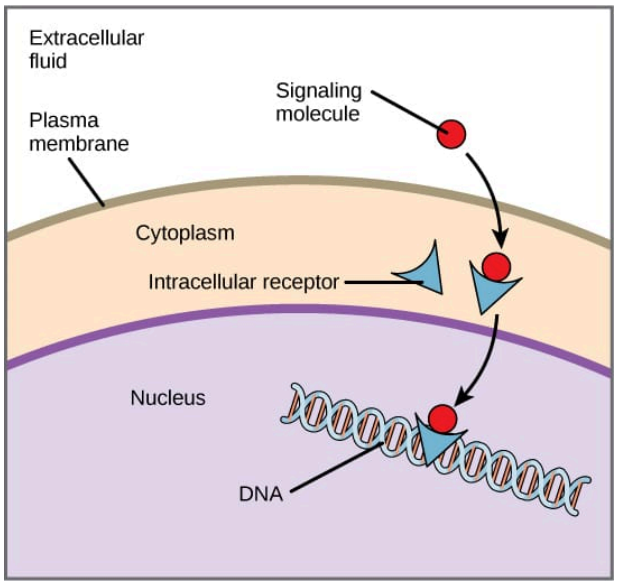
For a cell to respond to a signal, it must detect it. This detection is carried out by receptors, which are specialized proteins either on the cell surface or inside the cell. The molecules that bind to receptors are called ligands.

A ligand is a molecule that binds specifically to a receptor and can be thought of as signaling molecules. A ligand might be a small ion, a protein, a lipid, or another type of molecule. Some ligands are able to pass through the cell membrane, while others must interact with receptors on the outside of the cell. For example, small hydrophobic ligands, such as steroid hormones (e.g., estradiol, testosterone, and cholesterol), can diffuse directly through the plasma membrane and bind to internal receptors, often affecting gene expression. In contrast, water-soluble ligands like peptides and proteins are polar and cannot pass through the membrane; they bind to cell-surface receptors instead. Some ligands, like nitric oxide (NO), are gases that can cross membranes and act quickly over short distances, influencing processes like blood vessel dilation.

Internal receptors

Also known as intracellular or cytoplasmic receptors, these receptors are found inside the cell, usually in the cytoplasm. Only ligands that can cross the cell membrane—typically small, nonpolar molecules such as steroid hormones—can bind to these receptors.

Once inside the cell, the ligand binds to the receptor, causing a conformational change that often enables the receptor-ligand complex to enter the nucleus. There, it can bind to specific regions of DNA and influence gene expression by promoting or inhibiting the transcription of certain genes. This direct pathway allows internal receptors to have long-term effects on the cell's behavior (**Figure** 5.5.2). Internal receptors can directly influence gene expression (how much of a specific protein is produced from a gene) without having to pass the signal on to other receptors or messengers.



(**Figure** 5.5.2) Hydrophobic signaling molecules typically diffuse across the plasma membrane and interact with intracellular receptors in the cytoplasm. Many intracellular receptors are transcription factors that interact with DNA in the nucleus and regulate gene expression.

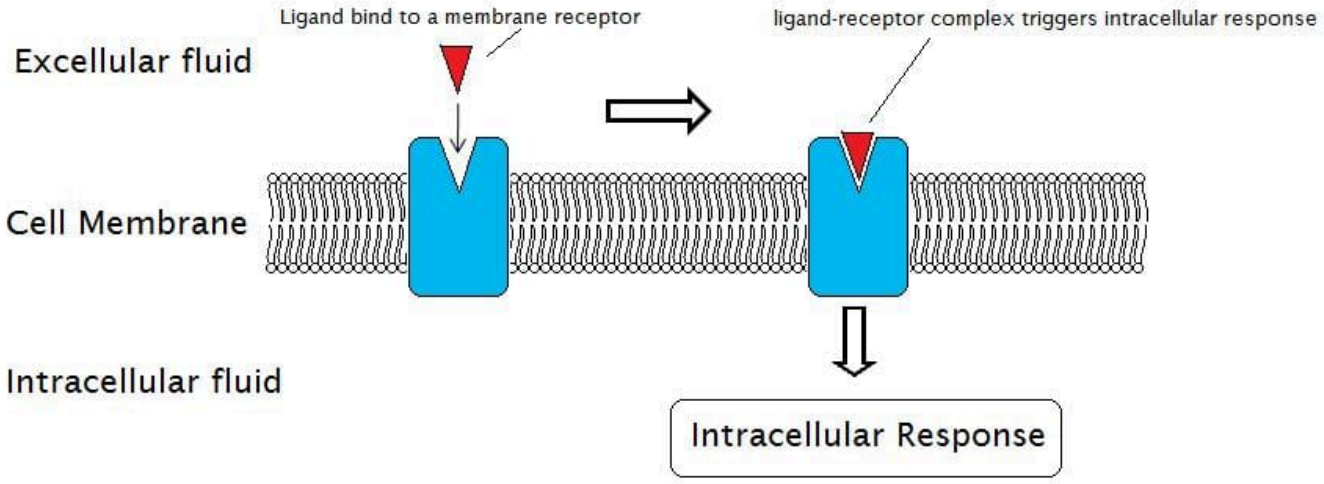
Cell-Surface Receptors

Cell-surface receptors, also known as transmembrane receptors, are proteins that are found attached to the cell membrane. These receptors bind to external ligand molecules (ligands that do not travel across the cell membrane). This type of receptor spans the plasma membrane and performs signal transduction, in which an extracellular signal is converted into an intercellular signal. Ligands that interact with cell-surface receptors do not have to enter the cell that they affect. Cell-surface receptors are also called cell-specific proteins or markers because they are specific to individual cell types.

Each cell-surface receptor has three main components:

- an external ligand-binding domain
- a hydrophobic membrane-spanning region
- an intracellular domain inside the cell

The size and extent of each of these domains vary widely, depending on the type of receptor.

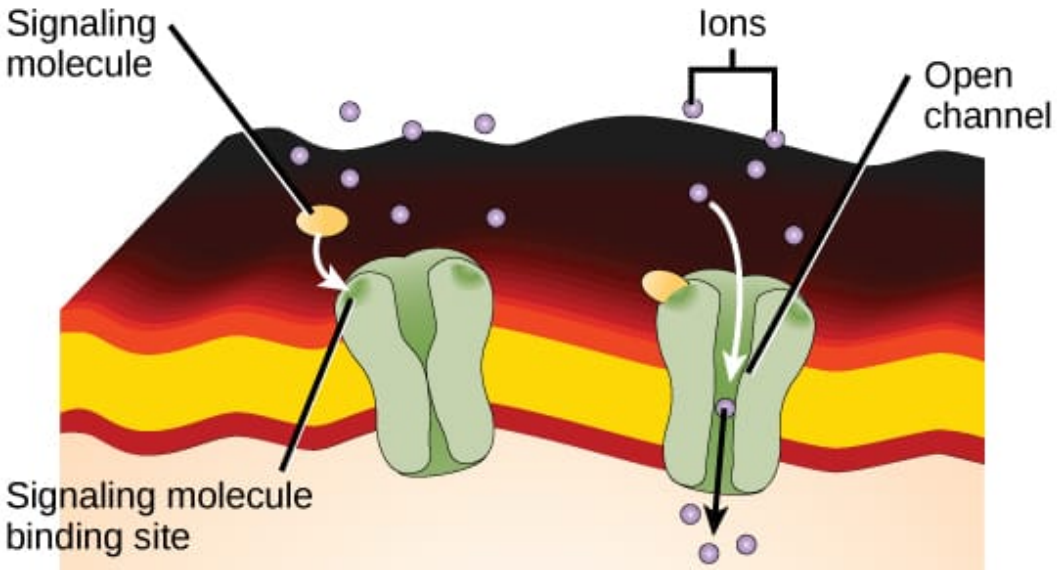


(**Figure** 5.5.3) Cell-surface receptors function by transmitting a signal through the cell membrane. The ligand does not directly enter the cell. Photo credit Laozhengzz; Wikimedia Commons.

Cell-surface receptors are involved in most of the signaling in multicellular organisms. There are three general categories of cell-surface receptors: ion channel-linked receptors, G-protein-linked receptors, and enzyme-linked receptors.

Ion channel-linked receptors

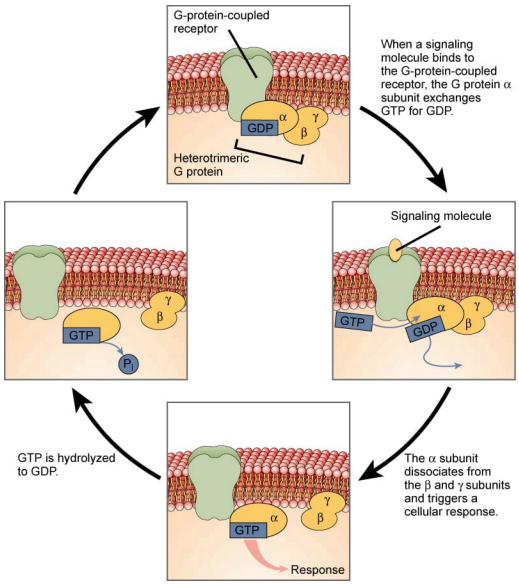
Ion channel-linked receptors bind a ligand and open a channel through the membrane that allows specific ions to pass through. To form a channel, this type of cell-surface receptor has an extensive membrane-spanning region. When a ligand binds to the extracellular region of the channel, there is a conformational change in the proteins structure that allows ions such as sodium, calcium, magnesium, and hydrogen to pass through (**Figure** 5.5.4).



(**Figure** 5.5.4) The diagram above shows gated ion channels forming a pore through the plasma membrane that opens when the signaling molecule binds. The open pore then allows ions to flow into or out of the cell.

G-protein-coupled receptors

G-protein-coupled receptors bind a ligand and activate a membrane protein called a G-protein. The activated G-protein then interacts with either an ion channel or an enzyme in the membrane (**Figure** 5.5.5). Before the ligand binds, the inactive G-protein can bind to a site on a specific receptor. Once the G-protein binds to the receptor, the G-protein changes shape, becomes active, and splits into two different subunits. One or both of these subunits may be able to activate other proteins as a result.



(**Figure** 5.5.5) The diagram shows the activation pathway for a heterotrimeric G-protein, which has three subunits: alpha beta, and gamma, all associated with the inside of the plasma membrane. When a signaling molecule binds to a G-protein-coupled receptor in the plasma membrane, a GDP molecule associated with the G-protein is exchanged for GTP. The subunits come apart from each other, and a cellular response is triggered either by one or both of the subunits. Hydrolysis of GTP to GDP terminates the signal.

Enzyme-linked receptors

Enzyme-linked receptors are cell-surface receptors with intracellular domains that are associated with an enzyme. In some cases, the intracellular domain of the receptor itself is an enzyme. Other enzyme-linked receptors have a small intracellular domain that interacts directly with an enzyme. When a ligand binds to the extracellular domain, a signal is transferred through the membrane, activating the enzyme. Activation of the enzyme sets off a chain of events within the cell that eventually leads to a response.

How Viruses Recognize a Host

Unlike living cells, many viruses do not have a plasma membrane or any of the structures necessary to sustain life. Some viruses are simply composed of an inert protein shell containing DNA or RNA. To reproduce, viruses must invade a living cell, which serves as a host, and then take over the hosts cellular apparatus. But how does a virus recognize its host?

Viruses often bind to cell-surface receptors on the host cell. For example, the virus that causes human influenza (flu) binds specifically to receptors on membranes of cells of the respiratory system. Chemical differences in the cell-surface receptors among hosts mean that a virus that infects a specific species (for example, humans) cannot infect another species (for example, chickens).

However, viruses have very small amounts of DNA or RNA compared to humans, and, as a result, viral reproduction can occur rapidly. Viral reproduction invariably produces errors that can lead to changes in newly produced viruses; these changes mean that the viral proteins that interact with cell-surface receptors may evolve in such a way that they can bind to receptors in a new host. Such changes happen randomly and quite often in the reproductive cycle of a virus, but the changes only matter if a virus with new binding properties comes into contact with a suitable host. In the case of influenza, this situation can occur in settings where animals and people are in close contact, such as poultry and swine farms (Sigalov, 2010). Once a virus jumps to a new host, it can spread quickly. Scientists watch newly appearing viruses (called emerging viruses) closely in the hope that such monitoring can reduce the likelihood of global viral epidemics.

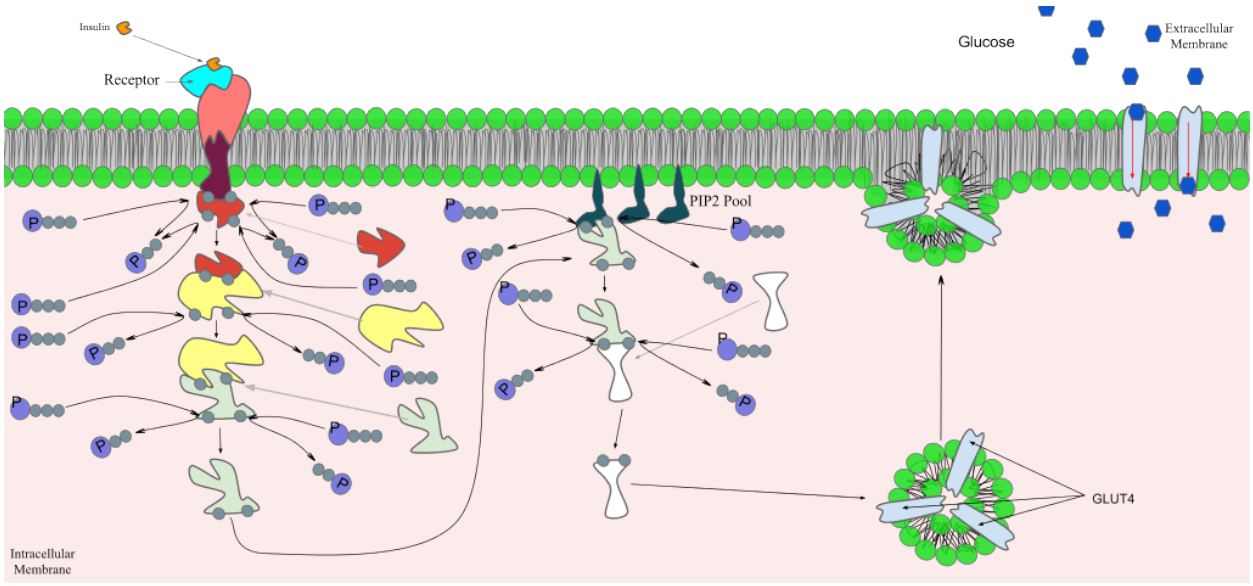
Propagation of the Signal

Once a ligand binds to a receptor, the signal is transmitted through the membrane and into the cytoplasm. Continuation of a signal in this manner is called signal transduction. Signal transduction only occurs with cell-surface receptors because internal receptors are able to interact directly with DNA in the nucleus to initiate protein synthesis.

Ligand Binding Initiates a Signaling Pathway

After the ligand binds to the cell-surface receptor, the activation of the receptor's intracellular components sets off a chain of events that is called a signaling pathway or a signaling cascade (**Figure** 5.5.6). Signaling pathways can get very complicated very quickly because most cellular proteins can affect different downstream events, depending on the conditions within the cell. A single pathway can branch off toward different endpoints based on the interplay between two or more signaling pathways, and the same ligands are often used to initiate different signals in different cell types. This variation in response is due to differences in protein expression in different cell types. Another complicating element is signal integration of the pathways, in which signals from two or more different cell-surface receptors merge to activate the same response in the cell. This process can ensure that multiple external requirements are met before a cell commits to a specific response.

The effects of extracellular signals can also be amplified by enzymatic cascades. At the initiation of the signal, a single ligand binds to a single receptor. However, activation of a receptor-linked enzyme can activate many copies of a component of the signaling cascade, which amplifies the signal.



(**Figure** 5.5.6) In this example of a signal transduction cascade, insulin serves as the ligand and activates a cascade that leads to activation of the GLUT4 glucose transporter on the cell membrane. Photo credit Luuis12321; Wikimedia Commons.

Methods of Intracellular Signaling

The activation of a signaling pathway depends on the modification of a cellular component by an enzyme. There are numerous types of enzymatic modifications that can occur, and they are recognized in turn by the next component downstream. The following are some of the more common events in intracellular signaling.

Phosphorylation

One of the most common chemical modifications that occurs in signaling pathways is the addition of a phosphate group (PO_4^{-3}) to a molecule such as a protein in a process called phosphorylation. The transfer of the phosphate is catalyzed by an enzyme called a kinase. Various kinases are named for the substrate they phosphorylate. Phosphorylation can create a binding site that interacts with downstream components in the signaling cascade. Phosphorylation may activate or inactivate enzymes, and the reversal of phosphorylation, dephosphorylation by a phosphatase, will reverse the effect.

Second Messengers

Second messengers are small molecules that help to spread a signal through the cytoplasm after a ligand binds to a receptor. They do this by altering the behavior of certain cellular proteins. Some examples of second messengers are cAMP (a modified version of AMP, which is related to ATP but only contains one phosphate) and calcium ions.

Summary

Cells must communicate to coordinate their functions, especially in complex organisms. Signals can be sent over short or long distances, and the receiving cell must have the right receptor to respond. These receptors may be located inside the cell or on the cell membrane.

Cell-surface receptors come in several forms, each with specialized functions that help the cell process and respond to signals in its environment. Together, these communication pathways allow cells to make decisions, work together, and adapt to changing conditions.

When a signaling molecule (ligand) binds to a cell-surface receptor, it triggers a process called signal transduction, which passes the signal into the cell. This starts a signaling pathway, a chain of events that leads to a specific response, like turning on a gene or moving a protein.

These pathways can be complex and often amplify the signal, meaning one signal can cause a big response. Inside the cell, signals are often passed along by adding phosphate groups to proteins (a process called phosphorylation) or by using second messengers like cAMP or calcium ions to spread the signal quickly.

Key Terms

autocrine signaling
: a form of signaling where a cell sends signals to itself

paracrine signaling
: signals sent to nearby cells

endocrine signaling
: long-distance signaling using hormones transported through the bloodstream

ligand
: a molecule that binds to a receptor to deliver a signal

receptor
: a protein that detects a ligand and initiates a response

signal transduction
: the process by which a cell responds to an external signa

ion channel-linked receptor
: a receptor that opens or closes an ion channel in response to ligand binding

g-protein-coupled receptor (GPCR)
: a receptor that activates intracellular enzymes in response to ligand binding

conformational change
: a change in the shape of a protein, for receptors it is often triggered by ligand binding

signal transduction
: the process by which a signal from outside the cell (such as a ligand binding to a receptor) is converted into a functional response inside the cell

signaling pathway
: a series of molecular events triggered by a signal, typically involving multiple proteins and reactions, that lead to a specific cellular response

signaling cascade
: another term for a signaling pathway, emphasizing the step-by-step nature of the process, where each step activates the next, often amplifying the original signa

phosphorylation
: the addition of a phosphate group to a molecule, usually a protein, which can activate or deactivate its function or help pass along a signal

kinase
: an enzyme that catalyzes (speeds up) the transfer of a phosphate group from ATP to another molecule, typically during signal transduction

second messengers
: small molecules inside the cell that help spread and amplify signals from receptors by influencing other molecules or proteins. Examples include cAMP and calcium ions

References

Text adapted from: OpenStax, Biology. OpenStax CNX. October 13, 2017. Find originals at: https://cnx.org/contents/GFy_h8cu@10.118:H4oMpCSi@8/Signaling-Molecules-and-Cellul#footnote1 [cnx.org] [cnx.org] and https://cnx.org/contents/GFy_h8cu@10...-of-the-Signal

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5.6: Cell Response

Learning Objectives

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

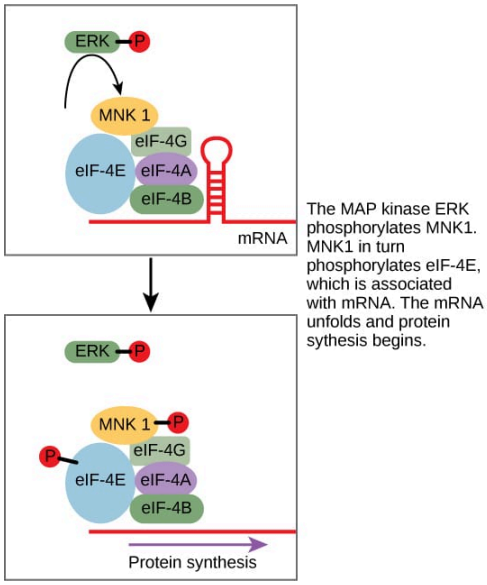
- Explain how signaling pathways can lead to changes in gene expression, metabolism, cell growth, and apoptosis
- Describe how external signals are converted into internal cellular changes
- Give examples of how the same signal can lead to different responses in different cells

Ligands which can enter the cell and bind to internal receptors are able to directly affect the cell's DNA and protein-producing machinery. Ligands which can not enter the cell bind to receptors in the plasma membrane and use signal transduction pathways to produce a variety of effects. The results of signaling pathways are extremely varied and depend on the type of cell involved as well as the external and internal conditions. A small sampling of responses is described below.

Gene Expression

Some signal transduction pathways regulate the production of mRNA. Others regulate the synthesis of proteins from mRNA by ribosomes. Typically these pathways increase gene expression so that more of a specific protein is produced from a gene, but some pathways do decrease gene expression.

An example of a protein that regulates translation in the nucleus is the MAP kinase ERK. ERK is activated in a phosphorylation cascade when epidermal growth factor (EGF) binds the EGF receptor. Upon phosphorylation, ERK enters the nucleus and activates a protein kinase that, in turn, regulates protein translation (**Figure** 5.6.1).



(**Figure** 5.6.1) This illustration shows the pathway by which ERK, a MAP kinase, activates protein synthesis. ERK phosphorylates MNK1, which in turn phosphorylates eIF-4E, an elongation initiation factor that, with other initiation factors, is associated with mRNA. When eIF-4E becomes phosphorylated, the mRNA unfolds, allowing protein synthesis in the nucleus to begin.

Increase in Cellular Metabolism

The result of another signaling pathway affects muscle cells. The activation of β-adrenergic receptors in muscle cells by adrenaline leads to an increase in cyclic AMP (cAMP) inside the cell. Also known as epinephrine, adrenaline is a hormone (produced by the adrenal gland attached to the kidney) that readies the body for short-term emergencies. Cyclic AMP activates

PKA (protein kinase A), which in turn phosphorylates two enzymes. The first enzyme promotes the degradation of glycogen by activating intermediate glycogen phosphorylase kinase (GPK) that in turn activates glycogen phosphorylase (GP) that catabolizes glycogen into glucose. (Recall that your body converts excess glucose to glycogen for short-term storage. When energy is needed, glycogen is quickly reconverted to glucose.) Phosphorylation of the second enzyme, glycogen synthase (GS), inhibits its ability to form glycogen from glucose. In this manner, a muscle cell obtains a ready pool of glucose by activating its formation via glycogen degradation and by inhibiting the use of glucose to form glycogen, thus preventing a futile cycle of glycogen degradation and synthesis. The glucose is then available for use by the muscle cell in response to a sudden surge of adrenaline—the "fight or flight" reflex.

Cell Growth

Cell signaling pathways also play a major role in cell division. Cells do not normally divide unless they are stimulated by signals from other cells. The ligands that promote cell growth are called growth factors. Most growth factors bind to cell-surface receptors that are linked to tyrosine kinases. These cell-surface receptors are called receptor tyrosine kinases (RTKs). Activation of RTKs initiates a signaling pathway that includes a G-protein called RAS, which activates the MAP kinase pathway described earlier. The enzyme MAP kinase then stimulates the expression of proteins that interact with other cellular components to initiate cell division.

Cell Death

When a cell is damaged, superfluous, or potentially dangerous to an organism, a cell can initiate a mechanism to trigger programmed cell death, or **apoptosis.** Apoptosis allows a cell to die in a controlled manner that prevents the release of potentially damaging molecules from inside the cell. There are many internal checkpoints that monitor a cell's health; if abnormalities are observed, a cell can spontaneously initiate the process of apoptosis. However, in some cases, such as a viral infection or uncontrolled cell division due to cancer, the cell's normal checks and balances fail. External signaling can also initiate apoptosis. For example, most normal animal cells have receptors that interact with the extracellular matrix, a network of glycoproteins that provides structural support for cells in an organism. The binding of cellular receptors to the extracellular matrix initiates a signaling cascade within the cell. However, if the cell moves away from the extracellular matrix, the signaling ceases, and the cell undergoes apoptosis. This system keeps cells from traveling through the body and proliferating out of control, as happens with tumor cells that metastasize.

Another example of external signaling that leads to apoptosis occurs in T-cell development. T-cells are immune cells that bind to foreign macromolecules and particles, and target them for destruction by the immune system. Normally, T-cells do not target "self" proteins (those of their own organism), a process that can lead to autoimmune diseases. In order to develop the ability to discriminate between self and non-self, immature T-cells undergo screening to determine whether they bind to so-called self proteins. If the T-cell receptor binds to self proteins, the cell initiates apoptosis to remove the potentially dangerous cell.

Apoptosis is also essential for normal embryological development. In vertebrates, for example, early stages of development include the formation of web-like tissue between individual fingers and toes (**Figure** 5.6.2). During the course of normal development, these unneeded cells must be eliminated, enabling fully separated fingers and toes to form. A cell signaling mechanism triggers apoptosis, which destroys the cells between the developing digits.



(**Figure** 5.6.2) The histological section of a foot of a 15-day-old mouse embryo, visualized using light microscopy, reveals areas of tissue between the toes, which apoptosis will eliminate before the mouse reaches its full gestational age at 27 days. (credit: modification of work by Michal Mañas)

Termination of the Signal Cascade

The aberrant signaling often seen in tumor cells is proof that the termination of a signal at the appropriate time can be just as important as the initiation of a signal. One method of stopping a specific signal is to degrade the ligand or remove it so that it can no longer access its receptor. One reason that hydrophobic hormones like estrogen and testosterone trigger long-lasting events is because they bind carrier proteins. These proteins allow the insoluble molecules to be soluble in blood, but they also protect the hormones from degradation by circulating enzymes.

Inside the cell, many different enzymes reverse the cellular modifications that result from signaling cascades. For example, phosphatases are enzymes that remove the phosphate group attached to proteins by kinases in a process called dephosphorylation. Cyclic AMP (cAMP) is degraded into AMP by phosphodiesterase, and the release of calcium stores is reversed by the Ca^{2+} pumps that are located in the external and internal membranes of the cell.

Summary

Once a cell detects a signal—such as a hormone or chemical messenger—it needs to respond appropriately. Depending on the type of signal and the kind of cell, the response can vary a lot. The signal might affect which proteins a cell makes, how it uses energy, whether it divides, or even whether it continues to live.

Key Terms

apoptosis
: programmed cell death, used to eliminate unnecessary or damaged cells

gene expression
: the process of turning genetic information into functional products like proteins

phosphorylation
: the addition of a phosphate group to a molecule, often used to activate proteins

kinase
: an enzyme that adds phosphate groups to other molecule

References

OpenStax, Biology. OpenStax CNX. October 13, 2017. Access for free at https://cnx.org/contents/GFy_h8cu@10...-to-the-Signal

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6: INTRODUCTION TO CELLULAR DIVISION AND DNA

6.1: The Cell Cycle
6.2: The Structure of DNA
6.3: DNA Replication

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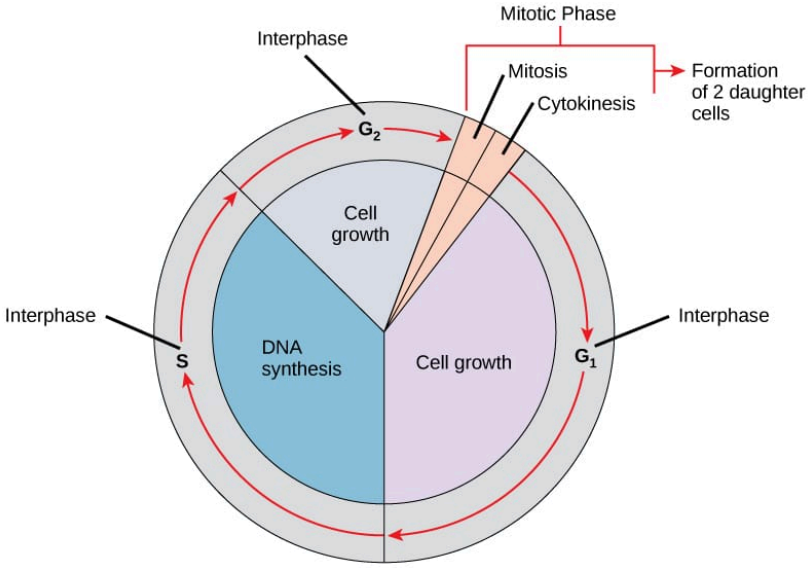
6.1: The Cell Cycle

Learning Objectives

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

- Identify the major phases of the cell cycle (G1, S, G2, mitosis, and cytokinesis) and describe their key characteristics
- Explain the role of checkpoints in regulating the cell cycle and ensuring accurate cell division
- Assess the consequences of checkpoint failure on genomic stability

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** 6.1.1). 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** 6.1.1) 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.

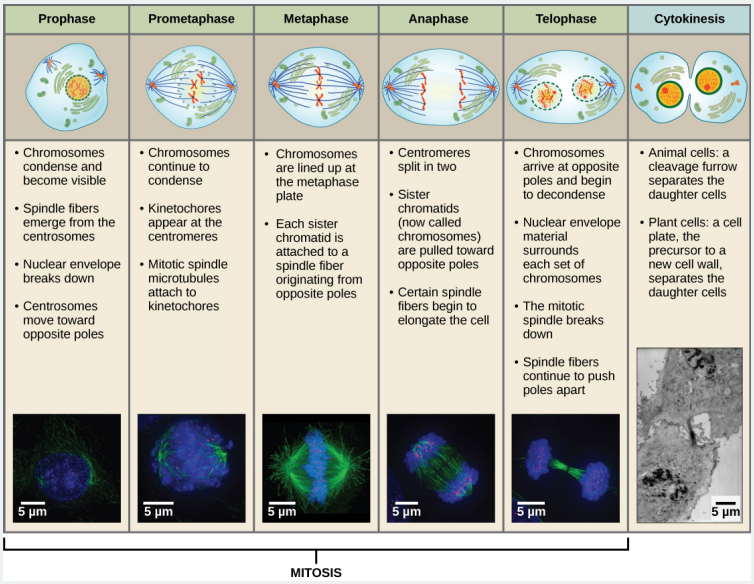
The Mitotic Phase

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** $6.1.2$**)**.

ART CONNECTION



Open for image description

This infographic illustrates the stages of mitosis and cytokinesis in a eukaryotic cell. The information is arranged in six vertical columns labeled **Prophase**, **Prometaphase**, **Metaphase**, **Anaphase**, **Telophase**, and **Cytokinesis**. Each column contains a diagram of the cell stage, descriptive text, and a microscope image with a scale bar labeled 5 µm.

Prophase

The cell is shown with condensed chromosomes visible inside the nucleus. Centrosomes and spindle fibers begin forming as the centrosomes move apart.

- Chromosomes condense and become visible
- Spindle fibers emerge from the centrosomes
- Nuclear envelope breaks down
- Centrosomes move toward opposite poles

The fluorescence microscope image below shows a cell with a large purple-stained nucleus surrounded by green filamentous structures.

Prometaphase

The nuclear envelope has disappeared. Condensed chromosomes are scattered throughout the cell, and spindle fibers extend from opposite poles toward the chromosomes.

- Chromosomes continue to condense
- Kinetochores appear at the centromeres
- Mitotic spindle microtubules attach to kinetochores

The microscope image shows condensed chromosomes stained purple and spindle fibers stained green.

Metaphase

Chromosomes are aligned at the center of the cell along the metaphase plate. Spindle fibers connect each chromosome to opposite poles.

- Chromosomes are lined up at the metaphase plate
- Each sister chromatid is attached to a spindle fiber originating from opposite poles

The microscope image shows chromosomes aligned in a straight line at the center of the cell, with spindle fibers extending from both poles.

Anaphase

Sister chromatids separate and move toward opposite poles while the cell elongates.

- Centromeres split in two
- Sister chromatids (now called chromosomes) are pulled toward opposite poles
- Certain spindle fibers begin to elongate the cell

The microscope image shows two groups of chromosomes moving apart toward opposite ends of the cell.

Telophase

Chromosomes have reached opposite poles and begin to decondense. New nuclear envelopes form around each set of chromosomes.

- Chromosomes arrive at opposite poles and begin to decondense
- Nuclear envelope material surrounds each set of chromosomes
- The mitotic spindle breaks down
- Spindle fibers continue to push poles apart

The microscope image shows two separate chromosome masses at opposite sides of the cell connected by remaining spindle structures.

Cytokinesis

Two daughter cells are formed as the cytoplasm divides. The illustration shows an animal cell pinching inward to separate into two cells.

- Animal cells: a cleavage furrow separates the daughter cells
- Plant cells: a cell plate, the precursor to a new cell wall, separates the daughter cells

The image below is a grayscale electron micrograph showing the division plane between two developing daughter cells.

Overall, the figure demonstrates the progression of cell division from chromosome condensation through chromosome alignment and separation, to the formation of two daughter cells through cytokinesis.

(**Figure** 6.1.2) This diagram shows the five phases of mitosis by light microscopy with fluorescence, and cytokinesis by a transmission electron microscope. During prophase, the chromosomes condense and become visible, spindle fibers emerge from the centrosomes, the centrosomes move toward opposite poles, and the nuclear envelope breaks down. During prometaphase, the chromosomes continue to condense and kinetochores appear at the centromeres. Mitotic spindle microtubules attach to the kinetochores. During metaphase, the centrosomes are at opposite poles of the cell. Chromosomes line up at the metaphase plate and each sister chromatid is attached to spindle fibers originating from the opposite poles. During anaphase, the centromeres split in two. The sister chromatids, which are now called chromosomes, move toward opposite poles of the cell. Certain spindle fibers lengthen, elongating the cell. During telophase, the chromosomes arrive at the opposite poles and begin to decondense. The nuclear envelope re-forms. During cytokinesis in animals, a cleavage furrow separates the two daughter cells. In plants, a cell plate—the precursor to a new cell wall—separates the two daughter cells.

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

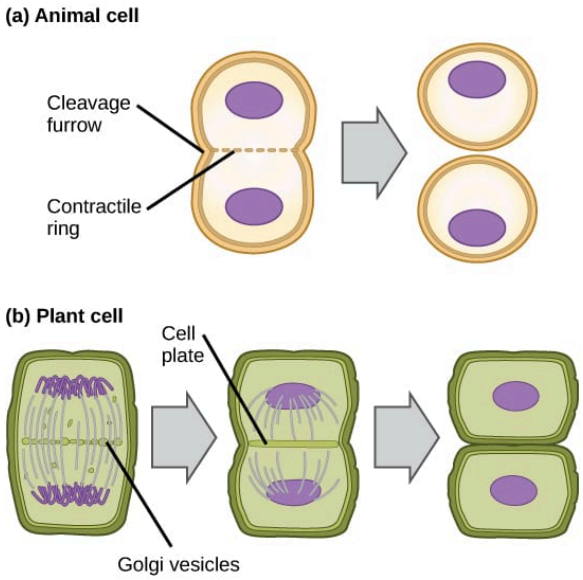
This page of videos illustrates different aspects of mitosis. Watch the video entitled “DIC microscopy of cell division in a newt lung cell” and identify the phases of mitosis.

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** 6.1.3).

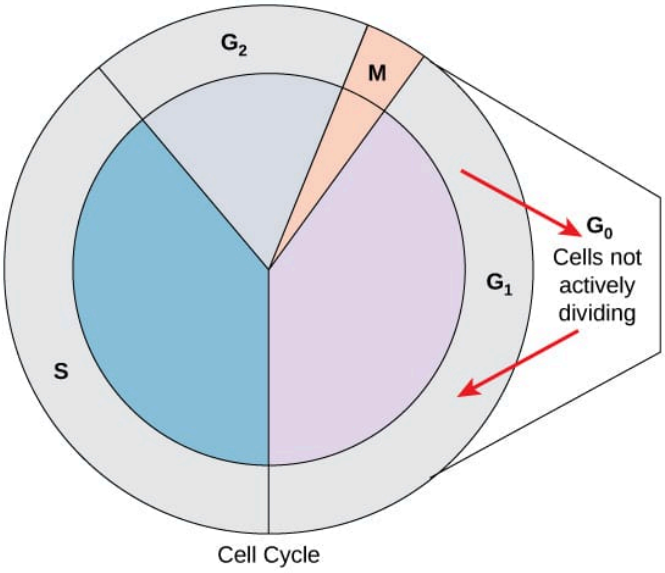
In plant cells, a cleavage furrow is not possible because of the rigid cell walls surrounding the plasma membrane. A new cell wall must form between the daughter cells. During interphase, the Golgi apparatus accumulates enzymes, structural proteins, and glucose molecules prior to breaking up into vesicles 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** 6.1.3).



(**Figure** 6.1.3) 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** 6.1.4).



(**Figure** 6.1.4) 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.

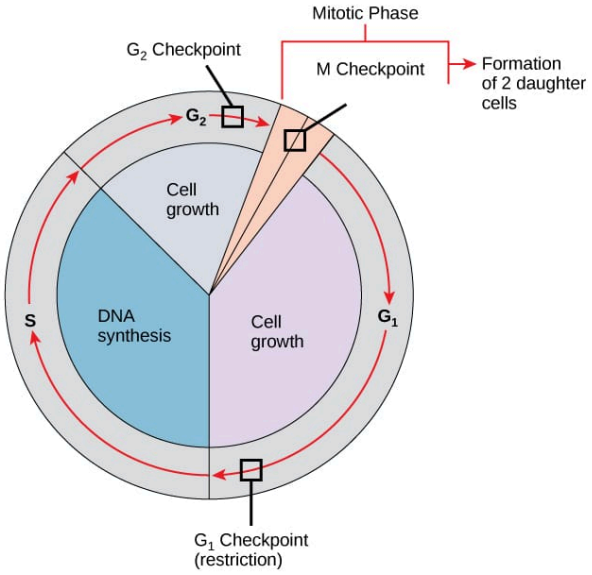
Control of the Cell Cycle

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** 6.1.5).



(**Figure** 6.1.5) 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 6.1.2: 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.

Key Terms

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

chromatin
DNA wrapped around proteins in a loose, threadlike form

chromosome
a tightly packed structure of DNA and proteins that carries genetic information

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

nuclear envelope
the double membrane that surrounds the nucleus

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

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6.2: The Structure of DNA

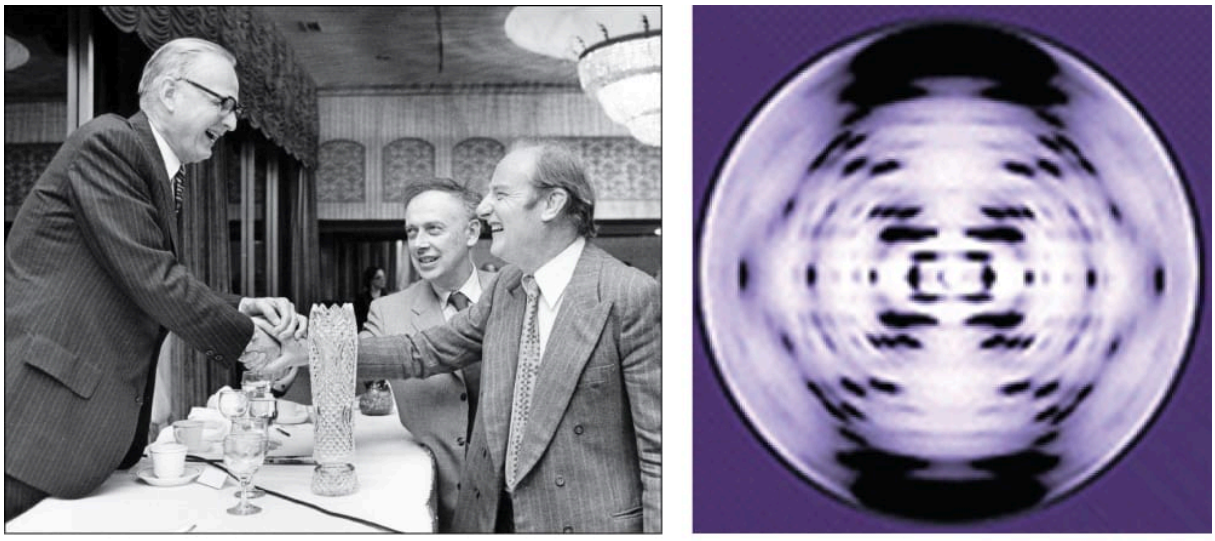
Learning Objectives

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

- Define the structure and components of DNA and RNA, including nucleotide structure, nitrogenous bases, and the sugar-phosphate backbone
- Recall the basic differences between DNA and RNA, including their sugar components, bases, and strand structures.Illustrate the antiparallel nature of DNA strands and how hydrogen bonding between base pairs maintains the double helix structure
- Compare and contrast the organization and packaging of DNA in prokaryotic vs. eukaryotic cells
- Interpret the levels of chromatin compaction and how DNA is packed into chromosomes within the nucleus
- Analyze how the structure of DNA enables accurate replication and transmission of genetic information.

Watson and Crick

In the 1950s, Francis Crick and James Watson worked together at the University of Cambridge, England, to determine the structure of DNA. Other scientists, such as Linus Pauling and Maurice Wilkins, were also actively exploring this field. Pauling had discovered the secondary structure of proteins using X-ray crystallography. X-ray crystallography is a method for investigating molecular structure by observing the patterns formed by X-rays shot through a crystal of the substance. The patterns give important information about the structure of the molecule of interest. In Wilkins' lab, researcher Rosalind Franklin was using X-ray crystallography to understand the structure of DNA. Watson and Crick were able to piece together the puzzle of the DNA molecule using Franklin's data (**Figure** 6.2.1). Watson and Crick also had key pieces of information available from other researchers such as Chargaff's rules. Chargaff had shown that of the four kinds of monomers (nucleotides) present in a DNA molecule, two types were always present in equal amounts and the remaining two types were also always present in equal amounts. This meant they were always paired in some way. In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine for their work in determining the structure of DNA.

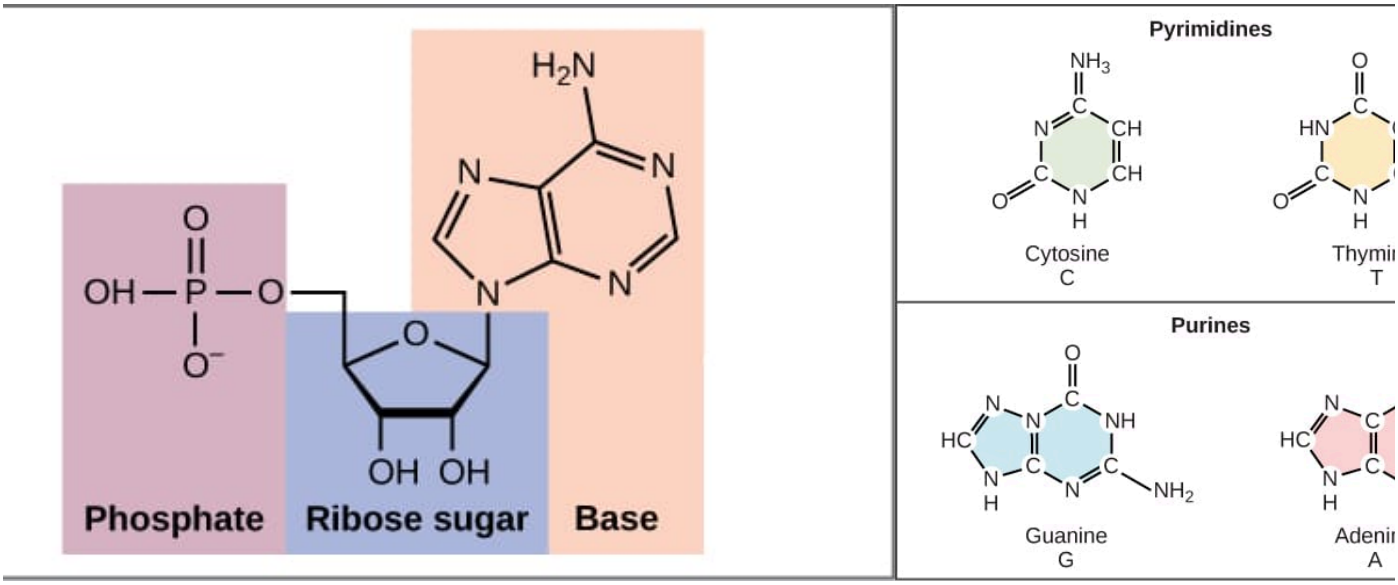


(a) (b)

(**Figure** 6.2.1) Pioneering scientists (**a**) James Watson and Francis Crick are pictured here with American geneticist Maclyn McCarty. Scientist Rosalind Franklin discovered (**b**) the X-ray diffraction pattern of DNA, which helped to elucidate its double helix structure. (credit a: modification of work by Marjorie McCarty; b: modification of work by NIH)

Now let's consider the structure of the two types of nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The building blocks of DNA are nucleotides, which are made up of three parts: a deoxyribose (5-carbon sugar), a phosphate group,

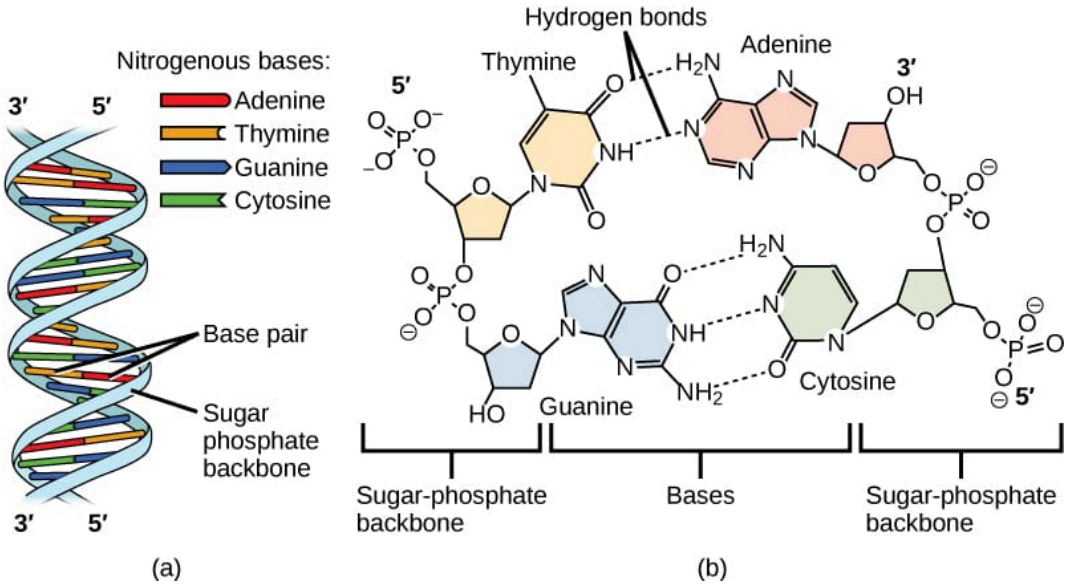
and a nitrogenous base (**Figure** 6.2.2). There are four types of nitrogenous bases in DNA. Adenine (A) and guanine (G) are double-ringed purines, and cytosine (C) and thymine (T) are smaller, single-ringed pyrimidines. The nucleotide is named according to the nitrogenous base it contains.



(**Figure** 6.2.2) (**left**) Each DNA nucleotide is made up of a deoxyribose sugar with a nitrogenous base attached at the 1' position and a phosphate group attached at the 5' position. (**right**) There are two kinds of nitrogenous bases: pyrimidines, which have one six-membered ring, and purines, which have a six-membered ring fused to a five-membered ring. Cytosine and thymine are pyrimidines. Guanine and adenine are purines.

The phosphate group of one nucleotide bonds covalently with the sugar molecule of the next nucleotide, and so on, forming a long polymer of nucleotide monomers. The sugar–phosphate groups line up in a "backbone" for each single strand of DNA, and the nucleotide bases stick out from this backbone. The carbon atoms of the five-carbon sugar are numbered clockwise from the oxygen as 1', 2', 3', 4', and 5' (1' is read as "one prime"). The phosphate group is attached to the 5' carbon of one nucleotide and the 3' carbon of the next nucleotide. In its natural state, each DNA molecule is actually composed of two single strands held together along their length with hydrogen bonds between the bases.

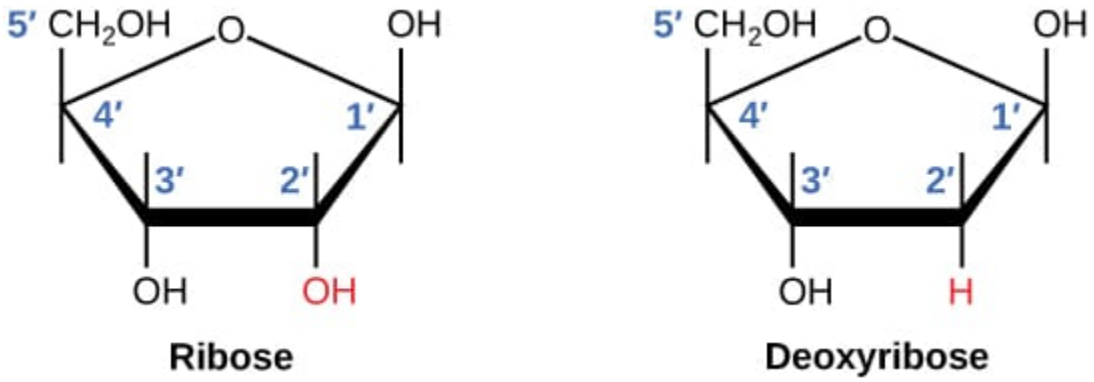
Watson and Crick proposed that the DNA is made up of two strands that are twisted around each other to form a right-handed helix, called a double helix. Base-pairing takes place between a purine and pyrimidine: namely, A pairs with T, and G pairs with C. In other words, adenine and thymine are complementary base pairs, and cytosine and guanine are also complementary base pairs. This is the basis for Chargaff's rule; because of their complementarity, there is as much adenine as thymine in a DNA molecule and as much guanine as cytosine. Adenine and thymine are connected by two hydrogen bonds, and cytosine and guanine are connected by three hydrogen bonds. The two strands are anti-parallel in nature; that is, one strand will have the 3' carbon of the sugar in the "upward" position, whereas the other strand will have the 5' carbon in the upward position. The diameter of the DNA double helix is uniform throughout because a purine (two rings) always pairs with a pyrimidine (one ring) and their combined lengths are always equal (**Figure** 6.2.3).



(**Figure** 6.2.3) DNA (**a**) forms a double stranded helix, which has a sugar phosphate backbone on the outside and nitrogenous base pairs on the inside. (**b**) Adenine pairs with thymine with 2 hydrogen bonds, and cytosine pairs with guanine with 3 hydrogen bonds. (credit a: modification of work by Jerome Walker, Dennis Myts)

The Structure of RNA

There is a second nucleic acid in all cells called ribonucleic acid, or RNA. Like DNA, RNA is a polymer of nucleotides. Each of the nucleotides in RNA is made up of a nitrogenous base, a five-carbon sugar, and a phosphate group. In the case of RNA, the five-carbon sugar is ribose, not deoxyribose. Ribose has a hydroxyl group at the 2' carbon, unlike deoxyribose, which has only a hydrogen atom (**Figure** 6.2.4).



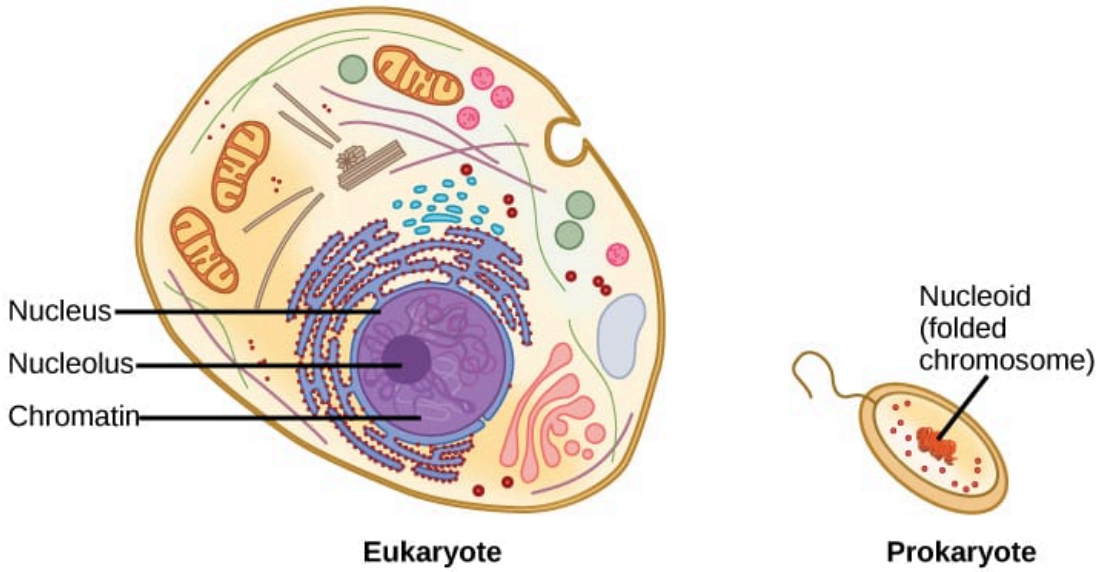
(**Figure** 6.2.4) The figure above shows the structure of ribose and deoxyribose sugars. In ribose, the OH at the 2' position is highlighted in red. In deoxyribose, the H at the 2' position is highlighted in red.

RNA nucleotides contain the nitrogenous bases adenine, cytosine, and guanine. However, they do not contain thymine, which is instead replaced by uracil, symbolized by a “U.” RNA exists as a single-stranded molecule rather than a double-stranded helix. Molecular biologists have named several kinds of RNA on the basis of their function. These include messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA)—molecules that are involved in the production of proteins from the DNA code.

How DNA Is Arranged in the Cell

DNA is a working molecule; it must be replicated when a cell is ready to divide, and it must be “read” to produce the molecules, such as proteins, to carry out the functions of the cell. For this reason, the DNA is protected and packaged in very specific ways. In addition, DNA molecules can be very long. Stretched end-to-end, the DNA molecules in a single human cell would come to a length of about 2 meters. Thus, the DNA for a cell must be packaged in a very ordered way to fit and function within a structure (the cell) that is not visible to the naked eye. The chromosomes of prokaryotes are much simpler than those of eukaryotes in many

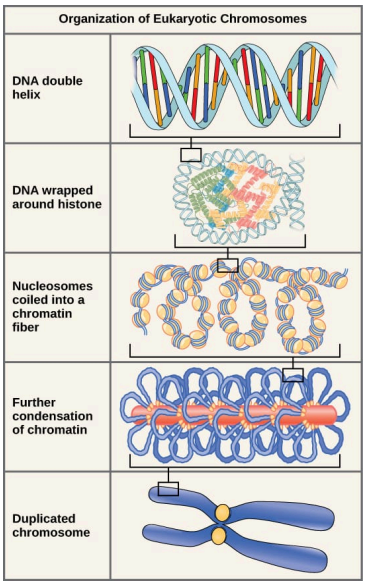
of their features (**Figure** 6.2.5). Most prokaryotes contain a single, circular chromosome that is found in an area in the cytoplasm called the nucleoid.



(**Figure** 6.2.5) The illustration above shows a eukaryotic cell, which has a membrane-bound nucleus containing chromatin and a nucleolus, and a prokaryotic cell, which has DNA contained in an area of the cytoplasm called the nucleoid. The prokaryotic cell is much smaller than the eukaryotic cell.

The size of the genome in one of the most well-studied prokaryotes, *Escherichia coli,* is 4.6 million base pairs, which would extend a distance of about 1.6 mm if stretched out. So how does this fit inside a small bacterial cell? The DNA is twisted beyond the double helix in what is known as supercoiling. Some proteins are known to be involved in the supercoiling; other proteins and enzymes help in maintaining the supercoiled structure.

Eukaryotes, whose chromosomes each consist of a linear DNA molecule, employ a different type of packing strategy to fit their DNA inside the nucleus (**Figure** 6.2.6). At the most basic level, DNA is wrapped around proteins known as histones to form structures called nucleosomes. The DNA is wrapped tightly around the histone core. This nucleosome is linked to the next one by a short strand of DNA that is free of histones. This is also known as the “beads on a string” structure; the nucleosomes are the “beads” and the short lengths of DNA between them are the “string.” The nucleosomes, with their DNA coiled around them, stack compactly onto each other to form a 30-nm–wide fiber. This fiber is further coiled into a thicker and more compact structure. At the metaphase stage of mitosis, when the chromosomes are lined up in the center of the cell, the chromosomes are at their most compacted. They are approximately 700 nm in width, and are found in association with scaffold proteins.



Open for image description This diagram, titled **"Organization of Eukaryotic Chromosomes,"** illustrates the progressive levels of DNA packaging inside a eukaryotic cell, beginning with the DNA double helix and ending with a condensed duplicated chromosome. Each stage is shown in a separate horizontal section, with brackets highlighting the portion of one level that is enlarged in the next level below.

DNA Double Helix

The top section shows the familiar double-helix structure of DNA. Two blue strands twist around one another to form a spiral ladder. Colored bars representing nitrogenous base pairs connect the strands across the center of the helix. A bracket beneath the helix indicates that a portion of the DNA molecule will be magnified in the next stage.

DNA Wrapped Around Histone

The second section shows DNA wrapped around a cluster of histone proteins. The light-blue DNA strand coils around a central protein core, forming a spool-like structure called a nucleosome. The DNA wraps around the histone complex approximately two times, creating the first level of DNA packaging. A bracket highlights a single nucleosome that will be enlarged in the next stage.

Nucleosomes Coiled into a Chromatin Fiber

The third section illustrates many nucleosomes connected by stretches of DNA. The nucleosomes appear as a series of bead-like structures arranged along the DNA strand. These beads loop and coil together to form a thicker chromatin fiber. A bracket identifies a region of the chromatin fiber that is further magnified in the next stage.

Further Condensation of Chromatin

The fourth section shows chromatin condensed into larger loops attached to a central protein scaffold. The blue chromatin fiber forms numerous loops extending outward from a thick orange-red core. This higher-order folding greatly compacts the DNA molecule. A bracket highlights one portion of the condensed chromatin that will be enlarged in the final stage.

Duplicated Chromosome

The bottom section shows a fully condensed duplicated chromosome. The chromosome consists of two identical sister chromatids connected at a central constricted region called the centromere. Each chromatid is depicted as a thick blue structure. Two yellow circular shapes at the center represent the centromere region where the sister chromatids remain joined. The chromosome has the characteristic X-shaped appearance typically seen during cell division.

Overall, the diagram demonstrates how a very long DNA molecule is progressively folded and compacted. The levels shown are: DNA double helix, DNA wrapped around histone proteins, nucleosomes coiled into chromatin fibers, further condensation of chromatin, and finally a duplicated chromosome visible during cell division.

(**Figure** 6.2.6) These figures illustrate the compaction of the eukaryotic chromosome. Starting with the DNA double helix, which wraps around histone proteins. The entire DNA molecule wraps around many clusters of histone proteins, forming a structure that looks like beads on a string. The chromatin is further condensed by wrapping around a protein core. The result is a compact chromosome, shown in duplicated form.

CONCEPT IN ACTION

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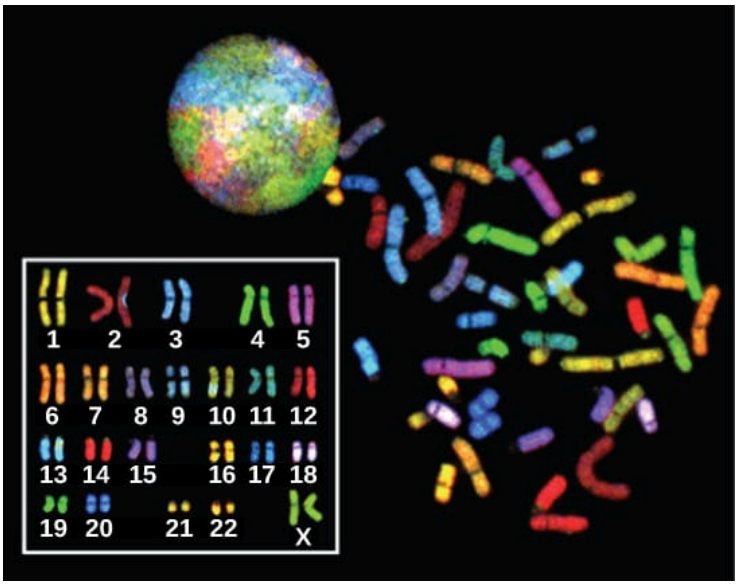
Watch this animation of DNA packaging.

The continuity of life from one cell to another has its foundation in the reproduction of cells by way of the cell cycle. The cell cycle is an orderly sequence of events in the life of a cell from the division of a single parent cell to produce two new daughter cells, to the subsequent division of those daughter cells. The mechanisms involved in the cell cycle are highly conserved across eukaryotes. Organisms as diverse as protists, plants, and animals employ similar steps.

Genomic DNA

Before discussing the steps a cell undertakes to replicate, a deeper understanding of the structure and function of a cell's genetic information is necessary. A cell's complete complement of DNA is called its genome. In prokaryotes, the genome is composed of a single, double-stranded DNA molecule in the form of a loop or circle. The region in the cell containing this genetic material is called a nucleoid. Some prokaryotes also have smaller loops of DNA called plasmids that are not essential for normal growth.

In eukaryotes, the genome comprises several double-stranded, linear DNA molecules (**Figure** 6.2.7) bound with proteins to form complexes called chromosomes. Each species of eukaryote has a characteristic number of chromosomes in the nuclei of its cells. Human body cells (somatic cells) have 46 chromosomes. A somatic cell contains two matched sets of chromosomes, a configuration known as diploid. The letter *n* is used to represent a single set of chromosomes; therefore a diploid organism is designated 2*n*. Human cells that contain one set of 23 chromosomes are called gametes, or sex cells; these eggs and sperm are designated *n*, or haploid.



(**Figure** 6.2.7) There are 23 pairs of homologous chromosomes in a female human somatic cell. These chromosomes are viewed within the nucleus (top), removed from a cell in mitosis (right), and arranged according to length (left) in an arrangement called a karyotype. In this image, the chromosomes were exposed to fluorescent stains to distinguish them. (credit: "718 Bot"/Wikimedia Commons, National Human Genome Research)

The matched pairs of chromosomes in a diploid organism are called homologous chromosomes. Homologous chromosomes are the same length and have specific nucleotide segments called genes in exactly the same location, or locus. Genes, the functional units of chromosomes, determine specific characteristics by coding for specific proteins. Traits are the different forms of a characteristic. For example, the shape of earlobes is a characteristic with traits of free or attached.

Each copy of the homologous pair of chromosomes originates from a different parent; therefore, the copies of each of the genes themselves may not be identical. The variation of individuals within a species is caused by the specific combination of the genes inherited from both parents. For example, there are three possible gene sequences on the human chromosome that codes for blood type: sequence A, sequence B, and sequence O. Because all diploid human cells have two copies of the chromosome that determines blood type, the blood type (the trait) is determined by which two versions of the marker gene are inherited. It is possible to have two copies of the same gene sequence, one on each homologous chromosome (for example, AA, BB, or OO), or two different sequences, such as AB.

Minor variations in traits such as those for blood type, eye color, and height contribute to the natural variation found within a species. The sex chromosomes, X and Y, are the single exception to the rule of homologous chromosomes; other than a small amount of homology that is necessary to reliably produce gametes, the genes found on the X and Y chromosomes are not the same.

Summary

The model of the double-helix structure of DNA was proposed by Watson and Crick. The DNA molecule is a polymer of nucleotides. Each nucleotide is composed of a nitrogenous base, a five-carbon sugar (deoxyribose), and a phosphate group. There are four nitrogenous bases in DNA, two purines (adenine and guanine) and two pyrimidines (cytosine and thymine). A DNA molecule is composed of two strands. Each strand is composed of nucleotides bonded together covalently between the phosphate group of one and the deoxyribose sugar of the next. From this backbone extend the bases. The bases of one strand bond to the bases of the second strand with hydrogen bonds. Adenine always bonds with thymine, and cytosine always bonds with guanine. The bonding causes the two strands to spiral around each other in a shape called a double helix. Ribonucleic acid (RNA) is a second nucleic acid found in cells. RNA is a single-stranded polymer of nucleotides. It also differs from DNA in that it contains the sugar ribose, rather than deoxyribose, and the nucleotide uracil rather than thymine. Various RNA molecules function in the process of forming proteins from the genetic code in DNA.

Prokaryotes contain a single, double-stranded circular chromosome. Eukaryotes contain double-stranded linear DNA molecules packaged into chromosomes. The DNA helix is wrapped around proteins to form nucleosomes. The protein coils are further coiled, and during mitosis and meiosis, the chromosomes become even more greatly coiled to facilitate their movement. Chromosomes

have two distinct regions which can be distinguished by staining, reflecting different degrees of packaging and determined by whether the DNA in a region is being expressed (euchromatin) or not (heterochromatin).

Prokaryotes have a single loop chromosome, whereas eukaryotes have multiple, linear chromosomes surrounded by a nuclear membrane. Human somatic cells have 46 chromosomes consisting of two sets of 22 homologous chromosomes and a pair of nonhomologous sex chromosomes. This is the 2*n,* or diploid, state. Human gametes have 23 chromosomes or one complete set of chromosomes. This is the *n,* or haploid, state. Genes are segments of DNA that code for a specific protein or RNA molecule. An organism's traits are determined in large part by the genes inherited from each parent, but also by the environment that they experience. Genes are expressed as characteristics of the organism and each characteristic may have different variants called traits that are caused by differences in the DNA sequence for a gene.

Key Terms

anti-parallel
: DNA is a double stranded molecule and the strands run in opposite direction

base pairing
: refers to the specific hydrogen bonding between complementary nitrogenous bases on opposite strands of a double-stranded nucleic acid (such as DNA)

complementary bases
: pairs of nitrogenous bases that form specific hydrogen bonds with each other in nucleic acids, helping stabilize the structure of the molecule. In DNA, adenine (A) always pairs with thymine (T), and guanine (G) always pairs with cytosine (C). In RNA, adenine pairs with uracil (U) instead of thymine

deoxyribose
: a five-carbon sugar molecule with a hydrogen atom rather than a hydroxyl group in the 2' position; the sugar component of DNA nucleotides

diploid
: describes a cell, nucleus, or organism containing two sets of chromosomes (2*n*)

double helix
: the molecular shape of DNA in which two strands of nucleotides wind around each other in a spiral shape

gamete
: a haploid reproductive cell or sex cell (sperm or egg)

gene
: the physical and functional unit of heredity; a sequence of DNA that codes for a specific peptide or RNA molecule

genome
: the entire genetic complement (DNA) of an organism

haploid
: describes a cell, nucleus, or organism containing one set of chromosomes (*n*)

histone
: a protein that DNA wraps around to form chromatin

homologous chromosomes
: chromosomes of the same length with genes in the same location; diploid organisms have pairs of homologous chromosomes, and the members of each pair come from different parents

karyotype
: a visual display of an individual's chromosomes arranged by size and shape

locus
: the position of a gene on a chromosome

nitrogenous base
: a nitrogen-containing molecule that acts as a base; often referring to one of the purine or pyrimidine components of nucleic acids

nucleosome
: a structural unit of chromatin made of DNA wrapped around histone proteins

phosphate group
: a molecular group consisting of a central phosphorus atom bound to four oxygen atoms

sugar phosphate backbone
: DNA structure consists of two strands, each with a backbone of alternating phosphate and sugar groups

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6.3: DNA Replication

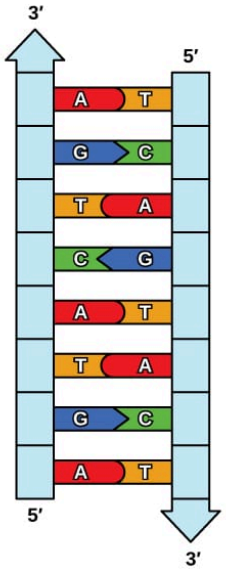
Learning Objectives

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

- Explain key terms involved in DNA replication, such as complementary base pairing, replication fork, Okazaki fragments, and telomerase.
- Explain the semiconservative model of DNA replication and how complementary base pairing ensures accurate copying of genetic information.
- Illustrate the steps of DNA replication, including initiation, elongation, and termination, using labeled diagrams or models.
- Compare and contrast DNA replication in prokaryotic and eukaryotic cells, highlighting differences in origin of replication, speed, and chromosomal structure.

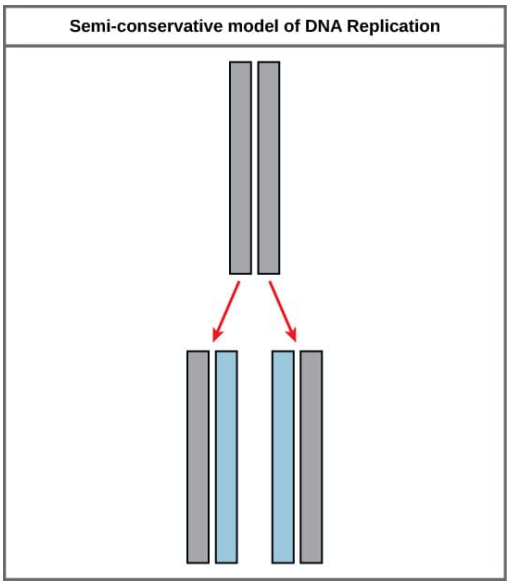
When a cell divides, it is important that each daughter cell receives an identical copy of the DNA. This is accomplished by the process of DNA replication. The replication of DNA occurs during the synthesis phase, or S phase, of the cell cycle, before the cell enters mitosis or meiosis.

The elucidation of the structure of the double helix provided a hint as to how DNA is copied. Recall that adenine nucleotides pair with thymine nucleotides, and cytosine with guanine. This means that the two strands are complementary to each other. For example, a strand of DNA with a nucleotide sequence of AGTCATGA will have a complementary strand with the sequence TCAGTACT (**Figure** 6.3.1).



(**Figure** 6.3.1) The figure shows the ladder-like structure of DNA. The two strands of DNA are complementary, meaning the sequence of bases in one strand can be used to create the correct sequence of bases in the other strand.

Because of the complementarity of the two strands, having one strand means that it is possible to recreate the other strand. This model for replication suggests that the two strands of the double helix separate during replication, and each strand serves as a template from which the new complementary strand is copied (**Figure** 6.3.2).



(**Figure** 6.3.2) The semiconservative model of DNA replication is shown. The original DNA strands are gray. They split during replication, and act as a template for the newly synthesized DNA in blue.

During DNA replication, each of the two strands that make up the double helix serves as a template from which new strands are copied. The new strand will be complementary to the parental or “old” strand. Each new double strand consists of one parental strand and one new daughter strand. This is known as semiconservative replication. When two DNA copies are formed, they have an identical sequence of nucleotide bases and are divided equally into two daughter cells.

DNA Replication in Eukaryotes

Because eukaryotic genomes are very complex, DNA replication is a very complicated process that involves several enzymes and other proteins. It occurs in three main stages: initiation, elongation, and termination.

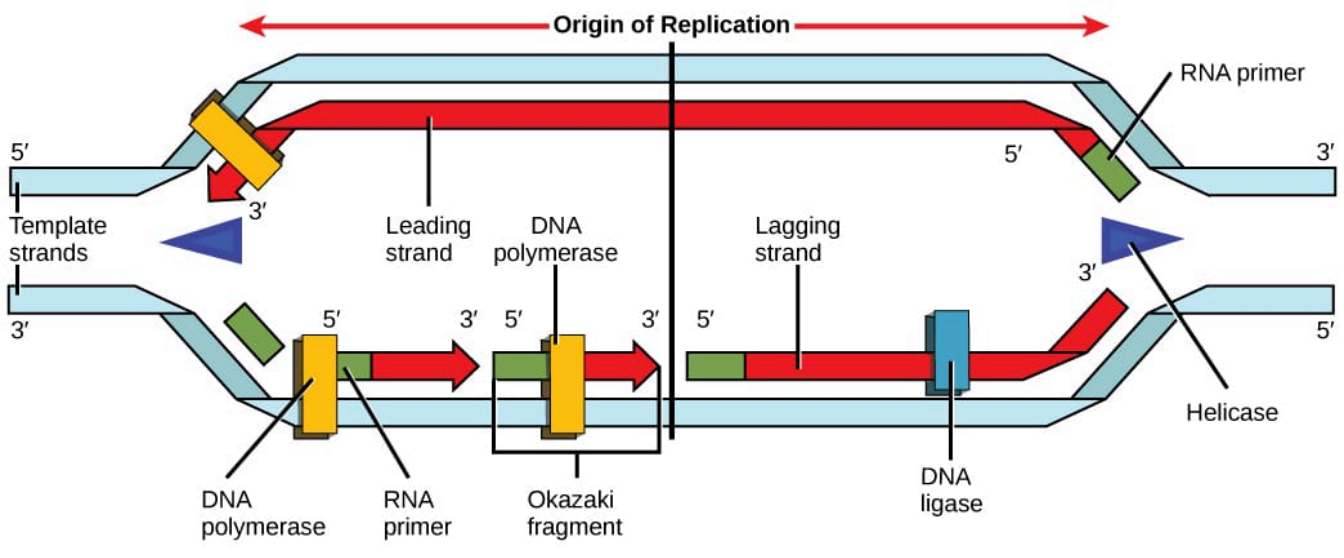
Recall that eukaryotic DNA is bound to proteins known as histones to form structures called nucleosomes. During initiation, the DNA is made accessible to the proteins and enzymes involved in the replication process. How does the replication machinery know where on the DNA double helix to begin? It turns out that there are specific nucleotide sequences called origins of replication at which replication begins. Certain proteins bind to the origin of replication while an enzyme called helicase unwinds and opens up the DNA helix. As the DNA opens up, Y-shaped structures called replication forks are formed (**Figure** 6.3.3). Two replication forks are formed at the origin of replication, and these get extended in both directions as replication proceeds. There are multiple origins of replication on the eukaryotic chromosome, such that replication can occur simultaneously from several places in the genome.

During elongation, an enzyme called DNA polymerase adds DNA nucleotides to the 3' end of the template. Because DNA polymerase can only add new nucleotides at the end of a backbone, a primer sequence, which provides this starting point, is added with complementary RNA nucleotides. This primer is removed later, and the nucleotides are replaced with DNA nucleotides. One strand, which is complementary to the parental DNA strand, is synthesized continuously toward the replication fork so the polymerase can add nucleotides in this direction. This continuously synthesized strand is known as the leading strand. Because DNA polymerase can only synthesize DNA in a 5' to 3' direction, the other new strand is put together in short pieces called Okazaki fragments. The Okazaki fragments each require a primer made of RNA to start the synthesis. The strand with the Okazaki fragments is known as the lagging strand. As synthesis proceeds, an enzyme removes the RNA primer, which is then replaced with DNA nucleotides, and the gaps between fragments are sealed by an enzyme called DNA ligase.

The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication.
2. New bases are added to the complementary parental strands. One new strand is made continuously, while the other strand is made in pieces.
3. Primers are removed, new DNA nucleotides are put in place of the primers and the backbone is sealed by DNA ligase.

ART CONNECTION

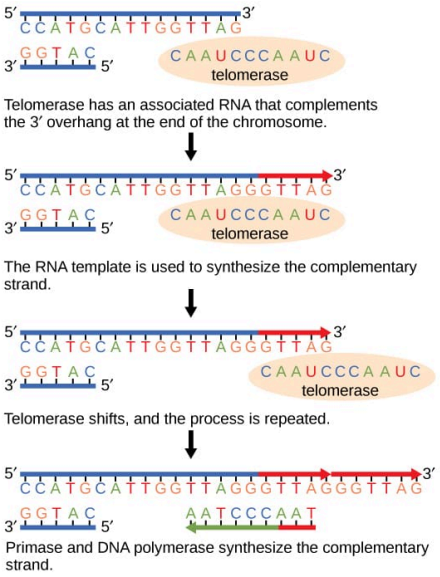


(**Figure** 6.3.3) The illustration shows a replication bubble. Helicase unwinds the helix. An RNA primer starts the synthesis, and DNA polymerase extends the DNA strand from the RNA primer. DNA synthesis occurs only in the 5' to 3' direction. On the leading strand, DNA synthesis occurs continuously. On the lagging strand, DNA synthesis restarts many times as the helix unwinds, resulting in many short fragments called Okazaki fragments. The DNA fragments are joined by DNA ligase (not shown).

You isolate a cell strain in which the joining together of Okazaki fragments is impaired and suspect that a mutation has occurred in an enzyme found at the replication fork. Which enzyme is most likely to be mutated?

Telomere Replication

Because eukaryotic chromosomes are linear, DNA replication comes to the end of a line in eukaryotic chromosomes. As you have learned, the DNA polymerase enzyme can add nucleotides in only one direction. In the leading strand, synthesis continues until the end of the chromosome is reached; however, on the lagging strand there is no place for a primer to be made for the DNA fragment to be copied at the end of the chromosome. This presents a problem for the cell because the ends remain unpaired, and over time these ends get progressively shorter as cells continue to divide. The ends of the linear chromosomes are known as telomeres, which have repetitive sequences that do not code for a particular gene. As a consequence, it is telomeres that are shortened with each round of DNA replication instead of genes. For example, in humans, a six base-pair sequence, TTAGGG, is repeated 100 to 1000 times. The discovery of the enzyme telomerase (**Figure** 6.3.4) helped in the understanding of how chromosome ends are maintained. The telomerase attaches to the end of the chromosome, and complementary bases to the RNA template are added on the end of the DNA strand. Once the lagging strand template is sufficiently elongated, DNA polymerase can now add nucleotides that are complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.



Open image description This diagram illustrates how the enzyme telomerase extends the ends of chromosomes, called telomeres, so that chromosome ends can be fully replicated. The figure is organized as a sequence of four steps connected by downward-pointing arrows.

Step 1: Telomerase Binds to the Chromosome End

At the top of the figure, a DNA molecule is shown with a double-stranded region on the left and a single-stranded 3′ overhang extending from the upper strand on the right. The upper DNA strand is labeled from **5′ to 3′**, and the lower strand is labeled from **3′ to 5′**.

The DNA sequence shown on the upper strand is:

5′ CCATGCATTGGTTAG 3′

The lower complementary strand is shorter and contains the sequence:

3′ GGTAC 5′

An oval labeled **telomerase** is positioned adjacent to the exposed 3′ end. Inside the telomerase enzyme is an RNA template with the sequence:

CAAUCCCAAUC

Text beneath the diagram reads:

> Telomerase has an associated RNA that complements the 3′ overhang at the end of the chromosome.

Step 2: Extension of the Telomere

In the second panel, telomerase remains attached to the chromosome end. The RNA template is used to extend the upper DNA strand by adding nucleotides to the 3′ end. The newly synthesized DNA is shown in red.

The extended upper strand sequence is:

5′ CCATGCATTGGTTAGGGTTAG 3′

The lower strand remains unchanged.

Text beneath the panel reads:

> The RNA template is used to synthesize the complementary strand.

Step 3: Telomerase Shifts and Repeats the Process

In the third panel, telomerase has moved farther toward the newly extended chromosome end. The enzyme's RNA template aligns with the new DNA sequence so that another round of extension can occur.

The upper DNA strand remains:

5′ CCATGCATTGGTTAGGGTTAG 3′

Text beneath the panel reads:

> Telomerase shifts, and the process is repeated.

Step 4: Completion of DNA Synthesis

In the final panel, the chromosome has been extended further. Additional telomere repeats have been added to the upper strand, creating a longer 3′ overhang. The upper strand sequence now appears as:

5′ CCATGCATTGGTTAGGGTTAGGGTTAG 3′

Below the extended region, a short newly synthesized DNA segment is shown in green and red with the sequence:

AATCCCAAT

Green arrows indicate synthesis of the complementary strand by conventional DNA replication machinery.

Text beneath the panel reads:

> Primase and DNA polymerase synthesize the complementary strand.

Overall, the figure demonstrates how telomerase uses an internal RNA template to add repeated DNA sequences to the 3′ end of a chromosome. After telomerase extends the telomere, primase and DNA polymerase synthesize the complementary strand, helping prevent the progressive shortening of chromosomes during DNA replication.

(**Figure** 6.3.4) The ends of linear chromosomes are maintained by the action of the telomerase enzyme. Telomerase has an associated RNA that complements the 5' overhang at the end of the chromosome. The RNA template is used to synthesize the complementary strand. Telomerase then shifts, and the process is repeated. Next, primase and DNA polymerase synthesize the rest of the complementary strand.

Telomerase is typically found to be active in germ cells, adult stem cells, and some cancer cells. For her discovery of telomerase and its action, Elizabeth Blackburn (**Figure** 6.3.5) received the Nobel Prize for Medicine and Physiology in 2009.



(**Figure** 6.3.5) Elizabeth Blackburn, 2009 Nobel Laureate, was the scientist who discovered how telomerase works. (credit: U.S. Embassy, Stockholm, Sweden)

Telomerase is not active in adult somatic cells. Adult somatic cells that undergo cell division continue to have their telomeres shortened. This essentially means that telomere shortening is associated with aging. In 2010, scientists found that telomerase can reverse some age-related conditions in mice, and this may have potential in regenerative medicine.[1] Telomerase-deficient mice were used in these studies; these mice have tissue atrophy, stem-cell depletion, organ system failure, and impaired tissue injury responses. Telomerase reactivation in these mice caused extension of telomeres, reduced DNA damage, reversed neurodegeneration, and improved functioning of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

DNA Replication in Prokaryotes

Recall that the prokaryotic chromosome is a circular molecule with a less extensive coiling structure than eukaryotic chromosomes. The eukaryotic chromosome is linear and highly coiled around proteins. While there are many similarities in the DNA replication process, these structural differences necessitate some differences in the DNA replication process in these two life forms.

DNA replication has been extremely well-studied in prokaryotes, primarily because of the small size of the genome and large number of variants available. *Escherichia coli* has 4.6 million base pairs in a 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. Table 6.3.1 summarizes the differences between prokaryotic and eukaryotic replications.

*(**Table** 6.3.1) Differences between Prokaryotic and Eukaryotic Replications*

Property	Prokaryotes	Eukaryotes
Origin of replication	Single	Multiple
Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
Chromosome structure	circular	linear
Telomerase	Not present	Present

CONCEPT IN ACTION

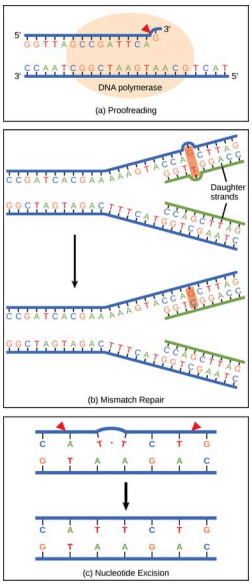
This interactive element is not available in this format. Visit the link or scan the QR code to access it online.

View this YouTube Video online

View this animation of DNA replication.

DNA Repair

DNA polymerase can make mistakes while adding nucleotides. It edits the DNA by proofreading every newly added base. Incorrect bases are removed and replaced by the correct base, and then polymerization continues (**Figure** $6.3.6$**a**). Most mistakes are corrected during replication, although when this does not happen, the mismatch repair mechanism is employed. Mismatch repair enzymes recognize the wrongly incorporated base and excise it from the DNA, replacing it with the correct base (**Figure** $6.3.6$**b**). In yet another type of repair, nucleotide excision repair, the DNA double strand is unwound and separated, the incorrect bases are removed along with a few bases on the 5' and 3' end, and these are replaced by copying the template with the help of DNA polymerase (**Figure** $6.3.6$**c**). Nucleotide excision repair is particularly important in correcting thymine dimers, which are primarily caused by ultraviolet light. In a thymine dimer, two thymine nucleotides adjacent to each other on one strand are covalently bonded to each other rather than their complementary bases. If the dimer is not removed and repaired it will lead to a mutation. Individuals with flaws in their nucleotide excision repair genes show extreme sensitivity to sunlight and develop skin cancers early in life.



Open image description

This figure illustrates three mechanisms that cells use to identify and repair errors in DNA: **(a) proofreading by DNA polymerase**, **(b) mismatch repair**, and **(c) nucleotide excision repair**. Each panel shows DNA strands with colored letters representing nucleotide bases and highlights the location of DNA damage or an incorrectly paired nucleotide.

(a) Proofreading

The first panel shows a DNA molecule being copied by DNA polymerase during DNA replication. The template strand runs along the bottom and is paired with a newly synthesized strand on top.

A large oval labeled **"DNA polymerase"** surrounds the replication site. Near the growing end of the new strand, a nucleotide labeled **G** is shown incorrectly positioned at the 3′ end of the newly synthesized DNA. A curved blue arrow and a red arrow point backward, illustrating the enzyme's proofreading activity.

The diagram shows DNA polymerase checking the most recently added nucleotide. When an incorrect base is detected, the enzyme removes it before continuing DNA synthesis. The figure emphasizes that proofreading occurs immediately during replication, helping maintain the accuracy of DNA copying.

(b) Mismatch Repair

The second panel illustrates mismatch repair after DNA replication has already occurred. The DNA is shown as a replication fork that has produced two daughter DNA molecules.

The label **"Daughter strands"** points to newly synthesized strands shown in green. One daughter strand contains an incorrectly paired nucleotide highlighted within a shaded area. The mismatched base is enclosed within a repair complex, indicating that the error has been detected.

A large downward arrow separates the upper and lower parts of the panel, showing the repair process. In the upper image, the mismatch is present. In the lower image, the incorrect nucleotide has been removed and replaced with the correct complementary base.

The figure demonstrates that mismatch repair proteins recognize errors that escaped proofreading, remove a section of the newly synthesized strand containing the mismatch, and replace it with correctly paired nucleotides using the original strand as a template.

(c) Nucleotide Excision Repair

The third panel illustrates nucleotide excision repair. A short DNA double strand is shown with a distortion in the upper strand. The damaged region is located between two adjacent thymine bases and is depicted as a bulge in the DNA strand.

Two red arrows point toward the damaged site from opposite sides, indicating where repair enzymes cut the DNA. The affected bases are shown as:

Upper strand: **C A T-T C T G**
Lower strand: **G T A A G A C**

The adjacent thymine bases are joined together, representing a thymine dimer caused by ultraviolet radiation.

A large black downward arrow indicates the repair process. In the lower image, the damaged section has been removed and replaced with correctly paired nucleotides. The DNA strand is restored to its normal structure without the bulge.

This panel demonstrates how nucleotide excision repair removes damaged sections of DNA, including thymine dimers, and fills the resulting gap using the undamaged strand as a template.

Overall, the figure compares three levels of DNA error correction. Proofreading corrects mistakes immediately as DNA is synthesized, mismatch repair fixes errors that remain after replication, and nucleotide excision repair removes damaged DNA segments such as UV-induced thymine dimers and replaces them with the correct sequence.

(**Figure** 6.3.6) Proofreading by DNA polymerase (a) corrects errors during replication. In mismatch repair (b), the incorrectly added base is detected after replication. The mismatch repair proteins detect this base and remove it from the newly synthesized strand by nuclease action. The gap is now filled with the correctly paired base. Nucleotide excision (c) repairs thymine dimers. When exposed to UV, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced.

Most mistakes are corrected; if they are not, they may result in a mutation—defined as a permanent change in the DNA sequence. Mutations in repair genes may lead to serious consequences like cancer.

Summary

DNA replicates by a semi-conservative method in which each of the two parental DNA strands act as a template for new DNA to be synthesized. After replication, each DNA has one parental or “old” strand, and one daughter or “new” strand.

Replication in eukaryotes starts at multiple origins of replication, while replication in prokaryotes starts from a single origin of replication. The DNA is opened with enzymes, resulting in the formation of the replication fork. Primase synthesizes an RNA primer to initiate synthesis by DNA polymerase, which can add nucleotides in only one direction. One strand is synthesized continuously in the direction of the replication fork; this is called the leading strand. The other strand is synthesized in a direction away from the replication fork, in short stretches of DNA known as Okazaki fragments. This strand is known as the lagging strand. Once replication is completed, the RNA primers are replaced by DNA nucleotides and the DNA is sealed with DNA ligase.

The ends of eukaryotic chromosomes pose a problem, as polymerase is unable to extend them without a primer. Telomerase, an enzyme with an inbuilt RNA template, extends the ends by copying the RNA template and extending one end of the chromosome. DNA polymerase can then extend the DNA using the primer. In this way, the ends of the chromosomes are protected. Cells have mechanisms for repairing DNA when it becomes damaged or errors are made in replication. These mechanisms include mismatch repair to replace nucleotides that are paired with a non-complementary base and nucleotide excision repair, which removes bases that are damaged such as thymine dimers.

Art Connections

Figure 6.3.3: You isolate a cell strain in which the joining together of Okazaki fragments is impaired and suspect that a mutation has occurred in an enzyme found at the replication fork. Which enzyme is most likely to be mutated?

Answer

Ligase, as this enzyme joins together Okazaki fragments.

Footnotes

1. 1 Mariella Jaskelioff, et al., "Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice," *Nature*, 469 (2011):102–7.

Key Terms

DNA ligase
the enzyme that catalyzes the joining of DNA fragments together

DNA polymerase
an enzyme that synthesizes a new strand of DNA complementary to a template strand

helicase
an enzyme that helps to open up the DNA helix during DNA replication by breaking the hydrogen bonds

lagging strand
during replication of the 3' to 5' strand, the strand that is replicated in short fragments and away from the replication fork

leading strand
the strand that is synthesized continuously in the 5' to 3' direction that is synthesized in the direction of the replication fork

mismatch repair
a form of DNA repair in which non-complementary nucleotides are recognized, excised, and replaced with correct nucleotides

mutation
a permanent variation in the nucleotide sequence of a genome

nucleotide excision repair
a form of DNA repair in which the DNA molecule is unwound and separated in the region of the nucleotide damage, the damaged nucleotides are removed and replaced with new nucleotides using the complementary strand, and the DNA strand is resealed and allowed to rejoin its complement

Okazaki fragments
the DNA fragments that are synthesized in short stretches on the lagging strand

primer
a short stretch of RNA nucleotides that is required to initiate replication and allow DNA polymerase to bind and begin replication

replication fork
the Y-shaped structure formed during the initiation of replication

semiconservative replication
the method used to replicate DNA in which the double-stranded molecule is separated and each strand acts as a template for a new strand to be synthesized, so the resulting DNA molecules are composed of one new strand of nucleotides and one old strand of nucleotides

telomerase
an enzyme that contains a catalytic part and an inbuilt RNA template; it functions to maintain telomeres at chromosome ends

telomere
: the DNA at the end of linear chromosomes

Contributors and Attributions

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7: PATTERNS OF INHERITANCE

7.1: Mendel's Experiments
7.2: Laws of Inheritance
7.3: Extensions of the Laws of Inheritance
7.4: Patterns of Inheritance (Exercises)

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7.1: Mendel's Experiments

Learning Objectives

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

- Describe the expected outcomes of monohybrid crosses involving dominant and recessive traits
- Differentiate between continuous and discontinuous variation and explain how Mendel's choice of traits impacted the interpretation of inheritance
- Assess the historical significance of Mendel's findings and explain why his work was initially overlooked and later recognized as foundational in genetics

Johann Gregor Mendel

Johann Gregor Mendel (1822–1884) (**Figure** 7.1.1) was a lifelong learner, teacher, scientist, and man of faith. As a young adult, he joined the Augustinian Abbey of St. Thomas in Brno in what is now the Czech Republic. Supported by the monastery, he taught physics, botany, and natural science courses at the secondary and university levels. In 1856, he began a decade-long research pursuit involving inheritance patterns in honeybees and plants, ultimately settling on pea plants as his primary model system (a system with convenient characteristics that is used to study a specific biological phenomenon to gain understanding to be applied to other systems). In 1865, Mendel presented the results of his experiments with nearly 30,000 pea plants to the local natural history society. He demonstrated that traits are transmitted faithfully from parents to offspring in specific patterns. In 1866, he published his work, *Experiments in Plant Hybridization,*[1] in the proceedings of the Natural History Society of Brünn.



Figure 7.1.1: Johann Gregor Mendel set the framework for the study of genetics.

Mendel's work went virtually unnoticed by the scientific community, which incorrectly believed that the process of inheritance involved a blending of parental traits that produced an intermediate physical appearance in offspring. This hypothetical process appeared to be correct because of what we know now as continuous variation. Continuous variation is the range of small differences we see among individuals in a characteristic like human height. It does appear that offspring are a "blend" of their parents' traits when we look at characteristics that exhibit continuous variation. Mendel worked instead with traits that show discontinuous variation. Discontinuous variation is the variation seen among individuals when each individual shows one of two—or a very few—easily distinguishable traits, such as violet or white flowers. Mendel's choice of these kinds of traits allowed him to see experimentally that the traits were not blended in the offspring as would have been expected at the time, but that they were inherited as distinct traits. In 1868, Mendel became abbot of the monastery and exchanged his scientific pursuits for

his pastoral duties. He was not recognized for his extraordinary scientific contributions during his lifetime; in fact, it was not until 1900 that his work was rediscovered, reproduced, and revitalized by scientists on the brink of discovering the chromosomal basis of heredity.

Mendel's Crosses

Mendel's seminal work was accomplished using the garden pea, *Pisum sativum*, to study inheritance. This species naturally self-fertilizes, meaning that pollen encounters ova within the same flower. The flower petals remain sealed tightly until pollination is completed to prevent the pollination of other plants. The result is highly inbred, or "true-breeding," pea plants. These are plants that always produce offspring that look like the parent. By experimenting with true-breeding pea plants, Mendel avoided the appearance of unexpected traits in offspring that might occur if the plants were not true breeding. The garden pea also grows to maturity within one season, meaning that several generations could be evaluated over a relatively short time. Finally, large quantities of garden peas could be cultivated simultaneously, allowing Mendel to conclude that his results did not come about simply by chance.

Mendel performed hybridizations, which involve mating two true-breeding individuals that have different traits. In the pea, which is naturally self-pollinating, this is done by manually transferring pollen from the anther of a mature pea plant of one variety to the stigma of a separate mature pea plant of the second variety.

Plants used in first-generation crosses were called P, or parental generation, plants (**Figure** 7.1.2). Mendel collected the seeds produced by the P plants that resulted from each cross and grew them the following season. These offspring were called the F_1, or the first filial (filial = daughter or son), generation. Once Mendel examined the characteristics in the F_1 generation of plants, he allowed them to self-fertilize naturally. He then collected and grew the seeds from the F_1 plants to produce the F_2, or second filial, generation. Mendel's experiments extended beyond the F_2 generation to the F_3 generation, F_4 generation, and so on, but it was the ratio of characteristics in the P, F_1, and F_2 generations that were the most intriguing and became the basis of Mendel's postulates.

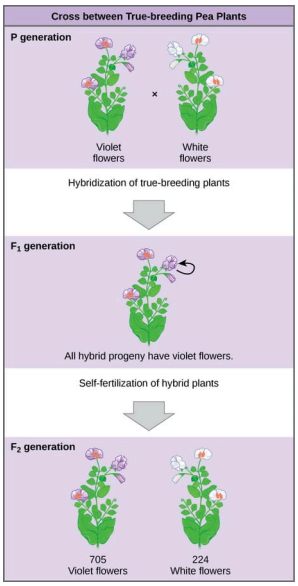


Figure 7.1.2: Mendel's process for performing crosses included examining flower color. The diagram shows a cross between pea plants that are true-breeding for purple flower color and plants that are true-breeding for white flower color. This cross-fertilization of the P generation resulted in an F_{1} generation with all violet flowers. Self-fertilization of the F_{1} generation resulted in an F_{2} generation that consisted of 705 plants with violet flowers, and 224 plants with white flowers.

Garden Pea Characteristics Revealed the Basics of Heredity

In his 1865 publication, Mendel reported the results of his crosses involving seven different characteristics, each with two contrasting traits. A trait is defined as a variation in the physical appearance of a heritable characteristic. The characteristics included plant height, seed texture, seed color, flower color, pea-pod size, pea-pod color, and flower position. For the

characteristic of flower color, for example, the two contrasting traits were white versus violet. To fully examine each characteristic, Mendel generated large numbers of F_1 and F_2 plants and reported results from thousands of F_2 plants.

What results did Mendel find in his crosses for flower color? First, Mendel confirmed that he was using plants that bred true for white or violet flower color. Irrespective of the number of generations that Mendel examined, all self-crossed offspring of parents with white flowers had white flowers, and all self-crossed offspring of parents with violet flowers had violet flowers. In addition, Mendel confirmed that, other than flower color, the pea plants were physically identical. This was an important check to make sure that the two varieties of pea plants only differed with respect to one trait, flower color.

Once these validations were complete, Mendel applied the pollen from a plant with violet flowers to the stigma of a plant with white flowers. After gathering and sowing the seeds that resulted from this cross, Mendel found that 100 percent of the F_1 hybrid generation had violet flowers. Conventional wisdom at that time would have predicted the hybrid flowers to be pale violet or for hybrid plants to have equal numbers of white and violet flowers. In other words, the contrasting parental traits were expected to blend in the offspring. Instead, Mendel's results demonstrated that the white flower trait had completely disappeared in the F_1 generation.

Importantly, Mendel did not stop his experimentation there. He allowed the F_1 plants to self-fertilize and found that 705 plants in the F_2 generation had violet flowers and 224 had white flowers. This was a ratio of 3.15 violet flowers to one white flower, or approximately 3:1. When Mendel transferred pollen from a plant with violet flowers to the stigma of a plant with white flowers and vice versa, he obtained approximately the same ratio irrespective of which parent—male or female—contributed which trait. This is called a reciprocal cross—a paired cross in which the respective traits of the male and female in one cross become the respective traits of the female and male in the other cross. For the other six characteristics that Mendel examined, the F_1 and F_2 generations behaved in the same way that they behaved for flower color. One of the two traits would disappear completely from the F_1 generation, only to reappear in the F_2 generation at a ratio of roughly 3:1 (**Figure** 7.1.3).

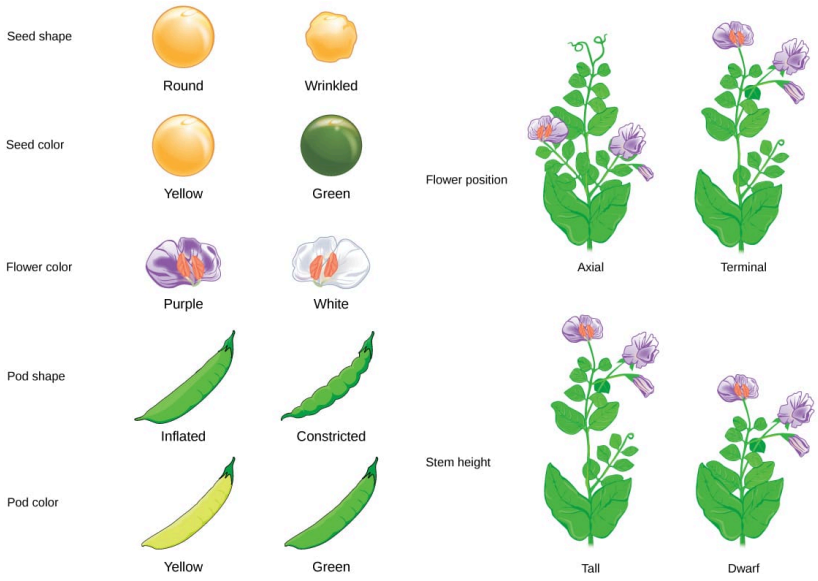


Figure 7.1.3: Illustrated are the seven pea plant characteristics identified by Mendel. The flowers can be purple or white. The peas can be yellow or green, or smooth or wrinkled. The pea pods can be inflated or constricted, or yellow or green. The flower position can be axial or terminal. The stem length can be tall or dwarf.

Upon compiling his results for many thousands of plants, Mendel concluded that the characteristics could be divided into expressed and latent traits. He called these dominant and recessive traits, respectively. Dominant traits are those that are inherited unchanged in a hybridization. Recessive traits become latent, or disappear in the offspring of a hybridization. The recessive trait does, however, reappear in the progeny of the hybrid offspring. An example of a dominant trait is the violet-colored flower trait. For this same characteristic (flower color), white-colored flowers are a recessive trait. The fact that the recessive trait reappeared in the F_2 generation meant that the traits remained separate (and were not blended) in the plants of the F_1 generation. Mendel proposed that this was because the plants possessed two copies of the trait for the flower-color characteristic, and that each parent transmitted one of their two copies to their offspring, where they came together. Moreover, the physical observation of a dominant trait could mean that the genetic composition of the organism included two dominant versions of the characteristic, or that it included one dominant and one recessive version. Conversely, the observation of a recessive trait meant

that the organism lacked any dominant versions of this characteristic.

Summary

Working with garden pea plants, Mendel found that crosses between parents that differed for one trait produced F_1 offspring that all expressed one parent's traits. The traits that were visible in the F_1 generation are referred to as dominant, and traits that disappear in the F_1 generation are described as recessive. When the F_1 plants in Mendel's experiment were self-crossed, the F_2 offspring exhibited the dominant trait or the recessive trait in a 3:1 ratio, confirming that the recessive trait had been transmitted faithfully from the original P parent. Reciprocal crosses generated identical F_1 and F_2 offspring ratios. By examining sample sizes, Mendel showed that traits were inherited as independent events.

Footnotes

1. 1 Johann Gregor Mendel, "Versuche über Pflanzenhybriden." *Verhandlungen des naturforschenden Vereines in Brünn*, Bd. IV für das Jahr, 1865 Abhandlungen (1866):3–47. [for English translation, see www.mendelweb.org/Mendel.plain.html]

Key Terms

continuous variation
: a variation in a characteristic in which individuals show a range of traits with small differences between them

discontinuous variation
: a variation in a characteristic in which individuals show two, or a few, traits with large differences between them

dominant
: describes a trait that masks the expression of another trait when both versions of the gene are present in an individual

F_1
: the first filial generation in a cross; the offspring of the parental generation

F_2
: the second filial generation produced when F_1 individuals are self-crossed or fertilized with each other

hybridization
: the process of mating two individuals that differ, with the goal of achieving a certain characteristic in their offspring

model system
: a species or biological system used to study a specific biological phenomenon to gain understanding that will be applied to other species

P
: the parental generation in a cross

recessive
: describes a trait whose expression is masked by another trait when the alleles for both traits are present in an individual

reciprocal cross
: a paired cross in which the respective traits of the male and female in one cross become the respective traits of the female and male in the other cross

self-fertilization
: when an organism uses its own gametes to produce offspring. Commonly seen with plants

trait
: a variation in an inherited characteristic

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7.2: Laws of Inheritance

Learning Objectives

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

- Define key terms such as gene, allele, genotype, phenotype, homozygous, and heterozygous as used in Mendelian genetics
- Explain Mendel's laws of dominance, segregation, and independent assortment and how they relate to the behavior of chromosomes during meiosis
- Use Punnett squares to predict genotypic and phenotypic ratios for monohybrid and dihybrid crosses
- Differentiate between the phenotypic outcomes of homozygous and heterozygous organisms and interpret how these outcomes validate Mendel's laws
- Assess the value of test crosses in determining unknown genotypes and justify predictions using genetic reasoning and probabilities.

The seven characteristics that Mendel evaluated in his pea plants were each expressed as one of two versions, or traits. Mendel deduced from his results that each individual had two discrete copies of the characteristic that are passed individually to offspring. We now call those two copies genes, which are carried on chromosomes. The reason we have two copies of each gene is that we inherit one from each parent. In fact, it is the chromosomes we inherit and the two copies of each gene are located on paired chromosomes. Recall that in meiosis these chromosomes are separated out into haploid gametes. This separation, or segregation, of the homologous chromosomes means also that only one of the copies of the gene gets moved into a gamete. The offspring are formed when that gamete unites with one from another parent and the two copies of each gene (and chromosome) are restored.

For cases in which a single gene controls a single characteristic, a diploid organism has two genetic copies that may or may not encode the same version of that characteristic. For example, one individual may carry a gene that determines white flower color and a gene that determines violet flower color. Gene variants that arise by mutation and exist at the same relative locations on homologous chromosomes are called alleles. Mendel examined the inheritance of genes with just two allele forms, but it is common to encounter more than two alleles for any given gene in a natural population.

Phenotypes and Genotypes

Two alleles for a given gene in a diploid organism are expressed and interact to produce physical characteristics. The observable traits expressed by an organism are referred to as its phenotype. An organism's underlying genetic makeup, consisting of both the physically visible and the non-expressed alleles, is called its genotype. Mendel's hybridization experiments demonstrate the difference between phenotype and genotype. For example, the phenotypes that Mendel observed in his crosses between pea plants with differing traits are connected to the diploid genotypes of the plants in the P, F_1, and F_2 generations. We will use a second trait that Mendel investigated, seed color, as an example. Seed color is governed by a single gene with two alleles. The yellow-seed allele is dominant and the green-seed allele is recessive. When true-breeding plants were cross-fertilized, in which one parent had yellow seeds and one had green seeds, all of the F_1hybrid offspring had yellow seeds. That is, the hybrid offspring were phenotypically identical to the true-breeding parent with yellow seeds. However, we know that the allele donated by the parent with green seeds was not simply lost because it reappeared in some of the F_2 offspring (**Figure** 7.2.1). Therefore, the F_1 plants must have been genotypically different from the parent with yellow seeds.

The P plants that Mendel used in his experiments were each homozygous for the trait he was studying. Diploid organisms that are homozygous for a gene have two identical alleles, one on each of their homologous chromosomes. The genotype is often written as *YY* or *yy*, for which each letter represents one of the two alleles in the genotype. The dominant allele is capitalized and the recessive allele is lower case. The letter used for the gene (seed color in this case) is usually related to the dominant trait (yellow allele, in this case, or "*Y*"). Mendel's parental pea plants always bred true because both produced gametes carried the same allele. When P plants with contrasting traits were cross-fertilized, all of the offspring were heterozygous for the contrasting trait, meaning their genotype had different alleles for the gene being examined. For example, the F_1 yellow plants that received a *Y* allele from their yellow parent and a *y* allele from their green parent had the genotype *Yy*.

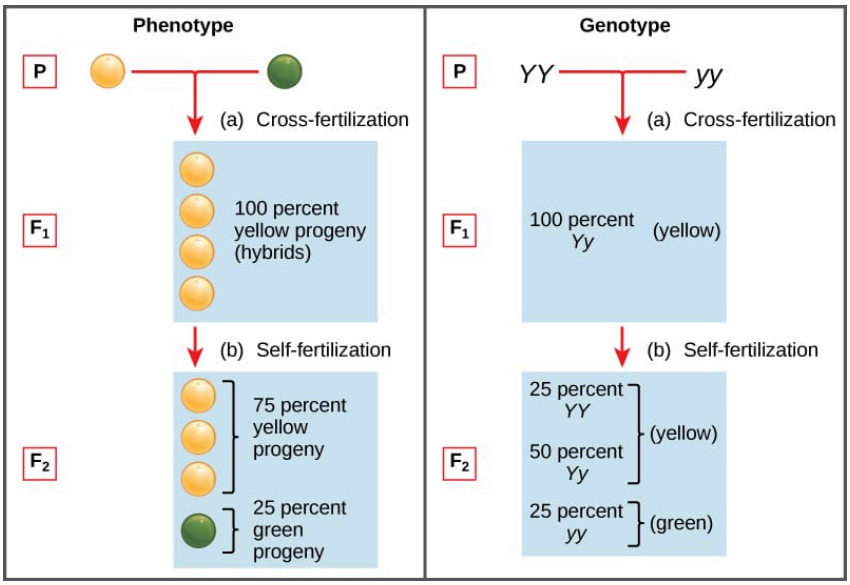


Figure 7.2.1: Phenotypes are physical expressions of traits that are transmitted by alleles. A graphic with 2 columns, the first with the heading "Phenotype" and the second with the heading "Genotype." Capital letters represent dominant alleles and lowercase letters represent recessive alleles. The phenotypic ratios are the ratios of visible characteristics. The genotypic ratios are the ratios of gene combinations in the offspring, and these are not always distinguishable in the phenotypes.

In the phenotype column, one yellow pea plant cross-fertilizes with one green pea plant. The first generation of offspring is 100 percent yellow pea plants. After self-fertilization of these yellow pea offspring, 75 percent of the second generation offspring have yellow peas and 25 percent have green peas. The genotype column shows the first generation offspring as 100 percent Yy, and the second generation as 25 percent YY, 50 percent Yy, and 25 percent yy.

Law of Dominance

Our discussion of homozygous and heterozygous organisms brings us to why the F_1 heterozygous offspring were identical to one of the parents, rather than expressing both alleles. In all seven pea-plant characteristics, one of the two contrasting alleles was dominant, and the other was recessive. Mendel called the dominant allele the expressed unit factor; the recessive allele was referred to as the latent unit factor. We now know that these so-called unit factors are actually genes on homologous chromosomes. For a gene that is expressed in a dominant and recessive pattern, homozygous dominant and heterozygous organisms will look identical (that is, they will have different genotypes but the same phenotype), and the recessive allele will only be observed in homozygous recessive individuals (**Table** 7.2.1).

Table 7.2.1***:*** *Correspondence between Genotype and Phenotype for a Dominant-Recessive Characteristic*

	Homozygous	Heterozygous	Homozygous
Genotype	*YY*	*Yy*	*yy*
Phenotype	yellow	yellow	green

Mendel's law of dominance states that in a heterozygote, one trait will conceal the presence of another trait for the same characteristic. For example, when crossing true-breeding violet-flowered plants with true-breeding white-flowered plants, all of the offspring were violet-flowered, even though they all had one allele for violet and one allele for white. Rather than both alleles contributing to a phenotype, the dominant allele will be expressed exclusively. The recessive allele will remain latent, but will be transmitted to offspring in the same manner as that by which the dominant allele is transmitted. The recessive trait will only be expressed by offspring that have two copies of this allele (**Figure** 7.2.2), and these offspring will breed true when self-crossed.

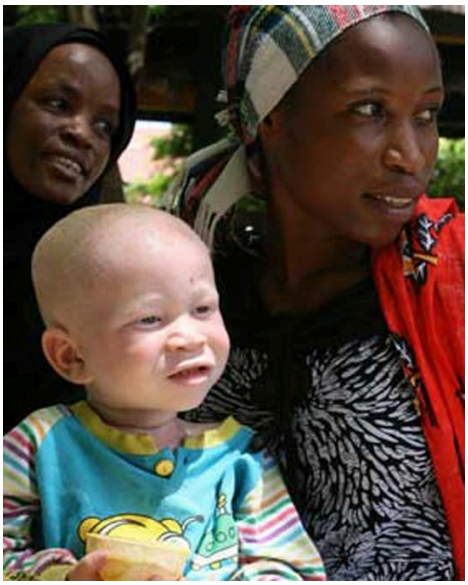


Figure 7.2.2: The allele for albinism, expressed here in humans, is recessive. Both of this child's parents carried the recessive allele.

Monohybrid Cross and the Punnett Square

When fertilization occurs between two true-breeding parents that differ by only the characteristic being studied, the process is called a monohybrid cross, and the resulting offspring are called monohybrids. Mendel performed seven types of monohybrid crosses, each involving contrasting traits for different characteristics. Out of these crosses, all of the F_1 offspring had the phenotype of one parent, and the F_2 offspring had a 3:1 phenotypic ratio. On the basis of these results, Mendel postulated that each parent in the monohybrid cross contributed one of two paired unit factors to each offspring, and every possible combination of unit factors was equally likely.

The results of Mendel's research can be explained in terms of probabilities, which are mathematical measures of likelihood. The probability of an event is calculated by the number of times the event occurs divided by the total number of opportunities for the event to occur. A probability of one (100 percent) for some event indicates that it is guaranteed to occur, whereas a probability of zero (0 percent) indicates that it is guaranteed to not occur, and a probability of 0.5 (50 percent) means it has an equal chance of occurring or not occurring.

To demonstrate this with a monohybrid cross, consider the case of true-breeding pea plants with yellow versus green seeds. The dominant seed color is yellow; therefore, the parental genotypes were *YY* for the plants with yellow seeds and *yy* for the plants with green seeds. A Punnett square, devised by the British geneticist Reginald Punnett, is useful for determining probabilities because it is drawn to predict all possible outcomes of all possible random fertilization events and their expected frequencies. **Figure** 7.2.3 shows a Punnett square for a cross between a plant with yellow peas and one with green peas. To prepare a Punnett square, all possible combinations of the parental alleles (the genotypes of the gametes) are listed along the top (for one parent) and side (for the other parent) of a grid. The combinations of egg and sperm gametes are then made in the boxes in the table on the basis of which alleles are combining. Each box then represents the diploid genotype of a zygote, or fertilized egg. Because each possibility is equally likely, genotypic ratios can be determined from a Punnett square. If the pattern of inheritance (dominant and recessive) is known, the phenotypic ratios can be inferred as well. For a monohybrid cross of two true-breeding parents, each parent contributes one type of allele. In this case, only one genotype is possible in the F_1 offspring. All offspring are *Yy* and have yellow seeds.

ART CONNECTION

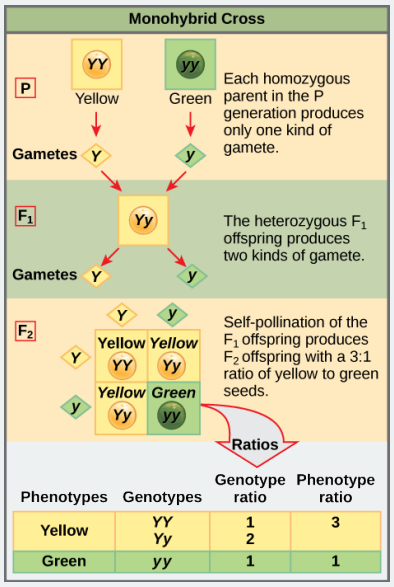


Figure 7.2.3**:** This Punnett square shows the cross between plants with yellow seeds and green seeds. The cross between the true-breeding P plants produces F_1 heterozygotes that can be self-fertilized. The self-cross of the F_1 generation can be analyzed with a Punnett square to predict the genotypes of the F_2 generation. Given an inheritance pattern of dominant–recessive, the genotypic and phenotypic ratios can then be determined.

In pea plants, round peas (*R*) are dominant to wrinkled peas (*r*). You do a test cross between a pea plant with wrinkled peas (genotype *rr*) and a plant of unknown genotype that has round peas. You end up with three plants, all which have round peas. From this data, can you tell if the parent plant is homozygous dominant or heterozygous?

When the F_1 offspring are crossed with each other, each has an equal probability of contributing either a *Y* or a *y* to the F_2 offspring. The result is a 1 in 4 (25 percent) probability of both parents contributing a *Y*, resulting in an offspring with a yellow phenotype; a 25 percent probability of parent A contributing a *Y* and parent B a *y*, resulting in offspring with a yellow phenotype; a 25 percent probability of parent A contributing a *y* and parent B a *Y*, also resulting in a yellow phenotype; and a (25 percent) probability of both parents contributing a *y*, resulting in a green phenotype. When counting all four possible outcomes, there is a 3 in 4 probability of offspring having the yellow phenotype and a 1 in 4 probability of offspring having the green phenotype. This explains why the results of Mendel's F_2 generation occurred in a 3:1 phenotypic ratio. Using large numbers of crosses, Mendel was able to calculate probabilities, found that they fit the model of inheritance, and use these to predict the outcomes of other crosses.

Law of Segregation

Observing that true-breeding pea plants with contrasting traits gave rise to F_1 generations that all expressed the dominant trait and F_2 generations that expressed the dominant and recessive traits in a 3:1 ratio, Mendel proposed the law of segregation. This law states that paired unit factors (genes) must segregate equally into gametes such that offspring have an equal likelihood of inheriting either factor. For the F_2 generation of a monohybrid cross, the following three possible combinations of genotypes result: homozygous dominant, heterozygous, or homozygous recessive. Because heterozygotes could arise from two different pathways (receiving one dominant and one recessive allele from either parent), and because heterozygotes and homozygous dominant individuals are phenotypically identical, the law supports Mendel's observed 3:1 phenotypic ratio. The equal segregation of alleles is the reason we can apply the Punnett square to accurately predict the offspring of parents with known genotypes. The physical basis of Mendel's law of segregation is the first division of meiosis in which the homologous chromosomes with their different versions of each gene are segregated into daughter nuclei. This process was not understood by the scientific community during Mendel's lifetime (**Figure** 7.2.4).

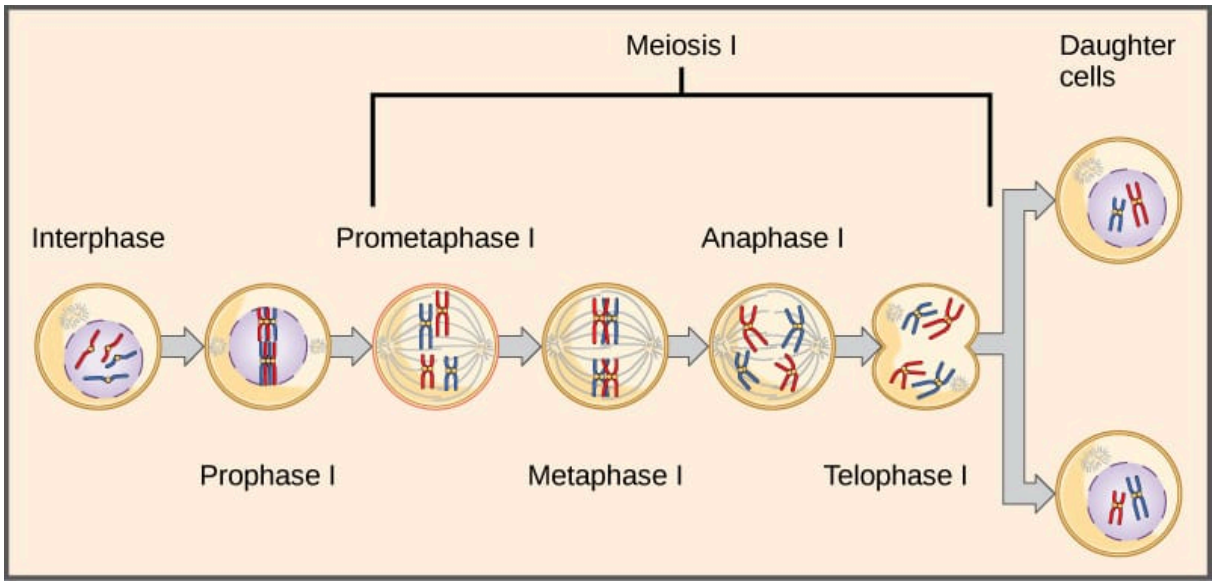


Figure 7.2.4: The first division in meiosis is shown. Homologous pairs of chromosomes line up at the metaphase plate during metaphase I of meiosis. The homologous chromosomes with their different versions of each gene are segregated into daughter nuclei.

Test Cross

Beyond predicting the offspring of a cross between known homozygous or heterozygous parents, Mendel also developed a way to determine whether an organism that expressed a dominant trait was a heterozygote or a homozygote. Called the test cross, this technique is still used by plant and animal breeders. In a test cross, the dominant-expressing organism is crossed with an organism that is homozygous recessive for the same characteristic. If the dominant-expressing organism is a homozygote, then all F_1 offspring will be heterozygotes expressing the dominant trait (**Figure** 7.2.5). Alternatively, if the dominant-expressing organism is a heterozygote, the F_1 offspring will exhibit a 1:1 ratio of heterozygotes and recessive homozygotes (**Figure** 7.2.5). The test cross further validates Mendel's postulate that pairs of unit factors segregate equally.

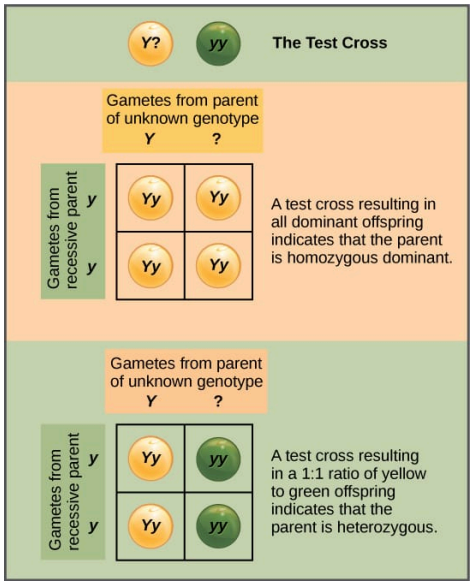


Figure 7.2.5: A test cross can be performed to determine whether an organism expressing a dominant trait is a homozygote or a heterozygote. Shown is an example of a test cross; a parent with a dominant phenotype but unknown genotype is crossed with a recessive parent. If the parent with the unknown phenotype is homozygous dominant, all the resulting offspring will have at least one dominant allele. If the parent with the unknown phenotype is heterozygous, 50 percent of the offspring will inherit a recessive allele from both parents and will have the recessive phenotype.

Law of Independent Assortment

Mendel's law of independent assortment states that genes do not influence each other with regard to the sorting of alleles into gametes, and every possible combination of alleles for every gene is equally likely to occur. Independent assortment of genes can be illustrated by the dihybrid cross, a cross between two true-breeding parents that express different traits for two characteristics. Consider the characteristics of seed color and seed texture for two pea plants, one that has wrinkled, green seeds (*rryy*) and another that has round, yellow seeds (*RRYY*). Because each parent is homozygous, the law of segregation indicates that the gametes for the wrinkled–green plant all are *ry*, and the gametes for the round–yellow plant are all *RY*. Therefore, the F_1 generation of offspring all are *RrYy* (**Figure** 7.2.6).

ART CONNECTION

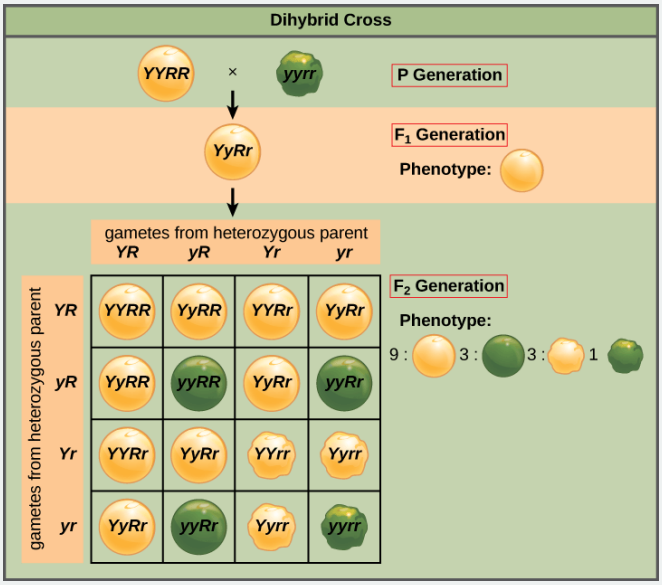


Figure 7.2.6: The resulting 9:3:3:1 F_2 phenotypic ratio is obtained using a Punnett square.

This illustration shows a dihybrid cross between pea plants that involves the genes for the seed color and texture. In the P generation, a plant that has the homozygous dominant phenotype of yellow, round peas is crossed with a plant with the homozygous recessive phenotype of green, wrinkled peas. The P cross produces F_1 offspring that are all heterozygous for both characteristics. Self-pollination of the F_1 generation results in F_2 offspring with a phenotypic ratio of 9:3:3:1 for round–yellow, round–green, wrinkled–yellow, and wrinkled–green peas, respectively.

In pea plants, purple flowers (*P*) are dominant to white (*p*), and yellow peas (*Y*) are dominant to green (*y*). What are the possible genotypes and phenotypes for a cross between *PpYY* and *ppYy* pea plants? How many squares would you need to complete a Punnett square analysis of this cross?

The gametes produced by the F_1 individuals must have one allele from each of the two genes. For example, a gamete could get an *R* allele for the seed shape gene and either a *Y* or a *y* allele for the seed color gene. It cannot get both an *R* and an *r* allele; each gamete can have only one allele per gene. The law of independent assortment states that a gamete into which an *r* allele is sorted would be equally likely to contain either a *Y* or a *y* allele. Thus, there are four equally likely gametes that can be formed when the *RrYy* heterozygote is self-crossed, as follows: *RY*, *rY*, *Ry*, and *ry*. Arranging these gametes along the top and left of a 4 × 4 Punnett square (**Figure** 7.2.6) gives us 16 equally likely genotypic combinations. From these genotypes, we find a phenotypic ratio of 9 round–yellow:3 round–green:3 wrinkled–yellow:1 wrinkled–green (**Figure** 7.2.6). These are the offspring ratios we would expect, assuming we performed the crosses with a large enough sample size.

The physical basis for the law of independent assortment also lies in meiosis I, in which the different homologous pairs line up in random orientations. Each gamete can contain any combination of paternal and maternal chromosomes (and therefore the genes on them) because the orientation of tetrads on the metaphase plane is random (**Figure** 7.2.7).

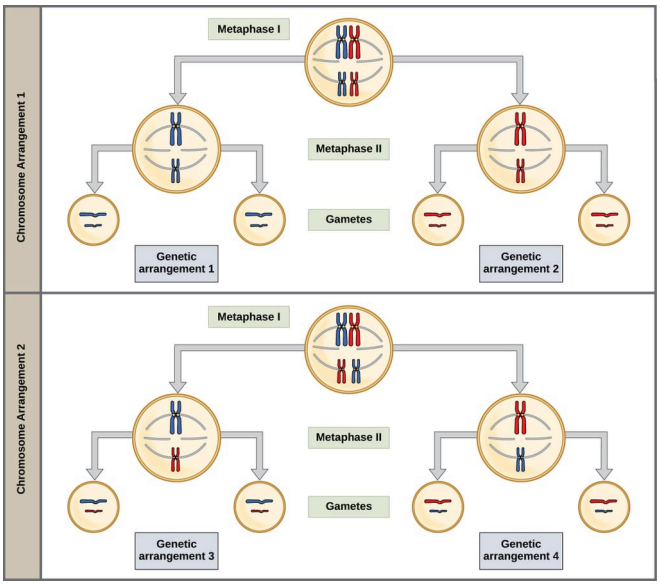


Figure 7.2.7: The random segregation into daughter nuclei that happens during the first division in meiosis can lead to a variety of possible genetic arrangements.

Shown are homologous pairs of chromosomes lined up at the metaphase plate during metaphase I of meiosis. The homologous chromosomes, with their different versions of each gene, are randomly segregated into daughter nuclei, resulting in a variety of possible genetic arrangements. In this example, the 4 possible genetic arrangements are shown.

Summary

When true-breeding, or homozygous, individuals that differ for a certain trait are crossed, all of the offspring will be heterozygous for that trait. If the traits are inherited as dominant and recessive, the F_1 offspring will all exhibit the same phenotype as the parent homozygous for the dominant trait. If these heterozygous offspring are self-crossed, the resulting F_2 offspring will be equally likely to inherit gametes carrying the dominant or recessive trait, giving rise to offspring of which one quarter are homozygous dominant, half are heterozygous, and one quarter are homozygous recessive. Because homozygous dominant and heterozygous individuals are phenotypically identical, the observed traits in the F_2 offspring will exhibit a ratio of three dominant to one recessive.

Mendel postulated that genes (characteristics) are inherited as pairs of alleles (traits) that behave in a dominant and recessive pattern. Alleles segregate into gametes such that each gamete is equally likely to receive either one of the two alleles present in a diploid individual. In addition, genes are assorted into gametes independently of one another. That is, in general, alleles are not more likely to segregate into a gamete with a particular allele of another gene.

Art Connections

Figure 7.2.3: In pea plants, round peas (*R*) are dominant to wrinkled peas (*r*). You do a test cross between a pea plant with wrinkled peas (genotype *rr*) and a plant of unknown genotype that has round peas. You end up with three plants, all which have round peas. From this data, can you tell if the parent plant is homozygous dominant or heterozygous?

Answer

You cannot be sure if the plant is homozygous or heterozygous as the data set is too small: by random chance, all three plants might have acquired only the dominant gene even if the recessive one is present.

Figure \({7{.}2{.}\eatSpaces{6} \endSpaces}\): In pea plants, purple flowers (*P*) are dominant to white (*p*), and yellow peas (*Y*) are dominant to green (*y*). What are the possible genotypes and phenotypes for a cross between *PpYY* and *ppYy* pea plants? How many squares would you need to complete a Punnett square analysis of this cross?

Answer

The possible genotypes are *PpYY*, *PpYy*, *ppYY*, and *ppYy*. The former two genotypes would result in plants with purple flowers and yellow peas, while the latter two genotypes would result in plants with white flowers with yellow peas, for a 1:1 ratio of each phenotype. You only need a 2 × 2 Punnett square (four squares total) to do this analysis because two of the alleles are homozygous.

Key Terms

allele
one of two or more variants of a gene that determines a particular trait for a characteristic

dihybrid
the result of a cross between two true-breeding parents that express different traits for two characteristics

genotype
the underlying genetic makeup, consisting of both physically visible and non-expressed alleles, of an organism

haploid
a cell with one set of chromosomes

heterozygous
having two different alleles for a given gene on the homologous chromosomes

homozygous
having two identical alleles for a given gene on the homologous chromosomes

law of dominance
in a heterozygote, one trait will conceal the presence of another trait for the same characteristic

law of independent assortment
genes do not influence each other with regard to sorting of alleles into gametes; every possible combination of alleles is equally likely to occur

law of segregation
paired unit factors (i.e., genes) segregate equally into gametes such that offspring have an equal likelihood of inheriting any combination of factors

monohybrid
the result of a cross between two true-breeding parents that express different traits for only one characteristic

phenotype
the observable traits expressed by an organism

Punnett square
a visual representation of a cross between two individuals in which the gametes of each individual are denoted along the top and side of a grid, respectively, and the possible zygotic genotypes are recombined at each box in the grid

test cross
a cross between a dominant expressing individual with an unknown genotype and a homozygous recessive individual; the offspring phenotypes indicate whether the unknown parent is heterozygous or homozygous for the dominant trait

Contributors and Attributions

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7.3: Extensions of the Laws of Inheritance

Learning Objectives

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

- Explain the differences between complete dominance, incomplete dominance, and codominance using genetic crosses and phenotypic outcomes
- Describe how multiple alleles influence trait variation within a population, using the ABO blood group system as an example
- Analyze inheritance patterns of sex-linked traits and predict outcomes of genetic crosses involving X-linked alleles

Mendel studied traits with only one mode of inheritance in pea plants. The inheritance of the traits he studied all followed the relatively simple pattern of dominant and recessive alleles for a single characteristic. There are several important modes of inheritance, discovered after Mendel's work, that do not follow the dominant and recessive, single-gene model.

Alternatives to Dominance and Recessiveness

Mendel's experiments with pea plants suggested that: 1) two types of "units" or alleles exist for every gene; 2) alleles maintain their integrity in each generation (no blending); and 3) in the presence of the dominant allele, the recessive allele is hidden, with no contribution to the phenotype. Therefore, recessive alleles can be "carried" and not expressed by individuals. Such heterozygous individuals are sometimes referred to as "carriers." Since then, genetic studies in other organisms have shown that much more complexity exists, but that the fundamental principles of Mendelian genetics still hold true. In the sections to follow, we consider some of the extensions of Mendelism.

Incomplete Dominance

Mendel's results, demonstrating that traits are inherited as dominant and recessive pairs, contradicted the view at that time that offspring exhibited a blend of their parents' traits. However, the heterozygote phenotype occasionally does appear to be intermediate between the two parents. For example, in the snapdragon, *Antirrhinum majus* (**Figure** 7.3.1), a cross between a homozygous parent with white flowers (C^WC^W) and a homozygous parent with red flowers (C^RC^R) will produce offspring with pink flowers (C^RC^W). (Note that different genotypic abbreviations are used for Mendelian extensions to distinguish these patterns from simple dominance and recessiveness.) This pattern of inheritance is described as incomplete dominance, meaning that one of the alleles appears in the phenotype in the heterozygote, but not to the exclusion of the other, which can also be seen. The allele for red flowers is incompletely dominant over the allele for white flowers. However, the results of a heterozygote self-cross can still be predicted, just as with Mendelian dominant and recessive crosses. In this case, the genotypic ratio would be 1 C^RC^R:2 C^RC^W:1 C^WC^W, and the phenotypic ratio would be 1:2:1 for red:pink:white. The basis for the intermediate color in the heterozygote is simply that the pigment produced by the red allele (anthocyanin) is diluted in the heterozygote and therefore appears pink because of the white background of the flower petals.



Figure 7.3.1: These pink flowers of a heterozygote snapdragon result from incomplete dominance. (credit: "storebukkebruse"/Flickr)

Codominance

A variation on incomplete dominance is codominance, in which both alleles for the same characteristic are simultaneously expressed in the heterozygote. An example of codominance occurs in the ABO blood groups of humans. The A and B alleles are expressed in the form of A or B molecules present on the surface of red blood cells. Homozygotes (I^AI^A and I^BI^B) express either the A or the B phenotype, and heterozygotes (I^AI^B) express both phenotypes equally. The I^AI^B individual has blood type AB. In a self-cross between heterozygotes expressing a codominant trait, the three possible offspring genotypes are phenotypically distinct. However, the 1:2:1 genotypic ratio characteristic of a Mendelian monohybrid cross still applies (**Figure** 7.3.2).

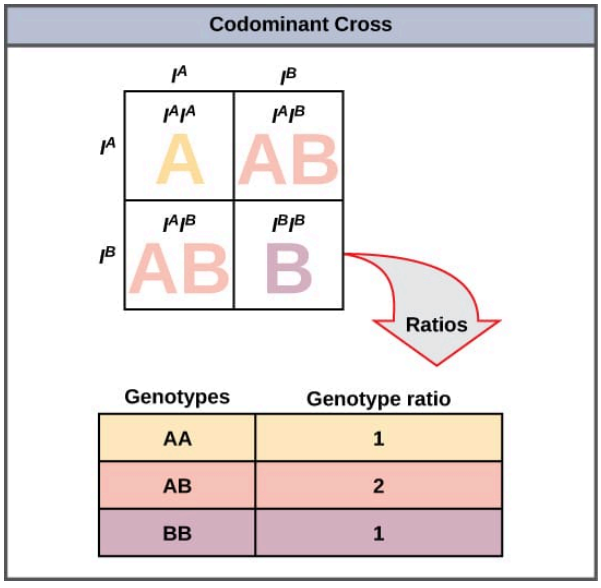


Figure 7.3.2: This Punnett square shows an AB/AB blood type cross. The offspring will have AA, AB, and BB blood types in a ratio of 1 to 2 to 1.

Multiple Alleles

Mendel implied that only two alleles, one dominant and one recessive, could exist for a given gene. We now know that this is an oversimplification. Although individual humans (and all diploid organisms) can only have two alleles for a given gene, multiple alleles may exist at the population level, such that many combinations of two alleles are observed. Note that when many alleles exist for the same gene, the convention is to denote the most common phenotype or genotype in the natural population as the wild

type (often abbreviated “+”). All other phenotypes or genotypes are considered variants (mutants) of this typical form, meaning they deviate from the wild type. The variant may be recessive or dominant to the wild-type allele.

An example of multiple alleles is the ABO blood-type system in humans. In this case, there are three alleles circulating in the population. The I^A allele codes for A molecules on the red blood cells, the I^B allele codes for B molecules on the surface of red blood cells, and the *i* allele codes for no molecules on the red blood cells. In this case, the I^A and I^B alleles are codominant with each other and are both dominant over the *i* allele. Although there are three alleles present in a population, each individual only gets two of the alleles from their parents. This produces the genotypes and phenotypes shown in **Figure** 7.3.3. Notice that instead of three genotypes, there are six different genotypes when there are three alleles. The number of possible phenotypes depends on the dominance relationships between the three alleles.

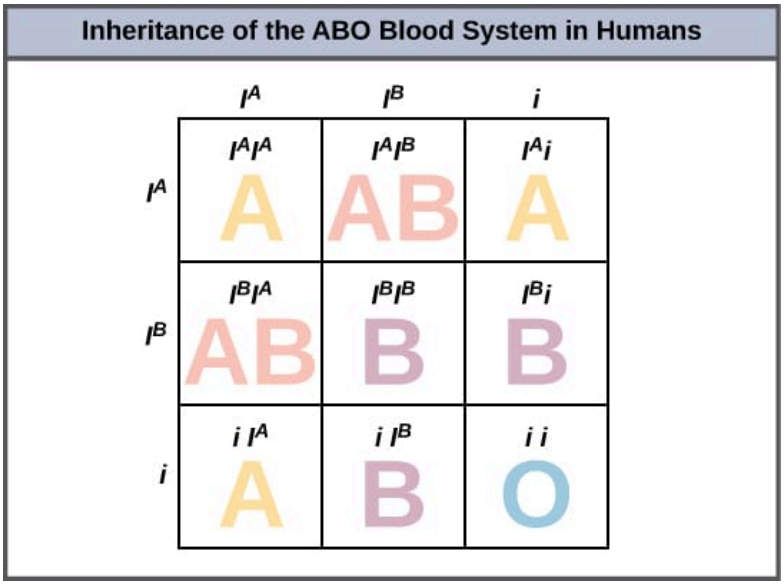


Figure 7.3.3: Inheritance of the ABO blood system in humans is shown. Resulting genotypes and blood types: I^AI^A and I^Ai produce type A blood, I^BI^B and I^Bi produce type B blood, I^AI^B produces type AB blood, and ii produces type O blood. The diagram illustrates the codominance of I^A and I^B and the recessive inheritance of i.

EVOLUTION IN ACTION: Multiple Alleles Confer Drug Resistance in the Malaria Parasite

Malaria is a parasitic disease in humans that is transmitted by infected female mosquitoes, including *Anopheles gambiae*, and is characterized by cyclic high fevers, chills, flu-like symptoms, and severe anemia. *Plasmodium falciparum* and *P. vivax* are the most common causative agents of malaria, and *P. falciparum* is the most deadly. When promptly and correctly treated, *P. falciparum* malaria has a mortality rate of 0.1 percent. However, in some parts of the world, the parasite has evolved resistance to commonly used malaria treatments, so the most effective malarial treatments can vary by geographic region.

In Southeast Asia, Africa, and South America, *P. falciparum* has developed resistance to the anti-malarial drugs chloroquine, mefloquine, and sulfadoxine-pyrimethamine. *P. falciparum*, which is haploid during the life stage in which it is infective to humans, has evolved multiple drug-resistant mutant alleles of the *dhps* gene. Varying degrees of sulfadoxine resistance are associated with each of these alleles. Being haploid, *P. falciparum* needs only one drug-resistant allele to express this trait.

In Southeast Asia, different sulfadoxine-resistant alleles of the *dhps* gene are localized to different geographic regions. This is a common evolutionary phenomenon that comes about because drug-resistant mutants arise in a population and interbreed with other *P. falciparum* isolates in close proximity. Sulfadoxine-resistant parasites cause considerable human hardship in regions in which this drug is widely used as an over-the-counter malaria remedy. As is common with pathogens that multiply to large numbers within an infection cycle, *P. falciparum* evolves relatively rapidly (over a decade or so) in response to the selective pressure of commonly used anti-malarial drugs. For this reason, scientists must constantly work to develop new drugs or drug combinations to combat the worldwide malaria burden.[1]

Sex-Linked Traits

In humans, as well as in many other animals and some plants, the sex of the individual is determined by sex chromosomes—one pair of non-homologous chromosomes. Until now, we have only considered inheritance patterns among non-sex chromosomes, or autosomes. In addition to 22 homologous pairs of autosomes, human females have a homologous pair of X chromosomes, whereas human males have an XY chromosome pair. Although the Y chromosome contains a small region of similarity to the X chromosome so that they can pair during meiosis, the Y chromosome is much shorter and contains fewer genes. When a gene being examined is present on the X, but not the Y, chromosome, it is X-linked.

Eye color in *Drosophila*, the common fruit fly, was the first X-linked trait to be identified. Thomas Hunt Morgan mapped this trait to the X chromosome in 1910. Like humans, *Drosophila* males have an XY chromosome pair, and females are XX. In flies the wild-type eye color is red (X^W) and is dominant to white eye color (X^w) (**Figure** 7.3.4). Because of the location of the eye-color gene, reciprocal crosses do not produce the same offspring ratios. Males are said to be hemizygous, in that they have only one allele for any X-linked characteristic. Hemizygosity makes descriptions of dominance and recessiveness irrelevant for XY males. *Drosophila* males lack the white gene on the Y chromosome; that is, their genotype can only be X^WY or X^wY. In contrast, females have two allele copies of this gene and can be X^WX^W, X^WX^w, or X^wX^w.



Figure 7.3.4: In *Drosophila*, the gene for eye color is located on the X chromosome. Red eye color is wild-type and is dominant to white eye color.

In an X-linked cross, the genotypes of F_1 and F_2 offspring depend on whether the recessive trait was expressed by the male or the female in the P generation. With respect to *Drosophila* eye color, when the P male expresses the white-eye phenotype and the female is homozygously red-eyed, all members of the F_1 generation exhibit red eyes (**Figure** 7.3.5). The F_1 females are heterozygous (X^WX^w), and the males are all X^WY, having received their X chromosome from the homozygous dominant P female and their Y chromosome from the P male. A subsequent cross between the X^WX^w female and the X^WY male would produce only red-eyed females (with X^WX^W or X^WX^w genotypes) and both red- and white-eyed males (with X^WY or X^wY genotypes). Now, consider a cross between a homozygous white-eyed female and a male with red eyes. The F_1 generation would exhibit only heterozygous red-eyed females (X^WX^w) and only white-eyed males (X^wY). Half of the F_2 females would be red-eyed (X^WX^w) and half would be white-eyed (X^wX^w). Similarly, half of the F_2 males would be red-eyed (X^WY) and half would be white-eyed (X^wY).

ART CONNECTION

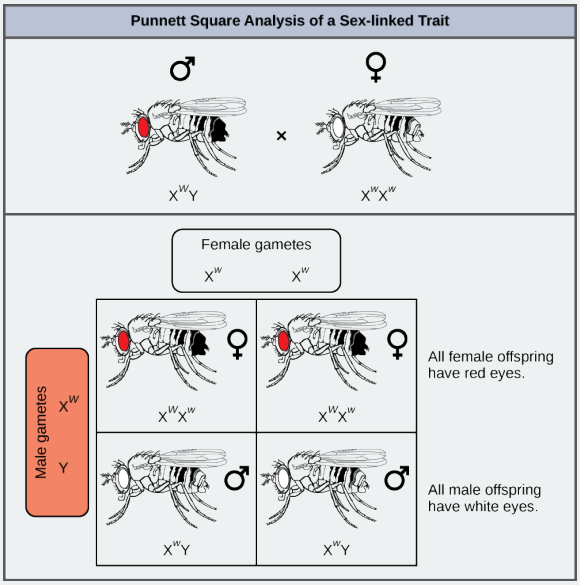


Figure 7.3.5: Crosses involving sex-linked traits often give rise to different phenotypes for the different sexes of offspring, as is the case for this cross involving red and white eye color in *Drosophila*. In the diagram, *w* is the white-eye mutant allele and *W* is the wild-type, red-eye allele. A red-eyed male fruit fly with the genotype X^{w}Y is crossed with a white-eyed female fruit fly with the genotype X^{w}X^{w}. All of the female offspring acquire a dominant X^{W} allele from the father and a recessive X^{w} allele from the mother, and are therefore heterozygous dominant with red eye color. All the male offspring acquire a recessive X^{w} allele from the mother and a Y chromosome from the father and are therefore hemizygous recessive with white eye color.

What ratio of offspring would result from a cross between a white-eyed male and a female that is heterozygous for red eye color?

Discoveries in fruit fly genetics can be applied to human genetics. When a female parent is homozygous for a recessive X-linked trait, she will pass the trait on to 100 percent of her male offspring, because the males will receive the Y chromosome from the male parent. In humans, the alleles for certain conditions (some color-blindness, hemophilia, and muscular dystrophy) are X-linked. Females who are heterozygous for these diseases are said to be carriers and may not exhibit any phenotypic effects. These females will pass the disease to half of their sons and will pass carrier status to half of their daughters; therefore, X-linked traits appear more frequently in males than females.

In some groups of organisms with sex chromosomes, the sex with the non-homologous sex chromosomes is the female rather than the male. This is the case for all birds. In this case, sex-linked traits will be more likely to appear in the female, in whom they are hemizygous.

CONCEPT IN ACTION

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

Watch the video above to learn more about sex-linked traits.

Linked Genes Violate the Law of Independent Assortment

Although all of Mendel's pea plant characteristics behaved according to the law of independent assortment, we now know that

some allele combinations are not inherited independently of each other. Genes that are located on separate, non-homologous chromosomes will always sort independently. However, each chromosome contains hundreds or thousands of genes, organized linearly on chromosomes like beads on a string. The segregation of alleles into gametes can be influenced by linkage, in which genes that are located physically close to each other on the same chromosome are more likely to be inherited as a pair. However, because of the process of recombination, or "crossover," it is possible for two genes on the same chromosome to behave independently, or as if they are not linked. To understand this, let us consider the biological basis of gene linkage and recombination.

Homologous chromosomes possess the same genes in the same order, though the specific alleles of the gene can be different on each of the two chromosomes. Recall that during interphase and prophase I of meiosis, homologous chromosomes first replicate and then synapse, with like genes on the homologs aligning with each other. At this stage, segments of homologous chromosomes exchange linear segments of genetic material (**Figure** 7.3.6). This process is called recombination, or crossover, and it is a common genetic process. Because the genes are aligned during recombination, the gene order is not altered. Instead, the result of recombination is that maternal and paternal alleles are combined onto the same chromosome. Across a given chromosome, several recombination events may occur, causing extensive shuffling of alleles.

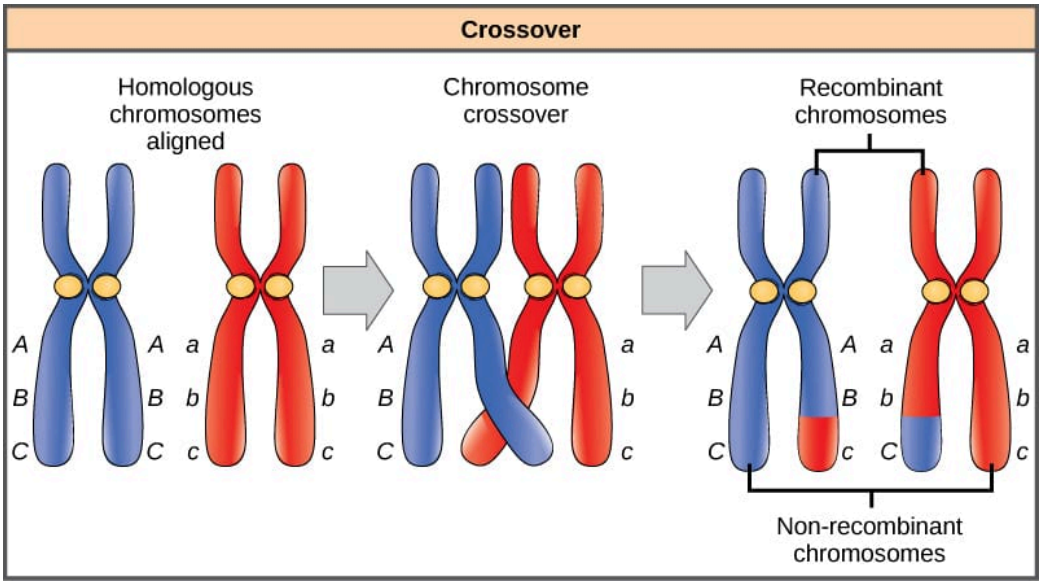


Figure 7.3.6: The process of crossover, or recombination, occurs when two homologous chromosomes align and exchange a segment of genetic material. One of the pair has the alleles ABC and the other has the alleles abc. During meiosis, crossover occurs between two of the chromosomes and genetic material is exchanged, resulting in one recombinant chromosome that has the alleles ABc and another that has the alleles abC. The other two chromosomes are non-recombinant and have the same arrangement of genes as before meiosis.

When two genes are located on the same chromosome, they are considered linked, and their alleles tend to be transmitted through meiosis together. To exemplify this, imagine a dihybrid cross involving flower color and plant height in which the genes are next to each other on the chromosome. If one homologous chromosome has alleles for tall plants and red flowers, and the other chromosome has genes for short plants and yellow flowers, then when the gametes are formed, the tall and red alleles will tend to go together into a gamete and the short and yellow alleles will go into other gametes. These are called the parental genotypes because they have been inherited intact from the parents of the individual producing gametes. But unlike if the genes were on different chromosomes, there will be no gametes with tall and yellow alleles and no gametes with short and red alleles. If you create a Punnett square with these gametes, you will see that the classical Mendelian prediction of a 9:3:3:1 outcome of a dihybrid cross would not apply. As the distance between two genes increases, the probability of one or more crossovers between them increases and the genes behave more like they are on separate chromosomes. Geneticists have used the proportion of recombinant gametes (the ones not like the parents) as a measure of how far apart genes are on a chromosome. Using this information, they have constructed linkage maps of genes on chromosomes for well-studied organisms, including humans.

Mendel's seminal publication makes no mention of linkage, and many researchers have questioned whether he encountered linkage but chose not to publish those crosses out of concern that they would invalidate his independent assortment postulate. The garden pea has seven chromosomes, and some have suggested that his choice of seven characteristics was not a coincidence. However, even if the genes he examined were not located on separate chromosomes, it is possible that he simply did not observe linkage because of the extensive shuffling effects of recombination.

Epistasis

Mendel's studies in pea plants implied that the sum of an individual's phenotype was controlled by genes (or as he called them, unit factors), such that every characteristic was distinctly and completely controlled by a single gene. In fact, single observable characteristics are almost always under the influence of multiple genes (each with two or more alleles) acting in unison. For example, at least eight genes contribute to eye color in humans.

CONCEPT IN ACTION



Eye color in humans is determined by multiple alleles. Use the Eye Color Calculator to predict the eye color of children from parental eye color.

In some cases, several genes can contribute to aspects of a common phenotype without their gene products ever directly interacting. In the case of organ development, for instance, genes may be expressed sequentially, with each gene adding to the complexity and specificity of the organ. Genes may function in complementary or synergistic fashions, such that two or more genes expressed simultaneously affect a phenotype. An apparent example of this occurs with human skin color, which appears to involve the action of at least three (and probably more) genes. Cases in which inheritance for a characteristic like skin color or human height depend on the combined effects of numerous genes are called polygenic inheritance.

Genes may also oppose each other, with one gene suppressing the expression of another. In epistasis, the interaction between genes is antagonistic, such that one gene masks or interferes with the expression of another. "Epistasis" is a word composed of Greek roots meaning "standing upon." The alleles that are being masked or silenced are said to be hypostatic to the epistatic alleles that are doing the masking. Often the biochemical basis of epistasis is a gene pathway in which expression of one gene is dependent on the function of a gene that precedes or follows it in the pathway.

An example of epistasis is pigmentation in mice. The wild-type coat color, agouti (*AA*) is dominant to solid-colored fur (*aa*). However, a separate gene *C*, when present as the recessive homozygote (*cc*), negates any expression of pigment from the *A* gene and results in an albino mouse (Figure 7.3.7). Therefore, the genotypes *AAcc*, *Aacc*, and *aacc* all produce the same albino phenotype. A cross between heterozygotes for both genes (*AaCc* x *AaCc*) would generate offspring with a phenotypic ratio of 9 agouti:3 black:4 albino (**Figure** 7.3.7). In this case, the *C* gene is epistatic to the *A* gene.

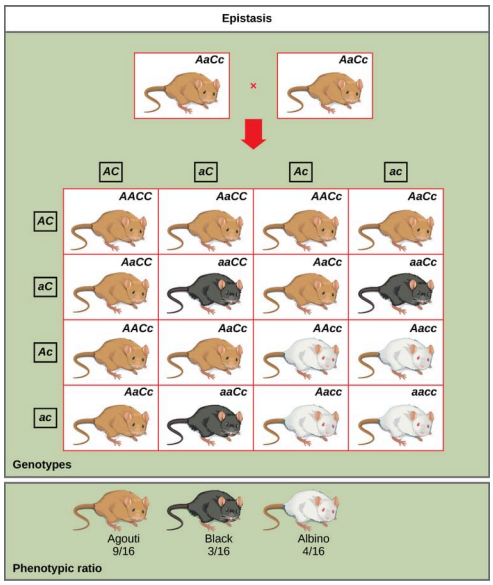


Figure 7.3.7: In this example of epistasis, one gene (*C*) masks the expression of another (*A*) for coat color. When the *C* allele is present, coat color is expressed; when it is absent (*cc*), no coat color is expressed. Coat color depends on the *A* gene, which shows dominance, with the recessive homozygote showing a different phenotype than the heterozygote or dominant homozygote.

A cross between two agouti mice with the heterozygous genotype AaCc is shown. Each mouse produces four different kinds of gametes (AC, aC, Ac, and ac). A 4 × 4 Punnett square is used to determine the genotypic ratio of the offspring. The phenotypic ratio is 9/16 agouti, 3/16 black, and 4/16 white.

Summary

Alleles do not always behave in dominant and recessive patterns. Incomplete dominance describes situations in which the heterozygote exhibits a phenotype that is intermediate between the homozygous phenotypes. Codominance describes the simultaneous expression of both of the alleles in the heterozygote. Although diploid organisms can only have two alleles for any given gene, it is common for more than two alleles for a gene to exist in a population. In humans, as in many animals and some plants, females have two X chromosomes and males have one X and one Y chromosome. Genes that are present on the X but not the Y chromosome are said to be X-linked, such that males only inherit one allele for the gene, and females inherit two.

According to Mendel's law of independent assortment, genes sort independently of each other into gametes during meiosis. This occurs because chromosomes, on which the genes reside, assort independently during meiosis and crossovers cause most genes on the same chromosomes to also behave independently. When genes are located in close proximity on the same chromosome, their alleles tend to be inherited together. This results in offspring ratios that violate Mendel's law of independent assortment. However, recombination serves to exchange genetic material on homologous chromosomes such that maternal and paternal alleles may be recombined on the same chromosome. This is why alleles on a given chromosome are not always inherited together. Recombination is a random event occurring anywhere on a chromosome. Therefore, genes that are far apart on the same chromosome are likely to still assort independently because of recombination events that occurred in the intervening chromosomal space.

Whether or not they are sorting independently, genes may interact at the level of gene products, such that the expression of an allele for one gene masks or modifies the expression of an allele for a different gene. This is called epistasis.

Art Connections

Figure 7.3.5: What ratio of offspring would result from a cross between a white-eyed male and a female that is heterozygous for red eye color?

Answer

Half of the female offspring would be heterozygous ($X^{W}X^{w}$) with red eyes, and half would be homozygous recessive ($X^{w}X^{w}$) with white eyes. Half of the male offspring would be hemizygous dominant ($X^{W}Y$) with red eyes, and half

would be hemizygous recessive (X^wY) with white eyes.

Footnotes

1. 1 Sumiti Vinayak et al., "Origin and Evolution of Sulfadoxine Resistant *Plasmodium falciparum*," *PLoS Pathogens* 6 (2010): e1000830.

Key Terms

allele
a version of a gene

codominance
in a heterozygote, complete and simultaneous expression of both alleles for the same characteristic

epistasis
an interaction between genes such that one gene masks or interferes with the expression of another

genotype
the genetic makeup of an organism bases on the alleles they have for traits

hemizygous
the presence of only one allele for a characteristic, as in X-linkage; hemizygosity makes descriptions of dominance and recessiveness irrelevant

incomplete dominance
in a heterozygote, expression of two contrasting alleles such that the individual displays an intermediate phenotype

linkage
a phenomenon in which alleles that are located in close proximity to each other on the same chromosome are more likely to be inherited together

phenotype
the observable traits of an organism

recombination
the process during meiosis in which homologous chromosomes exchange linear segments of genetic material, thereby dramatically increasing genetic variation in the offspring and separating linked genes

wild type
the most commonly occurring genotype or phenotype for a given characteristic found in a population

X-linked
a gene present on the X chromosome, but not the Y chromosome

Contributors and Attributions

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7.4: Patterns of Inheritance (Exercises)

7.1: Mendel's Experiments

Multiple Choice

Imagine that you are performing a cross involving seed color in garden pea plants. What traits would you expect to observe in the F_1 offspring if you cross true-breeding parents with green seeds and yellow seeds? Yellow seed color is dominant over green.

A. only yellow-green seeds
B. only yellow seeds
C. 1:1 yellow seeds:green seeds
D. 1:3 green seeds:yellow seeds

Answer

B

Imagine that you are performing a cross involving seed texture in garden pea plants. You cross true-breeding round and wrinkled parents to obtain F_1 offspring. Which of the following experimental results in terms of numbers of plants are closest to what you expect in the F_2 progeny?

A. 810 round seeds
B. 810 wrinkled seeds
C. 405:395 round seeds:wrinkled seeds
D. 610:190 round seeds:wrinkled seeds

Answer

D

Free Response

Describe one of the reasons that made the garden pea an excellent choice of model system for studying inheritance.

Answer

The garden pea has flowers that close tightly during self-pollination. This helps to prevent accidental or unintentional fertilizations that could have diminished the accuracy of Mendel's data.

7.2: Laws of Inheritance

Multiple Choice

The observable traits expressed by an organism are described as its _____.

A. phenotype
B. genotype
C. alleles
D. zygote

Answer

A

A recessive trait will be observed in individuals that are _____ for that trait.

A. heterozygous
B. homozygous or heterozygous
C. homozygousdiploid

Answer

C

What are the types of gametes that can be produced by an individual with the genotype *AaBb*?

A. *Aa*, *Bb*
B. *AA*, *aa*, *BB*, *bb*
C. *AB*, *Ab*, *aB*, *ab*
D. *AB*, *ab*

Answer

C

What is the reason for doing a test cross?

A. to identify heterozygous individuals with the dominant phenotype
B. to determine which allele is dominant and which is recessive
C. to identify homozygous recessive individuals in the F_2
D. to determine if two genes assort independently

Answer

A

Free Response

Use a Punnett square to predict the offspring in a cross between a dwarf pea plant (homozygous recessive) and a tall pea plant (heterozygous). What is the phenotypic ratio of the offspring?

Answer

The Punnett square would be 2 × 2 and will have *T* and *T* along the top and *T* and *t* along the left side. Clockwise from the top left, the genotypes listed within the boxes will be *Tt*, *Tt*, *tt*, and *tt*. The phenotypic ratio will be 1 tall:1 dwarf.

Use a Punnett square to predict the offspring in a cross between a tall pea plant (heterozygous) and a tall pea plant (heterozygous). What is the genotypic ratio of the offspring?

Answer

The Punnett square will be 2 × 2 and will have *T* and *t* along the top and *T* and *t* along the left side. Clockwise from the top left, the genotypes listed within the boxes will be *TT*, *Tt*, *Tt*, and *tt*. The genotypic ratio will be 1*TT*:2*Tt*:1*tt*.

7.3: Extensions of the Laws of Inheritance

Multiple Choice

If black and white true-breeding mice are mated and the result is all gray offspring, what inheritance pattern would this be indicative of?

A. dominance
B. codominance
C. multiple alleles
D. incomplete dominance

Answer

D

The ABO blood groups in humans are expressed as the I^A, I^B, and i alleles. The I^A allele encodes the A blood group antigen, I^B encodes B, and i encodes O. Both A and B are dominant to O. If a heterozygous blood type A parent (I^Ai) and a heterozygous blood type B parent (I^Bi) mate, one quarter of their offspring are expected to have the AB blood type (I^AI^B) in which both antigens are expressed equally. Therefore, ABO blood groups are an example of:

A. multiple alleles and incomplete dominance
B. codominance and incomplete dominance
C. incomplete dominance only
D. multiple alleles and codominance

Answer

D

In a cross between a homozygous red-eyed female fruit fly and a white-eyed male fruit fly, what is the expected outcome?

A. all white-eyed male offspring
B. all white-eyed female offspring
C. all red-eyed offspring
D. half white-eyed make offspring

Answer

C

When a population has a gene with four alleles circulating, how many possible genotypes are there?

A. 3
B. 6
C. 10
D. 16

Answer

C

Free Response

Can a male be a carrier of red-green color blindness?

Answer

No, males can only express color blindness and cannot carry it because an individual needs two X chromosomes to be a carrier.

Could an individual with blood type O (genotype *ii*) be a legitimate child of parents in which one parent had blood type A and the other parent had blood type B?

Answer

Yes this child could have come from these parents. The child would have inherited an *i* allele from each parent and for this to happen the type A parent had to have genotype I^Ai and the type b parent had to have genotype I^Bi.

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8: GENOTYPE TO PHENOTYPE AND MUTATIONS

8.1: Central Dogma - From Genes to Proteins
8.2: Transcription
8.3: Translation
8.4: How Gene Mutations Occur
8.5: Types of Mutations
8.6: Introduction to Genetic Disorders
8.7: Genetic Engineering Explained - Micropigs
8.8: Molecular Biology (Exercises)

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- 9: Molecular Biology by OpenStax is licensed CC BY 4.0.

8.1: Central Dogma - From Genes to Proteins

Learning Objectives

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

- Summarize the central dogma of molecular biology as the flow of genetic information from DNA to RNA to protein
- Recognize that only specific genes are used when needed, and that gene expression involves accessing packaged DNA within chromosomes
- Use real-world examples to connect alleles with differences in protein function, such as in blood type or sickle cell disease.

From Instructions to Action: How Cells Use DNA to Build Life

Have you ever wondered how your body knows what to make in order to grow, heal, or simply stay alive? From the enzymes that digest your food to the hemoglobin that carries oxygen in your blood, your body is constantly building complex molecules that keep it functioning. But how does it know what to make—and how to make it?

The answer lies in your genes. Genes are segments of DNA that contain the instructions for building proteins, the molecules responsible for nearly every task your cells perform. The process of using the information in DNA to build proteins is called gene expression, and it is central to how all living organisms function.

Gene expression allows the sequence of nucleotides in DNA to determine the sequence of amino acids in a protein. This flow of information happens in two major steps. First, during transcription, the DNA sequence of a gene is copied into a temporary message called RNA. Then, during translation, that RNA message is used as a guide to assemble a specific chain of amino acids, which folds into a functional protein. This directional flow—from DNA to RNA to protein—is known as the central dogma of molecular biology, and it explains how genetic information becomes biological function.

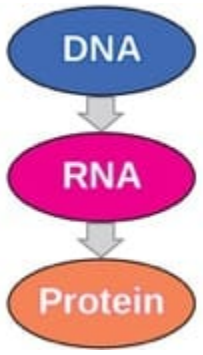


Figure 8.1.1**:** The Central Dogma – DNA is used to make RNA is used to make protein.

But where do these genes reside? Let's zoom out for a moment and revisit some foundational concepts that help us understand how gene expression works in the broader context of the cell and the organism.

The Genome: Your Complete Instruction Manual

Every cell in your body (with a few exceptions) contains the same complete set of DNA, known as your genome. Your genome is like a vast instruction manual that contains all the information needed to build and maintain "you." But just like you wouldn't read an entire manual every time you need to know how to do one specific task, your cells don't use every piece of DNA all the time. They refer to specific parts of chromosomes that are part of the genome when certain functions are needed. These specific segments are your genes—discrete stretches of DNA that encode the information needed to build a particular protein or RNA molecule and the basic unit of genetic information used in gene expression. For reference, the human genome contains about

20,000 to 25,000 genes.

Chromosomes: Packaging the Genome

The human genome consists of about 3 billion base pairs! To fit the roughly 2 meters of DNA into the nucleus of a microscopic cell, the DNA is tightly packaged into structures called chromosomes. Humans have 46 chromosomes, arranged in 23 pairs. One set comes from your mother, and one set comes from your father.

Each chromosome contains hundreds to thousands of genes arranged along its length. You can think of chromosomes like volumes in an encyclopedia set, with each gene being a single entry. Together, the chromosomes organize and protect DNA and help ensure that it is accurately copied and distributed when cells divide.

However, this packaging is not just for storage—it plays a critical role in whether and how genes are expressed. DNA that is tightly coiled around proteins (called histones) becomes less accessible to the cellular machinery that reads genes. In contrast, loosely packed regions of DNA are more open to being read and expressed. In this way, the structure and organization of chromosomes serve as a layer of control over gene expression, determining which parts of the genome are available to be used at any given time.

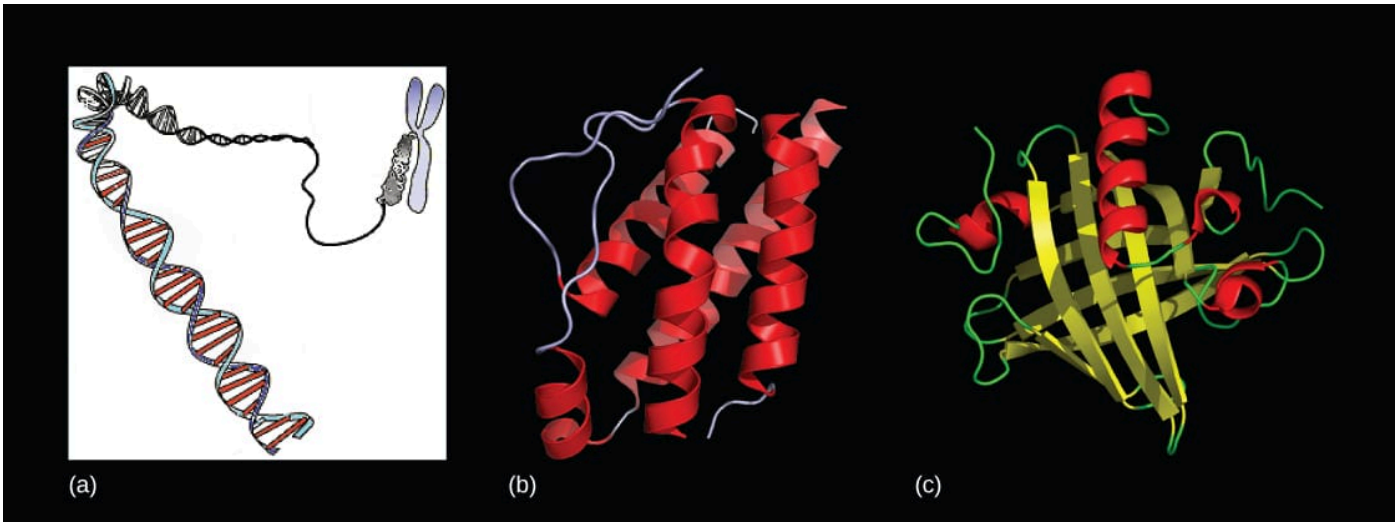


Figure 8.1.2**:** Genes, which are carried on (a) chromosomes, are linearly organized instructions for making the RNA and protein molecules that are necessary for all of the processes of life. The (b) interleukin-2 protein and (c) alpha-2u-globulin protein are just two examples of the array of different molecular structures that are encoded by genes. (credit "chromosome: National Human Genome Research Institute; credit "interleukin-2": Ramin Herati/Created from PDB 1M47 and rendered with Pymol; credit "alpha-2u-globulin": Darren Logan/rendered with AISMIG)

And this brings us to an important point: gene expression—the process of completing the central dogma—requires more than just having a gene. It requires a suite of proteins and molecular machines to locate, access, and decode the genetic information. DNA that is tightly packaged may be hidden from these proteins, while accessible regions of DNA can be transcribed into RNA. In this sense, the physical structure of the chromosome determines whether the genetic instructions are even available for use. As we expand on each stage of the central dogma, we will be highlighting the molecular machinery involved at each stage.

Connection: Gene Expression - From genotype to phenotype

Alleles: Variations on a Theme

While most people have the same genes in the same locations on the same chromosomes, there can be small differences in the exact DNA sequence of those genes. These different versions of a gene are called alleles. For example, everyone has the gene for hemoglobin, but there are several alleles of that gene, some of which affect how well it functions.

Alleles are part of what makes each person unique. They can influence everything from blood type to disease risk to how we respond to medications. When we talk about gene expression, we are usually referring to whether and how a particular allele is being used to build a protein. Some alleles may be more active than others or may result in slightly different versions of the same protein.

To appreciate the importance of alleles in gene expression, consider sickle cell anemia. This condition arises from a single change in the DNA sequence of the hemoglobin gene. People with two copies of this altered allele produce a defective version of the hemoglobin protein that causes red blood cells to take on a sickle shape. These misshapen cells don't carry oxygen efficiently and can block blood vessels, leading to pain and other complications. Here, a small change in the DNA—a different allele—leads to the production of a different protein with serious consequences.

Or think about blood type. Your ABO blood type is determined by a gene that has three common alleles: A, B, and O. The A and B alleles each produce a slightly different protein on the surface of red blood cells, while the O allele produces a non-functional version of the protein. Depending on which combination of alleles you inherit from your parents, your red blood cells will display type A, type B, type AB, or no surface protein at all (type O). These differences influence who you can safely receive blood from in a transfusion.

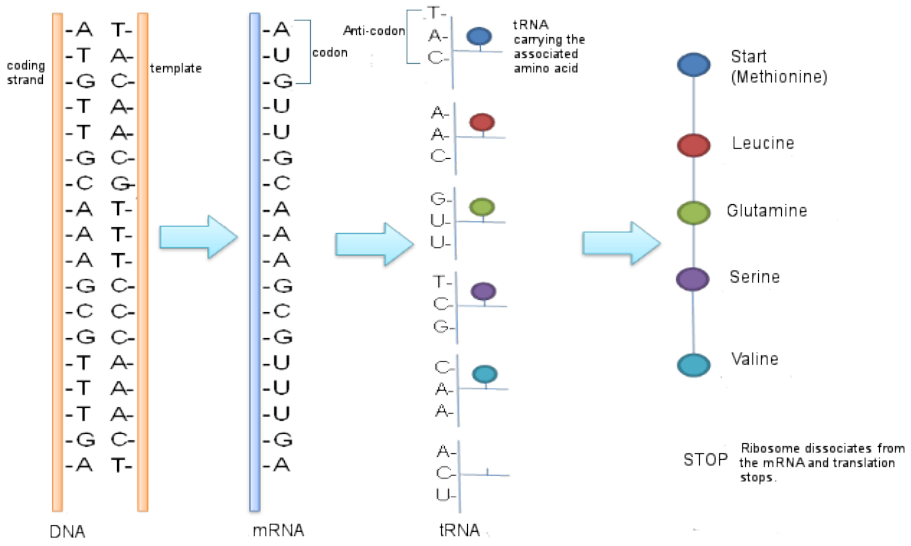
These examples underscore a central idea: the proteins made through gene expression go on to influence everything about an organism—from the structure of its cells to its ability to fight disease or metabolize food. And the exact form of each protein often depends on the allele from which it was made.

The Central Dogma: A Framework for Understanding

The central dogma of molecular biology provides a roadmap for how information flows within a cell: DNA → RNA → Protein. This concept is essential to biology because it explains how genetic information is transformed into the functional components of life.

Let's break this down:

- DNA stores the instructions. It's like the hard drive of the cell.
- RNA is the temporary message that carries instructions from DNA to the site of protein synthesis. It's like copying a recipe onto a sticky note to bring it into the kitchen.
- Protein is the final product—the molecule that actually does the work, whether it's speeding up chemical reactions, providing structure, or sending signals.



Open image description

This diagram illustrates the flow of genetic information from DNA to messenger RNA (mRNA) and then to a chain of amino acids during protein synthesis. The process is shown from left to right in four stages connected by large blue arrows.

DNA Template

On the far left is a double-stranded DNA molecule represented as two vertical strands. The strand on the left is labeled **"coding strand"** and the strand on the right is labeled **"template"**. Individual nucleotide bases are shown as letters connected between the two strands.

The coding strand sequence is:

A T G T A G C A A A T G C C T T T A G A

The complementary template strand is:

T A C A T C G T T T A C G G A A A T C T

The label **"DNA"** appears beneath the molecule.

Messenger RNA (mRNA)

A blue arrow points to a single-stranded RNA molecule labeled **"mRNA"**. The RNA strand is shown vertically and contains uracil (U) instead of thymine (T).

The mRNA sequence is:

A U G U U G C A A A G C G U U U G A

A bracket labels the first three nucleotides, **AUG**, as a **codon**.

Transfer RNA (tRNA)

A second blue arrow points to a column of transfer RNA molecules labeled **"tRNA"**. Each tRNA is shown as a small horizontal structure with a colored circular amino acid attached to one end and a three-letter anticodon at the other end.

A bracket labels the bases on the tRNA as an **"Anti-codon."** The figure also includes the label **"tRNA carrying the associated**

amino acid."

The tRNA molecules pair with the mRNA codons through complementary base pairing and carry specific amino acids corresponding to each codon.

Polypeptide Chain Formation

A third blue arrow points to the completed amino acid chain. The amino acids are represented as colored circles connected by a vertical line, illustrating the growing polypeptide.

From top to bottom, the amino acids shown are:

- Blue circle: **Start (Methionine)**
- Red circle: **Leucine**
- Green circle: **Glutamine**
- Purple circle: **Serine**
- Teal circle: **Valine**

Beneath the chain is the label:

> STOP

Additional text states:

> Ribosome dissociates from the mRNA and translation stops.

Overall, the figure demonstrates gene expression by showing how the information stored in a DNA sequence is transcribed into mRNA, translated through interactions with tRNA anticodons, and assembled into a chain of amino acids that forms a protein.

Figure 8.1.3**:** An illustration of protein synthesis. It starts with DNA and proceeds to mRNA, then tRNA, and then ends with a protein. (“Overview of Protein Synthesis” by Becky Boone is licensed under CC BY-SA 2.0)

This flow of information is directional. While there are some exceptions (like reverse transcription in viruses), the rule in most cells is that information goes from DNA to RNA to protein, not the other way around.

Why This Matters

Understanding gene expression helps answer fundamental questions in biology and medicine. Why does a skin cell look and act differently from a neuron, even though they have the same DNA? How do hormones trigger changes in growth or mood? Why do certain genetic mutations lead to disease?

All of these questions come back to gene expression.

Moreover, many modern biotechnologies—from gene therapies to CRISPR to cancer immunotherapies—are built around our ability to manipulate gene expression. When we understand how genes are expressed, we can begin to understand how to modify or correct gene activity when things go wrong.

The journey from gene to protein is complex and tightly controlled within each cell. It consists of two major steps: **transcription** and **translation.** Together, transcription and translation are known as **gene expression**.

Key Terms

allele
: a version of a gene. Different alleles can produce variations in the protein made from a gene, leading to different traits or health outcomes

central dogma
: the basic framework of molecular biology that describes the flow of genetic information from DNA to RNA to

protein

chromosome
: a tightly packaged structure of DNA and proteins found in the nucleus of cells. Humans have 46 chromosomes

gene
: a segment of DNA that contains the instructions to build a specific protein or RNA molecule

gene expression
: the process by which the information in a gene is used to make a functional product, usually a protein

genome
: the complete set of genetic material (DNA) in an organism. It includes all of the genes and other DNA sequences needed for life

histone
: a protein that DNA wraps around to form chromatin. This packaging can control access to genes and affect whether they are expressed

transcription
: the first step of gene expression in which a gene's DNA sequence is copied into RNA

translation
: the second step of gene expression in which the RNA message is used to assemble a chain of amino acids, forming a protein

References

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8.2: Transcription

Learning Objectives

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

- Explain the process of transcription, including the roles of the promoter, RNA polymerase, and the steps of initiation, elongation, and termination
- Distinguish between transcription in prokaryotes and eukaryotes, including where it occurs and how it is coupled (or not) with translation
- Summarize the key processing steps of eukaryotic mRNA, including the addition of a 5' cap, poly-A tail, and the removal of introns through splicing

In both prokaryotes and eukaryotes, the second function of DNA (the first was replication) is to provide the information needed to construct the proteins necessary so that the cell can perform all of its functions. To do this, the DNA is “read” or transcribed into an mRNA molecule. The mRNA then provides the code to form a protein by a process called translation. Through the processes of transcription and translation, a protein is built with a specific sequence of amino acids that was originally encoded in the DNA. This module discusses the details of transcription.

The Central Dogma: DNA Encodes RNA; RNA Encodes Protein

The flow of genetic information in cells from DNA to mRNA to protein is described by the central dogma (**Figure** 8.2.1), which states that genes specify the sequences of mRNAs, which in turn specify the sequences of proteins.

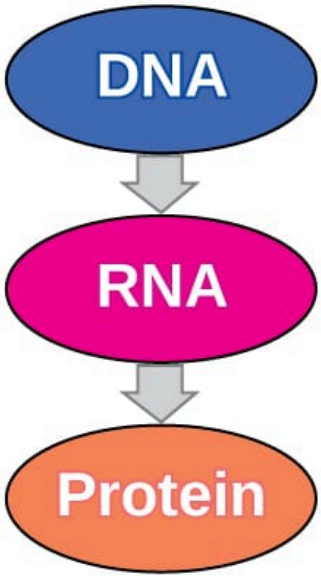


Figure 8.2.1: The central dogma states that DNA encodes RNA, which in turn encodes protein.

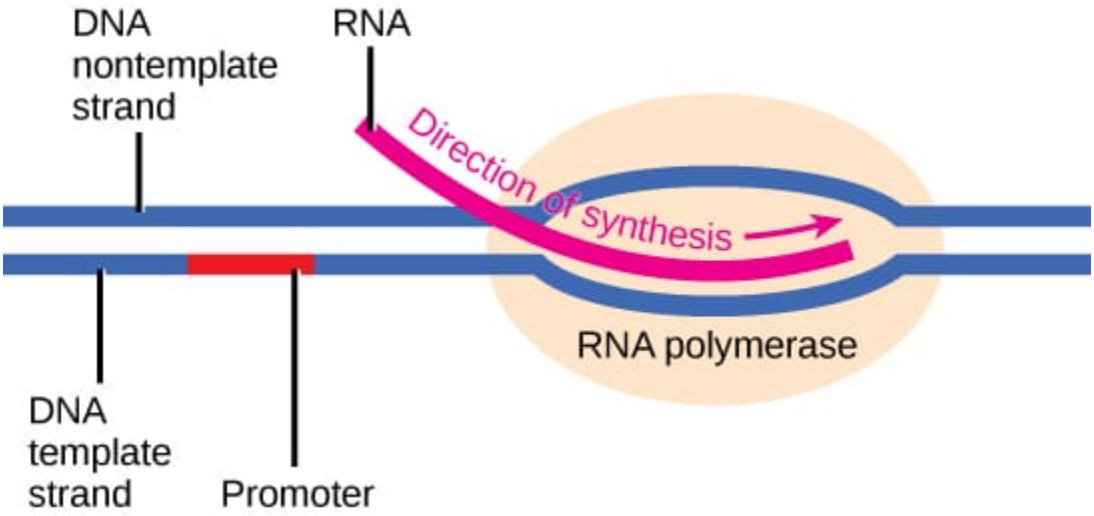
The copying of DNA to mRNA is relatively straightforward, with one nucleotide being added to the mRNA strand for every complementary nucleotide read in the DNA strand. The translation to protein is more complex because groups of three mRNA nucleotides correspond to one amino acid of the protein sequence. However, as we shall see in the next module, the translation to protein is still systematic, such that nucleotides 1 to 3 correspond to amino acid 1, nucleotides 4 to 6 correspond to amino acid 2, and so on.

Transcription: from DNA to mRNA

Both prokaryotes and eukaryotes perform fundamentally the same process of transcription, with the important difference of the membrane-bound nucleus in eukaryotes. With the genes bound in the nucleus, transcription occurs in the nucleus of the cell and the mRNA transcript must be transported to the cytoplasm. The prokaryotes, which include bacteria and archaea, lack membrane-bound nuclei and other organelles, and transcription occurs in the cytoplasm of the cell. In both prokaryotes and eukaryotes, transcription occurs in three main stages: initiation, elongation, and termination.

Initiation

Transcription requires the DNA double helix to partially unwind in the region of mRNA synthesis. The region of unwinding is called a transcription bubble. The DNA sequence onto which the proteins and enzymes involved in transcription bind to initiate the process is called a promoter. In most cases, promoters exist upstream of the genes they regulate. The specific sequence of a promoter is very important because it determines whether the corresponding gene is transcribed all of the time, some of the time, or hardly at all (**Figure** 8.2.2).

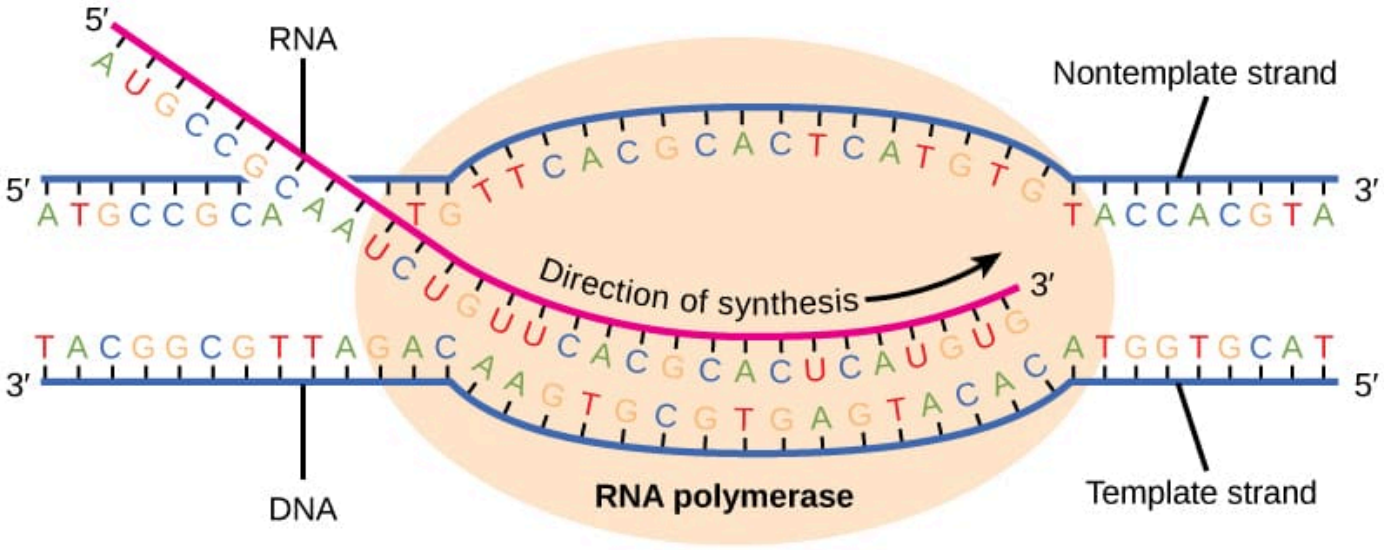


Open image description Diagram of transcription showing RNA synthesis from a DNA template. A double-stranded DNA molecule enters an RNA polymerase enzyme, depicted as a tan oval surrounding an opened section of the DNA. The upper DNA strand is labeled 'DNA nontemplate strand' and the lower strand is labeled 'DNA template strand.' A short red segment on the template strand is labeled 'Promoter.' A pink curved arrow labeled 'Direction of synthesis' shows an RNA strand being produced from the template strand as it exits the enzyme. The RNA strand extends away from the transcription bubble while the DNA strands rejoin behind the RNA polymerase.

Figure 8.2.2: The initiation of transcription begins when DNA is unwound, forming a transcription bubble. Enzymes and other proteins involved in transcription bind at the promoter section in red on the template strand. Downstream of the promoter is an RNA polymerase where RNA is being synthesized.

Elongation

Transcription always proceeds from one of the two DNA strands, which is called the template strand. The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the nontemplate strand, with the exception that RNA contains a uracil (U) in place of the thymine (T) found in DNA. During elongation, an enzyme called RNA polymerase proceeds along the DNA template adding nucleotides by base pairing with the DNA template in a manner similar to DNA replication, with the difference that an RNA strand is being synthesized that does not remain bound to the DNA template. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it (**Figure** 8.2.3).



Open image description

This diagram illustrates the process of transcription, in which RNA polymerase uses one strand of DNA as a template to synthesize a complementary RNA molecule. The figure shows a portion of DNA opened within the RNA polymerase enzyme, creating a transcription bubble where RNA synthesis occurs.

A large tan-colored oval labeled **"RNA polymerase"** surrounds the active transcription site in the center of the image. A double-stranded DNA molecule enters the enzyme from the left and exits on the right.

The upper DNA strand is labeled **"Nontemplate strand"**. It runs from **5′** at the left side of the image to **3′** at the right side. The lower DNA strand is labeled **"Template strand"**. It runs in the opposite direction, from **3′** on the left to **5′** on the right.

Within the transcription bubble, the two DNA strands are separated, exposing their nucleotide bases. Individual DNA nucleotides are shown as colored letters representing adenine (A), thymine (T), cytosine (C), and guanine (G). Outside the transcription bubble, the DNA strands are paired together in their normal double-stranded form.

A pink RNA strand emerges from the enzyme and extends upward and to the left. The RNA strand is labeled **"RNA"** and has a **5′** end at its free end. The RNA strand contains nucleotides represented by the letters A, U, C, and G, with uracil (U) replacing thymine (T) as is characteristic of RNA.

Inside the transcription bubble, the newly synthesized RNA is temporarily paired with the DNA template strand through complementary base pairing. The growing end of the RNA strand remains within the enzyme. The RNA's **3′** end is shown near the right side of the transcription bubble, where new nucleotides are being added.

A curved black arrow labeled **"Direction of synthesis"** points toward the right side of the figure. The arrow indicates the movement of RNA polymerase along the DNA template strand and the direction in which the RNA molecule is elongated.

The diagram demonstrates that RNA polymerase reads the DNA template strand while synthesizing a complementary RNA transcript. As the enzyme moves along the DNA, the RNA strand grows at its 3′ end, exits the enzyme, and the DNA strands rejoin behind the transcription bubble.

Figure 8.2.3: During elongation, RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to 3' direction, and unwinds then rewinds the DNA as it is read.

Termination

Once a gene is transcribed, the prokaryotic polymerase needs to be instructed to dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals, but both involve repeated nucleotide sequences in the DNA template that result in RNA polymerase stalling, leaving the DNA template, and freeing the mRNA transcript.

On termination, the process of transcription is complete. In a prokaryotic cell, by the time termination occurs, the transcript would already have been used to partially synthesize numerous copies of the encoded protein because these processes can occur concurrently using multiple ribosomes (polyribosomes) (**Figure** 8.2.4). In contrast, the presence of a nucleus in eukaryotic cells precludes simultaneous transcription and translation.

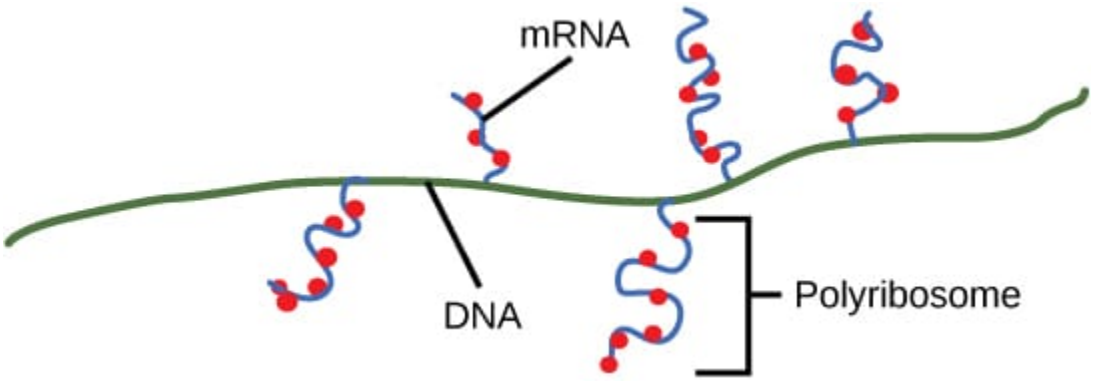


Figure 8.2.4: Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

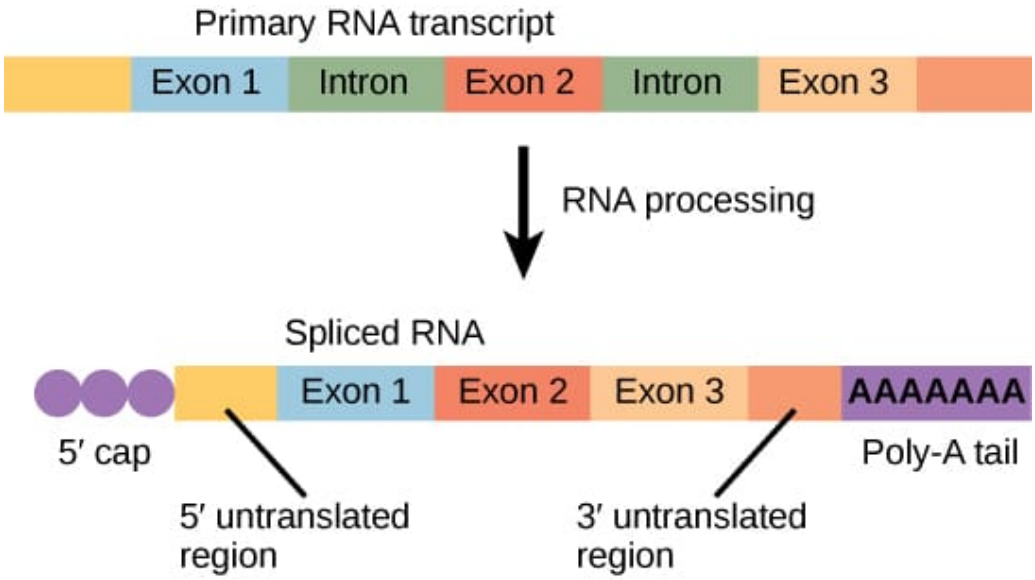
Eukaryotic RNA Processing

The newly transcribed eukaryotic mRNAs must undergo several processing steps before they can be transferred from the nucleus to the cytoplasm and translated into a protein. The additional steps involved in eukaryotic mRNA maturation create a molecule that is much more stable than a prokaryotic mRNA. For example, eukaryotic mRNAs last for several hours, whereas the typical prokaryotic mRNA lasts no more than five seconds.

The mRNA transcript is first coated in RNA-stabilizing proteins to prevent it from degrading while it is processed and exported out of the nucleus. This occurs while the pre-mRNA still is being synthesized by adding a special nucleotide “cap” to the 5' end of the growing transcript. In addition to preventing degradation, factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

Once elongation is complete, an enzyme then adds a string of approximately 200 adenine residues to the 3' end, called the poly-A tail. This modification further protects the pre-mRNA from degradation and signals to cellular factors that the transcript needs to be exported to the cytoplasm.

Eukaryotic genes are composed of protein-coding sequences called exons (*ex*-on signifies that they are *ex*pressed) and *int*ervening sequences called introns (*int*-ron denotes their *int*ervening role). Introns are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins. It is essential that all of a pre-mRNA’s introns be completely and precisely removed before protein synthesis so that the exons join together to code for the correct amino acids. If the process errs by even a single nucleotide, the sequence of the rejoined exons would be shifted, and the resulting protein would be nonfunctional. The process of removing introns and reconnecting exons is called splicing (**Figure** 8.2.5). Introns are removed and degraded while the pre-mRNA is still in the nucleus.



Open image description

This diagram illustrates RNA processing, showing how a primary RNA transcript is modified to produce a mature messenger RNA (mRNA) molecule. The figure is organized in two horizontal levels connected by a downward arrow labeled **"RNA processing."**

At the top of the figure is a long horizontal bar labeled **"Primary RNA transcript."** The transcript is divided into alternating segments representing exons and introns.

From left to right, the labeled segments are:

- **Exon 1**, shown as a light blue block
- **Intron**, shown as a green block
- **Exon 2**, shown as a salmon-colored block
- **Intron**, shown as a green block
- **Exon 3**, shown as a light orange block

The transcript extends beyond the labeled regions at both ends, indicating additional RNA sequence outside the highlighted exons and introns.

A large black arrow points downward from the primary transcript to the processed RNA product below, representing the removal of introns and other RNA modifications.

The lower part of the figure is labeled **"Spliced RNA."** It shows the mature RNA molecule after processing.

At the far left end are three purple circular structures labeled **"5′ cap."** These represent the modified cap that is added to the beginning of the RNA molecule.

Immediately after the cap is a short yellow segment labeled **"5′ untranslated region."** This region remains in the mature RNA but is not translated into protein.

Following the untranslated region are the three exons joined together in sequence:

- **Exon 1** (light blue)
- **Exon 2** (salmon-colored)
- **Exon 3** (light orange)

The introns that were present in the primary transcript are no longer shown, indicating that they have been removed during splicing.

To the right of Exon 3 is a short tan segment labeled **"3′ untranslated region."** Like the 5′ untranslated region, this sequence

remains in the mature RNA but is not translated into protein.

At the far right end is a purple rectangular region labeled with multiple adenine nucleotides as:

AAAAAAAA

This region is labeled **"Poly-A tail."** It represents the string of adenine bases added to the 3′ end of the RNA during processing.

Overall, the diagram demonstrates that RNA processing converts a primary RNA transcript containing both exons and introns into a mature messenger RNA. During processing, introns are removed, exons are joined together, a 5′ cap is added to the beginning of the molecule, and a poly-A tail is added to the end.

Figure 8.2.5: Eukaryotic mRNA contains introns that must be spliced out. In the spliced transcript, the introns are removed and the exons are fused together. A 5' cap and 3' poly-A tail are also added.

Summary

In prokaryotes, mRNA synthesis is initiated at a promoter sequence on the DNA template. Elongation synthesizes new mRNA. Termination liberates the mRNA and occurs by mechanisms that stall the RNA polymerase and cause it to fall off the DNA template. Newly transcribed eukaryotic mRNAs are modified with a cap and a poly-A tail. These structures protect the mature mRNA from degradation and help export it from the nucleus. Eukaryotic mRNAs also undergo splicing, in which introns are removed and exons are reconnected with single-nucleotide accuracy. Only finished mRNAs are exported from the nucleus to the cytoplasm.

Key Terms

elongation (transcription)
: the stage where RNA polymerase adds nucleotides to build the RNA strand

exon
: a sequence present in protein-coding mRNA after completion of pre-mRNA splicing

initiation complex (transcription)
: when RNA polymerase binds to DNA and beings making RNA

intron
: non–protein-coding intervening sequences that are spliced from mRNA during processing

mRNA
: messenger RNA; a form of RNA that carries the nucleotide sequence code for a protein sequence that is translated into a polypeptide sequence

nontemplate strand
: the strand of DNA that is not used to transcribe mRNA; this strand is identical to the mRNA except that T nucleotides in the DNA are replaced by U nucleotides in the mRNA

promoter
: a sequence on DNA to which RNA polymerase and associated factors bind and initiate transcription

RNA polymerase
: an enzyme that synthesizes an RNA strand from a DNA template strand

splicing
: the process of removing introns and reconnecting exons in a pre-mRNA

template strand
: the strand of DNA that specifies the complementary mRNA molecule

termination (transcription)
: when transcription stops and the newly made RNA is released

transcription bubble
: the region of locally unwound DNA that allows for transcription of mRNA

Contributors and Attributions

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8.3: Translation

Learning Objectives

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

- Describe the role of translation in the central dogma of molecular biology
- Identify the main molecular components involved in protein synthesis, including mRNA, ribosomes, tRNA, and amino acids
- Explain the function and structure of ribosomes in both prokaryotic and eukaryotic cells.Interpret the genetic code, including the concept of codons and the roles of start and stop codons
- Outline the three stages of translation—initiation, elongation, and termination—and describe what happens at each step
- Explain how tRNA molecules translate mRNA codons into specific amino acids to build a polypeptide.

The synthesis of proteins is one of a cell's most energy-consuming metabolic processes. In turn, proteins account for more mass than any other component of living organisms (with the exception of water), and proteins perform a wide variety of the functions of a cell. The process of translation, or protein synthesis, involves decoding an mRNA message into a polypeptide product. Amino acids are covalently strung together in lengths ranging from approximately 50 amino acids to more than 1,000.

The Protein Synthesis Machinery

In addition to the mRNA template, many other molecules contribute to the process of translation. The composition of each component may vary across species; for instance, ribosomes may consist of different numbers of ribosomal RNAs (rRNA) and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors (**Figure** 8.3.1).

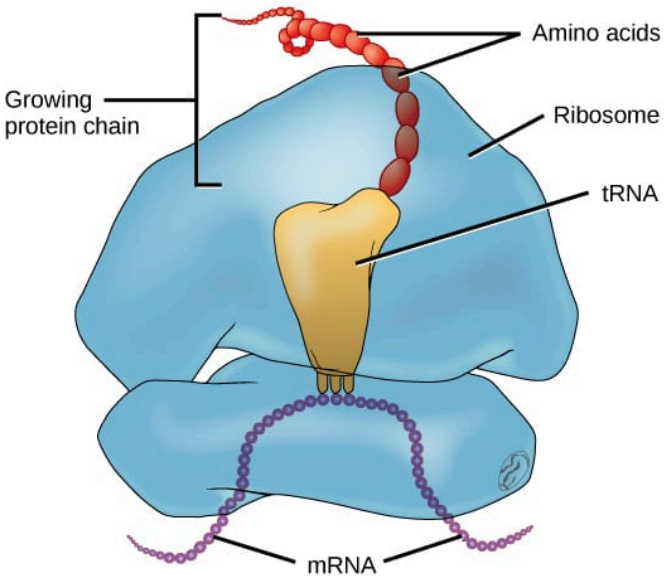


Figure 8.3.1: The protein synthesis machinery includes the large and small subunits of the ribosome, mRNA, and tRNA. Amino acids are emerging to form a protein chain.(credit: modification of work by NIGMS, NIH)

In *E. coli*, there are 200,000 ribosomes present in every cell at any given time. A ribosome is a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides. In eukaryotes, the nucleolus is completely specialized for the synthesis and assembly of rRNAs.

Ribosomes are located in the cytoplasm in prokaryotes and in the cytoplasm and endoplasmic reticulum of eukaryotes.

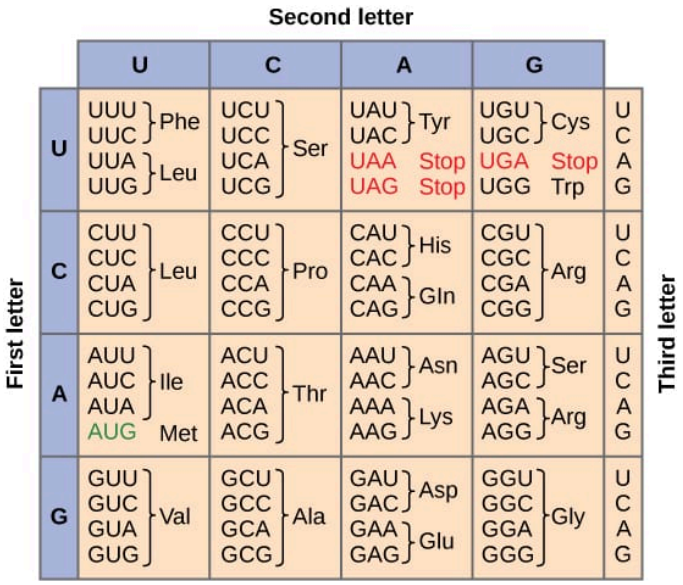
Ribosomes are made up of a large and a small subunit that come together for translation. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs, a type of RNA molecule that brings amino acids to the growing chain of the polypeptide. Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction.

Depending on the species, 40 to 60 types of tRNA exist in the cytoplasm. Serving as adaptors, specific tRNAs bind to sequences on the mRNA template and add the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually "translate" the language of RNA into the language of proteins. For each tRNA to function, it must have its specific amino acid bonded to it. In the process of tRNA "charging," each tRNA molecule is bonded to its correct amino acid.

The Genetic Code

To summarize what we know to this point, the cellular process of transcription generates messenger RNA (mRNA), a mobile molecular copy of one or more genes with an alphabet of A, C, G, and uracil (U). Translation of the mRNA template converts nucleotide-based genetic information into a protein product. Protein sequences consist of 20 commonly occurring amino acids; therefore, it can be said that the protein alphabet consists of 20 letters. Each amino acid is defined by a three-nucleotide sequence called the triplet codon. The relationship between a nucleotide codon and its corresponding amino acid is called the genetic code.

Given the different numbers of "letters" in the mRNA and protein "alphabets," combinations of nucleotides corresponded to single amino acids. Using a three-nucleotide code means that there are a total of 64 (4 × 4 × 4) possible combinations; therefore, a given amino acid is encoded by more than one nucleotide triplet (**Figure** 8.3.2).



Open image description

The left side of the chart is labeled **"First letter"** and lists the possible first nucleotide of the codon: U, C, A, and G. Across the top, the chart is labeled **"Second letter"** with columns for U, C, A, and G. Along the right side, the chart is labeled **"Third letter"**, indicating that the third nucleotide position can also be U, C, A, or G within each box.

First Letter U

When the first nucleotide is U:

- UUU and UUC code for **Phenylalanine (Phe)**.
- UUA and UUG code for **Leucine (Leu)**.
- UCU, UCC, UCA, and UCG code for **Serine (Ser)**.
- UAU and UAC code for **Tyrosine (Tyr)**.
- UAA and UAG are **Stop** codons.
- UGU and UGC code for **Cysteine (Cys)**.
- UGA is a **Stop** codon.
- UGG codes for **Tryptophan (Trp)**.

First Letter C

When the first nucleotide is C:

- CUU, CUC, CUA, and CUG code for **Leucine (Leu)**.
- CCU, CCC, CCA, and CCG code for **Proline (Pro)**.
- CAU and CAC code for **Histidine (His)**.
- CAA and CAG code for **Glutamine (Gln)**.
- CGU, CGC, CGA, and CGG code for **Arginine (Arg)**.

First Letter A

When the first nucleotide is A:

- AUU, AUC, and AUA code for **Isoleucine (Ile)**.
- **AUG**, highlighted in green, codes for **Methionine (Met)** and serves as the **start codon** for translation.
- ACU, ACC, ACA, and ACG code for **Threonine (Thr)**.
- AAU and AAC code for **Asparagine (Asn)**.

- AAA and AAG code for **Lysine (Lys)**.
- AGU and AGC code for **Serine (Ser)**.
- AGA and AGG code for **Arginine (Arg)**.

First Letter G

When the first nucleotide is G:

- GUU, GUC, GUA, and GUG code for **Valine (Val)**.
- GCU, GCC, GCA, and GCG code for **Alanine (Ala)**.
- GAU and GAC code for **Aspartic acid (Asp)**.
- GAA and GAG code for **Glutamic acid (Glu)**.
- GGU, GGC, GGA, and GGG code for **Glycine (Gly)**.

Stop codons are shown in red text and include **UAA**, **UAG**, and **UGA**. The start codon **AUG** is highlighted in green. The chart demonstrates the redundancy of the genetic code, where multiple codons can specify the same amino acid. It is used to determine which amino acid will be added to a growing protein based on the sequence of nucleotides in an mRNA molecule.

Figure 8.3.2: This figure shows the genetic code for all 64 codons. Sixty-two of these code for amino acids, and three are stop codons shown in red. The start codon, AUG, is colored green. (credit: modification of work by NIH)

Three of the 64 codons terminate protein synthesis and release the polypeptide from the translation machinery. These triplets are called stop codons. Another codon, AUG, also has a special function. In addition to specifying the amino acid methionine, it also serves as the start codon to initiate translation. The reading frame for translation is set by the AUG start codon near the 5' end of the mRNA. The genetic code is universal. With a few exceptions, virtually all species use the same genetic code for protein synthesis, which is powerful evidence that all life on Earth shares a common origin.

The Mechanism of Protein Synthesis

Just as with mRNA synthesis, protein synthesis can be divided into three phases: initiation, elongation, and termination. The process of translation is similar in prokaryotes and eukaryotes. Here we will explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between prokaryotic and eukaryotic translation.

Protein synthesis begins with the formation of an initiation complex. In *E. coli*, this complex involves the small ribosome subunit, the mRNA template, three initiation factors, and a special initiator tRNA. The initiator tRNA interacts with the AUG start codon, and links to a special form of the amino acid methionine that is typically removed from the polypeptide after translation is complete.

In prokaryotes and eukaryotes, the basics of polypeptide elongation are the same, so we will review elongation from the perspective of *E. coli*. The large ribosomal subunit of *E. coli* consists of three compartments: the A site binds incoming charged tRNAs (tRNAs with their attached specific amino acids). The P site binds charged tRNAs carrying amino acids that have formed bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The E site releases dissociated tRNAs so they can be recharged with free amino acids. The ribosome shifts one codon at a time, catalyzing each process that occurs in the three sites. With each step, a charged tRNA enters the complex, the polypeptide becomes one amino acid longer, and an uncharged tRNA departs. The energy for each bond between amino acids is derived from GTP, a molecule similar to ATP (**Figure** 8.3.3). Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200-amino acid polypeptide could be translated in just 10 seconds.

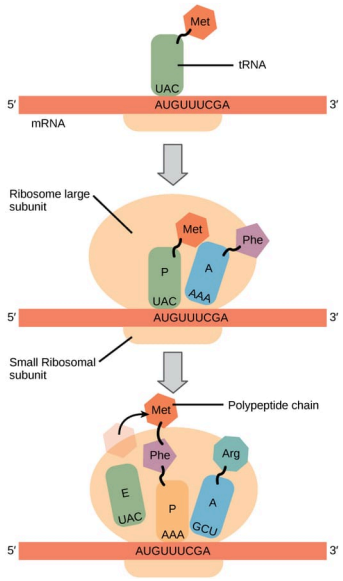


Figure 8.3.3: Translation begins when a tRNA anticodon recognizes the sequence AUG on the mRNA. The large ribosomal subunit joins the small subunit, and a second tRNA is recruited. As the mRNA moves relative to the ribosome, the polypeptide chain is formed between the first amino acid, which is at the P site, and the second amino acid, which is at the A site. The mRNA then shifts and the first tRNA is moved to the E site, where it dissociates from the ribosome. Entry of a release factor into the A site terminates translation and the components dissociate.

Termination of translation occurs when a stop codon (UAA, UAG, or UGA) is encountered. When the ribosome encounters the stop codon, the growing polypeptide is released and the ribosome subunits dissociate and leave the mRNA. After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction.

CONCEPT IN ACTION

Transcribe a gene and translate it to protein using complementary pairing and the genetic code at this Learn Genetics: Transcribe and Translate a Gene site.

Summary

The central dogma describes the flow of genetic information in the cell from genes to mRNA to proteins. Genes are used to make mRNA by the process of transcription; mRNA is used to synthesize proteins by the process of translation. The genetic code is the correspondence between the three-nucleotide mRNA codon and an amino acid. The genetic code is "translated" by the tRNA molecules, which associate a specific codon with a specific amino acid. The genetic code is degenerate because 64 triplet codons in mRNA specify only 20 amino acids and three stop codons. This means that more than one codon corresponds to an amino acid. Almost every species on the planet uses the same genetic code.

The players in translation include the mRNA template, ribosomes, tRNAs, and various enzymatic factors. The small ribosomal subunit binds to the mRNA template. Translation begins at the initiating AUG on the mRNA. The formation of bonds occurs between sequential amino acids specified by the mRNA template according to the genetic code. The ribosome accepts charged tRNAs, and as it steps along the mRNA, it catalyzes bonding between the new amino acid and the end of the growing polypeptide. The entire mRNA is translated in three-nucleotide "steps" of the ribosome. When a stop codon is encountered, a release factor binds and dissociates the components and frees the new protein.

Key Terms

anticodon
: a three-base sequence on tRNA that pairs with a codon on mRNA

codon
: three consecutive nucleotides in mRNA that specify the addition of a specific amino acid or the release of a polypeptide chain during translation

elongation (translation)
the stage in which amino acids are added one by one to the growing protein chain

genetic code
the amino acids that correspond to three-nucleotide codons of mRNA

initiation complex (translation)
the assembled group of mRNA, tRNA, and ribosomal subunits that begins translation

reading frame
is a way of dividing the sequence of nucleotides in a DNA or RNA molecule into a set of consecutive, non-overlapping triplets. In mRNA protein-coding reading frame typically is determined by the first three nucleotides with this sequence: AUG

rRNA
ribosomal RNA; molecules of RNA that combine to form part of the ribosome

stop codon
one of the three mRNA codons that specifies termination of translation

start codon
the AUG (or, rarely GUG) on an mRNA from which translation begins; always specifies the amino acid methionine

termination (translation)
when a stop codon is reached and the completed protein is released

transfer RNA
an RNA molecule that contains a specific three-nucleotide anticodon sequence to pair with the mRNA codon and also binds to a specific amino acid

Contributors and Attributions

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8.4: How Gene Mutations Occur

Learning Objectives

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

- Compare the different ways in which mutations arise
- Distinguish between inherited (germline) and acquired (somatic) mutations
- Recognize that mutations can have varying effects—from no impact to serious disease
- Relate mutations to real-world examples such as human traits, genetic disorders, and evolutionary change

When the Blueprint Changes: An Introduction to Gene Mutations

In the previous section, you learned how cells read the instructions in DNA to build proteins—tiny molecular machines that make life possible. This process, known as gene expression, is fundamental to how your body grows, repairs itself, digests food, responds to infections, and so much more. It's a remarkable system of biological communication, one that translates information written in the language of nucleotides into the language of amino acids, and ultimately into the proteins that do the work of life.

But what happens when the instructions themselves are flawed?

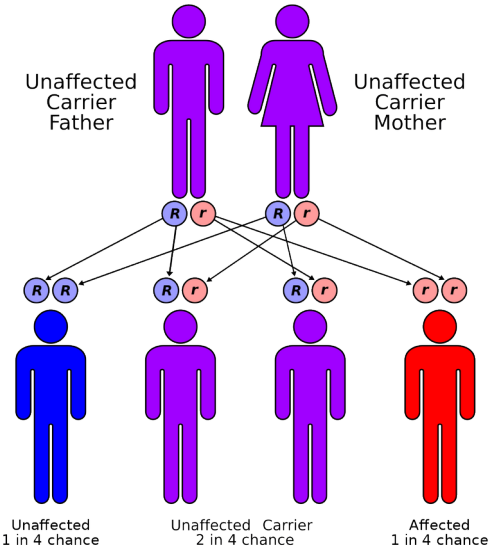
Imagine building a bookshelf from a set of blueprints. If one small line of the instructions is smudged, reversed, or missing entirely, the outcome might be a wobbly shelf—or no shelf at all. Similarly, when a gene contains an error in its DNA sequence—a mutation—the resulting protein may be altered, nonfunctional, or missing. This can lead to subtle changes in how cells function, or in some cases, to serious medical conditions. Some mutations are passed down from our parents and have been in our DNA since conception. Others arise later in life, as a result of environmental exposure, mistakes during DNA copying, or even by pure chance.

Though the word *mutation* might sound alarming—thanks in part to science fiction—it's important to understand that not all mutations are harmful. In fact, mutations are a natural part of biology. Some have no effect at all. Others contribute to the rich diversity of traits we see in human populations, such as differences in hair texture, skin color, or blood type. Still others may increase or decrease a person's risk for certain diseases. Learning how and why mutations happen gives us powerful insights into everything from genetic disorders to cancer, evolution, and even personalized medicine.

Now that you understand how genes direct the production of proteins, it's time to explore what happens when that process is disrupted. In the next section, we'll look more closely at the different kinds of mutations that can affect genes, where they come from, and how they can change the course of a protein's function—and sometimes, a person's life.

Gene mutations can be classified in two major ways:

- Hereditary mutations are inherited from a parent and are present throughout a person's life in virtually every cell in the body. These mutations are also called germline mutations because they are present in the parent's egg or sperm cells, which are also called germ cells. When an egg and a sperm cell unite, the resulting fertilized egg cell receives DNA from both parents. If this DNA has a mutation, the child that grows from the fertilized egg will have the mutation in every cell in their body. (**Figure** 8.4.1)
- Acquired (or somatic) mutations occur at some time during a person's life and are present only in certain cells, not in every cell in the body. These changes can be caused by environmental factors such as ultraviolet radiation from the sun, or can occur if a mistake is made as DNA copies itself during cell division. Importantly, acquired mutations in somatic cells (cells other than sperm and egg cells) cannot be passed on to the next generation. **Figure** 8.4.2



Open image description

This diagram illustrates the inheritance pattern of an autosomal recessive genetic trait when both parents are unaffected carriers. The figure shows how a carrier father and a carrier mother can produce offspring with different genotype combinations and probabilities.

At the top of the diagram are two purple human-shaped icons representing the parents. The figure on the left is labeled **"Unaffected Carrier Father"**, and the figure on the right is labeled **"Unaffected Carrier Mother."**

Below each parent are two colored circles representing their alleles. Each parent has one dominant allele labeled **R** shown in a blue circle and one recessive allele labeled **r** shown in a pink circle. This indicates that both parents have the genotype **Rr** and are carriers of the recessive trait but do not display the condition.

Black lines connect the parental alleles to the possible allele combinations in the offspring, illustrating the random inheritance of one allele from each parent.

Four possible offspring genotype combinations are shown across the bottom of the figure:

First Offspring: RR

On the far left, two blue circles each contain the letter **R**, representing the genotype **RR**. Beneath the genotype is a blue human-shaped icon labeled:

> Unaffected
> 1 in 4 chance

This offspring inherits a dominant allele from both parents and neither has the trait nor carries the recessive allele.

Second Offspring: Rr

To the right of the first offspring, one blue circle labeled **R** and one pink circle labeled **r** represent the genotype **Rr**. A purple human-shaped icon appears below, labeled:

> Unaffected Carrier
> 2 in 4 chance

This genotype represents a carrier who is unaffected but can pass the recessive allele to future offspring.

Third Offspring: Rr

The third offspring also has one blue **R** allele and one pink **r** allele, producing the genotype **Rr**. Another purple human-shaped icon appears below. Together with the previous Rr combination, these represent the two possible carrier outcomes.

Fourth Offspring: rr

On the far right, two pink circles each contain the letter **r**, representing the genotype **rr**. Below is a red human-shaped icon labeled:

> Affected
> 1 in 4 chance

This offspring inherits the recessive allele from both parents and expresses the recessive trait.

Overall, the diagram demonstrates the inheritance of a recessive trait when two carriers reproduce. The possible outcomes are:

- 25% (1 in 4) unaffected, non-carrier offspring (RR)
- 50% (2 in 4) unaffected carrier offspring (Rr)
- 25% (1 in 4) affected offspring (rr)

The figure visually represents the classic 1:2:1 genotype ratio and the corresponding 3:1 ratio of unaffected to affected offspring expected for a single-gene autosomal recessive inheritance pattern.

Figure 8.4.1**:** The red individual has inherited two mutated alleles of a gene from their parents. This is an example of a hereditary mutation. (CC BY-SA 3.0; Cburnett via Wikimedia Commons)

A tulip that is mostly red, with one petal that is half yellow.

Figure 8.4.2**:** The color variation seen in this tulip is caused by a somatic mutation – one which occurred early in the development of this individual flower. (CC BY-SA 3.0; Dmccabe via Wikimedia Commons)

Genetic changes that are described as de novo (new) mutations can be either hereditary or somatic. In some cases, the mutation occurs in a person's egg or sperm cell but is not present in any of the person's other cells. In other cases, the mutation occurs in the fertilized egg shortly after the egg and sperm cells unite. It is often impossible to tell exactly when a de novo mutation happened. As the fertilized egg divides, each resulting cell in the growing embryo will have the mutation. *De novo* mutations may explain genetic disorders in which an affected child has a mutation in every cell in the body but the parents do not, and there is no family history of the disorder.

Somatic mutations that happen in a single cell early in embryonic development can lead to a situation called mosaicism. These genetic changes are not present in a parent's egg or sperm cells, or in the fertilized egg, but happen a bit later when the embryo includes several cells. As all the cells divide during growth and development, cells that arise from the cell with the altered gene will have the mutation, while other cells will not. Depending on the mutation and how many cells are affected, mosaicism may or may not cause health problems.

Most disease-causing gene mutations are uncommon in the general population. However, other genetic changes occur more frequently. Genetic alterations that occur in more than 1 percent of the population are called polymorphisms. They are common enough to be considered a normal variation in the DNA. Polymorphisms are responsible for many of the normal differences between people such as eye color, hair color, and blood type. Although many polymorphisms have no negative effects on a person's health, some of these variations may influence the risk of developing certain disorders.

Summary

Gene expression is the process by which cells use the information in DNA to build proteins—critical molecules that support nearly every function in the body. However, the accuracy of this process depends on the integrity of the genetic instructions.

Mutations—changes in the DNA sequence—can alter how proteins are built, potentially affecting how the body functions. While some mutations are inherited and present from birth, others are acquired later in life due to environmental factors or errors during DNA replication.

Not all mutations are harmful; some have no effect at all or contribute to normal human variation. Understanding gene mutations is essential to exploring genetic disorders, human diversity, disease risk, and how genetic information is used in medicine.

Key Terms

acquired mutation
: a genetic change that occurs during a person's lifetime, affecting only certain cells. Not inherited or passed to offspring

genetic disorder
: a disease or condition caused by a mutation in one or more genes

germline mutation
: a mutation present in the egg or sperm that is passed from parent to offspring and found in all cells of the body

inherited mutation
: a genetic change passed from parents to their children through germline cells

mutation
: a permanent change in the DNA sequence of a gene that may or may not alter the function of the resulting protein

somatic mutation
: a mutation that occurs in a non-reproductive cell and is not passed to offspring

References

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8.5: Types of Mutations

Learning Objectives

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

- Define key types of gene mutations, including silent, missense, and nonsense mutations
- Predict the effects of specific mutations in gene coding regions on the protein encoded by the gene
- Compare and contrast frameshift mutations with point mutations in terms of their effects on protein structure

By definition, a mutation is a change in DNA sequence, which can occur in a number of ways. Gene mutations have varying effects on health, depending on where they occur and whether they alter the function of essential proteins. Three broad types of mutations are discussed below, although there are others they will not be discussed here.

A. DNA base substitutions

The simplest type of mutation is a substitution of one base for another in the DNA sequence. Substitutions most often arise as errors during DNA replication or repair. Depending on the location, a substitution may have no effect (silent), may change one amino acid (missense), or may introduce a stop codon (nonsense) (**Figure** 8.5.1). This will be discussed in further detail down below.



Figure 8.5.1: Example of DNA base substitution. In this example, the base G (GCTATA) is substituted for T, becoming GCTATT. Image by NIH-NHGRI.

B. Insertions & deletions

Insertions or deletions of DNA are other types of mutations that occur quite frequently. They can vary in size from one to thousands or more nucleotide bases. A deletion removes a piece of DNA (**Figure** 8.5.2). An insertion adds a piece of DNA (**Figure** 8.5.3). Insertions or deletions may be small (one or a few base pairs within a gene) or large (an entire gene, several genes, or a large section of a chromosome). In any of these cases, the protein made by the gene may not function properly.

Insertions and deletions that are in multiples of 3 are referred to as in-frame insertions and deletions and result in the addition or deletion of amino acids from the protein sequence.

When not in multiples of three, insertions and deletions lead to **frameshift mutations**, altering the amino acid sequence of the protein encoded by the mutated gene. As discussed farther bellow, their effects on gene function depend on the size of the insertion or deletion, and the location relative to the gene. (**Figure** 8.5.4)



Figure 8.5.2: In this example of deletion, the base A is deleted. ...GCTATACG... becomes ...GCTATCG... Image by NIH-NHGRI.



Figure 8.5.3: In this example of insertion, the base A is inserted between T and C. ...GCTATC... becomes ...GCTATAC... Image by NIH-NHGRI.

C. Larger-Scale DNA Mutations

While base substitutions, insertions, and deletions are some of the most common types of mutations, changes to DNA can occur on a larger scale as well. These mutations may involve the duplication or expansion of longer stretches of DNA, including entire genes or repetitive sequences. Unlike single-nucleotide changes, these types of mutations can have more widespread effects on gene expression and protein function. They are also often harder to detect and understand, since they can disrupt regulatory regions, alter gene dosage, or affect how genes are read during transcription.

- **Duplication:** A duplication consists of a piece of DNA that is abnormally copied one or more times, which can alter the size and structure of a gene. Duplications may involve a few bases or large stretches of DNA, sometimes including entire genes.This type of mutation may alter the function of the resulting protein. Charcot-Marie-Tooth (CMT) disease is the most frequent inherited neuropathy and is s often caused by duplication of the *PMP22* gene.
- **Repeat expansion:** Nucleotide repeats are short DNA sequences that are repeated a number of times in a row. For example, a trinucleotide repeat is made up of 3-base-pair sequences, and a tetranucleotide repeat is made up of 4-base-pair sequences. A repeat expansion is a mutation that increases the number of times that the short DNA sequence is repeated. This type of mutation can cause the resulting protein to function in a completely different way than it would have originally. Huntington's disease is caused by expanded CAG repeats in the *HTT* gene. People with 36 to 39 CAG repeats may or may not develop the signs and symptoms of Huntington's disease, while people with 40 or more repeats almost always develop the disorder. No cases of Huntington's disease have been diagnosed with a CAG repeat count below 36.

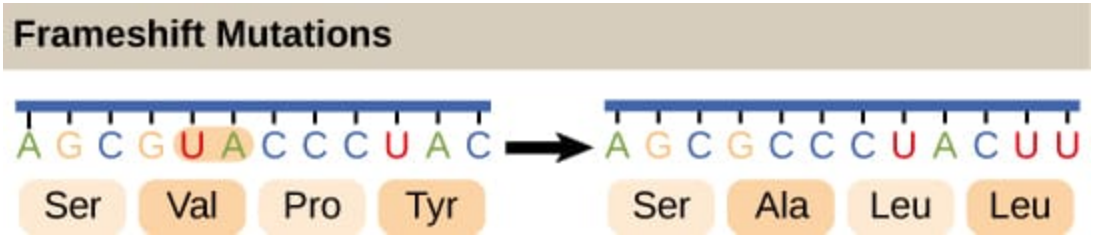
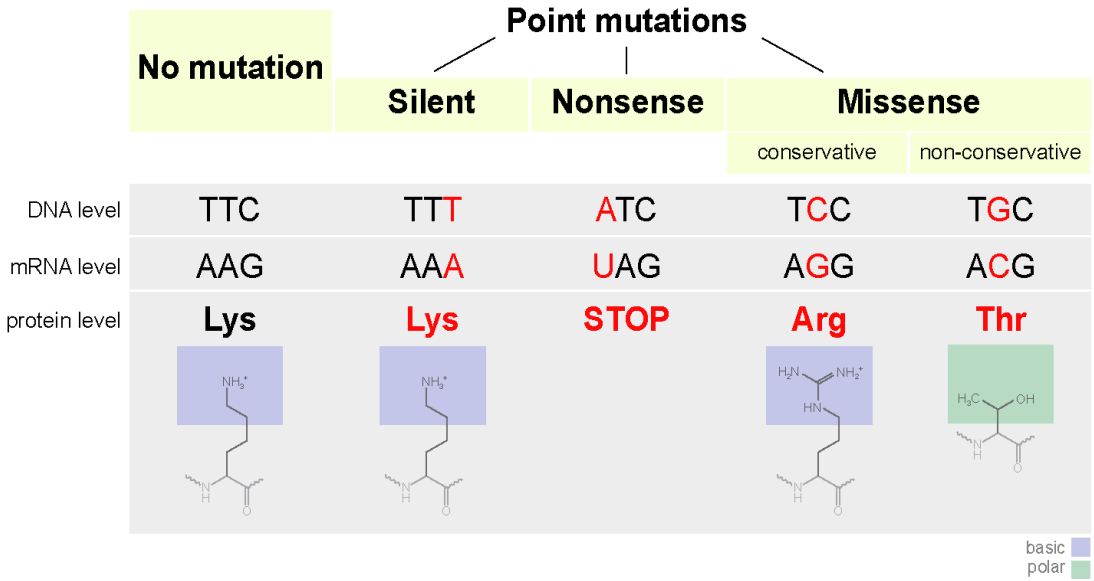


Figure 8.5.4**:** A frameshift mutation adds or deletes 1 or 2 bases. This results in a shift of the "reading frame" for the ribosome causing a drastic change in amino acid sequence. Photo Credit: OpenStax Biology. Effects of Mutations on Gene Function

- **Silent mutation:** Silent mutations cause a change in the sequence of bases in a DNA molecule, but do not result in a change in the amino acid sequence of a protein (**Figure** 8.5.5).
- **Missense mutation:** This type of mutation is a change in one DNA base pair that results in the substitution of one amino acid for another in the protein made by a gene (**Figure** 8.5.5**)**.
- **Nonsense mutation:** A nonsense mutation is also a change in one DNA base pair. Instead of substituting one amino acid for another, however, the altered DNA sequence prematurely signals the cell to stop building a protein (**Figure** 8.5.5). This type of mutation results in a shortened protein that may function improperly or not at all.



Click to open image description

This figure compares different types of point mutations and their effects on DNA, messenger RNA (mRNA), and the resulting protein. The diagram is arranged as a table with five columns labeled **No mutation**, **Silent**, **Nonsense**, and two types of **Missense** mutations: **conservative** and **non-conservative**.

The left side of the figure identifies three levels of comparison:

- **DNA level**
- **mRNA level**
- **protein level**

Nucleotides that differ from the original sequence are shown in red.

No Mutation

This column shows the original, unchanged sequence.

- **DNA codon:** TTC
- **mRNA codon:** AAG
- **Protein:** Lys (lysine)

Below the amino acid name is a structural formula of lysine. A small colored box in the background identifies lysine as a basic amino acid.

Silent Mutation

In the silent mutation example, a single nucleotide changes at the DNA level.

- **DNA codon:** TTT (the final T is highlighted in red)
- **mRNA codon:** AAA (the final A is highlighted in red)
- **Protein:** Lys (lysine)

Although the DNA and mRNA sequences differ from the original, the codon still specifies lysine. The amino acid remains unchanged, demonstrating that some mutations do not alter the protein sequence.

Nonsense Mutation

In the nonsense mutation example, a single nucleotide substitution converts a codon for an amino acid into a stop codon.

- **DNA codon:** ATC (the first A is highlighted in red)
- **mRNA codon:** UAG (the first U is highlighted in red)
- **Protein result:** STOP

The word **STOP** appears in large red letters, indicating that translation terminates prematurely and the protein would be truncated.

Missense Mutation (Conservative)

In this example, a nucleotide substitution changes one amino acid to another chemically similar amino acid.

- **DNA codon:** TCC (the middle C is highlighted in red)
- **mRNA codon:** AGG (the middle G is highlighted in red)
- **Protein:** Arg (arginine)

A structural formula of arginine appears below the amino acid name. Like lysine, arginine is shown with a colored background indicating it is a basic amino acid. Because both lysine and arginine have similar chemical properties, this mutation is classified as conservative.

Missense Mutation (Non-Conservative)

In the final example, a nucleotide substitution changes the amino acid to one with different chemical properties.

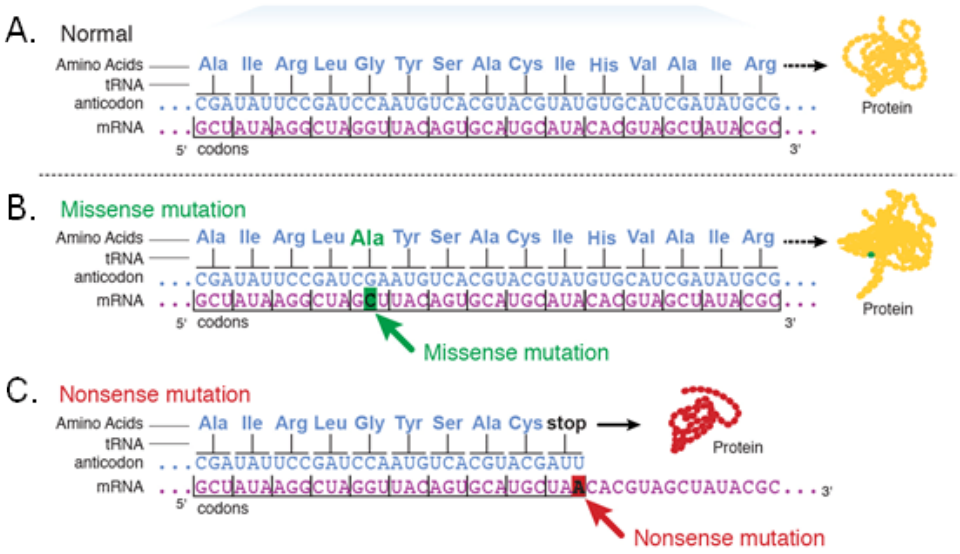
- **DNA codon:** TGC (the middle G is highlighted in red)
- **mRNA codon:** ACG (the middle C is highlighted in red)
- **Protein:** Thr (threonine)

A structural formula of threonine appears below the amino acid name with a different colored background indicating it is a polar amino acid. Because threonine has different chemical characteristics from lysine, this mutation is classified as non-conservative.

A small legend in the lower-right corner uses colored boxes to indicate amino acid properties. The purple color represents **basic** amino acids, while the green color represents **polar** amino acids.

Overall, the figure demonstrates that point mutations can have different consequences. A silent mutation changes the DNA sequence without changing the amino acid, a nonsense mutation creates a stop codon that ends protein synthesis, and missense mutations change one amino acid to another. Missense mutations may be conservative if the new amino acid has similar properties or non-conservative if its properties differ substantially from the original amino acid.

Figure 8.5.5**:** Some mutations do not change the sequence of amino acids in a protein. Some swap one amino acid for another. Others introduce an early stop codon into the sequence causing the protein to be truncated. (CC BY-SA 4.0; Jonsta247 via Wikimedia Commons)



Click to open image description

This figure compares the effects of a normal gene sequence, a missense mutation, and a nonsense mutation on the resulting protein. The diagram is divided into three horizontal panels labeled **A. Normal**, **B. Missense mutation**, and **C. Nonsense mutation**. Each panel shows an mRNA sequence, complementary tRNA anticodons, the amino acid sequence produced during translation, and a simplified drawing of the resulting protein.

A. Normal

The top panel illustrates translation of a normal mRNA sequence. Three rows are shown:

- **Amino Acids** (top row)
- **tRNA anticodon** (middle row)
- **mRNA** (bottom row)

The amino acid sequence produced from the normal mRNA is:

Ala → Ile → Arg → Leu → Gly → Tyr → Ser → Ala → Cys → Ile → His → Val → Ala → Ile → Arg

The corresponding mRNA strand is shown beneath the amino acids and is labeled from the 5′ end on the left to the 3′ end on the right. The sequence is organized into codons, each coding for one amino acid.

At the far right, a black arrow points to a compact yellow folded structure labeled **"Protein."** The tightly folded shape represents a correctly synthesized protein with its normal three-dimensional structure.

B. Missense Mutation

The middle panel shows the effect of a missense mutation. As in the first panel, amino acids, tRNA anticodons, and mRNA codons are displayed.

A single nucleotide in one codon has been altered. The changed nucleotide is highlighted in green, and a green arrow labeled **"Missense mutation"** points to this location in the mRNA sequence.

The amino acid sequence is almost identical to the normal sequence except for a single substitution:

Ala → Ile → Arg → Leu → **Ala** → Tyr → Ser → Ala → Cys → Ile → His → Val → Ala → Ile → Arg

The fifth amino acid is alanine instead of glycine. All subsequent amino acids remain unchanged.

At the far right, the resulting protein is shown as a yellow folded structure. Its shape differs somewhat from the normal protein, illustrating that replacing one amino acid can alter protein structure and potentially affect protein function.

C. Nonsense Mutation

The bottom panel illustrates a nonsense mutation. Again, amino acids, tRNA anticodons, and the mRNA sequence are shown.

A single nucleotide substitution is highlighted in red. A red arrow labeled **"Nonsense mutation"** points to the altered codon in the mRNA sequence.

In this case, the mutation converts a codon for an amino acid into a stop codon. The amino acid sequence is therefore shortened:

Ala → Ile → Arg → Leu → Gly → Tyr → Ser → Ala → Cys → **stop**

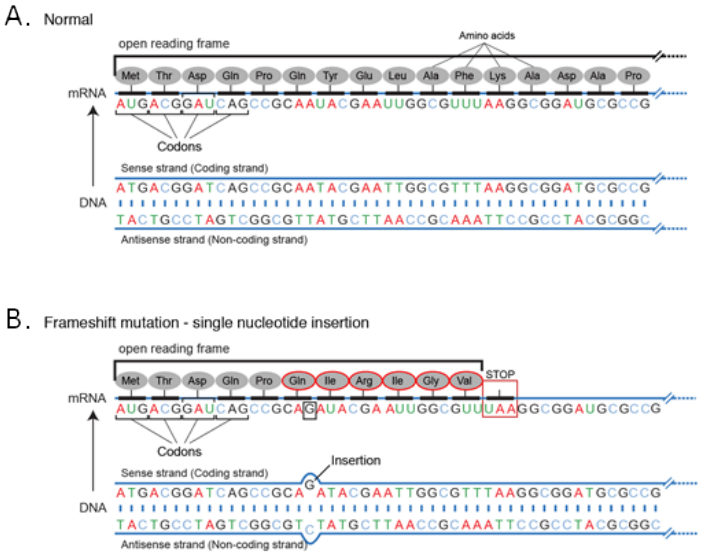
Translation terminates prematurely at the stop codon, preventing the remainder of the protein from being synthesized.

At the far right, the resulting protein is represented by a much smaller red folded structure labeled **"Protein."** The smaller size indicates that the protein is truncated because translation stopped early.

Overall, the figure demonstrates how different mutations can affect proteins. A missense mutation changes one amino acid while allowing translation to continue, potentially altering protein structure. A nonsense mutation creates a premature stop codon, resulting in a shortened protein that is often nonfunctional.

Figure 8.5.6: Impact of missense and nonsense mutations on gene function. The normal gene sequence is shown in part A. In part B, a missense mutation leads to replacement of the Gly by Ala. In part C, a nonsense mutation results in premature stop codon and shortening of the protein. Adapted from NIH-NHGRI.

- **Frameshift mutation:** This occurs when a DNA insertion or deletion changes the triplet reading frame used to translate the gene. Because codons are read in sets of three bases, any shift in this pattern can drastically alter the downstream amino acid sequence. The resulting protein is typically nonfunctional. Insertions, deletions, or duplications that are not in multiples of three can all cause frameshift mutations.(**Figure** 8.5.7).



Click to open image description

This figure compares a normal gene sequence with a frameshift mutation caused by the insertion of a single nucleotide. The diagram is divided into two panels labeled **A. Normal** and **B. Frameshift mutation - single nucleotide insertion**. Each panel shows DNA sequences, the corresponding mRNA sequence, codons, the resulting amino acid sequence, and the effect on the protein produced.

A. Normal

The top panel illustrates a gene being translated in its normal reading frame. The mRNA sequence is divided into groups of three nucleotides called codons, which are read sequentially during translation.

An arrow on the left indicates transcription from DNA to mRNA. Beneath the mRNA are two DNA strands:

- **Sense strand (coding strand)**
- **Antisense strand (non-coding strand)**

Above the mRNA is a label reading **"open reading frame"**, indicating that the codons are being read correctly from the start codon through the remainder of the sequence.

The amino acids produced from the normal sequence are shown as connected gray ovals labeled:

Met → Thr → Asp → Gln → Pro → Gln → Tyr → Glu → Leu → Ala → Phe → Lys → Ala → Asp → Ala → Pro

A bracket beneath the mRNA identifies the codons. The sequence continues uninterrupted, producing a full-length protein.

On the far right, a dashed arrow leads to a label reading **"Protein"**. The protein is represented by a compact yellow folded structure, indicating a complete protein synthesized from the correctly translated reading frame.

B. Frameshift Mutation - Single Nucleotide Insertion

The lower panel shows the consequences of inserting a single nucleotide into the DNA sequence. Most of the DNA and mRNA sequence initially resembles the normal example, but an additional nucleotide has been inserted.

A blue arrow labeled **"Insertion"** points to the specific location where the extra nucleotide has been added to the DNA sequence.

Because translation reads nucleotides in groups of three, the insertion shifts the reading frame for all downstream codons. The altered codons now specify a different sequence of amino acids.

The amino acid sequence produced after the insertion is:

Met → Thr → Asp → Gln → Pro → Gln → Ile → Arg → Ile → Gly → Val → STOP

Several amino acids after the insertion site are outlined in red, emphasizing that they differ from the amino acids in the normal sequence. The red circles surround:

Gln → Ile → Arg → Ile → Gly → Val

A stop codon appears prematurely and is labeled **"STOP"** in red. Translation ends at this point instead of continuing to produce the remainder of the normal protein sequence.

The resulting protein is not shown extending fully to the right as in the normal example. The diagram indicates that the frameshift mutation changes all downstream codons and causes early termination of translation, resulting in a shortened and potentially nonfunctional protein.

Overall, the figure demonstrates how the insertion of a single nucleotide can shift the reading frame of a gene. In the normal sequence, codons are read correctly to produce a complete protein. In the mutated sequence, the insertion changes every subsequent codon, alters multiple amino acids, creates a premature stop codon, and produces a truncated protein.

Figure 8.5.7: Impact of frameshift mutation on gene function. The normal gene sequence is shown in part A, with mRNA codons labeled. The insertion of the base G shown in part B changes the codons, creating a stop codon at UAA and resulting in a new protein. (Image by NIH-NHGRI.)

Amino acid additions or deletions

On the other hand, if insertions or deletions are in multiples of 3, amino acid additions or deletions can arise. Consider the following 3-base insertion (XXX):

Table shows codons UUU, CCC, XXX, AAA, GGG, and their corresponding amino acids aa1, aa2, aa5, aa3, aa4.

codons	UUU	CCC	XXX	AAA	GGG
amino acids	aa1	aa2	aa5	aa3	aa4

If such insertions or deletions occur in important regions of a protein, they might impair function.

Summary

Gene mutations are changes to the DNA sequence that can impact how proteins are made, potentially influencing an organism's traits, health, or development. While mutations can take many forms, they all share the common feature of altering the genetic instructions that guide protein synthesis.

Some mutations are small, involving just a single nucleotide, while others affect larger segments of DNA. A key difference lies in whether the mutation changes the actual protein product or leaves it functionally intact. Some mutations, like silent mutations, have no effect on the protein's structure or function, while others, such as frameshift or nonsense mutations, can drastically disrupt or eliminate protein production.

All mutations operate through the same fundamental mechanism: a change in the DNA code that may or may not affect the resulting protein's structure and function. Mutations are a natural part of genetic variation, but when they interfere with essential biological processes, they can lead to disease.

Key Terms

deletion mutation

a type of mutation in which one or more bases are removed from the DNA sequence, potentially altering protein structure and function

duplication mutation
: a mutation where a segment of DNA is copied one or more times, potentially changing the resulting protein's function

frameshift mutation
: a mutation caused by insertions or deletions that change the reading frame of a gene, often resulting in a nonfunctional protein

insertion mutation
: the addition of one or more nucleotides into a DNA sequence, which can disrupt protein coding

missense mutation
: a type of point mutation where one nucleotide change results in the substitution of one amino acid for another in the protein

mutation
: a change in the nucleotide sequence of DNA that may affect how proteins are made or function

nonsense mutation
: a point mutation that changes a codon into a premature stop codon, leading to a shortened, usually nonfunctional protein

point mutation
: a mutation affecting a single nucleotide in a DNA sequence; includes silent, missense, and nonsense mutations

repeat expansion
: a mutation where short DNA sequences are repeated more times than normal, potentially affecting protein function

silent mutation
: a type of point mutation where the DNA change does not alter the amino acid sequence of the protein

References

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8.6: Introduction to Genetic Disorders

Learning Objectives

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

- Explain how mutations in a gene can lead to changes in the structure and function of proteins
- Distinguish between single-gene disorders, multifactorial disorders, and chromosomal abnormalities
- Evaluate the role of genetic predisposition in complex diseases and how lifestyle choices may influence outcomes

To function correctly, each cell depends on thousands of proteins working in harmony, in the right places and at the right times. These proteins are the products of gene expression, the biological process by which the instructions stored in DNA are used to build the molecules that keep cells alive and functional. But what happens when those instructions are flawed? Sometimes, mutations in genes alter the instructions in ways that prevent a protein from being made correctly—or from being made at all. These changes can disrupt critical biological processes, resulting in a genetic disorder.

For example, cystic fibrosis is caused by mutations in a gene called CFTR. This gene normally provides instructions for making a protein that helps regulate salt and water balance in cells. However, certain mutations cause the protein to fold improperly and become nonfunctional. As a result, people with cystic fibrosis experience thick mucus buildup in their lungs and digestive systems, leading to serious health complications. It's important to note that everyone has the CFTR gene—what distinguishes those with the disease is having a mutated form of it.

Sometimes, mutations are so severe that they interfere with essential developmental processes and prevent an embryo from surviving to birth. These lethal mutations often affect genes that are crucial for early development. The fact that we don't see many of these conditions is not because they are rare, but because the embryos with them don't survive long enough to be born.

This section continues to build on our understanding of the central dogma of molecular biology by exploring what happens when the flow of information from DNA to protein is disrupted—and how this disruption plays out in real-world biological consequences.

Genetic Predispositions

A genetic predisposition (sometimes also called genetic susceptibility) is an increased likelihood of developing a particular disease based on a person's genetic makeup. A genetic predisposition results from specific genetic variations that are often inherited from a parent. These genetic changes contribute to the development of a disease but do not directly cause it. Some people with a predisposing genetic variation will never get the disease while others will, even within the same family.

Genetic variations can have large or small effects on the likelihood of developing a particular disease. For example, certain mutations in the *BRCA1* or *BRCA2* genes greatly increase a person's risk of developing breast cancer and ovarian cancer. Variations in other genes, such as *BARD1* and *BRIP1*, also increase breast cancer risk, but the contribution of these genetic changes to a person's overall risk appears to be much smaller.

Current research is focused on identifying genetic changes that have a small effect on disease risk but are common in the general population. Although each of these variations only slightly increases a person's risk, having changes in several different genes may combine to increase disease risk significantly. Changes in many genes, each with a small effect, may underlie susceptibility to many common diseases, including cancer, obesity, diabetes, heart disease, and mental illness.

In people with a genetic predisposition, the risk of disease can depend on multiple factors in addition to an identified genetic change. These include other genetic factors (sometimes called modifiers) as well as lifestyle and environmental factors. Although a person's genetic makeup cannot be altered, some lifestyle and environmental modifications (such as having more frequent disease screenings and maintaining a healthy weight) may be able to reduce disease risk in people with a genetic predisposition.

Multifactorial Disorders

Researchers are learning that nearly all conditions and diseases have a genetic component. Some disorders, such as sickle cell disease and cystic fibrosis, are caused by mutations in a single gene. The causes of many other disorders, however, are much more complex. Common medical problems such as heart disease, diabetes, and obesity do not have a single genetic cause—they are likely associated with the effects of multiple genes in combination with lifestyle and environmental factors. Conditions caused by many contributing factors are called complex or multifactorial disorders.

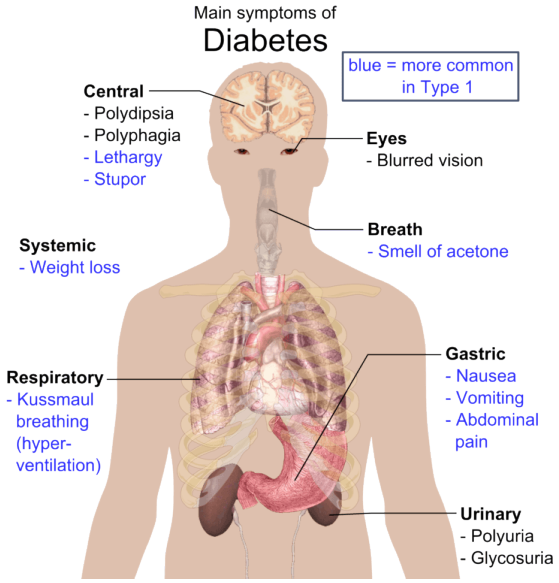


Figure 8.6.1**:** The main symptoms of diabetes, a multifactorial disorder.

Although complex disorders often cluster in families, they do not have a clear-cut pattern of inheritance. This makes it difficult to determine a person's risk of inheriting or passing on these disorders. Complex disorders are also difficult to study and treat because the specific factors that cause most of these disorders have not yet been identified. Researchers continue to look for major contributing genes for many common complex disorders.

Changes in Numbers of Gene Copies

People have two copies of most genes, one copy inherited from each parent. In some cases, however, the number of copies varies—meaning that a person can be born with one, three, or more copies of particular genes. Less commonly, one or more genes may be entirely missing. This type of genetic difference is known as copy number variation (CNV).

Copy number variation results from insertions, deletions, and duplications of large segments of DNA. These segments are big enough to include whole genes. Variation in gene copy number can influence the activity of genes and ultimately affect many body functions.

Researchers were surprised to learn that copy number variation accounts for a significant amount of genetic difference between people. More than 10 percent of human DNA appears to contain these differences in gene copy number. While much of this variation does not affect health or development, some differences likely influence a person's risk of disease and response to certain drugs. Future research will focus on the consequences of copy number variation in different parts of the genome and study the contribution of these variations to many types of disease.

Changes in Numbers of Chromosomes

Human cells normally contain 23 pairs of chromosomes, for a total of 46 chromosomes in each cell. A change in the number of chromosomes can cause problems with growth, development, and function of the body's systems. These changes can occur during the formation of reproductive cells (eggs and sperm), in early fetal development, or in any cell after birth. A gain or loss of chromosomes from the normal 46 is called aneuploidy.

A common form of aneuploidy is trisomy, or the presence of an extra chromosome in cells. “Tri-” is Greek for “three”; people with trisomy have three copies of a particular chromosome in cells instead of the normal two copies. Down syndrome is an example of a condition caused by trisomy. People with Down syndrome typically have three copies of chromosome 21 in each cell, for a total of 47 chromosomes per cell.



Figure 8.6.2**:** This karyotype, which is a picture of all the chromosomes from one individual, is from a person who has Trisomy 13. (CC BY-SA 3.0; CarloDiDio via Wikimedia Commons)

Monosomy, or the loss of one chromosome in cells, is another kind of aneuploidy. “Mono-” is Greek for “one”; people with monosomy have one copy of a particular chromosome in cells instead of the normal two copies. Turner syndrome is a condition caused by monosomy. Women with Turner syndrome usually have only one copy of the X chromosome in every cell, for a total of 45 chromosomes per cell.

Rarely, some cells end up with complete extra sets of chromosomes. Cells with one additional set of chromosomes, for a total of 69 chromosomes, are called **triploid.** Cells with two additional sets of chromosomes, for a total of 92 chromosomes, are called tetraploid. A condition in which every cell in the body has an extra set of chromosomes is not compatible with life.

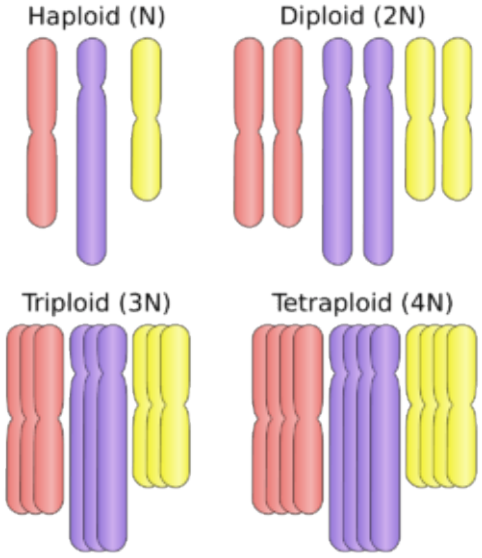


Figure 8.6.3**:** “Ploid” refers to the number of copies of each chromosome found in a somatic cell. (CC BY-SA 3.0; Ehamberg via Wikimedia Commons)



Figure 8.6.4**:** Human and other animal cells do not develop if they have an entire extra set of chromosomes. In contrast, plants often have entire copied sets of chromosomes. This strawberry is an example of a plant that is tetraploid. (CC BY-SA 3.0; David Monniaux via Wikimedia Commons)

In some cases, a change in the number of chromosomes occurs only in certain cells. When an individual has two or more cell populations with a different chromosomal makeup, this situation is called chromosomal mosaicism. Chromosomal mosaicism occurs from an error in cell division in cells other than eggs and sperm. Most commonly, some cells end up with one extra or missing chromosome (for a total of 45 or 47 chromosomes per cell), while other cells have the usual 46 chromosomes. Mosaic Turner syndrome is one example of chromosomal mosaicism. In females with this condition, some cells have 45 chromosomes because they are missing one copy of the X chromosome, while other cells have the usual number of chromosomes.

Many cancer cells also have changes in their number of chromosomes. These changes are not inherited; they occur in somatic cells (cells other than eggs or sperm) during the formation or progression of a cancerous tumor.

Do all mutations affect health and development?

No; only a small percentage of mutations cause genetic disorders—most have no impact on health or development. For example, some mutations alter a gene's DNA sequence but do not change the function of the protein made by the gene.

Often, gene mutations that could cause a genetic disorder are repaired by certain enzymes before the gene is expressed and an altered protein is produced. Each cell has a number of pathways through which enzymes recognize and repair mistakes in DNA. Because DNA can be damaged or mutated in many ways, DNA repair is an important process by which the body protects itself from disease.

A very small percentage of all mutations actually have a positive effect. These mutations lead to new versions of proteins that help an individual better adapt to changes in his or her environment. For instance, a well-known mutation in the CCR5 gene makes some people resistant to HIV infection. This genetic change prevents the virus from entering cells. Though rare, such advantageous mutations can offer protection against diseases and may become more common in populations over time.

Because a person's genetic code can have a large number of mutations with no effect on health, diagnosing genetic conditions can be difficult. Sometimes, genes thought to be related to a particular genetic condition have mutations, but whether these changes are involved in development of the condition has not been determined; these genetic changes are known as variants of unknown significance (VOUS). Sometimes, no mutations are found in suspected disease-related genes, but mutations are found in other genes whose relationship to a particular genetic condition is unknown. It is difficult to know whether these variants are involved in the disease.



Figure 8.6.5**:** This lobster contains a mutation that causes it to be blue. This is estimated to occur in roughly one in two million lobsters. (CC BY-SA 3.0; Steven G. Johnson via Wikimedia Commons)

Summary

Mutations in DNA can interfere with the production or function of proteins, leading to disruptions in normal biological processes and, in some cases, causing genetic disorders. Some disorders, such as cystic fibrosis or sickle cell disease, are caused by mutations in a single gene, while others, like heart disease, diabetes, and obesity, result from a combination of genetic and environmental factors and are classified as multifactorial disorders.

A genetic predisposition refers to an increased likelihood of developing a disease due to inherited genetic variations. However, the presence of a predisposition does not guarantee disease; factors such as lifestyle, environment, and additional genetic modifiers also play crucial roles.

Copy number variation (CNV), which involves the deletion or duplication of large segments of DNA, can influence gene dosage and affect physical traits or susceptibility to certain diseases. Changes in the number of chromosomes, including trisomy (an extra chromosome) or monosomy (a missing chromosome), can disrupt development and are responsible for conditions such as Down syndrome and Turner syndrome. In some individuals, not all cells have the same chromosomal makeup—a phenomenon known as chromosomal mosaicism.

Not all mutations are harmful. Many have no impact, some are corrected by cellular repair mechanisms, and a few may even be beneficial. Some mutations remain poorly understood, especially those classified as variants of unknown significance (VOUS), which may or may not contribute to disease.

Key Terms

aneuploidy
: a condition in which cells have an abnormal number of chromosomes, such as having one extra (trisomy) or one missing (monosomy)

chromosomal mosaicism
: a condition in which some cells in the body have different numbers or structures of chromosomes compared to others

copy number variation
: a type of genetic variation where individuals have one, three, or more copies of a gene instead of the typical two

genetic disorder
: a disease or condition caused by mutations in one or more genes or by chromosomal abnormalities

genetic predisposition
: an increased likelihood of developing a disease based on one's inherited genetic makeup

multifactorial disorder
: a disease caused by a combination of genetic, environmental, and lifestyle factors, rather than a mutation in a single gene

monosomy
: a type of aneuploidy in which one chromosome is missing from a pair

trisomy
: a form of aneuploidy where there are three copies of a particular chromosome instead of the usual two

variant of unknown significance
: a genetic change whose impact on disease or normal development is not yet known

References

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8.7: Genetic Engineering Explained - Micropigs

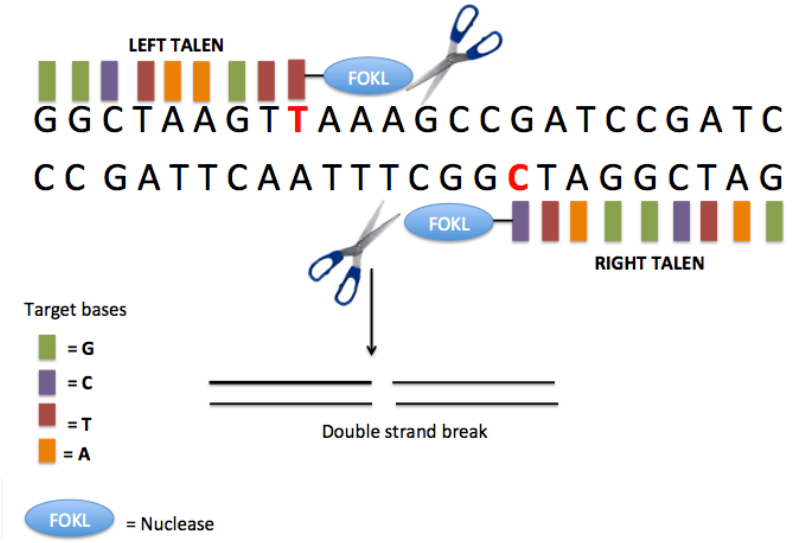
Learning Objectives

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

- Describe how TALEN technology can be used to make targeted changes to an organism's DNA
- Explain how growth hormone and the growth hormone receptor work together to regulate cell growth and development
- Predict how changes to the growth hormone receptor (GHR) gene can affect an organism's growth
- Discuss how gene-editing technologies such as TALENs can be used in biotechnology to study gene function and develop organisms with useful traits

TALENs Technique

Micropigs are tiny, genetically-edited pigs that have recently been developed by a Chinese genomics institute (Li, 2014). The Chinese scientists used a technique called TALENs to edit the genome of pig cells (**Figure** 8.7.1). Each cell inside a pig contains two copies of the growth hormone receptor gene: one from each of its parents. The TALENs technique was used to delete one of the two copies of this gene.



Click to open image description

This diagram illustrates how TALEN (Transcription Activator-Like Effector Nuclease) gene-editing technology creates a targeted double-strand break in DNA. The figure shows two engineered DNA-binding proteins, called left and right TALENs, binding to opposite sides of a specific DNA sequence and directing a nuclease enzyme to cut both strands of the DNA.

In the center of the figure is a double-stranded DNA molecule displayed as two horizontal rows of nucleotide letters. The top strand reads:

GGCTAAGTTAAAGCCGATCCGATC

The complementary bottom strand reads:

CCGATTCAATTTCGGCTAGGCTAG

Two nucleotides near the middle of the DNA sequence are highlighted in red to indicate the target region where cleavage will occur. The highlighted nucleotides are a red T on the upper strand and a red C on the lower strand.

Left TALEN

On the left side of the target DNA sequence is a structure labeled **"LEFT TALEN."** It is represented by a series of colored rectangular blocks positioned above the DNA strand. The blocks are colored green, olive, purple, red, orange, orange, olive, brown, and red.

Attached to the TALEN is a blue oval labeled **"FOKL"**, representing the FokI nuclease domain. A pair of scissors is positioned next to the FokI domain, symbolizing its DNA-cutting activity.

Right TALEN

On the right side of the target DNA sequence is a second structure labeled **"RIGHT TALEN."** This TALEN is also depicted as a series of colored rectangular blocks. The blocks extend outward from the DNA and are colored purple, brown, orange, olive, olive, purple, brown, orange, and olive.

Like the left TALEN, the right TALEN is attached to a blue oval labeled **"FOKL"** and is accompanied by a scissors icon representing the DNA-cutting function of the nuclease.

Target Base Recognition

A legend in the lower-left corner explains how the colored TALEN repeat units recognize specific DNA bases:

- Green = G
- Purple = C
- Brown-red = T
- Orange = A

This color coding illustrates how each TALEN can be engineered to bind a specific DNA sequence by arranging DNA-recognition modules in a particular order.

DNA Cleavage

Between the two TALEN proteins, the FokI nuclease domains are positioned so that they can work together. A black downward arrow points from the DNA target region toward two separated DNA fragments below.

Beneath the arrow are two pairs of horizontal black lines representing the DNA strands after they have been cut. The label below these fragments reads:

> Double strand break

At the bottom left of the figure, an additional legend identifies the blue oval:

> FOKL = Nuclease

Overall, the diagram demonstrates the mechanism of TALEN-mediated genome editing. Two custom-designed TALEN proteins bind to DNA sequences flanking a target site. Their attached FokI nuclease domains come together and cut both DNA strands at the target region, creating a double-strand break that can subsequently be repaired or modified by the cell.

Figure 8.7.1: General overview of the TALEN process. The left and right TALEN bind to a specific sequence of genomic DNA inside the nucleus of a cell. When they are correctly bound, nuclease enzymes (represented by scissors) cut the genomic DNA. The TALEN sequences can be edited by scientists to target different DNA sequences in the genome. (Photo credit: Ogletreerd, Wikimedia).

Growth Hormone

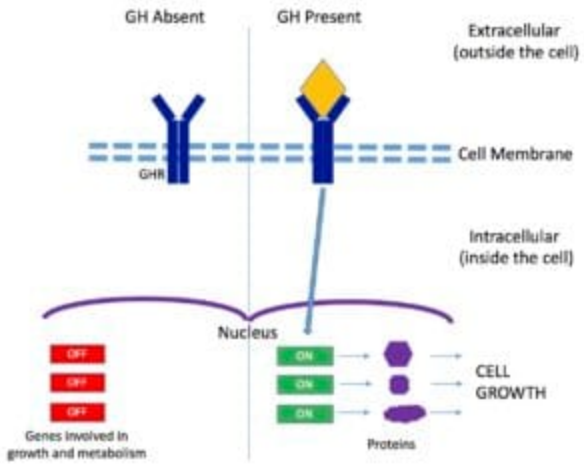
Growth hormone (GH) stimulates the growth of essentially all tissues within the body. GH is a 191 amino acid peptide (protein) hormone which is produced from the GH gene. Cells sense the presence of GH protein hormone with the growth hormone receptor (GHR) protein on the outside of the cell. GHR protein is produced from the GHR gene and is found on the cell membrane on the outside of cells.

The GHR protein has three major parts:

- An extracellular region that sticks out from the outside surface of the cell
- A transmembrane region that goes through the cell membrane and anchors the receptor to the membrane
- An intracellular region on the inside of the cell membrane that transmits signals to the interior of the cell.

The extracellular region binds (attaches) to GH, fitting together like a lock and its key. The binding of growth hormone transmits signals through the cell membrane to the intracellular region of the receptor (**Figure** 8.7.2). These signals “turn on” genes involved in growth and metabolism so that those genes are made into proteins. These proteins stimulate the growth and division of other cells in the organism.

If growth hormone is not present, the organism will not grow to full size. In humans, severe GH deficiency can lead to an adult height of only 4 feet tall. If growth hormone receptor is not present, the “grow” signal from the GH will not be transmitted inside of cells, so growth will not be stimulated (**Figure** 8.7.3).



Click to open image description

This diagram illustrates how growth hormone (GH) signaling affects gene expression and cell growth. The figure is divided into two side-by-side panels labeled **"GH Absent"** on the left and **"GH Present"** on the right. The diagram compares what happens when growth hormone is not bound to its receptor versus when growth hormone activates the receptor.

GH Absent

In the left panel, a blue Y-shaped protein is embedded in a horizontal cell membrane represented by two dashed blue lines. The receptor is labeled **"GHR"** (growth hormone receptor).

No growth hormone is attached to the receptor. Below the membrane is the intracellular portion of the cell, and above the membrane is the extracellular region.

A curved purple line represents the nucleus. Inside the nucleus are three red rectangular boxes labeled:

OFF
OFF
OFF

Beneath the boxes is the label:

Genes involved in growth and metabolism

The illustration indicates that, in the absence of growth hormone signaling, genes involved in growth and metabolism remain inactive.

GH Present

In the right panel, a yellow diamond-shaped molecule representing growth hormone is bound to the blue Y-shaped growth hormone receptor in the cell membrane.

At the top of the panel is the label:

Extracellular (outside the cell)

The area below the membrane is labeled:

Intracellular (inside the cell)

A blue signaling pathway extends from the activated receptor down toward the nucleus, representing transmission of the growth hormone signal into the cell.

Inside the nucleus, three green rectangular boxes are labeled:

ON
ON
ON

These boxes represent activated genes. Arrows point from the activated genes toward several purple protein shapes outside the nucleus.

The proteins are labeled:

Proteins

Additional arrows point from the proteins to the label:

CELL GROWTH

This portion of the diagram shows that activation of the growth hormone receptor initiates gene expression, leading to protein production and ultimately promoting cell growth.

Overall, the figure demonstrates that when growth hormone is absent, growth-related genes remain turned off. When growth hormone binds to its receptor, a signaling pathway activates genes involved in growth and metabolism, resulting in protein production and cell growth.

Figure 8.7.2**:** Growth hormone signaling pathway. When GH (growth hormone) binds to GHR (growth hormone receptor), a signal is sent through the cell membrane and into the nucleus of the cell. This signal turns on genes involved in cell metabolism and growth. (Photo credit: Lisa Bartee, 2017)



Figure 8.7.3**:** Micro pigs next to a tea cup. Image from Daily Mail article(opens in new window).

Summary

TALENs (Transcription Activator-Like Effector Nucleases) are a gene-editing technology that allows scientists to make precise changes to DNA. Two engineered TALEN proteins bind to specific DNA sequences on either side of a target gene. Each TALEN carries a FokI nuclease enzyme, which cuts both strands of the DNA when the two proteins bind correctly. The cell then repairs the break, allowing scientists to delete, insert, or modify genes. This technique was used to create micropigs by deleting one copy of the growth hormone receptor (GHR) gene, reducing growth. Normally, growth hormone (GH) binds to the GHR protein on the surface of cells, triggering signals that activate genes involved in growth and metabolism. These genes produce proteins that promote cell growth and division. If the GHR is absent or nonfunctional, cells cannot respond effectively to growth hormone,

resulting in reduced growth. TALENs demonstrate how targeted genome editing can be used to study gene function and develop animals with specific traits for research, agriculture, or potential medical applications.

Key Terms

TALENs (Transcription Activator-Like Effector Nucleases)
A gene-editing technology used to make precise changes to DNA.

Genome Editing
The process of altering an organism's DNA sequence.

Growth Hormone Receptor (GHR) Gene
The gene that encodes the receptor for growth hormone.

Growth Hormone (GH)
A protein hormone that stimulates growth and metabolism.

Growth Hormone Receptor (GHR)
A cell surface protein that binds growth hormone and initiates growth signaling.

DNA-Binding Protein
A protein engineered to recognize and bind a specific DNA sequence.

FokI Nuclease
The DNA-cutting enzyme attached to TALEN proteins.

Target DNA Sequence
The specific DNA region recognized by TALENs.

Double-Strand Break (DSB)
A cut through both strands of the DNA molecule.

DNA Repair
Cellular processes that repair broken DNA, allowing genes to be modified.

Gene Deletion (Knockout)
Removal or disruption of a gene to study its function or alter traits.

Signal Transduction
The process by which a signal at the cell surface is transmitted into the cell.

Gene Expression
The process of using genetic information to produce proteins.

Phenotype
Observable traits resulting from gene expression, such as reduced body size in micropigs.

Genetic Engineering
The deliberate modification of an organism's genetic material.

Biotechnology
The application of biological techniques, including gene editing, for research, medicine, and agriculture.

References

Li F, Li Y, Liu H, Zhang H, Liu C, Zhang X, Dou H, Yang W, Du Y. 2014 Sep. Production of GHR double-allelic knockout Bama pig by TALENs and handmade cloning. Yi Chuan: 36(9):903-11.

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8.8: Molecular Biology (Exercises)

8.1: The Structure of DNA

Multiple Choice

Which of the following does cytosine pair with?

A. guanine
B. thymine
C. adenine
D. a pyrimidine

Answer

A

Prokaryotes contain a _____chromosome, and eukaryotes contain _____ chromosomes.

A. single-stranded circular; single-stranded linear
B. single-stranded linear; single-stranded circular
C. double-stranded circular; double-stranded linear
D. double-stranded linear; double-stranded circular

Answer

C

Free Response

Describe the organization of the eukaryotic chromosome.

Answer

The DNA is wound around proteins called histones. The histones then stack together in a compact form that creates a fiber that is 30-nm thick. The fiber is further coiled for greater compactness. During metaphase of mitosis, the chromosome is at its most compact to facilitate chromosome movement. During interphase, there are denser areas of chromatin, called heterochromatin, that contain DNA that is not expressed, and less dense euchromatin that contains DNA that is expressed.

Describe the structure and complementary base pairing of DNA.

Answer

A single strand of DNA is a polymer of nucleic acids joined covalently between the phosphate group of one and the deoxyribose sugar of the next to form a “backbone” from which the nitrogenous bases stick out. In its natural state, DNA has two strands wound around each other in a double helix. The bases on each strand are bonded to each other with hydrogen bonds. Only specific bases bond with each other; adenine bonds with thymine, and cytosine bonds with guanine.

9.2: DNA Replication

Multiple Choice

DNA replicates by which of the following models?

A. conservative
B. semiconservative
C. dispersive
D. none of the above

Answer

B

The initial mechanism for repairing nucleotide errors in DNA is _____.

A. mismatch repair
B. DNA polymerase proofreading
C. nucleotide excision repair
D. thymine dimers

Answer

B

Free Response

How do the linear chromosomes in eukaryotes ensure that its ends are replicated completely?

Answer

Telomerase has an inbuilt RNA template that extends the 3' end, so a primer is synthesized and extended. Thus, the ends are protected.

9.3: Transcription

Multiple Choice

A promoter is _____.

A. a specific sequence of DNA nucleotides
B. a specific sequence of RNA nucleotides
C. a protein that binds to DNA
D. an enzyme that synthesizes RNA

Answer

A

Portions of eukaryotic mRNA sequence that are removed during RNA processing are _____.

A. exons
B. caps
C. poly-A tails
D. introns

Answer

D

9.4: Translation

Multiple Choice

The RNA components of ribosomes are synthesized in the _____.

A. cytoplasm
B. nucleus
C. nucleolus
D. endoplasmic reticulum

Answer

C

How long would the peptide be that is translated from this MRNA sequence: 5'-AUGGGCUACCGA-3'?

A. 0
B. 2
C. 3
D. 4

Answer

D

Free Response

Transcribe and translate the following DNA sequence (nontemplate strand): 5'-ATGGCCGGTTATTAAGCA-3'

Answer

The mRNA would be: 5'-AUGGCCGGUUAUUAAGCA-3'. The protein would be: MAGY. Even though there are six codons, the fifth codon corresponds to a stop, so the sixth codon would not be translated.

9.5: How Genes Are Regulated

Multiple Choice

Control of gene expression in eukaryotic cells occurs at which level(s)?

A. only the transcriptional level
B. epigenetic and transcriptional levels
C. epigenetic, transcriptional, and translational levels
D. epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels

Answer

D

Post-translational control refers to:

A. regulation of gene expression after transcription
B. regulation of gene expression after translation
C. control of epigenetic activation
D. period between transcription and translation

Answer

B

Free Response

Describe how controlling gene expression will alter the overall protein levels in the cell.

Answer

The cell controls which protein is expressed, and to what level that protein is expressed, in the cell. Prokaryotic cells alter the transcription rate to turn genes on or off. This method will increase or decrease protein levels in response to what is needed by the cell. Eukaryotic cells change the accessibility (epigenetic), transcription, or translation of a gene. This will alter the amount of RNA, and the lifespan of the RNA, to alter the amount of protein that exists. Eukaryotic cells also change the protein's translation to increase or decrease its overall levels. Eukaryotic organisms are much more complex and can manipulate protein levels by changing many stages in the process.

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9: REGULATION OF GENE EXPRESSION

Whereas each cell shares the same genome and DNA sequence, each cell does not turn on, or express, the same set of genes. Each cell type needs a different set of proteins to perform its function. Therefore, only a small subset of proteins is expressed in a cell. For the proteins to be expressed, the DNA must be transcribed into RNA and the RNA must be translated into protein. In a given cell type, not all genes encoded in the DNA are transcribed into RNA or translated into protein because specific cells in our body have specific functions. Specialized proteins that make up the eye (iris, lens, and cornea) are only expressed in the eye, whereas the specialized proteins in the heart (pacemaker cells, heart muscle, and valves) are only expressed in the heart. At any given time, only a subset of all of the genes encoded by our DNA are expressed and translated into proteins. The expression of specific genes is a highly regulated process with many levels and stages of control. This complexity ensures the proper expression in the proper cell at the proper time.

9.1: Introduction

9.2: Why Gene Regulation Matters—and How It Differs Between Prokaryotes and Eukaryotes

9.3: The Basics of Gene Regulation

9.4: Prokaryotic Gene Regulation

9.5: Eukaryotic Epigenetic Gene Regulation

9.6: Eukaryotic Transcription Gene Regulation

9.7: Eukaryotic Post-transcriptional Gene Regulation

9.8: Eukaryotic Post-translational Gene Regulation

9.9: Development

9.10: Stem Cells and Regenerative Medicine

9.11: Cancer and Gene Regulation

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9.1: Introduction

It might surprise you to learn that almost every cell in your body—whether it's in your brain, skin, liver, or muscles—contains the same DNA. But clearly, not all cells are the same. Eye cells help you see, while liver cells filter toxins and heart cells keep you alive with every beat. If they all have the same DNA, what makes them different? The answer lies not in which genes they have, but in which genes they use. Cells selectively turn genes on or off depending on what they need to do, when they need to do it, and where they are in the body. This selective use of genes is what we call gene regulation.

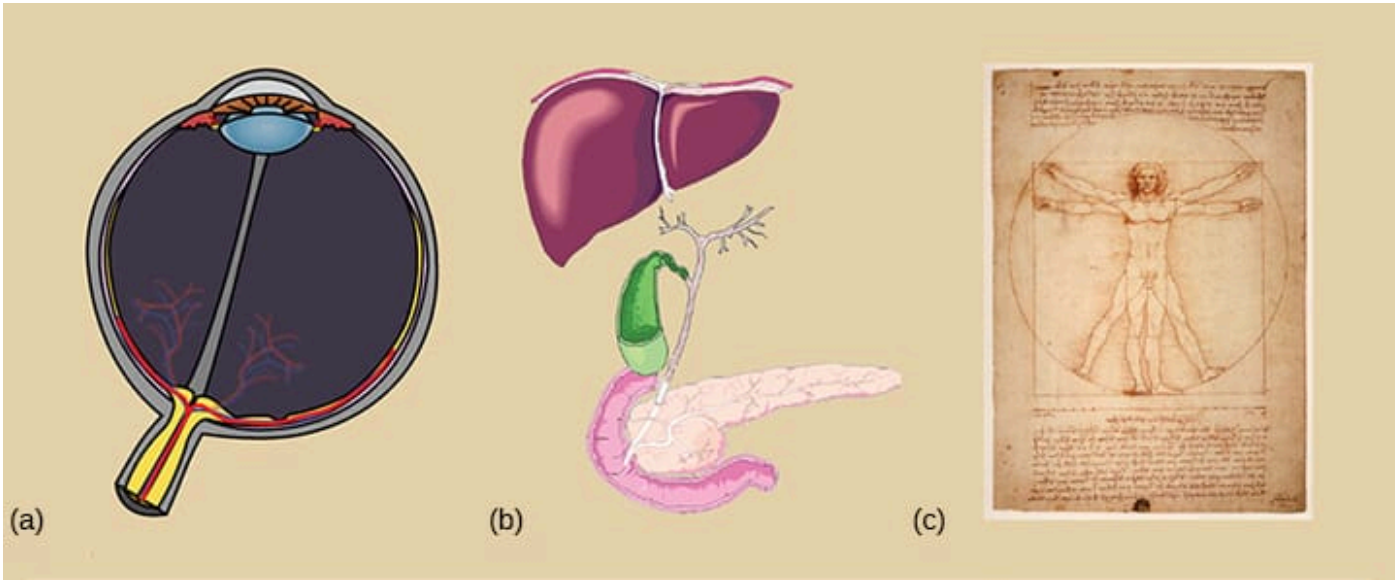


Figure 9.1.1: The genetic content of each somatic cell in an organism is the same, but not all genes are expressed in every cell. The control of which genes are expressed dictates whether a cell is, for example, (a) an eye cell or (b) a liver cell. It is the differential gene expression patterns that arise in different cells that give rise to (c) a complete organism.

Gene expression is not a one-size-fits-all or all-at-once process. It's tightly regulated and incredibly dynamic—cells can switch genes on or off in response to signals from their environment, their internal state, or developmental cues. This is why your skin tans in the sun, why your pancreas secretes insulin after a sugary snack, and why gene dysregulation can lead to diseases like cancer. It's also why red blood cells, which transport oxygen, eject their DNA entirely when they mature, and why certain immune cells rearrange their DNA to produce millions of different antibodies.

Understanding how gene expression works helps explain how complex life develops from a single cell, how our bodies adapt to change, and how scientists use this knowledge in biotechnology and medicine. From creating genetically engineered bacteria that produce insulin, to designing gene therapies that may one day correct genetic disorders at their source, the regulation of gene expression plays a central role in biology, medicine, and biotechnology.

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9.2: Why Gene Regulation Matters—and How It Differs Between Prokaryotes and Eukaryotes

Learning Objectives

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

- Discuss why every cell does not express all of its genes all of the time
- Describe how prokaryotic gene regulation occurs at the transcriptional level
- Discuss how eukaryotic gene regulation occurs at the epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels

In the last section, we introduced the idea that cells don't express every gene all the time. They carefully regulate gene expression, turning genes on or off as needed. Now, let's go deeper into why this regulation is so crucial and how it works differently depending on the type of organism.

Why Do Cells Regulate Gene Expression?

Cells regulate gene expression for several important reasons—some practical, others essential to survival. Let's unpack them.

- Energy Conservation: Making proteins takes a lot of energy. Every step—from copying DNA into RNA to building the protein itself—uses cellular resources. If a cell expressed all of its genes all the time, it would waste enormous amounts of energy on proteins it doesn't need. Regulation ensures that only the required genes are active, helping the cell use its energy wisely.
- Efficient Use of Space: Inside the nucleus of each cell, the DNA is tightly packed and wound around proteins. For a gene to be read and expressed, this DNA must be unwound and exposed. If every gene had to remain accessible at all times, cells would need to be much larger just to fit all the active DNA machinery. Instead, most genes remain tightly coiled and silent until needed—saving physical space.
- Environmental Responsiveness: Both single-celled and multicellular organisms must adapt to changes in their environment. For example, bacteria exposed to a new sugar source need to express the enzymes that help them digest it. Similarly, human cells exposed to stress, hormones, or temperature changes must adjust which genes are active. Gene regulation gives cells the flexibility to respond and survive. This relates to the chapter on how cells sense their environment.
- Functional Specialization: In multicellular organisms, cells often have specialized roles: skin cells protect, muscle cells contract, and neurons transmit signals. These cells all contain the same DNA, but they express different sets of genes. Gene regulation allows this differentiation to occur by turning on only the genes needed for a cell's specific function. Without this control, the body couldn't develop properly or maintain its organization. More on this topic in a later section on stem cells.
- Prevention of Harm: Regulation helps maintain order. If the wrong genes are turned on—or the right ones fail to turn off—serious problems can occur. For instance, unregulated cell division can lead to cancer. That's why cells have built-in checks to tightly control gene expression at multiple steps. More on this in a later section section on cancer.

A Tale of Two Cells: Why Gene Regulation Looks Different in Prokaryotes and Eukaryotes

Now that we understand why gene regulation is so important, we can explore how it works. The basic tools—like transcription factors and RNA polymerase—are shared across life. But the context in which they operate differs between prokaryotic and eukaryotic cells. These differences are rooted in two key areas:

Cell Structure

Prokaryotes (like bacteria) are simple, single-celled organisms that lack a nucleus. Their DNA floats freely in the cytoplasm, right alongside ribosomes and other molecular machinery. As a result, transcription (making RNA) and translation (making protein) can

happen at the same time and place—often even on the same RNA molecule **(Figure 9.2.1 Bacterial Cell)**.

Eukaryotes (like plants, animals, and fungi), on the other hand, have a nucleus that physically separates their DNA from the rest of the cell. Transcription happens inside the nucleus, but translation happens outside, in the cytoplasm. Because of this separation, eukaryotic gene expression involves more steps—and more opportunities for regulation **(Figure 9.2.1 Eukaryotic Cell)**.

Genome Organization

Prokaryotic genomes are smaller and more streamlined. They have relatively few genes, and most of their DNA is coding—that is, it contains instructions for making proteins. Because space is limited, prokaryotic genes are often organized into operons, groups of related genes that are turned on or off together.

Eukaryotic genomes are much larger and include a significant amount of non-coding DNA. These non-coding regions include regulatory sequences like enhancers and silencers, which play key roles in gene control. In addition, eukaryotic DNA is wrapped tightly around proteins called histones, forming chromatin—a structure that itself can block or allow access to genes.

Together, these differences explain why gene regulation in eukaryotes is generally more complex and occurs at multiple levels.

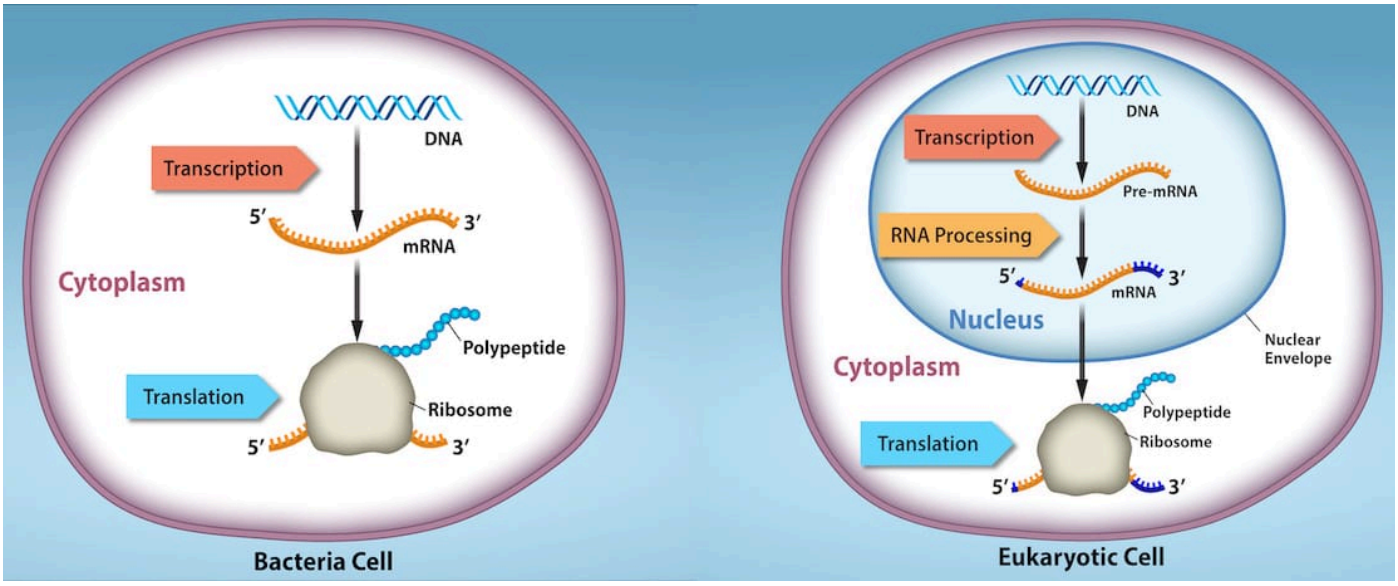


Figure 9.2.1: Regulation in prokaryotes (bacterial cells) and eukaryotes. A. Prokaryotic transcription and translation occur simultaneously in the cytoplasm as ribosomes attach to the mRNA as it is being transcribed from DNA, and regulation occurs primarily at the transcriptional level. B. Eukaryotic gene expression is regulated during transcription and RNA processing, which take place in the nucleus, and during protein translation, which takes place in the cytoplasm. Further regulation may occur through post-translational modifications of proteins in both prokaryotes and eukaryotes. Credit: Rao, A., Ryan, K. Fletcher, S. and Tag, A. Department of Biology, Texas A&M University.

When and Where Is Gene Expression Regulated?

Because of their structural and organizational differences, prokaryotes and eukaryotes emphasize different stages of regulation.

Gene Regulation in Prokaryotes: Transcription is the Main Gatekeeper

In prokaryotic cells, the primary control point is transcriptional regulation—controlling whether or not RNA is made from DNA. Once transcription begins, the rest of gene expression (translation into protein) tends to follow automatically. When the resulting protein is no longer needed, transcription stops. When more protein is required, more transcription occurs.

Because transcription and translation occur simultaneously in the cytoplasm, and there's little room or need for post-transcriptional control, most gene regulation in prokaryotes happens at the very start: deciding whether RNA polymerase can bind and initiate transcription.

Gene Regulation in Eukaryotes: Multiple Layers of Control

Eukaryotic cells, in contrast, have intracellular organelles that add to their complexity. In eukaryotic cells, the DNA is contained inside the cell's nucleus and there it is transcribed into RNA. The newly synthesized RNA is then transported out of the nucleus into the cytoplasm, where ribosomes translate the RNA into protein. The processes of transcription and translation are *physically separated* by the nuclear membrane; transcription occurs only within the nucleus, and translation occurs only outside the nucleus in the cytoplasm. The regulation of gene expression can occur at all stages of the process **(Figure** $9.2.1 Eukaryotic Cell$**)**.

As a result, eukaryotic gene regulation is more layered. Because of the extra steps required to go from DNA to protein, regulation can occur at several points along the way **(Figure** $9.2.2$**)**:

- Epigenetic regulation (chromatin modification): Regulation may occur when the DNA is uncoiled and loosened from nucleosomes to bind transcription factors
- Transcriptional regulation: This step is similar to prokaryotes but involves more factors, such as enhancers, silencers, and many transcription factors.
- Post-transcriptional regulation (RNA processing): After RNA is made, it must be processed (spliced, capped, etc.) before leaving the nucleus and going to the cytoplasm.
- Translational regulation – Once in the cytoplasm, the cell can regulate how efficiently or frequently an mRNA is translated into protein.
- Post-translational regulation – After a protein is made, it can be modified, transported to a specific location in the cel, stored for later use, activated, or broken down, affecting its function and lifespan.

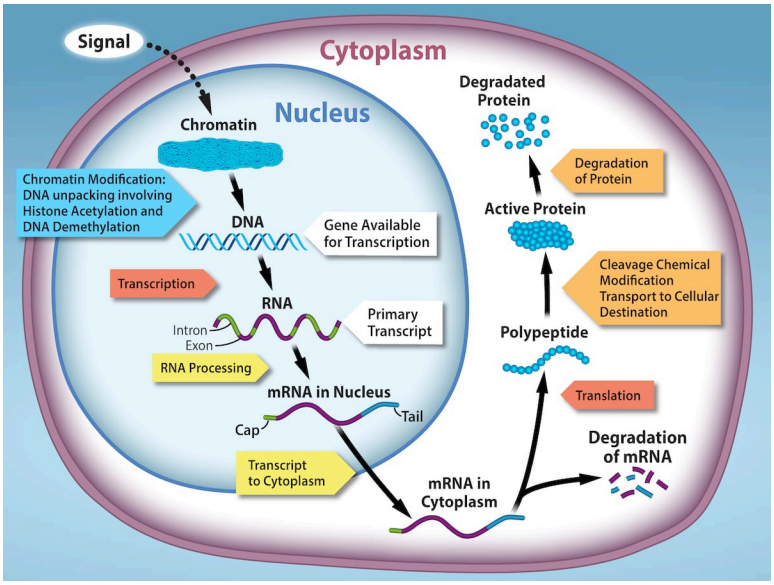


Figure $9.2.2$: Locations of gene regulation. The regulation of gene expression occurs at multiple steps going from DNA to the functional gene product, usually a protein. It begins with chromatin structure making the DNA more or less accessible for transcription by RNA polymerase. In eukaryotes, the primary mRNA transcript must be processed before it can be translated in the cytoplasm. The final level of active protein in the cell depends not only on the rate of synthesis, but also on the rate of degradation of mRNA and protein. Credit: Rao, A. and Ryan, K. Department of Biology, Texas A&M University.

The differences in the regulation of gene expression between prokaryotes and eukaryotes are summarized in **Table** $9.2.1$ The regulation of gene expression is discussed in detail in subsequent modules.

Prokaryotes: Lack a membrane-bound nucleus, DNA is found in the cytoplasm, RNA transcription and protein formation occur almost simultaneously, and gene expression is regulated primarily at the transcriptional level. Eukaryotes: Contain nucleus, DNA is confined to the nuclear compartment, RNA transcription occurs prior to protein formation, and it takes place in the nucleus, translation of RNA to protein occurs in the cytoplasm, and gene expression is regulated at many levels (epigenetic, transcriptional, post-transcriptional, translational, and post-translational).

Table 9.2.1 Differences in the Regulation of Gene Expression of Prokaryotic and Eukaryotic Organisms

Prokaryotic organisms	Eukaryotic organisms
Lack a membrane-bound nucleus	Contain nucleus
DNA is found in the cytoplasm	DNA is confined to the nuclear compartment
RNA transcription and protein formation occur almost simultaneously	RNA transcription occurs prior to protein formation, and it takes place in the nucleus. Translation of RNA to protein occurs in the cytoplasm.
Gene expression is regulated primarily at the transcriptional level	Gene expression is regulated at many levels (epigenetic, transcriptional, post-transcriptional, translational, and post-translational)

Understanding where and why gene regulation occurs lays the foundation for everything that follows. In the upcoming sections, we'll look more closely at the specific mechanisms used by prokaryotes and eukaryotes—like operons in bacteria, enhancers in eukaryotes, and how environmental signals can activate or silence genes.

By appreciating the differences in cell structure and genome organization, you'll better understand why gene regulation isn't one-size-fits-all, and why certain strategies make sense in some organisms but not others.

Gene regulation may seem like an abstract topic, but it's the basis for everything from how cells specialize in our bodies to how biotechnologists manipulate microbes to produce medicines. It's the language of control written into our DNA—and learning to read it is key to understanding life at the molecular level.

Evolution Connection

Evolution Connection - Evolution of Gene Regulation

Prokaryotic cells can only regulate gene expression by controlling the amount of transcription. As eukaryotic cells evolved, the complexity of the control of gene expression increased. For example, with the evolution of eukaryotic cells came compartmentalization of important cellular components and cellular processes. A nuclear region that contains the DNA was formed. Transcription and translation were physically separated into two different cellular compartments. It therefore became possible to control gene expression by regulating transcription in the nucleus, and also by controlling the RNA levels and protein translation present outside the nucleus.

Most gene regulation is done to conserve cell resources. However, other regulatory processes may be defensive. Cellular processes such as gene silencing developed to protect the cell from viral or parasitic infections. If the cell could quickly shut off gene expression for a short period of time, it would be able to survive an infection when other organisms could not. Therefore, the organism evolved a new process that helped it survive, and it was able to pass this new development to offspring.

Summary

While all cells within an organism contain the same DNA, not all cells within that organism express the same proteins. Prokaryotic organisms express most of their genes most of the time. However, some genes are expressed only when they are needed. Eukaryotic organisms, on the other hand, express only a subset of their genes in any given cell. To express a protein, the DNA is first transcribed into RNA, which is then translated into proteins, which are then targeted to specific cellular locations.

In prokaryotic cells, transcription and translation 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 mostly regulated at the transcriptional level (some epigenetic and post-translational regulation is also present), whereas in eukaryotic cells, gene expression is regulated at the epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels.

Key Terms

chromatin
: the complex of DNA and proteins (mainly histones) that packages eukaryotic DNA into a compact, organized structure within the nucleus

epigenetic regulation
: control of gene expression through structural changes to DNA or chromatin that affect accessibility without altering the DNA sequence

non-coding DNA
: regions of DNA that do not code for proteins but may contain regulatory elements that control gene expression

operon
: a cluster of genes in prokaryotes that are regulated and transcribed together as a single unit

post-transcriptional regulation
: control of gene expression after RNA is made but before it is translated, including splicing and export from the nucleus

post-translational regulation
: control of gene expression after a protein has been made, including modifications or degradation of the protein

regulatory DNA sequence
: non-coding segments of DNA that control the transcription of nearby genes (e.g., promoters, enhancers, silencers)

transcriptional regulation
: control of gene expression at the step where DNA is transcribed into RNA

translational regulation
: control of gene expression at the stage where RNA is translated into protein

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9.3: The Basics of Gene Regulation

Learning Objectives

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

- Describe the basic structure of a gene and the role of the promoter in initiating transcription
- Explain how transcription factors regulate gene expression through DNA binding, protein interactions, and signal responsiveness
- Distinguish between activators and repressors and their effects on transcription
- Define the function of rna polymerase and how it is guided to start transcription
- Explain how enhancers influence gene expression from a distance

Imagine a massive library. Each book in this library represents a different gene, and while the library holds the complete collection, not every book needs to be read at every moment. Some books are consulted only in emergencies. Others are read every day. And some might be useful only in certain rooms of the building. In a similar way, our cells contain all of our genes, but only some are "read" (or expressed) depending on the cell's needs, location, and external signals. This process of deciding which genes are turned "on" or "off" is called gene regulation.

At the heart of gene regulation is a process called transcription—the first step in gene expression, where the information in a gene (written in DNA) is copied into RNA. But just like a book needs a librarian to retrieve it and a reader to make sense of it, transcription involves a team of helpers that ensure the right genes are expressed at the right time.

To understand how this system works, we first need to look at how a gene is structured and how certain proteins help control access to it.

The Structure of a Gene: Where the Reading Begins

Think of a gene like a chapter in a book. Every chapter begins with a title page that signals where the reading starts. In the case of genes, this "start here" signal is a stretch of DNA called the promoter.

- Promoter: A specific DNA sequence found at the beginning of a gene. It acts as a landing site that marks where the machinery for transcription should begin copying the gene.

But a promoter by itself isn't enough. Following the promoter is the coding region of the gene—the actual part that carries the instructions for making a protein. On a DNA strand, regions (such as a promoter or a coding region) are said to be upstream of downstream. If the promoter is our point of reference, then the coding region is said to be downstream of the promoter. On the other hand, if our point of reference is the coding region, the promoter it is said to be upstream of the coding region.

The cell also needs to regulate whether that gene gets read at all. Now that we introduced the basic structure of a gene, we can introduce the proteins involved in regulating the expression of genes. That's where transcription factors come in.

Transcription Factors: The Gatekeepers of Gene Expression

Transcription factors are proteins that help determine whether a gene gets turned on or off. They act like skilled librarians, deciding which chapters of the genome should be opened and read—and which should remain closed.

To understand how they work, it helps to know that many proteins are built with distinct regions, called domains, that carry out specific tasks. For example, some proteins have a region that allows them to attach to other molecules, while another region might help send a message inside the cell. Transcription factors work in a similar way. Most have at least three important domains:

1. DNA-binding domain: This region allows the transcription factor to recognize and attach to a specific DNA sequence—often within or near the gene's promoter. The shape and chemical properties of the DNA-binding domain make it highly specific,

like a key that fits only one type of lock. The transcription factors DNA binding site, which are the regions of DNA recognized by transcription factors, are typically 10 nucleotides long. However, the length can vary from as few as 5 to over 30. This specificity ensures that different transcription factors can control different sets of genes.
2. Protein-binding domain: Transcription factors don't act alone. This domain allows them to interact with other proteins, including other transcription factors or enzymes involved in transcription. These interactions help form a functional team that regulates whether the gene is turned on or off.
3. Regulatory domain: This part of the transcription factor can be modified—turned "on" or "off"—by signals from outside the cell. These modifications (such as attaching a phosphate group) often come as a result of cell signaling pathways (such as those triggered by hormones, nutrients, or stress signals). In fact, many signals from outside the cell end up influencing which transcription factors are active inside the nucleus.

These three domains allow transcription factors to sense the state of the cell, bind the correct genes, and interact with other proteins to help control gene expression.

Activators and Repressors: Turning Genes On or Off

Some transcription factors serve as activators, helping turn a gene on by promoting transcription. Others act as repressors, keeping a gene off by blocking transcription.

You can think of this system like a dimmer switch for a light:

- Activators flip the switch and turn up the brightness.
- Repressors lower the light or keep it off entirely.

This fine-tuned control helps cells respond quickly and appropriately to changing conditions.

Both activators and repressors work by binding to DNA near the promoter or by interacting with the transcription machinery. Some can even recruit or block RNA polymerase, the enzyme responsible for making the RNA copy of the gene.

RNA Polymerase: The Copy Machine That Needs Instructions

Let's tie this back to what you already know: signal transduction. Cells constantly receive information from their environment—through hormones, nutrients, stress signals, and more. Many of these signals eventually lead to the activation or inhibition of transcription factors. Now that we've established the role of transcription factors and promoters, let's introduce the protein that does the actual "reading" of the gene: RNA polymerase.

RNA polymerase is the enzyme that copies the gene's DNA sequence into RNA. You can think of it as the copy machine in the library—it's the tool that turns a page of the genome into a usable message the cell can act on.

However, RNA polymerase doesn't automatically "know" where to start or when to begin. That's why transcription factors are so important—they help guide RNA polymerase to the right location on the DNA and either encourage or prevent it from making a copy of the gene.

In simpler organisms like bacteria, RNA polymerase can sometimes find the promoter and start transcription with only minimal assistance. But in more complex cells (like those in plants, fungi, and humans), RNA polymerase requires a whole team of transcription factors to locate the promoter, bind properly, and begin working. More on the regulation of gene expression in these two systems in the following sections.

If a repressor is in place, RNA polymerase might be blocked from binding to the promoter or from moving along the coding region of the gene. If an **activator** is present, it might help attract and stabilize RNA polymerase at the promoter, increasing the chances of transcription.

Enhancers: Long-Distance Boosters

Now that we've covered the basic setup, we can introduce a new player: the enhancer. Unlike promoters, which sit right at the start of a gene, enhancers (stretches of DNA with a specific sequence) can be located far away from the gene itself—sometimes thousands of base pairs upstream or downstream.

When certain transcription factors bind to these enhancers, the DNA loops around, bringing the enhancer closer to the gene's promoter. This physical interaction can help recruit RNA polymerase and other transcription factors, amplifying gene expression.

So even though enhancers are far from the gene's coding sequence, they act as remote control boosters—they work from a distance but can dramatically increase how strongly a gene is expressed.

Application

Everyday Examples of Gene Regulation in Action

Let's now look at some real-life scenarios that show how these components—promoters, transcription factors, RNA polymerase, activators, repressors, and enhancers—work together to control gene expression.

🔥 Example 1: Getting Warm in the Cold (Expanded)

Imagine stepping outside on a cold morning. Almost immediately, your body begins adjusting to keep you warm. This isn't just reflex—it's also a molecular process happening inside your cells.

One of the key players in warming your body is a special type of fat called brown fat (or brown adipose tissue). Unlike regular fat that mostly stores energy, brown fat is specialized for producing heat. But how does it do that?

When your skin senses cold, it sends signals to your brain, which in turn releases hormones that travel through your bloodstream. These hormones reach brown fat cells and trigger a chain reaction—a signaling pathway—inside the cell. Eventually, this pathway activates specific transcription factors.

These transcription factors enter the cell's nucleus and bind to genes that code for proteins involved in heat production. One such protein is called UCP1 (Uncoupling Protein 1), which plays a special role in the mitochondria—the part of the cell often referred to as the "powerhouse."

Normally, mitochondria use food molecules (like fats or sugars) to produce a chemical form of energy called ATP, which the cell uses to power its activities. But in brown fat, when UCP1 is present, it "uncouples" this energy production process. Instead of storing the energy as ATP, the mitochondria release it as heat—a process called non-shivering thermogenesis.

All of this depends on gene regulation: transcription factors bind to the gene's promoter, helping RNA polymerase produce the instructions for UCP1, leading to increased heat production.

🧪 Example 2: Fighting Off an Infection

Now imagine you get a small cut on your finger, and bacteria sneak in. Within moments, your immune system springs into action. Specialized white blood cells near the site of the injury detect the bacteria and send out alert signals in the form of chemical messengers called cytokines.

These cytokines travel to nearby immune cells and deliver a message: "Prepare to fight." Inside those immune cells, the message activates a series of internal signals that lead to the activation of transcription factors—such as one called NF-κB (you don't need to memorize the name, just know it's a common immune-related factor).

Once activated, this transcription factor travels into the nucleus and binds to genes involved in the immune response. These include genes that:

- Produce inflammatory molecules that help coordinate defense
- Attract more immune cells to the site
- And build receptors to detect and neutralize invaders

All of this happens quickly. Your immune cells, which previously had those genes "off," now have them turned "on"—producing new proteins in response to a real-time threat. As a result, your body fights the infection more effectively.

🍞 Example 3: Responding to a Meal

Let's say you just ate a slice of bread. Bread is rich in carbohydrates, which your body breaks down into glucose, a sugar that enters your bloodstream. Your body now needs to manage that sugar, so it doesn't overwhelm your system.

In response, your pancreas releases the hormone insulin, which helps cells absorb the glucose from the blood. When insulin reaches liver and muscle cells, it binds to receptors on their surfaces, triggering a signal inside the cells.

This signal turns on transcription factors that enter the nucleus and activate genes involved in storing glucose. One of these genes' codes for glycogen synthase, an enzyme that helps convert glucose into glycogen—a storage form of sugar.

With this gene turned on, your cells begin producing glycogen synthase protein. The protein then goes to work, assembling glucose molecules into glycogen so they can be stored for later use.

Understanding how genes are turned on or off—and the roles of transcription factors, promoters, enhancers, and RNA polymerase—is essential for understanding how cells function. These same components are the foundation for many modern biotechnologies that seek to harness or control gene expression.

Over the next few sections, we'll explore how this process plays out in both prokaryotic (bacterial) and eukaryotic (plant, animal, and fungal) cells. While the core components are shared, the ways they are organized and regulated vary— and understanding those differences will deepen our grasp of how gene regulation works across biological systems

Summary

Gene regulation controls when and how genes are expressed, allowing cells to respond to their environment. Each gene contains a promoter that signals where transcription should begin, and the presence and activation statuse of transcription factors determine whether the gene is turned on or off. These proteins bind specific dna sequences and interact with other molecules, including RNA polymerase—the enzyme that copies dna into RNA. Activators promote gene expression, while repressors block it.

Some genes also use enhancers, DNA sequences that act from a distance to boost transcription. Gene regulation enables precise cellular responses, as seen in examples like heat production, immune responses, and sugar storage.

Key Terms

activator
: a protein that increases gene expression by helping initiate transcription

coding region
: the part of a gene that contains instructions for making a protein

DNA-binding domain
: a part of a transcription factor that allows it to recognize and attach to specific DNA sequences

enhancer
: a distant DNA sequence that boosts the expression of a gene by interacting with activators and looping toward the promoter

gene
: a segment of DNA that carries the instructions for making a specific protein

promoter
: a DNA sequence at the start of a gene that signals where transcription should begin

protein-binding domain
: a region of a transcription factor that allows it to interact with other proteins

repressor
: a protein that prevents or decreases gene expression, often by blocking transcription

RNA polymerase
: the enzyme that reads the DNA sequence of a gene and makes an RNA copy

transcription factor
: a protein that regulates gene expression by binding to specific DNA sequences and interacting with other regulatory proteins

upstream/downstream
: terms describing locations along DNA relative to a point of refrence; if point of reference is the start of the gene upstream is before the gene, downstream is after

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9.4: Prokaryotic Gene Regulation

Learning Objectives

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

- Describe the steps involved in prokaryotic gene regulation
- Explain the roles of activators, inducers, and repressors in gene regulation

The DNA of prokaryotes is organized into a circular chromosome, supercoiled within the nucleoid region of the cell cytoplasm. Proteins that are needed for a specific function, or that are involved in the same biochemical pathway, are encoded together in blocks called operons. For example, all of the genes needed to use lactose as an energy source are coded next to each other in the lactose (or *lac*) operon, and transcribed into a single mRNA.

In prokaryotic cells, there are three types of regulatory molecules that can affect the expression of operons: repressors, activators, and inducers. Repressors and activators are proteins produced in the cell. Both repressors and activators regulate gene expression by binding to specific DNA sites *adjacent* to the genes they control. *In general, activators bind to the promoter site, while repressors bind to operator regions*. Repressors prevent transcription of a gene in response to an external stimulus, whereas activators increase the transcription of a gene in response to an external stimulus. Inducers are small molecules that may be produced by the cell or that are in the cell's environment. Inducers either activate or repress transcription depending on the needs of the cell and the availability of substrate.

The *trp* Operon: A Repressible Operon

Bacteria such as *Escherichia coli* need amino acids to survive, and are able to synthesize many of them. Tryptophan is one such amino acid that *E. coli* can either ingest from the environment or synthesize using enzymes that are encoded by five genes. These five genes are next to each other in what is called the tryptophan (*trp*) operon (**Figure** 9.4.1). The genes are transcribed into a single mRNA, which is then translated to produce all five enzymes. If tryptophan is present in the environment, then *E. coli* does not need to synthesize it and the *trp* operon is switched off. However, when tryptophan availability is low, the switch controlling the operon is turned on, the mRNA is transcribed, the enzyme proteins are translated, and tryptophan is synthesized.

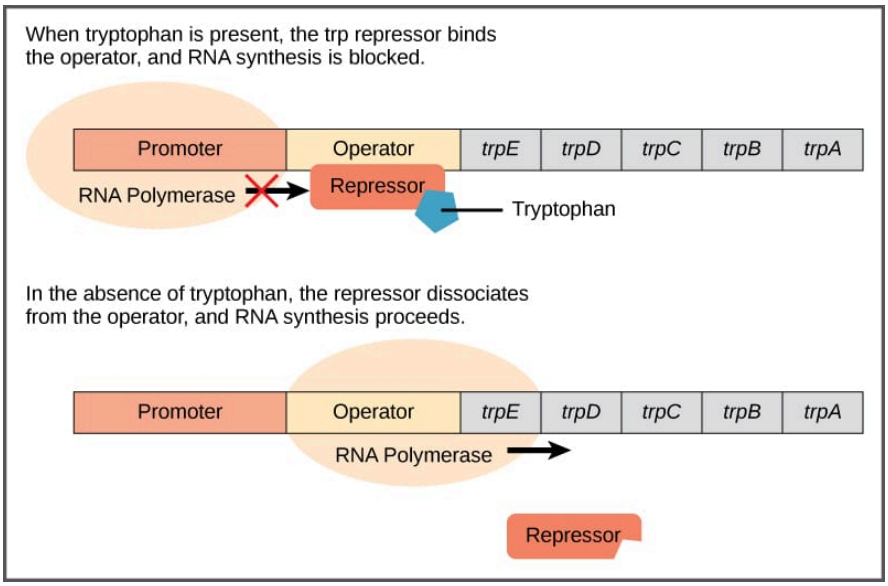


Figure 9.4.1: The trp operon has a promoter, an operator, and five genes named trpE; trpD, trpC, trpB, and trpA that are located in sequential order on the DNA. RNA polymerase binds to the promoter. When tryptophan is present, the trp repressor binds the operator and prevents the RNA polymerase from moving past the operator; therefore, RNA synthesis is blocked. In the absence of tryptophan, the repressor dissociates from the operator. RNA polymerase can now slide past the operator, and transcription begins.

The *trp* operon includes three important regions: the coding region, the *trp* operator and the *trp* promoter. The coding region includes the genes for the five tryptophan biosynthesis enzymes. Just before the coding region is the transcriptional start site. The promoter sequence, to which RNA polymerase binds to initiate transcription, is before or "upstream" of the transcriptional start site. Between the promoter and the transcriptional start site is the operator region.

The *trp* operator contains the DNA code to which the *trp* repressor protein can bind. However, the repressor alone cannot bind to the operator. When tryptophan is present in the cell, two tryptophan molecules bind to the *trp* repressor, which changes the shape of the repressor protein to a form that can bind to the *trp* operator. Binding of the tryptophan–repressor complex at the operator physically prevents the RNA polymerase from binding to the promoter and transcribing the downstream genes.

When tryptophan is not present in the cell, the repressor by itself does not bind to the operator, the polymerase can transcribe the enzyme genes, and tryptophan is synthesized. Because the repressor protein actively binds to the operator to keep the genes turned off, the *trp* operon is said to be *negatively regulated* and the proteins that bind to the operator to silence *trp* expression are negative regulators.

Link to Learning

Watch this video to learn more about the *trp* operon.

Click to view content(opens in new window)

Interactive Content

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View this Interactive Content online

Catabolite Activator Protein (CAP): A Transcriptional Activator

Just as the *trp* operon is negatively regulated by tryptophan molecules, there are proteins that bind to the promoter sequences that act as positive regulators to turn genes on and activate them. For example, when glucose is scarce, *E. coli* bacteria can turn to other sugar sources for fuel. To do this, new genes to process these alternate sugars must be transcribed. When glucose levels drop, cyclic AMP (cAMP) begins to accumulate in the cell. The cAMP molecule is a signaling molecule that is involved in glucose and energy metabolism in *E. coli*. Accumulating cAMP binds to the positive regulator catabolite activator protein (CAP), a protein that binds to the promoters of operons which control the processing of alternative sugars. When cAMP binds to CAP, the complex then binds to the promoter region of the genes that are needed to use the alternate sugar sources **Figure** 9.4.2. In these operons, a CAP-binding site is located upstream of the RNA-polymerase-binding site in the promoter. CAP binding stabilizes the binding of RNA polymerase to the promoter region and increases transcription of the associated protein-coding genes.

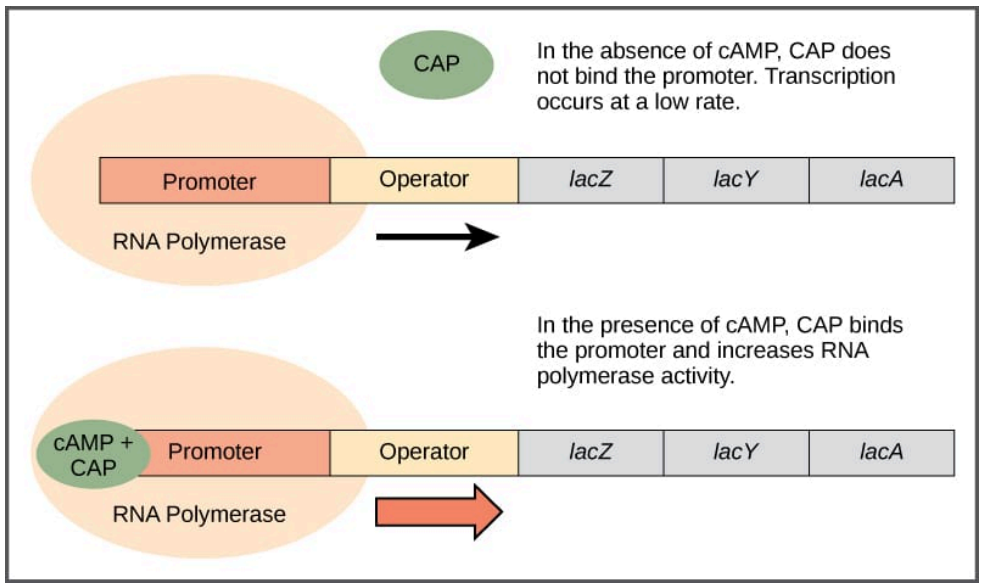


Figure 9.4.2: The lac operon consists of a promoter, an operator, and three genes named lacZ, lacY, and lacA that are located in sequential order on the DNA. In the absence of cAMP, the CAP protein does not bind the DNA. RNA polymerase binds the promoter, and transcription occurs at a slow rate. In the presence of cAMP, a CAP, cAMP complex binds to the promoter and increases RNA polymerase activity. As a result, the rate of RNA synthesis is increased.

The *lac* Operon: An Inducible Operon

The third type of gene regulation in prokaryotic cells occurs through *inducible operons*, which have proteins that bind to activate or repress transcription depending on the local environment and the needs of the cell. The *lac* operon is a typical inducible operon. As mentioned previously, *E. coli* is able to use other sugars as energy sources when glucose concentrations are low. One such sugar source is lactose. The *lac* operon encodes the genes necessary to acquire and process the lactose from the local environment. The Z gene of the *lac* operon encodes beta-galactosidase, which breaks lactose down to glucose and galactose.

However, for the *lac* operon to be activated, two conditions must be met. First, the level of glucose must be very low or non-existent. Second, lactose must be present. Only when glucose is absent and lactose is present will the *lac* operon be transcribed (**Figure** 9.4.3). In the absence of glucose, the binding of the CAP protein makes transcription of the *lac* operon more effective. When lactose is present, its metabolite, allolactose, binds to the *lac* repressor and changes its shape so that it cannot bind to the *lac* operator to prevent transcription. This combination of conditions makes sense for the cell, because it would be energetically wasteful to synthesize the enzymes to process lactose if glucose was plentiful or lactose was not available. It should be mentioned that the lac operon is transcribed at a very low rate even when glucose is present and lactose absent.

Visual Connection

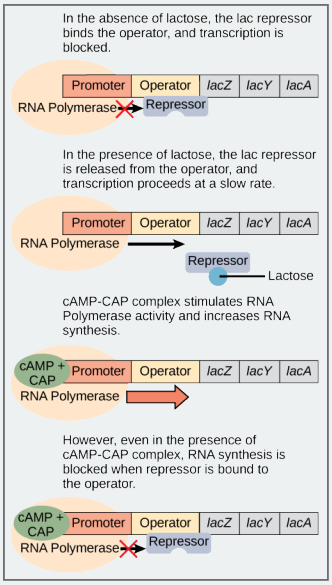


Figure 9.4.3 Regulation of the *lac* operon. Transcription of the *lac* operon is carefully regulated so that its expression only occurs when glucose is limited and lactose is present to serve as an alternative fuel source. The lac operon consists of a promoter, an operator, and three genes named lacZ, lacY, and lacA. RNA polymerase binds to the promoter. In the absence of lactose, the lac repressor binds to the operator and prevents RNA polymerase from transcribing the operon. In the presence of lactose, the repressor is released from the operator, and transcription proceeds at a slow rate. Binding of the cAMP plus sign CAP complex to the promoter stimulates RNA polymerase activity and increases RNA synthesis. However, even in the presence of the cAMP plus sign CAP complex, RNA synthesis is blocked if the repressor binds to the promoter.

In *E. coli*, the *trp* operon is on by default, while the *lac* operon is off. Why do you think this is the case?

If glucose is present, then CAP fails to bind to the promoter sequence to activate transcription. If lactose is absent, then the repressor binds to the operator to prevent transcription. If either of these conditions is met, then transcription remains off. Only when glucose is absent and lactose is present is the *lac* operon transcribed (**Table** 9.4.1).

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	-	-	+	No
+	-	+	-	Some
-	+	-	+	No
-	+	+	-	Yes

Table 9.4.1: Signals that Induce or Repress Transcription of the *lac* Operon.

Link to Learning

Watch an animated tutorial about the workings of *lac* operon here.

Click to view content(opens in new window)

Interactive Content

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Summary

Prokaryotic cells regulate gene expression by controlling transcription through groups of related genes called operons. An operon contains multiple genes that are transcribed together into a single mRNA, allowing bacteria to quickly respond to environmental changes. Three main regulatory molecules are involved: repressors, which block transcription; activators, which increase transcription; and inducers, small molecules that activate or inhibit these regulatory proteins. The trp operon is a repressible operon that controls tryptophan synthesis. When tryptophan is abundant, it binds to a repressor protein, allowing it to block transcription and prevent unnecessary enzyme production. When tryptophan is scarce, the operon is expressed. In contrast, the lac operon is an inducible operon that enables lactose metabolism. It is activated only when lactose is present and glucose is absent. Under low-glucose conditions, the CAP-cAMP complex enhances RNA polymerase binding, while lactose inactivates the lac repressor, allowing transcription. These regulatory mechanisms help bacteria conserve energy by producing proteins only when they are needed.

Key Terms

activator
: a protein that increases gene transcription when bound to DNA

catabolite
: a molecule produced when larger molecules are broken down

inducible operon
: a gene system that is normally off but can be turned on when needed

operator
: a segment of DNA where regulatory proteins bind to control gene expression

operon
: a group of related genes (found mainly in bacteria) that are controlled together by a single on/off switch, allowing the cell to turn several genes on or off at the same time

proteins
: are molecules made of amino acids that performs most of the cells work

repressible operon
: a gene system that is normally on but can be turned off when enough product is present

repressor
: a protein that binds to DNA to block transcription

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9.5: Eukaryotic Epigenetic Gene Regulation

Learning Objectives

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

- Explain how chromatin remodeling influences access to DNA for transcription
- Describe how access to DNA is controlled by histone modification
- Describe how DNA methylation is related to epigenetic gene changes

In previous sections, we learned that gene regulation allows cells to control which proteins they make, when, and how much. This ability is vital for saving energy, managing resources, and allowing cells to perform specialized functions. In prokaryotic cells like bacteria, most gene regulation happens by controlling whether transcription starts at all. But in eukaryotic cells—those with a nucleus and a more complex internal structure—gene regulation is more layered and more intricate.

One of the earliest and most important layers of control in eukaryotic gene expression is epigenetic regulation. This type of regulation helps determine which parts of the DNA are open and accessible for transcription and which parts are closed off. What's fascinating is that epigenetic changes do not alter the DNA sequence itself. Instead, they change how the DNA is packaged, shaping the physical structure of chromosomes in a way that determines whether a gene is turned “on” or “off.” This allows cells to inherit and maintain patterns of gene expression—even across generations of cells—without changing the actual genetic code.

Access to DNA: The Role of Chromatin and Histones

Imagine trying to read a book that’s been tightly sealed shut. No matter how interesting the content is, you can’t read the pages unless the cover is opened and the pages are loosened. DNA works the same way. In the nucleus of a eukaryotic cell, DNA is tightly wound and folded into structures called chromatin to fit inside the tiny space. This folding is organized and not random—it's carefully structured to allow only the necessary sections of DNA to be accessible in each type of cell.

The first level of this organization is the wrapping of DNA around special proteins called histones. Together, a histone with its coiled DNA forms a unit called a nucleosome. **Figure** $9.5.1a$. Under the electron microscope, this winding of DNA around histone proteins to form nucleosomes looks like small beads on a string **Figure** $9.5.1b$.

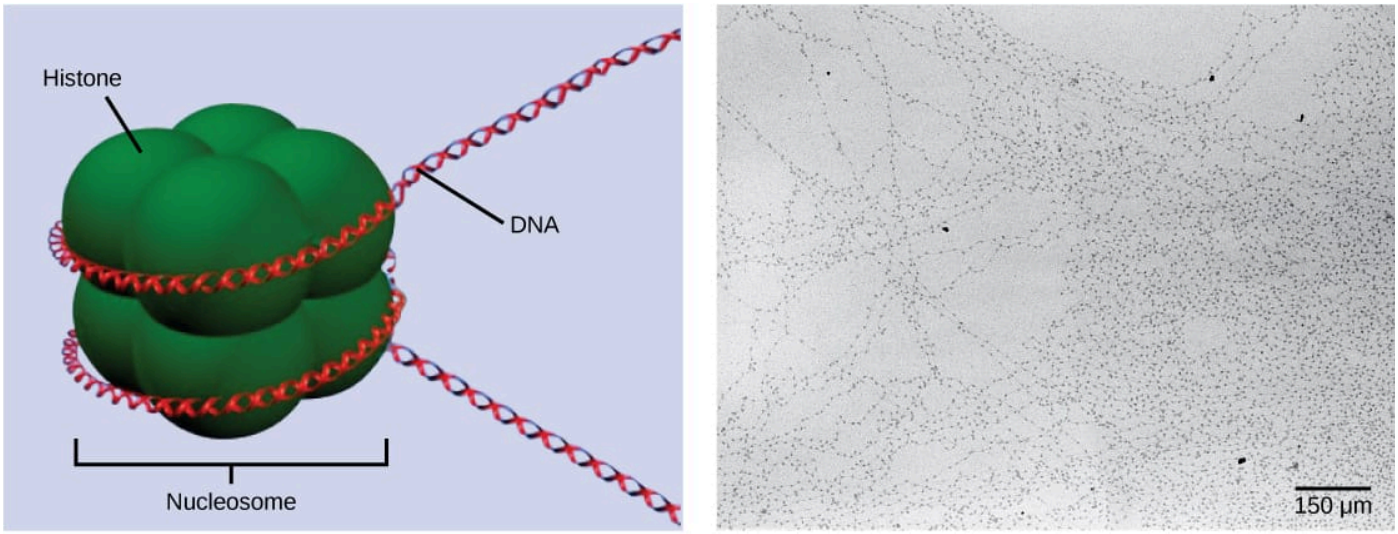


Figure 9.5.1: DNA is folded around histone proteins to create (a) nucleosome complexes composed of spherical histone proteins that are fused together. A double-stranded D N A helix wraps around the nucleosome twice. Free DNA extends from either end of the nucleosome. These nucleosomes control the access of proteins to the underlying DNA. When viewed through an electron microscope (b), the nucleosomes look like beads on a string connected by free DNA. (credit “micrograph”: modification of work by Chris Woodcock.

These beads aren't locked in place—they can slide along the DNA. This movement is known as chromatin remodeling. When the histones shift away from a gene’s promoter region, they expose the DNA so that RNA polymerase and other transcription factors can bind and begin transcription. When nucleosomes remain tightly packed around a gene, they block access and the gene stays silent (**Figure** 9.5.1**)**.

Histone Modifications: Chemical Tags

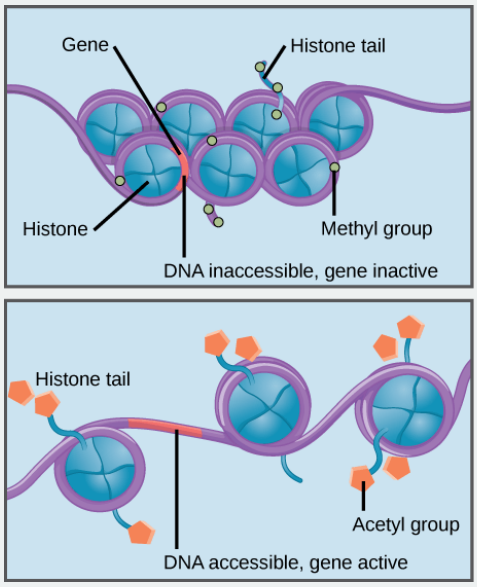
To better understand how histones can receive and respond to these signals, it helps to remember that histones are proteins, and like many proteins, they are made up of functional domains—each with a specific role. First, histones contain a DNA-binding domain, which allows them to interact with the negatively charged DNA. This interaction is possible because histones are rich in positively charged amino acids, helping them wrap DNA tightly like a magnetic spool. Second, histones have protein-protein interaction domains that enable eight histone proteins to assemble into a nucleosome—the bead-like core that DNA wraps around. These interactions are carefully coordinated to create a stable structure that can hold DNA in place.

Finally, each histone has tail regions—small extensions that stick out from the nucleosome structure. These histone tails are crucial because they are the sites of chemical modification. While there are a number of chemical modifications used to regulate chromatin remodeling, we will only discuss two key types of modifications. They are:

- Acetylation – the addition of an acetyl group to the histone tail. This loosens the DNA-histone interaction, making the DNA easier to access.
- Methylation – the addition of a methyl group to the histone tail. This generally tightens the DNA, making it harder to access.

These modifications are shown in **Figure** 9.5.2.

How are these modifications able to control how closely the histone proteins associate with the DNA is surprisingly simply. DNA is a negatively charged molecule and unmodified histones are positively charged; therefore, changes in the charge of the histone will change how tightly wound the DNA molecule will be. By adding chemical modifications like acetyl groups, the charge becomes less positive, and the binding of DNA to the histones is relaxed. Altering the location of nucleosomes and the tightness of histone binding opens some regions of chromatin to transcription and closes others.



Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

Figure 9.5.2: Nucleosomes made of histones can slide along DNA. Each histone has a tail, when DNA and the histone tails are methylated, the nucleosomes pack tightly together. When nucleosomes are spaced closely together (top), transcription factors cannot bind and gene expression is turned off. When the histone tails are acetylated, they are spaced far apart (bottom), asnd the DNA is exposed. Transcription factors can bind, allowing gene expression to occur. Modifications to the histones and DNA affect nucleosome spacing.

In females, one of the two X chromosomes is inactivated during embryonic development because of epigenetic changes to the chromatin. What impact do you think these changes would have on nucleosome packing?

DNA Methylation: Silencing at the Source

Histones aren't the only part of chromatin that can be tagged. The DNA itself can also be modified through a process called DNA methylation, where a small methyl group is added to a cytosine base in the DNA—often at locations called CpG islands. These stretches of DNA are typically found in promoter regions. When methyl groups are added here, the gene is often silenced—like putting a sticky note over the "on" switch.

This silencing plays a critical role in development, cell differentiation, and even X-chromosome inactivation in females, where one of the two X chromosomes in each cell is mostly shut down. These changes can sometimes be passed down from parent to offspring, a phenomenon known as genomic imprinting. Intriguingly, factors like diet, aging, stress, or environmental exposure can influence DNA methylation, affecting gene expression in ways that may last a lifetime—or beyond.

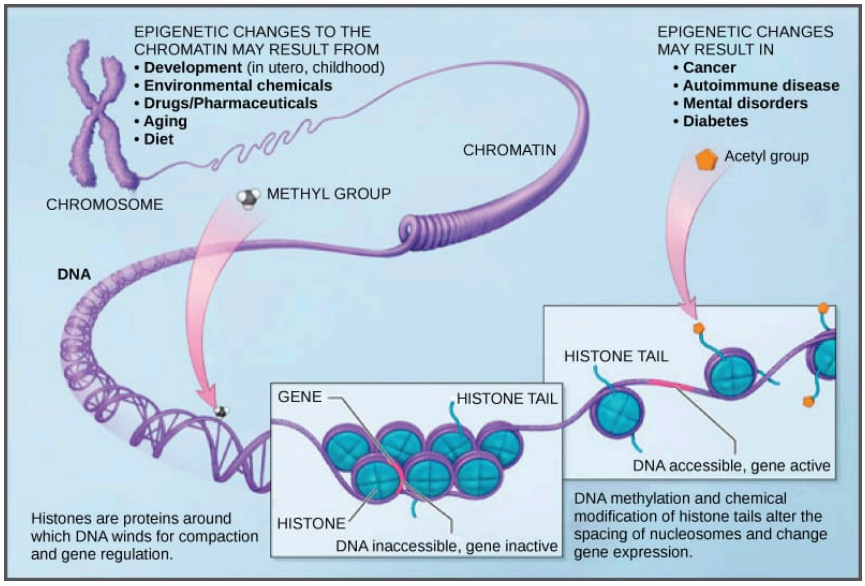


Figure 9.5.3: Illustration shows a chromosome that is partially unraveled and magnified, revealing histone proteins wound around the DNA double helix. Histones are proteins around which DNA winds for compaction and gene regulation. Methylation of DNA and chemical modification of histone tails are known as epigenetic changes. Epigenetic changes alter the spacing of nucleosomes and change gene expression. They may result from development, either in utero or in childhood, environmental chemicals, drugs, aging, or diet, and can cause cancer, autoimmune disease, mental disorders, and diabetes. (credit: modification of work by NIH)

Epigenetic changes are not permanent, although they often persist through multiple rounds of cell division and may even cross generational lines. Chromatin remodeling alters the chromosomal structure (open or closed) as needed. If a gene is to be transcribed, the histone proteins and DNA in the chromosomal region encoding that gene are modified in a way that opens the promoter region to allow RNA polymerase and other proteins, called transcription factors, to bind and initiate transcription. If a gene is to remain turned off, or silenced, the histone proteins and DNA have different modifications that signal a closed chromosomal configuration. In this closed configuration, the RNA polymerase and transcription factors do not have access to the DNA and transcription cannot occur **Figure** 9.5.3.

The Dynamic Nature of Epigenetic Regulation

One of the most amazing features of epigenetic regulation is that it's reversible. These chemical tags can be added or removed in response to a cell's needs or its environment. Epigenetic changes don't rewrite the genetic instructions—they change how and when those instructions are read.

Think of DNA as an enormous instruction manual containing all the information a cell might ever need. Epigenetic regulation doesn't rip out pages or change the wording—it simply adds bookmarks and sticky notes, highlighting which sections should be read and which should stay closed, depending on the cell's needs. This flexible system makes it possible for different cell types—like muscle cells, skin cells, and neurons—to all use the same DNA in very different ways. Just as you wouldn't read every page of a manual at once, cells only access the genes they need at a given time. But here's the key connection: only the sections of DNA that are "unwrapped" from histones—that is, located in loose, open chromatin—are available for transcription. Epigenetic regulation doesn't initiate transcription itself, but it determines which genes are even eligible to be transcribed. In this way, epigenetic control acts as the first gatekeeper, setting the stage for everything that follows in gene expression.

Link to Learning

View this video that describes how epigenetic regulation controls gene expression.

YouTube Video

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Connection

A Patchwork of Color: Calico Cats and the Power of Permanence

We often describe epigenetic changes as reversible, meaning they can be added or removed depending on a cell's environment or needs. But there's one fascinating and beautiful exception that demonstrates how epigenetics can also create permanent and visible effects—and it comes in the form of calico cats.

Calico cats are almost always female and have distinctive patchy fur, with areas of black, orange, and white. The reason for this patchwork coloring lies in a process called X-chromosome inactivation, a dramatic example of long-term epigenetic gene silencing. In mammals, females have two X chromosomes (XX), while males have only one (XY). But having two active copies of the X chromosome in every cell would create an imbalance. To correct for this, one of the X chromosomes in each female cell is randomly and permanently turned off during early development. This is an epigenetic process: the DNA on the silenced X chromosome is wrapped so tightly in chromatin and heavily methylated that it can no longer be transcribed.

What's truly mind-blowing is that once a particular X chromosome is turned off in a cell, all of its descendant cells will keep the same X silenced. In calico cats, the genes responsible for black and orange fur color are located on the X chromosome. Because X inactivation happens randomly in each cell early in development, some patches of fur express the orange version of the gene and others express the black version, depending on which X chromosome remained active.

So while epigenetic changes are often reversible, X-chromosome inactivation is a notable exception. It shows how epigenetics can create lasting, visual differences in an organism—even when all the DNA is the same.

Summary

In eukaryotic cells, the first stage of gene-expression control occurs at the epigenetic level. Epigenetic mechanisms control access to the chromosomal region to allow genes to be turned on or off. Chromatin remodeling controls how DNA is packed into the nucleus by regulating how tightly the DNA is wound around histone proteins. The DNA itself may be methylated to selectively silence genes. The addition or removal of chemical modifications (or flags) to histone proteins or DNA signals the cell to open or close a chromosomal region. Therefore, eukaryotic cells can control whether a gene is expressed by controlling accessibility to the binding of RNA polymerase and its transcription factors.

Key Terms

acetylation
: the addition of an acetyl group to histone proteins that loosens the dna-histone interaction and promotes gene expression

chromatin
: the structure formed by DNA and proteins (mainly histones) that packages eukaryotic DNA in the nucleus

chromatin remodeling
: the process of repositioning or restructuring nucleosomes to regulate access to DNA

CpG island
: a region of DNA with a high frequency of cytosine and guanine nucleotides, often located near gene promoters and

targeted for DNA methylation

DNA methylation
: the addition of methyl groups to DNA, usually cytosine bases, that generally leads to gene silencing

epigenetic regulation
: control of gene expression that occurs without changing the dna sequence, often through chemical modifications of DNA or histone proteins

genomic imprinting
: a genetic phenomenon in which certain genes are expressed in a parent-of-origin-specific manner due to epigenetic marks

histone
: a protein that dna wraps around to form nucleosomes in chromatin

histone tail
: the flexible end of a histone protein that extends from the nucleosome and can be chemically modified to influence chromatin structure

methylation
: the addition of a methyl group, which generally tightens chromatin and reduces gene expression

nucleosome
: the basic unit of chromatin, consisting of dna wrapped around a core of histone proteins

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9.6: Eukaryotic Transcription Gene Regulation

Learning Objectives

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

- Discuss the role of transcription factors in gene regulation
- Explain how enhancers and repressors regulate gene expression

In eukaryotic cells, the first major step of gene expression—transcription—can only begin when a set of conditions are just right. The DNA must be unwrapped from its tightly packed chromatin, which we explored in the section on epigenetic regulation. Once a gene is located in a region of "open" chromatin, the cell can begin preparing for transcription. But unlike prokaryotic cells, where the RNA polymerase enzyme can more easily access DNA, eukaryotic cells require a complex team of helper proteins to get transcription started.

These helper proteins are known as transcription factors, and they are the molecular matchmakers of gene expression. They help RNA polymerase, the enzyme that reads DNA to create RNA, find the right starting point on the gene and set the stage for transcription.

Link to Learning

View the process of transcription—the making of RNA from a DNA template.

YouTube Video

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The Promoter and the Transcription Machinery

Genes are organized to make the control of gene expression easier. It's a highly tuned process that lets cells respond to signals, differentiate into specific types, and regulate the timing and intensity of gene expression. Whether the gene codes for insulin, a digestive enzyme, or a developmental protein, it all starts with the promoter, the consensus sequence, and the transcription factors that bind there.

The promoter region is immediately upstream of the coding sequence and acts as the primary landing site for the transcription machinery. Its job is to attract the proteins—most importantly transcription factors—needed to start transcription. But not all promoters are built the same. Some are short, containing only a few nucleotides. Others stretch across hundreds of bases, offering more surface area for protein interactions. In general, the longer and more complex the promoter, the more opportunities it provides for transcription factors to bind and regulate gene expression with precision. This variability helps explain why some genes are tightly regulated while others are more universally expressed.

By now, we've seen that transcription factors (as with all proteins) are proteins that use functional domains to do their jobs. As we have discussed previously, one key domain, the DNA-binding domain, is shaped in a way that lets it recognize and bind to a very specific stretch of DNA. This brings us to a crucial concept: the consensus sequence.

A consensus sequence is the particular DNA code that a transcription factor looks for—its molecular "keyword." These sequences are typically found in or near promoter regions and act like precise docking signals. Because transcription factors must locate the right promoter out of thousands scattered throughout the genome, their DNA-binding domains are tuned to recognize only their target consensus sequences. This binding specificity is essential, especially when you consider that a single cell may contain hundreds or even thousands of different transcription factors, each with its own targets. The system only works because of the precise match between a transcription factor and its consensus sequence.

Connection

Mutations and Consensus Sequences

You've learned that mutations are changes in the DNA sequence, and we often think of them in terms of how they alter the instructions for building a protein. But what happens when a mutation occurs not in the gene itself, but in the promoter region, where the consensus sequence resides?

Because transcription factors rely on the exact sequence of nucleotides in the consensus site to recognize where to bind, even a single-letter change in this region can have a big impact. If a mutation changes just one base in the consensus sequence, it might prevent the transcription factor from recognizing and binding to it at all. That means the gene might no longer be turned on properly—or it could be turned on too much or at the wrong time. This doesn't change the protein's structure directly, but it can change how much of that protein is made, which in many cases can be just as significant.

This is a powerful example of how mutations can influence gene expression, not by rewriting the instructions, but by altering the regulatory "switches" that control when and how those instructions are used. Understanding this helps scientists uncover how subtle changes in DNA—far from the coding region—can still contribute to health issues like cancer or developmental disorders.

Below are some examples of diseases and conditions associated with mutations in the promoter sequence:

β-thalassemia

This blood disorder is often caused by mutations in the promoter region of the beta-globin gene, which reduce the expression of the β-globin protein needed to make hemoglobin. Even though the protein sequence might be perfectly normal, not enough of it is produced, leading to anemia and fatigue due to poor oxygen transport.

Fragile X syndrome

In this condition, a gene called FMR1 becomes silenced due to abnormal DNA methylation (an epigenetic change) in its promoter region. As a result, the FMRP protein—important for brain development—is not made, leading to intellectual disability and behavioral challenges.

HER2-positive breast cancer

In some breast cancers, the HER2 gene is overexpressed, meaning too much of the HER2 protein is made. HER2 is a growth-promoting receptor on the surface of cells. When its levels are too high, cells receive constant "grow!" signals, which contributes to uncontrolled cell division and tumor growth.

Gilbert's syndrome

This mild liver condition is caused by a reduced expression of the UGT1A1 enzyme, which helps process bilirubin. A mutation in the gene's promoter region lowers transcription, leading to occasional jaundice due to inefficient bilirubin breakdown.

Lactose intolerance (adult-onset)

In many people, a regulatory change upstream of the lactase gene (which digests milk sugar) reduces expression of the gene after childhood. While not a disease, this is a common evolutionarily driven change in gene expression, showing how gene regulation can shift across a lifetime.

But binding DNA is only the beginning. Most transcription factors also contain domains that allow them to interact with other proteins, including other transcription factors and eventually RNA polymerase. This means that transcription doesn't start with a

single protein, but rather through a coordinated cascade: one transcription factor binds to its consensus sequence and recruits others; together they form a complex network of interactions. The specific combination of transcription factors present—or absent—in a given cell at a given time determines whether this cascade successfully forms. Only when the full team is assembled can RNA polymerase be properly positioned and activated to begin transcription.

Enhancers and Transcription

So far, we've seen how transcription begins at a promoter—a landing pad for the general transcription machinery. But gene regulation often requires more than just a local launch site. In many eukaryotic genes, transcription is boosted or fine-tuned by additional DNA regions known as enhancers.

Enhancers are like remote control switches. They don't need to sit right next to the gene they regulate. In fact, they can be found upstream, downstream, inside the gene, or even thousands of nucleotides away. Despite their distance, they still manage to influence the promoter directly.

How is that possible? When specific transcription factors bind to enhancer regions, they undergo a shape change (called a conformational change) that allows them to interact with the transcription factors and RNA polymerase assembled at the promoter. To make this happen, the DNA bends and loops, bringing the enhancer region physically close to the promoter. DNA-bending proteins help guide this elegant folding process, turning what looks like a linear sequence on paper into a three-dimensional bridge between distant sites.

Just like promoters, enhancers have their own unique consensus sequences—short, recognizable stretches of DNA where only the right transcription factor will bind. Because these enhancer-binding transcription factors are different from those that bind the core promoter, they represent another layer of regulation. There are hundreds or thousands of different transcription factors in a single cell, and the presence—or absence—of specific ones determines which enhancers are active. This helps explain why two genes with identical promoters can behave very differently in different cell types: they may be connected to entirely different enhancer regions.

The flexibility this provides is astonishing. One gene can be expressed in a nerve cell, silenced in a skin cell, and activated in a liver cell—all because of which enhancer sequences are present and which transcription factors are available. This is how the same DNA blueprint can lead to such diverse outcomes across the human body.

(Figure $9.6.1$**)** illustrates how enhancer sequences, transcription factors, and mediator proteins all come together, creating a looped structure that connects the enhancer to the promoter and allows the gene to be turned on at just the right moment and intensity.

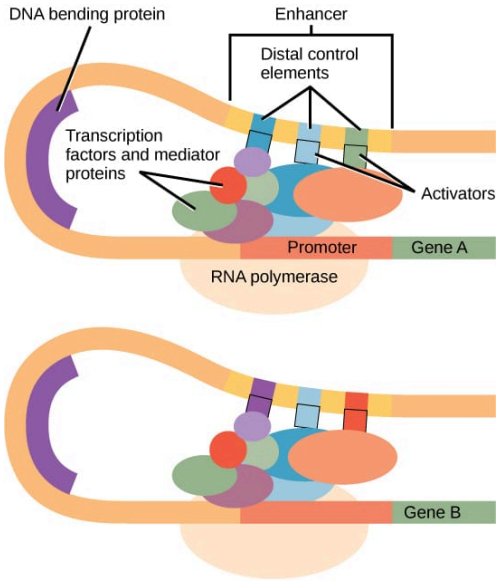


Figure 9.6.1: Interaction between proteins at the promoter and enhancer sites. An enhancer is a DNA sequence that promotes transcription. Each enhancer is made up of short DNA sequences called distal control elements. The DNA folds over itself, bringing the enhancer next to the promoter. Transcription factors and mediator proteins are sandwiched between the promoter and the enhancer. Activators bound to the distal control elements interact with mediator proteins and transcription factors. RNA polymerase binds the complex, allowing transcription to begin. Two different genes may have the same promoter but different distal control elements, enabling differential gene expression.

Turning Genes Off: Transcriptional Repressors

Just as some transcription factors activate genes, others serve as the brakes. These are called transcriptional repressors, and they prevent transcription from happening. They can bind to promoters or enhancer regions and physically block activators or the transcription machinery. Sometimes, repressors compete for binding sites; other times, they alter the shape of the DNA to make it less accessible. Like activators, they also respond to internal or external signals, making them part of the cell's way of responding to changing conditions.

Connection

Why Can't You Make Insulin in Your Skin?

Let's think about something puzzling: every cell in your body (with very few exceptions) contains the same DNA. So why do your pancreatic cells make insulin, but your skin cells do not?

The answer lies in transcriptional regulation.

The insulin gene has a promoter, just like every other gene. But it also has enhancers that are only recognized by specific transcription factors found in pancreatic cells. Those transcription factors bind to the enhancer, recruit general transcription factors and RNA polymerase, and switch on the insulin gene. In skin cells, those transcription factors are absent. Even though the DNA is the same, the insulin gene never gets transcribed—because the right combination of transcription factors and chromatin state simply isn't present.

This kind of system helps maintain cell identity and function. It's like giving every cell the same cookbook, but only certain chefs have the key to unlock specific recipes.

Summary

In eukaryotic cells, transcription is highly regulated and involves a combination of general and specific transcription factors. General transcription factors bind near the gene's promoter to help RNA polymerase begin transcription. Specific transcription factors bind to enhancer sequences located far from the gene and help amplify transcription by looping the DNA to interact with

the core transcriptional machinery. Transcriptional repressors can bind to either promoter or enhancer regions to block gene expression. This system of on/off switches ensures that only the right genes are expressed in the right cells at the right times, allowing for the complexity and specialization of multicellular life.

Key Terms

activator
: a specific transcription factor that increases gene expression by binding to enhancer sequences

enhancer
: a DNA sequence that can increase the rate of transcription, even if located far from the gene it regulates

promoter
: a sequence of DNA located upstream of a gene where RNA polymerase and transcription factors bind to begin transcription

repressors
: transcription factors that block transcription either by preventing activators from binding or by interfering with the transcription machinery

transcription factor
: a protein that helps regulate gene expression by binding to specific DNA sequences and interacting with the transcription machinery

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9.7: Eukaryotic Post-transcriptional Gene Regulation

Learning Objectives

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

- Explain the process and significance of RNA splicing
- Describe the role of alternative splicing in generating protein diversity
- Identify how mRNA stability affects protein production

By the time an RNA molecule is transcribed from DNA, it holds all the instructions needed to build a protein. But those instructions aren't quite ready to be read just yet. Like a rough draft that needs editing before it becomes a final report, newly made RNA—called pre-mRNA—undergoes several crucial modifications before it can leave the nucleus and be translated into a protein in the cytoplasm.

This stage of regulation is called post-transcriptional control. It happens after RNA has been transcribed, but before it's translated. Just like turning a faucet controls how much water flows out, post-transcriptional control helps determine how much of a protein gets made—or if it gets made at all. If the RNA is not correctly processed, transported, or protected, the protein it codes for may never be produced.

RNA Splicing, the First Stage of Post-transcriptional Control

In eukaryotic cells, the genes that code for proteins are sometimes interrupted by sequences called introns. These are non-coding segments—think of them as deleted scenes in a film. The coding regions, called exons, are the parts that will be stitched together to make the final instructions for a protein.

Before RNA leaves the nucleus, if the gene contains introns then it goes through a process called splicing **(Figure** 9.7.1**)**. Splicing removes the introns and joins the exons together. This editing is done by spliceosomes, large protein-RNA complexes that act like molecular scissors and tape.

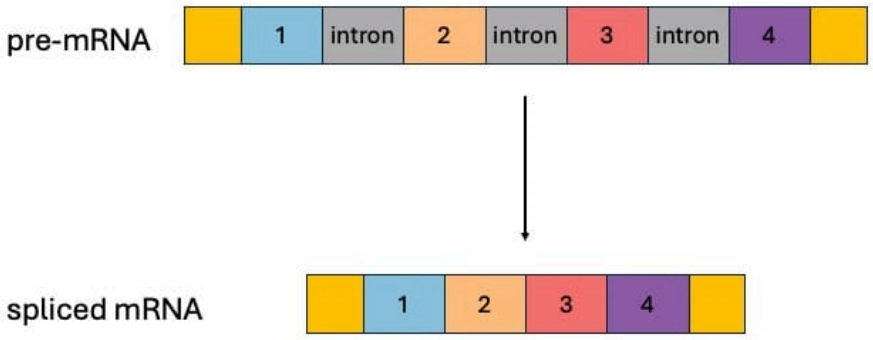


Figure 9.7.1**:** Splicing results in the removal of introns from the pre RNA.

Evolution Connection

Alternative RNA Splicing

In the 1970s, something interesting was found to occur with the processing of pre-mRNA: the same pre-mRNA can be spliced in more than one way, producing different versions of the final mRNA. This process, called alternative splicing, allows a single gene to give rise to multiple proteins—like a song remixed into different genres depending on the occasion.

Alternative RNA splicing is a mechanism that allows different protein products to be produced from one gene when different combinations of exons are combined to form the mRNA. **Figure** 9.7.2 shows how a single pre-mRNA molecule can produce different proteins by including or skipping certain exons.

Alternative splicing is now understood to be a common mechanism of gene regulation in eukaryotes; according to one estimate, 70 percent of genes in humans are expressed as multiple proteins through alternative splicing. Although there are multiple ways to alternatively splice RNA transcripts, the original 5'-3' order of the exons is *always conserved*. That is, a transcript with exons 1 2 3 4 5 6 7 might be spliced 1 2 4 5 6 7 or 1 2 3 6 7, but never 1 2 5 4 3 6 7.

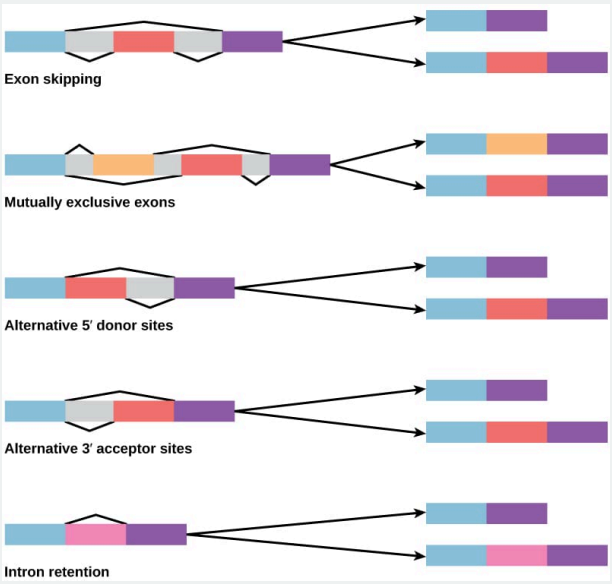


Figure 9.7.2: There are five basic modes of alternative splicing. When exon skipping occurs, an exon is spliced out in one mature mRNA product and retained in another. When mutually exclusive exons are present in the pre-mRNA, only one is retained in the mature mRNA. When an alternative 5’ donor site is present, the location of the 5’ splice site is variable. When an alternative 3’ acceptor site is present, the location of the 3’ splice site is variable. Intron retention results in an intron being retained in one mature mRNA and spliced out in another.

How could alternative splicing evolve? Introns have a beginning- and ending-recognition sequence; it is easy to imagine the failure of the splicing mechanism to identify the end of an intron and instead 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 intron 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 abnormal splicing rather than mutations in a coding sequence. Such mutations have been inked to diseases like spinal muscular atrophy and certain cancers.

For example, scientists have found that amelanism (a form of albinism) in corn snakes is caused by a transposable element inserted into an intron of the OCA2 gene. The extra DNA disrupts normal splicing, resulting in a nonfunctional protein involved in pigment production—hence the altered color patterns.

Figure 9.7.2 illustrates the different forms of alternative splicing, including skipped exons, retained introns, or shifted splice sites.

Each variation results in a different protein output.

Link to Learning

Visualize how mRNA splicing happens by watching the process in action in this video.

YouTube Video

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

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RNA Stability and the Journey to the Cytoplasm

Even after splicing, a newly made mRNA isn't quite ready to be read by the ribosome. It still needs protection. Think of mRNA as a message written on a delicate piece of paper that must travel a long and bumpy road through the cell. Without an envelope, the message would be torn up before reaching its destination. The cell solves this problem by placing two protective caps at each end of the mRNA before it exits the nucleus.

At the 5' end, the mRNA receives a 5' cap, which is a modified guanine nucleotide (specifically, a methylated guanosine triphosphate, or GTP). Interestingly, this cap is added "backwards"—the 5' carbon of the cap nucleotide is linked to the 5' carbon of the first nucleotide of the RNA via three phosphate groups. This unusual orientation makes it harder for degrading enzymes, called exonucleases, to break it down.

At the 3' end, the mRNA is given a poly-A tail—a long string of adenine nucleotides that acts like bubble wrap, cushioning the RNA and helping it survive the cytoplasmic environment. Together, the 5' cap and poly-A tail prevent premature degradation and help the cell recognize this RNA as "ready for translation."

Once the capped and tailed mRNA is transported into the cytoplasm, how long it stays intact becomes an important regulatory step. Some mRNAs are short-lived, quickly broken down to ensure only a small burst of protein is made. Others are very stable, sticking around for longer periods and producing more protein. This characteristic is known as RNA stability.

Controlling RNA's Lifespan

After mRNA reaches the cytoplasm, proteins called RNA-binding proteins (RBPs) help determine its fate. These proteins don't bind just anywhere—they attach specifically to the untranslated regions (UTRs) of the RNA. These UTRs are sequences located at both ends of the RNA but outside the actual coding region. The 5' UTR lies before the protein-coding sequence, and the 3' UTR follows it **(Figure** $9.7.3$**)**.

The UTRs don't code for protein themselves, but they play a crucial role in controlling when, where, and how much protein is made from that mRNA. RBPs recognize specific sequences or structures in these UTRs using a specialized part of their structure called an RNA-binding domain. This is somewhat similar to the DNA-binding domains found on transcription factors—but instead of binding to DNA to regulate transcription, RBPs bind to RNA to regulate translation, localization, and stability of the transcript.

Some RBPs stabilize mRNA, helping it stick around longer in the cytoplasm. Others signal that it's time to degrade the RNA, effectively reducing protein production. The presence—or absence—of these proteins influences the lifespan of an mRNA and how much protein ultimately gets made.

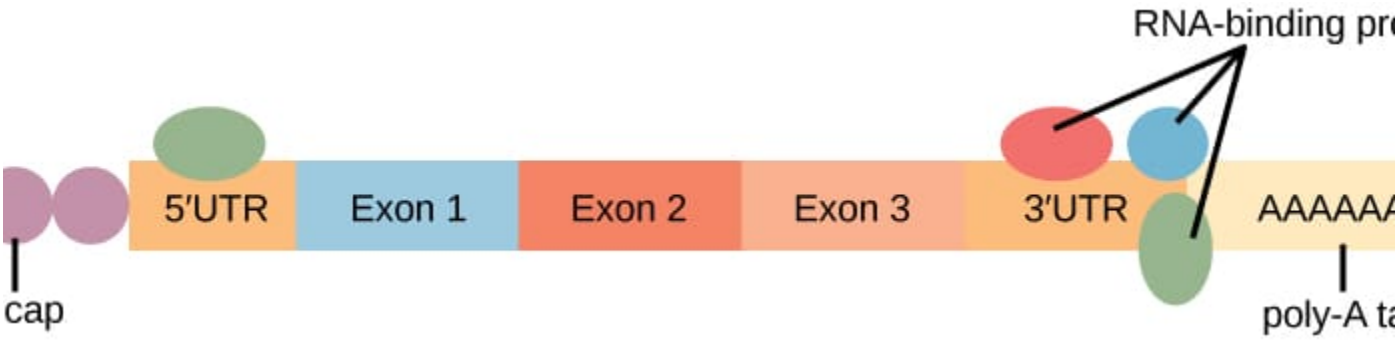


Figure 9.7.3: RNA-binding proteins. The protein-coding region of this processed mRNA is flanked by 5' and 3' untranslated regions (UTRs). The presence of RNA-binding proteins at the 5' or 3' UTR influences the stability of the RNA molecule.

RNA Stability and microRNAs

Another important layer of post-transcriptional control comes from microRNAs (miRNAs). These are very short RNA molecules—just 21 to 24 nucleotides long—that play a big role in silencing gene expression.Here's how it works: after being trimmed into shape by a protein called Dicer, miRNAs become part of a complex called RISC (RNA-induced silencing complex). The miRNA guides RISC to bind a specific mRNA sequence—like a lock and key. Once bound, the complex can either block translation or cause the mRNA to be degraded.

This is a powerful regulatory tool. For example, some cancers are caused by too much or too little miRNA activity. A drop in miRNAs that suppress cell division can lead to uncontrolled growth—a hallmark of cancer.

Summary

After transcription, the RNA molecule still has a long journey before it becomes a protein. It must first be processed through splicing, where introns are removed and exons are joined together. Through alternative splicing, cells can generate many different proteins from a single gene. Once processed, the mRNA is capped, tailed, and exported to the cytoplasm.

Here, the message's stability—how long it survives—plays a key role in determining how much protein is produced. RNA-binding proteins and microRNAs fine-tune this process, either protecting the mRNA or marking it for destruction. Post-transcriptional control ensures that the right proteins are made at the right time and in the right amounts, adding another elegant layer of gene regulation.

Key Terms

alternative splicing
: a process by which different combinations of exons are joined together to produce multiple mRNA variants from a single gene

exon
: a coding segment of an RNA transcript that is retained and translated into protein

intron
: a non-coding segment of an RNA transcript that is removed during splicing

mircoRNA (miRNA)
: a short RNA molecule that binds to target mRNA and inhibits translation or promotes degradation

post-transcriptional control
: regulation of gene expression that occurs after RNA is transcribed but before it is translated

pre-mRNA
: the initial RNA transcript that includes both exons and introns and requires processing

RNA-binding protein (RBP)
: a protein that binds to mRNA and influences its stability, localization, or translation

RNA stability

: the length of time an mRNA remains intact in the cytoplasm, which affects how much protein is produced

spliceosome
: a complex of proteins and RNA that removes introns from pre-mRNA

untranslated region (UTR)
: segments of mRNA located before (5') and after (3') the coding region that regulate mRNA function

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9.8: Eukaryotic Post-translational Gene Regulation

Learning Objectives

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

- Understand the process of translation and discuss its key factors
- Describe how the initiation complex controls translation
- Explain the different ways in which the post-translational control of gene expression takes place

Regulating the Regulators: Post-Translational Modifications and Gene Expression

After a protein is built through translation, the story isn't over. In fact, for many proteins—especially those involved in regulating gene expression—translation is just the beginning. Once a protein is produced, it can undergo a wide variety of changes, known as post-translational modifications (PTMs). These modifications are like finishing touches that determine where a protein goes, how long it lasts, how active it is, or even whether it functions at all.

In the context of gene expression, PTMs provide yet another critical layer of regulation. Many of the proteins we've talked about in this chapter—transcription factors, chromatin remodeling enzymes, RNA-binding proteins—can themselves be regulated through PTMs. These modifications act like molecular switches, tuning protein activity up or down, changing their location in the cell, or marking them for recycling and degradation. But it's not just proteins involved in gene regulation. PTMs are used across the entire spectrum of cell biology—from metabolism and DNA repair to cell signaling and immune responses. They are among the most versatile and powerful tools the cell uses to fine-tune its internal environment.

Types of Post-Translational Modifications

Some of the most common post-translational modifications include:

- Phosphorylation: The addition of a phosphate group (usually from ATP) to a protein, typically on the amino acids serine, threonine, or tyrosine. Phosphorylation can turn a protein "on" or "off," change its shape, or influence what other molecules it can bind. For example, phosphorylation can activate or deactivate transcription factors, determine whether chromatin remodeling proteins access DNA, or influence proteins involved in translation. This type of modification is a key player in cell signaling pathways—such as those responding to stress or growth signals—and is central to regulating processes like metabolism and gene expression.
- Ubiquitination: This is the addition of a small protein called ubiquitin to a target protein. When multiple ubiquitin molecules are added in a chain, it signals that the protein should be destroyed by a special protein complex called the proteasome. Think of ubiquitin as a "recycling tag." This process is essential for controlling protein levels inside the cell. If a transcription factor has done its job and is no longer needed, tagging it with ubiquitin ensures it doesn't hang around too long, which could cause harmful effects like uncontrolled gene activation. **(Figure** $9.8.1$**)**
- Acetylation and Methylation: These are chemical additions of acetyl or methyl groups, often to specific amino acids on proteins. You've already seen how acetylation of histones opens up chromatin to make genes more accessible, while methylation can either activate or repress gene expression depending on where it happens. But these same types of modifications can also happen to non-histone proteins, including transcription factors and RNA-binding proteins, altering their function in subtle yet powerful ways.
- Other Modifications: There are many other types of PTMs including sumoylation, glycosylation, and lipidation. Each of these can change how a protein behaves, whom it interacts with, and how it contributes to a given cellular process.

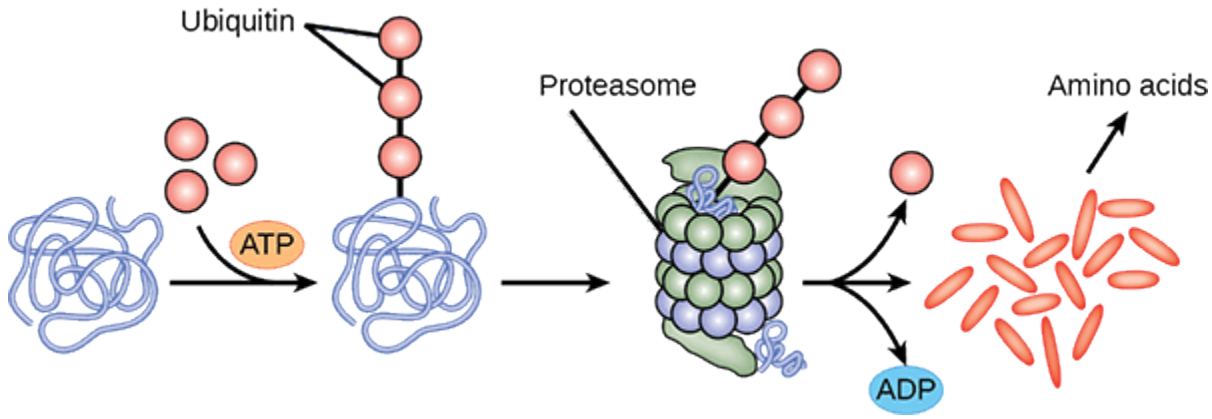


Figure 9.8.1: Proteins with ubiquitin tags are marked for degradation within the proteasome. The tagged protein is then fed into the hollow tube of a proteasome. The proteasome degrades the protein. This produces ADP and amino acids, and the ubiquitin is also released.

Application

Post-translational modifications in action

Let's look at two real-world examples.

During times of cellular stress—such as when oxygen is low or the cell is damaged—cells need to shift their gene expression quickly. A transcription factor called HIF-1 (hypoxia-inducible factor) is usually kept at low levels. However, when oxygen drops, post-translational modifications stabilize HIF-1, preventing its degradation. As a result, HIF-1 builds up and turns on genes that help the cell survive low oxygen conditions. When oxygen returns, ubiquitination tags HIF-1 for degradation, switching those genes back off.

Another example involves the p53 protein, sometimes called the "guardian of the genome." It plays a key role in monitoring DNA damage. In healthy cells, p53 is kept in check through continuous degradation. But when DNA is damaged, it's quickly stabilized through phosphorylation and acetylation, allowing it to activate genes that pause the cell cycle or trigger cell death. This flexible regulation, dependent on PTMs, helps the cell avoid passing on genetic mistakes.

These examples highlight how PTMs are essential not just for protein activity, but for the dynamic responsiveness of cells. Cells don't want to transcribe and translate new proteins every time conditions change. It's much faster to regulate the proteins they already have—fine-tuning them as needed. This makes post-translational control one of the fastest ways a cell can adjust to its environment.

Summary

Post-translational modifications offer cells a powerful way to regulate protein function quickly and efficiently. Proteins involved in gene regulation—such as transcription factors, chromatin remodelers, and RNA-binding proteins—can be activated, inhibited, stabilized, or destroyed through PTMs like phosphorylation, acetylation, methylation, and ubiquitination. These changes are often triggered by signals in the environment, allowing the cell to adapt rapidly without having to make entirely new proteins. But PTMs are not limited to gene regulation—they influence virtually all aspects of cellular function. Understanding how proteins are modified after they're made helps illuminate one of the most flexible and responsive layers of gene expression control.

Key Terms

post-translational modification (PTM)
: a chemical change to a protein after it has been made that can alter its activity, location, or lifespan

proteasome
: a protein complex in cells that breaks down proteins that have been tagged for degradation

ubiquitin
: a small protein that attaches to other proteins to mark them for degradation in the proteasome

ubiquitination
: the process of adding ubiquitin to a protein to signal its destruction

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9.9: Development

Learning Objectives

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

- Describe how the regulation of gene expression contributes to cell differentiation during early development.
- Explain the processes of differentiation and morphogenesis and how they contribute to the formation of tissues and organs.
- Distinguish between the three germ layers and list the general tissues/organs each layer gives rise to.

By now, you've learned that gene expression isn't just about turning genes "on" or "off"—it's a tightly regulated process that controls when, where, and how much of a gene product (usually a protein) is made. This control is especially important in living organisms because every cell in your body contains the same DNA, yet not all cells are the same. A muscle cell looks and behaves very differently from a nerve cell, even though they both contain identical genetic instructions. How does that happen?

To understand this, we need to zoom out and explore how gene regulation plays a central role in one of the most remarkable biological processes: development—the transformation of a single fertilized egg into a complex organism with many different cell types, tissues, and organs, all arranged in the right places.

The Beginning: A Single Cell with Endless Possibilities

All multicellular organisms, including humans, begin life as a zygote—a single cell formed when a sperm cell fertilizes an egg. This zygote contains all the genetic information needed to build an entire organism. But having the information isn't enough. What matters is how that information is used—and that's where gene regulation comes in.

As the zygote begins to divide, it produces a cluster of genetically identical cells. However, over time, these cells begin to behave differently. Some become skin cells, others muscle cells, others neurons. This process of cells becoming specialized in structure and function is called differentiation.

Differentiation is guided by differences in gene expression. Although all cells have the same DNA, different genes are turned on or off in different cell types. For example, a red blood cell expresses genes needed to produce hemoglobin, while a liver cell expresses genes that help break down toxins. These differences are regulated by molecules inside the cell (like transcription factors) and by signals from surrounding cells and the environment.

Building the Body: Morphogenesis and the Body Plan

Once cells begin to differentiate, the next step is arranging them into tissues and organs in the right places. This process is called morphogenesis , which literally means "the beginning of shape." Morphogenesis involves changes in cell shape, movement, and organization to form the structures of the developing organism.

Think of it like constructing a building. Differentiation gives you the specialized workers (electricians, plumbers, carpenters), while morphogenesis is the process of getting them to the right place at the right time so the structure comes together properly.

One of the most important aspects of morphogenesis is establishing the organism's body plan—a blueprint of how the body will be organized. This includes the placement of the head and tail, front and back, left and right, and where arms, legs, and organs will form. The body plan is established early in development and is highly conserved across animal species, meaning that many of the same genetic mechanisms are used by flies, frogs, mice, and humans.

The Role of Homeotic Genes

One of the most fascinating discoveries in developmental biology is the role of homeotic genes, also known as Hox genes, in

determining the body plan. These genes act like master switches that control the identity of different body parts along the head-to-tail axis.

Homeotic genes are a group of transcription factors that are turned on in specific regions of the embryo. They don't build structures themselves, but they control the expression of other genes that do. For example, a certain Hox gene might activate genes that tell cells in the middle of the embryo to become part of the spinal cord, while another Hox gene might activate genes that tell cells near the top to form the head.

What's especially remarkable is how similar these genes are across species. Scientists have found that fruit flies and humans share many of the same homeotic genes, even though the organisms look very different. This suggests that the basic genetic instructions for building body plans have been around for hundreds of millions of years.

Development in Mammals: A Closer Look

In mammals like humans, early development follows a general pattern but with some unique twists. After the zygote forms, it begins a series of rapid cell divisions known as cleavage, leading to a hollow ball of cells called a blastocyst. Inside the blastocyst is a small cluster of cells called the inner cell mass, which will eventually give rise to the embryo.

One of the first major events in development is gastrulation, a process that reorganizes the embryo into three distinct layers of cells called germ layer. These layers are:

- Ectoderm (outer layer): becomes the skin, brain, and nervous system.
- Mesoderm (middle layer): becomes muscles, bones, blood, and other internal organs.
- Endoderm (inner layer): becomes the lining of the digestive and respiratory systems.

Gastrulation is a key moment in development because it sets the stage for organ formation. Each germ layer will give rise to specific tissues and organs, guided again by tightly controlled patterns of gene expression.

How Do Cells Know What to Become?

One question students often ask is: If all cells have the same DNA, how do they "know" what to become? The answer lies in a combination of internal and external signals.

- Internal cues include the presence of specific transcription factors inherited during cell division.
- External cues come from neighboring cells or the environment, often in the form of signaling molecules that bind to receptors and trigger changes in gene expression.

Importantly, once a cell has fully differentiated, it usually cannot go back to an earlier state. A muscle cell, for example, typically can't become a nerve cell. However, some cells retain the potential to become many different types of cells—and these are known as stem cells. Stem cells are special because they can divide and produce both more stem cells (self-renewal) and differentiated cells (specialization). Some stem cells can become nearly any type of cell in the body, while others are more limited in their potential. They play a vital role in development, tissue repair, and are at the heart of exciting biotechnological advances.

You've already seen how tightly gene expression is controlled to guide development. In the next section, we'll dive deeper into how stem cells maintain their flexibility, how they can be directed to form specific cell types, and why they hold such promise for medicine.

Summary

Development begins with a single cell—the zygote—which contains all the genetic information needed to build a complex organism. Through a series of rapid divisions called cleavage, this single cell produces many smaller cells, eventually forming a hollow structure known as the blastocyst. Inside the blastocyst lies a cluster of cells that will develop into the embryo. Although all of these cells contain the same DNA, they begin to express different genes as development progresses. This selective gene expression is what drives differentiation, allowing cells to take on specialized roles like muscle, nerve, or skin cells.

As cells differentiate, they must also be arranged into the correct locations and structures within the body. This process, known as morphogenesis, shapes tissues and organs and helps establish the organism's overall body plan—including where the head, tail,

and limbs will form. Much of this organization is controlled by homeotic (Hox) genes, which act like genetic switches to turn on other genes in specific regions of the body. These genes are remarkably conserved across species, highlighting their fundamental role in development.

One of the most significant events in early development is gastrulation, when the embryo reorganizes into three primary germ layers: the ectoderm, mesoderm, and endoderm. These layers will give rise to all the organs and systems in the body, from the nervous system to the digestive tract. Throughout these stages, both internal factors (like inherited molecules) and external signals (from neighboring cells) influence which genes are expressed, guiding each cell along a particular developmental path.

While many cells become locked into their specialized roles, some remain flexible—able to divide and potentially become a variety of different cell types. These are known as stem cells, and they are central to both development and the future of biomedical research. In the next section, we'll explore what makes stem cells unique and why they hold so much promise for science and medicine.

Key Terms

blastocysts
: a hollow, fluid-filled structure formed during early development in mammals, following cleavage. It contains an outer layer of cells that will help form the placenta and an inner cell mass that will develop into the embryo itself

body plan
: the general layout of an organism's structure, including the arrangement of body parts along the head-to-tail, front-to-back, and left-to-right axes

cleavage
: a series of rapid cell divisions that occur in the early embryo after fertilization

differentiation
: the biological process through which cells become specialized in structure and function by expressing different sets of genes

ectoderm
: the outermost germ layer; gives rise to the skin, brain, and nervous system

endoderm
: the innermost germ layer; gives rise to the linings of the digestive and respiratory systems and associated organs

gastrulation
: a critical early developmental process in which the embryo reorganizes to form three distinct germ layers (ectoderm, mesoderm, and endoderm)

germ layer
: one of the three primary layers of cells formed during gastrulation (ectoderm, mesoderm, endoderm) that give rise to all the tissues and organs in the body.

homeotic genes (also called Hox genes)
: a group of genes that control the development of body structures in specific locations; they act as master regulators that turn on other genes during early development

mesoderm
: the middle germ layer; gives rise to muscles, bones, blood, and many internal organs

morphogenesis
: the developmental process that gives shape to tissues and organs in the body by organizing cells into complex structures

zygote
: the single cell formed when a sperm fertilizes an egg; it contains all the genetic information needed to develop into a complete organism

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9.10: Stem Cells and Regenerative Medicine

Learning Objectives

- Define stem cells and explain their unique properties.
- Describe how stem cells differentiate into specialized cells.
- Differentiate between embryonic stem cells and induced pluripotent stem cells, including their applications and ethical considerations.

What Are Stem Cells?

Imagine you're building a house. You need different kinds of workers-electricians, plumbers, carpenters-to complete specific jobs. But before those specialists arrive, you start with a general contractor who can coordinate and adapt to whatever task is needed. In your body, stem cells are like that general contractor. They haven't committed to a specific job yet, but they have the potential to become almost any kind of cell, depending on the body's needs.

Stem cells are special because they can do two things that most other cells in your body cannot

1. Self-renew - They can divide and create exact copies of themselves over and over again, which is essential for maintaining a supply of these versatile cells.
2. Differentiate - They can transform into more specialized cells like nerve cells, skin cells, or muscle cells.

To better understand how this works, it's helpful to compare stem cells to somatic (body) cells, which are the fully differentiated cells that make up your tissues and organs. Once a somatic cell becomes specialized-for example, a skin cell-it reaches a state called terminal differentiation. This means it has committed to its role and cannot go back or become a different type of cell. Just as an electrician cannot suddenly switch to being a plumber without retraining, a skin cell cannot become a nerve or muscle cell. Its job is fixed.

In contrast, stem cells remain in a more flexible state. They're like employees at a job training center who can be assigned new roles based on what the body needs. This is why they play such a critical role in growth, development, and healing.

Your body uses stem cells naturally during development-starting from a single fertilized egg that divides and eventually forms all the tissues and organs of a human body. But their importance doesn't end at birth. Throughout life, stem cells help maintain and repair tissues. When you cut your skin or bruise a muscle, adult stem cells help replace damaged cells with new ones. (**Figure** 9.10.1)

Scientists are now learning how to harness this natural repair system to treat diseases and injuries more effectively. The ability to guide stem cells to become specific cell types like insulin-producing cells for diabetes or nerve cells for spinal cord injuries-is one of the most exciting frontiers in modern medicine. Understanding how stem cells work, and how they differ from other cells in the body, is the first step toward unlocking their full potential in regenerative medicine.

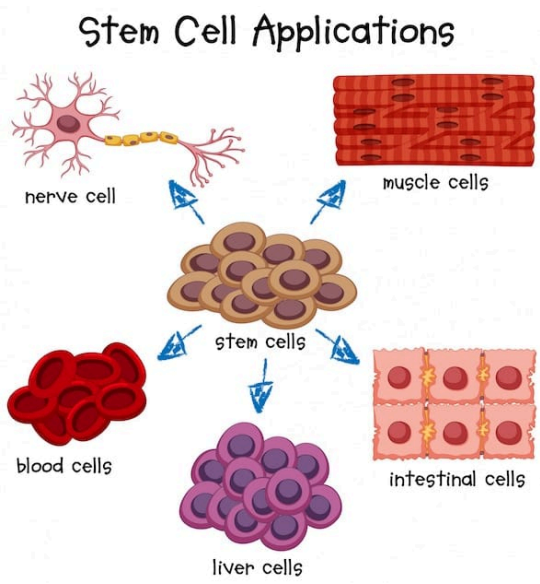


Figure 9.10.1**:** Stem cells differentiating into other specific cells, a key part of regenerative medicine. Image from https://www.freepik.com/author/brgfx

Types of Stem Cells

Not all stem cells have the same potential. Some are like blank slates, able to become any cell in the body, while others are more specialized. One way scientists classify stem cells is by how many different kinds of cells they can become. To make this idea easier to understand, let's use a classroom analogy.

- Totipotent Stem Cells: These are like students who haven't picked a major and can choose any career—even ones that support others, like a school counselor or principal. Totipotent stem cells exist only in the very early stages of development, right after fertilization. A zygote (a fertilized egg) is totipotent—it can give rise to every cell in the body, including the cells that form the placenta and other structures that support the embryo.
- Pluripotent Stem Cells: These students have chosen a broad major—say, "life sciences"—and can enter nearly any career within that field, but not support staff roles. In biological terms, they can become any cell in the body but not placental cells. Embryonic stem cells (ESCs) are pluripotent and have become a cornerstone of biomedical research due to their ability to differentiate into nearly any cell type in the body.
- Multipotent Stem Cells: These students have narrowed it down even further—they've picked a department like healthcare and can pursue related professions, such as nurse, doctor, or physical therapist, but not jobs outside that field. Multipotent stem cells are more restricted in their potential but still very useful. A well-known example is the hematopoietic stem cell, found in bone marrow. These cells can become all the different types of blood cells, including red blood cells (which carry oxygen), white blood cells (which fight infection), and platelets (which help blood clot). However, they cannot become nerve or skin cells. Hematopoietic stem cells are used in bone marrow transplants to treat conditions like leukemia, where the patient's own blood-forming stem cells are damaged or destroyed.
- For instance, in a bone marrow transplant, a patient receives healthy hematopoietic stem cells, often from a donor. These cells then settle into the patient's bone marrow and begin producing healthy blood cells. This process can effectively "reset" the patient's blood system and is a life-saving treatment for many blood-related diseases. The targeted flexibility of multipotent stem cells makes them ideal for therapies where only certain types of cells need to be replaced or regenerated.
- Unipotent Stem Cells: These are the hyper-focused students. They've picked one career path—like becoming a dentist—and they stick to it. Unipotent stem cells can only become one type of cell, but they can still self-renew. For example, skin stem cells can only become new skin cells. Even though their potential is limited, they're essential for the continuous maintenance and repair of specific tissues.

Understanding this classification helps scientists decide which types of stem cells are best suited for different types of therapy or research. If you need to replace many types of cells, pluripotent stem cells might be the right choice. If you only need blood cells, multipotent hematopoietic stem cells may be the most effective and safest option.

How Do Stem Cells Turn Into Specialized Cells?

The process of differentiation—when a stem cell becomes a specialized cell—is like a decision tree. Picture a fork in the road where a traveler (the stem cell) chooses one path over another based on signs, road conditions, and guidance from locals. In the body, those "signs" are chemical signals, like hormones and growth factors, and the "locals" are neighboring cells that help guide the decision.

Here's how it typically works:

1. Receiving Signals: The stem cell is exposed to molecules in its environment. These could come from nearby cells, blood, or tissue damage. These molecules "whisper" instructions, telling the cell what kind of role might be needed.
2. Turning On Genes: Inside the stem cell, certain genes are switched on or off in response to those external signals. These genes determine the cell's identity—for example, a gene might instruct the cell to start building proteins found only in muscle cells.
3. Changing Form and Function: As gene expression changes, so does the cell's structure. Its shape, contents, and surface proteins begin to reflect its new role.
4. Becoming Specialized: Eventually, the cell becomes fully specialized. A stem cell that once had endless possibilities is now a red blood cell, a nerve cell, or a skin cell—with a specific job and function.

Understanding differentiation is crucial in regenerative medicine because doctors and scientists must figure out how to guide stem cells to become exactly the right type of cell needed for therapy.

Regenerative Medicine: Using Stem Cells to Heal

Imagine if your body came with a repair manual and spare parts. That's the idea behind regenerative medicine—a field in biotechnology that aims to help the body heal itself using its own cells, or new cells grown in a lab. Rather than simply treating symptoms, regenerative medicine seeks to repair, replace, or regenerate damaged tissues or organs.

- Stem cells are the star players in this field because of their flexibility and regenerative power. Here are a few examples of how they're used:
- Bone Marrow Transplants: This is one of the oldest and most successful examples of regenerative medicine. Patients with leukemia or other blood diseases receive stem cells from healthy donors to rebuild their blood and immune systems.
- Burn and Skin Treatments: Stem cells can help regenerate skin for burn victims. In some cases, doctors can grow sheets of skin in the lab from a patient's own stem cells, then graft the new skin back onto the wound.
- Heart Repair: After a heart attack, part of the heart muscle is permanently damaged. Researchers are studying how to inject stem cells into the heart to regenerate that tissue and restore function.
- Spinal Cord Injuries: Scientists are exploring ways to help paralyzed patients regain movement by regenerating damaged nerve tissue using stem cells.
- Diabetes Research: For people with type 1 diabetes, the body's insulin-producing cells are destroyed. Researchers are working to turn stem cells into new insulin-producing cells that can be transplanted into patients.

Table 9.10.1: Examples of stem cells used in regenerative medicine to repair or replace damaged tissue include: bone marrow transplants using hematopoietic cells to replace blood cells; burn treatments using skin stem cells to regrow skin; heart repair using cardiac stem cells to restore heart muscles after heart attacks; spinal cord therapy using neural stem cells to treat paralysis; and diabetes research using iPSCs to produce insulin.

Application	Stem Cell Type	Purpose
Bone Marrow Transplants	Hematopoietic	Replace blood cells in leukemia
Burn Treatments	Skin stem cells	Regrow skin layers

Application	Stem Cell Type	Purpose
Heart Repair	Cardiac stem cells	Restore heart muscle after heart attacks
Spinal Cord Therapy	Neural stem cells	Treat paralysis
Diabetes Research	iPSCs	Produce insulin-producing cells

Although many of these treatments are still in development, they show how stem cells could revolutionize healthcare by offering long-term solutions instead of temporary fixes.

Embryonic Stem Cells and Ethical Concerns

Embryonic stem cells (ESCs) were the first type of human stem cell discovered that could become virtually any type of cell in the body—a property known as pluripotency. Isolated in the late 1990s, these cells are obtained from embryos at the blastocyst stage, just a few days after fertilization. At this early phase, the embryo is a tiny, hollow ball of cells, smaller than a grain of sand, but rich in developmental potential. The inner cell mass of this structure contains the pluripotent cells that researchers extract to grow in laboratory settings **(Figure** 9.10.2**)**.

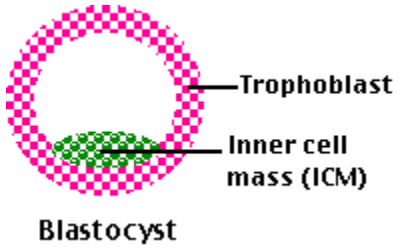


Figure 9.10.2**:** Blastocyst structure. Image from Biology Kinball

To picture their value, imagine being handed a blank canvas that can become any painting imaginable. ESCs are nature's blank canvas: they can develop into heart cells, brain cells, liver cells, and more. This flexibility has enormous potential in medicine. For example, in the study of Parkinson's disease, researchers have worked on turning ESCs into dopaminergic neurons—the specific brain cells that die off in this condition. If these lab-grown neurons could be transplanted into patients' brains and integrate successfully, they could restore lost motor function and improve quality of life.

ESCs have also been used in pioneering efforts to treat spinal cord injuries, blindness, and type 1 diabetes in animal models and early human trials. Because of their ability to create virtually any cell type, they have helped researchers model diseases in the lab, screen drugs, and test how certain genes influence development.

However, despite their scientific promise, ESCs are at the center of ethical debates. The core issue is that retrieving these cells typically involves the destruction of a human embryo. For some, this raises moral questions about the value and status of embryonic life. Is it ethical to destroy a potential life, even if it is just a few days old, in pursuit of cures that could save or improve other lives? For others, the context is different: many embryos used for research are donated from fertility clinics and would otherwise be discarded. Supporters argue that if these embryos can contribute to life-saving research, their use is not only justifiable, but valuable.

These contrasting views have led to wide variation in laws and public policy. In some countries, embryonic stem cell research is well-funded and supported, while in others, it faces strict limits or outright bans. In the United States, for example, federal funding for ESC research has fluctuated over the years depending on the political climate, creating uncertainty for researchers and limiting the pace of progress.

These ethical and political complexities helped catalyze one of the most significant breakthroughs in modern biology: the creation of induced pluripotent stem cells (iPSCs). These cells provide an alternative that mimics the properties of ESCs without relying on embryos—a discovery that reshaped the future of regenerative medicine and helped bridge the divide between scientific progress and ethical responsibility.

Connection

Resetting Identity: Transcription Factors and the Creation of iPSCs

In 2007, scientists made a groundbreaking discovery that would forever change the field of stem cell research. They learned how to reprogram adult cells—such as those from the skin or blood—to behave like embryonic stem cells. These lab-generated cells, known as induced pluripotent stem cells (iPSCs), are remarkable because they regain pluripotency, the ability to develop into nearly any cell type in the body.

But how does a specialized skin cell, which has long since committed to its identity, become so flexible again? The answer ties directly into the heart of gene regulation: transcription factors.

Scientists achieved this transformation by introducing a very specific set of transcription factors into adult cells. These transcription factors acted like molecular switches, flipping key developmental genes back on and turning off those that locked the cell into its previous identity. This delicate balance of activation and repression illustrates several key principles of gene regulation discussed earlier in this chapter:

- The presence or absence of transcription factors determines which genes are expressed in a cell at any given time.
- Each transcription factor has specific DNA-binding domains that recognize particular consensus sequences, allowing it to bind to target genes with precision.
- The combined actions of many transcription factors—some activating, others repressing—work in a kind of molecular cascade to remodel gene expression patterns across the entire genome.

In the case of iPSCs, researchers identified four crucial transcription factors that, when working together, were sufficient to reset the gene expression landscape of a mature cell. It's as if these proteins knew how to "turn back time" for the cell—not by changing its DNA sequence, but by reprogramming how its genes were read and expressed.

To understand the power of this process, imagine taking an old house and stripping it down to its foundation so it can be rebuilt into anything: a school, a hospital, or a museum. With iPSCs, scientists can essentially do the same with adult cells—reset them and guide them into new, specialized forms depending on the body's needs.

This breakthrough revolutionized medicine. For instance, in 2019, researchers used iPSCs to develop retinal cells as a treatment for age-related macular degeneration, a condition that causes vision loss in older adults. Skin cells taken from a patient were reprogrammed into iPSCs and then converted into the very eye cells lost in the disease. Because these cells originated from the patient's own tissue, they were a perfect genetic match—dramatically reducing the risk of immune rejection.

iPSCs offer both ethical and medical advantages. Prior to this technology, pluripotent stem cells could only be obtained from human embryos, a process that sparked legal and ethical debates. iPSCs sidestep this issue entirely. Medically, they hold enormous potential for personalized therapies, since patient-specific cells can be used to model diseases or develop tailor-made treatments. Scientists can now create cell models for conditions like Alzheimer's, diabetes, and heart disease—all using a patient's own genetic material.

In essence, iPSCs are a living demonstration of just how powerful and precise transcriptional regulation can be. Through the carefully orchestrated use of transcription factors, scientists can rewrite the molecular identity of a cell—offering a glimpse into a future where regenerative medicine and personalized healthcare are not only possible, but increasingly within reach.

A Game-Changer: Induced Pluripotent Stem Cells (iPSCs)

In 2007, scientists made a groundbreaking discovery: they learned how to reprogram adult cells, such as those from the skin or blood, to behave like embryonic stem cells. These reprogrammed cells are known as induced pluripotent stem cells, or iPSCs. Using a set of carefully selected genes, researchers were able to reset adult cells back to an earlier, more flexible state known as

pluripotency, giving these cells the ability to develop into almost any cell type in the body.

This achievement fundamentally changed the landscape of stem cell research. Before this, scientists were limited by the need to use embryos to obtain pluripotent stem cells. iPSCs removed that dependency and opened new possibilities. To understand the power of this process, imagine taking an old house and gutting it down to the foundation so it can be rebuilt into anything: a school, a hospital, or a museum. With iPSCs, scientists can essentially do the same with adult cells—reset them and guide them into new specialized forms.

One major breakthrough came in 2019, when scientists used iPSCs to develop retinal cells for treating age-related macular degeneration, a condition that causes vision loss in older adults. In this clinical trial, the patient's own skin cells were reprogrammed into iPSCs, which were then converted into the type of eye cells lost in the disease. When implanted into the eye, these cells offered the potential to slow or even reverse the progression of blindness. Because the cells were genetically matched to the patient, there was little risk of rejection by the immune system.

iPSCs offer both ethical and medical advantages, making them one of the most promising tools in regenerative medicine. Ethically, iPSCs eliminate the need to destroy human embryos, which has been a major source of public concern and legal restriction in many countries. Medically, their ability to be derived from a patient's own tissue reduces the likelihood of immune rejection, increases compatibility, and allows for the development of personalized therapies. Additionally, iPSCs make it possible for researchers to create disease-specific cell models in the lab—offering new ways to study conditions like Alzheimer's, diabetes, and heart disease in a dish. These combined benefits make iPSCs a vital innovation in modern biology, enabling safer, more adaptable, and ethically responsible advances in regenerative medicine.

Gene Editing Meets Regenerative Medicine: Introduction to CRISPR

As researchers explore ways to grow new tissues or replace faulty ones, they often run into a central problem: what if the cells being used still carry the genetic defect that caused the disease in the first place? This is where a powerful new tool comes in: CRISPR.

CRISPR (short for Clustered Regularly Interspaced Short Palindromic Repeats) is a breakthrough gene-editing technology that allows scientists to precisely change DNA inside living cells. Imagine having a "find and replace" function for the genetic code—CRISPR lets researchers cut out problematic genes and replace or fix them.

Here's how it works:

1. Scientists design a small piece of RNA (a guide molecule) that matches the part of the DNA they want to change.
2. This RNA guides an enzyme (called Cas9) to the exact location in the genome.
3. Cas9 acts like molecular scissors, cutting the DNA at that spot.
4. The cell then repairs the DNA, often using a template provided by scientists to insert the correct version of the gene.

The applications of CRISPR are enormous. For example:

- In cancer therapy, scientists are editing immune cells to better recognize and attack cancer cells.
- In trials for genetic disorders like cystic fibrosis, CRISPR has been used to correct the mutation in lung cells, improving symptoms and lung function.
- In the future, CRISPR might be used to correct inherited conditions like sickle cell anemia or muscular dystrophy before symptoms even appear.

CRISPR represents the intersection of genomics and regenerative medicine, allowing scientists not only to grow replacement cells but to ensure those cells don't carry harmful genetic defects. While you'll learn more about CRISPR in an upcoming chapter, it's important to understand how it is already changing what's possible in the world of biotechnology.

Summary

Stem cells are unique cells in the body that have two key abilities: they can self-renew (make copies of themselves) and differentiate (turn into specialized cells). These features make them vital for development, tissue maintenance, and healing. Unlike somatic cells, which are terminally differentiated and locked into their specific roles, stem cells remain flexible.

Scientists classify stem cells based on how many types of cells they can become. Totipotent stem cells, like the zygote, can

become any cell in the body—including support structures like the placenta. Pluripotent stem cells, such as embryonic stem cells, can become nearly any body cell but not placental cells. Multipotent stem cells, like hematopoietic stem cells from bone marrow, can develop into related cells within a specific group (e.g., different types of blood cells). Unipotent stem cells are limited to producing only one type of cell but play a crucial role in tissue repair.

Stem cells are essential to regenerative medicine, which aims to treat or cure diseases by repairing damaged tissues using the body's own natural repair system. Understanding the types and abilities of stem cells helps scientists choose the best cells for different types of treatments.

Key Terms

cell differentiation
: the the process by which a stem cell becomes a more specialized cell type with a specific function (e.g., nerve, skin, or blood cell)

CRISPR
: a powerful genome-editing tool used for precise DNA modifications

blastocyst
: a very early embryo with an inner group of cells that can form the body

embyronic stem cells (ESC)
: a pluripotent stem cell derived from early-stage embryos that can become nearly any cell in the body

hematopoietic stem cell
: a type of multipotent stem cell found in bone marrow that can develop into various types of blood cells

induced pluripotent stem cell (iPSC)
: a mature cell reprogrammed to behave like a stem cell that can become any one of many cell types

multipotent stem cell
: a stem cell that can become a limited range of related cell types (e.g., blood cells) but not any type of cell in the body

pluripotent stem cell
: a stem cell that can develop into nearly any cell type in the body but not support cells like the placenta

regenerative medicine
: a field of medicine that uses stem cells, gene therapy, and tissue engineering to repair or replace damaged tissues and organs

self-renewal
: the ability of a stem cell to divide and produce identical copies of itself over time

somatic cell
: a fully specialized (differentiated) cell that makes up tissues and organs in the body and cannot change into other cell types

stem cell
: an unspecialized cell that can self-renew and differentiate into one or more specialized cell types

terminal differentiation
: the final stage of cell specialization, after which a cell cannot change into another type

totipotent stem cell
: a stem cell that can become any cell type in the body, including placental support structures. The zygote is an example

unipotent stem cell
: a stem cell that can become only one specific type of cell but can still self-renew

zygote
: a fertilized egg cell that is totipotent and represents the earliest stage of human development

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9.11: Cancer and Gene Regulation

Learning Objectives

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

- Describe how changes to gene expression can cause cancer
- Differentiate between tumor suppressor genes and proto-oncogenes and their roles in cell cycle regulation.
- Discuss how understanding regulation of gene expression can lead to better drug design

Throughout this chapter, we've seen that cancer is a highly individualized and multifactorial disease. Even among patients diagnosed with the same type of cancer, such as lung or breast cancer, the molecular details—what genes are mutated, which proteins are altered, and how the cells behave—can vary dramatically from person to person. These differences help explain how tumors can share the same hallmarks yet arise through diverse biological pathways.

Understanding cancer at its molecular level is crucial because it reveals how seemingly small changes inside cells can produce profound effects on how those cells grow, survive, and interact with their environment. Cancer is not just a disease of rapidly dividing cells—it is fundamentally a disease driven by changes in molecules: DNA mutations, alterations in proteins, disrupted signaling pathways, and errors in the cellular systems that normally maintain order.

Imagine two people both diagnosed with liver cancer. Even if the cancer originates in the same type of liver cell, the genetic and environmental paths leading to tumor formation can be entirely different. One person's cancer might result from inherited mutations compounded by decades of smoking, while another's could be tied to viral infections or exposure to environmental toxins. These unique molecular journeys mean that a drug effective for one patient may fail in another with the same diagnosis. It also underscores a key challenge in cancer therapy: knowing precisely what molecular changes are driving an individual's tumor.

At the core of many of these molecular changes are alterations in the proteins that regulate fundamental processes like the cell cycle, DNA repair, and cell survival. In normal cells, these proteins act as finely tuned switches and signals, maintaining balance between growth and restraint. In cancer, however, mutations can lock these molecular switches in the "on" or "off" position, leading to unregulated cell division, evasion of cell death, and accumulation of further damage.

These molecular disruptions connect directly to the hallmarks of cancer we've discussed previously. Here we'll explore specific examples of how these molecular mechanisms go awry—such as mutations in proteins that normally act as tumor suppressors or those that function as accelerators of cell growth. Understanding these details provides insight not only into how cancer develops but also into how targeted therapies might work to restore balance in diseased cells.

Tumor Suppressor Genes and Oncogenes

The molecular basis of cancer often involves damage to key proteins that either keep cell growth in check or help promote it at the right times. Two groups of genes—tumor suppressor genes and proto-oncogenes—are central to this balance and illustrate how molecular changes drive cancer's hallmarks.

Tumor suppressor genes act like the brakes of a car, ensuring cells don't divide uncontrollably or survive when they shouldn't. One well-known example is p53, often called the "guardian of the genome." Under normal conditions, p53 senses DNA damage or cellular stress and can either halt the cell cycle to allow repairs or trigger apoptosis if the damage is beyond repair. When p53 is mutated—a change found in over half of all human cancers—it loses this protective role. Cells with DNA damage keep dividing, accumulating further mutations that promote cancer progression. It's akin to cutting the brakes in a speeding car, leaving it unable to stop.

Other tumor suppressors include RB (retinoblastoma protein), which controls the transition between phases of the cell cycle, and BRCA1 and BRCA2, which help repair DNA double-strand breaks. Mutations in BRCA1 or BRCA2 impair this critical repair process, making cells more likely to acquire further genetic errors. This molecular vulnerability explains why inherited mutations in these genes sharply increase the risk of breast and ovarian cancers.

On the opposite side are proto-oncogenes, which normally function like the gas pedal of a car—promoting cell growth and division when needed. When proto-oncogenes become mutated or overly active, they transform into oncogenes that push cells to divide uncontrollably, even without proper signals.

A classic example is the myc gene, which encodes a transcription factor turning on many genes involved in cell growth. In Burkitt's lymphoma, a chromosomal rearrangement places myc under control of a highly active promoter, causing it to drive excessive proliferation of immune cells.

Another frequent player is RAS, a gene that encodes a signaling protein helping relay growth signals from the cell surface to the nucleus. A single point mutation in RAS can lock it in an active state, continuously sending growth signals even without external cues. Mutated RAS proteins are found in various cancers, including pancreatic, colon, and lung cancers.

Ultimately, the molecular basis of cancer often involves a "double hit": tumor suppressor genes losing function (removing the brakes), and proto-oncogenes becoming hyperactive oncogenes (stomping the gas pedal). This imbalance pushes cells toward unchecked growth and survival, creating the conditions for cancer to develop and progress.

Gene Expression Changes: From Transcription to Translation

Gene expression is a multi-step process, and cancer can result from disruptions at any of these stages.

At the transcriptional level, cancer can involve mutations in transcription factors or the proteins that help initiate gene expression. For example, some breast cancers overexpress the epidermal growth factor receptor (EGFR). This leads to activation of downstream transcription factors that ramp up the expression of growth-related genes. Targeted therapies like anti-EGFR drugs are designed to block this signaling and slow tumor growth.

At the post-transcriptional level, molecules called microRNAs (miRNAs) help fine-tune gene expression by binding to mRNA transcripts and preventing them from being translated into proteins. In cancer, the levels of specific miRNAs can be altered—either too high or too low—resulting in unstable or excessive gene expression. Unique miRNA profiles are being studied as potential tools for diagnosis and treatment.

At the translational and post-translational levels, additional changes can influence cancer development. Proteins might be made in higher amounts, folded incorrectly, or modified in ways that alter their function. One example is the c-FLIP protein, which helps regulate apoptosis. In normal cells, both forms of c-FLIP (long and short) balance cell death signals. In some colon cancers, overexpression of the long form promotes growth instead of death—tipping the scale toward cancer.

These layers of regulation illustrate just how many opportunities there are for things to go wrong—and how cancer often arises not from a single mutation, but from a series of missteps in gene expression control.

Epigenetic Changes in Cancer

Some changes in gene expression don't involve mutations at all. Epigenetic modifications alter how DNA is packaged in the cell and how accessible genes are for transcription. Common epigenetic changes in cancer include DNA methylation (adding chemical tags to DNA that silence genes) and histone deacetylation (removing acetyl groups from histone proteins, which tightens DNA packaging and reduces gene expression).

These changes can silence tumor suppressor genes without changing the DNA sequence itself. The good news is that epigenetic changes are reversible. Drugs are being developed that target the enzymes responsible for these modifications, with the goal of "reactivating" silenced genes and restoring normal cell function.

Targeted Therapies and the Future of Personalized Medicine

As we've seen, cancer is ultimately a disease of molecular malfunction. Whether through genetic mutation, protein misfolding, altered signaling, or epigenetic disruption, the root cause of cancer lies in the dysfunction of key cellular components. Once we understand which proteins are defective and how, we gain insight into how the system—our cells—has gone wrong.

This molecular understanding forms the foundation of personalized medicine. Rather than relying on one-size-fits-all treatments, scientists and clinicians are now working to match therapies to the unique molecular profile of each tumor. This approach is

already improving patient outcomes by targeting the specific drivers of a person's cancer while minimizing harm to healthy cells.

Some targeted therapies block overactive signaling pathways. For example, HER2-positive breast cancers overproduce the HER2 receptor protein, which fuels aggressive cell growth. Drugs like trastuzumab (Herceptin) directly target this protein, reducing proliferation. Other strategies exploit vulnerabilities in DNA repair. Patients with BRCA1 or BRCA2 mutations, who already have impaired DNA repair mechanisms, can benefit from PARP inhibitors—drugs that further block repair and push cancer cells toward death.

Hormone-driven cancers also benefit from targeted interventions. Estrogen receptor-positive (ER+) breast cancers rely on estrogen signals to grow, and treatments like tamoxifen can block this pathway. Even triple-negative breast cancers, which lack all three common receptors (HER2, ER, PR), are now being parsed into molecular subtypes, offering new angles for therapy development.

Ultimately, by mapping how cancer cells are miswired, we can design therapies to correct or exploit those molecular flaws. This not only increases the chance of successful treatment, but also avoids wasting time and resources on ineffective options. Precision medicine helps ensure that each patient receives the right therapy for their specific disease—a shift that holds the promise of better outcomes and fewer side effects.

As we continue to unravel the layers of molecular regulation in cancer, new insights will lead to smarter, more individualized treatments. The future of cancer care lies not just in fighting disease, but in understanding it—one molecule at a time.

New Drugs to Combat Cancer: Targeted Therapies

Scientists are using what is known about the regulation of gene expression in disease states, including cancer, to develop new ways to treat and prevent disease development. Many scientists are designing drugs on the basis of the gene expression patterns within individual tumors. This idea, that therapy and medicines can be tailored to an individual, has given rise to the field of personalized medicine. With an increased understanding of gene regulation and gene function, medicines can be designed to specifically target diseased cells without harming healthy cells. Some new medicines, called targeted therapies, have exploited the over expression of a specific protein or the mutation of a gene to develop a new medication to treat disease. One such example is the use of anti-EGF receptor medications to treat the subset of breast cancer tumors that have very high levels of the EGF protein. Undoubtedly, more targeted therapies will be developed as scientists learn more about how gene expression changes can cause cancer.

Career Connection

Clinical Trial Coordinator

A clinical trial coordinator is the person managing the proceedings of the clinical trial. This job includes coordinating patient schedules and appointments, maintaining detailed notes, building the database to track patients (especially for long-term follow-up studies), ensuring proper documentation has been acquired and accepted, and working with the nurses and doctors to facilitate the trial and publication of the results. A clinical trial coordinator may have a science background, like a nursing degree, or other certification. People who have worked in science labs or in clinical offices are also qualified to become a clinical trial coordinator. These jobs are generally in hospitals; however, some clinics and doctor's offices also conduct clinical trials and may hire a coordinator.

Summary

Cancer is not a single disease but a diverse collection of conditions that share a key feature: uncontrolled cell division. At the molecular level, every case of cancer is unique, influenced by a combination of genetic mutations, environmental factors, and lifestyle choices. A major contributor to this loss of control is the dysregulation of gene expression—errors in how genes are turned on, off, or processed.

Gene expression can be disrupted at many stages, from transcription and RNA processing to protein synthesis and modification. For example, tumor suppressor genes act as cellular brakes, halting division when problems are detected. In contrast, proto-oncogenes function as accelerators and, when mutated, can become oncogenes that drive excessive growth.

Other disruptions include changes in transcription factor activity, altered microRNA levels, and errors in protein folding or modification. Even reversible epigenetic changes, like DNA methylation and histone deacetylation, can silence genes that normally protect cells from becoming cancerous.

Understanding these molecular mechanisms has revolutionized treatment. Targeted therapies now use this knowledge to block cancer-specific pathways, offering more personalized, effective approaches. In breast cancer, for instance, molecular markers guide therapy and highlight how modern cancer treatment is increasingly shaped by our understanding of gene expression.

Key Terms

apoptosis
: programmed cell death; a natural mechanism by which cells self-destruct when damaged or no longer needed

cancer
: a group of diseases characterized by uncontrolled cell division and the potential to invade or spread to other parts of the body

epigenetics
: heritable changes in gene expression that do not involve changes to the DNA sequence

microRNA (miRNA)
: small RNA molecules that regulate gene expression by degrading or blocking the translation of mRNA

oncogene
: a mutated or overactive proto-oncogene that contributes to uncontrolled cell division

post-translational modification
: changes to proteins after synthesis, such as phosphorylation or methylation, that alter function or activity

proto-oncogene
: a gene that normally promotes cell growth; when mutated, it can become an oncogene

tumor suppressor gene
: a gene that inhibits cell division or triggers apoptosis; loss of function can lead to cancer

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10: CELL CYCLE AND CANCER

10.1: Cancer
10.2: The Cell Cycle
10.3: Cancer and the Cell Cycle
10.4: Hallmarks of Cancer
10.5: Cancer Treatments

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10.1: Cancer

Learning Objectives

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

- Explain why cancer is considered a collection of diseases rather than a single disease.
- Describe how inherited and acquired DNA mutations contribute to the development of cancer.
- Distinguish between benign and malignant tumors and explain how metastasis contributes to cancer severity.
- Describe how environmental exposures and lifestyle factors can increase the likelihood of DNA mutations that may lead to cancer.

Cancer

When people hear the word cancer, they often picture a single disease with a clear cause. But cancer is not a single disease—it is a category that includes many related conditions. What all cancers have in common is that they begin when the regulatory systems that normally control how and when cells divide break down. In other words, cancer is a disease of gene regulation gone wrong. Whether it arises in lung tissue, breast tissue, or the colon, cancer begins when the genetic instructions that normally keep cell growth in check are altered.

Those genetic instructions are stored in DNA. Genes are segments of DNA that contain the instructions for making proteins, many of which help regulate when cells grow, divide, repair damage, or die. When the DNA sequence of a gene changes, the protein it produces may no longer function properly. If enough mutations affect genes that control cell growth, normal regulation can break down and cancer may develop. Because proteins perform most of the work inside a cell, mutations that alter important proteins can change how cells behave. Mutations affecting proteins involved in cell growth, DNA repair, or cell death are especially important in the development of cancer.

These disruptions are usually caused by mutations—changes in the DNA sequence of genes involved in cell cycle regulation, DNA repair, or cellular identity. Most mutations that lead to cancer are acquired during a person's lifetime rather than inherited. They can result from external factors like smoking, ultraviolet (UV) light, indoor tanning, certain chemicals called carcinogens, or certain viruses, but they can also arise spontaneously when the cell makes mistakes during DNA replication.

Carcinogens are physical, chemical, or biological agents that increase the likelihood that DNA will become damaged or mutated. For example, ultraviolet (UV) radiation from sunlight or tanning beds can damage DNA in skin cells, while chemicals in tobacco smoke can damage DNA in lung cells. Some viruses can also contribute to cancer by disrupting normal cellular processes or promoting long-term inflammation. Although cells possess sophisticated DNA repair systems that continuously detect and repair damaged DNA, not all damage is successfully repaired. Over time, unrepaired DNA damage can accumulate as mutations, increasing the risk that a normal cell will become cancerous.

In rare cases, individuals inherit mutations in key regulatory genes, which increases their risk of developing cancer—but even in those cases, additional mutations over time are needed for cancer to develop.

It is important to recognize that most cancers do not result from a single mutation. Instead, they develop after multiple mutations gradually accumulate in the same cell over many years. This helps explain why the risk of many cancers increases with age and why lifestyle choices that reduce DNA damage—such as avoiding tobacco products, limiting excessive sun exposure, and receiving recommended vaccines against cancer-causing viruses—can lower cancer risk.

Cancer causing microbes

Although cancer is most often associated with inherited or acquired DNA mutations, some microorganisms can also contribute to cancer development. These microorganisms generally do not "cause" cancer directly. Instead, they increase the likelihood that cancer will develop by damaging DNA, disrupting normal gene regulation, or promoting chronic inflammation.

Certain viruses, such as human papillomavirus (HPV) and hepatitis B virus (HBV), can increase the risk of cancer by introducing changes that disrupt normal gene regulation or by promoting chronic inflammation. For example, persistent HPV infection can lead to cervical cancer, while chronic HBV infection increases the risk of liver cancer. These viruses alter the normal behavior of infected cells, allowing mutations to accumulate more readily over time.

Approximately 15–20% of cancers worldwide are associated with infectious agents. In later chapters, you will learn how viruses and other microorganisms interact with host cells and how some of these interactions can contribute to diseases such as cancer.

Most cancers therefore result from the gradual accumulation of somatic mutations - DNA mutations acquired throughout a person's lifetime rather than from a single mutation or inherited genetic condition. This is why the risk of many cancers increases with age—the longer we live, the more opportunities there are for mutations to accumulate in our cells.

Can DNA Mutations Help Explain Why We Age?

Every day, the cells in your body acquire small changes in their DNA called somatic mutations. These mutations are not inherited from your parents. Instead, they arise throughout life as cells divide or when DNA is damaged by factors such as ultraviolet (UV) radiation, chemicals in tobacco smoke, or simply by the normal processes of cellular metabolism. Most of these mutations are harmless, and many are repaired before they can cause problems. However, some persist and gradually accumulate over time.

Scientists have long known that somatic mutations play a major role in the development of cancer. More recently, researchers have begun asking a broader question:

Could the gradual accumulation of DNA mutations also contribute to the aging process?

To investigate this question, researchers developed a mathematical model that estimated how long humans might live if every known hallmark of aging could somehow be eliminated except the accumulation of somatic mutations. Their model suggested that mutations alone could still limit the median human lifespan to approximately 146–194 years, with the exact value depending on the assumptions of the model. The study also found that organs such as the brain and heart, whose cells are rarely replaced, may be more vulnerable to the long-term accumulation of mutations than tissues such as the liver, where damaged cells can be replaced more readily.

It is important to understand what this study does—and does not—show. The researchers were not predicting that humans will someday routinely live for 150 to 200 years. Instead, they used a theoretical model to estimate the contribution of one biological process—the accumulation of somatic mutations—to aging. Their results suggest that DNA mutations are an important contributor to aging, but they are only one of many biological processes involved. Other hallmarks of aging, including changes in mitochondria, protein homeostasis, epigenetics, and cellular communication, also play important roles.

This study highlights an important theme in both cancer biology and aging research: our DNA is continually changing throughout life. Most mutations never cause disease, but a small number can alter how cells function. In some cells, these mutations may contribute to cancer. Across many cells and tissues over decades, they may also contribute to the gradual decline associated with aging. Understanding how mutations accumulate—and how cells repair DNA damage—is an active area of research that could one day lead to new strategies for preventing disease and promoting healthy aging.

Although the word tumor is often used interchangeably with cancer, not all tumors are cancerous. A tumor is simply a mass of cells that forms when cells divide more than they should or fail to die when they normally would **(Figure** $10.1.1$**)**. While tumors often result from mutations that disrupt the normal regulation of cell growth, not every tumor becomes cancerous. When a mutation occurs in a gene that normally limits cell division, those controls can be lost, and the affected cell begins dividing more rapidly. If the resulting tumor stays in one place, grows slowly, and is enclosed in a protective sheath, it is considered a benign tumor. However, if additional mutations accumulate that affect genes regulating cell adhesion, motility, or growth signals, the tumor may become malignant. Malignant tumors grow faster, invade surrounding tissues, and can eventually metastasize, spreading through the bloodstream or lymphatic system to distant parts of the body. This gradual progression from a benign growth to an invasive cancer illustrates that cancer typically develops through multiple genetic changes rather than a single mutation.

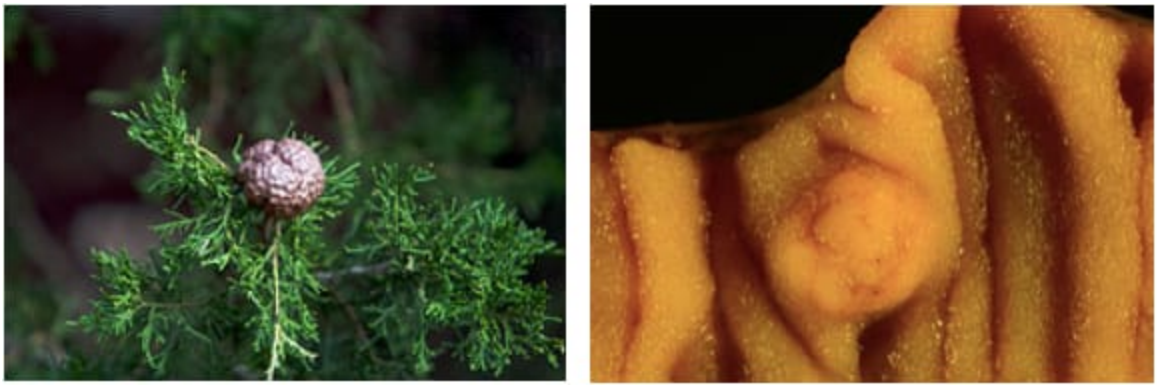


Figure 10.1.1: (left) A tumor on a cypress branch. (right) A tumor of the small intestine. Cypress tumor photo by W. Calder, cc licensed 2009. Small bowel tumor by E. Uthman, public domain 1999.

From a cellular perspective, cancer cells look and behave very differently from healthy cells, largely due to failures in the cellular control systems that regulate growth and division. Normally, cell division is tightly controlled by a network of signals that includes input from neighboring cells and internal checkpoints that monitor DNA integrity. In adult tissues, contact inhibition is one such regulatory mechanism—it prevents cells from dividing once they sense they are surrounded. But cancer cells often carry mutations in genes that override this inhibition. As a result, they continue dividing even when they shouldn't, forming dense piles of cells when grown in a lab dish. **(Figure** 10.1.2**)** This loss of contact inhibition is one of several characteristics that distinguish cancer cells from normal healthy cells.

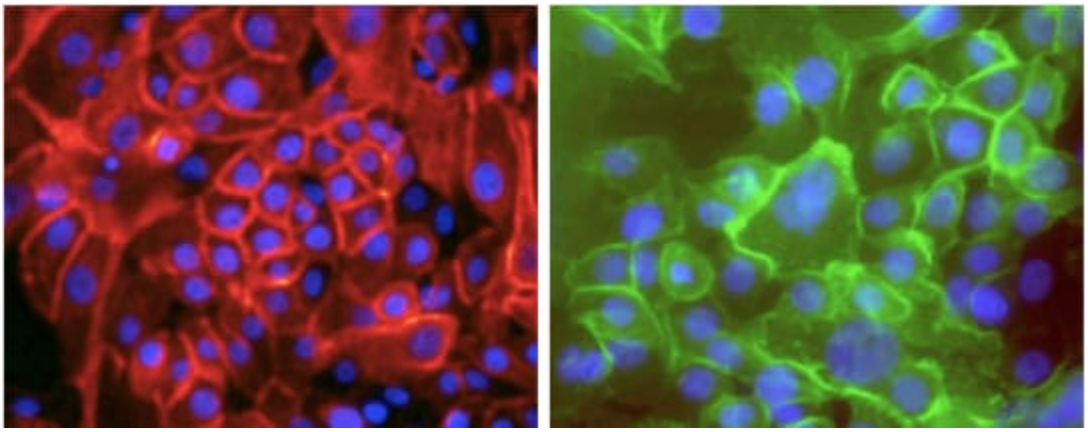


Figure 10.1.2: Normal human breast cells in culture at left. At right, similar cultured cells that have been transformed (i.e. they are now cancerous). Note the irregularity of both cell and nuclear morphology. Membranes are arbitrarily stained in different colors; chromosomes are stained blue in both panels. Photos from Ince et al, Cancer Cell 12:160-170, 2007.

In a developing embryo, cells begin identical and become specialized for certain functions through a process called differentiation. Differentiation is controlled by changes in gene expression that allow different genes to be turned on or off depending on a cell's function. As cells become specialized, they turn on specific sets of genes needed for their roles—for example, muscle cells activate muscle-specific genes, while liver cells turn on genes for detoxification. In cancer, this regulatory program is disrupted.

In cancer, this regulatory program is disrupted. Cancer cells often lose many of the characteristics that make them specialized. Instead, they behave more like immature cells that continue dividing, ignore signals that normally limit growth, and are better able to invade surrounding tissues. This loss of specialization contributes to the aggressive behavior seen in many cancers.

Because cancer is caused by multiple genetic changes, it tends to develop gradually. Scientists often track the mutations involved in different types of cancers. These studies have shown that cancer usually develops through a series of mutations rather than from a single genetic change. One well-studied example is colon cancer, which tends to follow a relatively predictable path involving multiple mutations over time **(Figure** 10.1.3**)**. In the early stages, mutations may arise in a gene called APC, which normally helps regulate the cell cycle. As additional mutations accumulate—such as in RAS, DCC, TP53, and PRL3—the risk of developing a full-blown, invasive cancer increases.

Each of these genes plays a different role in keeping cells in check. Some normally promote division only when it's appropriate, while others act more like brakes, preventing division when the DNA is damaged or the cell is too old. You can think of these genes as the accelerator and brake pedals of a car. Healthy cells need both systems working together to carefully regulate growth. When the accelerator gets stuck or the brakes fail, the result is uncontrolled growth. As additional mutations accumulate, more of the normal safeguards that regulate cell growth are lost, allowing cells to divide uncontrollably and eventually become invasive. This will be further discussed in the next few sections.

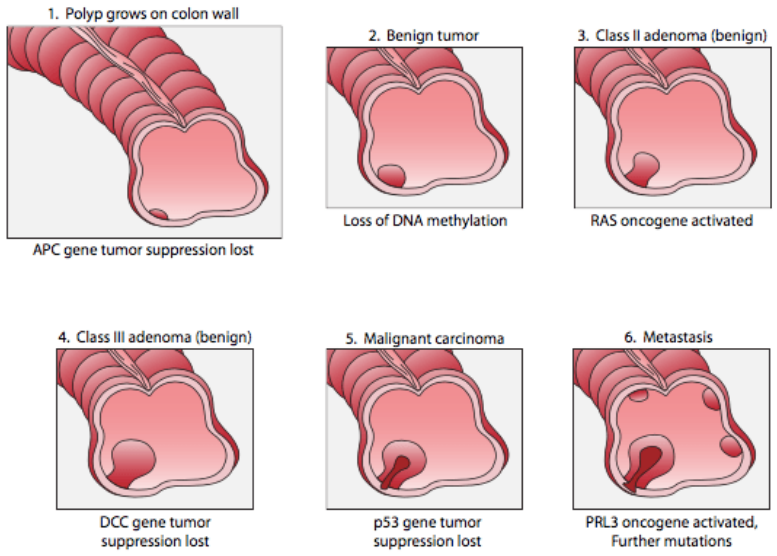


Figure 10.1.3: Development of colon cancer takes time and multiple mutations.

1. Polyp grows on colon wall: A small polyp protrudes from the colon lining. Caption: “APC gene tumor suppression lost.”
2. Benign tumor: The polyp enlarges. Caption: “Loss of DNA methylation.”
3. Class II adenoma (benign): The growth becomes larger and more prominent. Caption: “RAS oncogene activated.”
4. Class III adenoma (benign): The tumor continues to expand into the colon cavity. Caption: “DCC gene tumor suppression lost.”
5. Malignant carcinoma: The tumor develops cancerous characteristics and invades deeper tissue. Caption: “p53 gene tumor suppression lost.”
6. Metastasis: Multiple tumor sites are visible, indicating spread beyond the original location. Caption: “PRL3 oncogene activated, more DNA methylation."

It’s important to remember that even cancers in the same tissue type can look very different at the molecular level. Two people with breast cancer might have tumors caused by mutations in completely different genes. As a result, one treatment might work for one person but not the other. By identifying the specific genetic changes within a tumor, doctors can select treatments that are more likely to be effective for that individual patient. This approach, known as personalized medicine (or precision medicine), has become a major focus of modern cancer research and therapy. Advances in DNA sequencing and biotechnology now allow physicians to identify many of the mutations present in a patient's tumor, helping guide treatment decisions and improve outcomes.

Ultimately, cancer is a disease of failed cellular regulation that begins with changes in DNA. As mutations accumulate over time, they disrupt the genes responsible for controlling cell growth, DNA repair, differentiation, and cell survival. Eventually, the normal systems that maintain healthy tissues can no longer function properly, allowing cancer cells to grow, invade surrounding tissues, and sometimes spread throughout the body. Understanding the mechanisms behind this disease not only helps us treat it more effectively, but also deepens our appreciation of how healthy cells cooperate to keep our bodies functioning. In the following sections, we will examine the specific genes and molecular pathways that regulate the cell cycle and explore how mutations in these pathways drive cancer development.

Summary

Cancer is a disease caused by the accumulation of mutations in DNA that disrupt the normal regulation of cell growth, division, and survival. Genes are segments of DNA that provide instructions for making proteins, and mutations can alter how these

proteins function. Most cancer-causing mutations are acquired during a person's lifetime through normal DNA replication errors or exposure to environmental factors such as ultraviolet radiation, tobacco smoke, certain microorganisms, and other carcinogens, although some people inherit mutations that increase their risk of developing cancer. Because cancer usually results from multiple mutations that accumulate over many years, it develops gradually rather than from a single genetic change. Cancer cells lose normal growth controls, become less specialized, and may invade surrounding tissues or spread to distant organs through metastasis. Because the specific genetic changes vary between individuals and cancer types, treatment is increasingly tailored to the molecular characteristics of each tumor.

Key Terms

acquired mutation (somatic mutation)
: a mutation that develops during a person's lifetime in a particular cell and is not inherited from a parent

benign tumor
: a non-invasive, localized tumor that grows slowly

cancer
: a collection of diseases involving uncontrolled cell division due to genetic mutations

carcinogen
: a physical, chemical, or biological agent that increases the risk of cancer by damaging DNA or increasing the likelihood of mutations

contact inhibition
: a regulatory mechanism that stops normal cells from dividing once they fill available space

de-differentiation
: the process by which cancer cells lose their specialized features and become more primitive

differentiation
: the process by which cells become specialized for specific functions

DNA damage
: physical or chemical changes to DNA that can lead to mutations if not properly repaired by the cell

DNA repair
: the collection of cellular mechanisms that detect and repair damaged DNA, helping maintain the integrity of the genome

gene regulation
: the process by which cells control when and how strongly genes are expressed, allowing different cell types to perform specialized functions

inherited mutation (germline mutation)
: a mutation passed from parent to child that is present in nearly every cell of the body

malignant tumor
: a cancerous tumor that invades surrounding tissues and can metastasize

metastasis
: the spread of cancer cells from the original site to distant tissues via the blood or lymphatic system

mutation
: a change in the DNA sequence that can affect gene function

personalized medicine
: an approach to healthcare that uses the genetic characteristics of a patient's disease to guide prevention, diagnosis, or treatment

regulatory gene
: a gene whose protein product helps control important cellular processes such as cell growth, DNA repair,

differentiation, or cell death

somatic mutations
: a change in the DNA of a body cell that happens after conception, meaning it affects only the individual and cannot be passed on to their children

tumor
: a mass of abnormally growing cells; may be benign or malignant

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10.2: The Cell Cycle

Learning Objectives

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

- Describe the major phases of the cell cycle and explain their roles in growth and division
- Identify and describe the function of the G_1, G_2, and M checkpoints
- Understand how disruptions in cell cycle regulation contribute to diseases like cancer

In our previous section, we learned how cancer results from a breakdown in the regulatory machinery that controls when, how, and if a cell divides. To understand how these systems fail, we first need to know how healthy cells normally progress through the cell cycle—the well-ordered, gene-regulated process that ensures accurate growth, DNA replication, and division. The cell cycle isn't just a timeline of events—it's a choreography guided by proteins whose activity is carefully turned on and off at precise moments. When gene regulation fails—through inherited or acquired mutations—this choreography can collapse, ultimately contributing to cancer.

The cell cycle is an ordered series of stages a cell passes through to produce two new, genetically identical daughter cells. It consists of two main phases: interphase (preparing for division) and the mitotic phase (carrying out division) **(Figure** 10.2.1**)**.

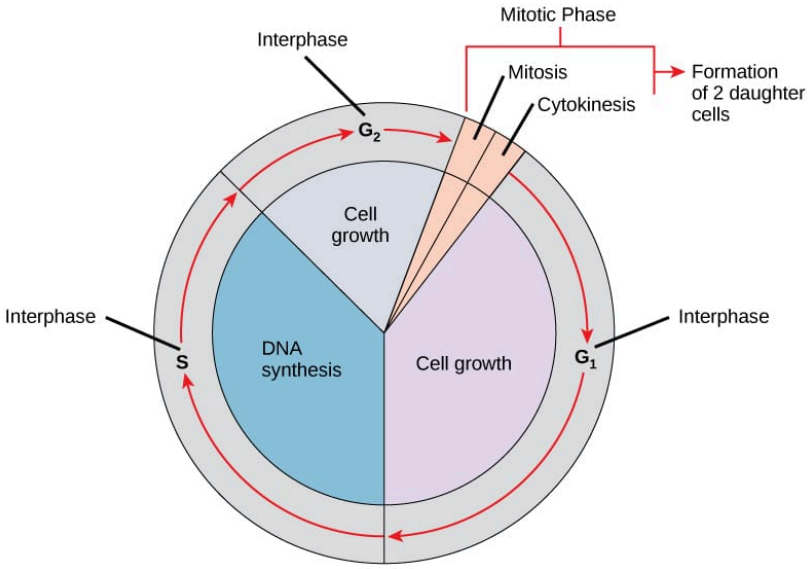


Figure 10.2.1: A cell moves through a series of phases in an orderly manner. During interphase, G_1 involves cell growth and protein synthesis, the S phase involves DNA replication and the replication of the centrosome, and G_2 involves further growth and protein synthesis. The mitotic phase follows 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.

What happens: The cell grows, making proteins that will be used for the next stage of the cell cycle as well as duplicating organelles that will eventually need to be divided into each daughter cell. It stockpiles energy and materials needed for DNA replication.

Why regulation matters: Before DNA is copied, regulatory proteins check for DNA damage and ensure the cell is big enough and healthy enough. If proteins that detect DNA damage detect problems with the DNA, the cell pauses here—preventing mutations from being passed on.

S Phase

The next stage of interphase is called the S phase, for synthesis, as this is the stage when a copy of the genetic material is made if the cell gets past G_1 phase.

What happens here: DNA replication results in the formation of two identical copies of each chromosome—sister chromatids—that are firmly attached at the centromere region.

Why regulation matters: DNA is monitored again during and after the S phase to ensure there are no problems with the newly synthesized DNA. Multiple DNA-repair proteins monitor and correct replication mistakes. Failure to detect and repair issues with the DNA at this stage could contribute to the development of cancer if they affect key genes (like APC, TP53, or RAS; mentioned in the previous section).

G_2 Phase

In the G_2 phase, or second gap, the cell replenishes its energy stores and prepares for the next phase of the cell cycle involved with chromosome manipulation.

What happens: More growth and protein production occur. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic spindle. Proteins necessary for the next phase of the cell cycle are synthesized. Finally, the cell checks that all DNA was replicated correctly and that no damage is present before division begins

Why regulation matters: This is the last chance the cell has to assess the integrity of DNA before the process of cell division beings in the next phage. As such, proteins involved in monitoring for DNA damage (e.g., p53) assess DNA integrity. If issues are found, the cell halts here to repair before entering mitosis. Mutation in such checkpoints lets damaged cells proceed to divide, increasing the risk of cancerous growth.

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** 10.2.2).

ART CONNECTION

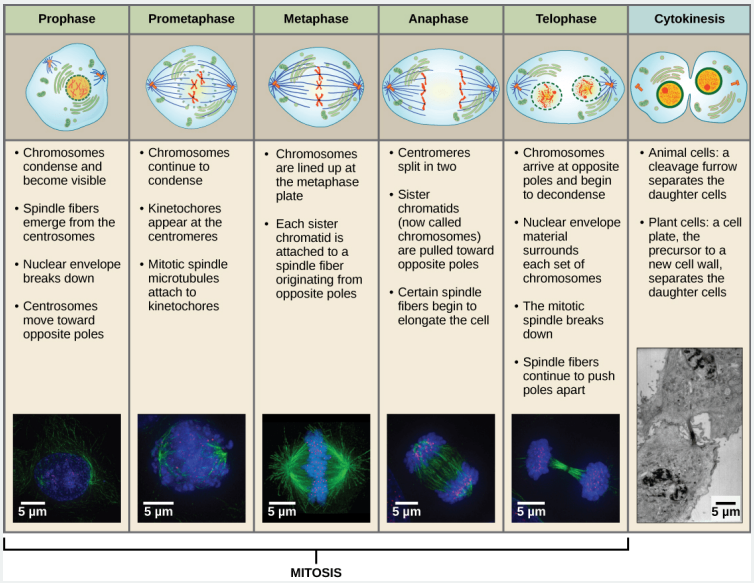


Figure 10.2.2: Animal cell mitosis is divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase—visualized here by light microscopy with fluorescence. Mitosis is usually accompanied by cytokinesis, shown here by a transmission electron microscope. During prophase, the chromosomes condense and become visible, spindle fibers emerge from the centrosomes, the centrosomes move toward opposite poles, and the nuclear envelope breaks down. During prometaphase, the chromosomes continue to condense and kinetochores appear at the centromeres. Mitotic spindle microtubules attach to the kinetochores. During metaphase, the centrosomes are at opposite poles of the cell. Chromosomes line up at the metaphase plate and each sister chromatid is attached to spindle fibers originating from the opposite poles. During anaphase, the centromeres split in two. The sister chromatids, which are now called chromosomes, move toward opposite poles of the cell. Certain spindle fibers lengthen, elongating the cell. During telophase, the chromosomes arrive at the opposite poles and begin to decondense. The nuclear envelope re-forms. During cytokinesis in animals, a cleavage furrow separates the two daughter cells. In plants, a cell plate—the precursor to a new cell wall—separates the two daughter cells. (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.

Prophase

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.

Prometaphase

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.

Metaphase

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.

Anaphase

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.

Telophase

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** 10.2.3**).**

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** 10.2.3**).**

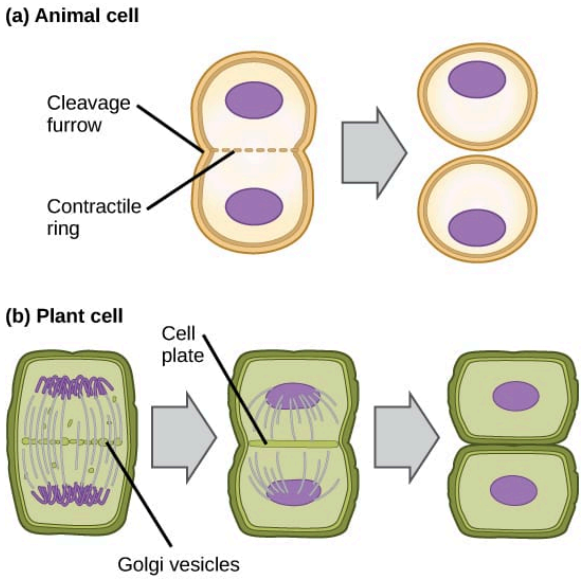


Figure 10.2.3: This illustration shows cytokinesis in a typical animal cell and a typical plant cell. In part (a), a cleavage furrow forms at the former metaphase plate in the animal cell. The plasma membrane is drawn in by a ring of actin fibers contracting just inside the membrane. The cleavage furrow 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** 10.2.4**)**.

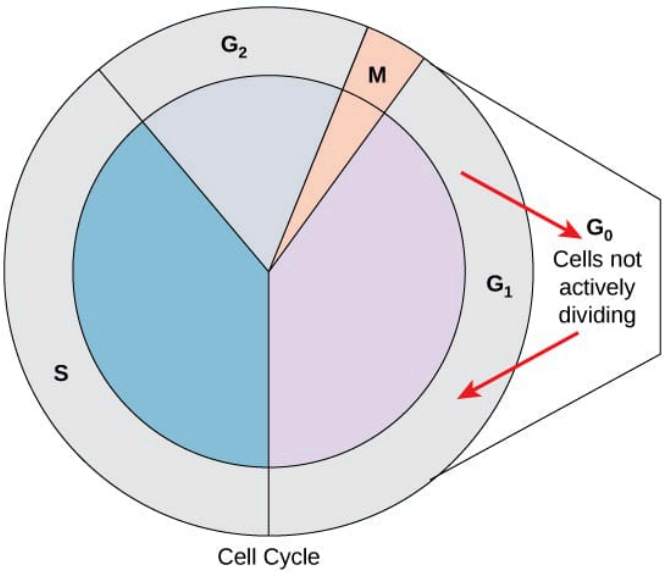


Figure 10.2.4: The cell cycle is shown in a circular graphic, with four stages. The S stage accounts for about 40 percent of the cycle. The G2 stage accounts for about 19 percent. Mitosis accounts for 2 percent, and G1 accounts for 39 percent. Cells that are not actively preparing to divide enter an 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.

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.

Concept Connection

Why Do Some Cells Stop Dividing—Forever?

In our earlier exploration of stem cells, we learned that they have the unique ability to divide and develop into many types of cells. Once cells specialize, however—like becoming a nerve cell or a heart muscle cell—they often stop dividing permanently. These are called terminally differentiated cells. In essence, terminally differentiated cells are in G0 phase.

We also learned that cells can respond to environmental cues, including signals that tell them to grow or divide. So here's a question worth pausing to consider:

- If the environment sends out signals to divide, why don't terminally differentiated cells like neurons or heart muscle cells respond and start dividing again?
- What's stopping cells in G0 phase?

The Answer Lies in Gene Regulation and Cellular Identity

When a cell becomes fully specialized, it changes in ways that go far beyond just shape or function. It also changes what genes it uses—what's turned on or off inside.

One major change is that these cells reduce or completely stop producing the receptors that would normally detect signals from the environment telling them to grow. Without those receptors, the signal may be out there—but the cell simply doesn't "hear" it.

Even if a small signal did get through, many of the internal signaling molecules and switches that the cell would need to respond are also missing as they are not actively expressed. It's like trying to answer a phone call when your phone is off and the battery is dead. Someone is trying to give you a message—but there's no way to receive or respond to it.

This built-in silence is a protective feature. It keeps terminally differentiated cells focused on their jobs (like conducting nerve impulses or contracting the heart) and prevents them from dividing when they shouldn't.

What If That Silence Is Broken?

Sometimes, something goes wrong. A mutation or a mistake in gene regulation might restore sensitivity to those growth signals, by re-activating parts of the signaling machinery the cell normally keeps shut off for growth receptors.

Now imagine a neuron in the brain—or a heart cell—starting to divide when it's not supposed to. It's no longer just doing its specialized job. Instead, it may start growing uncontrollably and interfere with the function of surrounding tissue.

This is one of the ways cancer can begin: when cells that are meant to stay quiet and stable start responding to signals they were meant to ignore.

Checkpoints: Control of the Cell Cycle

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** $10.2.5$**)**.

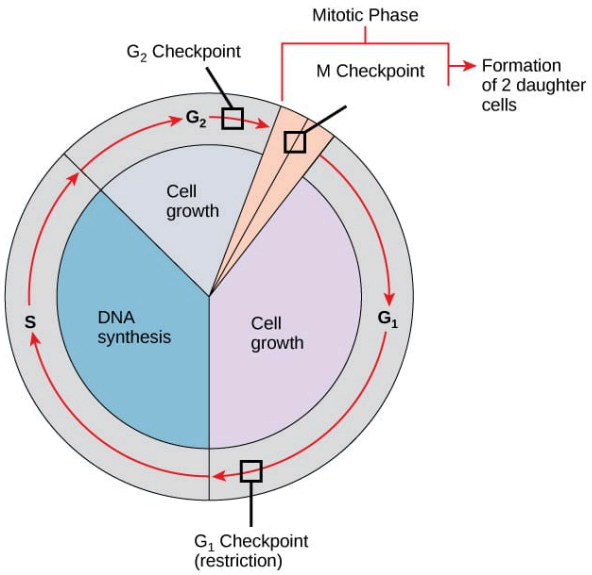


Figure 10.2.5: The cell cycle is controlled at three checkpoints. Integrity of the DNA is assessed at the G_1 checkpoint. Proper chromosome duplication is assessed at the G_2 checkpoint. Attachment of each kinetochore to a spindle fiber is assessed at the M checkpoint.

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, DNA integrity is of utmost importance and so 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 mitotic spindle. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until 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. If mutations occur in the genes that regulate these stages or checkpoints, cells may divide uncontrollably or with errors—paving the way for cancer development.

Key Terms

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

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10.3: Cancer and the Cell Cycle

Learning Objectives

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

- Explain how proto-oncogenes, tumor suppressor genes, and apoptosis work together to regulate the cell cycle.
- Predict how mutations in proto-oncogenes or tumor suppressor genes affect cell division and contribute to cancer development.
- Explain why cancer develops through the progressive failure of multiple cellular safeguards rather than from a single mutation.

Cancer is a collective name for many different diseases caused by a common mechanism: uncontrolled cell division. Despite the redundancy and overlapping levels of cell-cycle control, errors occur. One of the critical processes monitored by the cell-cycle checkpoint surveillance mechanism is the proper replication of DNA during the S phase. Even when all of the cell-cycle controls are fully functional, a small percentage of replication errors (mutations) will be passed on to the daughter cells. If one of these changes to the DNA nucleotide sequence occurs within a gene, a gene mutation results.

All cancers begin when a gene mutation gives rise to a faulty protein that participates in the process of cell reproduction. These mutations may affect proteins that promote cell division, slow the cell cycle, repair damaged DNA, or trigger apoptosis. The change in the cell that results from the malformed protein may be minor. Even minor mistakes, however, may allow subsequent mistakes to occur more readily. Over and over, small, uncorrected errors are passed from parent cell to daughter cells and accumulate as each generation of cells produces more non-functional proteins from uncorrected DNA damage. Eventually, the pace of the cell cycle speeds up as the effectiveness of the control and repair mechanisms decreases. Uncontrolled growth of the mutated cells outpaces the growth of normal cells in the area, and a tumor can result.

Having explored how gene expression is regulated and how its misregulation can disrupt the cell cycle, we now turn to two major categories of genes that play a direct role in cancer development: proto-oncogenes and tumor suppressor genes. These genes form a critical part of the cell's internal control system—some act as the accelerator, others function as the brakes **(Figure** $10.3.1a$ **)**. In addition to regulating the cell cycle itself, this internal system also includes mechanisms that can trigger apoptosis, or programmed cell death. Apoptosis serves as a safeguard to eliminate cells that have suffered irreparable DNA damage or other severe defects, thereby preventing them from continuing to divide and potentially forming tumors. When mutations or regulatory changes affect proto-oncogenes, tumor suppressor genes, or the pathways controlling apoptosis, the balance between cell growth, inhibition, and cell death can collapse. This breakdown leads to unchecked cell division, the survival of damaged cells that should have been eliminated, accumulation of further genetic damage, and ultimately, cancer.

Understanding how these genes function—and how their disruption contributes to tumor formation—helps explain why cancer is not caused by a single event, but rather by the stepwise failure of safeguards that normally preserve genomic stability. In the sections that follow, we'll build on this foundation to explore the hallmarks of cancer, and later, the multi-layered gene regulation processes that can further distort cell behavior. Together, these topics will help clarify how molecular changes give rise to the complexity and diversity seen in human cancers.

Proto-oncogenes

Proto-oncogenes are normal genes that promote cell growth and division when appropriate. They produce proteins that drive progression through the cell cycle, such as cyclins, cyclin-dependent kinases (CDKs), and various growth factor receptors. Under normal circumstances, proto-oncogenes are tightly regulated—activated only when needed and promptly shut off afterward.

Consider what might happen to the cell cycle in a cell with a recently acquired oncogene. In most instances, the alteration of the DNA sequence will result in a less functional (or non-functional) protein. The result is detrimental to the cell and will likely prevent the cell from completing the cell cycle; however, the organism is not harmed because the mutation will not be propagated.

Occasionally, however, a gene mutation causes a change that increases the activity of a positive regulator. For example, a mutation that allows CDK, a protein involved in cell-cycle regulation, to be activated before it should be could push the cell cycle

past a checkpoint before all of the required conditions are met. If the resulting daughter cells are too damaged to undertake further cell divisions, the mutation would not be propagated and no harm comes to the organism. However, if the atypical daughter cells are able to divide further, the subsequent generation of cells will likely accumulate even more mutations, some possibly in additional genes that regulate the cell cycle.

The CDK example is only one of many genes that are considered proto-oncogenes. In addition to the cell-cycle regulatory proteins, any protein that influences the cycle can be altered in such a way as to override cell-cycle checkpoints. Once a proto-oncogene has been altered so that it continually promotes cell division, it is then called an oncogene.

Tumor Suppressor Genes

Just as cars have brakes to prevent speeding, cells have genes that act like brakes to slow or stop cell division when necessary **(Figure** $10.3.1a$**)**. Tumor suppressor genes are genes that code for the negative regulator proteins, the type of regulator that—when activated—can prevent the cell from undergoing uncontrolled division. The collective function of the best-understood tumor suppressor gene proteins, retinoblastoma protein (RB1), p53, and p21, is to put up a roadblock to cell-cycle progress until certain events are completed. A cell that carries a mutated form of a negative regulator might not be able to halt the cell cycle if there is a problem. Instead of pausing to repair damaged DNA or eliminating the damaged cell, it continues dividing. The result is that cells continue dividing with damaged DNA, and the mutation load escalates rapidly which contributes to the development of cancer.

Mutated p53 genes have been identified in more than half of all human tumor cells. This discovery is not surprising in light of the multiple roles that the p53 protein plays at the G_1 checkpoint. The p53 protein activates other genes whose products halt the cell cycle (allowing time for DNA repair), activates genes whose products participate in DNA repair, or activates genes that initiate cell death (apoptosis) when DNA damage cannot be repaired. A damaged p53 gene can result in the cell behaving as if there are no mutations **(Figure** $10.3.1b$**)**. This allows cells to divide, propagating the mutation in daughter cells and allowing the accumulation of new mutations. In addition, the damaged version of p53 found in cancer cells cannot trigger apoptosis, allowing genetically damaged cells that should have died to survive and continue dividing.

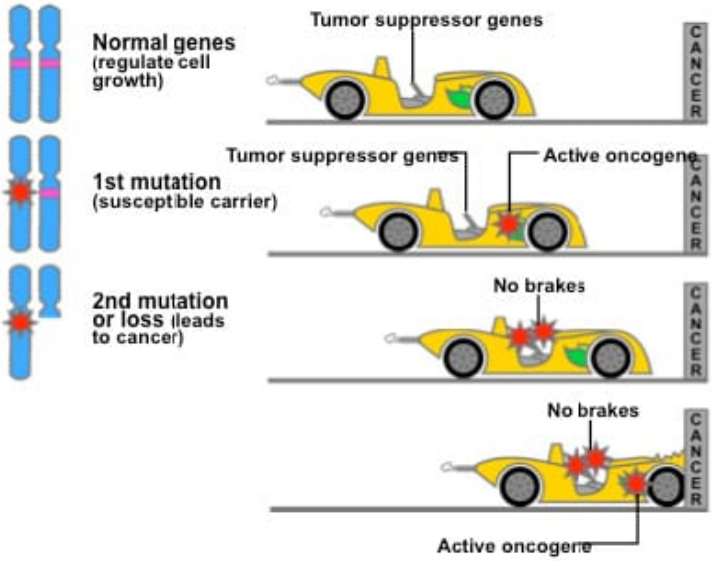
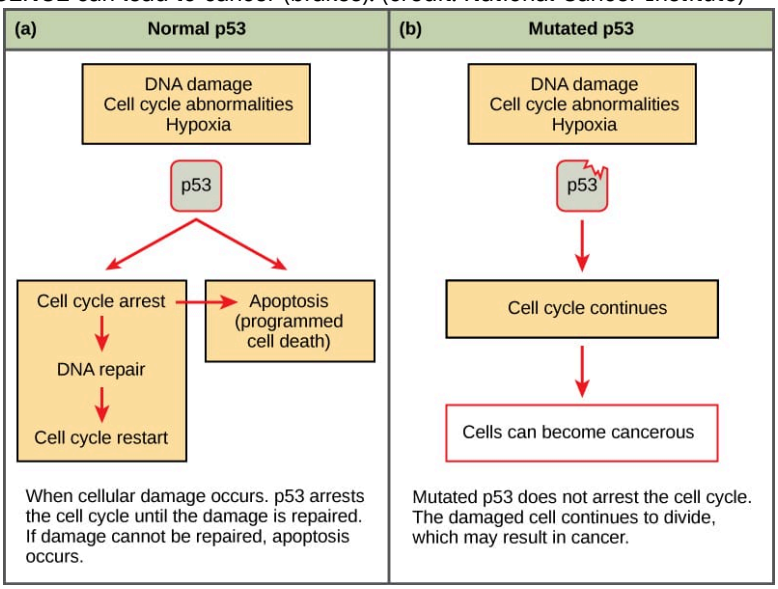


Figure
10.3.1*a*:
Oncogenes are mutated genes whose PRESENCE can stimulate the development of cancer (accelerator). Tumor suppressor genes are normal genes whose ABSENCE can lead to cancer (brakes). (credit: National Cancer Institute)



Click to open image description

In panel (a), Normal p53, a box at the top lists three conditions that can trigger a cellular response: DNA damage, cell cycle abnormalities, and hypoxia (low oxygen). A box labeled p53 appears below these conditions. Two arrows extend from p53. One arrow points to a box labeled Cell cycle arrest. Within this box, downward arrows indicate that cell cycle arrest allows DNA repair, followed by cell cycle restart if repair is successful. The second arrow from p53 points to a box labeled Apoptosis (programmed cell death). An additional arrow from the cell cycle arrest pathway leads to apoptosis, indicating that if DNA damage cannot be repaired, the cell is directed to undergo programmed cell death. Text beneath the diagram states that when cellular damage occurs, p53 stops the cell cycle until the damage is repaired, and if the damage cannot be repaired, apoptosis occurs.

In panel (b), Mutated p53, the same cellular stresses appear at the top: DNA damage, cell cycle abnormalities, and hypoxia. Below them is a box labeled p53 marked with a jagged symbol indicating mutation or malfunction. Unlike the normal pathway, a single arrow extends downward to a box labeled Cell cycle continues. Another arrow points from this box to a final box labeled Cells can become cancerous. The diagram shows that defective p53 does not halt the cell cycle, does not initiate DNA repair, and does not trigger apoptosis. As a result, damaged cells continue dividing and may accumulate mutations that lead to cancer. Text beneath the diagram explains that mutated p53 fails to arrest the cell cycle, allowing damaged cells to continue dividing, which may result

in cancer.

Figure 10.3.1b: (a) The role of p53 is to monitor DNA. If damage is detected, p53 triggers repair mechanisms. If repairs are unsuccessful, p53 signals apoptosis. (b) A cell with an abnormal p53 protein cannot repair damaged DNA and cannot signal apoptosis. Cells with abnormal p53 can become cancerous. (credit: modification of work by Thierry Soussi)

CONCEPT IN ACTION

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Go to this website to watch an animation of how cancer results from errors in the cell cycle.

Summary

Cancer develops when mutations disrupt the genes that normally regulate the cell cycle. Proto-oncogenes promote cell division, while tumor suppressor genes slow or stop cell division and help maintain genomic stability. When mutations activate proto-oncogenes, inactivate tumor suppressor genes, or disable apoptosis, damaged cells continue dividing and accumulate additional mutations, increasing the likelihood of cancer development.

Key Terms

apoptosis
: programmed cell death; a controlled process that eliminates damaged or unnecessary cells to maintain tissue health and prevent tumor formation

oncogene
: a mutated version of a proto-oncogene, which allows for uncontrolled progression of the cell cycle, or uncontrolled cell reproduction

proto-oncogene
: a normal gene that controls cell division by regulating the cell cycle that becomes an oncogene if it is mutated

tumor suppressor gene
: a gene that codes for regulator proteins that prevent the cell from undergoing uncontrolled division

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10.4: Hallmarks of Cancer

Learning Objectives

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

- Describe the major hallmarks of cancer and explain how they enable tumor growth and progression.
- Relate the hallmarks of cancer to the genetic and cellular changes discussed in previous sections.
- Explain how understanding the hallmarks of cancer has led to the development of targeted cancer therapies.

In the previous sections, we explored how gene expression is tightly regulated and how disruptions in this regulation can tip the balance of cell growth and division. We learned about proto-oncogenes, which normally promote healthy cell cycle progression, and tumor suppressor genes, which act as brakes to prevent unchecked cell growth. We also saw how apoptosis, or programmed cell death, serves as a crucial safeguard to remove cells that might become harmful.

Despite the multiple layers of surveillance and control built into cellular processes, cancer can still arise when enough of these systems fail. Importantly, cancer is not a single disease but a broad category of disorders that share common biological features. These shared characteristics help explain why different types of cancer—even those originating in different tissues—can behave in similar ways.

This brings us to a powerful framework for understanding cancer biology: the hallmarks of cancer. This concept, first proposed by scientists Douglas Hanahan and Robert Weinberg, outlines the fundamental biological capabilities that cancer cells acquire during their development. These hallmarks provide a common framework for understanding cancer, uniting diverse genetic changes and molecular disruptions into a shared set of characteristics. Although individual cancers may acquire these hallmarks in different ways, nearly all successful cancers possess many or all of these capabilities. In this section, we'll explore each hallmark and examine how these traits contribute to the development and progression of cancer.

Uncontrolled Cell Division

A defining feature of cancer cells is their ability to divide without the normal checks and balances. In healthy tissues, cells only divide when needed—to replace dead cells, repair injuries, or support growth. Signals from neighboring cells, hormones, and internal cellular mechanisms tightly regulate this process.

Cancer cells, however, evade growth suppression and stimulate their own proliferation. This happens in part through mutations in proto-oncogenes and tumor suppressor genes, as discussed earlier.

- Example: A mutation that converts the proto-oncogene RAS into its oncogenic form locks it in an “always on” state, continuously signaling the cell to divide—even without external growth signals. RAS mutations are found in many cancers, including pancreatic and colorectal cancers.
- Tumor suppressor loss: Tumor suppressors like p53 or RB1 normally halt the cell cycle if DNA damage is detected. If these genes are mutated and lose function, damaged cells proceed through the cycle unchecked, leading to accumulation of further mutations.

Think of normal cell division as a car controlled by both the gas pedal and the brakes. Cancer cells have the accelerator stuck down (activated oncogenes) and the brakes cut (inactive tumor suppressor genes).

This hallmark forms the foundation for many of the others. As cells divide more rapidly, they accumulate additional mutations that increase the likelihood of acquiring even more cancer-promoting characteristics.

Evasion of Apoptosis

Another key hallmark of cancer is evasion of apoptosis, the cell's built-in suicide program. In healthy tissues, apoptosis removes cells that are damaged, infected, or no longer needed. This process protects the body from cells that might become dangerous if

allowed to survive.

Cancer cells often acquire changes that disable apoptosis, allowing them to survive despite severe DNA damage or other abnormalities.

- p53 and apoptosis: The p53 protein plays a central role in apoptosis. When DNA is damaged, p53 can trigger cell death if repairs aren't successful. Many cancers have mutations in p53, meaning the cell can no longer initiate apoptosis in response to DNA damage. As a result, mutated cells persist and divide instead of dying.
- Bcl-2 family proteins: Another example is the Bcl-2 family of proteins, which regulate the pathways leading to apoptosis. Some cancers, like certain lymphomas, overproduce Bcl-2, which blocks the cell's suicide pathways and promotes survival even when the cell is abnormal.

Avoiding apoptosis gives cancer cells a tremendous survival advantage. Instead of being eliminated, damaged cells continue dividing, accumulating additional mutations that promote cancer progression.

Concept in Action

Watch this video describing how scientists can use our undertanding of p53 to help target and destroy cancer cells

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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Stimulating Their Own Blood Supply (Angiogenesis)

For a tumor to grow beyond a tiny cluster of cells (usually about 1–2 mm in diameter), it needs a steady supply of oxygen and nutrients. Normal tissues receive these supplies through networks of blood vessels. Cancer cells solve this problem by stimulating angiogenesis, the growth of new blood vessels toward the tumor.

- VEGF signaling: A major player in angiogenesis is a protein called vascular endothelial growth factor (VEGF). Many tumors produce high levels of VEGF, which signals nearby blood vessels to sprout new branches toward the growing cancer. These new vessels deliver nutrients and oxygen and carry away waste products, helping the tumor expand.
- Examples: In colorectal cancer, overproduction of VEGF promotes robust blood vessel growth, making tumors more aggressive. Some treatments for cancers, like bevacizumab (Avastin), are designed to block VEGF and starve the tumor of its blood supply.

However, tumor blood vessels are often abnormal. They tend to be leaky, disorganized, and fragile, which not only supports tumor growth but also makes it easier for cancer cells to enter the bloodstream during metastasis.

Invasion and Metastasis

One of the most dangerous aspects of cancer is its ability to spread from the original site to other parts of the body—a process called metastasis. While a benign tumor grows locally, a malignant tumor can invade nearby tissues and eventually migrate to distant organs.

The process of metastasis involves several steps:

1. Local invasion: Cancer cells break away from the primary tumor and invade surrounding tissue. They often produce enzymes that digest the extracellular matrix, clearing a path for movement.
2. Intravasation: Cancer cells enter blood or lymphatic vessels.
3. Circulation: They travel through the bloodstream or lymphatic system, often facing hostile conditions like immune attacks.
4. Extravasation: Cells exit the bloodstream into new tissue.

5. Colonization: Finally, they establish a new tumor in the distant site.

- Example: Breast cancer cells often metastasize to the bones, lungs, liver, or brain. Prostate cancer frequently spreads to bones.

Each of these steps presents challenges that relatively few cancer cells successfully overcome. Nevertheless, even a small number of surviving metastatic cells can establish life-threatening secondary tumors.

Resistance to Growth-Inhibition Signals

Normal cells respond to signals telling them when to grow—and importantly, when to stop growing. These growth-inhibitory signals help maintain the architecture and proper function of tissues.

Cancer cells, however, often ignore these inhibitory signals. They become insensitive to molecules that would normally restrain cell division or promote differentiation (the process by which cells become specialized).

- TGF-β pathway: One key pathway involves transforming growth factor-beta (TGF-β), which usually inhibits cell proliferation. Many cancer cells lose responsiveness to TGF-β, allowing unchecked growth.
- Contact inhibition: Normal cells exhibit contact inhibition—they stop dividing when they become crowded and touch neighboring cells. Cancer cells frequently lose this trait, continuing to pile up on one another and forming disorganized masses.

Together with uncontrolled proliferation, this loss of growth inhibition allows tumors to expand rapidly and disrupt normal tissue organization.

Cellular Immortality: Unlimited replicative potential

Normal human cells are not immortal. Most have a built-in limit on how many times they can divide—a phenomenon known as the Hayflick limit. This limit is largely controlled by structures called telomeres, which are repetitive DNA sequences at the ends of chromosomes.

Each time a cell divides, its telomeres shorten. When they become too short, the cell either stops dividing (enters senescence) or undergoes apoptosis. This helps prevent old or damaged cells from persisting indefinitely.

Cancer cells often overcome this limit by activating an enzyme called telomerase, which rebuilds telomeres and allows cells to divide endlessly.

- Example: In more than 80–90% of human tumors, telomerase is highly active. This enables cells to maintain their telomeres and achieve “immortality,” a crucial step in cancer progression.

This hallmark not only supports continuous tumor growth but also provides additional opportunities for mutations to accumulate, further driving cancer evolution.

Now that we've explored the hallmarks that define cancer's behavior, we're ready to examine how these traits emerge at the molecular level. In the next section, we'll explore how changes in epigenetic regulation, transcription, RNA processing, and protein regulation contribute to these hallmarks and how biotechnology is helping scientists develop increasingly targeted cancer therapies.

Summary

The hallmarks of cancer describe the common biological capabilities that enable cancer cells to survive, grow, and spread. Although different cancers arise through different genetic mutations, they often acquire the same hallmarks, including uncontrolled cell division, resistance to apoptosis, angiogenesis, metastasis, resistance to growth-inhibitory signals, and unlimited replicative potential. Understanding these shared characteristics has transformed cancer research and led to the development of targeted therapies designed to interfere with these hallmark processes.

Key Terms

angiogenesis
: the process by which new blood vessels form from existing vessels; in cancer, tumors stimulate angiogenesis to ensure a supply of oxygen and nutrients

apoptosis
: programmed cell death that eliminates damaged, unneeded, or potentially dangerous cells in an organized manner

Bcl-2
: a protein that helps cells avoid programmed cell death

cellular immortality
: the ability of cells to divide indefinitely, often achieved in cancer cells through activation of telomerase

contact inhibition
: a property of normal cells in which they stop dividing when they come into contact with neighboring cells; lost in many cancer cells

hallmarks of cancer
: a set of common biological capabilities acquired by cancer cells that enable them to survive, grow, invade tissues, and spread throughout the body

Hayflick limit
: the number of times a normal human cell population can divide before cell division stops, due to telomere shortening

invasion
: the ability of cancer cells to penetrate surrounding tissues beyond their tissue of origin

intravasation
: the process by which cancer cells enter the bloodstream or lymphatic system

metastasis
: the spread of cancer cells from the original tumor site to distant organs or tissues

p53
: a protein that helps prevent damaged cells from dividing; often called the "guardian of the genome"

RAS
: a signaling molecule that regulates cell growth; mutations can lead to cancer

telomerase
: an enzyme that maintains telomere length, allowing cells to divide indefinitely; highly active in many cancers

telomeres
: repetitive DNA sequences at chromosome ends that protect genetic material and shorten with each cell division

TGF-β
: a signaling molecule that regulates cell growth and immune responses

VEGF (vascular endothelial growth factor)
a protein produced by tumors to stimulate angiogenesis

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10.5: Cancer Treatments

Learning Objectives

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

- Explain how alterations in gene regulation at multiple levels contribute to cancer development.
- Describe how biotechnology is used to identify molecular changes in cancer cells and guide treatment decisions.
- Explain how targeted therapies and personalized medicine use knowledge of gene regulation to improve cancer treatment.

Cancer: Disease of Altered Gene Expression

Throughout this chapter, we have seen that cancer develops through the gradual accumulation of DNA mutations that disrupt the normal regulation of cell growth, division, and survival. These mutations alter the activity of proteins that regulate the cell cycle, allowing cells to acquire the hallmarks of cancer. However, DNA mutations are only part of the story. Ultimately, cancer develops because these genetic changes alter how genes are expressed and how proteins function within the cell.

Modern biotechnology has transformed our understanding of these molecular changes. Today, scientists can analyze DNA sequences, measure gene expression, identify abnormal proteins, and detect epigenetic changes within individual tumors. These technologies allow researchers and physicians to better understand why cancers develop, predict how they may behave, and select therapies that specifically target the molecular abnormalities driving a patient's disease.

Cancer can therefore be viewed as a disease of altered gene regulation. Changes in gene expression can occur at nearly every step of the pathway that converts genetic information into functional proteins. Mutations may alter the DNA sequence itself, while changes in epigenetic regulation, transcription, RNA processing, translation, or post-translational modification can all influence which proteins are produced and how they function. Different cancers disrupt different parts of this pathway, but the result is the same: proteins that normally regulate cell growth, DNA repair, apoptosis, or differentiation no longer function properly.

Because these changes occur at multiple levels, researchers investigate each step of gene regulation for potential biomarkers that can improve diagnosis or serve as targets for new therapies.

Link to Learning

Watch this animation to learn more about the use of p53 in fighting cancer.

YouTube Video

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Cancer and Epigenetic Alterations

Not all changes that contribute to cancer involve mutations in DNA. Some involve epigenetic modifications that alter whether genes are turned on or off without changing the DNA sequence itself. These changes can silence important tumor suppressor genes or activate genes that promote cancer development.

Silencing genes through epigenetic mechanisms is common in many cancers. DNA within promoter regions may become methylated, while histone proteins lose acetyl groups. Together, these modifications reduce gene expression and can prevent cells from producing proteins needed to regulate growth or repair DNA.

Unlike DNA mutations, many epigenetic modifications are reversible. This has made epigenetics an exciting area of biotechnology research. Scientists have developed drugs that inhibit DNA methyltransferases or histone deacetylases, allowing previously silenced genes to become active again. These therapies demonstrate that understanding gene regulation can lead directly to new treatment strategies.

Cancer and Transcriptional Control

Cancer can also result from changes in transcription—the process of copying DNA into RNA. Many cancers contain mutations that increase the activity of transcription factors or signaling pathways that regulate gene expression. These changes can dramatically increase the production of proteins that stimulate cell growth and division.

One example is the epidermal growth factor receptor (EGFR) signaling pathway. Some cancers produce unusually high levels of EGFR, leading to continuous activation of genes involved in cell proliferation. Biotechnology has enabled scientists to develop drugs that specifically block EGFR signaling, slowing the growth of tumors that depend on this pathway. Rather than affecting every rapidly dividing cell, these targeted therapies focus on the molecular abnormalities driving a particular cancer.

Cancer and Post-transcriptional Control

Gene regulation continues even after RNA has been produced. Small RNA molecules called microRNAs (miRNAs) help determine whether messenger RNAs are translated into proteins or degraded before they can be used.

Many cancers produce abnormal patterns of miRNA expression. Some miRNAs are overproduced, reducing the expression of proteins that normally suppress tumors, while others are underproduced, allowing cancer-promoting proteins to accumulate.

Researchers are now exploring whether specific miRNA "signatures" can serve as biomarkers for early cancer detection or as targets for future therapies.

Cancer and Translational and Post-translational Regulation

The final stages of gene expression also influence cancer development. Proteins can be produced in abnormal amounts, modified by phosphorylation, or processed into alternative forms that behave differently from their normal counterparts.

For example, cyclin proteins, which regulate progression through the cell cycle, are controlled by phosphorylation. Abnormal phosphorylation can keep these proteins active longer than they should be, allowing cells to continue dividing despite DNA damage.

Similarly, different forms of the c-FLIP protein can either promote apoptosis or allow damaged cells to survive. In some colon cancers, expression shifts toward the form that prevents cell death, contributing to continued tumor growth.

These examples illustrate that cancer is not simply a disease of mutated genes—it is also a disease of altered protein activity.

Biotechnology, Targeted Therapies, and Personalized Medicine

Perhaps the greatest impact of understanding gene regulation has been the development of targeted therapies. Traditional chemotherapy attacks rapidly dividing cells, affecting both cancerous and healthy tissues. In contrast, targeted therapies are designed to interfere with specific molecules or signaling pathways that are essential for a particular cancer.

Examples include drugs that block EGFR, inhibit overactive protein kinases, or restore signaling pathways that normally limit cell growth. Before these treatments are prescribed, many patients undergo molecular testing to determine whether their tumors contain the specific genetic or molecular changes that the therapy targets.

This approach has led to the growth of personalized medicine, in which treatments are selected based on the unique molecular

characteristics of an individual's tumor rather than solely on where the cancer originated in the body.

Advances in DNA sequencing, molecular diagnostics, and biotechnology continue to improve our ability to identify these molecular differences. As researchers gain a deeper understanding of how gene regulation becomes disrupted in cancer, new therapies are being developed that are increasingly precise, effective, and tailored to individual patients.

Career Connection

Clinical Trial Coordinator

A clinical trial coordinator is the person managing the proceedings of the clinical trial. This job includes coordinating patient schedules and appointments, maintaining detailed notes, building the database to track patients (especially for long-term follow-up studies), ensuring proper documentation has been acquired and accepted, and working with the nurses and doctors to facilitate the trial and publication of the results. A clinical trial coordinator may have a science background, like a nursing degree, or other certification. People who have worked in science labs or in clinical offices are also qualified to become a clinical trial coordinator. These jobs are generally in hospitals; however, some clinics and doctor's offices also conduct clinical trials and may hire a coordinator.

Summary

Cancer develops because mutations and changes in gene regulation alter the expression and function of proteins that control cell growth, DNA repair, and cell survival. Modern biotechnology allows scientists to identify these molecular changes, leading to more accurate diagnosis, improved understanding of cancer biology, and the development of targeted therapies and personalized medicine. As our knowledge of gene regulation continues to expand, so does our ability to develop more effective strategies for preventing, detecting, and treating cancer.

Key Terms

DNA methylation
: An epigenetic modification involving the addition of methyl groups to DNA that can reduce or silence gene expression.

epigenetic modification
: A change in gene activity that does not alter the DNA sequence but affects how genes are turned on or off.

gene silencing
: The process of preventing a gene from being expressed or producing its RNA/protein product.

histone deacetylation
: An epigenetic process that removes acetyl groups from histone proteins, often resulting in tighter DNA packaging and reduced gene expression.

microRNA (miRNA)
: A small RNA molecule that regulates gene expression by binding to messenger RNA and affecting its stability or translation.

post-translational modification
: A chemical change made to a protein after it is produced that can alter its activity, location, or function.

targeted therapy
: A treatment designed to specifically block molecules or pathways involved in disease while minimizing effects on healthy cells.

therapeutic target
: A specific molecule, gene, or pathway that can be acted upon by a drug to treat a disease.

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11: IMMUNE SYSTEM

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11.1: Overview of the Immune System

Learning Objectives

- Distinguish between innate and adaptive immunity

Biotech Focus

From Bacterial Defense to Biotech Breakthrough

Interestingly, the rudimentary immune system found in bacteria isn't just a biological curiosity—it's the foundation of one of the most powerful tools in modern biotechnology: CRISPR-Cas9. This system, which bacteria use to recognize and cut up viral DNA from invading bacteriophages, functions like a genetic memory bank and molecular scalpel combined. Scientists studying this mechanism realized that if bacteria could be programmed to target specific DNA sequences, perhaps we could use a similar system to edit genes in other organisms. That insight led to the development of CRISPR-Cas9 as a precise, customizable gene-editing tool. In 2020, researchers Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for their work on this groundbreaking discovery. Today, CRISPR-Cas9 is being used in a wide range of applications—from engineering disease-resistant crops and developing new cancer therapies to exploring potential cures for genetic disorders like sickle cell disease and muscular dystrophy. It has opened the door to discussions about the future of medicine, agriculture, and even bioethics. While we'll explore CRISPR-Cas9 in greater depth later in this course, it's worth noting that this revolutionary technology originated not in a high-tech lab, but in the ancient, microbial struggle for survival. What began as a bacterial defense mechanism has become one of humanity's most promising scientific tools.

Listen to Jennifer Doudna as she discusses Genome Editing with CRISPR-Cas Systems: Challenges and Opportunities.

The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue.

Pathogens can rapidly evolve and adapt to avoid detection and neutralization by the immune system. As a result, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms, such as bacteria, possess a rudimentary immune system in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms including include phagocytosis, antimicrobial peptides called defensins, and the complement system, which evolved in ancient eukaryotes and remain in modern descendants, such as plants and insects. Jawed vertebrates have even more sophisticated defense mechanisms, including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.

Innate and Adaptive Immunity

Our immune system is what keeps us alive in a world teeming with harmful microbes. Every day, we encounter pathogens—viruses, bacteria, and other microorganisms that can cause disease. Thankfully, our bodies have evolved a powerful and multilayered defense system that protects us from these invisible invaders. This system is generally divided into two parts: **innate immunity** and **adaptive immunity**.

Innate immunity is our first line of defense. It's fast and non-specific, meaning it responds the same way to all pathogens, regardless of what they are. You can think of it like a fence around your property—it doesn't matter who approaches, the fence blocks everyone. Physical barriers like our skin, enzymes in our tears and saliva, and specialized immune cells all belong to this category. This type of immunity is ancient and found across nearly all living organisms, including plants and invertebrates.

Adaptive immunity, on the other hand, is more like having a security guard at the gate of that fence. This system can **recognize** and **remember** specific pathogens. It's slower to respond at first, but it learns and adapts. If the same pathogen invades again,

the immune system is ready with a faster, stronger response. This ability to “remember” is the basis for how vaccines work.

Before birth, your body surveys all of its cells and tags them as “self.” Any unfamiliar (non-self) substances—like invading microbes—can trigger the immune system to respond. Both innate and adaptive immunity rely on this ability to distinguish between self and non-self.

The immune response often begins when a pathogen manages to get past physical barriers like skin or mucous membranes. Once inside, the innate immune system reacts immediately to try to neutralize the threat. If the pathogen evades this initial response, the adaptive immune system kicks in to finish the job. This layered response is shown in **Figure** 11.1.1.

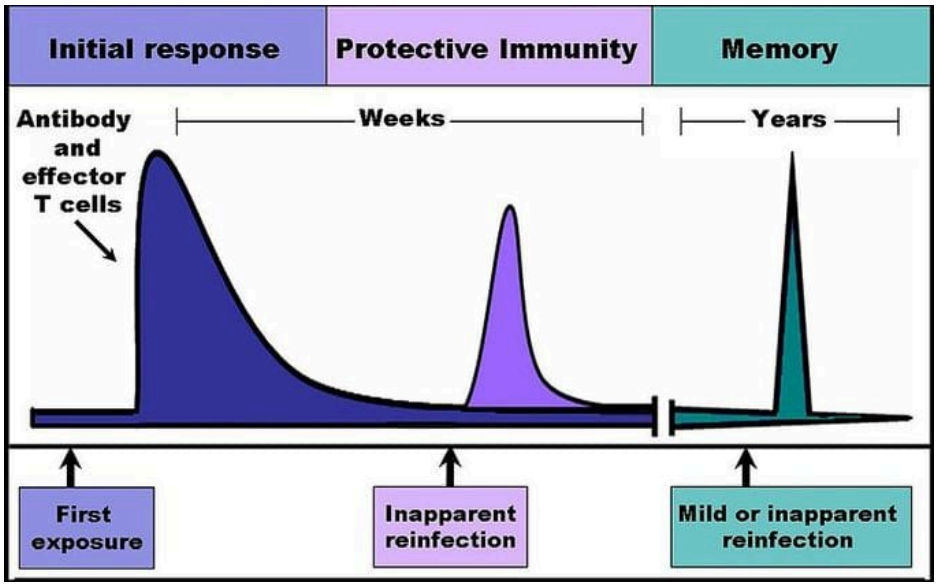


Figure 11.1.1: The Time Course of an Immune Response: Immune reactants, such as antibodies and effector T-cells, work to eliminate an infection, and their levels and activity rapidly increase following an encounter with an infectious agent, whether that agent is a pathogen or a vaccine. For several weeks these reactants remain in the serum and lymphatic tissues and provide protective immunity against reinfection by the same agent. During an early reinfection, few outward symptoms of illness are present, but the levels of immune reactants increase and are detectable in the blood and/or lymph. Following clearance of the infection, antibody level and effector T cell activity gradually declines. Because immunological memory has developed, reinfection at later times leads to a rapid increase in antibody production and effector T cell activity. These later infections can be mild or even inapparent.

Innate Immunity

Innate, or nonspecific, immunity is the natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches. Microbes first encounter the epithelial layers (physical barriers that line our skin and mucous membranes). Subsequent general defenses include secreted chemical signals (cytokines), antimicrobial substances, fever, and phagocytic activity associated with the inflammatory response. The phagocytes express cell surface receptors that can bind and respond to common molecular patterns expressed on the surface of invading microbes. Through these approaches, innate immunity can prevent the colonization, entry, and spread of microbes **(Figure** 11.1.2**)**.

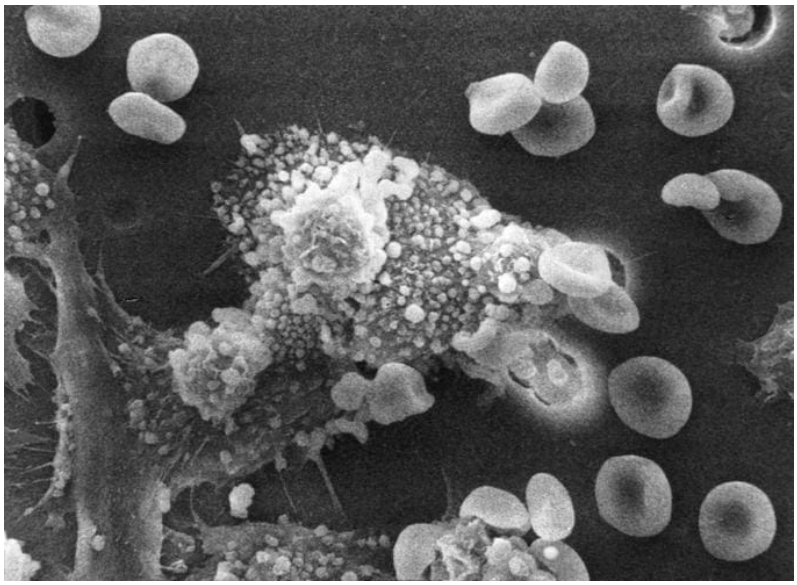


Figure 11.1.2: An animal's immune response to a foreign body: Macrophages begin to fuse with, and inject its toxins into, the cancer cell. The cell starts rounding up and loses its spikes. As the macrophage cell becomes smooth. The cancer cell appears lumpy in the last stage before it dies. These lumps are actually the macrophages fused within the cancer cell. The cancer cell then loses its morphology, shrinks up and dies. Photo magnification: 3: x8,000 Type: B & W print

Adaptive Immunity

Adaptive immunity is often sub-divided into two major types depending on how the immunity was introduced. Naturally acquired immunity occurs through contact with a disease causing agent, when the contact was not deliberate, whereas artificially acquired immunity develops only through deliberate actions such as vaccination. Both naturally and artificially acquired immunity can be further subdivided depending on whether immunity is induced in the host or passively transferred from an immune host. Passive immunity is acquired through transfer of antibodies or activated T cells from an immune host, and is short lived—usually lasting only a few months. Active immunity is induced in the host itself by antigen, and lasts much longer, sometimes the entire lifetime **(Figure** 11.1.3**)**.

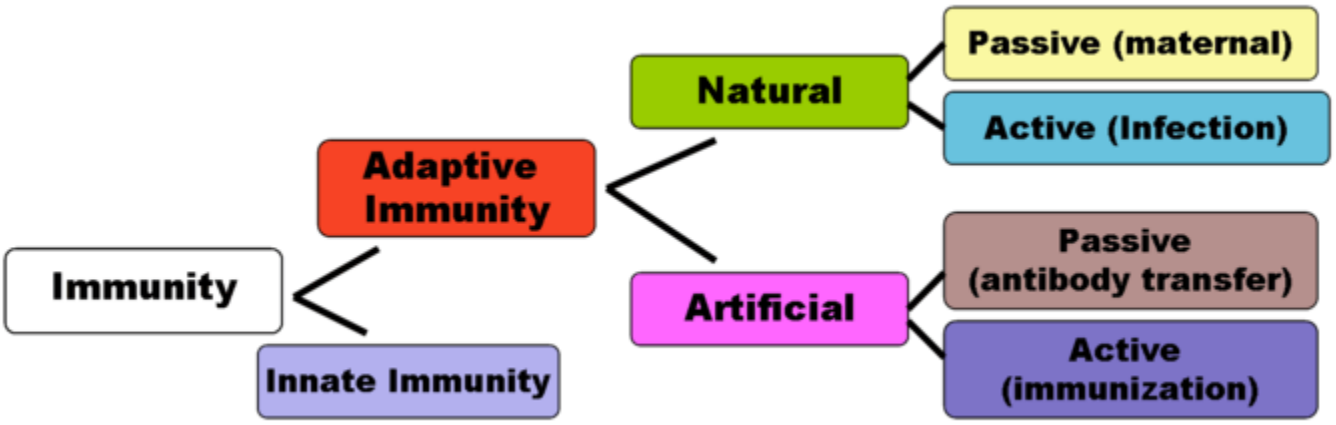


Figure 11.1.3: Immunity: Natural immunity occurs through contact with a disease causing agent, when the contact was not deliberate, whereas artificial immunity develops only through deliberate actions of exposure. Both natural and artificial immunity can be further subdivided, depending on the amount of time the protection lasts. Passive immunity is short lived, and usually lasts only a few months, whereas protection via active immunity lasts much longer, and is sometimes life-long.

A further subdivision of adaptive immunity is characterized by the cells involved; humoral immunity is the aspect of immunity that is mediated by secreted antibodies, whereas the protection provided by cell-mediated immunity involves T lymphocytes alone. Humoral immunity is active when the organism generates its own antibodies, and passive when antibodies are transferred

between individuals. Similarly, cell-mediated immunity is active when the organism's own T cells are stimulated and passive when T cells come from another organism.

The table below summarizes some of the key differences between the two systems which will be discussed in more detail throughout this chapter:

Innate immune system: Response is non-specific, exposure leads to immediate maximal response, cell-mediated and humoral components, no immunological memory, found in nearly all forms of life. Adaptive immune system: Pathogen and antigen specific response, lag time between exposure and maximal response, cell-mediated and humoral components, exposure leads to immunological memory, found only in jawed vertebrates.

Components of the immune system

Innate immune system	Adaptive immune system
Response is non-specific	Pathogen and antigen specific response
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Exposure leads to immunological memory
Found in nearly all forms of life	Found only in jawed vertebrates

In short, immunity refers to the body's ability to defend itself against infection and disease. It involves both non-specific (innate) and specific (adaptive) components, and can develop naturally—through infection or exposure—or artificially, such as through vaccination.

Summary

- Pathogens can rapidly evolve and adapt to avoid detection and neutralization by the immune system. As a result, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. The immune system protects from infection with layered defenses of increasing specificity.
- Physical barriers prevent pathogens from entering the organism. If these barriers are breached, the innate immune system provides an immediate, non-specific response. If pathogens successfully evade the innate response, vertebrates possess a second layer of protection, the adaptive immune system.
- Immunity is a biological term that describes a state of having sufficient biological defences to avoid infection, disease, or other unwanted biological invasion.
- Innate, or nonspecific, immunity is the natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches.
- Adaptive immunity is often sub-divided into two major types acording to how the immunity was introduced. Naturally acquired immunity occurs through non-deliberate contact with a disease causing agent, whereas artificially acquired immunity develops through deliberate actions such as vaccination.
- Immunology is a branch of biomedical science that covers the study of all aspects of the immune system in all organisms. It deals with the physiological functioning of the immune system in states of both health and disease.

Key Terms

adaptive (acquired) immunity
: the creation of immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination

innate immunity
: the natural resistance with which a person is born. It provides resistance through several physical, chemical, and cellular approaches

self molecules
: those components of an organism's body that can be distinguished by the immune system from foreign substances.

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11.2: Cells and Organs of the Immune System

Learning Objectives

- Recognize the cells and organs of the immune system and their functions

Immune System Cells

Leukocytes (white blood cells) are immune system cells involved in defending the body against infectious disease and foreign materials. Five different types of leukocytes exist, all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. The innate leukocytes include the phagocytes, mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are important mediators in the activation of the adaptive immune system.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte. During the acute phase of inflammation neutrophils migrate toward the site of inflammation and are usually the first cells to arrive at the scene of infection. Macrophages reside within tissues and produce a wide array of chemicals. They also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system. Dendritic cells are phagocytes in tissues that are in contact with the external environment, and are located mainly in the skin, nose, lungs, stomach, and intestines. These cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T-cells, one of the key cell types of the adaptive immune system.

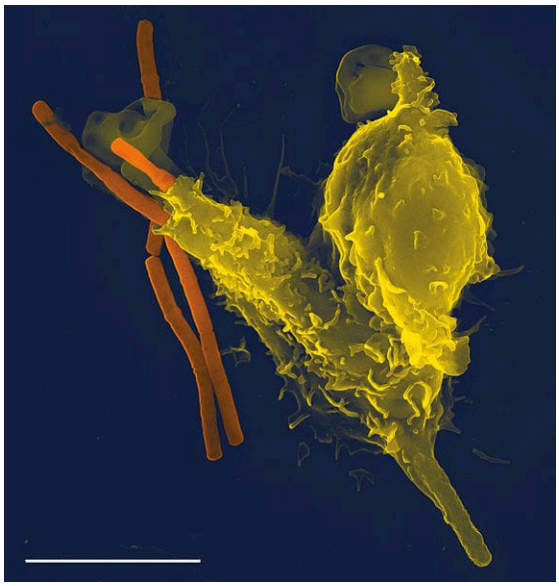


Figure 11.2.1: A Phagocyte in Action: Neutrophil (yellow) engulfing anthrax bacteria (orange). Taken with a Leo 1550 scanning electron microscope. Scale bar is 5 micrometers.

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis.

Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites, and play a role in allergic reactions, such as asthma.

Natural killer cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses.

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.

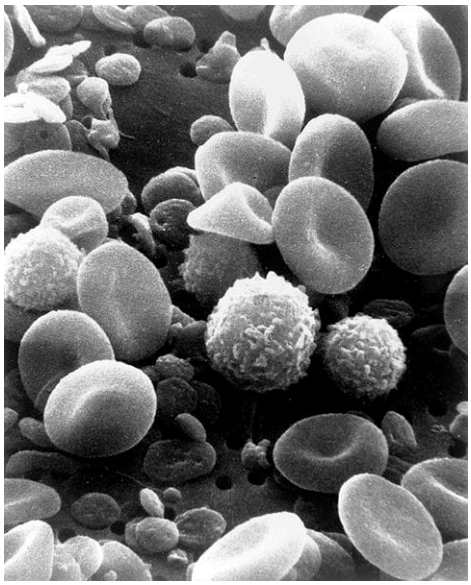


Figure 11.2.2: Blood Cells: Red blood cells, several white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.

T cells recognize a “non-self” target, such as a pathogen, only after antigens have been processed and presented in combination with a “self” receptor, called a major histocompatibility complex (MHC) molecule. There are two major subtypes of T cells: the killer T cell, which kills cells that are infected with viruses (and other pathogens) or are otherwise damaged or dysfunctional, and the helper T cell, which regulates both innate and adaptive immune responses and helps determine which immune responses the body makes to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly. A third, minor subtype are the γ T cells that recognize intact antigens not bound to MHC receptors.

In contrast, the B cell antigen-specific receptor is an antibody molecule on the B cell surface, which recognizes whole pathogens without any need for antigen processing. Each lineage of B cell expresses a different antibody, so the complete set of B cell antigen receptors represent all the antibodies that the body can manufacture.

Immune System Organs

The key primary lymphoid organs of the immune system include the thymus and bone marrow, as well as secondary lymphatic tissues including spleen, tonsils, lymph vessels, lymph nodes, adenoids, skin, and liver **(Figure** 11.2.3**)**.

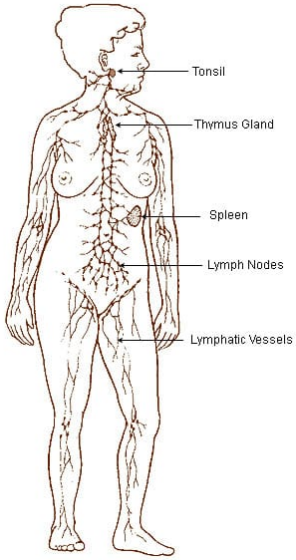


Figure 11.2.3: The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels that carry a clear fluid called lymph.

The thymus “educates” T cells and provides an inductive environment for the development of T cells from hematopoietic progenitor cells. The thymus is largest and most active during the neonatal and pre-adolescent periods of development. By the early teens, the thymus begins to atrophy and thymic stroma is replaced by adipose tissue. Nevertheless, residual T-lymphopoiesis continues throughout adult life.

Bone marrow is the flexible tissue found in the interior of bones. In humans, red blood cells are produced in the heads of long bones. The red bone marrow is a key element of the lymphatic system, being one of the primary lymphoid organs that generate lymphocytes from immature hematopoietic progenitor cells. Bone marrow and thymus constitute the primary lymphoid tissues involved in the production and early selection of lymphocytes.

The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels that carry a clear fluid, called lymph, unidirectionally towards the heart. The lymphatic system has multiple interrelated functions including the transportation of white blood cells to and from the lymph nodes into the bones, and the transportation of antigen -presenting cells (such as dendritic cells) to the lymph nodes where an immune response is stimulated. Lymphoid tissue is found in many organs, particularly the lymph nodes **(Figure** $11.2.4a$**)**.

Lymph nodes are distributed widely throughout areas of the body, including the armpit and stomach, and linked by lymphatic vessels. Lymph nodes are garrisons of B, T and other immune cells. Lymph nodes act as filters or traps for foreign particles and are important in the proper functioning of the immune system. They are packed tightly with the white blood cells, called lymphocytes and macrophages **(Figure** $11.2.4b$**)**.

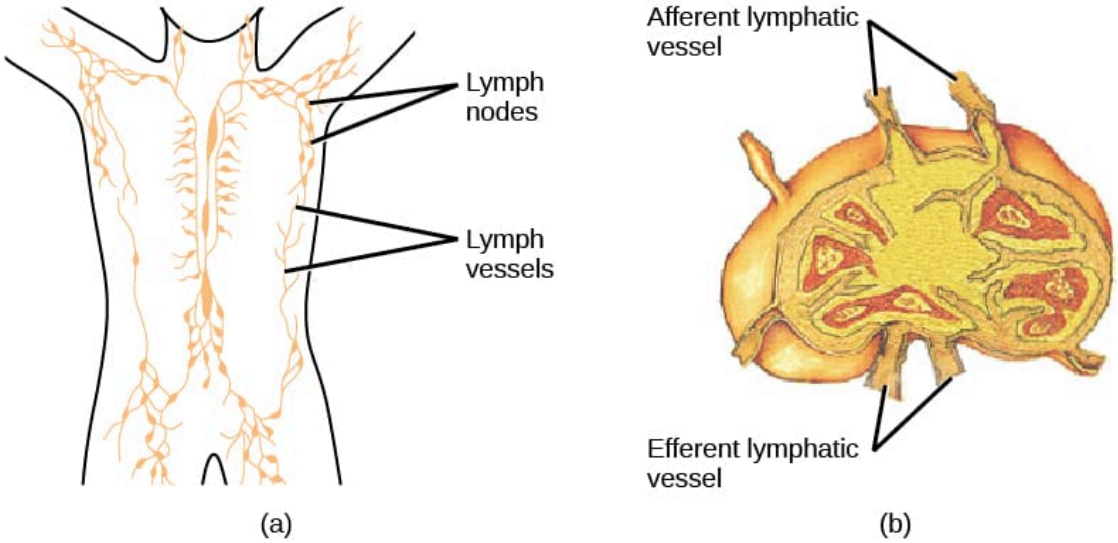


Figure 11.2.4: (a) Lymphatic vessels carry a clear fluid called lymph throughout the body. The liquid passes through (b) lymph nodes that filter the lymph that enters the node through afferent vessels and leaves through efferent vessels; lymph nodes are filled with lymphocytes that purge infecting cells. (credit a: modification of work by NIH; credit b: modification of work by NCI, NIH)

The spleen is similar in structure to a large lymph node and acts primarily as a blood filter, as shown in **Figure** 11.2.5. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation.

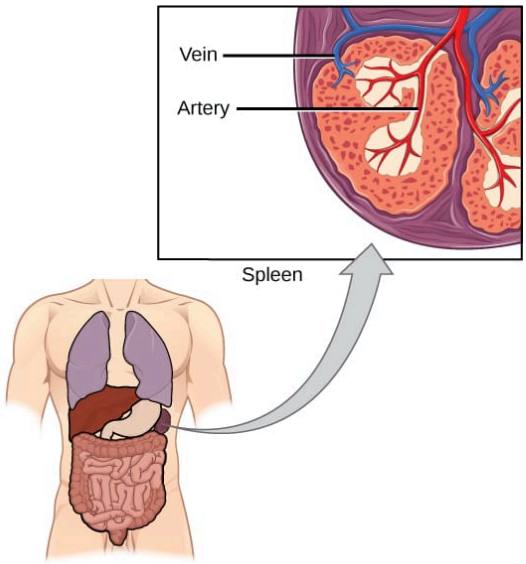


Figure 11.2.5: The spleen functions to immunologically filter the blood and allow for communication between cells corresponding to the innate and adaptive immune responses. An inset diagram shows arteries and veins extending into the tissue of the spleen. (credit: modification of work by NCI, NIH)

The palatine tonsils and the nasopharyngeal tonsil are lymphoepithelial tissues located near the oropharynx and nasopharynx. These immunocompetent tissues are the immune system's first line of defense against ingested or inhaled foreign pathogens. The fundamental immunological roles of tonsils aren't yet understood.

The skin is one of the most important parts of the body because it interfaces with the environment, and is the first line of defense from external factors, acting as an anatomical barrier from pathogens and damage between the internal and external environment in bodily defense. Langerhans cells in the skin are part of the adaptive immune system.

The liver has a wide range of functions, including immunological effects—the reticuloendothelial system of the liver contains many

immunologically active cells, acting as a “sieve” for antigens carried to it via the portal system.

Summary

The key primary lymphoid organs of the immune system are the thymus and bone marrow, and secondary lymphatic tissues such as spleen, tonsils, lymph vessels, lymph nodes, adenoids, and skin and liver. Leukocytes (white blood cells) act like independent, single-celled organisms and are the second arm of the innate immune system. The innate leukocytes include the phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are also important mediators in the activation of the adaptive immune system.

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow. The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels. The lymphatic system has multiple functions such as the transportation of white blood cells to and from the lymph nodes into the bones.

Key Terms

B cells
: a white blood cell that produces antibodies

basal cell
: a stem-like skin cell found at the base of the epidermis

helper T cell
: a T cell that coordinates immune responses by activating other immune cells

hematopoietic stem cells
: a stem cell in bone marrow that produces all blood cells

killer T cell (cytotoxic T cell)
: a T cell that destroys infected or cancerous cells

lymph node
: a small organ that filters lymph and houses immune cells

lymphocytes
: a lymphocyte is a type of white blood cell in the vertebrate immune system. The three major types of lymphocyte are T cells, B cells and natural killer (NK) cells. T cells (thymus cells) and B cells (bursa-derived cells) are the major cellular components of the adaptive immune response

leukocytes
: cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist

macrophage
: a large immune cell that engulfs pathogens and helps activate other immune cells

major histocompatibility complex (MHC)
: molecules that display protein fragments on cell surfaces to help the immune system recognize threats

mast cell
: an immune cell involved in inflammation and allergic responses

natural killer cell
: an immune cell that destroys virus-infected or abnormal cells

neutrophil
: a fast-acting white blood cell that engulfs and destroys invading microbes

phagocyte
: any cell that engulfs and digests foreign particles or microbes

spleen
: an organ that filters blood and helps activate immune response

T cell
: a white blood cell that helps regulate immunity and kill infected cells

tonsils
: immune tissues in the throat that help protect against inhaled or swallowed pathogens

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11.3: Innate Immunity

Learning Objectives

- Explain how the key physical and chemical barriers that serve as the body's first line of defense against pathogens help prevent infection
- Compare the defenses of the innate immune system, including the roles of inflammation, phagocytosis, natural killer cells, cytokines, and the complement system
- Describe how the immune system distinguishes self from non-self, and why this distinction is critical for mounting an effective immune response

The vertebrate, including human, immune system is a complex multilayered system for defending against external and internal threats to the integrity of the body. The system can be divided into two types of defense systems: the innate immune system, which is nonspecific toward a particular kind of pathogen, and the adaptive immune system, which is specific **(Figure** 11.3.1**)**. Innate immunity is not caused by an infection or vaccination and depends initially on physical and chemical barriers that work on all pathogens, sometimes called the first line of defense. The second line of defense of the innate system includes chemical signals that produce inflammation and fever responses as well as mobilizing protective cells and other chemical defenses. The adaptive immune system mounts a highly specific response to substances and organisms that do not belong in the body. The adaptive system takes longer to respond and has a memory system that allows it to respond with greater intensity should the body reencounter a pathogen even years later.

Vertebrate Immunity		
Innate Immune System		Adaptive Immune System
Physical Barriers	Internal Defenses	
• Skin, hair, cilia • Mucus membranes • Mucus and chemical secretions • Digestive enzymes in mouth • Stomach acid	• Inflammatory response • Complement proteins • Phagocytic cells • Natural killer (NK) cells	• Antibodies and the humoral immune response • Cell-mediated immune response • Memory response

Figure 11.3.1: There are two main parts to the vertebrate immune system. The innate immune system, which is made up of physical barriers and internal defenses, responds to all pathogens. Under physical barriers are: skin, hairs, cilia, mucus membranes, mucus and chemical secretions, digestive enzymes in mouth, and stomach acid. Under internal defenses are: inflammatory response, complement proteins, phagocytic cells, and natural killer (NK) cells. The adaptive immune system is highly specific, including: antibodies and the humoral immune response, cell-mediated immune response, and memory response.

External and Chemical Barriers

The body has significant physical barriers to potential pathogens. The skin contains the protein keratin, which resists physical entry into cells. Other body surfaces, particularly those associated with body openings, are protected by the mucous membranes. The sticky mucus provides a physical trap for pathogens, preventing their movement deeper into the body. The openings of the body, such as the nose and ears, are protected by hairs that catch pathogens, and the mucous membranes of the upper respiratory tract have cilia that constantly move pathogens trapped in the mucus coat up to the mouth.

The skin and mucous membranes also create a chemical environment that is hostile to many microorganisms. The surface of the skin is acidic, which prevents bacterial growth. Saliva, mucus, and the tears of the eye contain an enzyme that breaks down bacterial cell walls. The stomach secretions create a highly acidic environment, which kills many pathogens entering the digestive

system.

Finally, the surface of the body and the lower digestive system have a community of microorganisms such as bacteria, archaea, and fungi that coexist without harming the body. There is evidence that these organisms are highly beneficial to their host, combating disease-causing organisms and outcompeting them for nutritional resources provided by the host body. Despite these defenses, pathogens may enter the body through skin abrasions or punctures, or by collecting on mucosal surfaces in large numbers that overcome the protections of mucus or cilia.

Internal Defenses

When pathogens enter the body, the innate immune system responds with a variety of internal defenses. These include the inflammatory response, phagocytosis, natural killer cells, and the complement system. White blood cells in the blood and lymph recognize pathogens as foreign to the body. A white blood cell is larger than a red blood cell, is nucleated, and is typically able to move using amoeboid locomotion. Because they can move on their own, white blood cells can leave the blood to go to infected tissues. For example, a monocyte is a type of white blood cell that circulates in the blood and lymph and develops into a macrophage after it moves into infected tissue. A macrophage is a large cell that engulfs foreign particles and pathogens. Mast cells are produced in the same way as white blood cells, but unlike circulating white blood cells, mast cells take up residence in connective tissues and especially mucosal tissues. They are responsible for releasing chemicals in response to physical injury. They also play a role in the allergic response, which will be discussed later in the chapter.

When a pathogen is recognized as foreign, chemicals called cytokines are released. A cytokine is a chemical messenger that regulates cell differentiation (form and function), proliferation (production), and gene expression to produce a variety of immune responses. Approximately 40 types of cytokines exist in humans. In addition to being released from white blood cells after pathogen recognition, cytokines are also released by the infected cells and bind to nearby uninfected cells, inducing those cells to release cytokines. This positive feedback loop results in a burst of cytokine production.

One class of early-acting cytokines is the interferons, which are released by infected cells as a warning to nearby uninfected cells. An interferon is a small protein that signals a viral infection to other cells. The interferons stimulate uninfected cells to produce compounds that interfere with viral replication. Interferons also activate macrophages and other cells.

The Inflammatory Response and Phagocytosis

The first cytokines to be produced encourage inflammation, a localized redness, swelling, heat, and pain. Inflammation is a response to physical trauma, such as a cut or a blow, chemical irritation, and infection by pathogens (viruses, bacteria, or fungi). The chemical signals that trigger an inflammatory response enter the extracellular fluid and cause capillaries to dilate (expand) and capillary walls to become more permeable, or leaky. The serum and other compounds leaking from capillaries cause swelling of the area, which in turn causes pain. Various kinds of white blood cells are attracted to the area of inflammation. The types of white blood cells that arrive at an inflamed site depend on the nature of the injury or infecting pathogen. For example, a neutrophil is an early arriving white blood cell that engulfs and digests pathogens. Neutrophils are the most abundant white blood cells of the immune system **(Figure** 11.3.2**)**. Macrophages follow neutrophils and take over the phagocytosis function and are involved in the resolution of an inflamed site, cleaning up cell debris and pathogens.

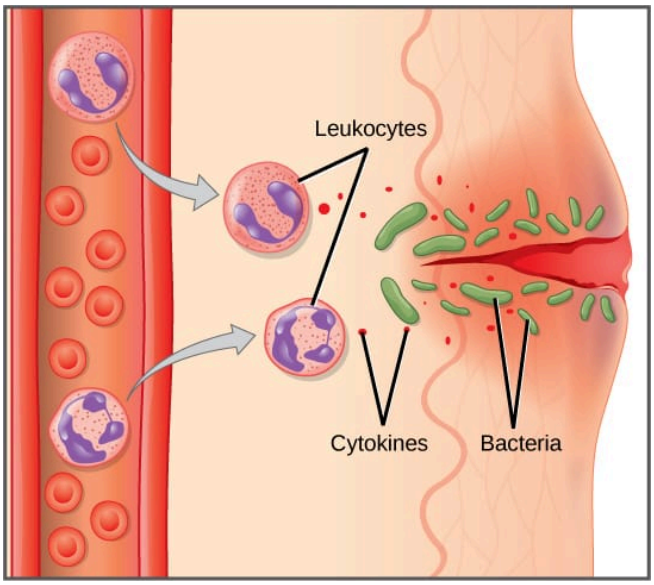


Figure 11.3.2: White blood cells (leukocytes) release chemicals to stimulate the inflammatory response following a cut in the skin.

Illustration shows a capillary near the surface of skin that has a cut in it. Bacteria have penetrated the skin around the cut. In response, mass cells in the lower part of the skin tissue release histamines, and dendritic cells release cytokines. The histamines cause the capillary to become permeable. Neutrophils and monocytes exit the capillary into the damaged skin. Both the neutrophil and macrophage release cytokines and consumes bacteria by phagocytosis.

Cytokines also send feedback to cells of the nervous system to bring about the overall symptoms of feeling sick, which include lethargy, muscle pain, and nausea. Cytokines also increase the core body temperature, causing a fever. The elevated temperatures of a fever inhibit the growth of pathogens and speed up cellular repair processes. For these reasons, suppression of fevers should be limited to those that are dangerously high.

CONCEPT IN ACTION

Check out this 23-second, stop-motion video showing a neutrophil that searches and engulfs fungus spores during an elapsed time of 79 minutes.

Natural Killer Cells

A lymphocyte is a white blood cell that contains a large nucleus **(Figure** 11.3.3**)**. Most lymphocytes are associated with the adaptive immune response, but infected cells are identified and destroyed by natural killer cells, the only lymphocytes of the innate immune system. A natural killer (NK) cell is a lymphocyte that can kill cells infected with viruses (or cancerous cells). NK cells identify intracellular infections, especially from viruses, by the altered expression of major histocompatibility class (MHC) I molecules on the surface of infected cells. MHC class I molecules are proteins on the surfaces of all nucleated cells that provide a sample of the cell's internal environment at any given time. Unhealthy cells, whether infected or cancerous, display an altered MHC class I complement on their cell surfaces.

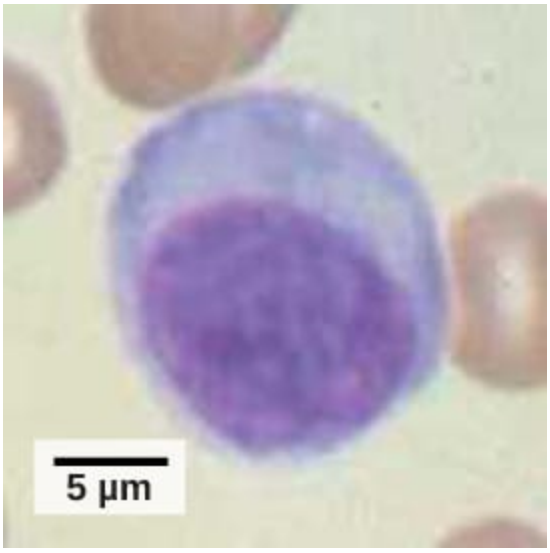


Figure 11.3.3: Lymphocytes, such as NK cells, are characterized by their large nuclei that actively absorb Wright stain and therefore appear dark colored under a microscope. (credit: scale-bar data from Matt Russell)

After the NK cell detects an infected or tumor cell, it induces programmed cell death, or apoptosis. Phagocytic cells then come along and digest the cell debris left behind. NK cells are constantly patrolling the body and are an effective mechanism for controlling potential infections and preventing cancer progression. The various types of immune cells are shown in **Figure** 11.3.4.

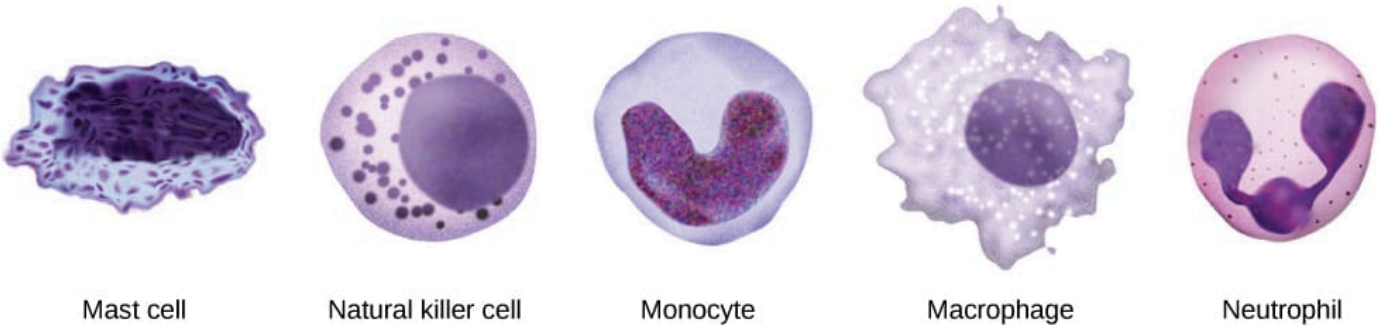


Figure 11.3.4: Cells involved in the innate immune response include mast cells, natural killer cells, and white blood cells, such as monocytes, macrophages and neutrophils.

Complement

An array of approximately 20 types of proteins, called a complement system, is also activated by infection or the activity of the cells of the adaptive immune system and functions to destroy extracellular pathogens. Liver cells and macrophages synthesize inactive forms of complement proteins continuously; these proteins are abundant in the blood serum and are capable of responding immediately to infecting microorganisms. The complement system is so named because it is complementary to the innate and adaptive immune system. Complement proteins bind to the surfaces of microorganisms and are particularly attracted to pathogens that are already tagged by the adaptive immune system. This “tagging” involves the attachment of specific proteins called antibodies (discussed in detail later) to the pathogen. When they attach, the antibodies change shape providing a binding site for one of the complement proteins. After the first few complement proteins bind, a cascade of binding in a specific sequence of proteins follows in which the pathogen rapidly becomes coated in complement proteins.

Complement proteins perform several functions, one of which is to serve as a marker to indicate the presence of a pathogen to phagocytic cells and enhance engulfment. Certain complement proteins can combine to open pores in microbial cell membranes and cause lysis of the cells.

Summary

The innate immune system consists first of physical and chemical barriers to infection including the skin and mucous membranes

and their secretions, ciliated surfaces, and body hairs. The second line of defense is an internal defense system designed to counter pathogenic threats that bypass the physical and chemical barriers of the body. Using a combination of cellular and molecular responses, the innate immune system identifies the nature of a pathogen and responds with inflammation, phagocytosis, cytokine release, destruction by NK cells, or the complement system.

Key Terms

complement system
: an array of approximately 20 soluble proteins of the innate immune system that enhance phagocytosis, bore holes in pathogens, and recruit lymphocytes

cytokine
: a chemical messenger that regulates cell differentiation, proliferation, and gene expression to effect immune responses

inflammation
: the localized redness, swelling, heat, and pain that results from the movement of leukocytes through opened capillaries to a site of infection

innate immunity
: an immunity that occurs naturally because of genetic factors or physiology, and is not caused by infection or vaccination

interferon
: a cytokine that inhibits viral replication

lymphocyte
: a type of white blood cell that includes natural killer cells of the innate immune system and B and T cells of the adaptive immune system

macrophage
: a large phagocytic cell that engulfs foreign particles and pathogens

major histocompatibility class (MHC) I
: a group of proteins found on the surface of all nucleated cells that signals to immune cells whether the cell is normal or is infected or cancerous; it also provides the appropriate sites into which antigens can be loaded for recognition by lymphocytes

mast cell
: a leukocyte that produces inflammatory molecules, such as histamine, in response to large pathogens

monocyte
: a type of white blood cell that circulates in the blood and lymph and differentiates into a macrophage after it moves into infected tissue

natural killer (NK) cell
: a lymphocyte that can kill cells infected with viruses or tumor cells

neutrophil
: a phagocytic leukocyte that engulfs and digests pathogens

white blood cell
: a nucleated cell found in the blood that is a part of the immune system; also called leukocytes

Contributors and Attributions

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11.4: Physical Defenses

Learning Objectives

- Describe the various physical barriers and mechanical defenses that protect the human body against infection and disease
- Describe the role of microbiota as a first-line defense against infection and disease

Nonspecific innate immunity can be characterized as a multifaceted system of defenses that targets invading pathogens in a nonspecific manner. In this chapter, we have divided the numerous defenses that make up this system into three categories: physical defenses, chemical defenses, and cellular defenses. However, it is important to keep in mind that these defenses do not function independently, and the categories often overlap. **Table** 11.4.1 provides an overview of the nonspecific defenses discussed in this chapter.

Table 11.4.1*: Overview of Nonspecific Innate Immune Defenses*

Physical defenses	• Physical barriers • Mechanical defenses • Microbiome
Chemical defenses	• Chemicals and enzymes in body fluids • Antimicrobial peptides • Plasma protein mediators • Cytokines • Inflammation-eliciting mediators
Cellular defenses	• Granulocytes • Agranulocytes

Physical defenses provide the body's most basic form of nonspecific defense. They include physical barriers to microbes, such as the skin and mucous membranes, as well as mechanical defenses that physically remove microbes and debris from areas of the body where they might cause harm or infection. In addition, the microbiome provides a measure of physical protection against disease, as microbes of the normal microbiota compete with pathogens for nutrients and cellular binding sites necessary to cause infection.

Physical Barriers

Physical barriers play an important role in preventing microbes from reaching tissues that are susceptible to infection. At the cellular level, barriers consist of cells that are tightly joined to prevent invaders from crossing through to deeper tissue. For example, the endothelial cells that line blood vessels have very tight cell-to-cell junctions, blocking microbes from gaining access to the bloodstream. Cell junctions are generally composed of cell membrane proteins that may connect with the extracellular matrix or with complementary proteins from neighboring cells. Tissues in various parts of the body have different types of cell junctions. These include tight junctions, desmosomes, and gap junctions, as illustrated in **Figure** 11.4.1. Invading microorganisms may attempt to break down these substances chemically, using enzymes such as proteases that can cause structural damage to create a point of entry for pathogens.

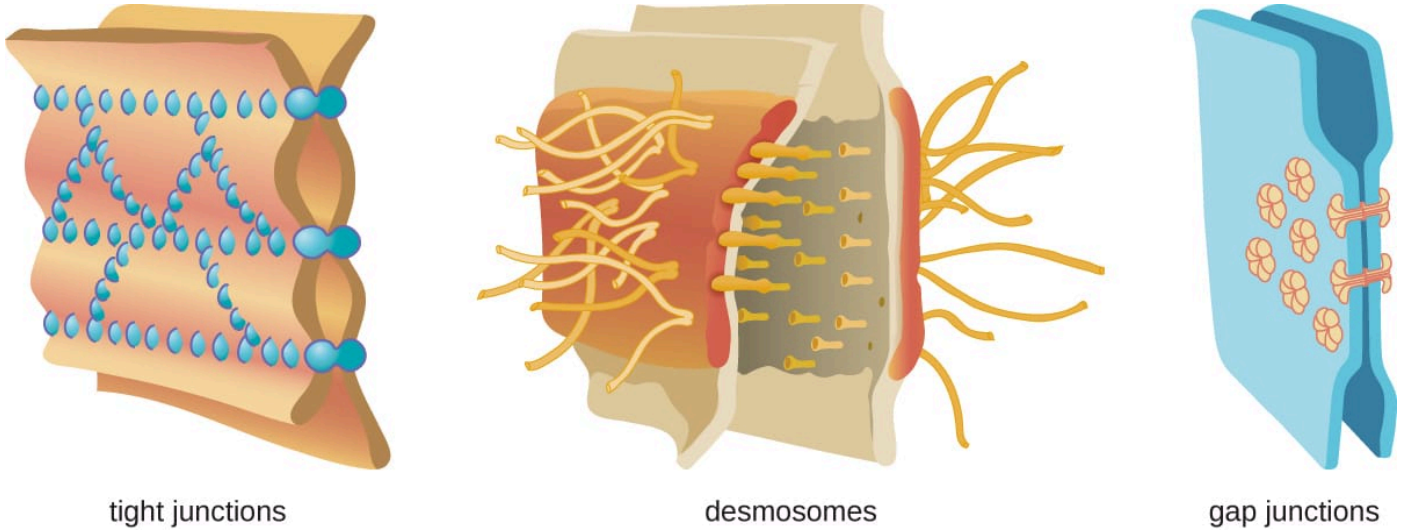


Figure 11.4.1: There are multiple types of cell junctions in human tissue, three of which are shown here. Tight junctions rivet two adjacent cells together, preventing or limiting material exchange through the spaces between them. Desmosomes have intermediate fibers that act like shoelaces, tying two cells together, allowing small materials to pass through the resulting spaces. Gap junctions are channels between two cells that permit their communication via signals. (credit: modification of work by Mariana Ruiz Villareal)

The Skin Barrier

One of the body's most important physical barriers is the skin barrier, which is composed of three layers of closely packed cells. The thin upper layer is called the epidermis. A second, thicker layer, called the dermis, contains hair follicles, sweat glands, nerves, and blood vessels. A layer of fatty tissue called the hypodermis lies beneath the dermis and contains blood and lymph vessels **(Figure** 11.4.2**)**.

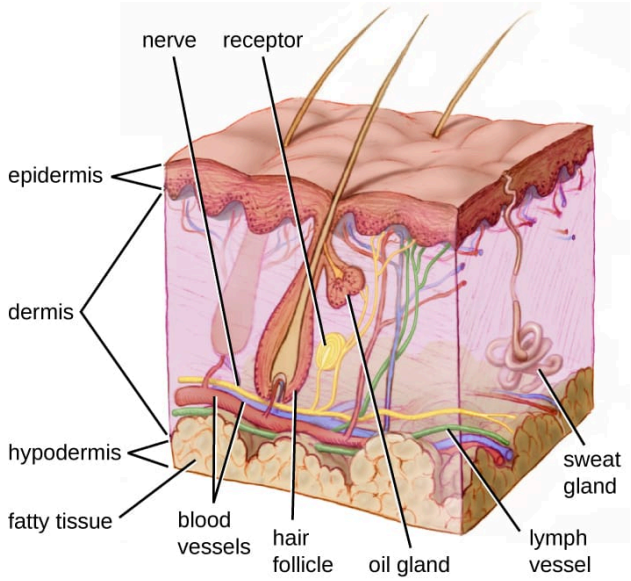


Figure 11.4.2: Human skin has three layers, the epidermis, the dermis, and the hypodermis, which provide a thick barrier between microbes outside the body and deeper tissues. The hypodermis is mostly made up of large circular cells (fatty tissue). The next layer is the dermis. At the bottom of the dermis are blood vessels, lymph vessels, and nerves, all of which run throughout the dermis. Sweat glands are coiled tubes that lead to the surface. Hair follicles are thick vase-shaped structures containing a hair; an oil gland is attached to the hair follicle. The top layer is the epidermis and is made of many layers of flat cells. Dead skin cells on the surface of the epidermis are continually shed, taking with them microbes on the skin's surface. (credit: modification of work by National Institutes of Health)

A diagram of a section of skin.

The topmost layer of skin, the epidermis, consists of cells that are packed with keratin. These dead cells remain as a tightly connected, dense layer of protein-filled cell husks on the surface of the skin. The keratin makes the skin's surface mechanically

tough and resistant to degradation by bacterial enzymes. Fatty acids on the skin's surface create a dry, salty, and acidic environment that inhibits the growth of some microbes and is highly resistant to breakdown by bacterial enzymes. In addition, the dead cells of the epidermis are frequently shed, along with any microbes that may be clinging to them. Shed skin cells are continually replaced with new cells from below, providing a new barrier that will soon be shed in the same way.

Infections can occur when the skin barrier is compromised or broken. A wound can serve as a point of entry for opportunistic pathogens, which can infect the skin tissue surrounding the wound and possibly spread to deeper tissues.

Every Rose Has its Thorn

Mike, a gardener from southern California, recently noticed a small red bump on his left forearm. Initially, he did not think much of it, but soon it grew larger and then ulcerated (opened up), becoming a painful lesion that extended across a large part of his forearm **(Figure** 11.4.3**)**. He went to an urgent care facility, where a physician asked about his occupation. When he said he was a landscaper, the physician immediately suspected a case of sporotrichosis, a type of fungal infection known as rose gardener's disease because it often afflicts landscapers and gardening enthusiasts.

Under most conditions, fungi cannot produce skin infections in healthy individuals. Fungi grow filaments known as hyphae, which are not particularly invasive and can be easily kept at bay by the physical barriers of the skin and mucous membranes. However, small wounds in the skin, such as those caused by thorns, can provide an opening for opportunistic pathogens like *Sporothrix schenkii,* a soil-dwelling fungus and the causative agent of rose gardener's disease. Once it breaches the skin barrier, *S. schenkii* can infect the skin and underlying tissues, producing ulcerated lesions like Mike's. Compounding matters, other pathogens may enter the infected tissue, causing secondary bacterial infections.

Luckily, rose gardener's disease is treatable. Mike's physician wrote him a prescription for some antifungal drugs as well as a course of antibiotics to combat secondary bacterial infections. His lesions eventually healed, and Mike returned to work with a new appreciation for gloves and protective clothing.



Figure 11.4.3: Rose gardener's disease can occur when the fungus *Sporothrix schenkii* breaches the skin through small cuts, such as might be inflicted by thorns. (credit left: modification of work by Elisa Self; credit right: modification of work by Centers for Disease Control and Prevention)

Mucous Membranes

The mucous membranes lining the nose, mouth, lungs, and urinary and digestive tracts provide another nonspecific barrier against potential pathogens. Mucous membranes consist of a layer of epithelial cells bound by tight junctions. The epithelial cells secrete a moist, sticky substance called mucus, which covers and protects the more fragile cell layers beneath it and traps debris and particulate matter, including microbes. Mucus secretions also contain antimicrobial peptides.

In many regions of the body, mechanical actions serve to flush mucus (along with trapped or dead microbes) out of the body or away from potential sites of infection. For example, in the respiratory system, inhalation can bring microbes, dust, mold spores, and other small airborne debris into the body. This debris becomes trapped in the mucus lining the respiratory tract, a layer known as the mucociliary blanket. The epithelial cells lining the upper parts of the respiratory tract are called ciliated epithelial cells because they have hair-like appendages known as cilia. Movement of the cilia propels debris-laden mucus out and away from the

lungs. The expelled mucus is then swallowed and destroyed in the stomach, or coughed up, or sneezed out **(Figure** 11.4.4**)**. This system of removal is often called the mucociliary escalator.

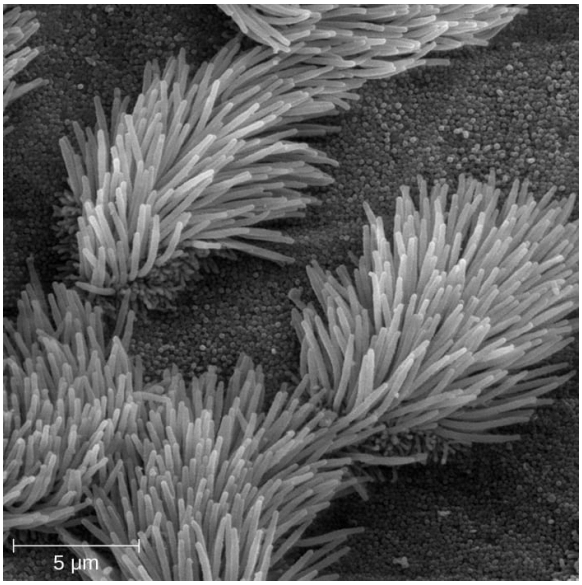


Figure 11.4.4: This scanning electron micrograph shows ciliated and nonciliated epithelial cells from the human trachea. The mucociliary escalator pushes mucus away from the lungs, along with any debris or microorganisms that may be trapped in the sticky mucus, and the mucus moves up to the esophagus where it can be removed by swallowing. Each hair is about 5 µm long; each tuft is about 10 µm in diameter.

The mucociliary escalator is such an effective barrier to microbes that the lungs, the lowermost (and most sensitive) portion of the respiratory tract, were long considered to be a sterile environment in healthy individuals. Only recently has research suggested that healthy lungs may have a small normal microbiota. Disruption of the mucociliary escalator by the damaging effects of smoking or diseases such as cystic fibrosis can lead to increased colonization of bacteria in the lower respiratory tract and frequent infections, which highlights the importance of this physical barrier to host defenses.

Like the respiratory tract, the digestive tract is a portal of entry through which microbes enter the body, and the mucous membranes lining the digestive tract provide a nonspecific physical barrier against ingested microbes. The intestinal tract is lined with epithelial cells, interspersed with mucus-secreting goblet cells **(Figure** 11.4.5**)**. This mucus mixes with material received from the stomach, trapping foodborne microbes and debris. The mechanical action of peristalsis, a series of muscular contractions in the digestive tract, moves the sloughed mucus and other material through the intestines, rectum, and anus, excreting the material in feces.

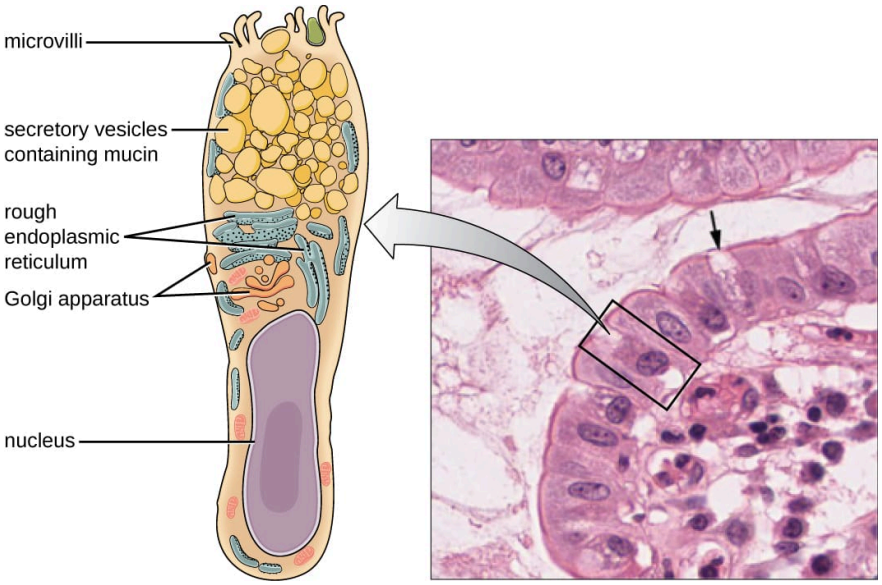


Figure 11.4.5: Goblet cells produce and secrete mucus. The bottom of the cell is filled with a nucleus. The top shows the Golgi apparatus (folds of membranes), rough endoplasmic reticulum (folds of membranes with dots), secretory vesicles containing mucin (large bubbles), and microvilli (finger-like projections at the top). Right is a micrograph of two goblet cells within a row of epithelial cells. The epithelial cells are rectangular with a large nucleus visible. The goblet cells are thinner and have a clear (uncolored) top. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Endothelia

The epithelial cells lining the urogenital tract, blood vessels, lymphatic vessels, and certain other tissues are known as endothelia. These tightly packed cells provide a particularly effective frontline barrier against invaders. The endothelia of the blood-brain barrier, for example, protect the central nervous system (CNS), which consists of the brain and the spinal cord. The CNS is one of the most sensitive and important areas of the body, as microbial infection of the CNS can quickly lead to serious and often fatal inflammation. The cell junctions in the blood vessels traveling through the CNS are some of the tightest and toughest in the body, preventing any transient microbes in the bloodstream from entering the CNS. This keeps the cerebrospinal fluid that surrounds and bathes the brain and spinal cord sterile under normal conditions.

Mechanical Defenses

In addition to physical barriers that keep microbes out, the body has a number of mechanical defenses that physically remove pathogens from the body, preventing them from taking up residence. We have already discussed several examples of mechanical defenses, including the shedding of skin cells, the expulsion of mucus via the mucociliary escalator, and the excretion of feces through intestinal peristalsis. Other important examples of mechanical defenses include the flushing action of urine and tears, which both serve to carry microbes away from the body. The flushing action of urine is largely responsible for the normally sterile environment of the urinary tract, which includes the kidneys, ureters, and urinary bladder. Urine passing out of the body washes out transient microorganisms, preventing them from taking up residence. The eyes also have physical barriers and mechanical mechanisms for preventing infections. The eyelashes and eyelids prevent dust and airborne microorganisms from reaching the surface of the eye. Any microbes or debris that make it past these physical barriers may be flushed out by the mechanical action of blinking, which bathes the eye in tears, washing debris away **(Figure** 11.4.6**)**.

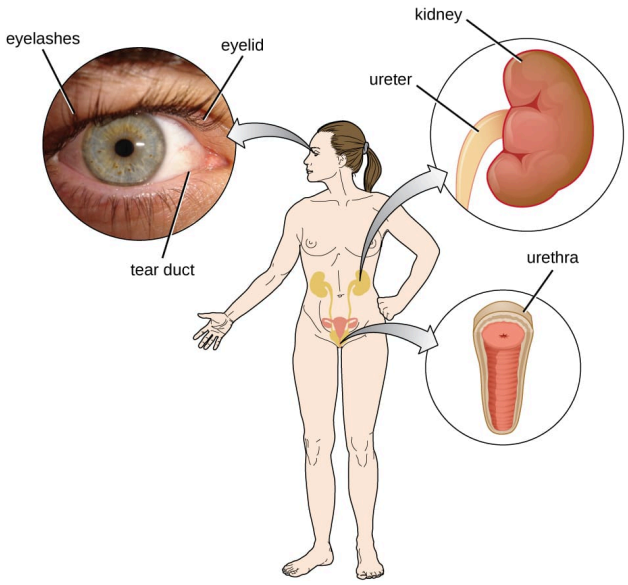


Figure 11.4.6: A diagram of a person. Tears flush microbes away from the surface of the eye. Urine washes microbes out of the urinary tract as it passes through; as a result, the urinary system is normally sterile.

Microbiome

In various regions of the body, resident microbiota serve as an important first-line defense against invading pathogens. Through their occupation of cellular binding sites and competition for available nutrients, the resident microbiota prevent the critical early steps of pathogen attachment and proliferation required for the establishment of an infection. For example, in the vagina, members of the resident microbiota compete with opportunistic pathogens like the yeast *Candida*. This competition prevents infections by limiting the availability of nutrients, thus inhibiting the growth of *Candida,* keeping its population in check. Similar competitions occur between the microbiota and potential pathogens on the skin, in the upper respiratory tract, and in the gastrointestinal tract. As will be discussed later in this chapter, the resident microbiota also contribute to the chemical defenses of the innate nonspecific host defenses.

The importance of the normal microbiota in host defenses is highlighted by the increased susceptibility to infectious diseases when the microbiota is disrupted or eliminated. Treatment with antibiotics can significantly deplete the normal microbiota of the gastrointestinal tract, providing an advantage for pathogenic bacteria to colonize and cause diarrheal infection. In the case of diarrhea caused by *Clostridium difficile*, the infection can be severe and potentially lethal. One strategy for treating *C. difficile* infections is fecal transplantation, which involves the transfer of fecal material from a donor (screened for potential pathogens) into the intestines of the recipient patient as a method of restoring the normal microbiota and combating *C. difficile* infections.

Table 11.4.2 provides a summary of the physical defenses discussed in this section.

Table 11.4.2*: Physical Defenses of Nonspecific Innate Immunity*

Defense	Examples	Function
Cellular barriers	Skin, mucous membranes, endothelial cells	Deny entry to pathogens
Mechanical defenses	Shedding of skin cells, mucociliary sweeping, peristalsis, flushing action of urine and tears	Remove pathogens from potential sites of infection
Microbiome	Resident bacteria of the skin, upper respiratory tract, gastrointestinal tract, and genitourinary tract	Compete with pathogens for cellular binding sites and nutrients

Summary

Nonspecific innate immunity serves as the body's first line of defense against infection by preventing the entry of microbes and promoting their removal before they can cause harm. This defense relies on a combination of physical barriers, mechanical

processes, and the resident microbiome. The skin, mucous membranes, and endothelia throughout the body form protective barriers that block microbes from reaching potential sites of infection, with tight cell junctions further limiting their ability to pass through tissues. Microbes that become trapped in dead skin cells or mucus are cleared from the body through mechanical actions such as the shedding of skin cells, mucociliary sweeping, coughing, peristalsis, and the flushing of bodily fluids like urine and tears. In addition, the normal microbiota contribute to innate defense by occupying available binding sites and competing with pathogens for nutrients, making it more difficult for harmful microbes to establish infections.

Key Terms

cell junctions
: structures that connect neighboring cells and allow communication or strong attachment

cilia
: hair-like structures on cells that move mucus and trapped particles

epidermis
: the outer layer of skin

epithelial cells
: cells that form protective layers on surfaces such as skin and the lining of organs

microbiome
: the community of microorganisms living in and on the body

mucociliary escalator
: the system in the respiratory tract where cilia move mucus upward to keep airways clear

mucus
: a thick, slippery substance that traps dust and microbes

peristalsis
: wave-like muscle contractions that move food through the digestive tract

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11.5: Chemical Defenses

Learning Objectives

- Describe how enzymes in body fluids provide protection against infection or disease
- List and describe the function of antimicrobial peptides, complement components, cytokines, and acute-phase proteins
- Describe similarities and differences among classic, alternate, and lectin complement pathways

In addition to physical defenses, the innate nonspecific immune system uses a number of chemical mediators that inhibit microbial invaders. The term “chemical mediators” encompasses a wide array of substances found in various body fluids and tissues throughout the body. Chemical mediators may work alone or in conjunction with each other to inhibit microbial colonization and infection.

Some chemical mediators are endogenously produced, meaning they are produced by human body cells; others are produced exogenously, meaning that they are produced by certain microbes that are part of the microbiome. Some mediators are produced continually, bathing the area in the antimicrobial substance; others are produced or activated primarily in response to some stimulus, such as the presence of microbes.

Chemical and Enzymatic Mediators Found in Body Fluids

Fluids produced by the skin include examples of both endogenous and exogenous mediators. Sebaceous glands in the dermis secrete an oil called sebum that is released onto the skin surface through hair follicles. This sebum is an endogenous mediator, providing an additional layer of defense by helping seal off the pore of the hair follicle, preventing bacteria on the skin’s surface from invading sweat glands and surrounding tissue **(Figure** 11.5.1**)**. Certain members of the microbiome, such as the bacterium *Propionibacterium acnes* and the fungus *Malassezia*, among others, can use lipase enzymes to degrade sebum, using it as a food source. This produces oleic acid, which creates a mildly acidic environment on the surface of the skin that is inhospitable to many pathogenic microbes. Oleic acid is an example of an exogenously produced mediator because it is produced by resident microbes and not directly by body cells.

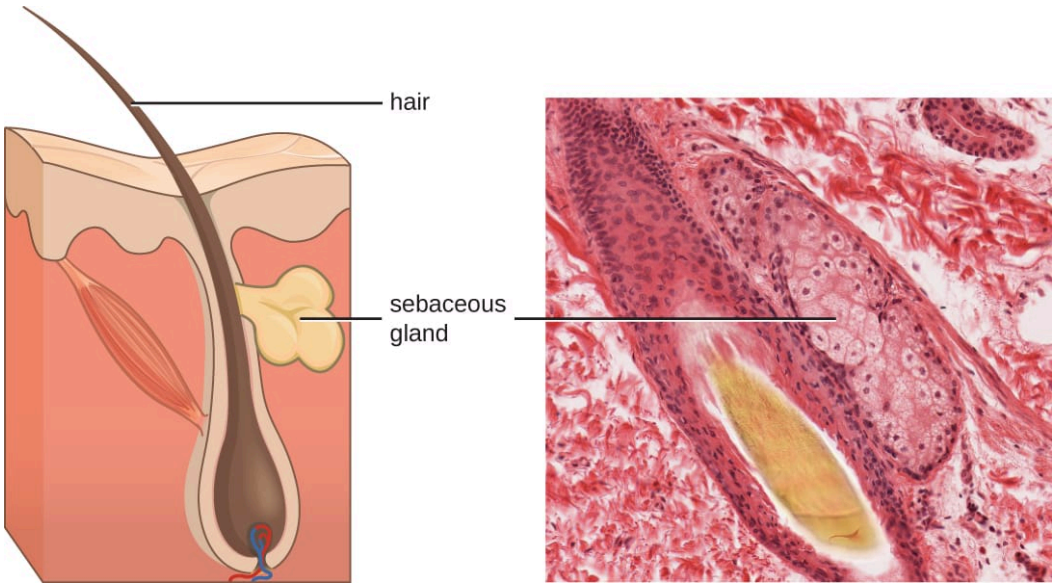


Figure 11.5.1: Sebaceous glands secrete sebum, a chemical mediator that lubricates and protect the skin from invading microbes. Sebum is also a food source for resident microbes that produce oleic acid, an exogenously produced mediator. (credit micrograph: Micrograph provided by the Regents of University of Michigan Medical School © 2012)

Environmental factors that affect the microbiota of the skin can have a direct impact on the production of chemical mediators. Low humidity or decreased sebum production, for example, could make the skin less habitable for microbes that produce oleic

acid, thus making the skin more susceptible to pathogens normally inhibited by the skin's low pH. Many skin moisturizers are formulated to counter such effects by restoring moisture and essential oils to the skin.

The digestive tract also produces a large number of chemical mediators that inhibit or kill microbes. In the oral cavity, saliva contains mediators such as lactoperoxidase enzymes, and mucus secreted by the esophagus contains the antibacterial enzyme lysozyme. In the stomach, highly acidic gastric fluid kills most microbes. In the lower digestive tract, the intestines have pancreatic and intestinal enzymes, antibacterial peptides (cryptins), bile produced from the liver, and specialized Paneth cells that produce lysozyme. Together, these mediators are able to eliminate most pathogens that manage to survive the acidic environment of the stomach.

In the urinary tract, urine flushes microbes out of the body during urination. Furthermore, the slight acidity of urine (the average pH is about 6) inhibits the growth of many microbes and potential pathogens in the urinary tract.

The female reproductive system employs lactate, an exogenously produced chemical mediator, to inhibit microbial growth. The cells and tissue layers composing the vagina produce glycogen, a branched and more complex polymer of glucose. Lactobacilli in the area ferment glycogen to produce lactate, lowering the pH in the vagina and inhibiting transient microbiota, opportunistic pathogens like *Candida* (a yeast associated with vaginal infections), and other pathogens responsible for sexually transmitted diseases.

In the eyes, tears contain the chemical mediators lysozyme and lactoferrin, both of which are capable of eliminating microbes that have found their way to the surface of the eyes. Lysozyme cleaves the bond between NAG and NAM in peptidoglycan, a component of the cell wall in bacteria. It is more effective against gram-positive bacteria, which lack the protective outer membrane associated with gram-negative bacteria. Lactoferrin inhibits microbial growth by chemically binding and sequestering iron. This effectually starves many microbes that require iron for growth.

In the ears, cerumen (earwax) exhibits antimicrobial properties due to the presence of fatty acids, which lower the pH to between 3 and 5.

The respiratory tract uses various chemical mediators in the nasal passages, trachea, and lungs. The mucus produced in the nasal passages contains a mix of antimicrobial molecules similar to those found in tears and saliva (e.g., lysozyme, lactoferrin, lactoperoxidase). Secretions in the trachea and lungs also contain lysozyme and lactoferrin, as well as a diverse group of additional chemical mediators, such as the lipoprotein complex called surfactant, which has antibacterial properties.

Exercise 11.5.1

1. Explain the difference between endogenous and exogenous mediators
2. Describe how pH affects antimicrobial defenses

Antimicrobial Peptides

The antimicrobial peptides (AMPs) are a special class of nonspecific cell-derived mediators with broad-spectrum antimicrobial properties. Some AMPs are produced routinely by the body, whereas others are primarily produced (or produced in greater quantities) in response to the presence of an invading pathogen. Research has begun exploring how AMPs can be used in the diagnosis and treatment of disease.

AMPs may induce cell damage in microorganisms in a variety of ways, including by inflicting damage to membranes, destroying DNA and RNA, or interfering with cell-wall synthesis. Depending on the specific antimicrobial mechanism, a particular AMP may inhibit only certain groups of microbes (e.g., gram-positive or gram-negative bacteria) or it may be more broadly effective against bacteria, fungi, protozoa, and viruses. Many AMPs are found on the skin, but they can also be found in other regions of the body.

A family of AMPs called defensins can be produced by epithelial cells throughout the body as well as by cellular defenses such as macrophages and neutrophils (see Cellular Defenses). Defensins may be secreted or act inside host cells; they combat microorganisms by damaging their plasma membranes. AMPs called bacteriocins are produced exogenously by certain members of the resident microbiota within the gastrointestinal tract. The genes coding for these types of AMPs are often carried on plasmids and can be passed between different species within the resident microbiota through lateral or horizontal gene transfer.

There are numerous other AMPs throughout the body. The characteristics of a few of the more significant AMPs are summarized

in **Table** 11.5.1.

Table 11.5.1*: Characteristics of Selected Antimicrobial Peptides (AMPs)*

AMP	Secreted by	Body site	Pathogens inhibited	Mode of action
Bacteriocins	Resident microbiota	Gastrointestinal tract	Bacteria	Disrupt membrane
Cathelicidin	Epithelial cells, macrophages, and other cell types	Skin	Bacteria and fungi	Disrupts membrane
Defensins	Epithelial cells, macrophages, neutrophils	Throughout the body	Fungi, bacteria, and many viruses	Disrupt membrane
Dermicidin	Sweat glands	Skin	Bacteria and fungi	Disrupts membrane integrity and ion channels
Histatins	Salivary glands	Oral cavity	Fungi	Disrupt intracellular function

Exercise 11.5.2

Why are antimicrobial peptides (AMPs) considered nonspecific defenses?

Plasma Protein Mediators

Many nonspecific innate immune factors are found in plasma, the fluid portion of blood. Plasma contains electrolytes, sugars, lipids, and proteins, each of which helps to maintain homeostasis (i.e., stable internal body functioning), and contains the proteins involved in the clotting of blood. Additional proteins found in blood plasma, such as acute-phase proteins, complement proteins, and cytokines, are involved in the nonspecific innate immune response.

Plasma versus serum

There are two terms for the fluid portion of blood: plasma and serum. How do they differ if they are both fluid and lack cells? The fluid portion of blood left over after coagulation (blood cell clotting) has taken place is serum. Although molecules such as many vitamins, electrolytes, certain sugars, complement proteins, and antibodies are still present in serum, clotting factors are largely depleted. Plasma, conversely, still contains all the clotting elements. To obtain plasma from blood, an anticoagulant must be used to prevent clotting. Examples of anticoagulants include heparin and ethylene diamine tetraacetic acid (EDTA). Because clotting is inhibited, once obtained, the sample must be gently spun down in a centrifuge. The heavier, denser blood cells form a pellet at the bottom of a centrifuge tube, while the fluid plasma portion, which is lighter and less dense, remains above the cell pellet.

Acute-Phase Proteins

The acute-phase proteins are another class of antimicrobial mediators. Acute-phase proteins are primarily produced in the liver and secreted into the blood in response to inflammatory molecules from the immune system. Examples of acute-phase proteins include C-reactive protein, serum amyloid A, ferritin, transferrin, fibrinogen, and mannose-binding lectin. Each of these proteins has a different chemical structure and inhibits or destroys microbes in some way **(Table** 11.5.1**)**.

Table 11.5.2*: Some Acute-Phase Proteins and Their Functions*

Some Acute-Phase Proteins and Their Functions	
C-reactive protein	Coats bacteria (opsonization), preparing them for ingestion by phagocytes
Serum amyloid A	
Ferritin	Bind and sequester iron, thereby inhibiting the growth of pathogens

Some Acute-Phase Proteins and Their Functions	
Transferrin	
Fibrinogen	Involved in formation of blood clots that trap bacterial pathogens
Mannose-binding lectin	Activates complement cascade

The Complement System

The complement system is a group of plasma protein mediators that can act as an innate nonspecific defense while also serving to connect innate and adaptive immunity (discussed in the next chapter). The complement system is composed of more than 30 proteins (including C1 through C9) that normally circulate as precursor proteins in blood. These precursor proteins become activated when stimulated or triggered by a variety of factors, including the presence of microorganisms. Complement proteins are considered part of innate nonspecific immunity because they are always present in the blood and tissue fluids, allowing them to be activated quickly. Also, when activated through the alternative pathway (described later in this section), complement proteins target pathogens in a nonspecific manner.

The process by which circulating complement precursors become functional is called complement activation. This process is a cascade that can be triggered by one of three different mechanisms, known as the alternative, classical, and lectin pathways.

The alternative pathway is initiated by the spontaneous activation of the complement protein C3. The hydrolysis of C3 produces two products, C3a and C3b. When no invader microbes are present, C3b is very quickly degraded in a hydrolysis reaction using the water in the blood. However, if invading microbes are present, C3b attaches to the surface of these microbes. Once attached, C3b will recruit other complement proteins in a cascade **(Figure** 11.5.2**)**.

The classical pathway provides a more efficient mechanism of activating the complement cascade, but it depends upon the production of antibodies by the specific adaptive immune defenses. To initiate the classical pathway, a specific antibody must first bind to the pathogen to form an antibody-antigen complex. This activates the first protein in the complement cascade, the C1 complex. The C1 complex is a multipart protein complex, and each component participates in the full activation of the overall complex. Following recruitment and activation of the C1 complex, the remaining classical pathway complement proteins are recruited and activated in a cascading sequence **(Figure** 11.5.2**)**.

The lectin activation pathway is similar to the classical pathway, but it is triggered by the binding of mannose-binding lectin, an acute-phase protein, to carbohydrates on the microbial surface. Like other acute-phase proteins, lectins are produced by liver cells and are commonly upregulated in response to inflammatory signals received by the body during an infection **(Figure** 11.5.2 **)**.

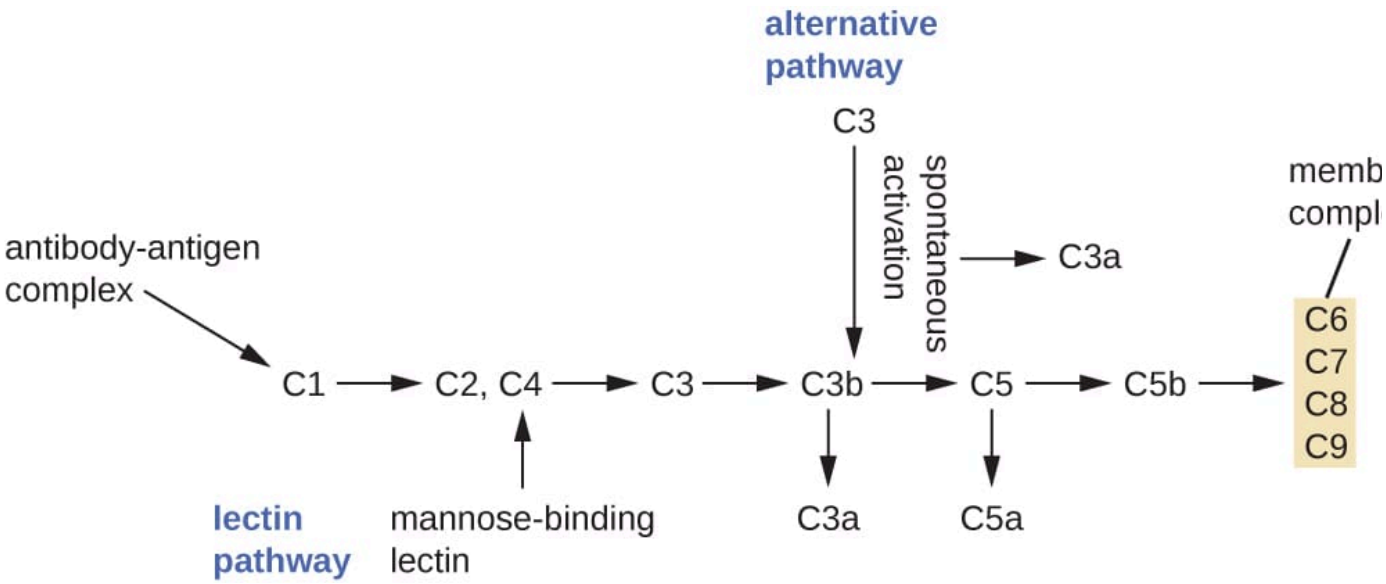


Figure 11.5.2: The three complement activation pathways have different triggers, as shown here, but all three result in the activation of the complement protein C3, which produces C3a and C3b. At the top is the invading pathogen. Two antibodies bind to an antigen on the surface of the pathogen. C1 binds to the antigen-antibody complex. This is labeled the classic pathway. C1 causes C2 and C4 to be cut into two pieces. Parts of C2 and C4 bind together to form C3 convertase. The alternate pathway also leads to C3 convertase but does so directly. C3 convertase then cuts C3 in two and one of these binds to C3 convertase. The resulting enzyme is called C5 convertase. C5 convertase lyses C5 into two pieces. One of the C5 pieces joins other complement proteins (C6, C7, C8 and C9) to create a pore through the membrane of the invading cell. This pore kills the cell. Endogenous proteins on the host cell protect the host membrane from the complement proteins.

Although each complement activation pathway is initiated in a different way, they all provide the same protective outcomes: opsonization, inflammation, chemotaxis, and cytolysis. The term opsonization refers to the coating of a pathogen by a chemical substance (called an opsonin) that allows phagocytic cells to recognize, engulf, and destroy it more easily. Opsonins from the complement cascade include C1q, C3b, and C4b. Additional important opsonins include mannose-binding proteins and antibodies. The complement fragments C3a and C5a are well-characterized anaphylatoxins with potent proinflammatory functions. Anaphylatoxins activate mast cells, causing degranulation and the release of inflammatory chemical signals, including mediators that cause vasodilation and increased vascular permeability. C5a is also one of the most potent chemoattractants for neutrophils and other white blood cells, cellular defenses that will be discussed in the next section.

The complement proteins C6, C7, C8, and C9 assemble into a membrane attack complex (MAC), which allows C9 to polymerize into pores in the membranes of gram-negative bacteria. These pores allow water, ions, and other molecules to move freely in and out of the targeted cells, eventually leading to cell lysis and death of the pathogen **(Figure** 11.5.2**)**. However, the MAC is only effective against gram-negative bacteria; it cannot penetrate the thick layer of peptidoglycan associated with cell walls of gram-positive bacteria. Since the MAC does not pose a lethal threat to gram-positive bacterial pathogens, complement-mediated opsonization is more important for their clearance.

Cytokines

Cytokines are soluble proteins that act as communication signals between cells. In a nonspecific innate immune response, various cytokines may be released to stimulate production of chemical mediators or other cell functions, such as cell proliferation, cell differentiation, inhibition of cell division, apoptosis, and chemotaxis.

When a cytokine binds to its target receptor, the effect can vary widely depending on the type of cytokine and the type of cell or receptor to which it has bound. The function of a particular cytokine can be described as autocrine, paracrine, or endocrine **(Figure** 11.5.3**)**. In autocrine function, the same cell that releases the cytokine is the recipient of the signal; in other words, autocrine function is a form of self-stimulation by a cell. In contrast, paracrine function involves the release of cytokines from one cell to other nearby cells, stimulating some response from the recipient cells. Last, endocrine function occurs when cells release cytokines into the bloodstream to be carried to target cells much farther away.

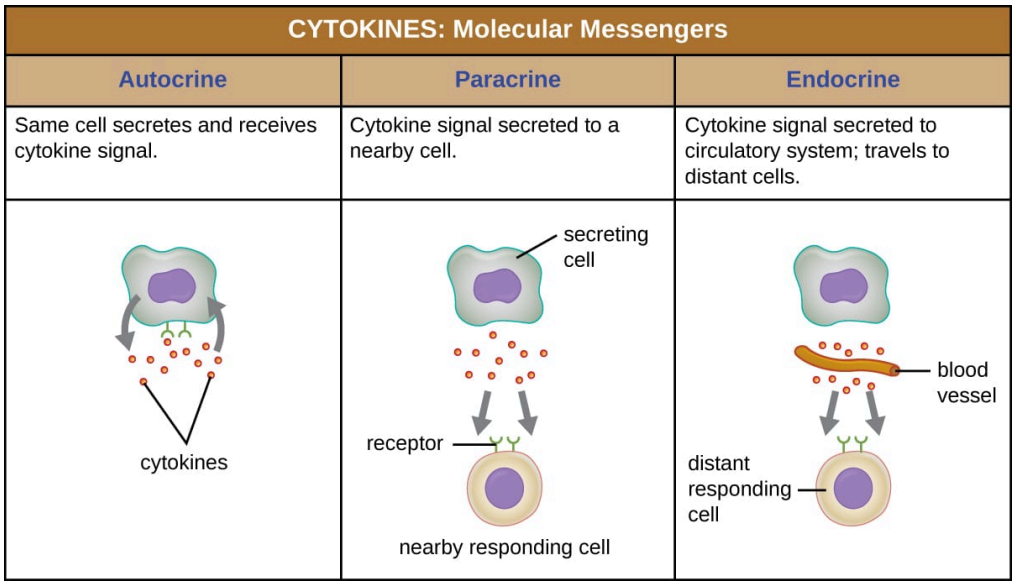


Figure 11.5.3: Cytokines are molecular messengers. In autocrine signaling the same cell secretes and receives cytokine signals. In paracrine signaling cytokine signals are secreted to a nearby cell that has receptors for the molecules. In endocrine signaling cytokine signals are secreted to the circulatory system and travel to distant cells.

Three important classes of cytokines are the interleukins, chemokines, and interferons. The interleukins were originally thought to be produced only by leukocytes (white blood cells) and to only stimulate leukocytes, thus the reasons for their name. Although interleukins are involved in modulating almost every function of the immune system, their role in the body is not restricted to immunity. Interleukins are also produced by and stimulate a variety of cells unrelated to immune defenses.

The chemokines are chemotactic factors that recruit leukocytes to sites of infection, tissue damage, and inflammation. In contrast to more general chemotactic factors, like complement factor C5a, chemokines are very specific in the subsets of leukocytes they recruit.

Interferons are a diverse group of immune signaling molecules and are especially important in our defense against viruses. Type I interferons (interferon-α and interferon-β) are produced and released by cells infected with virus. These interferons stimulate nearby cells to stop production of mRNA, destroy RNA already produced, and reduce protein synthesis. These cellular changes inhibit viral replication and production of mature virus, slowing the spread of the virus. Type I interferons also stimulate various immune cells involved in viral clearance to more aggressively attack virus-infected cells. Type II interferon (interferon-γ) is an important activator of immune cells **(Figure** 11.5.4**)**.

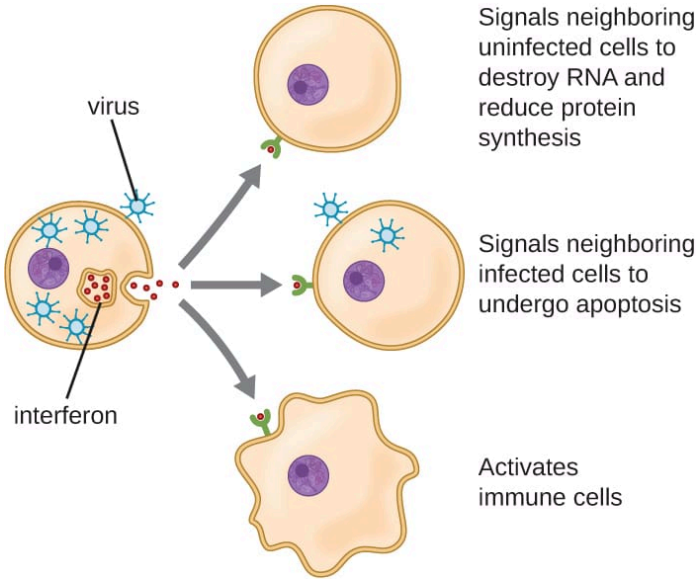


Figure 11.5.4: Interferons are cytokines released by a cell infected with a virus. Interferon-α and interferon-β signal uninfected neighboring cells to inhibit mRNA synthesis, destroy RNA, and reduce protein synthesis (top arrow). Interferon-α and interferon-β also promote apoptosis in cells infected with the virus (middle arrow). Interferon-γ alerts neighboring immune cells to an attack (bottom arrow). Although interferons do not cure the cell releasing them or other infected cells, which will soon die, their release may prevent additional cells from becoming infected, thus stemming the infection.

Inflammation-Eliciting Mediators

Many of the chemical mediators discussed in this section contribute in some way to inflammation and fever, which are nonspecific immune responses discussed in more detail in Inflammation and Fever. Cytokines stimulate the production of acute-phase proteins such as C-reactive protein and mannose-binding lectin in the liver. These acute-phase proteins act as opsonins, activating complement cascades through the lectin pathway.

Some cytokines also bind mast cells and basophils, inducing them to release histamine, a proinflammatory compound. Histamine receptors are found on a variety of cells and mediate proinflammatory events, such as bronchoconstriction (tightening of the airways) and smooth muscle contraction.

In addition to histamine, mast cells may release other chemical mediators, such as leukotrienes. Leukotrienes are lipid-based proinflammatory mediators that are produced from the metabolism of arachidonic acid in the cell membrane of leukocytes and tissue cells. Compared with the proinflammatory effects of histamine, those of leukotrienes are more potent and longer lasting. Together, these chemical mediators can induce coughing, vomiting, and diarrhea, which serve to expel pathogens from the body.

Certain cytokines also stimulate the production of prostaglandins, chemical mediators that promote the inflammatory effects of kinins and histamines. Prostaglandins can also help to set the body temperature higher, leading to fever, which promotes the activities of white blood cells and slightly inhibits the growth of pathogenic microbes (see Inflammation and Fever).

Another inflammatory mediator, bradykinin, contributes to edema, which occurs when fluids and leukocytes leak out of the bloodstream and into tissues. It binds to receptors on cells in the capillary walls, causing the capillaries to dilate and become more permeable to fluids.

Exercise 11.5.3

1. What do the three complement activation pathways have in common?
2. Explain autocrine, paracrine, and endocrine signals.
3. Name two important inflammation-eliciting mediators.

Table 11.5.3 provides a summary of the chemical defenses discussed in this section.

Table 11.5.3: Chemical Defenses of Nonspecific Innate Immunity

Defense	Examples	Function
Chemicals and enzymes in body fluids	Sebum from sebaceous glands	Provides oil barrier protecting hair follicle pores from pathogens
	Oleic acid from sebum and skin microbiota	Lowers pH to inhibit pathogens
	Lysozyme in secretions	Kills bacteria by attacking cell wall
	Acid in stomach, urine, and vagina	Inhibits or kills bacteria
	Digestive enzymes and bile	Kill bacteria
	Lactoferrin and transferrin	Bind and sequester iron, inhibiting bacterial growth
	Surfactant in lungs	Kills bacteria
Antimicrobial peptides	Defensins, bacteriocins, dermicidin, cathelicidin, histatins,	Kill bacteria by attacking membranes or interfering with cell functions
Plasma protein mediators	Acute-phase proteins (C-reactive protein, serum amyloid A, ferritin, fibrinogen, transferrin, and mannose-binding lectin)	Inhibit the growth of bacteria and assist in the trapping and killing of bacteria
	Complements C3b and C4b	Opsonization of pathogens to aid phagocytosis
	Complement C5a	Chemoattractant for phagocytes
	Complements C3a and C5a	Proinflammatory anaphylatoxins
Cytokines	Interleukins	Stimulate and modulate most functions of immune system
	Chemokines	Recruit white blood cells to infected area
	Interferons	Alert cells to viral infection, induce apoptosis of virus-infected cells, induce antiviral defenses in infected and nearby uninfected cells, stimulate immune cells to attack virus-infected cells
Inflammation-eliciting mediators	Histamine	Promotes vasodilation, bronchoconstriction, smooth muscle contraction, increased secretion and mucus production
	Leukotrienes	Promote inflammation; stronger and longer lasting than histamine
	Prostaglandins	Promote inflammation and fever
	Bradykinin	Increases vasodilation and vascular permeability, leading to edema

Summary

The innate nonspecific immune system uses a wide variety of chemical mediators—both endogenous (produced by body cells) and exogenous (produced by resident microbes)—to prevent microbial colonization and infection. These mediators are found in many body fluids such as sebum, saliva, tears, gastric fluids, urine, and mucus, where they act independently or together to create inhospitable environments for pathogens. Antimicrobial peptides (AMPs) like defensins and cathelicidins directly kill microbes by damaging membranes or disrupting cell functions. Plasma proteins, including acute-phase proteins and the complement system, enhance innate immunity through inflammation, opsonization, and cytolysis. The complement system, activated by different pathways (e.g., lectin pathway), enhances immunity via opsonization, inflammation, and direct killing through the membrane attack complex (MAC). Cytokines are key signaling proteins that regulate immune responses and include interleukins, chemokines, and interferons, each with distinct roles in communication and activation of immune defenses. Several mediators, such as histamine, leukotrienes, prostaglandins, and bradykinin, promote inflammation and contribute to pathogen removal through mechanisms like vasodilation, fever, and recruitment of immune cells.

Key Terms

antimicrobial peptides
nonspecific peptides (e.g., defensins, cathelicidins) that disrupt microbial membranes or interfere with microbial cell functions

autocrine signaling
cytokine signaling where the cell stimulates itself

chemokines
cytokines that recruit specific white blood cells to infection sites

chemotaxis
movement of a cell toward or away from chemical signals

chemical mediators
substances that inhibit microbial growth; can be endogenous or exogenous

complement system
plasma proteins that enhance immune functions such as opsonization and cytolysis

cytokines
signaling proteins that regulate immune responses, including interleukins, chemokines, and interferons

cytolysis
the breaking or bursting of a cell

defensins
AMPs that disrupt microbial membranes, produced by epithelial and immune cells

endocrine signaling
cytokine signaling that acts on distant cells via the bloodstream

endogenous mediator
a chemical defense substance produced by human body cells

exogenous mediator
a chemical defense substance produced by microbes in the microbiome

histamine
an inflammatory mediator that causes vasodilation and bronchoconstriction

inflammation-eliciting mediators
chemicals (e.g., histamine, leukotrienes) that trigger inflammation and related responses

interferons
cytokines that help inhibit viral replication and activate immune cells

interleukins
cytokines involved in modulating immune system functions

lactoferrin
a protein that binds iron to limit microbial growth

lectin pathway
complement activation pathway triggered by mannose-binding lectin

lysozyme
An enzyme that breaks down bacterial cell walls, especially in Gram-positive bacteria

membrane attack complex (MAC)
structure formed by complement proteins that lyses Gram-negative bacteria

opsonins
: molecules that coat pathogens to make them easier for immune cells to recognize and engulf

opsonization
: process where pathogens are coated with molecules to enhance phagocytosis

paracrine signaling
: cytokine signaling that acts on nearby cells

sebaceous gland
: a skin gland that produces oil to protect and moisturize the skin

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11.6: Cellular Defenses

Learning Objectives

- Describe the process of hematopoiesis and identify the bone marrow as the source of all white blood cells (leukocytes)
- Differentiate between the major types of granulocytes—neutrophils, eosinophils, and basophils—based on structure and function
- Explain the roles of monocytes, macrophages, and dendritic cells, including their phagocytic abilities and tissue localization
- Summarize the function of natural killer (NK) cells, including how they detect abnormal cells and induce apoptosis.

In the previous section, we discussed some of the chemical mediators found in plasma, the fluid portion of blood. The nonfluid portion of blood consists of various types of formed elements, so called because they are all formed from the same stem cells found in bone marrow. The three major categories of formed elements are: red blood cells (RBCs), also called erythrocytes; platelets, also called thrombocytes; and white blood cells (WBCs), also called leukocytes.

Red blood cells are primarily responsible for carrying oxygen to tissues. Platelets are cellular fragments that participate in blood clot formation and tissue repair. Several different types of WBCs participate in various nonspecific mechanisms of innate and adaptive immunity. In this section, we will focus primarily on the innate mechanisms of various types of WBCs.

Hematopoiesis

All of the formed elements of blood are derived from pluripotent hematopoietic stem cells (HSCs) in the bone marrow. As the HSCs make copies of themselves in the bone marrow, individual cells receive different cues from the body that control how they develop and mature. As a result, the HSCs differentiate into different types of blood cells that, once mature, circulate in peripheral blood. This process of differentiation, called hematopoiesis, is shown in more detail in **Figure** 11.6.1.

In terms of sheer numbers, the vast majority of HSCs become erythrocytes. Much smaller numbers become leukocytes and platelets. Leukocytes can be further subdivided into granulocytes, which are characterized by numerous granules visible in the cytoplasm, and agranulocytes, which lack granules. **Figure** 11.6.2 provides an overview of the various types of formed elements, including their relative numbers, primary function, and lifespans.

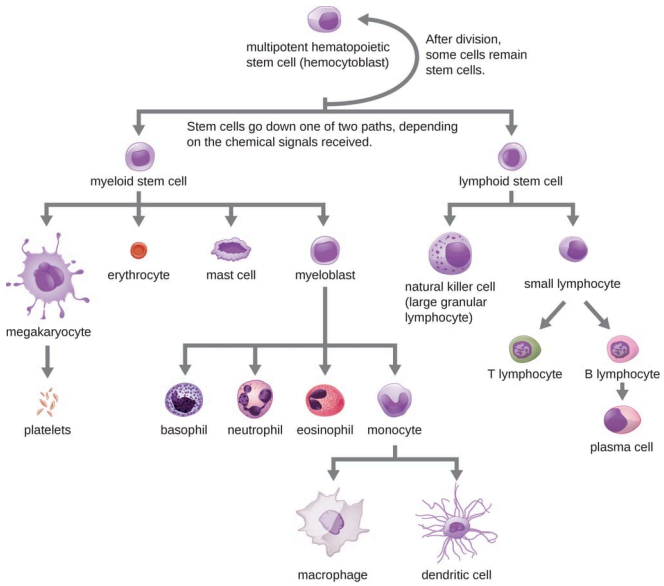


Figure 11.6.1: All the formed elements of the blood arise by differentiation of hematopoietic stem cells in the bone marrow. The flowchart shows the progression of development for formed elements of blood. At the top is a multipotent hematopoietic stem cell (hemocytoblast). This cell divides and after division some of the new cells remain stem cells. Others go down one of two paths depending on the chemical signals received. One path begins with lymphoid stem cells which can either become natural killer cells (large granular lymphocytes) or small lymphocytes. The natural killer cell is a medium-large purple cell. Small lymphocytes can either become T lymphocytes or B lymphoctyes. The T and B lymphocytes are medium size cells with a large nucleus. B lymphocytes become plasma cells which are medium size cells with a large nucleus. The other option for the stem cell is to become a myeloid stem cell. Myeloid stem cells follow one of four paths. One path leads to megakaryocyte which leads to platelets. Platelets are small flecks. The second path leads to erythrocyte. Erythrocytes are small donut shaped red cells. The third path leads to mast cells. The fourth path leads to basophil, neutrophil, eosinophil, or monocyte. Basophils are medium cells with many dark purple spots. Neutrophils are medium pink cells with a multi-lobbed nucleus. Eosinophils are medium size cells with many pink spots. Monocytes lead to macrophages or dendritic cells. Macrophages are large irregularly shaped cells. Dendritic cells have longer tendons branching off of them.

Formed Element	Major Subtypes		Numbers Present per Microliter (μL) and Mean (Range)	Appearance in a Standard Blood Smear	Summary of Functions	Comments
Erythrocytes (red blood cells)			5.2 million (4.4–6.0 million)	Flattened biconcave disk; no nucleus; pale red	Transport oxygen and some carbon dioxide between tissue and lungs	Lifespan of approximately 120 days
Leukocytes (white blood cells)			7000 (5000–10,000)	Obvious dark-staining nucleus	All function in body defenses	Exit capillaries and move into tissues; lifespan of usually a few hours or days
	Granulocytes, including neutrophils, eosinophils, and basophils	*Total leukocytes (%)*	4360 (1800–9950)	Abundant granules in cytoplasm; nucleus normally lobed	Nonspecific (innate) resistance to disease	Classified according to membrane-bound granules in cytoplasm
	Neutrophils	50–70	4150 (1800–7300)	Nucleus lobes increase with age; pale lilac granules	Phagocytic; particularly effective against bacteria; release cytotoxic chemicals from granules	Most common leukocyte; lifespan of minutes to days
	Eosinophils	1–3	165 (0–700)	Nucleus generally two-lobed; bright red-orange granules	Phagocytic cells; particularly effective with antigen-antibody complexes; release antihistamines; combat parasitic infections	Lifespan of minutes to days
	Basophils	<1	44 (0–150)	Nucleus generally two-lobed but difficult to see due to presence of heavy, dense, dark purple granules	Pro-inflammatory	Least common leukocyte; lifespan unknown
	Agranulocytes, including lymphocytes and monocytes		2640 (1700–4950)	Lack abundant granules in cytoplasm; have a simple-shaped nucleus that may be indented	Body defenses	Group consists of two major cell types from different lineages
	Lymphocytes	20–40	2185 (1500–4000)	Spherical cells with a single, often large, nucleus occupying much of the cell's volume; stains purple; seen in large (natural killer cells) and small (B and T cells) variants	Primarily specific (adaptive) immunity: T cells directly attack other cells (cellular immunity); B cells release antibodies (humoral immunity); natural killer cells are similar to T cells but nonspecific	Initial cells originate in bone marrow, but secondary production occurs in lymphatic tissue; several distinct subtypes; memory cells form after exposure to a pathogen and rapidly increase responses to subsequent exposure; lifespan of many years
	Monocytes	1–6	455 (200–950)	Largest leukocyte; has an indented or horseshoe-shaped nucleus	Very effective phagocytic cells engulfing pathogens or worn-out cells; also serve as antigen-presenting cells (APCs) or other components of the immune system	Produced in red bone marrow; referred to as macrophages and dendritic cells after leaving the circulation
Platelets			350,000 (150,000–500,000)	Cellular fragments surrounded by a plasma membrane and containing granules; stains purple	Hemostasis; release growth factors for repair and healing of tissue	Formed from megakaryocytes that remain in the red bone marrow and shed platelets into circulation

Figure 11.6.2 Formed elements of blood include erythrocytes (red blood cells), leukocytes (white blood cells), and platelets. Click to open description of the above table of the formed elements Top row reads: formed element, major subtypes, numbers present per microliter and mean, appearance in standard blood smear, summary of functions, and comments. The first row is for erythrocytes (red blood cells). There are 5.2 million per microliter of blood (ranging from 4.4 – 6 million). These cells are flattened biconcave disks with no nucleus and a pale red color. Their function is to transport oxygen and some carbon dioxide between tissue and lungs. Their lifespan is approximately 120 days. The set of rows is classified under leukocytes (white blood cells). Leukocytes as a group number 7000 per microliter of blood (ranging from 5000-10,000). Leukocytes have an obvious dark-staining nucleus and function in body defenses. They exit capillaries and move into tissues. Their lifespan is usually a few hours or days. Leukocytes are divided into two groups. The first is granulocytes including neutrophils, eosinophils, and basophils. The second is agranulocytes including lymphocytes and monocytes. Granulocytes number 4300 per microliter of blood (range of 1800-9950). Granulocytes have abundant granules in the cytoplasm and the nucleus is normally lobed. Granulocytes function in nonspecific (innate) resistance to disease and are classified according to membrane-bound granules in the cytoplasm. Neutrophils make up 50-70% of the total leukocytes and number 4150 per microliter of blood (range 1800-7300). Neutrophils have a nucleus with lobes that increase with age and pale lilac granules. They are phagocytic and particularly effective against bacteria; they release toxic chemicals from granules. Neutrophils are the most common leukocyte with a lifespan of minutes to days. Eosinophils make up 1-3% of total leukocytes. They number 165 per microliter of blood (range of 0 – 700). Eosinophils have a nucleus that is generally two-lobed and bright red-orange granules. They are phagocytic cells and particularly effective with antigen-antibody complexes. Eosinophils release antihistamines and increase allergies, they also help fight parasitic infections. Eosinophils have a lifespan of minutes to days. Basophils make up less than 1% of total leukocytes. They number 44 per microliter of blood (range 0 – 150). Basophils have a nucleus that is generally two lobed but difficult to see due to the presence of heavy, dense, dark purple granules. Basophils promote inflammation and are the least common leukocyte. Their lifespan is unknown. Agranulocytes (including lymphocytes and monocytes) number 2640 per microliter of blood (range 1700 – 4900). Agranulocytes lack abundant granules in the cytoplasm and have a simple-shaped nucleus that may be indented. They function in body defenses and are grouped into two major cell types from different lineages. Lymphocytes make up 20-40% of total leukocytes and number 2185 per microliter of blood (range 1500-4000). Lymphocytes are spherical cells with a single, often large, nucleus occupying much of the cell's volume. They stain purple and are seen in large (natural killer cells) and small (B and T cells) variants. Lymphocytes are primarily involved in specific (adaptive) immunity. T cells directly attack other cells (cellular immunity); natural killer cells are similar to T cells but nonspecific. Lymphocytes originate in bone marrow but secondary production occurs in lymphatic tissue. Several distinct subtypes. Memory cells form after exposure to a pathogen and rapidly increase responses to subsequent exposure. Lifespan of many years. Monocytes make up 1-6% of total leukocytes. They number 455 per microliter of blood (range 200-950). Monocytes are large leukocytes with an indented or horseshoe-shaped nucleus. They are very effective phagocytic cells engulfing pathogens or worn out cells and also serve as antigen presenting cells (APCs) or other components of the immune system. Monocytes are produced in red bone marrow and are referred to as macrophages and dendritic cells after leaving circulation. The last row of the table is for platelets. These number 350,000 per microliter of blood (range 150,000-500,000). Platelets are cellular fragments surrounded by a plasma membrane and containing granules. They stain purple. The function of platelets is hemostasis plus releasing growth factors for repair and healing of tissues. They are formed from megakaryocytes that remain in the red bone marrow and shed platelets into circulation.

Granulocytes

The various types of granulocytes can be distinguished from one another in a blood smear by the appearance of their nuclei and the contents of their granules, which confer different traits, functions, and staining properties. The neutrophils, also called polymorphonuclear neutrophils (PMNs), have a nucleus with three to five lobes and small, numerous, lilac-colored granules. Each lobe of the nucleus is connected by a thin strand of material to the other lobes. The eosinophils have fewer lobes in the nucleus

(typically 2–3) and larger granules that stain reddish-orange. The basophils have a two-lobed nucleus and large granules that stain dark blue or purple **(Figure** 11.6.3**)**.

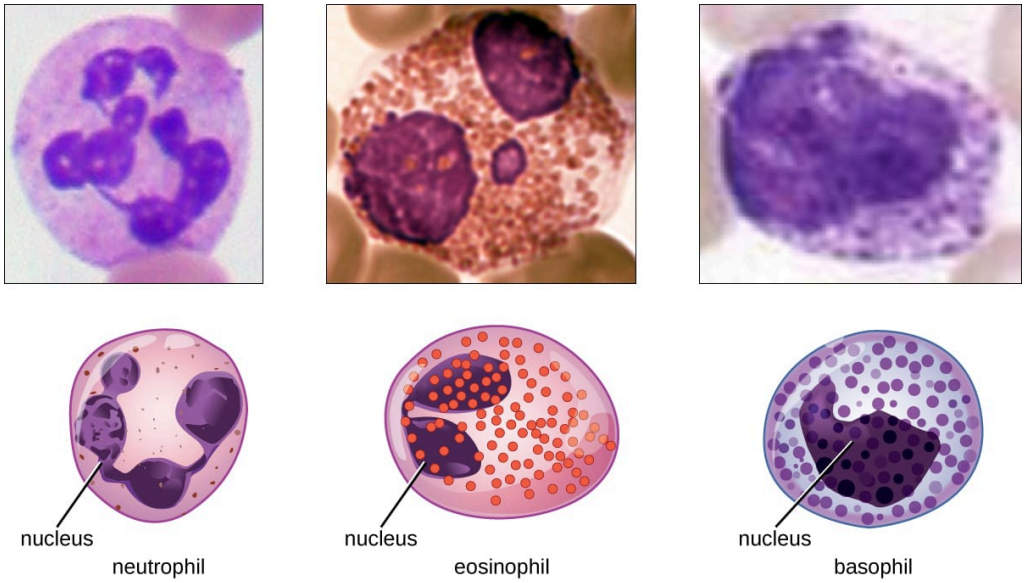


Figure 11.6.3: Granulocytes can be distinguished by the number of lobes in their nuclei and the staining properties of their granules. Neutrophils have a multi-lobed nucleus. Eosinophils have a two-lobed nucleus and distinct pink spots when stained. Basophils have a two-lobed nucleus and distinct purple spots when stained. (credit "neutrophil" micrograph: modification of work by Ed Uthman)

Neutrophils (PMNs)

Neutrophils (PMNs) are frequently involved in the elimination and destruction of extracellular bacteria. They are capable of migrating through the walls of blood vessels to areas of bacterial infection and tissue damage, where they seek out and kill infectious bacteria. PMN granules contain a variety of defensins and hydrolytic enzymes that help them destroy bacteria through phagocytosis (described in more detail in Pathogen Recognition and Phagocytosis) In addition, when many neutrophils are brought into an infected area, they can be stimulated to release toxic molecules into the surrounding tissue to better clear infectious agents. This is called degranulation.

Another mechanism used by neutrophils is neutrophil extracellular traps (NETs), which are extruded meshes of chromatin that are closely associated with antimicrobial granule proteins and components. Chromatin is DNA with associated proteins (usually histone proteins, around which DNA wraps for organization and packing within a cell). By creating and releasing a mesh or lattice-like structure of chromatin that is coupled with antimicrobial proteins, the neutrophils can mount a highly concentrated and efficient attack against nearby pathogens. Proteins frequently associated with NETs include lactoferrin, gelatinase, cathepsin G, and myeloperoxidase. Each has a different means of promoting antimicrobial activity, helping neutrophils eliminate pathogens. The toxic proteins in NETs may kill some of the body's own cells along with invading pathogens. However, this collateral damage can be repaired after the danger of the infection has been eliminated.

As neutrophils fight an infection, a visible accumulation of leukocytes, cellular debris, and bacteria at the site of infection can be observed. This buildup is what we call pus (also known as purulent or suppurative discharge or drainage). The presence of pus is a sign that the immune defenses have been activated against an infection; historically, some physicians believed that inducing pus formation could actually promote the healing of wounds. The practice of promoting "laudable pus" (by, for instance, wrapping a wound in greasy wool soaked in wine) dates back to the ancient physician Galen in the 2nd century AD, and was practiced in variant forms until the 17th century (though it was not universally accepted). Today, this method is no longer practiced because we now know that it is not effective. Although a small amount of pus formation can indicate a strong immune response, artificially inducing pus formation does not promote recovery.

Eosinophils

Eosinophils are granulocytes that protect against protozoa and helminths; they also play a role in allergic reactions. The granules of eosinophils, which readily absorb the acidic reddish dye eosin, contain histamine, degradative enzymes, and a compound known as major basic protein (MBP) **(Figure** 11.6.3**)**. MBP binds to the surface carbohydrates of parasites, and this binding is

associated with disruption of the cell membrane and membrane permeability.

Basophils

Basophils have cytoplasmic granules of varied size and are named for their granules' ability to absorb the basic dye methylene blue **(Figure** 11.6.3**)**. Their stimulation and degranulation can result from multiple triggering events. Activated complement fragments C3a and C5a, produced in the activation cascades of complement proteins, act as anaphylatoxins by inducing degranulation of basophils and inflammatory responses. This cell type is important in allergic reactions and other responses that involve inflammation. One of the most abundant components of basophil granules is histamine, which is released along with other chemical factors when the basophil is stimulated. These chemicals can be chemotactic and can help to open the gaps between cells in the blood vessels. Other mechanisms for basophil triggering require the assistance of antibodies, as discussed in B Lymphocytes and Humoral Immunity.

Mast Cells

Hematopoiesis also gives rise to mast cells, which appear to be derived from the same common myeloid progenitor cell as neutrophils, eosinophils, and basophils. Functionally, mast cells are very similar to basophils, containing many of the same components in their granules (e.g., histamine) and playing a similar role in allergic responses and other inflammatory reactions. However, unlike basophils, mast cells leave the circulating blood and are most frequently found residing in tissues. They are often associated with blood vessels and nerves or found close to surfaces that interface with the external environment, such as the skin and mucous membranes in various regions of the body **(Figure** 11.6.4**)**.

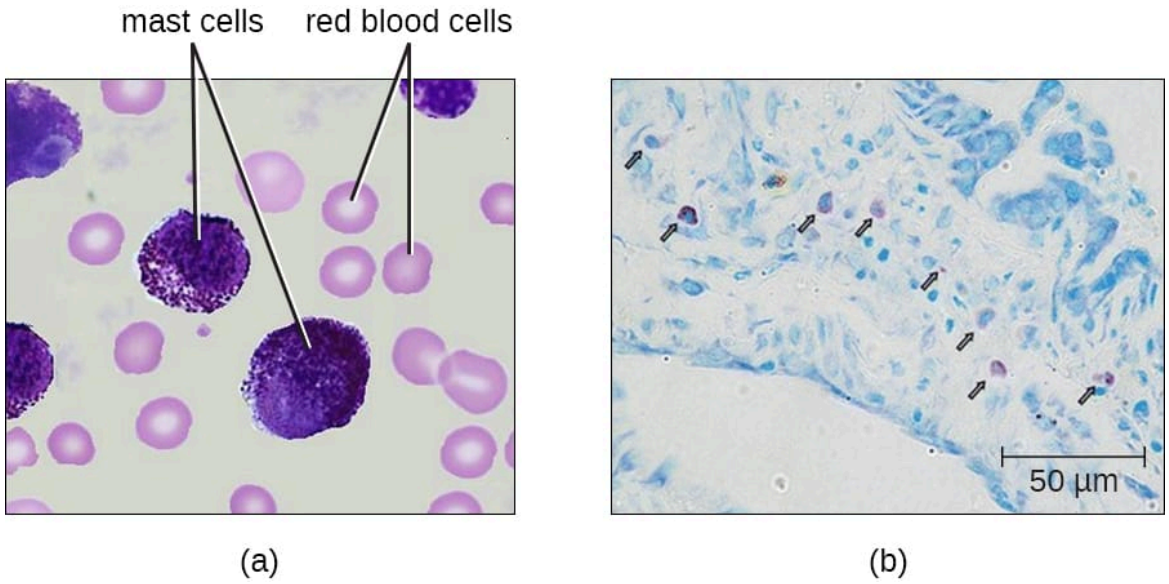


Figure 11.6.4: Mast cells function similarly to basophils by inducing and promoting inflammatory responses. (a) This figure shows mast cells in blood. In a blood smear, they are difficult to differentiate from basophils (b). Unlike basophils, mast cells migrate from the blood into various tissues. (credit right: modification of work by Greenland JR, Xu X, Sayah DM, Liu FC, Jones KD, Looney MR, Caughey GH)

Exercise 11.6.1

1. Describe the granules and nuclei of neutrophils, eosinophils, basophils, and mast cells.
2. Name three antimicrobial mechanisms of neutrophils

Agranulocytes

As their name suggests, agranulocytes lack visible granules in the cytoplasm. Agranulocytes can be categorized as lymphocytes or monocytes **(Figure** 11.6.2**)**. Among the lymphocytes are natural killer cells, which play an important role in nonspecific innate immune defenses. Lymphocytes also include the B cells and T cells, which are discussed in the next chapter because they are

central players in the specific adaptive immune defenses. The monocytes differentiate into macrophages and dendritic cells, which are collectively referred to as the mononuclear phagocyte system.

Natural Killer Cells

Most lymphocytes are primarily involved in the specific adaptive immune response, and thus will be discussed in the following chapter. An exception is the natural killer cells (NK cells); these mononuclear lymphocytes use nonspecific mechanisms to recognize and destroy cells that are abnormal in some way. Cancer cells and cells infected with viruses are two examples of cellular abnormalities that are targeted by NK cells. Recognition of such cells involves a complex process of identifying inhibitory and activating molecular markers on the surface of the target cell. Molecular markers that make up the major histocompatibility complex (MHC) are expressed by healthy cells as an indication of "self." This will be covered in more detail in next chapter. NK cells are able to recognize normal MHC markers on the surface of healthy cells, and these MHC markers serve as an inhibitory signal preventing NK cell activation. However, cancer cells and virus-infected cells actively diminish or eliminate expression of MHC markers on their surface. When these MHC markers are diminished or absent, the NK cell interprets this as an abnormality and a cell in distress. This is one part of the NK cell activation process **(Figure** 11.6.5**)**. NK cells are also activated by binding to activating molecular molecules on the target cell. These activating molecular molecules include "altered self" or "nonself" molecules. When a NK cell recognizes a decrease in inhibitory normal MHC molecules and an increase in activating molecules on the surface of a cell, the NK cell will be activated to eliminate the cell in distress.

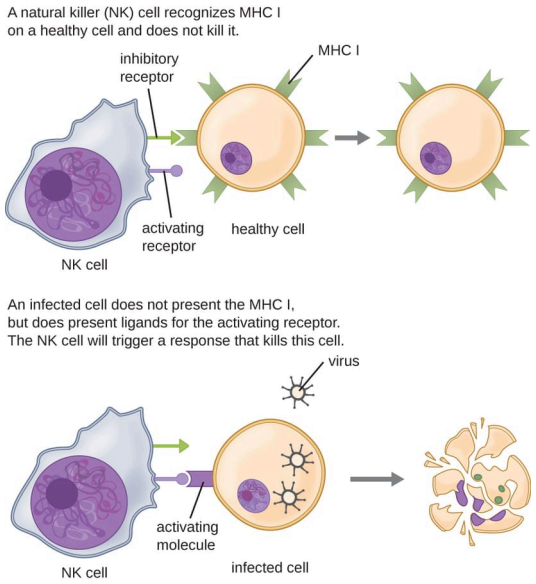


Figure 11.6.5: Natural killer (NK) cells are inhibited by the presence of the major histocompatibility cell (MHC) receptor on healthy cells. Cancer cells and virus-infected cells have reduced expression of MHC and increased expression of activating molecules. When a NK cell recognizes decreased MHC and increased activating molecules, it will kill the abnormal cell.

Once a cell has been recognized as a target, the NK cell can use several different mechanisms to kill its target. For example, it may express cytotoxic membrane proteins and cytokines that stimulate the target cell to undergo apoptosis, or controlled cell suicide. NK cells may also use perforin-mediated cytotoxicity to induce apoptosis in target cells. This mechanism relies on two toxins released from granules in the cytoplasm of the NK cell: perforin, a protein that creates pores in the target cell, and granzymes, proteases that enter through the pores into the target cell's cytoplasm, where they trigger a cascade of protein activation that leads to apoptosis. The NK cell binds to the abnormal target cell, releases its destructive payload, and detaches from the target cell. While the target cell undergoes apoptosis, the NK cell synthesizes more perforin and proteases to use on its next target.

NK cells contain these toxic compounds in granules in their cytoplasm. When stained, the granules are azurophilic and can be visualized under a light microscope **(Figure** 11.6.6**)**. Even though they have granules, NK cells are not considered granulocytes because their granules are far less numerous than those found in true granulocytes. Furthermore, NK cells have a different lineage than granulocytes, arising from lymphoid rather than myeloid stem cells **(Figure** 11.6.1**)**.

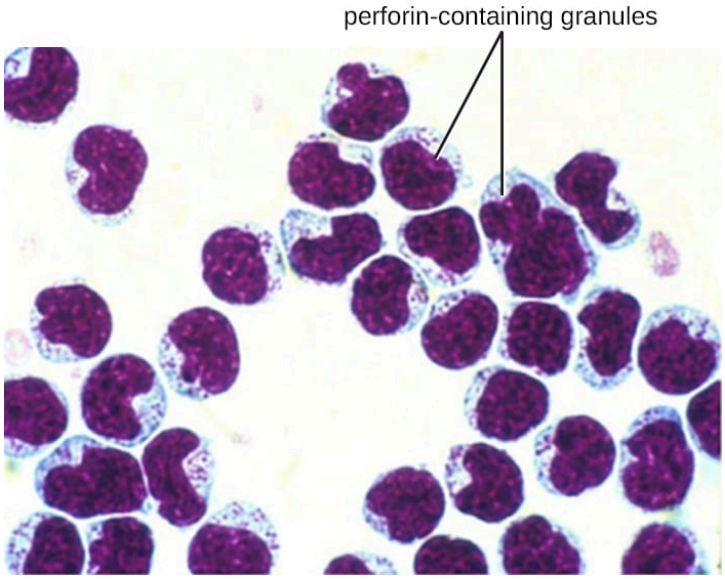


Figure 11.6.6: Many red blood cells with a single larger cell. The larger cell is pink with a purple region that fills nearly the entire cell. The purple region is labeled perforin-containing granules. (credit: modification of work by Rolstad B)

Monocytes

The largest of the white blood cells, monocytes have a nucleus that lacks lobes, and they also lack granules in the cytoplasm **(Figure** 11.6.7**)**. Nevertheless, they are effective phagocytes, engulfing pathogens and apoptotic cells to help fight infection.

When monocytes leave the bloodstream and enter a specific body tissue, they differentiate into tissue-specific phagocytes called macrophages and dendritic cells. They are particularly important residents of lymphoid tissue, as well as nonlymphoid sites and organs. Macrophages and dendritic cells can reside in body tissues for significant lengths of time. Macrophages in specific body tissues develop characteristics suited to the particular tissue. Not only do they provide immune protection for the tissue in which they reside but they also support normal function of their neighboring tissue cells through the production of cytokines. Macrophages are given tissue-specific names, and a few examples of tissue-specific macrophages are listed in **Table** 11.6.1. Dendritic cells are important sentinels residing in the skin and mucous membranes, which are portals of entry for many pathogens. Monocytes, macrophages, and dendritic cells are all highly phagocytic and important promoters of the immune response through their production and release of cytokines. These cells provide an essential bridge between innate and adaptive immune responses, as discussed in the next section as well as the next chapter.

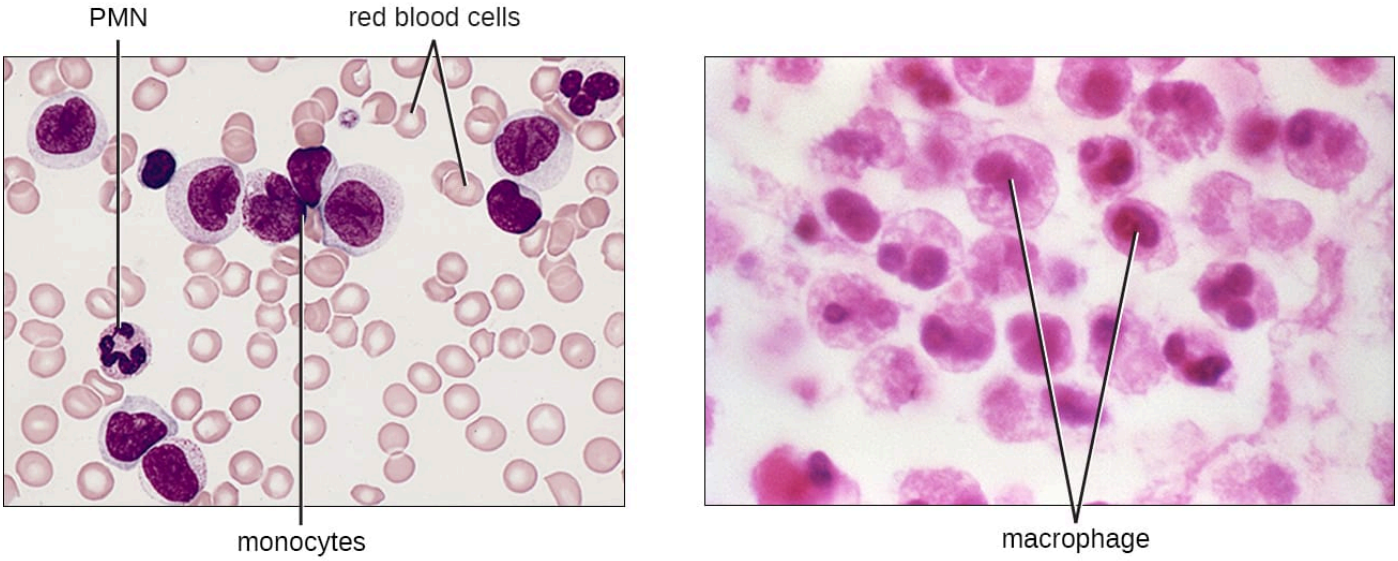


Figure 11.6.7: Monocytes are large, agranular white blood cells with a nucleus that lacks lobes. When monocytes leave the bloodstream, they differentiate and become macrophages with tissue-specific properties. Monocytes are large cells with a large purple nucleus. There is a cluster of them in a field of smaller red blood cells. A PMN is also visible with a dark, multi-lobed nucleus. Macrophages are large cells with a defined nucleus. (credit left: modification of work by Armed Forces Institute of Pathology; credit right: modification of work by Centers for Disease Control and Prevention)

Table 11.6.1: Macrophages Found in Various Body Tissues

Tissue	Macrophage
Brain and central nervous system	Microglial cells
Liver	Kupffer cells
Lungs	Alveolar macrophages (dust cells)
Peritoneal cavity	Peritoneal macrophages

Exercise 11.6.3

1. Describe the signals that activate natural killer cells.
2. What is the difference between monocytes and macrophages?

Summary

The innate immune system relies on white blood cells (leukocytes) derived from hematopoietic stem cells in the bone marrow, which differentiate via hematopoiesis into granulocytes, monocytes, macrophages, dendritic cells, mast cells, and natural killer (NK) cells. Granulocytes—including neutrophils, eosinophils, and basophils—are defined by their granules and lobed nuclei. Neutrophils are highly phagocytic, release toxic enzymes, and form neutrophil extracellular traps (NETs) to capture pathogens. Eosinophils target parasites and contribute to allergic responses. Basophils and mast cells release histamine and other mediators during inflammation and allergy, though mast cells reside in tissues. Basophils also release histamine during inflammation and are activated by complement proteins. Monocytes circulate in blood and differentiate into macrophages and dendritic cells, both highly phagocytic; dendritic cells also act as antigen-presenting sentinels in skin and mucosa. Natural killer (NK) cells are lymphocytes that identify infected or cancerous cells by detecting reduced MHC expression and increased activating molecules, then kill the target using perforin and granzymes to induce apoptosis. Together, these cellular defenses act quickly and nonspecifically to control infection and link to adaptive immunity.

Key Terms

apoptosis
programmed cell death triggered by NK cells to eliminate abnormal cells

basophils
granulocytes that promote inflammation and allergic responses via histamine release

bone marrow
site where hematopoietic stem cells reside and form all blood cells

dendritic cells
tissue-resident phagocytes derived from monocytes that present antigens

eosiphonils
granulocytes effective against parasites and involved in allergy

granulocytes
leukocytes characterized by visible granules and lobed nuclei (neutrophils, eosinophils, basophils)

hematopoiesis
process by which hematopoietic stem cells differentiate into all blood formed elements

hematopoietic stem cells
pluripotent bone marrow cells that give rise to blood cells

histamine
molecule released by basophils and mast cells that promotes inflammation

leukocytes
white blood cells involved in immune defense

macrophages
large phagocytic cells derived from monocytes that engulf pathogens

mast cells
tissue-resident cells similar to basophils that release histamine

monocytes
circulating cells that differentiate into macrophages and dendritic cells

natural killer (NK) cells
innate lymphocytes that kill infected or cancerous cells by sensing absent MHC I and inducing apoptosis

neutrophils
most abundant granulocytes; highly phagocytic, form NETs, and release destructive granules

neutrophil extracellular traps (NETs)
DNA-protein webs released by neutrophils to trap pathogens

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11.7: Pathogen Recognition and Phagocytosis

Learning Objectives

- Describe how leukocytes migrate from peripheral blood into infected tissues
- Explain the mechanisms by which leukocytes recognize pathogens
- Outline the process of phagocytosis and the mechanisms by which phagocytes destroy and degrade pathogens
- Discuss the antigen-presenting function of macrophages and dendritic cells and their connection to adaptive immunity

Several of the cell types discussed in the previous section can be described as phagocytes—cells whose main function is to seek, ingest, and kill pathogens. This process, called phagocytosis, was first observed in starfish in the 1880s by Nobel Prize-winning zoologist Ilya Metchnikoff (1845–1916), who made the connection to white blood cells (WBCs) in humans and other animals. At the time, Pasteur and other scientists believed that WBCs were spreading pathogens rather than killing them (which is true for some diseases, such as tuberculosis). But in most cases, phagocytes provide a strong, swift, and effective defense against a broad range of microbes, making them a critical component of innate nonspecific immunity. This section will focus on the mechanisms by which phagocytes are able to seek, recognize, and destroy pathogens.

Extravasation (Diapedesis) of Leukocytes

Some phagocytes are leukocytes (WBCs) that normally circulate in the bloodstream. To reach pathogens located in infected tissue, leukocytes must pass through the walls of small capillary blood vessels within tissues. This process, called extravasation, or diapedesis, is initiated by complement factor C5a, as well as cytokines released into the immediate vicinity by resident macrophages and tissue cells responding to the presence of the infectious agent **(Figure** 11.7.1**)**. Similar to C5a, many of these cytokines are proinflammatory and chemotactic, and they bind to cells of small capillary blood vessels, initiating a response in the endothelial cells lining the inside of the blood vessel walls. This response involves the upregulation and expression of various cellular adhesion molecules and receptors. Leukocytes passing through will stick slightly to the adhesion molecules, slowing down and rolling along the blood vessel walls near the infected area. When they reach a cellular junction, they will bind to even more of these adhesion molecules, flattening out and squeezing through the cellular junction in a process known as transendothelial migration. This mechanism of “rolling adhesion” allows leukocytes to exit the bloodstream and enter the infected areas, where they can begin phagocytosing the invading pathogens.

Note that extravasation does not occur in arteries or veins. These blood vessels are surrounded by thicker, multilayer protective walls, in contrast to the thin single-cell-layer walls of capillaries. Furthermore, the blood flow in arteries is too turbulent to allow for rolling adhesion. Also, some leukocytes tend to respond to an infection more quickly than others. The first to arrive typically are neutrophils, often within hours of a bacterial infection. By contract, monocytes may take several days to leave the bloodstream and differentiate into macrophages.

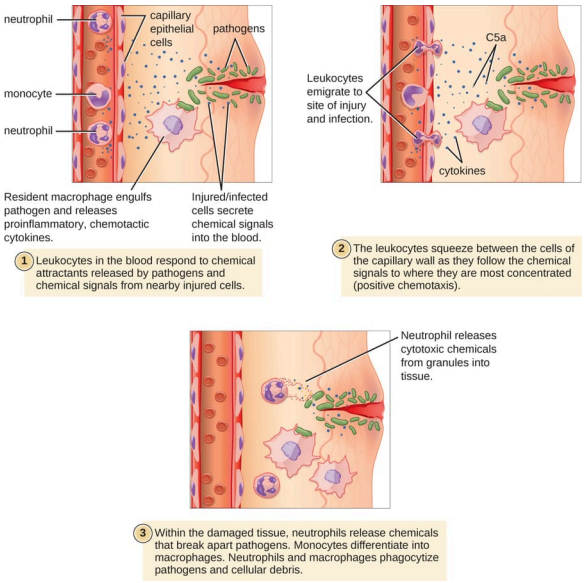


Figure 11.7.1: Leukocytes in the blood first respond to chemical attractants released by pathogens and chemical signals from nearby injured cells. An injury to the surface of the skin is labeled: injured/infected cells secrete chemical signals into the blood. Pathogens are present in the wound. Neutrophils and monocytes are in the blood stream; and the outside of the vessel is labeled capillary epithelial cells. A resident macrophage engulfs the pathogens and releases proinflammatory chemotactic cytokines. Then, the leukocytes squeeze between the cells of the capillary wall as they follow the chemical signals to where they are most concentrated (positive chemotaxis). The leukocytes emigrate to the site of injury and infection. The chemical signals present include C5a and cytokines. Finally, within the damaged tissue, neutrophils release chemicals that break apart pathogens. Monocytes differentiate into macrophages. Neutrophils and macrophages phagocytize pathogens and cellular debris. Neutrophils release cytotoxic chemicals from granules into tissue.

Link to Learning

Watch the following videos on leukocyte extravasation and leukocyte rolling to learn more.

Exercise 11.7.1

Explain the role of adhesion molecules in the process of extravasation.

Pathogen Recognition

As described in the previous section, opsonization of pathogens by antibody; complement factors C1q, C3b, and C4b; and lectins can assist phagocytic cells in recognition of pathogens and attachment to initiate phagocytosis. However, not all pathogen recognition is opsonin dependent. Phagocytes can also recognize molecular structures that are common to many groups of pathogenic microbes. Such structures are called pathogen-associated molecular patterns (PAMPs). Common PAMPs include the following:

- peptidoglycan, found in bacterial cell walls;
- flagellin, a protein found in bacterial flagella;
- lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria;
- lipopeptides, molecules expressed by most bacteria; and
- nucleic acids such as viral DNA or RNA.

Like numerous other PAMPs, these substances are integral to the structure of broad classes of microbes.

The structures that allow phagocytic cells to detect PAMPs are called pattern recognition receptors (PRRs). One group of PRRs is the toll-like receptors (TLRs), which bind to various PAMPs and communicate with the nucleus of the phagocyte to elicit a response. Many TLRs (and other PRRs) are located on the surface of a phagocyte, but some can also be found embedded in the membranes of interior compartments and organelles **(Figure** 11.7.2**)**. These interior PRRs can be useful for the binding and

recognition of intracellular pathogens that may have gained access to the inside of the cell before phagocytosis could take place. Viral nucleic acids, for example, might encounter an interior PRR, triggering production of the antiviral cytokine interferon.

In addition to providing the first step of pathogen recognition, the interaction between PAMPs and PRRs on macrophages provides an intracellular signal that activates the phagocyte, causing it to transition from a dormant state of readiness and slow proliferation to a state of hyperactivity, proliferation, production/secretion of cytokines, and enhanced intracellular killing. PRRs on macrophages also respond to chemical distress signals from damaged or stressed cells. This allows macrophages to extend their responses beyond protection from infectious diseases to a broader role in the inflammatory response initiated from injuries or other diseases.

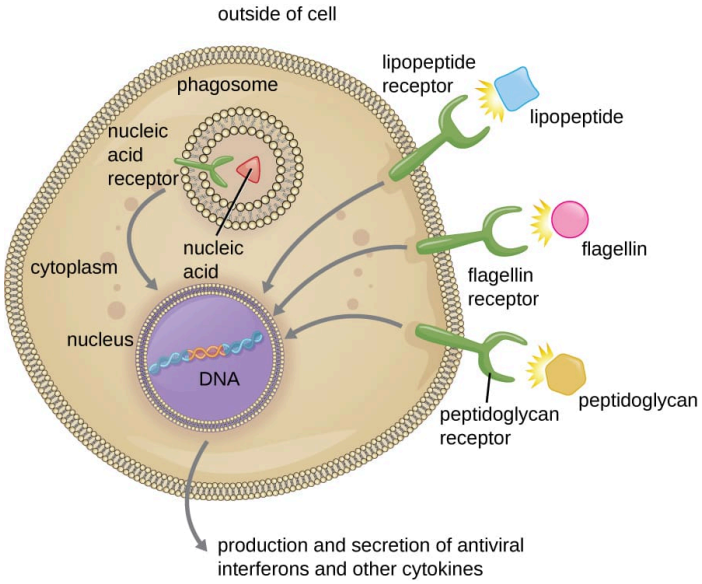


Figure 11.7.2: Phagocytic cells contain pattern recognition receptors (PRRs) capable of recognizing various pathogen-associated molecular patterns (PAMPs), such as:lipopeptide receptors, flagelin receptors, and peptidoglycan receptors. These PRRs can be found on the plasma membrane or in internal phagosomes. When a PRR recognizes a PAMP, it sends a signal to the nucleus that activates genes involved in phagocytosis, cellular proliferation, production and secretion of antiviral interferons and proinflammatory cytokines, and enhanced intracellular killing.

Exercise 11.7.2

1. Name four pathogen-associated molecular patterns (PAMPs).
2. Describe the process of phagocyte activation.

Pathogen Degradation

Once pathogen recognition and attachment occurs, the pathogen is engulfed in a vesicle and brought into the internal compartment of the phagocyte in a process called phagocytosis **(Figure** 11.7.3**)**. PRRs can aid in phagocytosis by first binding to the pathogen's surface, but phagocytes are also capable of engulfing nearby items even if they are not bound to specific receptors. To engulf the pathogen, the phagocyte forms a pseudopod that wraps around the pathogen and then pinches it off into a membrane vesicle called a phagosome. Acidification of the phagosome (pH decreases to the range of 4–5) provides an important early antibacterial mechanism. The phagosome containing the pathogen fuses with one or more lysosomes, forming a phagolysosome. Formation of the phagolysosome enhances the acidification, which is essential for activation of pH-dependent digestive lysosomal enzymes and production of hydrogen peroxide and toxic reactive oxygen species. Lysosomal enzymes such as lysozyme, phospholipase, and proteases digest the pathogen. Other enzymes are involved a respiratory burst. During the respiratory burst, phagocytes will increase their uptake and consumption of oxygen, but not for energy production. The increased oxygen consumption is focused on the production of superoxide anion, hydrogen peroxide, hydroxyl radicals, and other reactive oxygen species that are antibacterial.

In addition to the reactive oxygen species produced by the respiratory burst, reactive nitrogen compounds with cytotoxic (cell-killing) potential can also form. For example, nitric oxide can react with superoxide to form peroxynitrite, a highly reactive nitrogen

compound with degrading capabilities similar to those of the reactive oxygen species. Some phagocytes even contain an internal storehouse of microbicidal defensin proteins (e.g., neutrophil granules). These destructive forces can be released into the area around the cell to degrade microbes externally. Neutrophils, especially, can be quite efficient at this secondary antimicrobial mechanism.

Once degradation is complete, leftover waste products are excreted from the cell in an exocytic vesicle. However, it is important to note that not all remains of the pathogen are excreted as waste. Macrophages and dendritic cells are also antigen-presenting cells involved in the specific adaptive immune response. These cells further process the remains of the degraded pathogen and present key antigens (specific pathogen proteins) on their cellular surface. This is an important step for stimulation of some adaptive immune responses, as will be discussed in more detail in the next chapter.

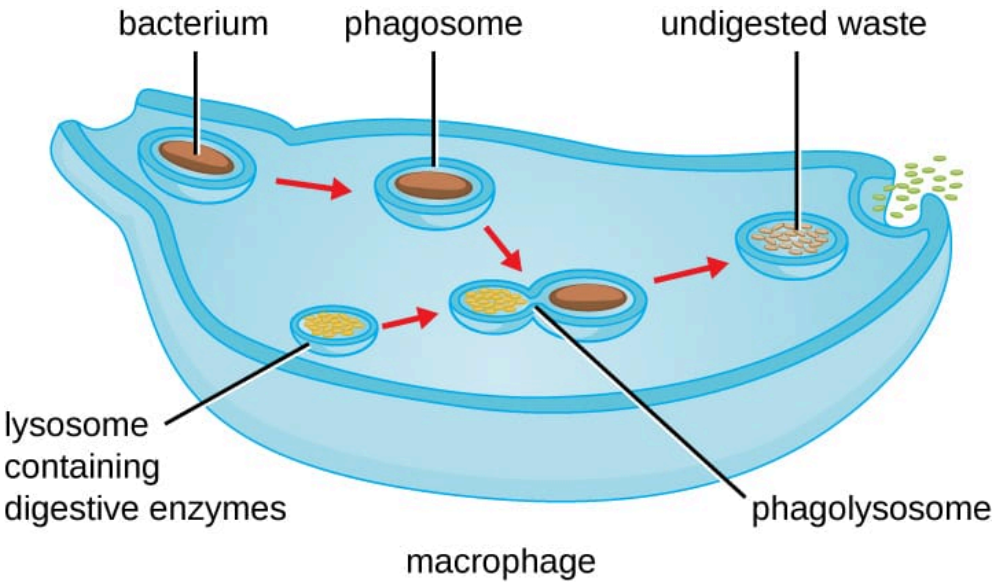


Figure 11.7.3: The stages of phagocytosis include the engulfment of a pathogen, the formation of a vesicle called a phagosome. This fuses with a lysosome which contains digestive enzymes. The resulting vesicle is labeled phagolysosome. Exocytosis removes the remaining debris.

Link to Learning

Watch a phagocyte chasing and engulfing a pathogen.

Exercise 11.7.3

What is the difference between a phagosome and a lysosome?

When Phagocytosis Fails

Although phagocytosis successfully destroys many pathogens, some are able to survive and even exploit this defense mechanism to multiply in the body and cause widespread infection. Protozoans of the genus *Leishmania* are one example. These obligate intracellular parasites are flagellates transmitted to humans by the bite of a sand fly. Infections cause serious and sometimes disfiguring sores and ulcers in the skin and other tissues **(Figure** 11.7.4**)**. Worldwide, an estimated 1.3 million people are newly infected with leishmaniasis annually.

Salivary peptides from the sand fly activate host macrophages at the site of their bite. The classic or alternate pathway for complement activation ensues with C3b opsonization of the parasite. *Leishmania* cells are phagocytosed, lose their flagella, and multiply in a form known as an amastigote (Leishman-Donovan body) within the phagolysosome. Although many other pathogens are destroyed in the phagolysosome, survival of the *Leishmania* amastigotes is maintained by the presence of surface lipophosphoglycan and acid phosphatase. These substances inhibit the macrophage respiratory burst and lysosomal

enzymes. The parasite then multiplies inside the cell and lyses the infected macrophage, releasing the amastigotes to infect other macrophages within the same host. Should another sand fly bite an infected person, it might ingest amastigotes and then transmit them to another individual through another bite.

There are several different forms of leishmaniasis. The most common is a localized cutaneous form of the illness caused by *L. tropica*, which typically resolves spontaneously over time but with some significant lymphocyte infiltration and permanent scarring. A mucocutaneous form of the disease, caused by *L. viannia brasilienfsis*, produces lesions in the tissue of the nose and mouth and can be life threatening. A visceral form of the illness can be caused by several of the different *Leishmania* species. It affects various organ systems and causes abnormal enlargement of the liver and spleen. Irregular fevers, anemia, liver dysfunction, and weight loss are all signs and symptoms of visceral leishmaniasis. If left untreated, it is typically fatal.

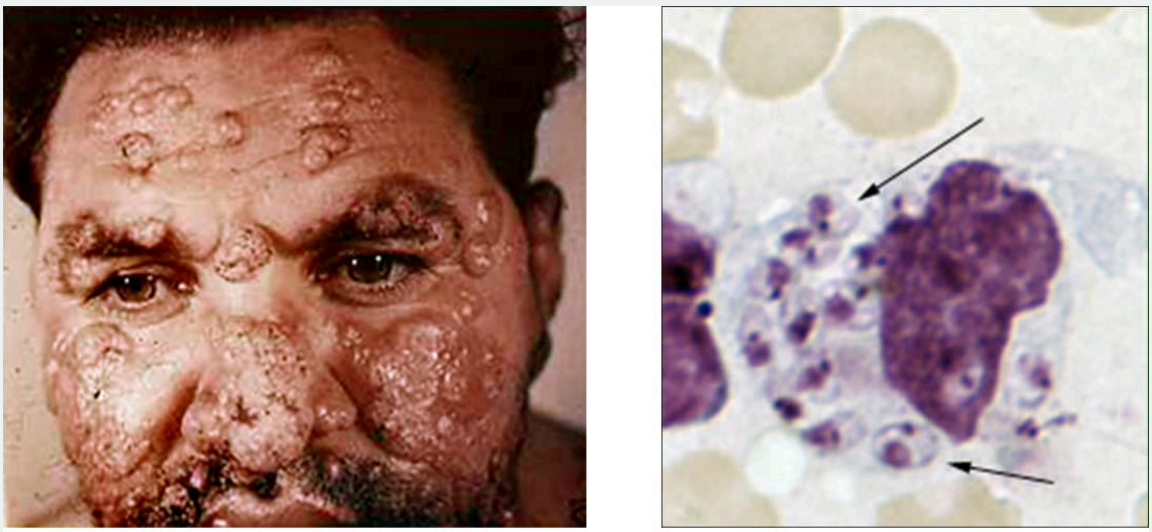


Figure 11.7.4: (a) Cutaneous leishmaniasis is a disfiguring disease caused by the intracellular flagellate *Leishmania tropica*, transmitted by the bite of a sand fly. (b) This light micrograph of a sample taken from a skin lesion shows a large cell, which is a macrophage infected with *L. tropica* amastigotes (arrows). The amastigotes have lost their flagella but their nuclei are visible. Soon the amastigotes will lyse the macrophage and be engulfed by other phagocytes, spreading the infection. (credit a: modification of work by Otis Historical Archives of "National Museum of Health & Medicine"; credit b: modification of work by Centers for Disease Control and Prevention)

Summary

Phagocytosis is the process by which certain phagocytes—such as neutrophils, monocytes, macrophages, and dendritic cells—seek, ingest, and destroy pathogens. To reach infection sites, circulating leukocytes undergo extravasation (diapedesis) by exiting capillaries through rolling adhesion and transendothelial migration, guided by cytokines and complement. Once at the infection site, phagocytes recognize pathogens using pattern recognition receptors (PRRs) that bind to pathogen-associated molecular patterns (PAMPs)—molecules commonly found in microbes, such as peptidoglycan, LPS, flagellin, lipopeptides, and viral nucleic acids. One major group of PRRs is the toll-like receptors (TLRs), which trigger activation of the phagocyte, leading to increased killing ability and cytokine secretion. Phagocytes engulf pathogens into phagosomes, which fuse with lysosomes to form phagolysosomes. Inside, lysosomal enzymes and acidification break down the pathogen, aided by reactive oxygen species (from a respiratory burst) and reactive nitrogen compounds like nitric oxide and peroxynitrite. Some phagocytes, especially neutrophils, also use defensins to kill microbes extracellularly. After degradation, waste is expelled, but macrophages and dendritic cells retain and present pathogen antigens, linking innate to adaptive immunity.

Key Terms

antigen
: a molecule or protein fragment from a pathogen presented by immune cells to activate adaptive immunity

cytokines
: signaling proteins released by cells, especially immune cells, to mediate and regulate immunity and inflammation

defensins
antimicrobial proteins stored in granules of some phagocytes, particularly neutrophils, that can destroy pathogens externally

diapedesis (extravasation)
the movement of leukocytes from the bloodstream into infected tissues by squeezing through capillary walls

flagellin
a protein component of bacterial flagella recognized as a PAMP by the immune system

lippeptides
bacterial surface molecules recognized as PAMPs by phagocytes

lipopolysaccaride (LPS)
a component of the outer membrane of gram-negative bacteria, commonly recognized as a PAMP

lysosome
an organelle containing digestive enzymes that fuse with phagosomes to form phagolysosomes

macrophage
a phagocytic cell derived from monocytes that engulfs and digests pathogens and presents antigens

monocyte
a circulating leukocyte that can migrate into tissues and differentiate into macrophages or dendritic ce

nitric oxide
a reactive nitrogen compound produced by phagocytes that contributes to the destruction of pathogens

pathogen-associated molecular patterns (PAMPs)
molecular structures found on many microbes that are recognized by PRRs

pattern recognition receptors (PRRs)
receptors on or inside phagocytes that detect PAMPs and trigger immune activation

peptidoglycan
a component of bacterial cell walls recognized as a PAMP

phagocyte
a cell capable of engulfing and destroying pathogens through phagocytosis

phagocytosis
the process by which phagocytes ingest and destroy pathogens

phagolysosome
a fusion of a phagosome and a lysosome where pathogen degradation occurs

phagosome
a membrane-bound vesicle formed around a pathogen during phagocytosis

reactive nitrogen compound
cytotoxic molecules (e.g., nitric oxide, peroxynitrite) used by phagocytes to destroy pathogens

reactive oxygen species (ROS)
toxic oxygen-derived molecules (e.g., hydrogen peroxide, superoxide) produced during respiratory bursts to kill pathogens

respiratory burst
a rapid increase in oxygen uptake by phagocytes to generate reactive oxygen

species for killing microbes

toll-like receptors (TLRs)
: a type of PRR that recognizes PAMPs and activates immune cell responses

transendothelial migration
: the process of leukocytes squeezing between capillary endothelial cells to reach infection sites

Footnotes

1. 1 World Health Organization. “Leishmaniasis.” 2016. http://www.who.int/mediacentre/factsheets/fs375/en/. [www.who.int].

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11.8: Adaptive Immunity Overview

Learning Objectives

- Define memory, primary response, secondary response, and specificity
- Distinguish between humoral and cellular immunity
- Differentiate between antigens, epitopes, and haptens
- Describe the structure and function of antibodies and distinguish between the different classes of antibodies

Adaptive Immunity

Adaptive immunity is defined by two important characteristics: specificity and memory. Specificity refers to the adaptive immune system's ability to target specific pathogens, and memory refers to its ability to quickly respond to pathogens to which it has previously been exposed. For example, when an individual recovers from chickenpox, the body develops a *memory* of the infection that will *specifically* protect it from the causative agent, the varicella-zoster virus, if it is exposed to the virus again later.

Specificity and memory are achieved by essentially programming certain cells involved in the immune response to respond rapidly to subsequent exposures of the pathogen. This programming occurs as a result of the first exposure to a pathogen or vaccine, which triggers a primary response. Subsequent exposures result in a secondary response that is faster and stronger as a result of the body's memory of the first exposure **(Figure** 11.8.1**)**. This secondary response, however, is specific to the pathogen in question. For example, exposure to one virus (e.g., varicella-zoster virus) will not provide protection against other viral diseases (e.g., measles, mumps, or polio).

Adaptive specific immunity involves the actions of two distinct cell types: B lymphocytes (B cells) and T lymphocytes (T cells). Although B cells and T cells arise from a common hematopoietic stem cell differentiation pathway, their sites of maturation and their roles in adaptive immunity are very different.

B cells mature in the bone marrow and are responsible for the production of glycoproteins called antibodies, or immunoglobulins. Antibodies are involved in the body's defense against pathogens and toxins in the extracellular environment. Mechanisms of adaptive specific immunity that involve B cells and antibody production are referred to as humoral immunity. The maturation of T cells occurs in the thymus. T cells function as the central orchestrator of both innate and adaptive immune responses. They are also responsible for destruction of cells infected with intracellular pathogens. The targeting and destruction of intracellular pathogens by T cells is called cell-mediated immunity, or cellular immunity.

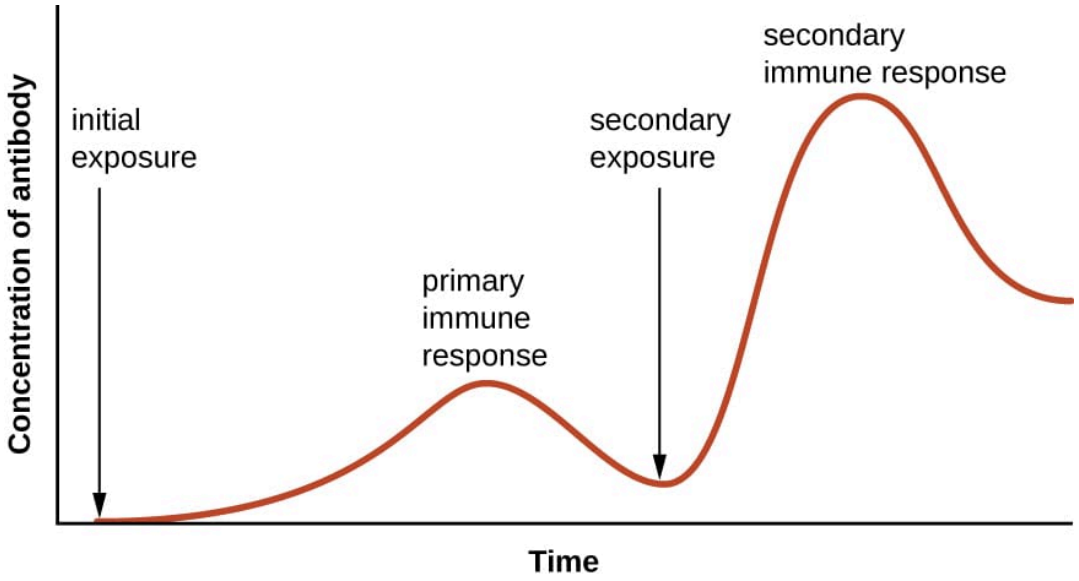


Figure 11.8.1: This graph illustrates the primary and secondary immune responses related to antibody production after an initial and secondary exposure to an antigen. A graph with time on the X axis and concentration of antibody on the Y axis. The concentration is near 0 at the initial exposure and increases during the primary immune response. The concentration then drop back down but remains above the level at initial exposure. The secondary exposure increases the concentration of antibody to higher levels than the primary response, even after dropping back down this count remains relatively high. The secondary response is faster.

Exercise 11.8.2

1. List the two defining characteristics of adaptive immunity.
2. Explain the difference between a primary and secondary immune response.
3. How do humoral and cellular immunity differ?

Antigens

Activation of the adaptive immune defenses is triggered by pathogen-specific molecular structures called antigens. Antigens are similar to the pathogen-associated molecular patterns (PAMPs) discussed in Pathogen Recognition and Phagocytosis; however, whereas PAMPs are molecular structures found on numerous pathogens, antigens are unique to a specific pathogen. The antigens that stimulate adaptive immunity to chickenpox, for example, are unique to the varicella-zoster virus but significantly different from the antigens associated with other viral pathogens.

The term *antigen* was initially used to describe molecules that stimulate the production of antibodies; in fact, the term comes from a combination of the words antibody and generator, and a molecule that stimulates antibody production is said to be antigenic. However, the role of antigens is not limited to humoral immunity and the production of antibodies; antigens also play an essential role in stimulating cellular immunity, and for this reason antigens are sometimes more accurately referred to as immunogens. In this text, however, we will typically refer to them as antigens.

Pathogens possess a variety of structures that may contain antigens. For example, antigens from bacterial cells may be associated with their capsules, cell walls, fimbriae, flagella, or pili. Bacterial antigens may also be associated with extracellular toxins and enzymes that they secrete. Viruses possess a variety of antigens associated with their capsids, envelopes, and the spike structures they use for attachment to cells.

Antigens may belong to any number of molecular classes, including carbohydrates, lipids, nucleic acids, proteins, and combinations of these molecules. Antigens of different classes vary in their ability to stimulate adaptive immune defenses as well as in the type of response they stimulate (humoral or cellular). The structural complexity of an antigenic molecule is an important factor in its antigenic potential. In general, more complex molecules are more effective as antigens. For example, the three-dimensional complex structure of proteins make them the most effective and potent antigens, capable of stimulating both humoral and cellular immunity. In comparison, carbohydrates are less complex in structure and therefore less effective as

antigens; they can only stimulate humoral immune defenses. Lipids and nucleic acids are the least antigenic molecules, and in some cases may only become antigenic when combined with proteins or carbohydrates to form glycolipids, lipoproteins, or nucleoproteins.

One reason the three-dimensional complexity of antigens is so important is that antibodies and T cells do not recognize and interact with an entire antigen but with smaller exposed regions on the surface of antigens called epitopes. A single antigen may possess several different epitopes **(Figure** 11.8.2**)**, and different antibodies may bind to different epitopes on the same antigen **(Figure** 11.8.3**)**. For example, the bacterial flagellum is a large, complex protein structure that can possess hundreds or even thousands of epitopes with unique three-dimensional structures. Moreover, flagella from different bacterial species (or even strains of the same species) contain unique epitopes that can only be bound by specific antibodies.

An antigen's size is another important factor in its antigenic potential. Whereas large antigenic structures like flagella possess multiple epitopes, some molecules are too small to be antigenic by themselves. Such molecules, called haptens, are essentially free epitopes that are not part of the complex three-dimensional structure of a larger antigen. For a hapten to become antigenic, it must first attach to a larger carrier molecule (usually a protein) to produce a conjugate antigen. The hapten-specific antibodies produced in response to the conjugate antigen are then able to interact with unconjugated free hapten molecules. Haptens are not known to be associated with any specific pathogens, but they are responsible for some allergic responses. For example, the hapten urushiol, a molecule found in the oil of plants that cause poison ivy, causes an immune response that can result in a severe rash (called contact dermatitis). Similarly, the hapten penicillin can cause allergic reactions to drugs in the penicillin class.

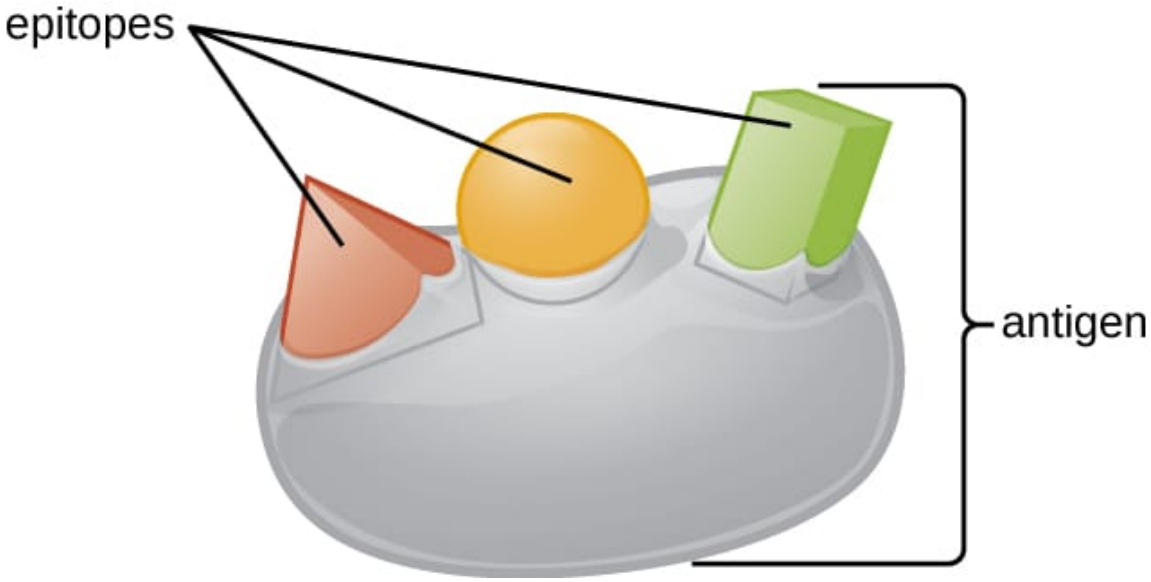


Figure 11.8.2: An antigen is a macromolecule that reacts with components of the immune system. A given antigen may contain several motifs that are recognized by immune cells.

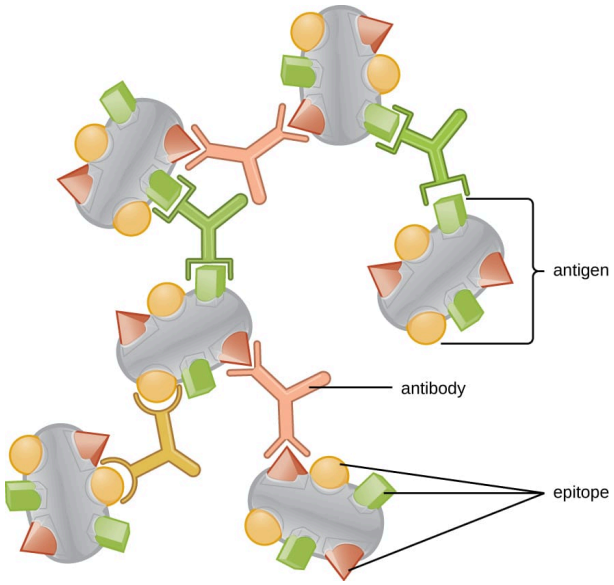


Figure 11.8.3: A typical protein antigen (shown as large spheres) has multiple epitopes, shown by the ability of three different antibodies to bind to different epitopes of the same antigen.

Exercise 11.8.3

1. What is the difference between an antigen and an epitope?
2. What factors affect an antigen's antigenic potential?
3. Why are haptens typically not antigenic, and how do they become antigenic?

Antibodies

Antibodies (also called immunoglobulins) are glycoproteins that are present in both the blood and tissue fluids. The basic structure of an antibody monomer consists of four protein chains held together by disulfide bonds **(Figure** 11.8.4**)**. A disulfide bond is a covalent bond between the sulfhydryl *R* groups found on two cysteine amino acids. The two largest chains are identical to each other and are called the heavy chains. The two smaller chains are also identical to each other and are called the light chains. Joined together, the heavy and light chains form a basic Y-shaped structure.

The two 'arms' of the Y-shaped antibody molecule are known as the Fab region, for "fragment of antigen binding." The far end of the Fab region is the variable region, which serves as the site of antigen binding. The amino acid sequence in the variable region dictates the three-dimensional structure, and thus the specific three-dimensional epitope to which the Fab region is capable of binding. Although the epitope specificity of the Fab regions is identical for each arm of a single antibody molecule, this region displays a high degree of variability between antibodies with different epitope specificities. Binding to the Fab region is necessary for neutralization of pathogens, agglutination or aggregation of pathogens, and antibody-dependent cell-mediated cytotoxicity.

The constant region of the antibody molecule includes the trunk of the Y and lower portion of each arm of the Y. The trunk of the Y is also called the Fc region, for "fragment of crystallization," and is the site of complement factor binding and binding to phagocytic cells during antibody-mediated opsonization.

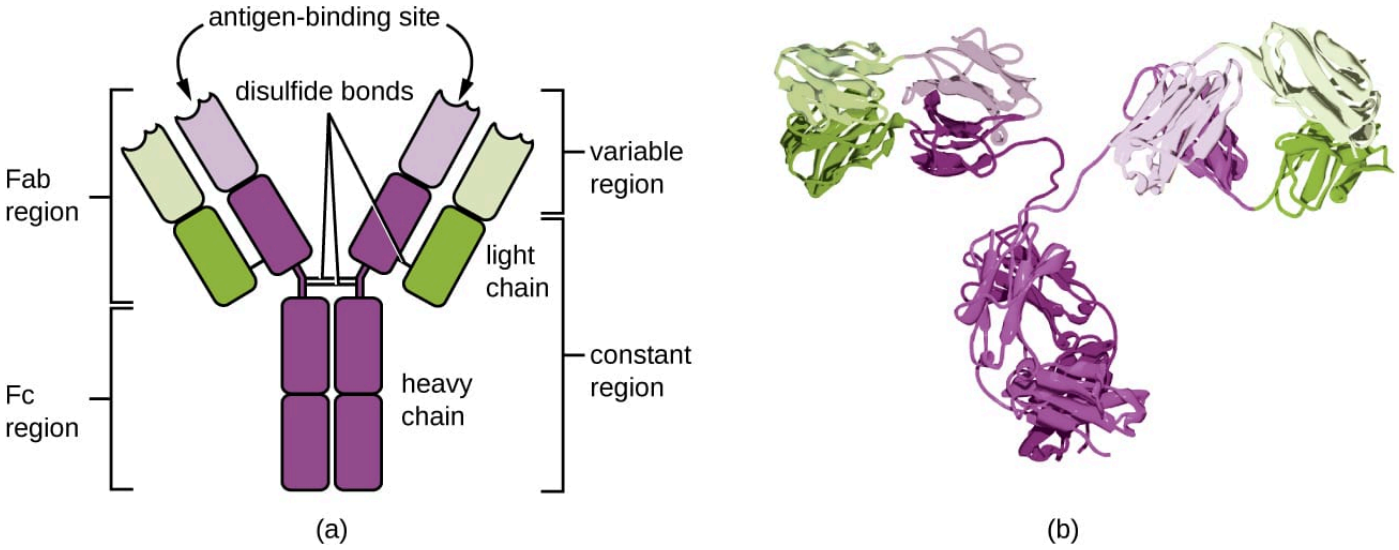


Figure 11.8.4: (a) An antibody is a Y shape made of four strands. The two inner strands form the actual Y shape and are the heavy chain two light chains sit on the outsides of the top regions of the Y. The bottom portion of the Y (made of only heavy chains) is called the Region. The Fc region along with half of the top portion of the Y (made of both light and heavy chains) is the constant regions. The var region is the very tips of the Y and is made of both light and heavy chains. The antigen binding site is in the variable region. Disulfide b hold the antigen's shape. **(b)** The corresponding three-dimensional structure of the antibody IgG. (credit b: modification of work by ˜ Vickers)

Exercise 11.8.4

Describe the different functions of the Fab region and the Fc region.

Antibody Classes

The constant region of an antibody molecule determines its class, or isotype. The five classes of antibodies are IgG, IgM, IgA, IgD, and IgE. Each class possesses unique heavy chains designated by Greek letters γ, μ, α, δ, and ε, respectively. Antibody classes also exhibit important differences in abundance in serum, arrangement, body sites of action, functional roles, and size **(Figure 11.8.5)**.

IgG is a monomer that is by far the most abundant antibody in human blood, accounting for about 80% of total serum antibody. IgG penetrates efficiently into tissue spaces, and is the only antibody class with the ability to cross the placental barrier, providing passive immunity to the developing fetus during pregnancy. IgG is also the most versatile antibody class in terms of its role in the body's defense against pathogens.

IgM is initially produced in a monomeric membrane-bound form that serves as an antigen-binding receptor on B cells. The secreted form of IgM assembles into a pentamer with five monomers of IgM bound together by a protein structure called the J chain. Although the location of the J chain relative to the Fc regions of the five monomers prevents IgM from performing some of the functions of IgG, the ten available Fab sites associated with a pentameric IgM make it an important antibody in the body's arsenal of defenses. IgM is the first antibody produced and secreted by B cells during the primary and secondary immune responses, making pathogen-specific IgM a valuable diagnostic marker during active or recent infections.

IgA accounts for about 13% of total serum antibody, and secretory IgA is the most common and abundant antibody class found in the mucus secretions that protect the mucous membranes. IgA can also be found in other secretions such as breast milk, tears, and saliva. Secretory IgA is assembled into a dimeric form with two monomers joined by a protein structure called the secretory component. One of the important functions of secretory IgA is to trap pathogens in mucus so that they can later be eliminated from the body.

Similar to IgM, IgD is a membrane-bound monomer found on the surface of B cells, where it serves as an antigen-binding receptor. However, IgD is not secreted by B cells, and only trace amounts are detected in serum. These trace amounts most likely

come from the degradation of old B cells and the release of IgD molecules from their cytoplasmic membranes.

IgE is the least abundant antibody class in serum. Like IgG, it is secreted as a monomer, but its role in adaptive immunity is restricted to anti-parasitic defenses. The Fc region of IgE binds to basophils and mast cells. The Fab region of the bound IgE then interacts with specific antigen epitopes, causing the cells to release potent pro-inflammatory mediators. The inflammatory reaction resulting from the activation of mast cells and basophils aids in the defense against parasites, but this reaction is also central to allergic reactions.

The Five Immunoglobulin (Ig) Classes					
Properties	**IgG monomer**	**IgM pentamer**	**Secretory IgA dimer**	**IgD monomer**	**IgE monomer**
Structure			 Secretory component		
Heavy chains	γ	μ	α	δ	ε
Number of antigen-binding sites	2	10	4	2	2
Molecular weight (Daltons)	150,000	900,000	385,000	180,000	200,000
Percentage of total antibody in serum	80%	6%	13% (monomer)	<1%	<1%
Crosses placenta	yes	no	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to	phagocytes				mast cells and basophils
Function	Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cytotoxicity.	Neutralization, agglutination, and complement activation. The monomer form serves as the B-cell receptor.	Neutralization and trapping of pathogens in mucus.	B-cell receptor.	Activation of basophils and mast cells against parasites and allergens.

Figure 11.8.5: The five Immunoglobulin classes: IgG monomer is a Y shaped molecule; the heavy chain is gamma, it has 2 antigen binding sites and a molecular weight of 150,000 daltons. It makes up 80% of the total antibodies in the serum. It crosses the placenta and fixes compliments and the Fc binds to phagocytes. It's functions are: Neutralization, agglutination, complement activation, opsonization, and antibody-dependent cell-mediated cytotoxicity.

IgM pentamer is 5 Y's bound at their bases; the heavy chain is mu, it has 10 antigen binding sites and a molecular weight of 900,000 daltons. It makes up 6% of the total antibodies in the serum. It does not cross the placenta but does fix compliments and the Fc does not bind to a particular cell type. It's functions are: Neutralization, agglutination, and complement activation. The monomer form serves as the B cell receptor.

IgA dimer is 2 Y's bound at their bases; the heavy chain is alpha, it has 4 antigen binding sites and a molecular weight of 385,000 daltons. It makes up 13% of the total antibodies in the serum. It does not cross the placenta nor fix compliments and the Fc does not bind to a particular cell type. It's functions are: Neutralization and trapping of pathogens in mucus.

IgD monomer is 1 Y; the heavy chain is delta, it has 2 antigen binding sites and a molecular weight of 180,000 daltons. It makes up <1% of the total antibodies in the serum. It does not cross the placenta nor fix compliments and the Fc does not bind to a particular cell type. It's functions are: B cell receptors.

IgE monomer is 1 Y; the heavy chain is epsilon, it has 2 antigen binding sites and a molecular weight of 200,000 daltons. It makes up <1% of the total antibodies in the serum. It does not cross the placenta nor fix compliments. The Fc binds to mast cells and basophils. It's functions are: Activation of basophils and mast cells against parasites and allergens.

Exercise 11.8.5

1. What part of an antibody molecule determines its class?
2. What class of antibody is involved in protection against parasites?
3. Describe the difference in structure between IgM and IgG.

Antigen-Antibody Interactions

Different classes of antibody play important roles in the body's defense against pathogens. These functions include neutralization of pathogens, opsonization for phagocytosis, agglutination, complement activation, and antibody-dependent cell-mediated cytotoxicity. For most of these functions, antibodies also provide an important link between adaptive specific immunity and innate nonspecific immunity.

Neutralization involves the binding of certain antibodies (IgG, IgM, or IgA) to epitopes on the surface of pathogens or toxins, preventing their attachment to cells. For example, Secretory IgA can bind to specific pathogens and block initial attachment to intestinal mucosal cells. Similarly, specific antibodies can bind to certain toxins, blocking them from attaching to target cells and thus neutralizing their toxic effects. Viruses can be neutralized and prevented from infecting a cell by the same mechanism **(Figure** 11.8.6**)**.

As described in Chemical Defenses, opsonization is the coating of a pathogen with molecules, such as complementfactors, C-reactive protein, and serum amyloid A, to assist in phagocyte binding to facilitate phagocytosis. IgG antibodies also serve as excellent opsonins, binding their Fab sites to specific epitopes on the surface of pathogens. Phagocytic cells such as macrophages, dendritic cells, and neutrophils have receptors on their surfaces that recognize and bind to the Fc portion of the IgG molecules; thus, IgG helps such phagocytes attach to and engulf the pathogens they have bound **(Figure** 11.8.7**)**.

Agglutination or aggregation involves the cross-linking of pathogens by antibodies to create large aggregates **(Figure** 11.8.8**)**. IgG has two Fab antigen-binding sites, which can bind to two separate pathogen cells, clumping them together. When multiple IgG antibodies are involved, large aggregates can develop; these aggregates are easier for the kidneys and spleen to filter from the blood and easier for phagocytes to ingest for destruction. The pentameric structure of IgM provides ten Fab binding sites per molecule, making it the most efficient antibody for agglutination.

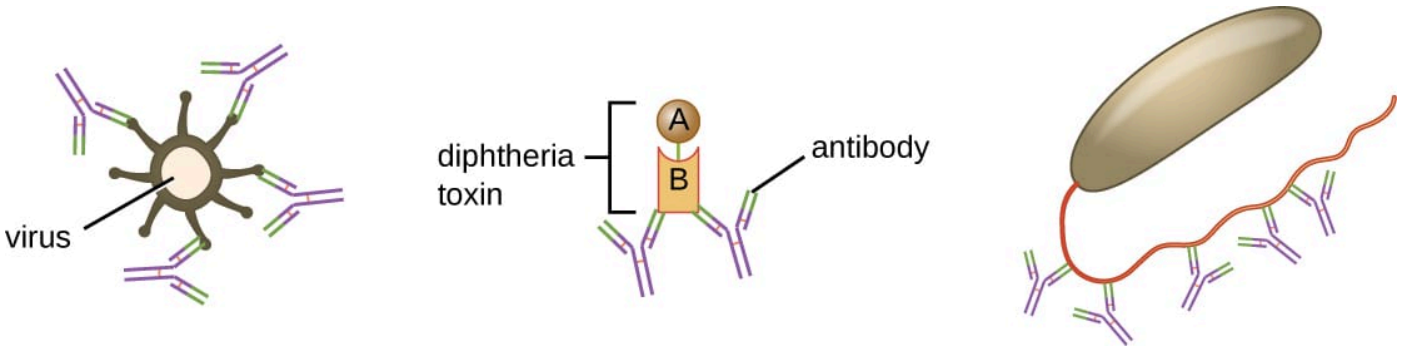


Figure 11.8.6: Neutralization involves the binding of specific antibodies to antigens found on bacteria, viruses, and toxins (like diptheria toxin shown above), preventing them from attaching to target cells.

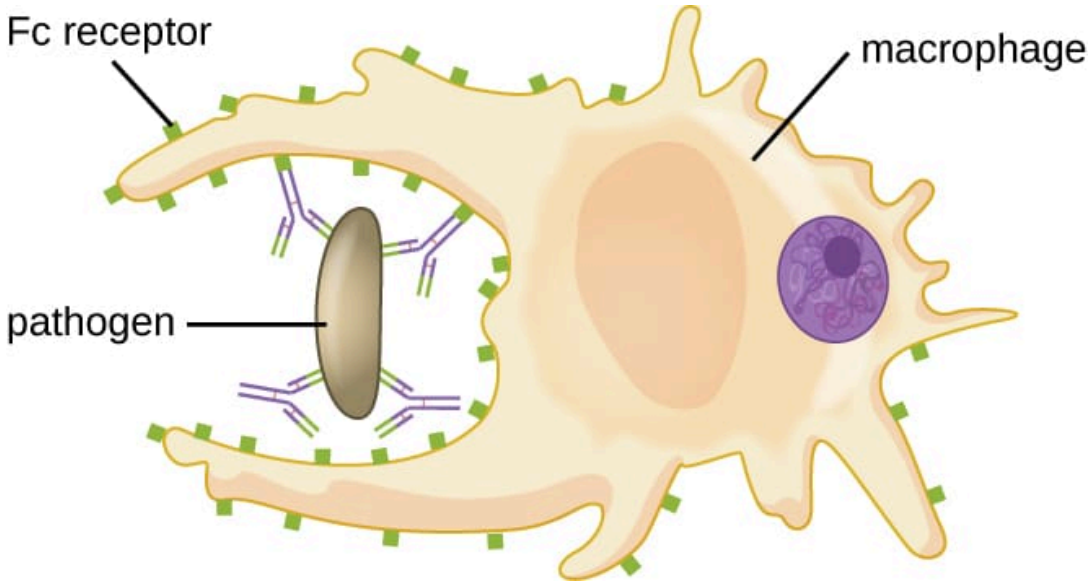


Figure 11.8.7: Antibodies serve as opsonins and inhibit infection by tagging pathogens for destruction by macrophages, dendritic cells, and neutrophils. These phagocytic cells use Fc receptors to bind to IgG-opsonized pathogens and initiate the first step of attachment before phagocytosis.

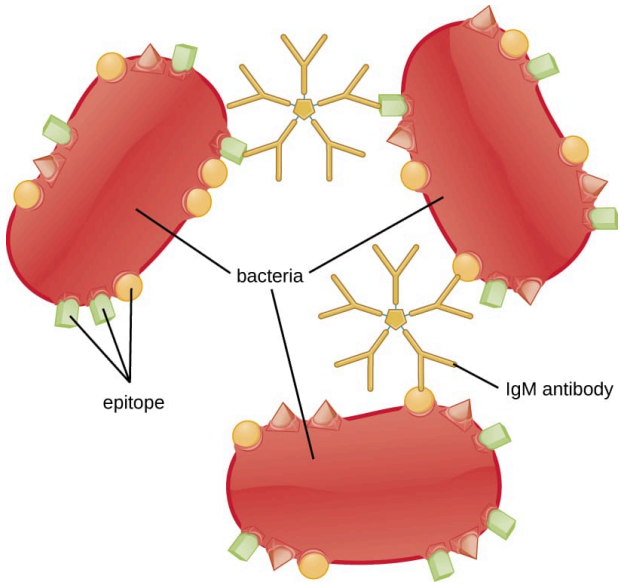


Figure 11.8.8: Antibodies, especially IgM antibodies, agglutinate bacteria by binding to epitopes on two or more bacteria simultaneously. Epitopes are shown as different shapes in the diagram above. When multiple pathogens and antibodies are present, aggregates form when the binding sites of antibodies bind with separate pathogens.

Another important function of antibodies is activation of the complement cascade. As discussed in the previous chapter, the complement system is an important component of the innate defenses, promoting the inflammatory response, recruiting phagocytes to site of infection, enhancing phagocytosis by opsonization, and killing gram-negative bacterial pathogens with the membrane attack complex (MAC). Complement activation can occur through three different pathways, but the most efficient is the classical pathway, which requires the initial binding of IgG or IgM antibodies to the surface of a pathogen cell, allowing for recruitment and activation of the C1 complex.

Yet another important function of antibodies is antibody-dependent cell-mediated cytotoxicity (ADCC), which enhances killing of pathogens that are too large to be phagocytosed. This process is best characterized for natural killer cells (NK cells), as shown in **Figure** 11.8.9, but it can also involve macrophages and eosinophils. ADCC occurs when the Fab region of an IgG antibody binds to a large pathogen; Fc receptors on effector cells (e.g., NK cells) then bind to the Fc region of the antibody, bringing them into close proximity with the target pathogen. The effector cell then secretes powerful cytotoxins (e.g., perforin and granzymes) that kill the pathogen.

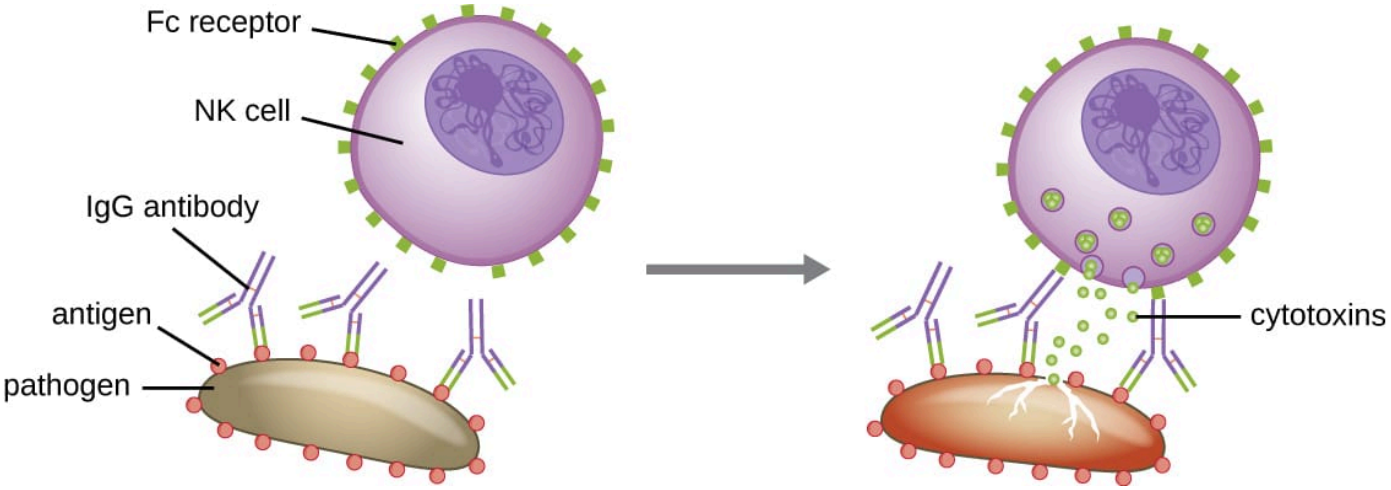


Figure 11.8.9: In this example of ADCC, antibodies bind to a large pathogenic cell that is too big for phagocytosis and then bind to Fc receptors on the membrane of a natural killer cell. This interaction brings the NK cell into close proximity, where it can kill the pathogen through release of lethal extracellular cytotoxins.

Exercise 11.8.6

1. Where is IgA normally found?
2. Which class of antibody crosses the placenta, providing protection to the fetus?
3. Compare the mechanisms of opsonization and antibody-dependent cell-mediated cytotoxicity.

Summary

Adaptive immunity is a highly specific and memory-driven arm of the immune system. It targets particular pathogens and responds more efficiently upon repeated exposure. The key components include B cells, which produce antibodies in humoral immunity, and T cells, which mediate cellular immunity. The adaptive response is triggered by antigens, unique pathogen-associated molecules recognized by immune cells. Antigens have distinct regions called epitopes, which bind to antibodies—glycoproteins with specific structural regions (Fab and Fc) that mediate a range of immune functions.

Antibodies exist in five classes—IgG, IgM, IgA, IgD, and IgE—each with distinct structures and roles. These antibodies neutralize pathogens, promote their phagocytosis via opsonization, clump them together in agglutination, activate the complement cascade, and trigger antibody-dependent cell-mediated cytotoxicity (ADCC). Some small molecules, called haptens, can become antigenic only when attached to larger proteins. The ability of antigens to stimulate the immune system depends on their size, complexity, and molecular class.

Key Terms

adaptive immunity
: a targeted immune response with memory, involving B cells and T cells

antibody-dependent cell-mediated cytotoxicity
: a process in which antibodies recruit immune cells (e.g., NK cells) to kill large

pathogens

agglutination
clumping of pathogens by antibodies to make them easier to eliminate

antibodies
Y-shaped glycoproteins secreted by B cells that bind to specific antigens

antigen
a molecule unique to a specific pathogen that stimulates an adaptive immune response

antigenic potential
the ability of a molecule to stimulate an immune response, influenced by size, complexity, and type

B cell
a lymphocyte that matures in the bone marrow and produces antibodies

cell-mediated immunity
immune response involving T cells that destroy infected or abnormal cells

complement activation
antibody-mediated recruitment of complement proteins to destroy pathogens

epitopes
specific regions on antigens that antibodies or T cells bind to

Fab region
the variable, antigen-binding part of an antibody molecule

Fc region
the constant part of an antibody that interacts with immune cell receptors and complement proteins

humoral immunity
antibody-mediated immune response involving B cells

hapten
a small molecule that becomes antigenic only when attached to a larger carrier protein

IgA
an antibody class found in mucous secretions and breast milk; functions in trapping pathogens

IgD
a membrane-bound antibody acting as a B cell receptor; not secreted in significant amounts

IgE
an antibody class involved in anti-parasitic defense and allergic responses

IgG
: the most abundant antibody in the blood, capable of crossing the placenta and performing many immune functions

IgM
: the first antibody produced during an immune response; forms a pentamer in its secreted form

immunogen
: a molecule that triggers an immune response; synonymous with antigen in most contexts

memory
: the ability of the adaptive immune system to respond more rapidly upon re-exposure to a specific pathogen

neutralization
: the binding of antibodies to pathogens or toxins, preventing their attachment to host cells

opsonization
: coating of pathogens with antibodies to enhance phagocytosis

primary response
: the initial immune response upon first exposure to an antigen

secondary response
: a faster, stronger immune response upon re-exposure to the same antigen

specificity
: the adaptive immune system's ability to target particular antigens

T cell
: a lymphocyte that matures in the thymus and mediates cellular immune responses

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11.9: Adaptive Immunity in Action

Learning Objectives

- Explain the roles and maturation pathways of B cells and T cells.
- Describe the processes of humoral and cell-mediated immune responses, including antibody production and T cell activation.
- Understand the concept of immunological memory and distinguish between primary and secondary immune responses.
- Identify the components and functions of the lymphatic and mucosal immune systems.
- Define immune tolerance and explain how the immune system avoids targeting self or harmless antigens

The adaptive, or acquired, immune response takes days or even weeks to become established—much longer than the innate response; however, adaptive immunity is more specific to an invading pathogen. Adaptive immunity is an immunity that occurs after exposure to an antigen either from a pathogen or a vaccination. An antigen is a molecule that stimulates a response in the immune system. This part of the immune system is activated when the innate immune response is insufficient to control an infection. In fact, without information from the innate immune system, the adaptive response could not be mobilized. There are two types of adaptive responses: the cell-mediated immune response, which is controlled by activated T cells, and the humoral immune response, which is controlled by activated B cells and antibodies. Activated T and B cells whose surface binding sites are specific to the molecules on the pathogen greatly increase in numbers and attack the invading pathogen. Their attack can kill pathogens directly or they can secrete antibodies that enhance the phagocytosis of pathogens and disrupt the infection. Adaptive immunity also involves a memory to give the host long-term protection from reinfection with the same type of pathogen; on reexposure, this host memory will facilitate a rapid and powerful response.

B and T Cells

Lymphocytes, which are white blood cells, are formed with other blood cells in the red bone marrow found in many flat bones, such as the shoulder or pelvic bones. The two types of lymphocytes of the adaptive immune response are B and T cells **(Figure** 11.9.1**)**. Whether an immature lymphocyte becomes a B cell or T cell depends on where in the body it matures. The B cells remain in the bone marrow to mature (hence the name “B” for “bone marrow”), while T cells migrate to the thymus, where they mature (hence the name “T” for “thymus”).

Maturation of a B or T cell involves becoming immunocompetent, meaning that it can recognize, by binding, a specific molecule or antigen (discussed below). During the maturation process, B and T cells that bind too strongly to the body’s own cells are eliminated in order to minimize an immune response against the body’s own tissues. Those cells that react weakly to the body’s own cells, but have highly specific receptors on their cell surfaces that allow them to recognize a foreign molecule, or antigen, remain. This process occurs during fetal development and continues throughout life. The specificity of this receptor is determined by the genetics of the individual and is present before a foreign molecule is introduced to the body or encountered. Thus, it is genetics and not experience that initially provides a vast array of cells, each capable of binding to a different specific foreign molecule. Once they are immunocompetent, the T and B cells will migrate to the spleen and lymph nodes where they will remain until they are called on during an infection. B cells are involved in the humoral immune response, which targets pathogens loose in blood and lymph, and T cells are involved in the cell-mediated immune response, which targets infected cells.

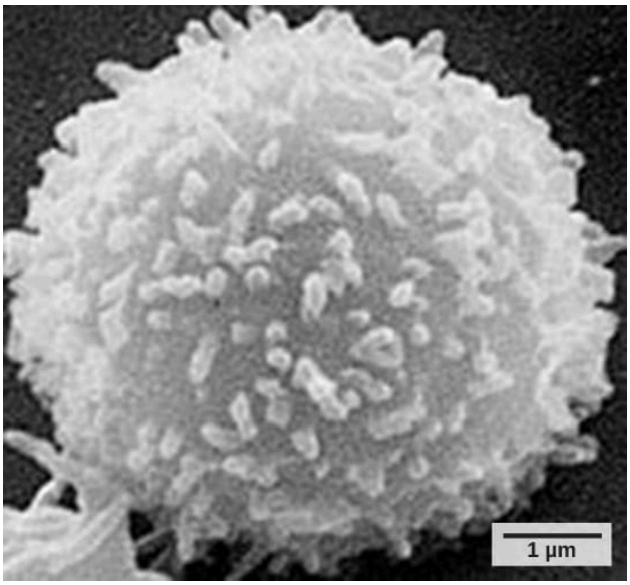


Figure 11.9.1: This scanning electron micrograph shows a T lymphocyte. T and B cells are indistinguishable by light microscopy but can be differentiated experimentally by probing their surface receptors. (credit: modification of work by NCI; scale-bar data from Matt Russell)

Humoral Immune Response

As mentioned, an antigen is a molecule that stimulates a response in the immune system. Not every molecule is antigenic. B cells participate in a chemical response to antigens present in the body by producing specific antibodies that circulate throughout the body and bind with the antigen whenever it is encountered. This is known as the humoral immune response. As discussed, during maturation of B cells, a set of highly specific B cells are produced that have many antigen receptor molecules in their membrane **(Figure** 11.9.2**)**.

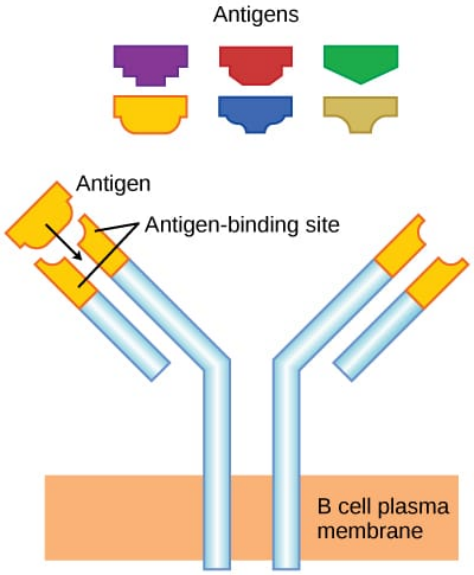


Figure 11.9.2: B cell receptors are embedded in the membranes of B cells and bind a variety of antigens through their variable regions. The upper portion of both ends of the Y is the variable region that makes up the antigen binding site.

Each B cell has only one kind of antigen receptor, which makes every B cell different. Once the B cells mature in the bone marrow, they migrate to lymph nodes or other lymphatic organs. When a B cell encounters the antigen that binds to its receptor, the antigen molecule is brought into the cell by endocytosis and reappears on the surface of the cell bound to an MHC class II molecule. When this process is complete, the B cell is sensitized. In most cases, the sensitized B cell must then encounter a specific kind of T cell, called a helper T cell, before it is activated. The helper T cell must already have been activated through an encounter with the antigen (discussed below).

The helper T cell binds to the antigen-MHC class II complex and is induced to release cytokines that induce the B cell to divide rapidly, which makes thousands of identical (clonal) cells. These daughter cells become either plasma cells or memory B cells. The memory B cells remain inactive at this point, until another later encounter with the antigen, caused by a reinfection by the same bacteria or virus, results in them dividing into a new population of plasma cells. The plasma cells, on the other hand, produce and secrete large quantities, up to 100 million molecules per hour, of antibody molecules. An antibody, also known as an immunoglobulin (Ig), is a protein that is produced by plasma cells after stimulation by an antigen. Antibodies are the agents of humoral immunity. Antibodies occur in the blood, in gastric and mucus secretions, and in breast milk. Antibodies in these bodily fluids can bind pathogens and mark them for destruction by phagocytes before they can infect cells.

These antibodies circulate in the blood stream and lymphatic system and bind with the antigen whenever it is encountered. The binding can fight infection in several ways. Antibodies can bind to viruses or bacteria and interfere with the chemical interactions required for them to infect or bind to other cells. The antibodies may create bridges between different particles containing antigenic sites clumping them all together and preventing their proper functioning. The antigen-antibody complex stimulates the complement system described previously, destroying the cell bearing the antigen. Phagocytic cells, such as those already described, are attracted by the antigen-antibody complexes, and phagocytosis is enhanced when the complexes are present. Finally, antibodies stimulate inflammation, and their presence in mucus and on the skin prevents pathogen attack.

Antibodies coat extracellular pathogens and neutralize them by blocking key sites on the pathogen that enhance their infectivity (such as receptors that “dock” pathogens on host cells) **(Figure** 11.9.3**)**. Antibody neutralization can prevent pathogens from entering and infecting host cells. The neutralized antibody-coated pathogens can then be filtered by the spleen and eliminated in urine or feces.

Antibodies also mark pathogens for destruction by phagocytic cells, such as macrophages or neutrophils, in a process called opsonization. In a process called complement fixation, some antibodies provide a place for complement proteins to bind. The combination of antibodies and complement promotes rapid clearing of pathogens.

The production of antibodies by plasma cells in response to an antigen is called active immunity and describes the host’s active response of the immune system to an infection or to a vaccination. There is also a passive immune response where antibodies come from an outside source, instead of the individual’s own plasma cells, and are introduced into the host. For example, antibodies circulating in a pregnant woman’s body move across the placenta into the developing fetus. The child benefits from the presence of these antibodies for up to several months after birth. In addition, a passive immune response is possible by injecting antibodies into an individual in the form of an antivenom to a snake-bite toxin or antibodies in blood serum to help fight a hepatitis infection. This gives immediate protection since the body does not need the time required to mount its own response.

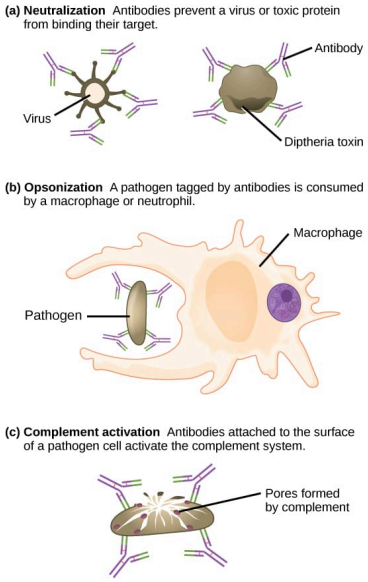


Figure 11.9.3: **(a)** Neutralization is a process where antibodies coat the surface of a virus or toxic protein, such as the diphtheria toxin, and prevent them from binding to their target. **(b)** Opsonization, a process by which a pathogen coated with antigens is consumed by a macrophage or neutrophil. **(c)** Complement activation is where antibodies attached to the surface of a pathogen cell activate the complement system. Pores are formed in the cell membrane, destroying the cell.

Cell-Mediated Immunity

Unlike B cells, T lymphocytes are unable to recognize pathogens without assistance. Instead, dendritic cells and macrophages first engulf and digest pathogens into hundreds or thousands of antigens. Then, an antigen-presenting cell (APC) detects, engulfs, and informs the adaptive immune response about an infection. When a pathogen is detected, these APCs will engulf and break it down through phagocytosis. Antigen fragments will then be transported to the surface of the APC, where they will serve as an indicator to other immune cells. A dendritic cell is an immune cell that mops up antigenic materials in its surroundings and presents them on its surface. Dendritic cells are located in the skin, the linings of the nose, lungs, stomach, and intestines. These positions are ideal locations to encounter invading pathogens. Once they are activated by pathogens and mature to become APCs they migrate to the spleen or a lymph node. Macrophages also function as APCs. After phagocytosis by a macrophage, the phagocytic vesicle fuses with an intracellular lysosome. Within the resulting phagolysosome, the components are broken down into fragments; the fragments are then loaded onto MHC class II molecules and are transported to the cell surface for antigen presentation **(Figure** 11.9.4**)**. Helper T cells cannot properly respond to an antigen unless it is processed and embedded in an MHC class II molecule. The APCs express MHC class II on their surfaces, and when combined with a foreign antigen, these complexes signal an invader.

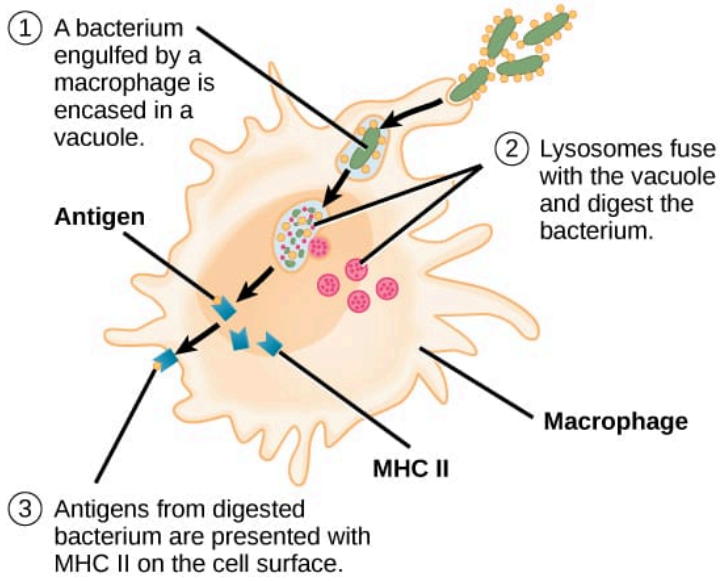


Figure 11.9.4: An antigen-presenting cell (APC), such as a macrophage, engulfs a foreign antigen, partially digests it in a lysosome, and then embeds it in an MHC class II molecule for presentation at the cell surface. Lymphocytes of the adaptive immune response must interact with antigen-embedded MHC class II molecules to mature into functional immune cells.

View this video to see how dendritic cells act as sentinels in the body's immune system.

T cells have many functions. Some respond to APCs of the innate immune system and indirectly induce immune responses by releasing cytokines. Others stimulate B cells to start the humoral response as described previously. Another type of T cell detects APC signals and directly kills the infected cells, while some are involved in suppressing inappropriate immune reactions to harmless or "self" antigens.

There are two main types of T cells: helper T lymphocytes (T_H) and the cytotoxic T lymphocytes (T_C). The T_H lymphocytes function indirectly to tell other immune cells about potential pathogens. T_H lymphocytes recognize specific antigens presented by the MHC class II complexes of APCs. There are two populations of T_H cells: T_H1 and T_H2. T_H1 cells secrete cytokines to enhance the activities of macrophages and other T cells. T_H2 cells stimulate naïve B cells to secrete antibodies. Whether a T_H1 or a T_H2 immune response develops depends on the specific types of cytokines secreted by cells of the innate immune system, which in turn depends on the nature of the invading pathogen.

Cytotoxic T cells (T_C) are the key component of the cell-mediated part of the adaptive immune system and attack and destroy infected cells. T_C cells are particularly important in protecting against viral infections; this is because viruses replicate within cells where they are shielded from extracellular contact with circulating antibodies. Once activated, the T_C creates a large clone of cells with one specific set of cell-surface receptors, as in the case with proliferation of activated B cells. As with B cells, the clone includes active T_C cells and inactive memory $T_C$cells. The resulting active T_C cells then identify infected host cells. Because

of the time required to generate a population of clonal T and B cells, there is a delay in the adaptive immune response compared to the innate immune response.

T_C cells attempt to identify and destroy infected cells before the pathogen can replicate and escape, thereby halting the progression of intracellular infections. T_C cells also support NK lymphocytes to destroy early cancers. Cytokines secreted by the T_H1 response that stimulates macrophages also stimulate T_C cells and enhance their ability to identify and destroy infected cells and tumors. A summary of how the humoral and cell-mediated immune responses are activated appears in **Figure** 11.9.5.

B plasma cells and T_C cells are collectively called effector cells because they are involved in “effecting” (bringing about) the immune response of killing pathogens and infected host cells.

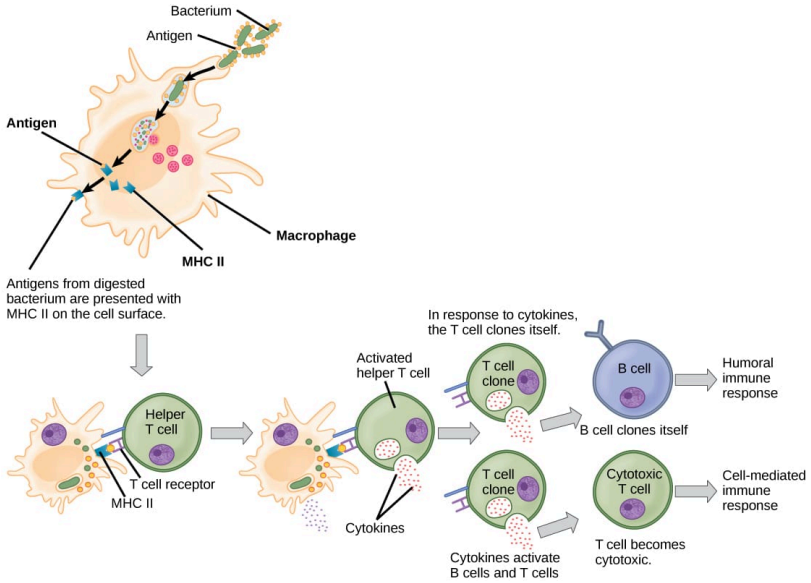


Figure 11.9.5: The first step shows a bacterium being engulfed by a macrophage. Lysosomes fuse with the vacuole containing the bacteria. The bacterium is digested, and antigens from the bacterium are attached to a MHC II molecule and presented on the cell surface. The a T cell receptor on the surface of the T cell binds the MHC II-antigen complex presented by the macrophage (also called an antigen-presenting cell). As a result, the helper T cell becomes activated and both the helper T cell and macrophage cell release cytokines. The cytokines induce the helper T cell to clone itself. The cloned helper T cells release different cytokines that activate B cells, causing them to clone and begin the humoral immune response; and other T cells, turning them into cytotoxic T cells and beginning the cell-mediated immune response.

Immunological Memory

The adaptive immune system has a memory component that allows for a rapid and large response upon reinvasion of the same pathogen. During the adaptive immune response to a pathogen that has not been encountered before, known as the primary immune response, plasma cells secreting antibodies and differentiated T cells increase, then plateau over time. As B and T cells mature into effector cells, a subset of the naïve populations differentiates into B and T memory cells with the same antigen specificities **(Figure** 11.9.6**)**. A memory cell is an antigen-specific B or T lymphocyte that does not differentiate into an effector cell during the primary immune response, but that can immediately become an effector cell on reexposure to the same pathogen. As the infection is cleared and pathogenic stimuli subside, the effectors are no longer needed and they undergo apoptosis. In contrast, the memory cells persist in the circulation.

ART CONNECTION

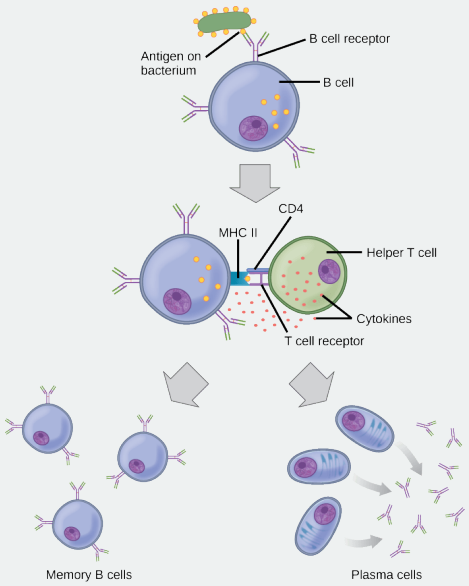


Figure 11.9.6: After initially binding an antigen to the B cell receptor, a B cell internalizes the antigen and presents it on MHC class II. A helper T cell recognizes the MHC class II- antigen complex and activates the B cell. As a result, memory B cells and plasma cells are made.

The Rh antigen is found on Rh-positive red blood cells. An Rh-negative female can usually carry an Rh-positive fetus to term without difficulty. However, if she has a second Rh-positive fetus, her body may launch an immune attack that causes hemolytic disease of the newborn. Why do you think hemolytic disease is only a problem during the second or subsequent pregnancies?

If the pathogen is never encountered again during the individual's lifetime, B and T memory cells will circulate for a few years or even several decades and will gradually die off, having never functioned as effector cells. However, if the host is re-exposed to the same pathogen type, circulating memory cells will immediately differentiate into plasma cells and T_C cells without input from APCs or T_H cells. This is known as the secondary immune response. One reason why the adaptive immune response is delayed is because it takes time for naïve B and T cells with the appropriate antigen specificities to be identified, activated, and proliferate. On reinfection, this step is skipped, and the result is a more rapid production of immune defenses. Memory B cells that differentiate into plasma cells output tens to hundreds-fold greater antibody amounts than were secreted during the primary response **(Figure** 11.9.7**)**. This rapid and dramatic antibody response may stop the infection before it can even become established, and the individual may not realize they had been exposed.

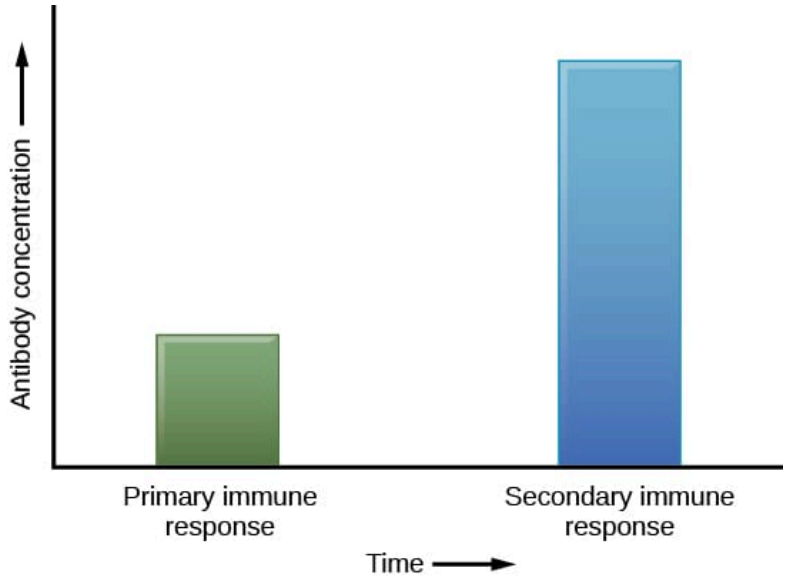


Figure 11.9.7: In the primary response to infection, antibodies are secreted first from plasma cells. Upon re-exposure to the same pathogen, memory cells differentiate into antibody-secreting plasma cells that output a greater amount of antibody for a longer period of time. The bar graph shows the antibody concentration for the secondary immune response is much greater than the primary immune response.

Vaccination is based on the knowledge that exposure to noninfectious antigens, derived from known pathogens, generates a mild primary immune response. The immune response to vaccination may not be perceived by the host as illness but still confers immune memory. When exposed to the corresponding pathogen to which an individual was vaccinated, the reaction is similar to a secondary exposure. Because each reinfection generates more memory cells and increased resistance to the pathogen, some vaccine courses involve one or more booster vaccinations to mimic repeat exposures.

The Lymphatic System

Lymph is the watery fluid that bathes tissues and organs and contains protective white blood cells but does not contain erythrocytes. Lymph moves about the body through the lymphatic system, which is made up of vessels, lymph ducts, lymph glands, and organs, such as tonsils, adenoids, thymus, and spleen.

Although the immune system is characterized by circulating cells throughout the body, the regulation, maturation, and intercommunication of immune factors occur at specific sites. The blood circulates immune cells, proteins, and other factors through the body. Approximately 0.1 percent of all cells in the blood are leukocytes, which include monocytes (the precursor of macrophages) and lymphocytes. Most cells in the blood are red blood cells. Cells of the immune system can travel between the distinct lymphatic and blood circulatory systems, which are separated by interstitial space, by a process called extravasation (passing through to surrounding tissue).

Recall that cells of the immune system originate from stem cells in the bone marrow. B cell maturation occurs in the bone marrow, whereas progenitor cells migrate from the bone marrow and develop and mature into naïve T cells in the organ called the thymus.

On maturation, T and B lymphocytes circulate to various destinations. Lymph nodes scattered throughout the body house large populations of T and B cells, dendritic cells, and macrophages **(Figure** 11.9.8**)**. Lymph gathers antigens as it drains from tissues. These antigens then are filtered through lymph nodes before the lymph is returned to circulation. APCs in the lymph nodes capture and process antigens and inform nearby lymphocytes about potential pathogens.

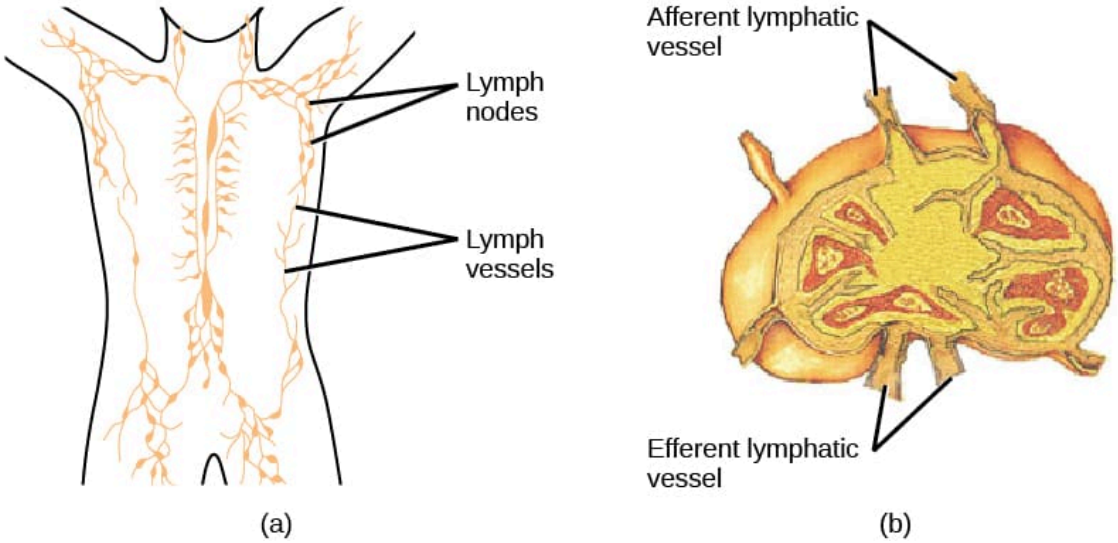


Figure 11.9.8: **(a)** Lymphatic vessels carry a clear fluid called lymph throughout the body. The liquid passes through **(b)** lymph nodes that filter the lymph that enters the node through afferent vessels and leaves through efferent vessels; lymph nodes are filled with lymphocytes that purge infecting cells. (credit a: modification of work by NIH; credit b: modification of work by NCI, NIH)

The spleen houses B and T cells, macrophages, dendritic cells, and NK cells **(Figure 11.9.9)**. The spleen is the site where APCs that have trapped foreign particles in the blood can communicate with lymphocytes. Antibodies are synthesized and secreted by activated plasma cells in the spleen, and the spleen filters foreign substances and antibody-complexed pathogens from the blood. Functionally, the spleen is to the blood as lymph nodes are to the lymph.

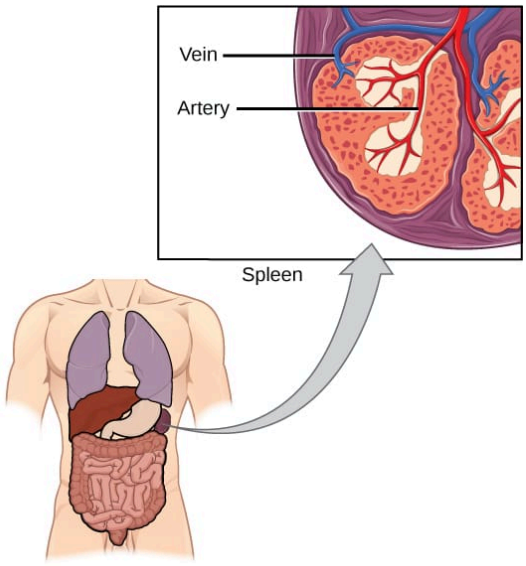


Figure 11.9.9: The spleen, located the upper left part of the abdomen, functions to immunologically filter the blood and allow for communication between cells corresponding to the innate and adaptive immune responses. (credit: modification of work by NCI, NIH)

Mucosal Immune System

The innate and adaptive immune responses compose the systemic immune system (affecting the whole body), which is distinct from the mucosal immune system. Mucosa associated lymphoid tissue (MALT) is a crucial component of a functional immune system because mucosal surfaces, such as the nasal passages, are the first tissues onto which inhaled or ingested pathogens are deposited. The mucosal tissue includes the mouth, pharynx, and esophagus, and the gastrointestinal, respiratory, and urogenital tracts.

Mucosal immunity is formed by MALT, which functions independently of the systemic immune system, and which has its own innate and adaptive components. MALT is a collection of lymphatic tissue that combines with epithelial tissue lining the mucosa throughout the body. This tissue functions as the immune barrier and response in areas of the body with direct contact to the external environment. The systemic and mucosal immune systems use many of the same cell types. Foreign particles that make their way to MALT are taken up by absorptive epithelial cells and delivered to APCs located directly below the mucosal tissue. APCs of the mucosal immune system are primarily dendritic cells, with B cells and macrophages having minor roles. Processed antigens displayed on APCs are detected by T cells in the MALT and at the tonsils, adenoids, appendix, or the mesenteric lymph nodes of the intestine. Activated T cells then migrate through the lymphatic system and into the circulatory system to mucosal sites of infection.

Immune Tolerance

The immune system has to be regulated to prevent wasteful, unnecessary responses to harmless substances, and more importantly, so that it does not attack "self." The acquired ability to prevent an unnecessary or harmful immune response to a detected foreign substance known not to cause disease, or self-antigens, is described as immune tolerance. The primary mechanism for developing immune tolerance to self-antigens occurs during the selection for weakly self-binding cells during T and B lymphocyte maturation. There are populations of T cells that suppress the immune response to self-antigens and that suppress the immune response after the infection has cleared to minimize host cell damage induced by inflammation and cell lysis. Immune tolerance is especially well developed in the mucosa of the upper digestive system because of the tremendous number of foreign substances (such as food proteins) that APCs of the oral cavity, pharynx, and gastrointestinal mucosa encounter. Immune tolerance is brought about by specialized APCs in the liver, lymph nodes, small intestine, and lung that present harmless antigens to a diverse population of regulatory T (T_{reg}) cells, specialized lymphocytes that suppress local inflammation and inhibit the secretion of stimulatory immune factors. The combined result of T_{reg} cells is to prevent immunologic activation and inflammation in undesired tissue compartments and to allow the immune system to focus on pathogens instead.

Summary

The adaptive immune response is a slower-acting, longer-lasting, and more specific response than the innate response. However, the adaptive response requires information from the innate immune system to function. APCs display antigens on MHC molecules to naïve T cells. T cells with cell-surface receptors that bind a specific antigen will bind to that APC. In response, the T cells differentiate and proliferate, becoming T_H cells or T_C cells. T_H cells stimulate B cells that have engulfed and presented pathogen-derived antigens. B cells differentiate into plasma cells that secrete antibodies, whereas T_C cells destroy infected or cancerous cells. Memory cells are produced by activated and proliferating B and T cells and persist after a primary exposure to a pathogen. If re-exposure occurs, memory cells differentiate into effector cells without input from the innate immune system. The mucosal immune system is largely independent of the systemic immune system but functions in parallel to protect the extensive mucosal surfaces of the body. Immune tolerance is brought about by T_{reg} cells to limit reactions to harmless antigens and the body's own molecules.

Art Connections

Figure 11.9.6**:** The Rh antigen is found on Rh-positive red blood cells. An Rh-negative female can usually carry an Rh-positive fetus to term without difficulty. However, if she has a second Rh-positive fetus, her body may launch an immune attack that causes hemolytic disease of the newborn. Why do you think hemolytic disease is only a problem during the second or subsequent pregnancies?

Answer

If the blood of the mother and fetus mixes, memory cells that recognize the Rh antigen of the fetus can form in the mother late in the first pregnancy. During subsequent pregnancies, these memory cells launch an immune attack on the fetal blood cells of an Rh-positive fetus. Injection of anti-Rh antibody during the first pregnancy prevents the immune response from occurring.

Key Terms

active immunity
an immunity that occurs as a result of the activity of the body's own cells rather than from antibodies acquired from an external source

adaptive immunity
a specific immune response that occurs after exposure to an antigen either from a pathogen or a vaccination

antibody
a protein that is produced by plasma cells after stimulation by an antigen; also known as an immunoglobulin

antigen
a macromolecule that reacts with cells of the immune system and which may or may not have a stimulatory effect

antigen-presenting cell (APC)
an immune cell that detects, engulfs, and informs the adaptive immune response about an infection by presenting the processed antigen on its cell surface

B cell
a lymphocyte that matures in the bone marrow

cell-mediated immune response
an adaptive immune response that is controlled by T cells

cytokine
signaling molecule released by immune cells to coordinate responses

cytotoxic T lymphocyte (T_C)
an adaptive immune cell that directly kills infected cells via enzymes, and that releases cytokines to enhance the immune response

dendritic cell
an immune cell that processes antigen material and presents it on the surface of its cell in MHC class II molecules and induces an immune response in other cells

effector cell
a mature immune cell, such as a plasma B cell or cytotoxic T cell, that performs immune functions

helper T lymphocyte (T_H)
a cell of the adaptive immune system that binds APCs via MHC class II molecules and stimulates B cells or secretes cytokines to initiate the immune response

humoral immune response
the adaptive immune response that is controlled by activated B cells and antibodies

immune tolerance
an acquired ability to prevent an unnecessary or harmful immune response to a detected foreign body known not to cause disease

lymph
the watery fluid present in the lymphatic circulatory system that bathes tissues and organs with protective white blood cells and does not contain erythrocytes

lymph node
an organ that filters lymph and facilitates communication between immune cells

lymphocyte
a type of white blood cell involved in the adaptive immune response, including B and T cells

major histocompatibility class (MHC) II molecule
: a protein found on the surface of antigen-presenting cells that signals to immune cells whether the cell is normal or is infected or cancerous; it provides the appropriate template into which antigens can be loaded for recognition by lymphocytes

mucosa-associated lymphoid tissue (MALT)
: lymphoid tissues associated with mucosal surfaces involved in localized immune responses

memory cell
: an antigen-specific B or T lymphocyte that does not differentiate into an effector cell during the primary immune response but that can immediately become an effector cell on reexposure to the same pathogen

neutralization
: the process by which antibodies block pathogens from binding to host cells

opsonization
: the process of coating pathogens with antibodies to enhance phagocytosis

passive immunity
: an immunity that does not result from the activity of the body's own immune cells but by transfer of antibodies from one individual to another

plasma cell
: an activated B cell that secretes antibodies

primary immune response
: the response of the adaptive immune system to the first exposure to an antigen

secondary immune response
: the response of the adaptive immune system to a second or later exposure to an antigen mediated by memory cells

T cell
: a lymphocyte that matures in the thymus gland

T helper 1 cell (T_H1)
: a helper T cell subtype that activates macrophages and cytotoxic T cells

T helper 2 cell (T_{H2})
: a helper T cell subtype that stimulates B cell activation and antibody production

T regulatory cell (T_{reg})
: T cell that suppresses immune responses and helps maintain immune tolerance

vaccination
: exposure to a harmless form of a pathogen to stimulate an immune response and develop memory

Contributors and Attributions

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11.10: Vaccines

Clinical Focus

When six-month-old Olivia spiked a high fever, became unusually lethargic, and began crying inconsolably, her parents rushed her to the emergency room. The attending physician, concerned by her symptoms and stiff neck, made a presumptive diagnosis of bacterial meningitis and began immediate treatment—well before lab results were returned. Olivia was admitted to the hospital and started on intravenous broad-spectrum antibiotics and rehydration therapy.

Over the next several days, her condition began to improve. Follow-up tests revealed no microbes in her blood or cerebrospinal fluid (CSF), and her white blood cell count gradually returned to normal. Meanwhile, lab analysis confirmed that the original CSF sample contained *Neisseria meningitidis*—the bacterium responsible for meningococcal meningitis.

Neisseria meningitidis is a dangerous pathogen that produces a polysaccharide capsule, allowing it to evade immune detection. It often targets infants around Olivia's age—just as the protective maternal antibodies acquired during pregnancy (particularly IgG) begin to wane. At this stage, infants have not yet developed the memory cells necessary to recognize and respond to capsule antigens, leaving them especially vulnerable. Tragically, infections like Olivia's are frequently fatal in infants because their adaptive immune systems are not yet equipped to mount an effective defense.

Yet cases like Olivia's are entirely preventable. A conjugate vaccine, designed specifically to overcome the immune system's limitations in early life, can protect against *N. meningitidis* and is approved for use in infants as young as two months. Despite this, current U.S. vaccination guidelines recommend routine administration of the meningococcal conjugate vaccine at age 11–12, with a booster at 16—well past the age of greatest vulnerability for infants like Olivia.

Learning Objectives

- Compare the various kinds of artificial immunity
- Differentiate between variolation and vaccination
- Describe how vaccines induce artificial active immunity and contribute to herd immunity.
- 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** 11.10.1**)**. 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 11.10.1: The four classifications of immunity. Passive and Natural: Immunity acquired from antibodies passed through breast milk or placenta. Passive and Artificial: Immunity gained through antibodies harvested from another animal. Active and Natural: Immunity Gained through illness and recovery. Active and Artificial: Immunity gained through a vaccine. (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 11.10.1

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 disproved. 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** 11.10.2**)**. 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 11.10.2: 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** 11.10.3**)**. The boy developed a mild infection that included a low-grade fever, discomfort in his axillae (armpit) and loss of appetite. When the boy was later infected with infectious samples from smallpox lesions, he did not contract smallpox.[3] This new approach was termed vaccination, a name deriving from the use of cowpox (Latin *vacca* meaning "cow") to protect against smallpox. Today, we know that Jenner's vaccine worked because the cowpox virus is genetically and antigenically related to the *Variola* viruses that caused smallpox. Exposure to cowpox antigens resulted in a primary response and the production of memory cells that identical or related epitopes of Variola virus upon a later exposure to smallpox.

The success of Jenner's smallpox vaccination led other scientists to develop vaccines for other diseases. Perhaps the most notable was Louis Pasteur, who developed vaccines for rabies, cholera, and anthrax. During the 20th and 21st centuries, effective vaccines were developed to prevent a wide range of diseases caused by viruses (e.g., chickenpox and shingles, hepatitis, measles, mumps, polio, and yellow fever) and bacteria (e.g., diphtheria, pneumococcal pneumonia, tetanus, and whooping cough).



Figure 11.10.3: **(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 11.10.2

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** 11.10.1.

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** 11.10.1 lists examples of live attenuated vaccines.

Inactivated Vaccines

Inactivated vaccines contain whole pathogens that have been killed or inactivated with heat, chemicals, or radiation. For inactivated vaccines to be effective, the inactivation process must not affect the structure of key antigens on the pathogen.

Because the pathogen is killed or inactive, inactivated vaccines do not produce an active infection, and the resulting immune response is weaker and less comprehensive than that provoked by a live attenuated vaccine. Typically the response involves only humoral immunity, and the pathogen cannot be transmitted to other individuals. In addition, inactivated vaccines usually require higher doses and multiple boosters, possibly causing inflammatory reactions at the site of injection.

Despite these disadvantages, inactivated vaccines do have the advantages of long-term storage stability and ease of transport. Also, there is no risk of causing severe active infections. However, inactivated vaccines are not without their side effects. **Table** 11.10.1 lists examples of inactivated vaccines.

Subunit Vaccines

Whereas live attenuated and inactive vaccines expose an individual to a weakened or dead pathogen, subunit vaccines only expose the patient to the key antigens of a pathogen—not whole cells or viruses. Subunit vaccines can be produced either by chemically degrading a pathogen and isolating its key antigens or by producing the antigens through genetic engineering. Because these vaccines contain only the essential antigens of a pathogen, the risk of side effects is relatively low. **Table** 11.10.1 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** 11.10.1 lists examples of toxoid vaccines.

Conjugate Vaccines

A conjugate vaccine is a type of subunit vaccine that consists of a protein conjugated to a capsule polysaccharide. Conjugate vaccines have been developed to enhance the efficacy of subunit vaccines against pathogens that have protective polysaccharide capsules that help them evade phagocytosis, causing invasive infections that can lead to meningitis and other serious conditions. The subunit vaccines against these pathogens introduce T-independent capsular polysaccharide antigens that result in the production of antibodies that can opsonize the capsule and thus combat the infection; however, children under the age of two years do not respond effectively to these vaccines. Children do respond effectively when vaccinated with the conjugate vaccine, in which a protein with T-dependent antigens is conjugated to the capsule polysaccharide. The conjugated protein-polysaccharide antigen stimulates production of antibodies against both the protein and the capsule polysaccharide. **Table** 11.10.1 lists examples of conjugate vaccines.

Table 11.10.1*: Classes of Vaccines*

Class	Description	Advantages	Disadvantages	Examples
Live attenuated	Weakened strain of whole pathogen	• Cellular and humoral immunity • Long-lasting immunity • Transmission to contacts	• Difficult to store and transport • Risk of infection in immunocompromised patients • Risk of reversion	Chickenpox, German measles, measles, mumps, tuberculosis, typhoid fever, yellow fever

Class	Description	Advantages	Disadvantages	Examples
Inactivated	Whole pathogen killed or inactivated with heat, chemicals, or radiation	• Ease of storage and transport • No risk of severe active infection	• Weaker immunity (humoral only) • Higher doses and more boosters required	Cholera, hepatitis A, influenza, plague, rabies
Subunit	Immunogenic antigens	Lower risk of side effects	• Limited longevity • Multiple doses required • No protection against antigenic variation	Anthrax, hepatitis B, influenza, meningitis, papillomavirus, pneumococcal pneumonia, whooping cough
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 • Better response in young children	• Costly to produce • No protection against antigenic variation • May interfere with other vaccines	Meningitis (*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitides*)

Exercise 11.10.3

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.

Link to Learning

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.

Summary

Vaccination is one of the most effective methods for disease prevention and functions by inducing artificial active immunity, enabling individuals to mount a rapid and strong immune response upon future exposure without first experiencing illness. Adaptive immunity can be categorized as active or passive and acquired naturally or artificially. Vaccines, as a form of artificial active immunity, differ in type: live attenuated, inactivated, subunit, toxoid, and conjugate vaccines each present antigens in specific ways to stimulate immune responses. Herd immunity protects populations by reducing the number of susceptible individuals, emphasizing the importance of widespread vaccination.

Historically, variolation introduced individuals to smallpox material to promote immunity but carried significant risk. Edward Jenner's development of vaccination using cowpox led to safer and more effective immunization practices. Despite the proven benefits, vaccination remains controversial in some circles due to misinformation, ethical concerns, or political beliefs, contributing to recent outbreaks of preventable diseases. This ongoing debate raises questions about individual rights, public health policy, and the responsibilities of healthcare providers.

Effective vaccines minimize side effects while providing lasting immunity. Their classification depends on how they present antigens and stimulate the immune system, with each type having specific advantages and limitations. Understanding these distinctions helps clarify vaccine function, development, and societal importance.

Key Terms

active immunity
: immunity resulting from the activation of an individual's own immune system, either through natural infection or vaccination

artificial active immunity
: immunity gained through exposure to a vaccine containing antigens or weakened/inactivated pathogens temporary

artificial passive immunity
: temporary immunity acquired by receiving antibodies from another individual or animal

attenuated
: weakened form of a pathogen used in vaccines to elicit an immune response without causing severe disease

conjugate vaccine
: a vaccine combining a capsule polysaccharide with a protein to improve immune response, especially in young children

cowpox
: a mild viral infection used by Edward Jenner in the first successful vaccination to

protect against smallpox

herd immunity
: indirect protection from infectious disease that occurs when a large percentage of a population is immune, reducing the spread of disease

humoral immunity
: part of the adaptive immune response involving B cells and the production of antibodies

inactivated vaccine
: a vaccine made with pathogens that have been killed or inactivated to prevent infection while still eliciting an immune response

memory cell
: long-lived immune cells that remain in the body after an infection or vaccination, enabling a faster secondary response upon re-exposure

natural active immunity
: immunity acquired through exposure to a pathogen and subsequent recovery

natural passive immunity
: immunity passed from mother to child via placenta (IgG) or breast milk (IgA)

passive immunity
: immunity gained by receiving antibodies from another individual rather than through the body's own immune response

subunit vaccine
: a vaccine composed only of specific antigens from a pathogen, not the entire organism

T-dependent antigen
: an antigen that requires help from T cells to elicit a full antibody response

T-independent antigen
: an antigen that can stimulate B cells directly without T-cell help

toxoid vaccine
: a vaccine containing inactivated bacterial toxins to stimulate antibody production and neutralize toxins

vaccination
: the administration of a vaccine to stimulate adaptive immunity against a pathogen

variolation
: an early method of immunization involving the deliberate infection with smallpox material to induce a milder form of the disease

Footnotes

1. 1 K. Mupapa, M. Massamba, K. Kibadi, K. Kivula, A. Bwaka, M. Kipasa, R. Colebunders, J. J. Muyembe-Tamfum. "Treatment of Ebola Hemorrhagic Fever with Blood Transfusions from Convalescent Patients." *Journal of Infectious Diseases* 179 Suppl.

(1999): S18–S23.
2. 2 Elizabeth Yale. “Why Anti-Vaccination Movements Can Never Be Tamed.” *Religion & Politics*, July 22, 2014. religionandpolitics.org/2014/...never-be-tamed.
3. 3 N. J. Willis. “Edward Jenner and the Eradication of Smallpox.” *Scottish Medical Journal* 42 (1997): 118–121.
4. 4 M. Alonso and J. C. Leong. “Licensed DNA Vaccines Against Infectious Hematopoietic Necrosis Virus (IHNV).” *Recent Patents on DNA & Gene Sequences (Discontinued)* 7 no. 1 (2013): 62–65, issn 1872-2156/2212-3431. doi 10.2174/1872215611307010009.
5. 5 S.B. Halstead and S. J. Thomas. “New Japanese Encephalitis Vaccines: Alternatives to Production in Mouse Brain.” *Expert Review of Vaccines* 10 no. 3 (2011): 355–64.

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12: BIOTECHNOLOGY AND DNA MANIPULATION

12.1: Introduction to Biotechnologies
12.2: PCR and Gel Electrophoresis
12.3: Principles of Mammalian Cell Culture
12.4: Principles of Genetic Engineering
12.5: Creating Recombinant DNA
12.6: Applications of Animal Biotechnology
12.7: Genetic Engineering in Animals

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12.1: Introduction to Biotechnologies

Learning Objectives

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

- Describe gel electrophoresis
- Explain molecular and reproductive cloning
- Describe biotechnology uses in medicine and agriculture

Biotechnology is the use of biological agents for technological advancement. Biotechnology was used for breeding livestock and crops long before people understood the scientific basis of these techniques. Since the discovery of the structure of DNA in 1953, the biotechnology field has grown rapidly through both academic research and private companies. The primary applications of this technology are in medicine (vaccine and antibiotic production) and agriculture (crop genetic modification in order to increase yields). Biotechnology also has many industrial applications, such as fermentation, treating oil spills, and producing biofuels (**Figure** 12.1.1**:**).

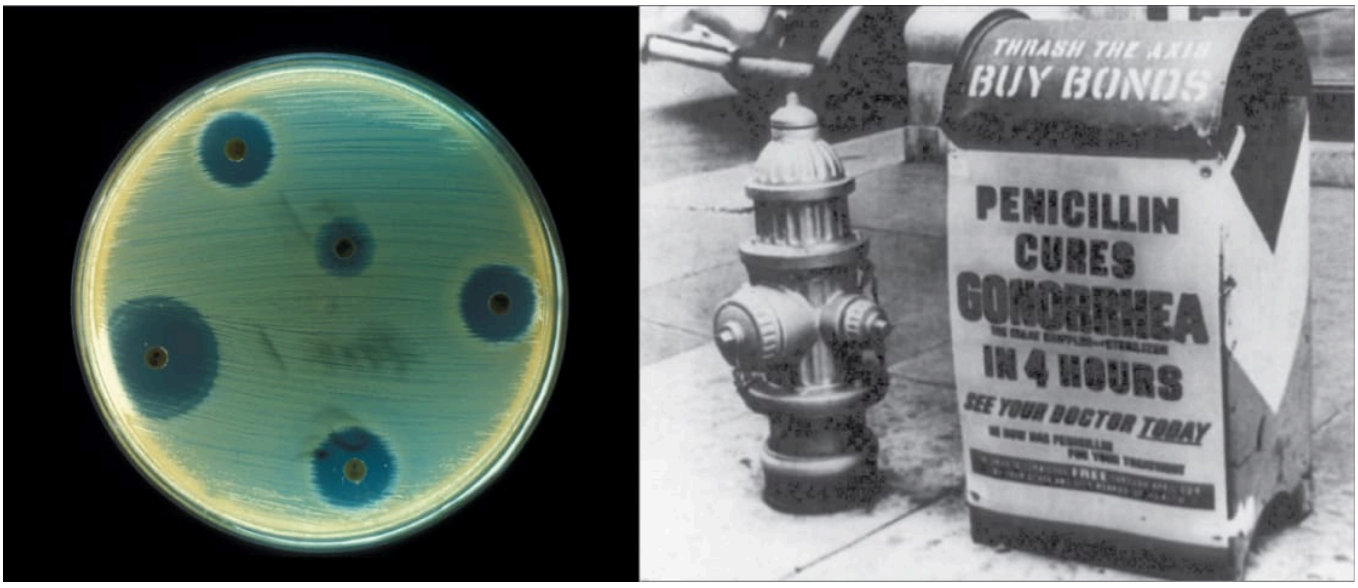


Figure
12.1.1**:**
Fungi, bacteria, and other organisms that have antimicrobial properties produce antibiotics. The image on the left shows b acteria growing everywhere on the plate except where discs containing antibiotic have been placed. These areas are completely devoid of bacterial growth
. The
first antibiotic discovered was penicillin, an advertisement is shown on the right, "Penicillin cures gonorrhea in 4 hours." Pharmaceutical companies now commercially produce and test antibiotics for their potential to inhibit bacterial growth. (credit "advertisement": modification of work by NIH; credit "test plate": modification of work by Don Stalons/CDC; scale-bar data from Matt Russell)

Basic Techniques to Manipulate Genetic Material (DNA and RNA)

To understand the basic techniques used to work with nucleic acids, remember that nucleic acids are macromolecules made of nucleotides (a sugar, a phosphate, and a nitrogenous base) linked by phosphodiester bonds. The phosphate groups on these molecules each have a net negative charge. An entire set of DNA molecules in the nucleus is called the genome. DNA has two complementary strands linked by hydrogen bonds between the paired bases. Exposure to high temperatures (DNA denaturation)

can separate the two strands and cooling can reanneal them. The DNA polymerase enzyme can replicate the DNA. Unlike DNA, which is located in the eukaryotic cells' nucleus, RNA molecules leave the nucleus. The most common type of RNA that researchers analyze is the messenger RNA (mRNA) because it represents the protein-coding genes that are actively expressed. However, RNA molecules present some other challenges to analysis, as they are often less stable than DNA.

DNA and RNA Extraction

To study or manipulate nucleic acids, one must first isolate or extract the DNA or RNA from the cells. Researchers use various techniques to extract different types of DNA **Figure** 12.1.2**:**. Most nucleic acid extraction techniques involve steps to break open the cell and use enzymatic reactions to destroy all macromolecules that are not desired (such as unwanted molecule degradation and separation from the DNA sample). A lysis buffer (a solution which is mostly a detergent) breaks cells. Note that lysis means "to split". These enzymes break apart lipid molecules in the cell membranes and nuclear membranes. Enzymes such as proteases that break down proteins inactivate macromolecules, and ribonucleases (RNAses) that break down RNA. Using alcohol precipitates the DNA. Human genomic DNA is usually visible as a gelatinous, white mass. One can store the DNA samples frozen at –80°C for several years.

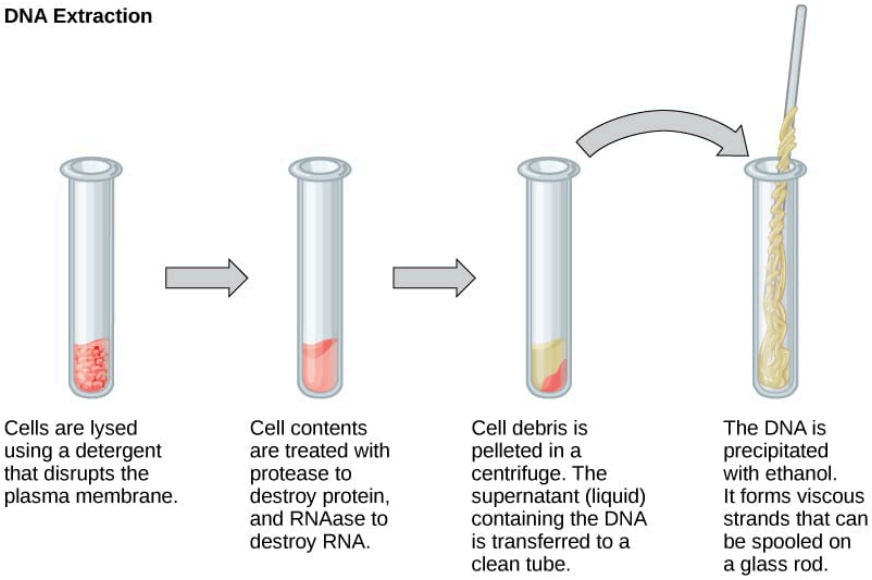


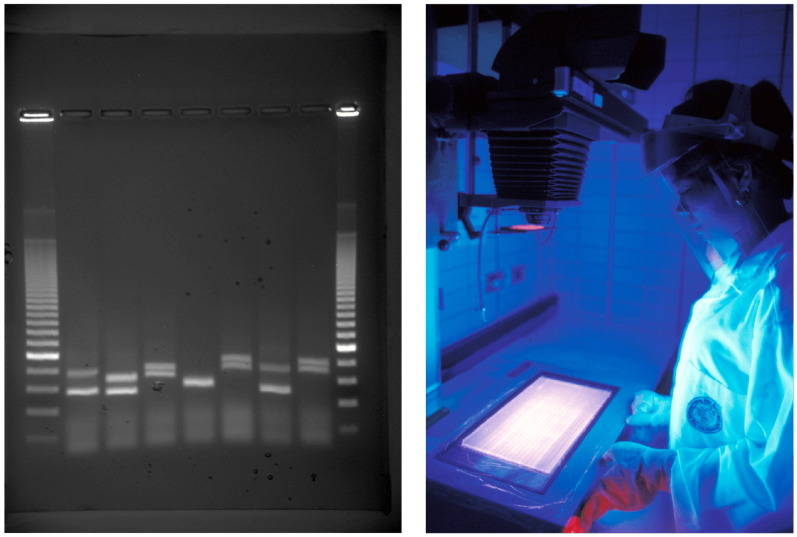
Figure 12.1.2**:** This diagram shows the basic method of DNA extraction. In the first step, cells in a test tube are lysed using a detergent that disrupts the plasma membrane. In the second step, cell contents are treated with protease to destroy protein, and RNAase to destroy R N A. The resulting slurry is centrifuged to pellet the cell debris. The supernatant, or liquid, containing the D N A is then transferred to a clean test tube. The D N A is precipitated with ethanol. It forms viscous, mucous-like strands that can be spooled on a glass rod.

Scientists perform RNA analysis to study gene expression patterns in cells. RNA is naturally very unstable because RNAses are commonly present in nature and very difficult to inactivate. Similar to DNA, RNA extraction involves using various buffers and enzymes to inactivate macromolecules and preserve the RNA.

Gel Electrophoresis

Because nucleic acids are negatively charged ions at neutral or basic pH in an aqueous environment, an electric field can mobilize them. Gel electrophoresis is a technique that scientists use to separate molecules on the basis of size, using this charge. One can separate the nucleic acids as whole chromosomes or fragments. The nucleic acids load into a slot near the semisolid, porous gel matrix's negative electrode, and pulled toward the positive electrode at the gel's opposite end. Smaller molecules move through the gel's pores faster than larger molecules. This difference in the migration rate separates the fragments on the basis of size. There are molecular weight standard samples that researchers can run alongside the molecules to provide a size comparison. We can observe nucleic acids in a gel matrix using various fluorescent or colored dyes. Distinct nucleic acid fragments appear as bands at specific distances from the gel's top (the negative electrode end) on the basis of their size (**Figure** 12.1.3**:**). A mixture of

genomic DNA fragments of varying sizes appear as a long smear; whereas, uncut genomic DNA is usually too large to run through the gel and forms a single large band at the gel's top.



(a) (b)

Figure 12.1.3**:** **(a)** Shown are DNA fragments from seven samples run on a gel, stained with a fluorescent dye, and viewed under UV light. The DNA appears as thin, white bands on a black background.; and **(b)** a researcher from International Rice Research Institute, reviewing DNA profiles using UV light. (credit: a: James Jacob, Tompkins Cortland Community College b: International Rice Research Institute)

Nucleic Acid Fragment Amplification by Polymerase Chain Reaction

Although genomic DNA is visible to the naked eye when it is extracted in bulk, DNA analysis often requires focusing on one or more specific genome regions. Polymerase chain reaction (PCR) is a technique that scientists use to amplify specific DNA regions for further analysis (**Figure** 12.1.4**:**). Researchers use PCR for many purposes in laboratories, such as cloning gene fragments to analyze genetic diseases, identifying contaminant foreign DNA in a sample, and amplifying DNA for sequencing. More practical applications include determining paternity and detecting genetic diseases.

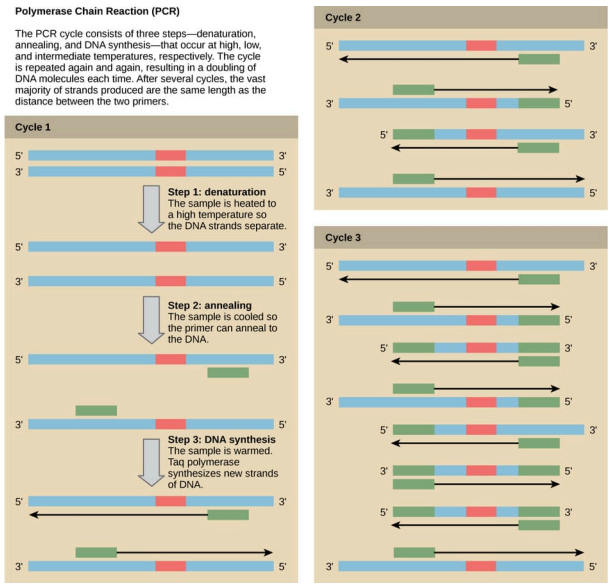


Figure
12.1.4**:**
Scientists use polymerase chain reaction, or PCR, to amplify a specific DNA sequence.

P C R consists of three steps; denaturation, annealing, and D N A synthesis; that occur at high, low, and intermediate temperatures. In step 1, the denaturation step, the sample is heated to a high temperature so the D N A strands separate. In step 2, annealing, the sample is cooled so two primers (short pieces of DNA complementary to the ends of the target sequence) can anneal to the two strands of D N A. The primers are spaced such that the sequence of interest between them will be amplified. In step 3, D N A synthesis, the sample is warmed to the optimal temperature for Taq polymerase, which synthesizes the complementary strand from the primer to the 3 prime end of the molecule.

This cycle is repeated again and again.

Taq polymerase is a DNA polymerase isolated from the thermostable bacterium *Thermus aquaticus* that is able to withstand the high temperatures that scientists use in PCR. *Thermus aquaticus* grows in the Lower Geyser Basin of Yellowstone National Park. Reverse transcriptase PCR (RT-PCR) is similar to PCR, but cDNA is made from an RNA template before PCR begins.

DNA fragments can also be amplified from an RNA template in a process called reverse transcriptase PCR (RT-PCR). The first step is to recreate the original DNA template strand (called cDNA) by applying DNA nucleotides to the mRNA. This process is called reverse transcription. This requires the presence of an enzyme called reverse transcriptase. After the cDNA is made, regular PCR can be used to amplify it.

Link to Learning

Deepen your understanding by watching this video of the polymerase chain reaction.

YouTube Video

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

Hybridization, Southern Blotting, and Northern Blotting

Scientists can probe nucleic acid samples, such as fragmented genomic DNA and RNA extracts, for the presence of certain sequences. Scientists design and label short DNA fragments, or probes with radioactive or fluorescent dyes to aid detection. Gel

electrophoresis separates the nucleic acid fragments according to their size. Scientists then transfer the fragments in the gel onto a nylon membrane in a procedure we call blotting (**Figure** 12.1.5**:**). Scientists can then probe the nucleic acid fragments that are bound to the membrane's surface with specific radioactively or fluorescently labeled probe sequences. When scientists transfer DNA to a nylon membrane, they refer to the technique as Southern blotting. When they transfer the RNA to a nylon membrane, they call it Northern blotting. Scientists use Southern blots to detect the presence of certain DNA sequences in a given genome, and Northern blots to detect gene expression.

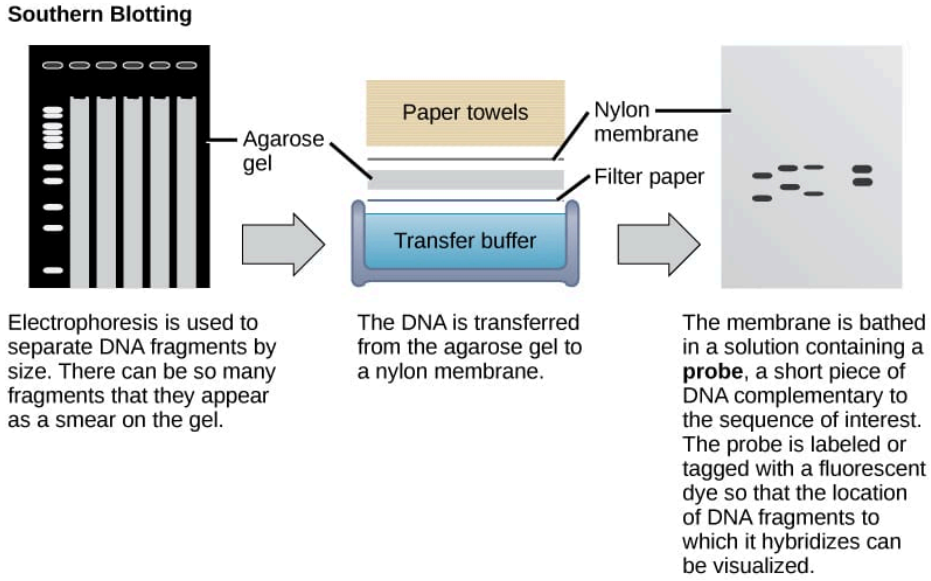


Figure 12.1.5**:** Scientists use Southern blotting to find a particular sequence in a DNA sample. Scientists separate DNA fragments on a gel, transfer them to a nylon membrane, and incubate them with a DNA probe complementary to the sequence of interest. Northern blotting is similar to Southern blotting, but scientists run RNA on the gel instead of DNA. In Western blotting, scientists run proteins on a gel and detect them using antibodies.

Molecular Cloning

In general, the word “cloning” means the creation of a perfect replica; however, in biology, the re-creation of a whole organism is referred to as “reproductive cloning.” Long before attempts were made to clone an entire organism, researchers learned how to reproduce desired regions or fragments of the genome, a process that is referred to as molecular cloning. The technique offered methods to create new medicines and to overcome difficulties with existing ones. When Lydia Villa-Komaroff, working in the Gilbert Lab at Harvard, published the first paper outlining the technique for producing synthetic insulin, diabetes researchers and patients received new hope in fighting the disease. Insulin at that time was only produced using pig and cow pancreases, and the life-saving substance was often in short supply. Synthetic insulin, once mass produced, would solve that problem for many patients. These early discoveries led to the "BioTech Boom," and spurred continued research and funding for newer and better ways to improve health.

Cloning small genome fragments allows researchers to manipulate and study specific genes (and their protein products), or noncoding regions in isolation. A plasmid, or vector, is a small circular DNA molecule that replicates independently of the chromosomal DNA. In cloning, scientists can use the plasmid molecules to provide a "folder" in which to insert a desired DNA fragment. Plasmids are usually introduced into a bacterial host for proliferation. In the bacterial context, scientists call the DNA fragment from the human genome (or the genome of another studied organism) foreign DNA, or a transgene, to differentiate it from the bacterium's DNA, or the host DNA.

Plasmids occur naturally in bacterial populations (such as *Escherichia coli*) and have genes that can contribute favorable traits to the organism, such as antibiotic resistance (the ability to be unaffected by antibiotics). Scientists have repurposed and engineered plasmids as vectors for molecular cloning and the large-scale production of important reagents, such as insulin and human growth hormone. An important feature of plasmid vectors is the ease with which scientists can introduce a foreign DNA fragment via the multiple cloning site (MCS). The MCS is a short DNA sequence containing multiple sites that different commonly available restriction endonucleases can cut. Restriction endonucleases recognize specific DNA sequences and cut them in a predictable manner. They are naturally produced by bacteria as a defense mechanism against foreign DNA. Many restriction

endonucleases make staggered cuts in the two DNA strands, such that the cut ends have a 2- or 4-base single-stranded overhang. Because these overhangs are capable of annealing with complementary overhangs, we call them “sticky ends.” Adding the enzyme DNA ligase permanently joins the DNA fragments via phosphodiester bonds. In this way, scientists can splice any DNA fragment generated by restriction endonuclease cleavage between the plasmid DNA's two ends that has been cut with the same restriction endonuclease (**Figure** 12.1.6).

Recombinant DNA Molecules

Plasmids with foreign DNA inserted into them are called recombinant DNA molecules because they are created artificially and do not occur in nature. They are also called chimeric molecules because the origin of different molecule parts of the molecules can be traced back to different species of biological organisms or even to chemical synthesis. We call proteins that are expressed from recombinant DNA molecules recombinant proteins. Not all recombinant plasmids are capable of expressing genes. The recombinant DNA may need to move into a different vector (or host) that is better designed for gene expression. Scientists may also engineer plasmids to express proteins only when certain environmental factors stimulate them, so they can control the recombinant proteins' expression.

Visual Connection

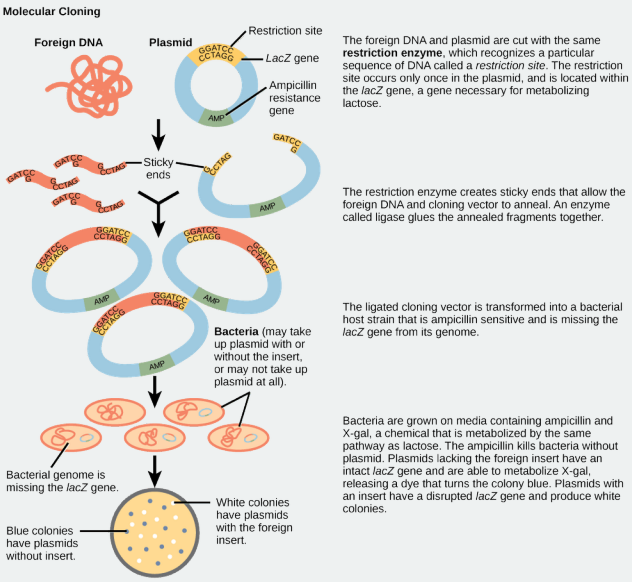


Figure 12.1.6: This figure illustrates the steps in molecular cloning into a plasmid called a cloning vector. The vector has a lac Z gene, which is necessary for metabolizing lactose, and a gene for ampicillin resistance. Within the lac Z gene are restriction sites, sequences of DNA cut by a particular restriction enzyme. The DNA to be cloned and the plasmid are both cut by the same restriction enzyme. The restriction enzyme staggers the cuts on the two strands of DNA, such that each strand has an overhanging single-stranded bit of DNA. On one strand, the sequence of the overhang is GATC, and on the other, the sequence is CTAG. These two sequences are complementary, and allow the fragment of foreign DNA to anneal with the plasmid. An enzyme called ligase joins the two pieces together. The ligated plasmid is then transformed into a bacterial strain that lacks the lac Z gene and is sensitive to the antibiotic ampicillin. The bacteria are plated on media containing ampicillin, so that only bacteria that have taking up the plasmid; which has an ampicillin resistance gene; will grow. The media also contains X gal, a chemical that is metabolized in the same way as lactose. Plasmids lacking the insert are able to metabolize X gal, releasing a dye from X gal that turns the colony blue. Plasmids with the insert have a disrupted lac Z gene and produce white colonies. Thus, colonies containing the cloned DNA can be selected on the basis of color.

You are working in a molecular biology lab and, unbeknownst to you, your lab partner left the foreign genomic DNA that you are planning to clone on the lab bench overnight instead of storing it in the freezer. As a result, it was degraded by nucleases, but still used in the experiment. The plasmid, on the other hand, is fine. What results would you expect from your molecular cloning experiment?

a. There will be no colonies on the bacterial plate.
b. There will be blue colonies only.

c. There will be blue and white colonies.
d. The will be white colonies only.

Link to Learning

View an animation of recombination in cloning from the DNA Learning Center.

Cellular Cloning

Unicellular organisms, such as bacteria and yeast, naturally produce clones of themselves when they replicate asexually by binary fission; this is known as cellular cloning. The nuclear DNA duplicates by the process of mitosis, which creates an exact replica of the genetic material.

Reproductive Cloning

Reproductive cloning is a method scientists use to clone or identically copy 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 a clone of either parent. Recent advances in biotechnology have made it possible to artificially induce mammal asexual reproduction in the laboratory.

Parthenogenesis, or “virgin birth,” occurs when an embryo grows and develops without egg fertilization. This is a form of asexual reproduction. An example of parthenogenesis occurs in species in which the female lays an egg and 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 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. Therefore, if we replace the egg cell's haploid nucleus with a diploid nucleus from the cell of any individual of the same species (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. Scientists can use it for either therapeutic cloning or reproductive cloning.

The first cloned animal was Dolly, a sheep born in 1996. The reproductive cloning success rate at the time was very low. Dolly lived for seven years and died of respiratory complications (**Figure** 12.1.7**:**). There is speculation that because the cell DNA belongs to an older individual, DNA's age may affect a cloned individual's life expectancy. Since Dolly, scientists have cloned successfully several animals such as horses, bulls, and goats, although these animals often exhibit facial, limb, and cardiac abnormalities. There have been attempts at producing cloned human embryos as sources of embryonic stem cells for therapeutic purposes. Therapeutic cloning produces stem cells in the attempt to remedy detrimental diseases or defects (unlike reproductive cloning, which aims to reproduce an organism). While some have met therapeutic cloning efforts with resistance because of bioethical considerations, , new discoveries have identified additional opportunities for stem cell therapies. For example, Freda Miller and Elaine Fuchs, working independently, discovered stem cells in different layers of the skin. These cells help the skin repair itself, and their discovery may have applications in treatments of skin disease and potentially other conditions, such as nerve damage.

Visual Connection

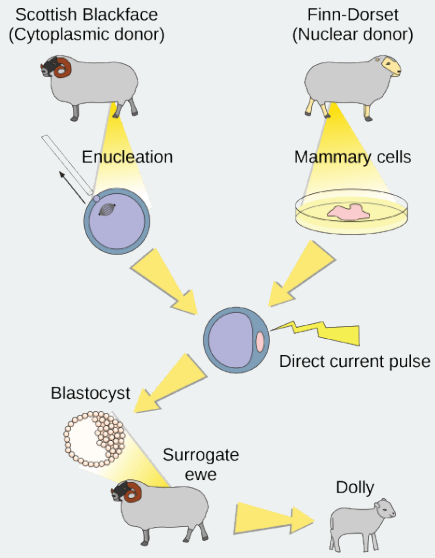


Figure 12.1.7: Dolly the sheep was the first mammal to be cloned. To create Dolly, they removed the nucleus from a donor egg cell. They then introduced the nucleus from a second sheep into the cell, which divided to the blastocyst stage before they implanted it in a surrogate mother. (credit: modification of work by "Squidonius"/Wikimedia Commons)

Do you think Dolly was a Finn-Dorset or a Scottish Blackface sheep?

Genetic Engineering

Genetic engineering is the alteration of an organism's genotype using recombinant DNA technology to modify an organism's DNA to achieve desirable traits. The addition of foreign DNA in the form of recombinant DNA vectors generated by molecular cloning is the most common method of genetic engineering. The organism that receives the recombinant DNA is a genetically modified organism (GMO). If the foreign DNA comes from a different species, the host organism is transgenic. Scientists have genetically modified bacteria, plants, and animals since the early 1970s for academic, medical, agricultural, and industrial purposes. In the US, GMOs such as Roundup-ready soybeans and borer-resistant corn are part of many common processed foods.

Gene Targeting

Although classical methods of studying gene function began with a given phenotype and determined the genetic basis of that phenotype, modern techniques allow researchers to start at the DNA sequence level and ask: "What does this gene or DNA element do?" This technique, reverse genetics, has resulted in reversing the classic genetic methodology. This method would be similar to damaging a body part to determine its function. An insect that loses a wing cannot fly, which means that the wing's function is flight. The classical genetic method would compare insects that cannot fly with insects that can fly, and observe that the non-flying insects have lost wings. Similarly, mutating or deleting genes provides researchers with clues about gene function. We collectively call the methods they use to disable gene function gene targeting. Gene targeting is the use of recombinant DNA vectors to alter a particular gene's expression, either by introducing mutations in a gene, or by eliminating a certain gene's expression by deleting a part or all of the gene sequence from the organism's genome.

Biotechnology in Medicine and Agriculture

It is easy to see how biotechnology can be used for medicinal purposes. Knowledge of the genetic makeup of our species, the

genetic basis of heritable diseases, and the invention of technology to manipulate and fix mutant genes provides methods to treat the disease. Biotechnology in agriculture can enhance resistance to disease, pest, and environmental stress, and improve both crop yield and quality.

Genetic Diagnosis and Gene Therapy

Scientists call the process of testing for suspected genetic defects before administering treatment genetic diagnosis by genetic testing. Depending on the inheritance patterns of a disease-causing gene, family members are advised to undergo genetic testing. For example, doctors usually advise people diagnosed with breast cancer to have a biopsy so that the medical team can determine the genetic basis of cancer development. Doctors base treatment plans on genetic test findings that determine the type of cancer. If inherited gene mutations cause the cancer, doctors also advise other female relatives to undergo genetic testing and periodic screening for breast cancer. Doctors also offer genetic testing for fetuses (or embryos with in vitro fertilization) to determine the presence or absence of disease-causing genes in families with specific debilitating diseases.

Gene therapy is a genetic engineering technique used to cure disease. In its simplest form, it involves the introduction of a good gene at a random location in the genome to aid the cure of a disease that is caused by a mutated gene. The good gene is usually introduced into diseased cells as part of a vector transmitted by a virus that can infect the host cell and deliver the foreign DNA (**Figure** 12.1.8**:**). More advanced forms of gene therapy try to correct the mutation at the original site in the genome, such as is the case with treatment of severe combined immunodeficiency (SCID).

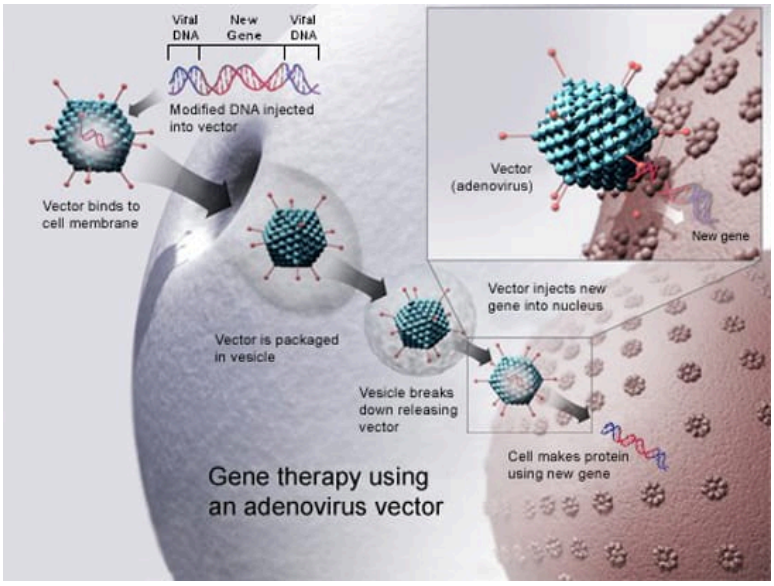


Figure
12.1.8**:**
Gene therapy using an adenovirus vector can be used to cure certain genetic diseases in which a person has a defective gene. To cure disease using an adenovirus vector, a new gene intended to replace a defective one is packaged with the adenovirus genome. The genes that make the virus pathogenic are removed. The modified DNA is put inside the virus' capsid, or protein coat. The person to be cured is infected with the modified virus. Viral DNA enters the nucleus, where the modified gene can replace the defective one.

(credit: NIH)

Production of Vaccines, Antibiotics, and Hormones

Traditional vaccination strategies use weakened or inactive forms of microorganisms to mount the initial immune response. Modern techniques use the genes of microorganisms cloned into vectors to mass produce the desired antigen. Doctors then introduce the antigen into the body to stimulate the primary immune response and trigger immune memory. The medical field has used genes cloned from the influenza virus to combat the constantly changing strains of this virus.

Antibiotics are a biotechnological product. Microorganisms, such as fungi, naturally produce them to attain an advantage over bacterial populations. Cultivating and manipulating fungal cells produces antibiotics.

Scientists used recombinant DNA technology to produce large-scale quantities of human insulin in *E. coli* as early as 1978. Previously, it was only possible to treat diabetes with pig insulin, which caused allergic reactions in humans because of differences in the gene product. In addition, doctors use human growth hormone (HGH) to treat growth disorders in children. Researchers cloned the HGH gene from a cDNA library and inserted it into *E. coli* cells by cloning it into a bacterial vector.

Transgenic Animals

Although several recombinant proteins in medicine are successfully produced in bacteria, some proteins require a eukaryotic animal host for proper processing. For this reason, the desired genes are cloned and expressed in animals, such as sheep, goats, chickens, and mice. We call animals that have been modified to express recombinant DNA transgenic animals. Several human proteins are expressed in transgenic sheep and goat milk, and some are expressed in chicken eggs. Scientists have used mice extensively for expressing and studying recombinant gene and mutation effects.

Transgenic Plants

Manipulating the DNA of plants (i.e., creating GMOs) has helped to create desirable traits, such as disease resistance, herbicide and pesticide resistance, better nutritional value, and better shelf-life (**Figure** 12.1.9**:**). Plants are the most important source of food for the human population. Farmers developed ways to select for plant varieties with desirable traits long before modern-day biotechnology practices were established.



Figure 12.1.9: Corn, a major agricultural crop used to create products for a variety of industries, is often modified through plant biotechnology. Photo shows corn cobs with different colors, including yellow, white, red, and a mixture of these colors. (credit: Keith Weller, USDA)

We call plants that have received recombinant DNA from other species transgenic plants. Because they are not natural, government agencies closely monitor transgenic plants and other GMOs to ensure that they are fit for human consumption and do not endanger other plant and animal life. Because foreign genes can spread to other species in the environment, extensive testing is required to ensure ecological stability. Staples like corn, potatoes, and tomatoes were the first crop plants that scientists genetically engineered.

Transformation of Plants Using *Agrobacterium tumefaciens*

Gene transfer occurs naturally between species in microbial populations. Many viruses that cause human diseases, such as cancer, act by incorporating their DNA into the human genome. In plants, tumors caused by the bacterium *Agrobacterium tumefaciens* occur by DNA transfer from the bacterium to the plant. Although the tumors do not kill the plants, they stunt the plants and they become more susceptible to harsh environmental conditions. *A. tumefaciens* affects many plants such as walnuts, grapes, nut trees, and beets. Artificially introducing DNA into plant cells is more challenging than in animal cells because of the thick plant cell wall.

Researchers used the natural transfer of DNA from *Agrobacterium* to a plant host to introduce DNA fragments of their choice into plant hosts. In nature, the disease-causing *A. tumefaciens* have a set of plasmids, Ti plasmids (tumor-inducing plasmids), that contain genes to produce tumors in plants. DNA from the Ti plasmid integrates into the infected plant cell's genome. Researchers manipulate the Ti plasmids to remove the tumor-causing genes and insert the desired DNA fragment for transfer into the plant genome. The Ti plasmids carry antibiotic resistance genes to aid selection and researchers can propagate them in *E. coli* cells as well.

The Organic Insecticide *Bacillus thuringiensis*

Bacillus thuringiensis (Bt) is a bacterium that produces protein crystals during sporulation that are toxic to many insect species that affect plants. Insects need to ingest Bt toxin in order to activate the toxin. Insects that have eaten Bt toxin stop feeding on the plants within a few hours. After the toxin activates in the insects' intestines, they die within a couple of days. Modern biotechnology has allowed plants to encode their own crystal Bt toxin that acts against insects. Scientists have cloned the crystal toxin genes from Bt and introduced them into plants. Bt toxin is safe for the environment, nontoxic to humans and other mammals, and organic farmers have approved it as a natural insecticide.

Flavr Savr Tomato

The first GM crop on the market was the Flavr Savr Tomato in 1994. Scientists used antisense RNA technology to slow the softening and rotting process caused by fungal infections, which led to increased shelf life of the GM tomatoes. Additional genetic modification improved the tomato's flavor. The Flavr Savr tomato did not successfully stay in the market because of problems maintaining and shipping the crop.

Summary

Biotechnology uses living organisms, cells, or DNA to develop useful products and technologies in medicine, agriculture, and industry. Molecular biologists first extract DNA or RNA from cells, then analyze it using techniques such as gel electrophoresis, which separates nucleic acids by size in an electric field. PCR (polymerase chain reaction) amplifies specific DNA sequences for research, disease diagnosis, and genetic testing, while Southern and Northern blotting identify specific DNA or RNA sequences. Molecular cloning involves inserting a gene into a plasmid vector, creating recombinant DNA that can be introduced into bacteria to produce proteins such as human insulin. In contrast, reproductive cloning creates a genetically identical organism through somatic cell nuclear transfer, as demonstrated by Dolly the sheep. Biotechnology also includes genetic engineering, which produces genetically modified organisms (GMOs) with beneficial traits. Medical applications include gene therapy, vaccines, antibiotics, and recombinant hormones, while agricultural applications include pest-resistant, herbicide-tolerant, and higher-yield crops. Together, these techniques have transformed biological research, healthcare, and food production.

Key Terms

adaptation
: characteristic that improves an organism's ability to survive and reproduce in a specific environment

adenovirus
: a virus commonly used as a vector in gene therapy to deliver a functional gene into human cells

agrobacterium tumefaciens
: a bacterium that naturally transfers DNA into plant cells and is used by scientists to genetically modify plants

ampicillin Resistance Gene
: a gene carried on plasmids that allows bacteria containing the plasmid to survive in the presence of the antibiotic ampicillin

antibiotics
: substances produced by microorganisms or made using biotechnology that inhibit or kill bacteria

antisense RNA
: a strand of RNA designed to bind to a specific mRNA and block protein production

bacillus thuringiensis (Bt)
: a bacterium that produces proteins toxic to certain insects and is used as a natural insecticide

BioTech boom
: a period of rapid growth in biotechnology research and industry following early successes such as synthetic insulin

biotechnology
: the use of biological systems or organisms to develop products or technologies for medicine, agriculture, or industry

blotting
: a laboratory technique that transfers DNA or RNA from a gel onto a membrane for detection

capsid
: the protein coat of a virus that encloses its genetic material

cellular cloning
: the production of genetically identical cells through asexual reproduction, such as bacterial cell division

cDNA (Complementary DNA)
: DNA synthesized from an RNA template using reverse transcriptase

cloning vector
: a DNA molecule, such as a plasmid, used to carry foreign DNA into a host cell

conjugation
: a process by which bacteria exchange genetic material through direct contact

denaturation
: the separation of DNA strands by heating during PCR

DNA extraction
: the process of isolating DNA from cells for analysis or manipulation

DNA ligase
: an enzyme that joins DNA fragments together by forming phosphodiester bonds

electrophoresis (gel electrophoresis)
: a technique that separates DNA or RNA fragments based on size using an electric field

enucleated egg
: an egg cell that has had its nucleus removed

Flavr Savr Tomato
: the first genetically modified crop sold commercially, engineered to slow ripening

foreign DNA (Transgene)
: DNA introduced into an organism from another species

gene targeting
: the use of biotechnology to alter or disable specific genes to study their function

gene therapy
: a medical technique that treats disease by adding, removing, or altering genes in a patient's cells

genome
: the complete set of DNA in an organism

genetic diagnosis
: testing used to identify genetic mutations associated with disease

genetic engineering
: the direct modification of an organism's DNA using biotechnology

genetically Modified organism (GMO)
: an organism whose DNA has been altered using genetic engineering techniques.

hybridization
: the binding of a labeled DNA or RNA probe to a complementary sequence

insulin
: a hormone involved in blood sugar regulation that is produced using recombinant DNA technology

lac Z gene
: a gene used in cloning experiments to identify bacteria that contain inserted DNA

lysis buffer
: a detergent-containing solution used to break open cells during DNA extraction

messenger RNA (mRNA)
: RNA that carries genetic instructions from DNA to ribosomes for protein production

microbiome (microbiota)
: the community of microorganisms that normally live in or on the body

molecular cloning
: the process of copying specific DNA fragments using plasmids and host cells

multiple cloning site (MCS)
: a region of a plasmid containing multiple restriction enzyme cut sites

northern blotting
: a technique used to detect specific RNA sequences

parthenogenesis
: a form of reproduction in which an organism develops from an unfertilized egg

PCR (polymerase chain reaction)
: a technique used to amplify specific DNA sequences

phosphodiester bond
: the chemical bond that links nucleotides together in DNA and RNA

plasmid
: a small, circular DNA molecule used as a vector in cloning

polymerase (Taq polymerase)
: an enzyme used in PCR to synthesize DNA at high temperatures

primer
: a short DNA sequence that initiates DNA synthesis during PCR

recombinant DNA
: DNA created by combining genetic material from different sources

recombinant protein
: a protein produced from recombinant DNA

regulation
: the control of biological processes to maintain proper function

reverse genetics
: a research approach that starts with a gene sequence and studies its function by altering it

reverse transcriptase
: an enzyme that converts RNA into DNA

RT-PCR (reverse transcriptase PCR)
: a technique used to amplify DNA made from RNA templates

selective media
: growth media containing antibiotics used to identify cells that have taken up plasmids

somatic cell nuclear transfer (SCNT)
: a cloning technique in which a nucleus from a body cell is transferred into an enucleated egg

southern blotting
: a method used to detect specific DNA sequences

sticky ends
: single-stranded DNA overhangs that allow DNA fragments to join together

therapeutic cloning
: cloning used to produce stem cells for medical treatment rather than to create an organism

Ti plasmid
: a tumor-inducing plasmid from *Agrobacterium tumefaciens* used to genetically modify plants

transformation
: the process of introducing foreign DNA into a cell

transgenic animal
: an animal that carries and expresses foreign DNA

transgenic plant
: a plant genetically modified to contain DNA from another species

vector
: a DNA carrier used to transfer genetic material into a host cell

western blotting
: a technique used to detect specific proteins using antibodies

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12.2: PCR and Gel Electrophoresis

Learning Objectives

At the completion of this PCR lesson, learners will be able to:

- List the 5 chemical components of a PCR reaction and describe their roles.
- List the functions of the 3 temperature cycles which are repeated during a PCR reaction.
- Describe the process of observing results and interpreting results of a PCR experiment.
- List possible uses of PCR in genetic testing and in research.

Introduction

The polymerase chain reaction laboratory technique is used in a variety of applications to make copies of a specific DNA sequence. This lesson describes how a PCR reaction works, what it accomplishes, and its basic requirements for success. Examples of interpreting results are given. PCR's strengths, weaknesses, and applications to plant biotechnology are explained.

The Discovery of PCR

In 1983, Kary Mullis was driving along a Californian mountain road late one night. As a molecular biologist, Dr. Mullis was imagining a better way to study DNA. This late-night thinking led to a revolutionary way to make laboratory copies of DNA molecules (Saiki et al. 1985, Mullis 1990). In the decades since, the polymerase chain reaction or PCR, has become the standard method used for detecting specific DNA or RNA sequences. Selling the equipment and reagent kits for PCR is a multi-billion-dollar business because DNA and RNA detection is critical information in many applications.

In Vitro vs. *In Vivo* Replication

PCR is an *In Vitro* process; a series of chemical reactions that happen outside of a living cell. This laboratory technique is modeled after an In vivo process, the living cell's natural ability to replicate DNA during normal cell cycles (see Lesson on DNA: The Genetic Material). Every living cell makes a duplicate copy of each chromosome before the cell is ready to divide. **Figure** 12.2.1 below illustrates the key parts of *In vivo* DNA replication that are the basis for PCR success.

There are other enzymes that play an important role in in vivo replication. However, PCR works as an *in vitro* DNA replication process by using just one of these enzymes. Mullis imagined a chemical reagent and a temperature change step in the method that could perform the work of the other two enzymes. It should be noted that because Dr. Kerry Mullis had learned about the details of *in vivo* DNA replication, he could create this science changing *in vitro* method.

Before reading the description of PCR components and processes, watching this video can help you visualize the importance of each step,

Name and Chemical Components of PCR

The name 'Polymerase Chain Reaction' represents the nature of the process. 'Polymerase' because DNA Polymerase III is required for constructing new DNA strands, just like in a living cell. 'Chain Reaction' describes repeating cycles of replication which target a specific segment of a chromosome and use a "copy the copies" progression each cycle that doubles the amount of DNA copies of a specific segment of DNA present each cycle. In just 20 cycles of the chain reaction, over one million (2^{20}) copies of that specific segment of DNA can be produced. This is enough DNA to see with your naked eye. The goal of PCR is to make millions of copies of a specific segment of DNA that all originate from a single DNA sample.

The five chemical components that must be added to a test tube for the PCR reaction to work, include a DNA template, DNA polymerase III enzyme, single stranded DNA primers, nucleotides, and reaction buffer.

1. The DNA template is a sample of DNA that contains the target sequence of DNA for copying.
2. DNA pol III. There are two requirements for a suitable DNA polymerase enzyme for PCR. First, the enzyme must have a good activity rate around 75°C. Second, the enzyme should be able to withstand temperatures of 95-100°C without denaturing and losing activity.
3. Two primers. Primers are short oligonucleotides of DNA, usually around 20 base pairs in length. Because the purpose of PCR is to amplify a specific section of DNA in the genome, such as a known gene, then primers of specific sequences must be used. The geneticist planning the PCR reaction will design a forward primer to bind to one strand and a reverse primer that complements and binds to the other strand. The primer design process to select forward and reverse primers is requiring appropriate genetics thinking and is describe later in this reading.
4. The four different deoxyribonucleotide triphosphates (dNTPs). Adenine (A), guanine (G), cytosine (C), and thymine (T) are needed to provide the building blocks for DNA replication. DNA polymerase will add each complentary base to the new growing DNA strand according to the original strand's sequence following normal A-T and C-G pairings.
5. Finally, a reaction buffer. This creates a stable pH and provides the Mg^{2+} cofactor needed for DNA pol III activity.

Three Temperature Cycles

A key insight to the success of PCR as an in vitro DNA replication process which generates millions of specific sequence copies was a three-temperature cycle which accomplish three parts of DNA replication: denaturation of the double stranded template, annealing of the primers to the single strands and extension of new strand synthesis by DNA pol III.

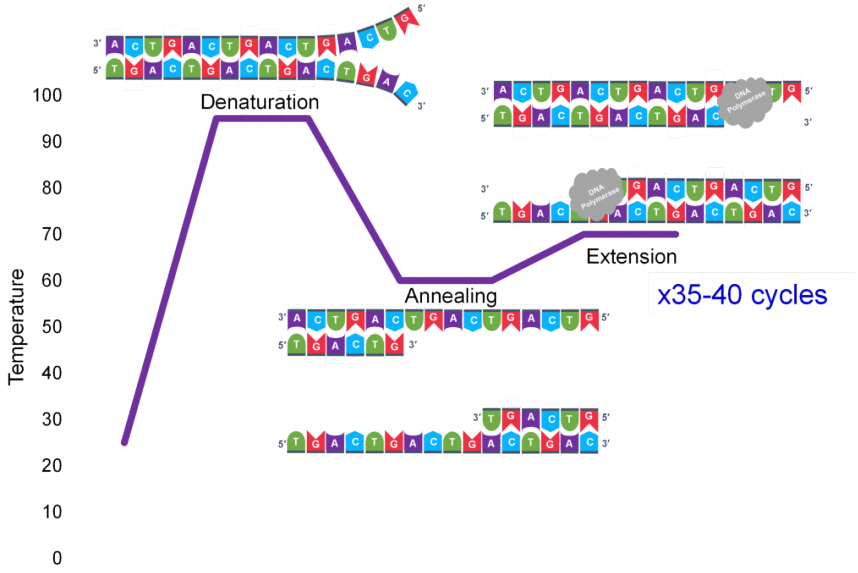


Figure 12.2.1: DNA strands during a PCR cycle. Under high temperature, denaturation occurs and "unzips" the strands. Annealing occurs at a much lower temperature, and extension happens at a temperature between the two. Image by Marjorie Hanneman.

In general, a single PCR run will undergo 25-35 cycles. The first step for a single cycle is the denaturation step, in which the double-stranded DNA template molecule (**Figure** 12.2.2) is made single-stranded(**Figure** 12.2.3). The temperature for this step is typically in the range of 95-100°C, near boiling. The high heat breaks the hydrogen bonds between the strands but does not break the sugar-phosphate bonds that hold the nucleotides of a single strand together (**Figure** 12.2.3).



Figure 12.2.2: DNA template in the PCR reaction test tube is a double stranded molecule. The targeted sequence of nucleotides for this illustration is shown. The template DNA could be fragments from the chromosome of various lengths. The 5′ and 3′ orientation of both strands is shown. Image by Marjorie Hanneman.

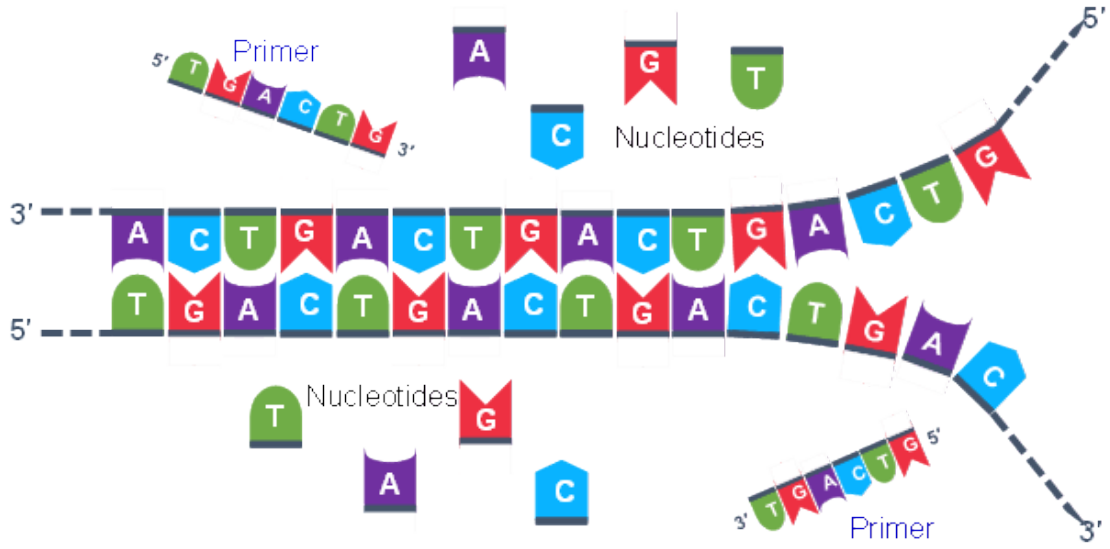


Figure 12.2.3: Denaturation: The hydrogen bonds holding the strands of the double stranded template together are broken by heating the test tube. Image by Marjorie Hanneman and Donald Lee.

Thousands of copies of the single stranded primers and the individual nucleotides were added to the test tube prior to beginning the cycles. Both the primers and nucleotides will become part of the new DNA strands. The second step in the PCR reaction is to cool the temperature in the test tube to 45-55°C. This is the primer annealing step in which the primers bind to complementary sequences in the single-stranded DNA template. The two primers are called the forward and the reverse primer and are designed because their sequences will target the desired segment of the DNA template for replication (**Figure** 12.2.4).

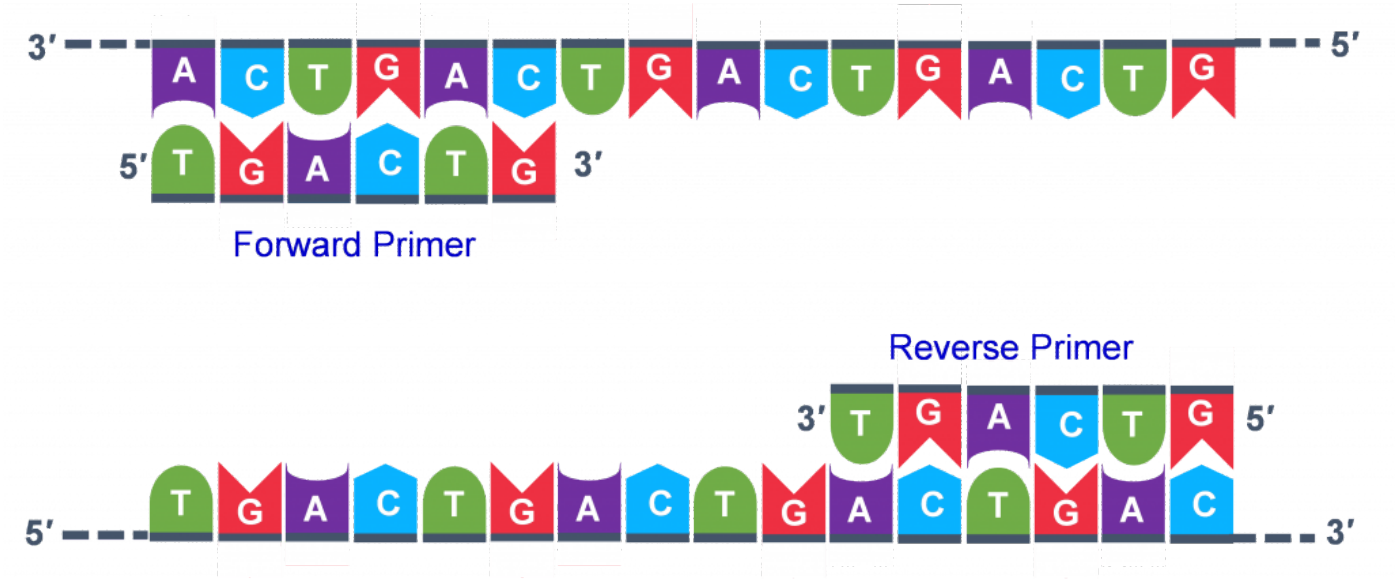


Figure 12.2.4: Annealing: The second step of PCR is to cool the test tube which allows the forward and reverse primer to bind to the template. The image above shows the forward primer one strand, and the reverse primer on the other strand. Image by Marjorie Hanneman and Donald Lee.

The geneticist planning the PCR analysis must “design” the forward and reverse primers and then buy them from a vendor who can synthesize single stranded DNA that has a specific sequence and length. The two most important criteria for primer design are the following.

1. One primer must have a sequence that complements one of the template strands and the other primer must be complementary to the other strand. BOTH strands need to be primed for the replication process.
2. The primers must bind so that their 3′ ends are ‘pointing’ in the direction of the other primer. This ensures that the sequence between the primers is replicated in the PCR cycles.

Extension: The final PCR step is when the DNA polymerase enzyme reads the template and connects new nucleotides to the primer's 3' end, extending a new complementary strand of DNA (**Figure** 12.2.5). Completion of the final step and the first cycle of PCR, will make two double stranded DNA copies from the original template DNA, doubling of the amount of DNA present. The test tube is heated to around 75°C, optimizing DNA pol. III activity and the newly synthesizing DNA strand is extended as the template strand is read by DNA pol. III. The Extension step will run for a few minutes and this step completes one PCR cycle.



Figure 12.2.5: Extension: This step is catalyzed by DNA pol III. The time allowed for this step determines how much of the template DNA pol III can read and replicate. The image shows DNA polymerase synthesizing DNA on both strands.Image by Marjorie Hanneman.

For cycle 2, the denaturation, annealing, and extension steps are repeated (**Figure** 12.2.6**a, b, and c**). This time, though there will be twice as many DNA template molecules compared to what there was at the beginning of cycle 1. Copies are being made by reading the original template and copies are made by reading the copies made in the previous cycle. Therefore, if everything is working correctly, the DNA replication in the test tube is a chain reaction where at the end of a cycle, there is double the amount of that DNA sequence as what was found at the beginning of the cycle.



Figure 12.2.6**a:** Cycle 1 is complete; two double stranded DNA molecules are made from the original double stranded template. Image by Marjorie Hanneman and Donald Lee.

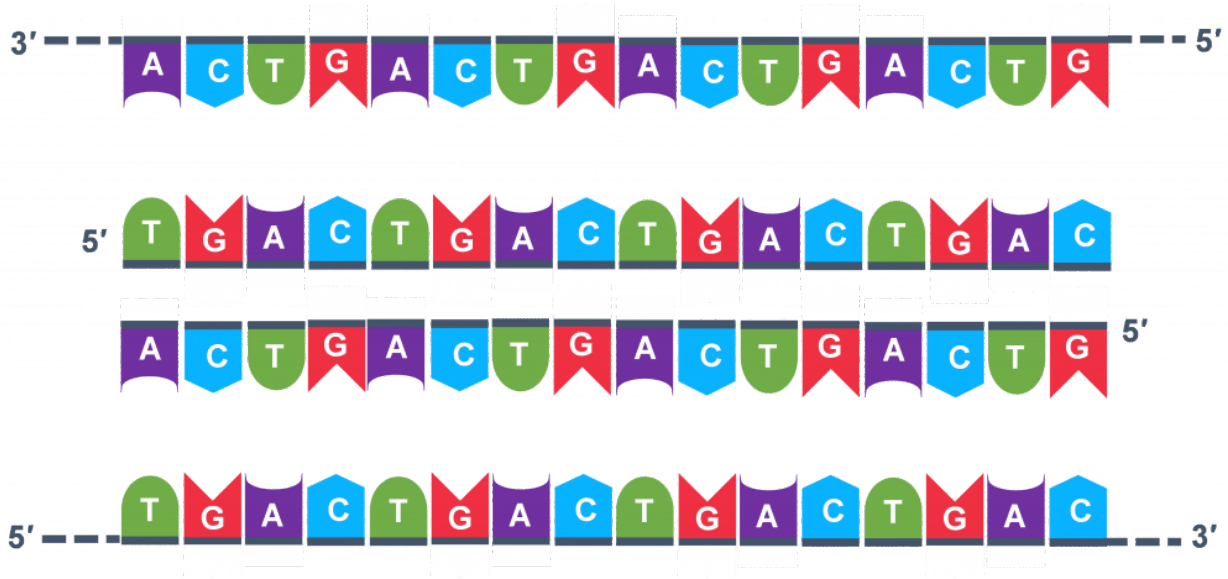


Figure 12.2.6b: Denaturation step generates four single strand templates. Image by Marjorie Hanneman

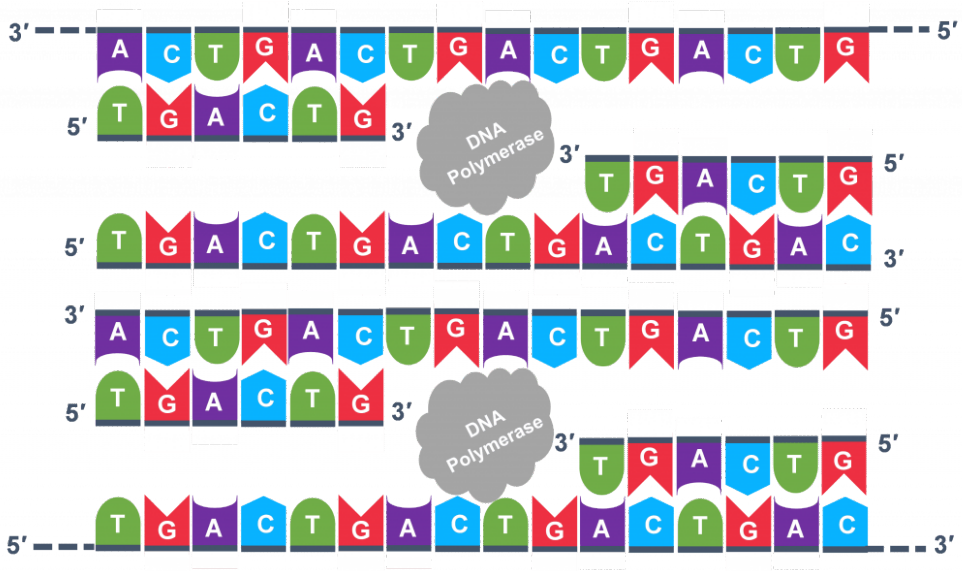


Figure 12.2.6c: Annealing and Extension. All templates are primed and four 3′ ends provide a place of DNA pol III extension. Image by Marjorie Hanneman

Because thousands of copies of the forward and reverse primer are added at the start of PCR, all the single strand templates, both the original, the copies in cycle 3 and beyond, and the copies of the copies made from previous cycles will be primed for the extension step of the cycles.

Thermal cycler



Figure 12.2.7: The thermal cycler is used to carry out PCR reaction. Image by Walter Suza.

When PCR was first invented, scientists used water baths set at different temperatures and the hand transfer of test tubes at timed intervals to run the PCR reaction. Once the technique became a proven technology for DNA analysis, engineers went to work to create PCR machines. The instrument which heats and cools the DNA samples is called a thermal cycler (**Figure** 12.2.7). Each small tube or sample well in a plate contains all the chemical components needed for a PCR reaction. Adding a specific sample to the reaction mix provides the template DNA. A thermal cycler can be programmed for specific temperatures and the amount of time spent at each temperature. The engineered design of thermal cyclers to maximize the accurate replication of the targeted DNA in a small sample volume with the minimum amount of time can be critical in many applications of PCR.

Taq DNA polymerase

When Dr. Kerry Mullis ran the first PCR experiments, he needed to add a new sample of DNA pol III after each denaturation step. This was because the high temperature needed to denature the double stranded DNA template also denatured the DNA pol III protein structure. The DNA pol III enzyme commonly available to molecular geneticists was from E. coli bacteria and this enzyme had no stability at near boiling temperatures. Fortunately, biologists had been investigating Thermus aquaticus, (Taq) a thermophilic eubacterium found in hot springs (Chien et al. 1976). The Taq version of DNA pol III does not easily denature in the hot temperatures required in PCR; plus, it has a good efficiency, able to add 60 base pairs/sec at 70°C. Like all other DNA polymerases, Taq DNA pol III cannot begin DNA replication without the addition of a starting primer. Thus, the discovery of Taq DNA pol III and the commercial availability of this enzyme made PCR a more reliable and doable technology which hastened its application to science investigation and diagnostic testing.

Visualizing the Results with Electrophoresis

Once a PCR reaction has been completed, we need to be able to see the results. To do this, a sample of the PCR mixture is loaded into an agarose gel for electrophoresis. The agarose gel contains a matrix of pores which enables it to separate DNA fragments based on their sizes. For details about setting up and running an electrophoresis gel, see Electrophoresis: How Scientist observe fragments of DNA(opens in new window)

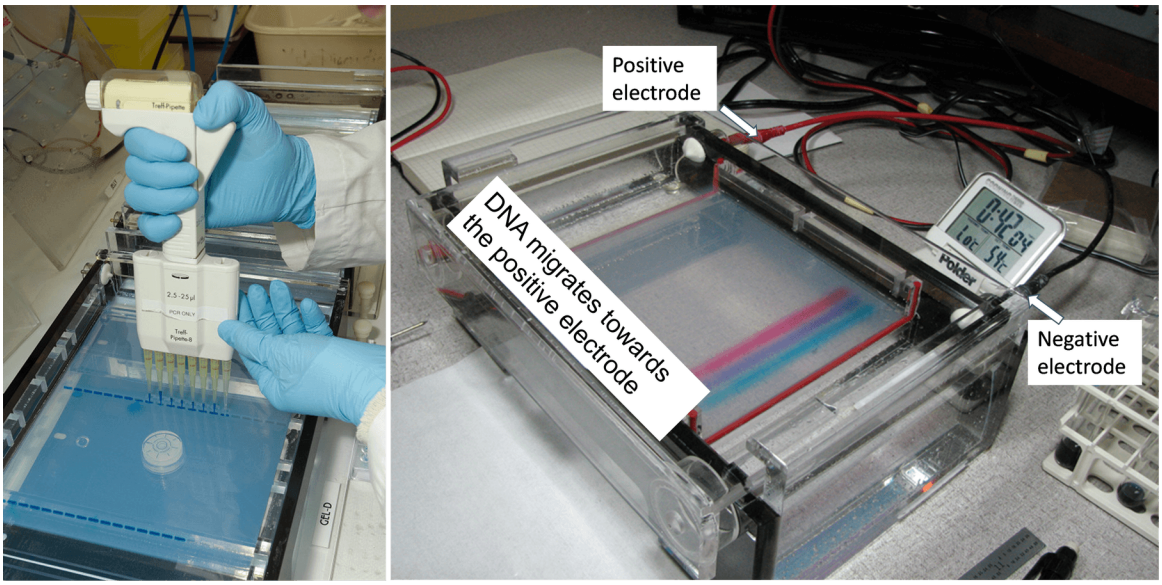


Figure 12.2.8: Electrophoresis: A gel electrophoresis set-up with agarose gel with DNA and loading dye on the left and the power supply on the right. The image on the left shows a scientist loading a gel, and the right shows the complete electrophoresis setup with positive and negative electrode. DNA migrates towards the positive electrode. Image Source: Michael, CC BY 2.0, via Wikimedia Commons and U. S. Department of Agriculture, CC BY 2.0, via Wikimedia Commons.

Figure 12.2.8 shows a picture of a gel electrophoresis gel that is running. The box on the right contains DNA loaded in the agarose gel. The gel placed in an aqueous solution of electrolytes. Depending on the type of dye used, color bands are a dye that was added to the PCR sample before it was loaded into the sample well. This allows for the tracking of the DNA's progression through the gel. Hooked up to this gel unit is an electrical power source which provides the force to move the DNA through the gel. Since DNA molecules are negatively charged, they will migrate towards the red, positive electrode. Shorter DNA fragments move faster than longer fragments through the pores in the gel.

After the gel is run, the DNA is stained with a chemical that binds specifically to DNA molecules and then will either reflect a specific color of visible light or fluoresce a specific color when viewed with ultraviolet light. A single 'band' contains 1000s of individual DNA fragments, all of the same length. **Figure** 12.2.10 illustrates the visual information that can be obtained from an electrophoresis gel after it has run. The electric current uniformly moves all the DNA fragments through a gel in the same direction. The sample wells at the top of the gel image thus establish lanes for the DNA samples to move.

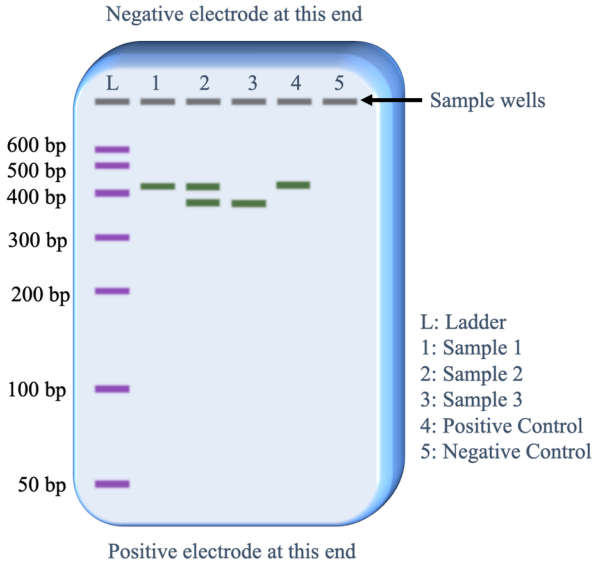


Figure 12.2.10 Depiction of an electrophoresis gel with six sample wells that were loaded with either a DNA size ladder (lane L) or a sample from a PCR run (1-5.) The gel was subjected to a DNA staining dye. Image by Marjorie Hanneman.

Below is a description of what information is revealed from each lane.

- Lane L: This was loaded with the DNA size ladder that contains copies of seven different lengths of DNA fragments. Commercial vendors of the DNA size ladder provide information on the lengths of each fragment in base pairs (bp). Running this lane provides an estimate of the DNA fragment lengths in the sample lanes (1-5).
- Lane 1: The PCR sample loaded in this lane has copies of a single length of DNA. The length is slightly more than 400 base pairs.
- Lane 2: The PCR sample loaded in this lane has copies of two lengths of DNA. One fragment is the same length as the fragments in lane 1 and the second fragment is slightly less than 400 bp.
- Lane 3: The PCR sample loaded in this lane has copies of one fragment that is the same length as the shorter fragments in lane 2.
- Lane 4: The positive control worked as predicted. The sample was set up with all the same reagents as the other PCR samples plus a DNA template that was known to contain the sequence targeted by the PCR primers that would generate a 410 base pair fragment.
- Lane 5: The negative control worked as predicted. The sample was set up with all the same reagents as the other PCR samples except no template DNA was added. Therefore, we would not expect the PCR reaction to work and the absence of a band of DNA fragments is as expected.

Because the positive and negative controls worked as expected, the biologist can be confident that the bands of DNA observed in lanes 1,2 and 3 reveal genetic information about the individual providing that DNA sample.

Advantages of PCR

PCR quickly became the method of choice for many types of DNA analysis because of several advantages over other DNA detection methods. The first; it is a simple procedure to set up and run. Fewer steps save time in getting a DNA analysis result. The second is the sensitivity. A very small amount of template DNA in the sample can be detected. Even just a few skin cells from one human hair contain enough DNA, making PCR useful in forensics. The third is that it can be designed to accurately differentiate genetic samples that are different by as little as single nucleotide in the targeted sequence. Finally, in application where large numbers of samples need to be subjected to the same PCR analysis, automation and robotic assistance allow the processing of many samples in a very short time. For example, an automated PCR would be critical for testing large numbers of people in hours during a pandemic.

Limitations of PCR

There are some drawbacks of using PCR that one should be aware of as well. First, the sequence of the gene or chromosome region being targeted is required. This limitation is rapidly diminishing as gene and genome sequencing technology and sharing of this sequence through Internet data bases has emerged as the norm in genetic analysis. Because of the size of the genomes of living things and a high conservation of gene sequences in many organisms, primers designed for a PCR test must be empirically tested with the proper controls. Biologists can easily generate false positive DNA from PCR that is caused by contamination or lack of specificity in primer design. There may also be a need to optimize concentrations of each chemical component. For example, changing the amount of DNA template, MgCl2 and Taq polymerase can affect both the quantity and quality of bands produced. Some studies have shown that even the brand of Taq polymerase can affect results (Holden et al. 2003). Likewise, the temperature cycles may need to be fine-tuned for a specific PCR test. Finally, as an in vitro DNA replication method, PCR cannot replicate entire chromosomes.

Summary

To briefly highlight the topics of this lesson, remember that PCR is a relatively easy lab technique which amplifies the amount of DNA present, much like living cells do in the beginning stages of a cell cycle. There are 5 chemical components of a PCR reaction: a DNA template, a DNA polymerase, primers, nucleotides, and a buffer. The 3 temperature steps for one cycle are the denaturation, primer annealing and extension steps. There are now many variations and uses of PCR ranging from forensics to genomic studies to identifying transgenic crops.

Key Terms

Polymerase Chain Reaction (PCR)
A laboratory technique used to amplify (make millions of copies of) a specific DNA sequence.

DNA template
The original DNA sample that contains the target sequence to be copied during PCR.

DNA polymerase
The enzyme that synthesizes new DNA strands by adding complementary nucleotides to a growing DNA strand.

Taq DNA polymerase
A heat-stable DNA polymerase isolated from *Thermus aquaticus* that remains active during PCR's high temperatures.

Primers
Short, single-stranded DNA sequences that bind to complementary regions of the template DNA and provide a starting point for DNA synthesis.

Forward primer
The primer that binds to one DNA strand and initiates DNA synthesis in one direction.

Reverse primer
The primer that binds to the complementary DNA strand, allowing replication of the opposite strand.

dNTPs (deoxyribonucleotide triphosphates)
The four DNA building blocks (A, T, C, and G) used to synthesize new DNA strands.

Reaction buffer
A solution that maintains the proper pH and provides magnesium ions (Mg^{2+}), which are required for DNA polymerase activity.

Denaturation
The first PCR step (95–100°C), where double-stranded DNA separates into single strands.

Annealing
The second PCR step (45–55°C), where primers bind to complementary sequences on the template DNA.

Extension (Elongation)
The third PCR step (~72–75°C), where DNA polymerase synthesizes new DNA strands by adding nucleotides.

PCR cycle
One complete round of denaturation, annealing, and extension that doubles the amount of target DNA.

Amplification
The exponential increase in the number of copies of a specific DNA sequence through repeated PCR cycles.

Electrophoresis (Gel electrophoresis)
A laboratory technique that separates DNA fragments by size using an electric field and an agarose gel.

Positive control
A PCR sample known to contain the target DNA sequence, confirming that the reaction worked correctly.

Negative control
A PCR sample lacking template DNA, used to detect contamination or false-positive results.

Target sequence
The specific DNA region selected for amplification by PCR.

Specificity
The ability of PCR primers to amplify only the intended DNA sequence.

Sensitivity
PCR's ability to detect very small amounts of DNA, even from tiny biological samples.

False positive
An incorrect result indicating the presence of target DNA when it is actually absent.

Lesson Activities

Watch these videos to learn more about PCR:

- Polymerase Chain Reaction (PCR) from DNA Learning Center(opens in new window)
- Polymerase Chain Reaction (from UNL)(opens in new window)

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12.3: 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.

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

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** 12.3.1).

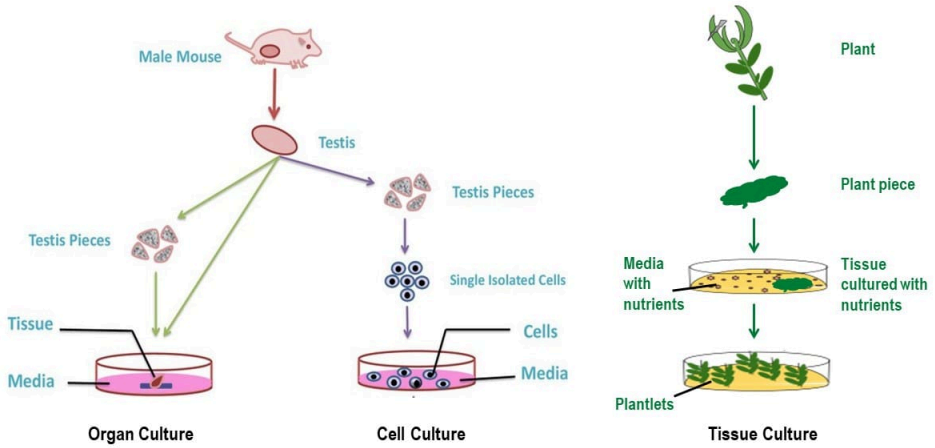


Figure 12.3.1**:** Cell vs. Organ vs. Tissue Culture. In organ culture, a tissue, like the testis, is isolated from an animal (such as a male mouse) and cut into pieces (testis pieces). The resulting tissue is placed into culture dishes with media for maintenance of the tissue. In cell culture, the tissue 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)

Types of Cultures

Adherent Culture

Adherent culture is a type of *in vitro* culture in which cells grow on a surface as a single layer of cells (**Figure** 12.3.2). 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

Suspension culture is a type of *in vitro* culture in which the cells grow suspended within the culture medium; no adherence is required (**Figure** 12.3.2). 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.

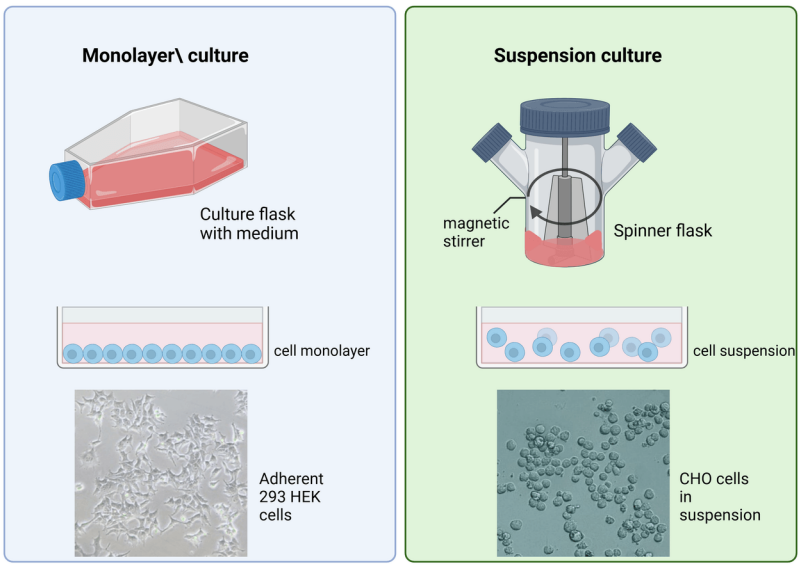


Figure 12.3.2: Adherent vs. Suspension Culture. In adherent culture (blue box on left), cells are grown specially-treated plastic-ware, like a culture flask, whose surfaces promote cell adherence (culture flask with medium). The cells in adherent culture will grow as a cell monolayer. Adherent 293 HEK 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

Organ culture is 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

Tissue culture is 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 12.3.1 below lists some of the more commonly cells cultured cells.

Table 12.3.1: ***Commonly Culture Cells***

Cell Abbreviation	Cell Name	Description
3T3	"**3**-day **T**ransfer **3**00,000 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
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 *in 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** 12.3.2 below compares a cell line with a cell strain.

Table 12.3.2*: 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** 12.3.3 below outlines some of the advantages and disadvantages of immortalized cells.

Table 12.3.3*: 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

Advantages	Disadvantages
well-studied and widely used in research; extensive data available on their growth, behavior, genetic profile, and functional characteristics	prone to contamination with mycoplasma
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** 12.3.3) 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 12.3.3: 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 12.3.4 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 12.3.4*: 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** 12.3.5. Biological labs are classified using these BSLs.

Table 12.3.5*: 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

Summary

Cell culture is the process of growing cells outside of an organism under controlled laboratory conditions, allowing researchers to study cell behavior, disease, development, and drug responses. Different types of culture include adherent cultures, in which cells attach to a surface; suspension cultures, in which cells grow freely in liquid media; organ culture, which maintains intact tissues or organs; and plant tissue culture. Cell cultures may begin as primary cultures, which contain a mixture of cells directly isolated from tissues, and can be expanded into more uniform cell lines or single-cell-derived cell strains. Some cell lines become immortalized through genetic changes, allowing indefinite growth and making them valuable for research, despite differing from normal cells. Successful cell culture requires carefully controlled temperature, pH, carbon dioxide, humidity, and nutrient-rich media. Researchers also follow strict biosafety practices, including appropriate personal protective equipment (PPE), biosafety cabinets, and Biosafety Level (BSL) guidelines to prevent contamination and protect personnel. Cell culture remains an essential tool in molecular biology, biotechnology, medicine, vaccine development, and pharmaceutical research.

Key Terms

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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12.4: 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.

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

Concept in Action:

Video: Genetic Engineering

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 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.

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** 12.4.1). 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.

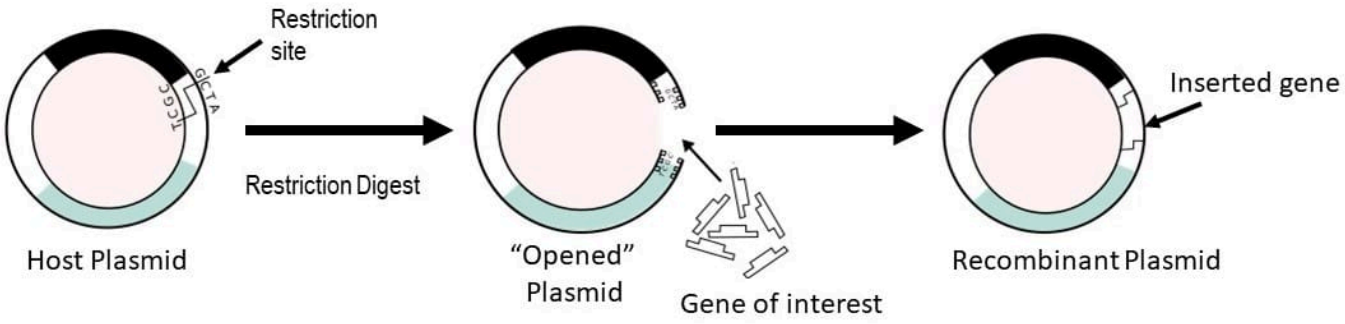


Figure 12.4.1: Creating recombinant DNA (rDNA) through DNA cloning. Left image: A host plasmid can be cut open or "cleaved" at a specific sequence of DNA (i.e., a restriction site) using a technique called a restriction digest. Center image: The restriction digest creates an "opened plasmid" into which 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** 12.4.2):

- 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** 12.4.2). 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.

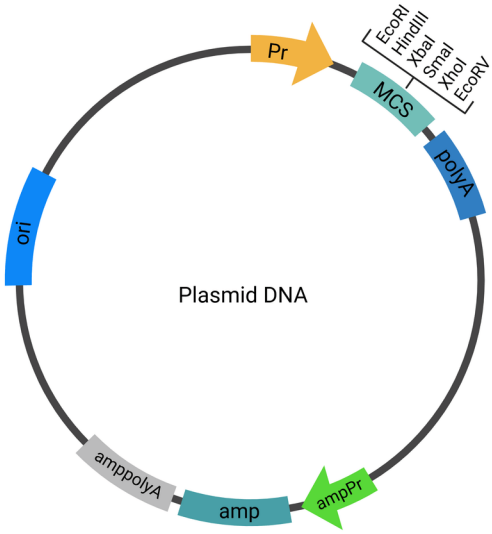


Figure 12.4.2: The plasmid. A plasmid is a small, circular piece of DNA that can be used for the insertion of pieces of DNA. Both cloning and expression plasmids have several basic regions in common. The multi-cloning site (MCS), comprised of multiple restriction sites (e.g. EcoRI, HindIII, XbaI, SmaI, 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** 12.4.3). The way a restriction enzyme cuts the DNA helix will determine how it is inserted into a vector, like a plasmid.

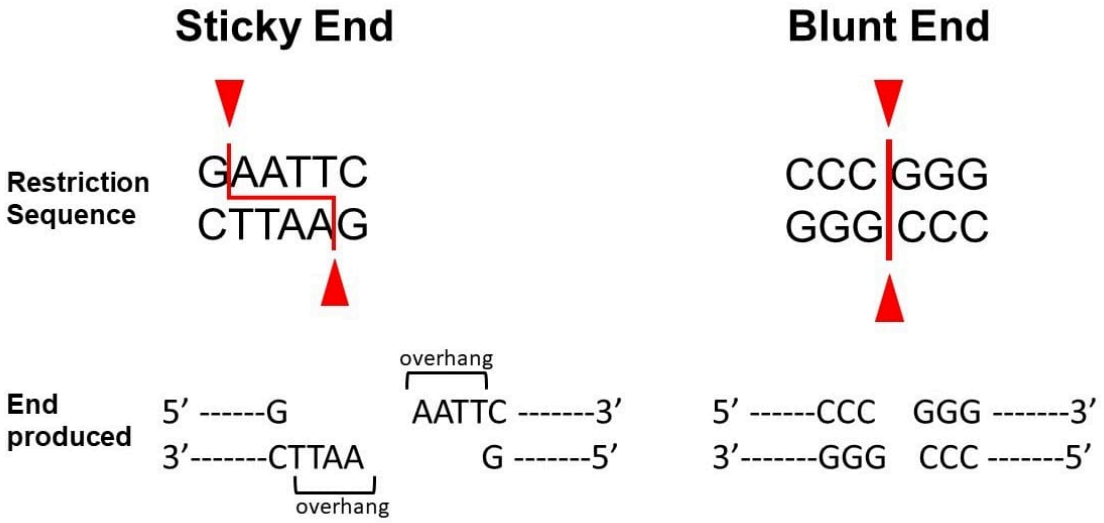


Figure 12.4.3**:** Sticky vs. Blunt ends. Restriction enzymes may cut the DNA of a restriction sequence (red arrows and lines) to produce overhangs known as "sticky ends" or blunted ends known as "blunt ends". The sticky end is a single-stranded DNA overhang (e.g., TTAA and AATT) that forms because the 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** 12.4.1 below.

Table 12.4.1: ***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'

Summary

Genetic engineering is the direct modification of an organism's DNA using laboratory techniques, allowing scientists to add, remove, or alter specific genes more quickly and precisely than traditional breeding. A major milestone was the development of recombinant human insulin, first produced in genetically engineered *E. coli* and approved for medical use in 1982, demonstrating the potential of biotechnology to produce important therapeutic proteins. Central to genetic engineering is recombinant DNA (rDNA) technology, in which a gene of interest is inserted into a DNA vector, such as a plasmid, and introduced into a host cell for replication or protein production. Larger DNA fragments can be cloned using cosmids or bacterial artificial chromosomes (BACs). Restriction enzymes, often called molecular scissors, recognize specific DNA sequences and cut DNA to create sticky or blunt ends that facilitate gene insertion. Together with techniques such as PCR, DNA sequencing, electrophoresis, and transformation, these tools enable advances in medicine, agriculture, research, and biotechnology.

Key Terms

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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12.5: 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.

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

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).

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).

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 12.5.1 and **Figure** 12.5.2 below shows such a directional cloning strategy using the restriction enzymes HindIII and PstI. In the first part of the procedure shown in **Figure** 12.5.1, 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).

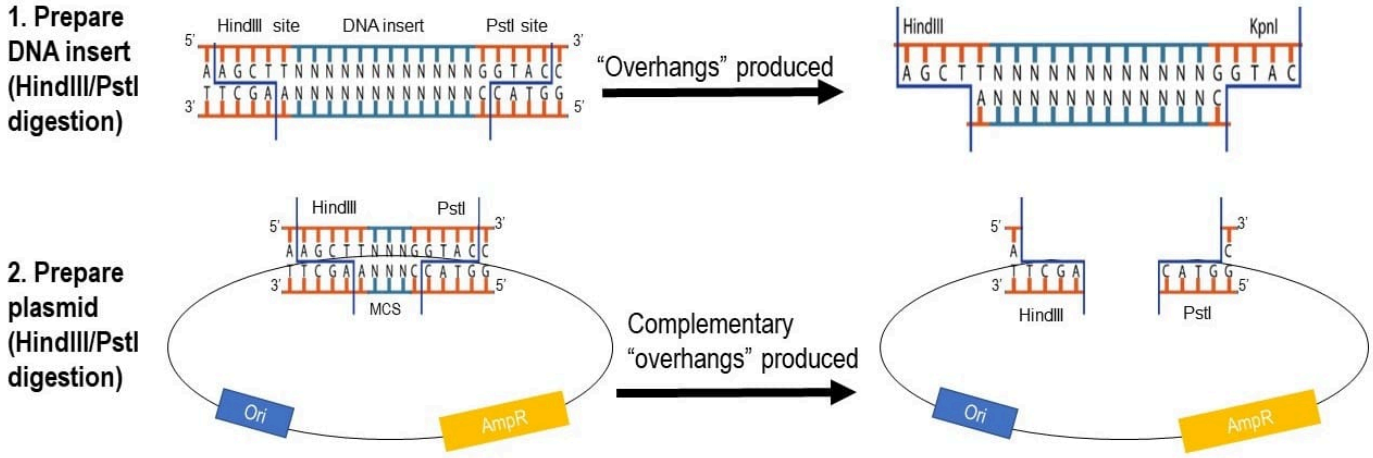


Figure 12.5.1: A directional cloning strategy: Part 1. The first steps of a directional cloning strategy using the restriction enzymes HindIII and PstI are: (1) Prepare the DNA insert (HindIII/PstI digestion) - digestion of insert DNA with HindIII and PstI produces overhangs of AGCT and GTAC, respectively; (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** 12.5.2**)**. 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.

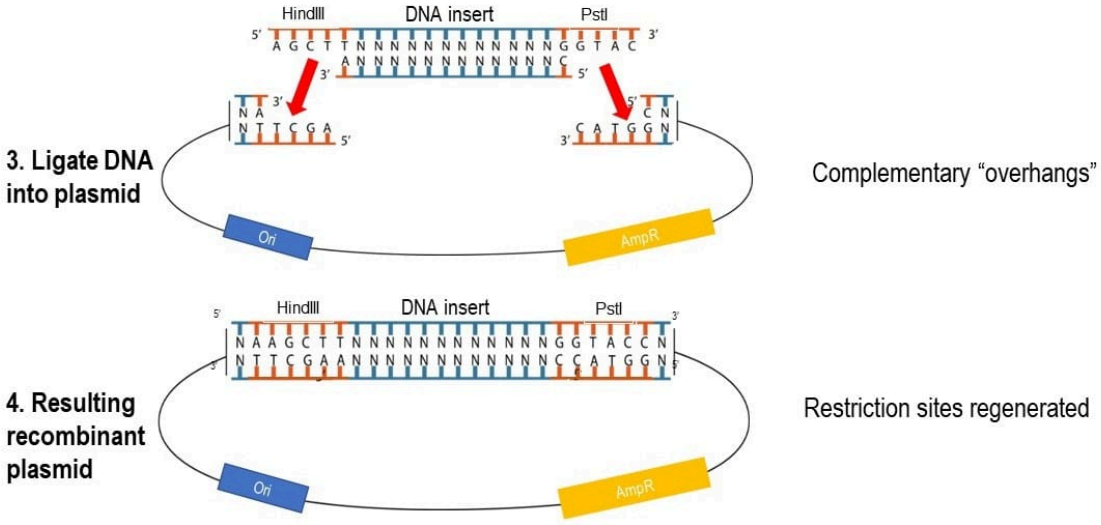


Figure 12.5.2: A directional cloning strategy: Part 2. The remaining steps of the directional cloning strategy are: (3) Ligate DNA into plasmid - ligation of DNA insert and plasmid is accomplished because the overhangs complement one another (AGCT and TCGA; GTAC and CATG). (4) The resulting recombinant 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** 12.5.3 **)**.

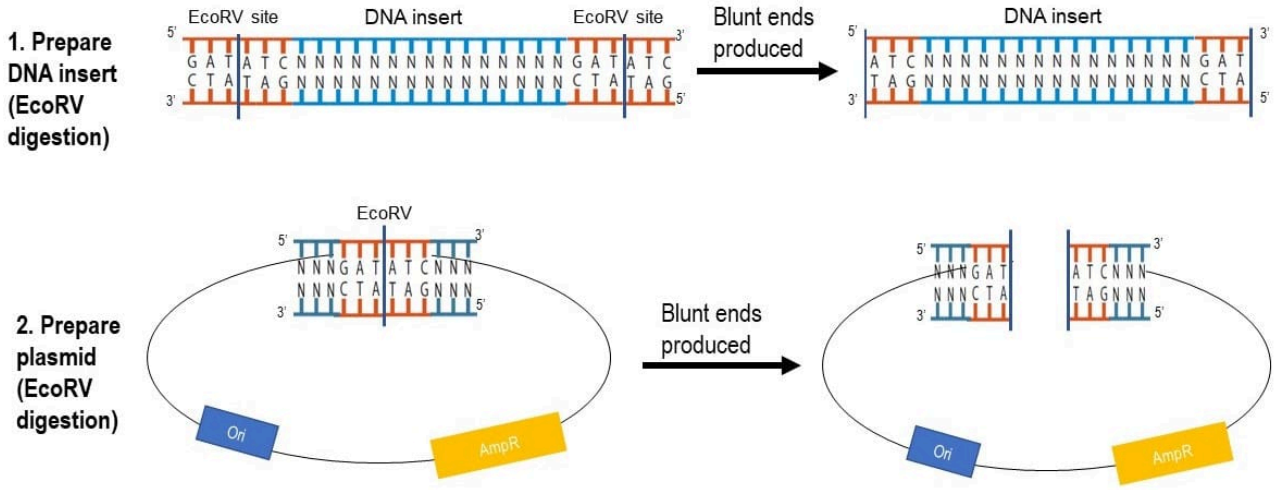


Figure 12.5.3: Blunt end cloning: Part 1. In "blunt end" cloning, both the DNA insert and plasmid are digested with a restriction enzyme that produces blunt ends. The first steps of a blunt end cloning strategy using the enzyme EcoRV are as follows: (1) Prepare the DNA insert (EcoRV digestion) - digestion 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** 12.5.4**)**.

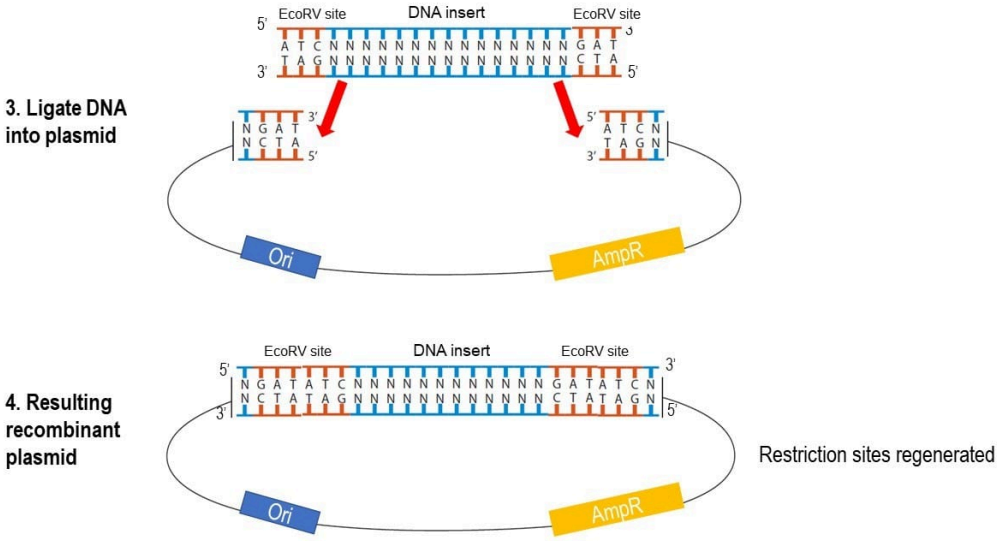


Figure 12.5.4: Blunt end cloning: Part 2. The remaining steps of a blunt end cloning strategy using the restriction enzyme EcoRV are: (3) Ligate DNA into plasmid - ligation of DNA insert and plasmid inserts the DNA into the plasmid. (4) The resulting recombinant plasmid contains the insert DNA and the 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** 12.5.5 below shows the correct cloning orientation of a DNA insert.

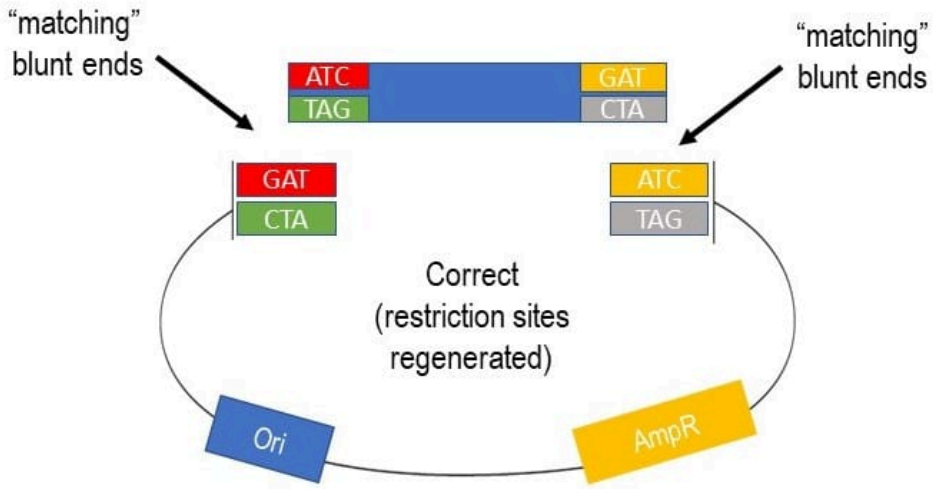


Figure 12.5.5:The correct blunt end cloning direction. The cloning of a DNA insert with blunt ends must be performed so that the restriction sites are regenerated. The sense and anti-sense strands of the DNA insert and plasmid digested with a blunt end restriction enzyme are shown with four different 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** 12.5.6**)**. 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.

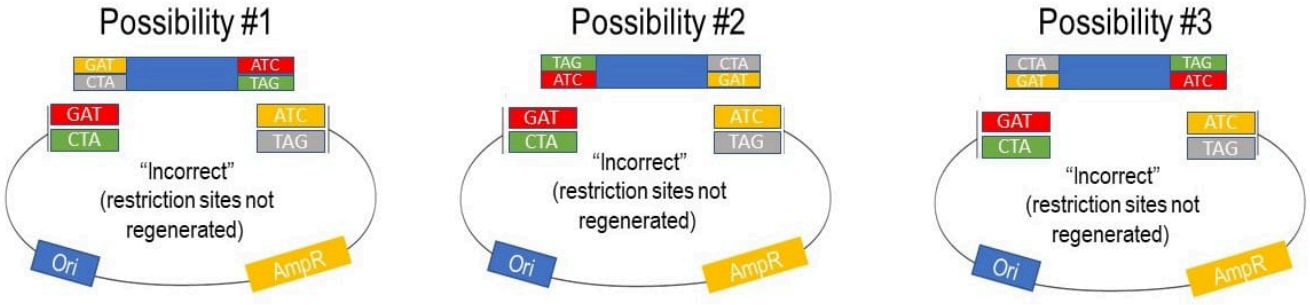


Figure 12.5.6: Incorrect blunt end cloning possibilities. Three possible cloning orientations are shown. Each of these are incorrect because they do not regenerate the restriction site sequence of GATATC at either end of the cloning site. (Blue box = Ori; origin of replication; yellow box = AmpR; ampicillin 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 YouTube Video 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 12.5.8)**. 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.

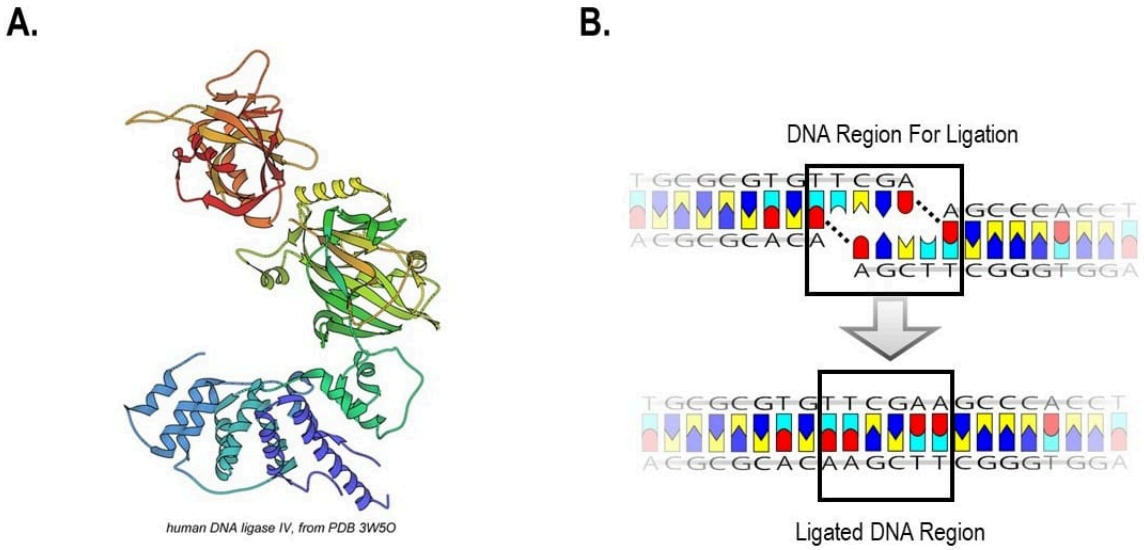


Figure 12.5.7: DNA ligation. (A) The ribbon structure of a human DNA ligase enzyme. (DNA ligase by Dcrjsr, CC BY-SA 4.0) (B) The ligation of DNA by DNA ligase requires the formation of phosphodiester bonds between adjacent nucleotides. Once ligated, the DNA helix is one continuous piece. In the top figure, 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** 12.5.8**)**. 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).

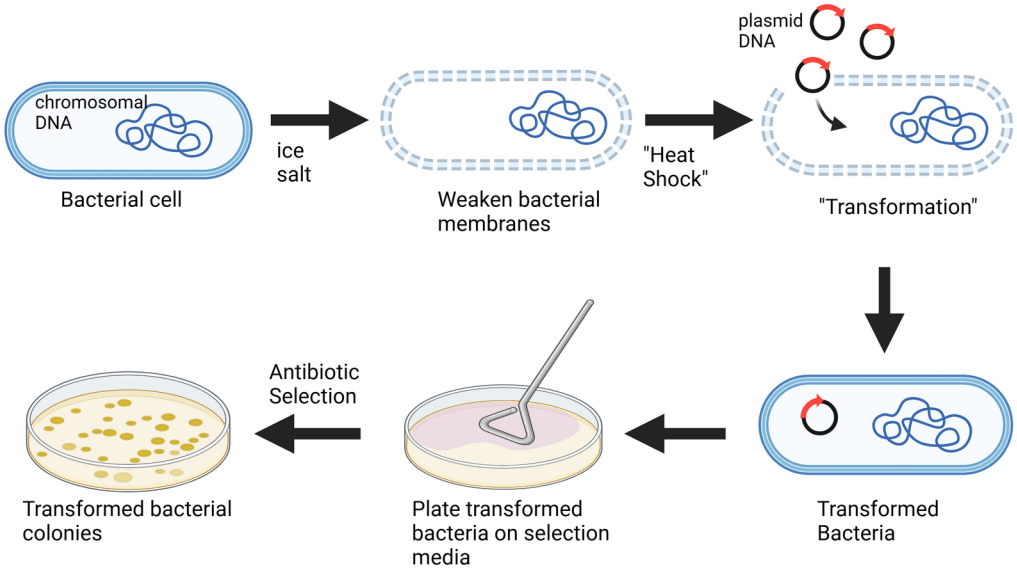


Figure 12.5.8: Transformation of bacterial cells. Bacteria are weakened using ice-cold salt solutions prior to incubation with recombinant plasmids. "Heat Shocking" the weakened bacteria allows for the entry of recombinant plasmids. Plating of transformed bacteria onto agar plates containing an antibiotic 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

Summary

DNA cloning (molecular cloning) is the process of creating recombinant DNA (rDNA) by inserting a DNA fragment from one organism into a plasmid, a small circular DNA molecule found in bacteria. This technology, first demonstrated in 1973 when scientists cloned a frog gene into *E. coli*, became a foundation of modern genetic engineering. The main steps include isolating the DNA of interest, cutting both the DNA and plasmid with restriction enzymes, joining them with DNA ligase to form a recombinant plasmid, and introducing the plasmid into bacteria through transformation. In directional cloning, different restriction enzymes create matching overhangs that ensure the DNA inserts in the correct orientation. In blunt-end cloning, no overhangs are produced, so the insert can join in multiple orientations and must be verified. After transformation, bacteria carrying recombinant plasmids are selected using antibiotic resistance markers, and the inserted DNA is confirmed using techniques such as PCR, DNA sequencing, or gel electrophoresis. This process enables scientists to replicate and study specific genes.

Key Terms

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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12.6: 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.

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

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.

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** 12.6.1). 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.

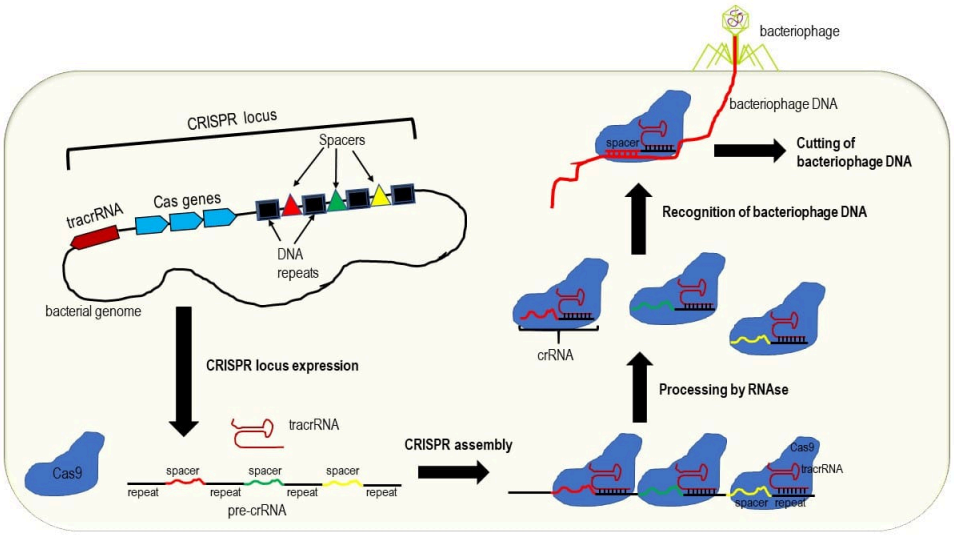


Figure 12.6.1: The CRISPR system of bacterial immunity. Adaptive immunity against bacteriophage infection is achieved in bacteria through the CRISPR system. The CRIPSR locus is comprised of several palindromic repeats of DNA (DNA repeats, black squares) found in between regions of integrated bacteriophage 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** 12.6.2). 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.

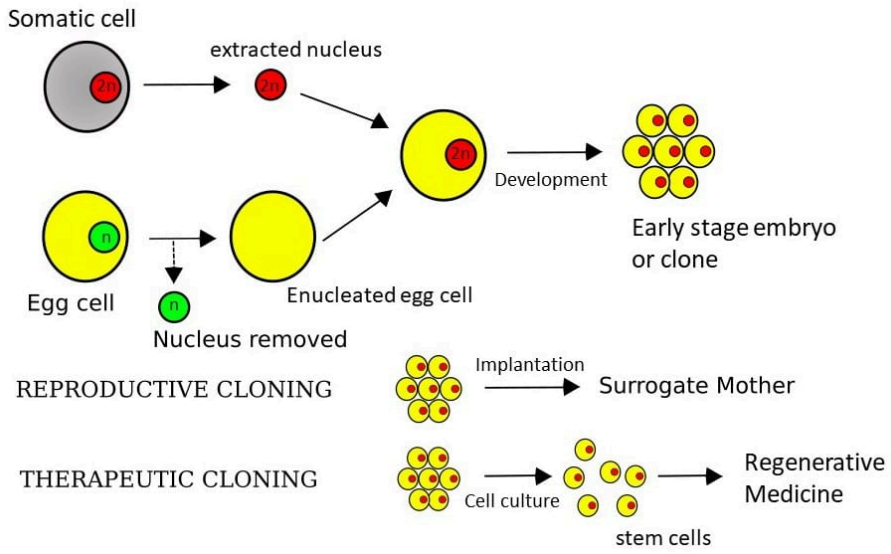


Figure 12.6.2: Somatic Cell Nuclear Transfer (SCNT). The diploid nucleus from a somatic cell with the desired genes is removed and inserted into a recipient egg cell in which the haploid nucleus has been removed. The genetically modified egg cell is then allowed to undergo development to form an early 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.

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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

Summary

Animal biotechnology uses genetic engineering and gene editing to modify animal genomes for applications in agriculture, medicine, and biotechnology. Scientists can add beneficial genes to improve traits such as growth, disease resistance, or production of therapeutic proteins, while gene silencing reduces the activity of harmful genes. Gene delivery can be achieved using viral vectors or synthetic carriers such as liposomes and nanoparticles. One of the most important gene-editing tools is CRISPR-Cas9, which uses a guide RNA to direct the Cas9 enzyme to a specific DNA sequence, allowing precise changes such as disabling or correcting genes. Another key technique is somatic cell nuclear transfer (SCNT), or cloning, in which the nucleus of a body cell is transferred into an egg cell without a nucleus to produce a genetically identical embryo. SCNT has been used for reproductive cloning, exemplified by Dolly the sheep, and for therapeutic cloning to generate stem cells for regenerative medicine. Together, these technologies have advanced livestock production, disease treatment, drug development, and biomedical research while also raising important ethical and regulatory considerations.

Key Terms

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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12.7: 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.

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

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.

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** 12.7.1). For more information about transfection, go to Chapter 13.10: Liposomal transfection into mammalian cells.

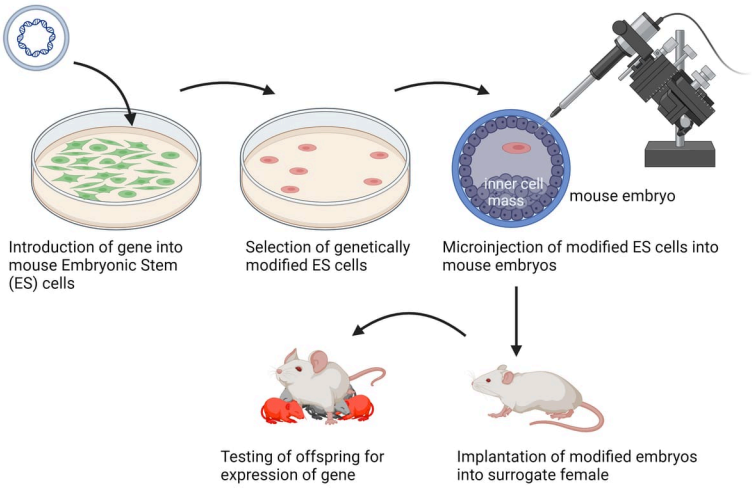


Figure 12.7.1: GMO animal production. One method of producing a genetically-modified organism (GMO) is shown in this figure. The gene is introduced into embryonic stem cells (ES cells) that have been isolated from the host organism, such as a mouse. The selection of genetically modified mouse ES cells 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** 12.7.2). 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.

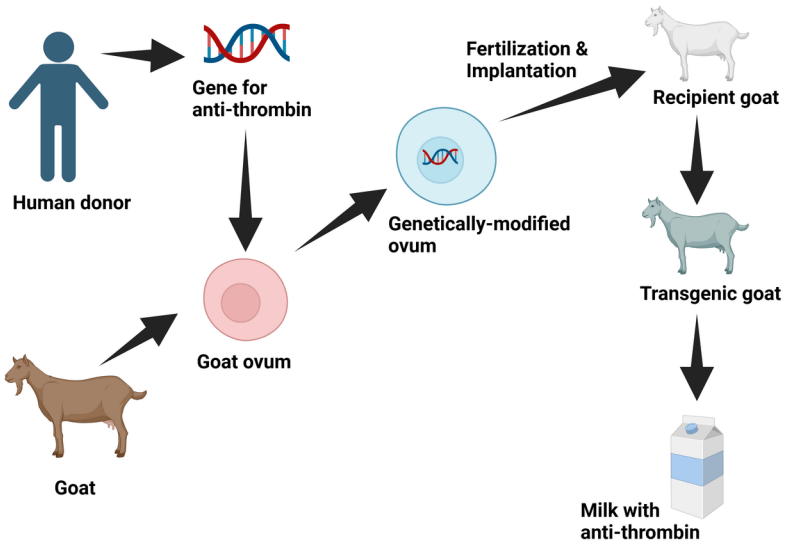


Figure 12.7.2: Biopharming of human anti-thrombin. The human anti-thrombin gene is isolated from a human donor and a goat is used for the donation of an ovum. The gene is microinjected into a goat ovum (pink cell) to created a genetically-modified ovum (blue cell). The ovum is then fertilized and implanted 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** 12.7.3). 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.

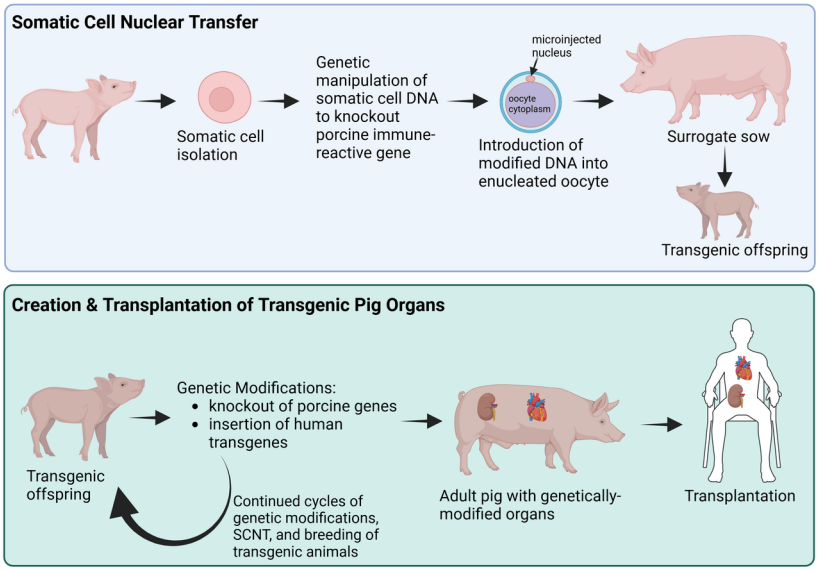


Figure 12.7.3: Xenotransplantation. The modification of pig organs for human transplantation is done in two phases: Somatic Cell Nuclear Transfer (top box in blue) and the creation and transplantation of transgenic pig organs (bottom box in green). In SCNT, a somatic cell (pink) is isolated from a pig. 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.

Summary

Genetically modified organisms (GMOs) are organisms whose DNA has been altered using genetic engineering. When a gene from another species is introduced, the organism is called transgenic. In animals, genetic engineering is used to improve agriculture, advance medical research, and develop new treatments. Scientists create GMOs by identifying a useful gene, copying it, inserting it into a vector such as a plasmid, and introducing it into animal cells or embryos using techniques such as transfection or CRISPR gene editing. Transgenic animals can serve as “bioreactors” that produce valuable human proteins, such

as anti-thrombin or insulin, in their milk or eggs, a process known as biopharming. Genetic engineering is also used to create disease-resistant livestock and genetically modified mosquitoes that reduce the spread of diseases like malaria and dengue. Another promising application is xenotransplantation, in which genetically modified pig organs are engineered to reduce immune rejection and potentially address shortages of human donor organs. While these technologies offer significant medical and agricultural benefits, they also raise important ethical, environmental, and regulatory questions.

Key Terms

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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13: DEVELOPMENT OF BIOTECHNOLOGY

13.1: Biotechnology- From DNA Manipulation to Innovative Solutions
13.2: Navigating the Lifecycles of Biotechnological Products

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13.1: Biotechnology- From DNA Manipulation to Innovative Solutions

Learning Objectives

- By the end of this chapter, students should be able to:
- Define biotechnology and explain its interdisciplinary nature.
- Describe key biomolecules (DNA, RNA, proteins) and their role in biotechnology.
- Explain the Central Dogma of Molecular Biology and its significance.
- Identify tools and techniques used in genetic engineering, such as recombinant DNA technology and plasmids.
- Discuss the applications of biotechnology in medicine, agriculture, and industry.

Biotechnology: From DNA Manipulation to Innovative Solutions

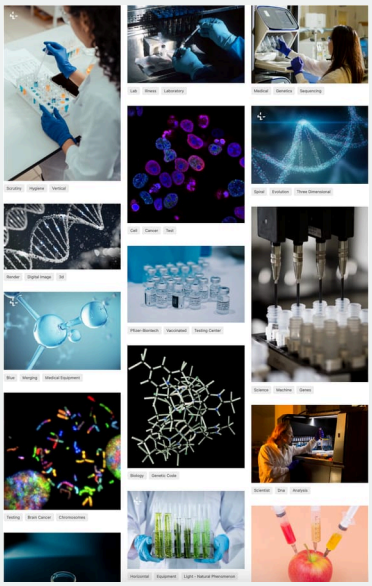


Figure 13.1.1: The type of images you would typically see when searching the word, "Biotechnology". Image source from [unsplash.com]

Embarking on a visual exploration of "biotechnology" through a Google search unveils a captivating landscape (**Figure** 13.1.1). The intricate process of individual development and the iconic representation of the DNA double helix often take center stage in these visuals. While DNA has become a symbol of biological science, it's essential to acknowledge that modern biotechnology extends far beyond the manipulation of DNA.

Definition: Biotechnology

In essence, biotechnology is a multidisciplinary field that encompasses the manipulation of biological molecules for various products and processes, utilizing genetic information for engineering solutions and advancements. Initially rooted in basic research, the biotechnological landscape has evolved into a multifaceted industry, reaching far beyond DNA manipulation to include a diverse array of protein-based products. From cancer therapeutics to everyday items like lens cleaning solutions, biotechnological products have filled various facets of our lives, propelling the field's remarkable growth.

The laboratory, as the birthplace of key biological molecules—DNA, RNA, and proteins—plays a crucial role in understanding biotechnology. DNA, or Deoxyribonucleic acid, is a molecule carrying genetic instructions for the development, functioning,

growth, and reproduction of all known living organisms encompassing prokaryotic cells (e.g., bacteria) and eukaryotic cells (e.g., plants and animals). It serves as a powerful tool in biotechnology, allowing the manipulation of genetic material. Organized into chromosomes, DNA contains numerous genes, the building blocks of heredity, carrying instructions for constructing and operating cells.

Proteins, the cornerstone of cellular structure and function, play a pivotal role in shaping the intricate tapestry of cellular life. Comprising amino acid building blocks, proteins exhibit a dynamic nature, forming complex three-dimensional structures essential for diverse cellular activities. In the realm of molecular biology, the Central Dogma of Biology delineates the flow of genetic information within a cell (**Figure** 13.1.2). Initiating this process is DNA, the master blueprint, where specific genes encode information for protein synthesis. This genetic code is transcribed into Messenger Ribonucleic Acid (mRNA), a key intermediary molecule, which carries the genetic instructions from the nucleus to the Ribosomes. Acting as cellular workbenches, ribosomes meticulously read the mRNA code, facilitating the translation of genetic information into the synthesis of proteins. This orchestrated dance of DNA, mRNA, and protein embodies the Central Dogma, portraying the fundamental process by which genetic information is transcribed and translated to craft the proteins essential for cellular functions.

What is the Central Dogma of Biology?

The Central Dogma of Molecular Biology is the process that describes how genetic information flows inside a cell to make proteins, which are essential for life. It explains how DNA → RNA → Protein in a step-by-step manner. This concept was first proposed by Francis Crick in 1957 and remains a fundamental principle in genetics and molecular biology. It helps us understand how cells function, how traits are inherited, and even how diseases like genetic disorders occur.

- Step 1: DNA – The Blueprint of Life: Imagine your body is like a giant factory, and every cell inside it is a tiny worker following specific instructions. These instructions are stored in the form of DNA (Deoxyribonucleic Acid), which is like a master blueprint. DNA is a double helix, meaning it looks like a twisted ladder. It is made up of four nucleotides (A, T, C, G), which act like letters in a code. The sequence of these letters (bases) determines what proteins your body will make. Example: Think of DNA like a recipe book. If you want to bake a cake, you need the recipe. In the same way, if your body needs a protein (like insulin to regulate blood sugar), it needs instructions from DNA. However, DNA cannot leave the nucleus of the cell, so it needs a messenger to carry the instructions to the rest of the cell.
- Step 2: Transcription – Copying the Recipe (DNA → RNA): Since DNA stays inside the nucleus, a copy of the information is made in the form of RNA (Ribonucleic Acid). This process is called transcription. An enzyme called RNA polymerase unzips the DNA and makes a copy of one of its strands. This copy is called messenger RNA (mRNA). mRNA is single-stranded and carries the same instructions as DNA, but with a small difference: Instead of T (Thymine), RNA has U (Uracil). So, A pairs with U instead of A pairing with T. Example: If DNA is a cookbook kept in a locked cabinet, RNA is like a handwritten note of one recipe that you can take into the kitchen. Now that we have the mRNA copy, it needs to travel out of the nucleus to a special structure called the ribosome, where proteins are made.
- Step 3: Translation – Making the Protein (RNA → Protein): Once the mRNA reaches a ribosome (the protein-making factory of the cell), the instructions are read and followed to build a protein. This step is called translation. The ribosome reads the mRNA in sets of three-letter "words" called codons. Each codon corresponds to a specific amino acid, the building blocks of proteins. Example: The mRNA sequence AUG codes for the amino acid Methionine, which always starts a protein chain. Another type of RNA called transfer RNA (tRNA) helps bring the correct amino acids to the ribosome. tRNA carries amino acids and matches them to the correct codon on the mRNA. This ensures the amino acids are put together in the correct order. As the ribosome moves along the mRNA, it links amino acids together to form a protein. This chain of amino acids folds into a specific shape that determines the protein's function. Example: Think of translation like making a necklace. Each bead (amino acid) must be placed in the correct order according to the instructions (mRNA) to create a complete piece of jewelry (a protein). Final Product: Proteins – The Workers of the Cell Once the protein is built, it folds into a specific shape and is ready to do its job. Proteins control almost everything in your body, such as: Enzymes (like amylase that helps digest food), hormones (like insulin that controls blood sugar), and structural proteins (like collagen that gives skin strength). Each protein's function depends on its shape, which is determined by the sequence of amino acids.

Summary of the Central Dogma

- DNA (genetic instructions) → Transcription → RNA (messenger copy)
- RNA → Translation → Protein (final product)
- Proteins control traits and cell functions.

This is how information in DNA ultimately determines everything from eye color to how your body fights infections.

Why is the Central Dogma Important?

It explains how genetic information is used. It helps scientists understand genetic diseases and how to treat them. It is the foundation for biotechnology, including making medicines like insulin and gene editing (CRISPR). The Central Dogma is like a biological instruction manual for life. It ensures that cells can create the proteins they need to survive and function properly. Understanding this process helps scientists develop new medical treatments, study genetics, and even create artificial life!

Real-World Connection

- Mutations: Sometimes, there are mistakes in DNA (like a typo in a recipe). This can lead to genetic diseases like sickle cell anemia.
- Genetic Engineering: Scientists can modify DNA to help cure diseases or create better crops.
- Viruses & COVID-19: Some viruses, like the coronavirus, use RNA instead of DNA and follow a slightly different process.

Recombinant DNA is a fundamental tool for biotechnologists navigating the intricacies of DNA, RNA, and proteins. Genetic engineering, often denoted as genetically modified (GM) or genetically engineered (GE) organisms, is the deliberate alteration of genetic material, deviating from natural processes like mating or recombination. Serving as a cornerstone in genetic engineering, recombinant DNA involves the artificial fusion of DNA from diverse sources. For example, a gene responsible for drought resistance from a desert plant might be combined with the DNA of a crop plant to confer similar resilience. This process allows scientists to harness the genetic diversity present across different organisms, introducing novel traits and capabilities into target organisms. This sophisticated technique, guided by molecular scissors (e.g. Restriction Enzymes)—enzymes precisely cutting DNA—facilitates the isolation and integration of specific genes into the DNA of another organism. This transformative approach not only plays a vital role in scientific progress but also serves as a bridge between the realms of nature and artificial genetic manipulation.

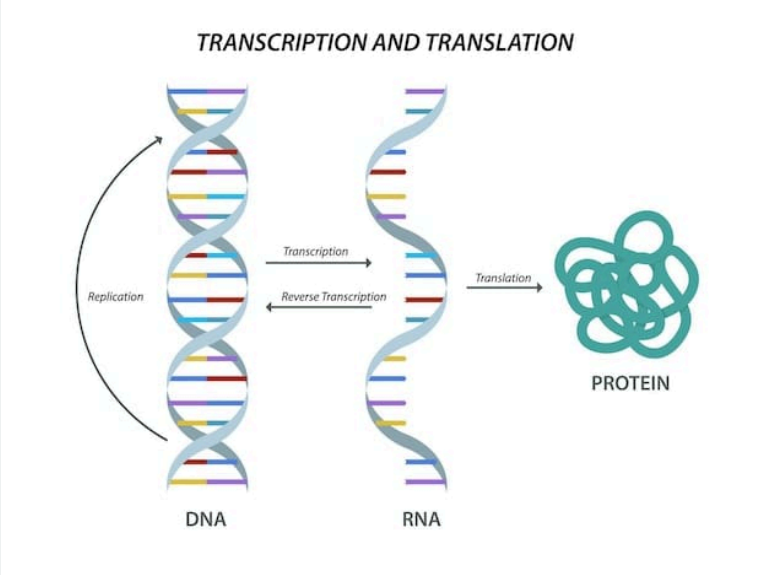


Figure 13.1.2: This is an image of the Central Dogma, showing DNA is transcribed into RNA, and then translated into proteins. Image source from [www.freepik.com]

Plasmids, small circular DNA molecules found in bacteria, can replicate independently of the bacterial chromosomal DNA (**Figure** 13.1.3). A vector, such as a plasmid or virus, serves as a vehicle to transfer genetic material into a host organism. This is vital for processes like transformation, where bacterial cells take up and express foreign genes facilitated by plasmids. Additionally, in transfection, foreign DNA is introduced into eukaryotic cells, enabling the expression of genes from the introduced DNA**.** This capability enables scientists to engineer desired traits in cells, such as producing insulin, a crucial development in medicine that has revolutionized diabetes treatment, impacting fields from basic research to biotechnology and medicine.

Cultured cells, grown in a controlled environment outside their natural habitat, serve as a vital canvas for various experimental purposes, including drug testing and studying disease mechanisms. Fermenters and bioreactors, specialized vessels in industrial

processes, play a pivotal role in growing microorganisms for the scalable production of products, such as enzymes, antibiotics, and biofuels. These vessels provide controlled conditions, optimizing the growth and productivity of microorganisms, and showcasing the versatility and significance of biotechnological processes in industrial settings.

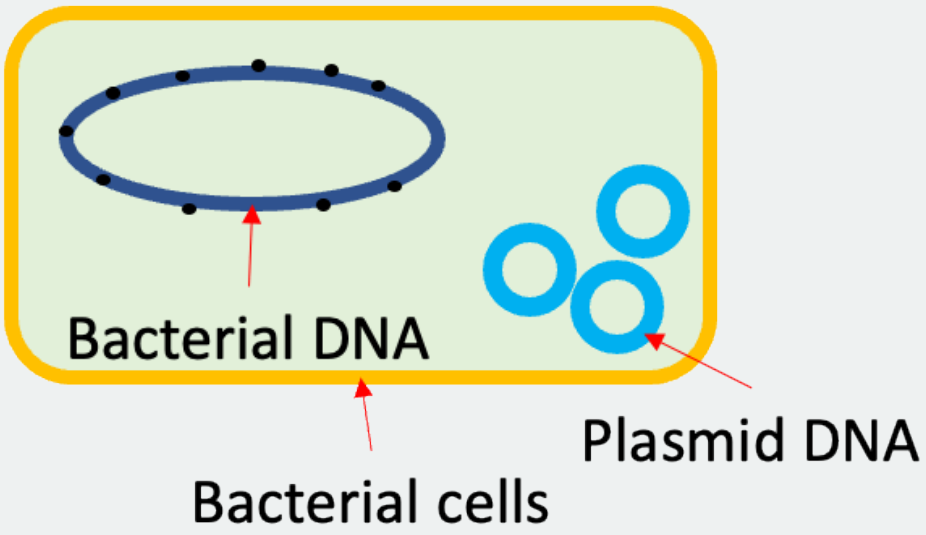


Figure 13.1.3: This is an image of a bacteria. It also shows a large circular bacterial DNA and a small circular plasmid DNA.

Biopharmaceuticals, commonly known as Biologics, represent the pinnacle of biotechnological innovation (**Figure** 13.1.4). These medicinal products are crafted using living cells or organisms through biotechnological processes. Examples include monoclonal antibodies like Rituximab, used in cancer treatment, and insulin produced by genetically modified bacteria. The significance of Biologics lies in their ability to offer targeted and personalized therapeutic interventions, addressing conditions like cancer, autoimmune diseases, and metabolic disorders. This marks a paradigm shift in medicine, providing more effective and tailored treatment options for patients.

What Are Biopharmaceuticals?
Biopharmaceuticals, also called biologic drugs, are medical treatments made using living cells, bacteria, or genetically modified organisms instead of synthetic chemicals. These drugs help treat diseases like cancer, diabetes, and autoimmune disorders, and they also include vaccines that protect against infections. Unlike traditional drugs (such as aspirin or ibuprofen) that are made using chemicals, biopharmaceuticals are proteins, antibodies, or even whole viruses that have been engineered to help the body heal or defend itself. Biopharmaceuticals can be grouped into different categories based on what they do and how they are made.

Types of Biopharmaceuticals

1. Monoclonal Antibodies (mAbs) – The “Smart Missiles” of Medicine: Monoclonal antibodies are engineered proteins that act like the body's own immune system to fight diseases. How do they work? These antibodies recognize and attach to specific targets (antigens) on viruses, bacteria, or cancer cells. Imagine your body is a battlefield, and antibodies are trained soldiers that recognize enemy invaders. Monoclonal antibodies are like special forces, designed to attack one specific enemy.
    - Herceptin (Trastuzumab): Used to treat breast cancer by blocking growth signals to cancer cells.
    - Rituximab: Used to treat autoimmune diseases and some types of cancer by attacking abnormal immune cells.
    - COVID-19 antibody treatments: Lab-made antibodies that help neutralize the virus and prevent severe infection.
2. Vaccines – Teaching the Body to Fight Infections: Vaccines are biological preparations that train the immune system to recognize and fight harmful pathogens before they cause illness. How do they work? They introduce a harmless version of a virus or bacteria (or a piece of it) to the immune system so it learns to fight the real infection. Think of vaccines like a "wanted poster" given to your immune system. They show what the enemy (virus/bacteria) looks like, so if the real threat appears, your body already knows how to fight it.
    - Live Attenuated Vaccines: Use a weakened form of the virus or bacteria (i.e., Measles, Mumps, and Rubella (MMR) vaccine)
    - Inactivated Vaccines: Use a killed version of the pathogen. (i.e., Polio vaccine)
    - mRNA Vaccines: Provide genetic instructions to make a harmless viral protein, which triggers immunity. (i.e., COVID-19

vaccines (Pfizer & Moderna))
    - Recombinant Protein Vaccines: Contain pieces of the virus (like a protein) instead of the whole pathogen. (i.e., Hepatitis B vaccine)
    - Viral Vector Vaccines: Use a harmless virus to deliver genetic material from the pathogen. (i.e., Johnson & Johnson COVID-19 vaccine)
3. Gene Therapy – Fixing Genetic Diseases: Gene therapy is a treatment that replaces, repairs, or modifies faulty genes to treat diseases. How does it work? Scientists use viruses as delivery vehicles to carry healthy genes into patient cells. Imagine your DNA is like a book, and a genetic disease is like a misprinted sentence. Gene therapy is like an editor who fixes the typo so the book can be read correctly.
    - Luxturna: A gene therapy for blindness caused by genetic mutations.
    - Zolgensma: Used to treat spinal muscular atrophy by replacing a missing gene.
4. Hormone-Based Biopharmaceuticals – Replacing What's Missing: Some diseases occur because the body does not make enough of a certain hormone. Biopharmaceuticals help replace missing hormones. If your body is like a car, hormones are like fuel. If there's not enough fuel, the car won't run properly. Insulin and growth hormones act like adding the missing fuel.
    - Insulin: Used to treat diabetes by controlling blood sugar levels.
    - Growth Hormone: Helps children with growth deficiencies.
5. Enzyme Replacement Therapy – Helping People with Genetic Disorders: Some people are born with genetic conditions where their body doesn't produce a necessary enzyme. How does it work? Scientists produce the missing enzyme in a lab and give it to patients. If your body is like a factory, enzymes are the workers. If a worker is missing, production stops. Enzyme therapy is like hiring new workers to restart production.
    - Lysosomal Storage Disorders: Enzyme therapies like Aldurazyme help break down waste inside cells.
    - Cystic Fibrosis Treatments: Help improve mucus clearance in the lungs.
6. Blood and Plasma-Based Biopharmaceuticals – Helping the Immune System: These treatments use human blood or plasma to help patients with immune deficiencies or blood disorders. Imagine a blood disorder is like a leaking pipe in your house. Plasma-based therapies work like a patch to stop the leak and restore normal function.
    - Clotting Factors: Used for hemophilia (a disorder where blood doesn't clot properly).
    - Immunoglobulins: Help boost the immune system in people with immune deficiencies.

Why Are Biopharmaceuticals Important?
Biopharmaceuticals have changed medicine by treating diseases that had no cure before, helping millions of people live longer and healthier lives, and improving cancer treatments and making vaccines safer and more effective Scientists are continuing to develop new biopharmaceuticals to fight diseases like Alzheimer's, HIV, and even cancer. Biopharmaceuticals represent the future of medicine. They are more advanced than traditional chemical drugs and offer targeted, effective treatments for diseases that were once thought to be untreatable. Scientists are continuously improving these medicines to make them safer, more affordable, and accessible to everyone.

Summary of Biopharmaceuticals

Table 13.1.1: Summary of Biopharmaceuticals

Type	How It Works	Example
Monoclonal Antibodies	Target specific proteins in diseases	Herceptin, Rituximab
Vaccines	Train immune system to recognize infections	COVID-19, MMR, Hepatitis B
Gene Therapy	Fixes or replaces faulty genes	Luxturna, Zolgensma
Hormone Therapy	Replaces missing hormones	Insulin, Growth hormone
Enzyme Replacement	Provides missing enzymes to patients	Aldurazyme, Cystic fibrosis treatments
Blood & Plasma Therapies	Helps immune system and blood disorders	Clotting factors, Immunoglobulins

The potential of manipulating DNA for commercial purposes became apparent in the early 1970s, leading to the inception of the modern biotechnology industry. Genentech, founded in 1976, achieved a groundbreaking feature by transferring the gene coding for human insulin into bacteria, revolutionizing the production of insulin for diabetic patients.

While pharmaceuticals and genetically modified crops are widely recognized applications of genetic engineering, recombinant DNA technology has quietly revolutionized manufacturing in various areas. One notable example is rennin, an enzyme crucial in cheese production. Traditionally obtained from the fourth stomach of unweaned calves, rennin is now synthesized by genetically

modified bacteria.

In the realm of household products, nearly all contemporary laundry detergents incorporate enzymes produced by genetically modified microorganisms, offering efficient stain removal. Enzymes such as lipases for breaking down oils, proteases for breaking down proteins, and amylases for breaking down starches are instrumental in these products. Subtilisin, a protease produced by genetically modified bacteria, is a common ingredient found in various household products, including laundry detergents and contact lens cleaners.

Biotechnology has also enhanced various industrial processes, including paper manufacturing. Enzymes produced by genetically modified bacteria can replace harsh chemicals traditionally used in paper production, providing a more environmentally friendly alternative. Moreover, genetically modified bacteria have demonstrated the ability to break down pollutants in contaminated soil and water.



Figure 13.1.4: This is an image of the different biopharmaceuticals, such as medications and vaccines. Image source from [www.freepik.com]

A promising frontier in biotechnology is the development of "bioplastics" that could potentially replace conventional plastics. Given the escalating environmental pollution resulting from the widespread production and disposal of plastic items, bioplastics offer a sustainable solution. By utilizing renewable feedstocks such as plants, industrial and food waste, and agricultural residues, bioplastics can be produced, reducing reliance on fossil fuel-derived ingredients used in conventional plastics.

In conclusion, biotechnology, rooted in basic research and DNA manipulation, has evolved into a dynamic industry with diverse applications. The manipulation of DNA, mRNA, and proteins, facilitated by sophisticated tools, has revolutionized fields ranging from medicine to food production. The use of genetically modified cells has not only transformed pharmaceuticals but has also led to innovations in agriculture and environmental sustainability. As we witness the ongoing advancements in biotechnology, it is evident that this field will continue to shape our future in unforeseen ways, offering solutions to global challenges and improving the quality of human life.

Summary

Biotechnology is the use of living organisms, cells, or biological molecules to develop products that improve medicine, agriculture, industry, and the environment. It combines knowledge from microbiology, genetics, molecular biology, chemistry, and engineering to solve real-world problems.

The foundation of biotechnology is the relationship between DNA, RNA, and proteins. DNA stores genetic information, RNA carries these instructions from DNA to the ribosome, and proteins perform most cellular functions. This process is described by the Central Dogma of Molecular Biology: DNA → RNA → Protein. Understanding this flow of genetic information helps explain inheritance, disease, and modern genetic technologies.

Scientists use tools such as recombinant DNA technology, restriction enzymes, and plasmids to modify genes and transfer DNA into cells. These techniques have made it possible to produce important medicines, including human insulin, and have advanced genetic research.

Biotechnology has many applications. In medicine, it is used to produce biopharmaceuticals, including vaccines, monoclonal antibodies, and gene therapies. In agriculture, it improves crop yield and disease resistance. In industry, microorganisms produce enzymes, antibiotics, biofuels, and biodegradable plastics. Biotechnology continues to transform healthcare and many other fields, making it an essential area of study for future healthcare professionals.

Key Terms

Biotechnology
The use of biological systems, organisms, or derivatives to develop or modify products for specific use.

DNA (Deoxyribonucleic Acid)
The molecule that carries genetic instructions for life.

Central Dogma of Molecular Biology
The process through which genetic information flows from DNA to RNA to protein.

Recombinant DNA
Artificially created DNA that combines sequences from different sources.

Plasmid
A small, circular DNA molecule found in bacteria, often used as a vector in genetic engineering.

Vector
A vehicle (e.g., plasmid, virus) used to transfer genetic material into a host cell.

Transformation
The uptake of foreign DNA by a bacterial cell.

Transfection
The introduction of foreign DNA into eukaryotic cells.

Biopharmaceuticals (Biologics)
Medicinal products derived from living organisms, such as monoclonal antibodies and insulin.

Bioplastics
Environmentally friendly plastics derived from renewable biological sources.

Post-Lecture Questions

1. How has biotechnology evolved from its early applications to modern innovations?
2. Why is the Central Dogma fundamental to understanding genetic engineering?
3. What are the ethical considerations of using genetically modified organisms (GMOs)?
4. How does recombinant DNA technology benefit medicine and industry?
5. Can biotechnology play a role in solving global environmental challenges? If so, how?
6. Discuss in small groups: What are the most impactful applications of biotechnology today?
7. Research a current biotechnology breakthrough and summarize its significance.
8. Create a short quiz reviewing key terms and concepts from the lecture.

Answer available at the end of this section.

Answers

Post-Lecture Questions

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13.2: Navigating the Lifecycles of Biotechnological Products

Learning Objectives

- Define the biotechnology product life cycle and its key stages.
- Explain the roles of Research and Development (R&D), scale-up, production, Quality Control (QC), Quality Assurance (QA), and regulatory affairs in biotechnology.
- Discuss the historical significance of insulin development in biotechnology and its impact on modern medicine.
- Describe the significance of intellectual property (IP) protection, including patents and trade secrets, in the biotechnology industry.
- Identify the challenges and ethical considerations in the commercialization of biotechnological products.

Navigating the Lifecycles of Biotechnological Products

In the landscape of biotechnology, products undergo a comprehensive biotechnology product life cycle, from conceptualization to market presence. This journey encompasses Research and Development (R&D), scale-up, production (aka manufacturing), quality control (QC), quality assurance (QA), and regulatory affairs staff, each playing a crucial role in transforming ideas into tangible, commercially viable entities.

Consider the pivotal moment in medical history in 1918 when Elizabeth Hughes, diagnosed with type 1 diabetes, received insulin extracted from an animal pancreas. This breakthrough marked the inception of "modern" biotechnology, emphasizing the intricate interplay between scientific achievement and commercial enterprise. Biotechnology operates at the intersection of science and business, with products like insulin showcasing its societal impact. While advancements save lives, societal challenges arise, such as pricing dynamics affecting accessibility. This chapter explores how biotechnological products evolve, urging awareness of the broader landscape where science converges with business.

The journey from an idea to a product is a detailed process, forming the life cycle of a product. It all starts in research labs, where discoveries spark ideas for potential products. These ideas then move into development, where they transition from being just concepts to becoming tangible realities. Once the development phase succeeds, the product moves into production, involving crucial stages like sales, expansion, and growth. This careful coordination is necessary to turn innovative ideas into biotechnological products ready for the market.

Biotechnology finds its roots in scientific exploration, often initiated by researchers in universities, colleges, and research institutes. In research labs, preliminary experiments lead to the exploration of new possibilities. Protecting intellectual property (IP) becomes crucial during this phase, covering the idea, potential product, applications, and manufacturing processes. Many biotech companies can trace their origins back to the efforts of research scientists, with a majority having scientists and technicians continuously exploring new ideas for potential products.

Entering the development phase means engaging in relentless testing and refining of a potential product to enhance its functionality. A key aspect of development is scaling up from small laboratory processes to larger, reproducible quantities. Collaboration is common, especially in pharmaceutical and biopharmaceutical companies, where multiple teams work together in the intricate process of product evolution.

Successfully moving through the development phase brings us to production, where a specialized unit handles tasks related to manufacturing, marketing, and sales. Methods in biotechnology differ, ranging from growing bacteria in small lab flasks to extracting products from cultures in large fermenters. In production, blueprints are transformed into actual products, making them ready and available in the market.

As a product goes through its stages, the teams responsible for ensuring its quality – Quality Control (QC) and Quality Assurance (QA) – adapt to make sure the product is excellent. QC involves keeping an eye on processes and running tests in the lab, making sure products have the necessary qualities before they're released. QA covers a wider range of activities, including carefully checking documentation and actively taking part in solving problems. When products are subject to government regulations, having a dedicated regulatory affairs team is crucial. This team works closely with regulatory agencies like the FDA to ensure the company follows all the strict requirements. Regulatory affairs personnel collaborate with quality teams, making sure the

company's quality systems align with the ever-changing regulatory rules.

Biotech companies go through a growth process similar to their innovative projects. In the beginning, startups concentrate on research and development, often with a small team managing various tasks. As they mature, specialized staff is brought in, and new areas like business development, marketing, sales, business divisions, dispensing, metrology, and clinical research management emerge to support different aspects of the company. This evolution shifts the culture from versatile employees in startups to specialized personnel in larger entities. Being flexible and adaptable becomes crucial for navigating this transformation. In the biotech industry, safeguarding intellectual property (IP) becomes crucial, covering valuable creations protected through various means. Inventions, from digital devices to groundbreaking biotech innovations, are particularly important. This next section delves into how IP is protected through patents and trade secrets (**Figure** 13.2.1).

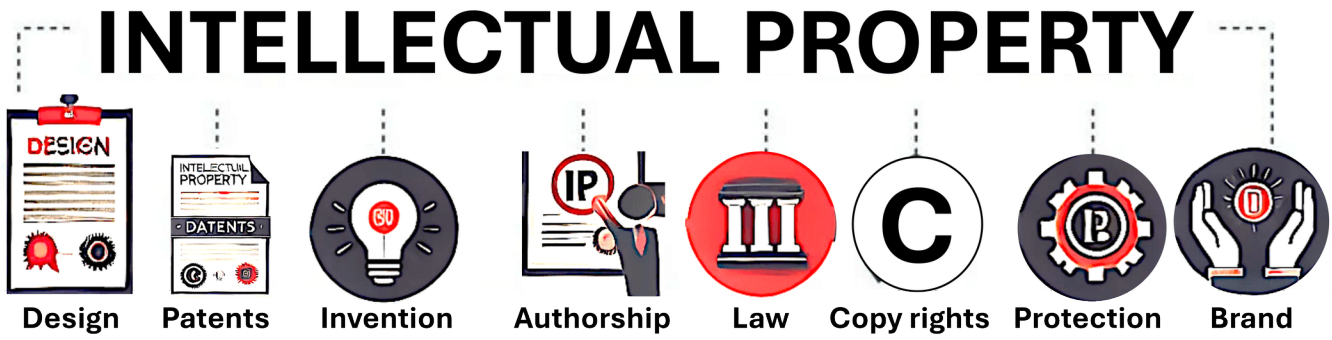


Figure 13.2.1: An illustration of the different types of intellectual property. Image generated via ChatGPT

What is Intellectual Property (IP)?

Intellectual Property (IP) refers to the legal rights that protect ideas, inventions, and creative works. In industries like electronics and software development, IP ensures that people who create new products, designs, and brands have control over how they are used and shared. Think of IP as a way to protect your hard work—just like how a musician copyrights a song, a tech company patents a new smartphone feature, or a business trademarks its logo.

Types of Intellectual Property

There are several types of IP, each serving a different purpose:

- Patents – Protect new inventions, such as a new type of battery for a phone or a faster computer chip. Patents give the inventor the right to stop others from making or selling their invention for a certain period (usually 20 years).
  - Example: If someone invents a new type of wireless charging system, they can patent it so others can't copy their technology without permission.
- Design Rights – Protect how a product looks, including its shape, patterns, and colors. These can be registered (officially documented) or unregistered (automatically protected).
  - Example: The unique curved shape of a Coca-Cola bottle is a registered design, meaning no other company can copy that exact look.
- Trademarks – Protect brand names, logos, and slogans that make a business recognizable.
  - Example: The Nike "swoosh" logo and the phrase "Just Do It" are trademarks that legally belong to Nike. No one else can use them without permission.

Why is Intellectual Property Important?

IP is essential because it gives inventors and companies a competitive edge. Imagine spending years developing a new app, only for someone else to copy it and take credit! Protecting IP ensures that the people who create something valuable get the rewards they deserve.

Challenges of Protecting Intellectual Property

Protecting IP isn't always easy, and it often requires legal experts. Some challenges include:

- International Protection: A patent in one country (like the UK) doesn't automatically apply in another country (like the U.S.), so companies often need multiple patents.
- Costs: Securing patents and trademarks can be expensive, so businesses must plan their budgets carefully.
- Piracy and Theft: Digital content (like music, movies, and software) can be illegally copied and shared, making it harder to enforce IP rights.

Intellectual Property in Research & Development (R&D)

In fields like electronics and software design, IP is created at different stages of innovation. These stages include:

- Background IP: Existing knowledge and ideas brought into a project before development begins.
  - Example: A company developing a new smartwatch might use existing research on battery life.
- Foreground IP: New knowledge or inventions created during a project.
  - Example: If a research team discovers a new way to make smartwatches waterproof, that new idea becomes foreground IP.
- Sideground IP: Ideas developed by project partners outside of the specific project but still relevant.
  - Example: A company working on a fitness tracker might already have separate research on heart rate sensors, which could be useful for the project.
- Postground IP: New knowledge created after a project ends.
  - Example: After launching a new phone, engineers may discover ways to improve its battery life, leading to future upgrades.

Intellectual Property is a crucial part of innovation. Whether you're an inventor, a business owner, or a software developer, protecting your IP ensures your hard work isn't stolen or misused. Understanding IP helps businesses grow, encourages creativity, and ensures fair competition in the market. Wouldn't it be frustrating if you spent months designing a cool new sneaker, only for someone else to copy your design and sell it as their own? That's why IP laws exist—to make sure creators and inventors get the credit and rewards they deserve!

Patents, agreements between the government and inventors, give exclusive rights, excluding others from using the invention. In exchange, the inventor discloses the invention, fostering innovation by spreading new ideas. In biotech, processes like manufacturing biopharmaceutical agents or genetically transforming bacteria can be patented. Granted by offices like the United States Patent and Trademark Office (USPTO), patents typically last 20 years from filing. After expiration, anyone can use the invention. Patents can be licensed, allowing the holder to permit others to use the invention for fees or a share of revenues. Disputes over patent infringement are common, often resolved in federal civil courts. The outcomes significantly impact a company's future, emphasizing the critical role of IP protection.

Trade secrets, unlike patents, aren't registered with the government. They're actively shielded through confidentiality and non-compete agreements. A nondisclosure agreement commits parties to uphold disclosed secret information confidentiality. Employees, interns, consultants, vendors, and anyone associated with the company usually sign such agreements, emphasizing the need for diligent confidentiality. A non-compete agreement ensures a party refrains from leaving and competing with the business for a stipulated duration. Violating these agreements can lead to legal consequences, highlighting their role in protecting proprietary knowledge crucial to a biotech company's success.

While patents are vital for safeguarding inventions and fostering innovation, issues arise in their interpretation and enforcement. Disagreements often revolve around the novelty and obviousness of an innovation. Patent disputes significantly impact a company's future and societal perspectives on innovation and accessibility.

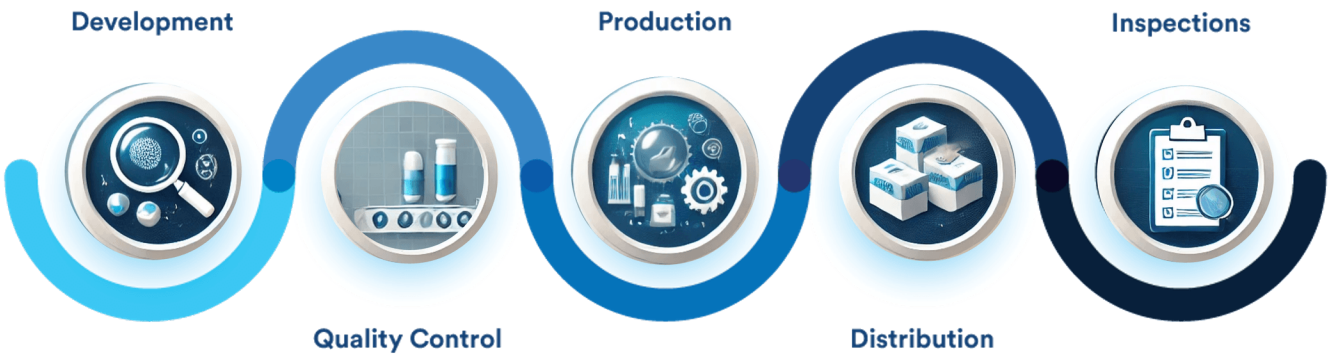


Figure 13.2.2: Illustration shows the types of quality assurance in Pharmaceutical companies. Image created using ChatGPT

Key Areas of Quality Assurance in Pharmaceuticals

Quality assurance has five major areas: Development, Quality Control, Production, Distribution, and Inspections. Each step helps ensure that medicines are safe, effective, and meet high standards before reaching patients (**Figure** 13.2.2).

1. Development
   - The quality process starts when a new medicine is being created. Scientists research and test different formulas to make sure the drug works as expected. During this stage, they follow strict guidelines, carefully record their experiments, and make sure their methods are accurate. By following these steps early on, they set the stage for a safe and effective product.
2. Quality Control
   - Once a drug is developed, it must be tested at every stage—before, during, and after production. In quality control labs, experts use advanced equipment to check if the medicine meets safety and quality standards. If something is wrong, they investigate and fix the problem before the medicine reaches the public.
3. Production
   - During manufacturing, strict rules ensure the drug is made the same way every time. Machines and equipment must be properly maintained, and workers are trained to follow exact procedures. This prevents mistakes and makes sure every batch of medicine is high quality.
4. Distribution
   - After production, medicines must be safely stored, transported, and delivered to hospitals, pharmacies, and patients. Temperature-sensitive drugs, like vaccines, need special handling so they don't spoil. Quality assurance in this stage ensures that the medicines remain safe and effective throughout the process.
5. Inspections
   - Government agencies check pharmaceutical companies to make sure they are following all regulations. These inspections look at the company's records, facilities, and production methods. If inspectors find any problems, the company must fix them to continue selling their products.

By following these quality assurance steps, pharmaceutical companies make sure the medicines we take are safe and effective

In conclusion, understanding intellectual property concepts is vital for biotechnologists, shaping the landscape of their careers. Patents and trade secrets protect valuable creations, fostering innovation and contributing to the growth of the biotechnology industry. Navigating this intricate landscape requires a keen understanding of the lifecycles of biotechnological products and the legal frameworks protecting their intellectual contributions.

Summary

Biotechnology products, such as vaccines and biologic medicines, follow a product life cycle from discovery to patient use. The process begins with Research and Development (R&D), where scientists identify and test new ideas. Successful products are scaled up for large-scale manufacturing and then produced under carefully controlled conditions.

Quality Control (QC) tests products to ensure they are safe, effective, and consistent, while Quality Assurance (QA) oversees manufacturing processes and documentation to maintain quality standards. Regulatory affairs specialists work with agencies such as the FDA to ensure products meet legal and safety requirements before they reach the public.

The development of recombinant human insulin demonstrated how biotechnology can transform healthcare by producing life-saving medicines. Protecting intellectual property (IP) through patents and trade secrets encourages innovation while allowing companies to recover development costs. Although biotechnology has improved healthcare, it also raises ethical questions about product pricing, accessibility, and balancing innovation with patient needs.

Key Terms

Biotechnology Product Life Cycle
The journey of a biotech product from idea conceptualization to market availability, including R&D, scale-up, production, and commercialization.

Research and Development (R&D)
The initial phase where scientific discoveries lead to potential product development.

Scale-Up
The transition from small-scale laboratory production to large-scale manufacturing.

Quality Control (QC)
A set of procedures to ensure the final product meets safety and efficacy standards.

Quality Assurance (QA)
A broader system that ensures compliance with regulations and documentation accuracy.

Regulatory Affairs
The department responsible for ensuring a biotech company complies with government regulations, such as those set by the FDA.

Intellectual Property (IP)
Legal rights that protect inventions, including patents and trade secrets.

Patent
A government-granted exclusive r ight to an invention for a set period, usually 20 years.

Trade Secret
Proprietary knowledge protected through confidentiality agreements rather than government registration.

Non-Disclosure Agreement (NDA)
A legal contract ensuring confidentiality between parties sharing proprietary information.

Non-Compete Agreement
A contract preventing employees or associates from joining competitors within a certain time frame after leaving a company.

Discussion Questions

1. Can you outline the key stages of the biotechnology product life cycle?
2. How does the historical development of insulin illustrate the interplay between science and business?
3. What are the roles of Quality Control, Quality Assurance, and Regulatory Affairs in biotech manufacturing?
4. Why is intellectual property protection critical in biotechnology, and how do patents and trade secrets differ?
5. What are some ethical and economic challenges in the commercialization of biotech products?
6. Why is scaling up production a crucial yet challenging step in biotech product development?
7. How do Quality Control and Quality Assurance differ in ensuring biotech product safety and efficacy?
8. What are some real-world examples of biotech companies facing patent disputes, and how were they resolved?
9. What ethical dilemmas arise in the commercialization of life-saving biotech products like insulin?

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14: MEDICAL BIOTECHNOLOGY – “RED” BIOTECHNOLOGY

14.1: Principles of Medical Biotechnology
14.2: Diagnostic Biotechnology
14.3: Therapeutic Biotechnology
14.4: Regenerative Medicine

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14.1: 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 14.1.1):

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

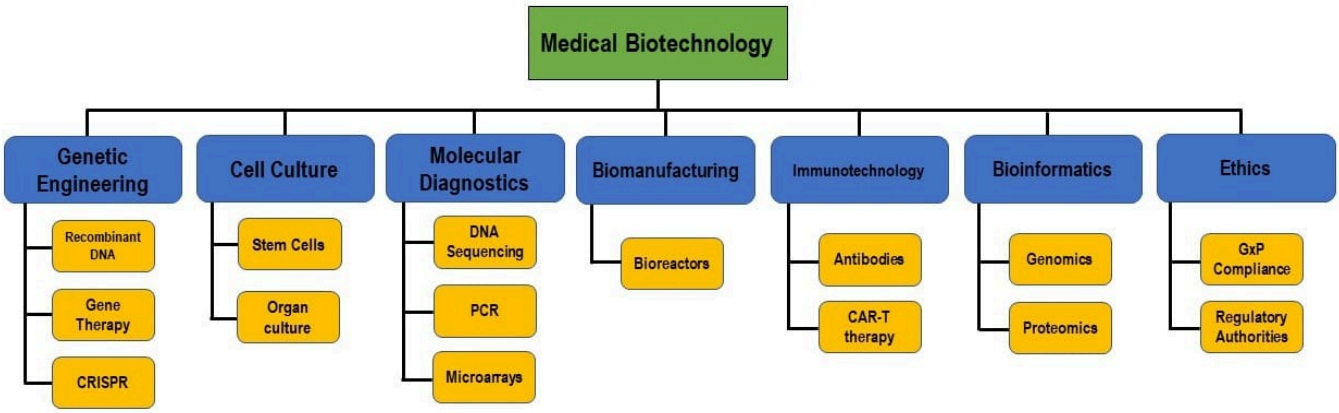


Figure 14.1.1: Medical Biotechnology Principles. (Medical Biotechnology by Patricia Zuk, CC BY 4.0)

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

Key Terms

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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14.2: 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.

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

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

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** 14.2.1). 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.

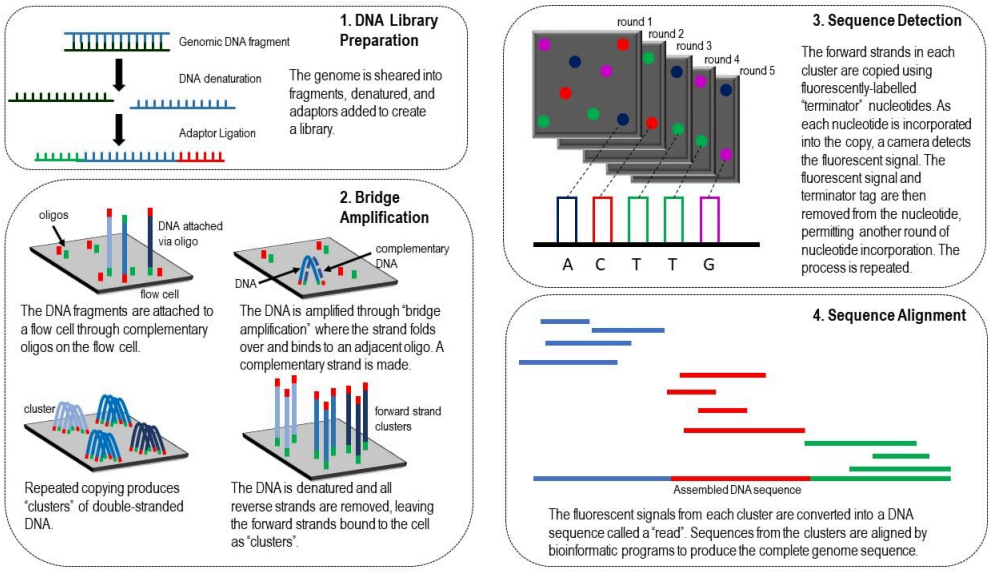


Figure 14.2.1**:** The Next Generation Sequencing (NGS) workflow. NGS can sequence multiple genomes in a day. (1) DNA Library Production: The genome is sheared into smaller fragments. Each fragment is flanked by two types of adaptors to create a DNA library for sequencing. (2) Bridge Amplification: Through 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** 14.2.2).

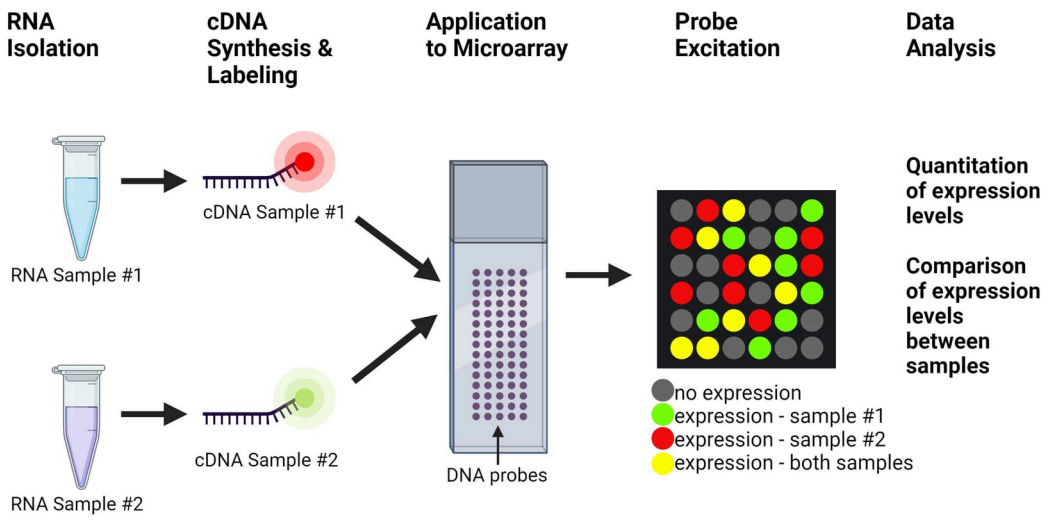


Figure 14.2.2: The steps of dual-color microarray analysis. (1) RNA Isolation: RNA is isolated from two different samples (RNA Sample #1 and RNA Sample #2). (2) cDNA Synthesis & Labeling: cDNA samples are synthesized from these RNA samples and labeled with different fluorescent molecules (cDNA sample #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** 14.2.3). 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.

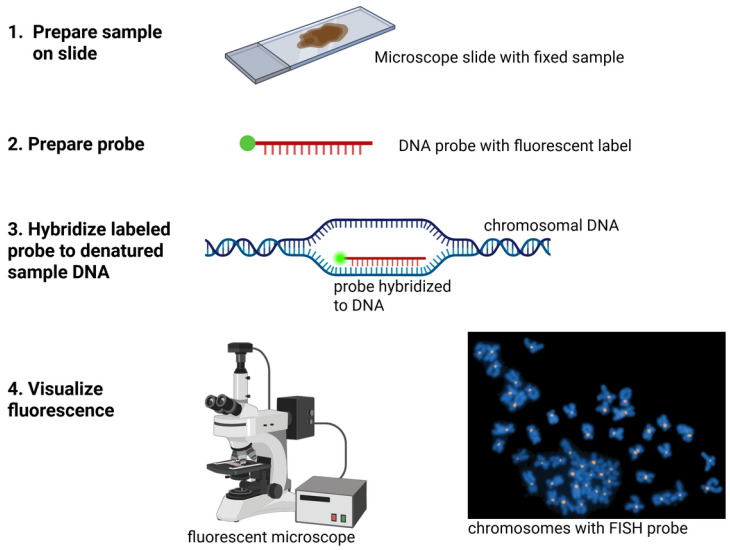


Figure 14.2.3: Fluorescent in situ hybridization (FISH). (1) Prepare sample on slide: samples a fixed to a microscope slide, permeabilized with detergent and the DNA in the sample denatured. (2) Prepare probe: DNA (or RNA) probes are synthesized to be complementary to a target DNA sequence in the sample 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** 14.2.4). 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.

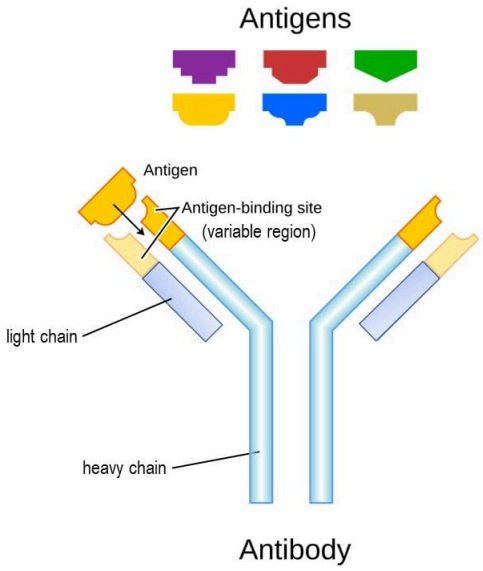


Figure 14.2.4: The structure of an antibody. The antibody is comprised of four polypeptides known as two heavy chains and two light chains. The ends of the light and heavy chains are known as the antigen-binding site or the variable region (shown in yellow). This variable region changes its shape to 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** 14.2.5), 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.

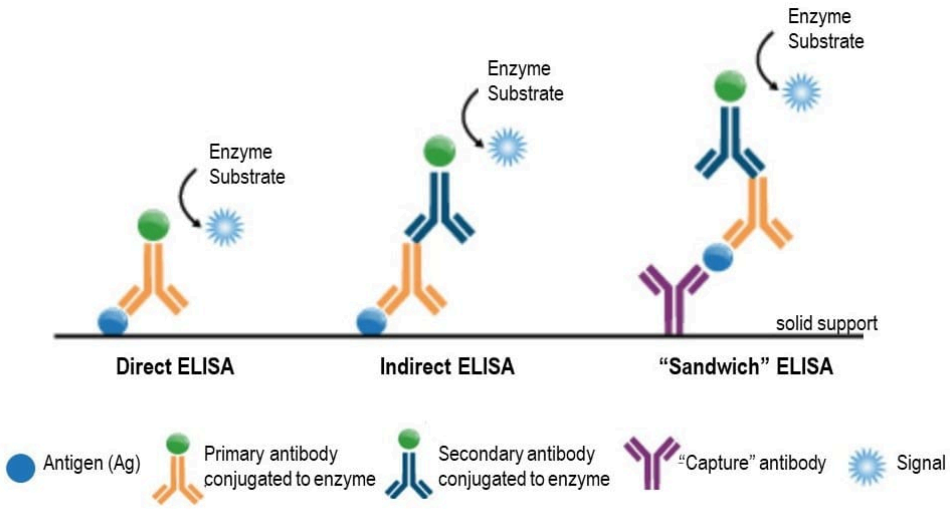


Figure 14.2.5: ELISA types. Some of the major types of ELISAs are direct, indirect, and "sandwich". In the direct ELISA, a primary antibody, coupled to an enzyme binds the antigen bound to the solid support. In the indirect ELISA, a primary antibody binds the antigen, which is then bound by the enzyme-coupled 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** 14.2.1.

Table 14.2.1*: 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

ELISA Step	Direct ELISA	Indirect ELISA	"Sandwich" ELISA
8. Substrate Addition	Yes	Yes	Yes
9. Measurement	Yes	Yes	Yes

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.

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** 14.2.6) 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

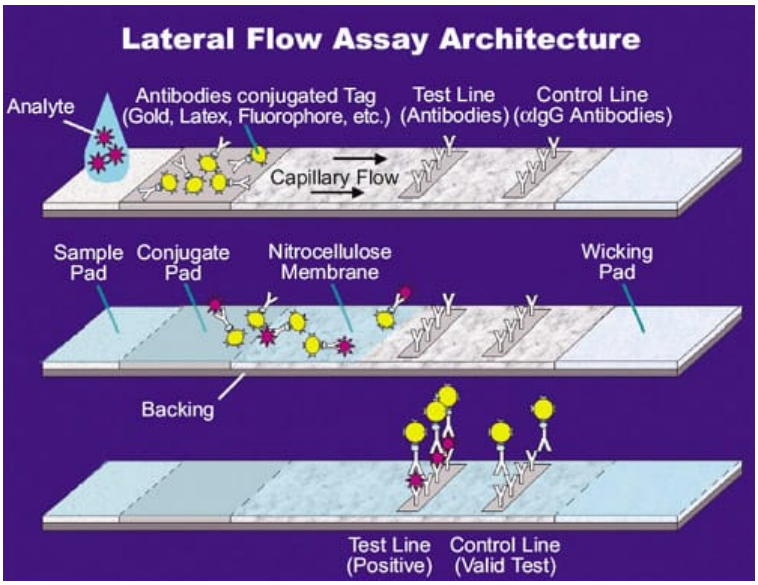


Figure 14.2.6: The lateral flow assay. The lateral flow assay (LFA) test strip is made of a sample pad, a conjugate pad containing antigen-specific antibodies conjugated to a tag like a gold nanoparticle, a nitrocellulose membrane with an antibody-bound test line, and a wicking pad. The sample (i.e. 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.

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** 14.2.7). 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.

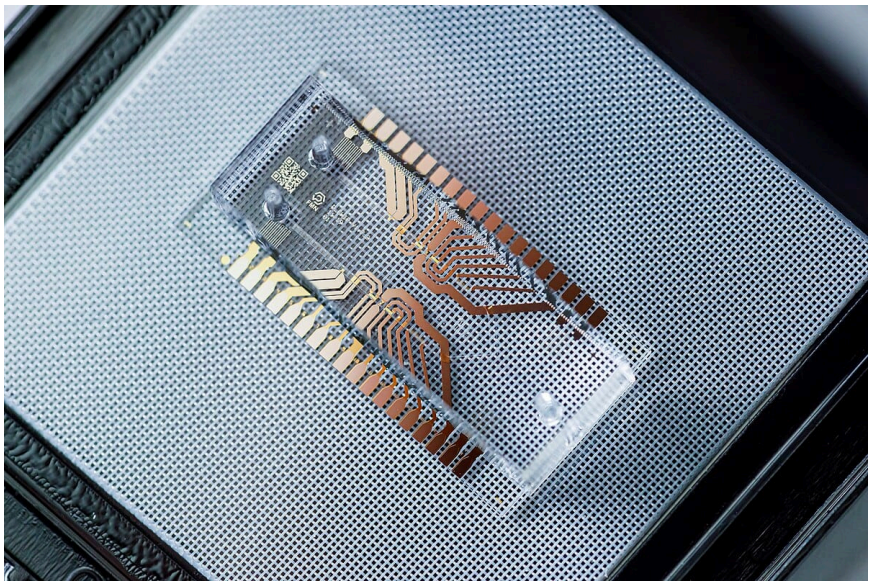


Figure 14.2.7: a Microfluidic chip. The microfluidic chip is a key element of lab-on-a-chip devices that are outside hospitals. The chip consists of multiple microfluidic channels and a detection system of metal electrodes on a glass substrate. (Microfluidic Chip by FMNLab, CC BY-SA 4.0)

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** 14.2.8) 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

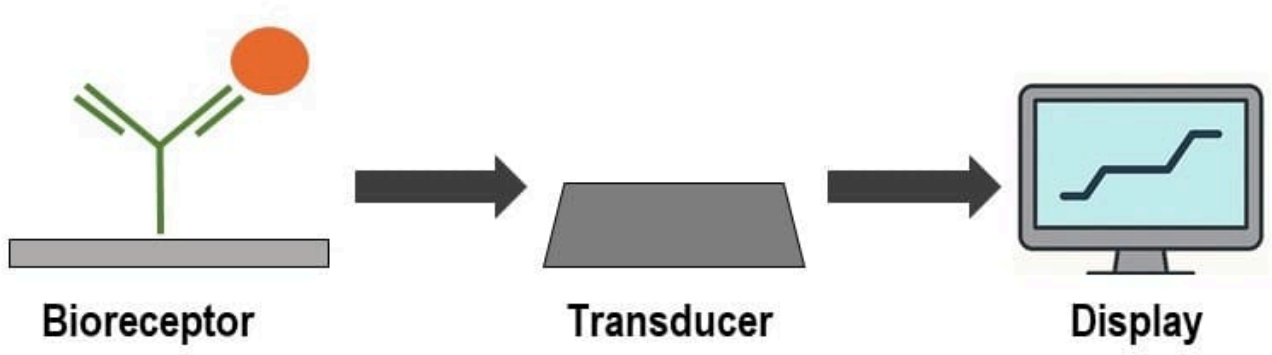


Figure 14.2.8: The biosensor. The biosensor is made of three components: the bioreceptor for detection of the target, the transducer which converts the detection into a measurable signal, and the display which displays the results (The Biosensor by Patricia Zuk, CC BY 4.0)

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.

Summary

Diagnostic biotechnology uses biological molecules and laboratory techniques to detect diseases, monitor health, and guide treatment decisions. Biomarkers—such as DNA, RNA, proteins, or hormones—help diagnose diseases, predict treatment responses, and monitor patient recovery.

Molecular diagnostic techniques, including PCR, next-generation sequencing (NGS), DNA microarrays, and fluorescent in situ hybridization (FISH), detect genetic changes associated with infections, inherited disorders, and cancers. Immunodiagnostic tests use antibodies to identify specific antigens or antibodies in patient samples. Common examples include the ELISA, which measures proteins or antibodies in blood, and lateral flow assays, such as home pregnancy tests and rapid COVID-19 tests.

Many of these technologies are used as point-of-care diagnostics, allowing healthcare providers to obtain results within minutes. Examples include glucose monitors, rapid test strips, biosensors, and lab-on-a-chip devices. These tools improve patient care by providing fast, accurate diagnoses and helping clinicians make timely treatment decisions. As diagnostic biotechnology continues to advance, it plays an increasingly important role in personalized medicine, infectious disease detection, and cancer diagnosis.

Key Terms

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.

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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14.3: 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.

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

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.

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 **C**ystic Fibrosis Transmembrane conductance Regulator (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** 14.3.1). 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.

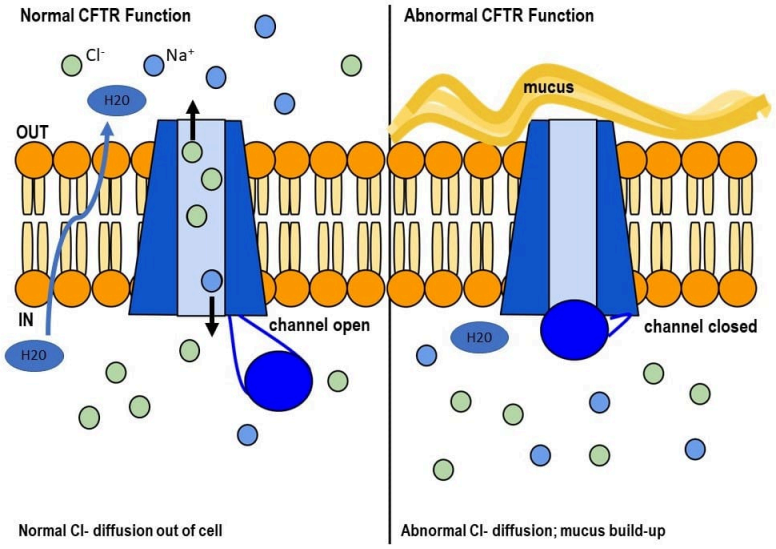


Figure 14.3.1: Cystic fibrosis (CF) and gene therapy. CF is caused by an abnormal CFTR channel protein. The CFTR controls the flow of sodium and chloride ions in and out of the epithelial cells lining the respiratory and digestive systems. In normal CFTR channel function (left panel), the channel is 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** 14.3.2). 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.

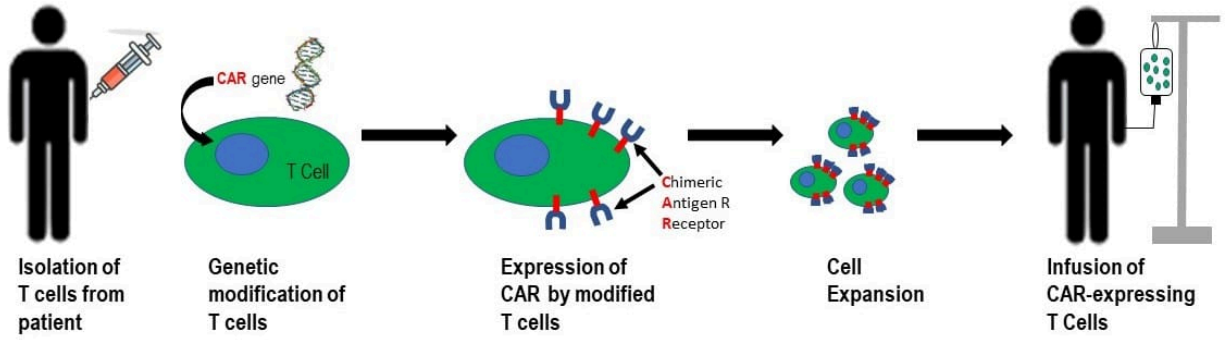


Figure 14.3.2: CAR-T gene therapy. T cells are isolated from a patient and genetically modified by introducing the gene for the Chimeric Antigen Receptor (CAR gene). Gene expression of this gene leads to the expression of the CAR on the surface of T cells. The modified T cells are expanded in number 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.

View this YouTube Video online

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** 14.3.3). 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.

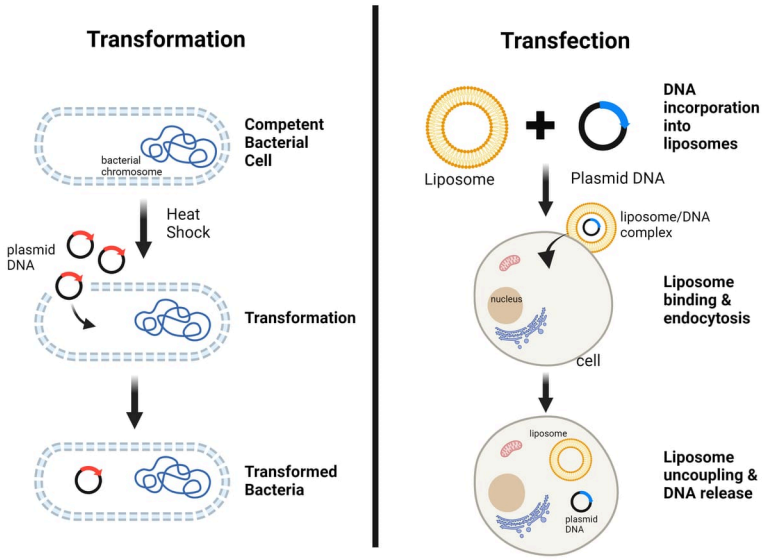


Figure 14.3.3: Transformation vs. Transfection. In transformation, competent bacterial cells undergo a "heat shock" to internalize DNA such as plasmid DNA. The transformed bacteria with its incorporated plasmid DNA begin expression of the new gene product. In transfection, DNA (e.g. plasmid DNA) is added 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** 14.3.4). 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.

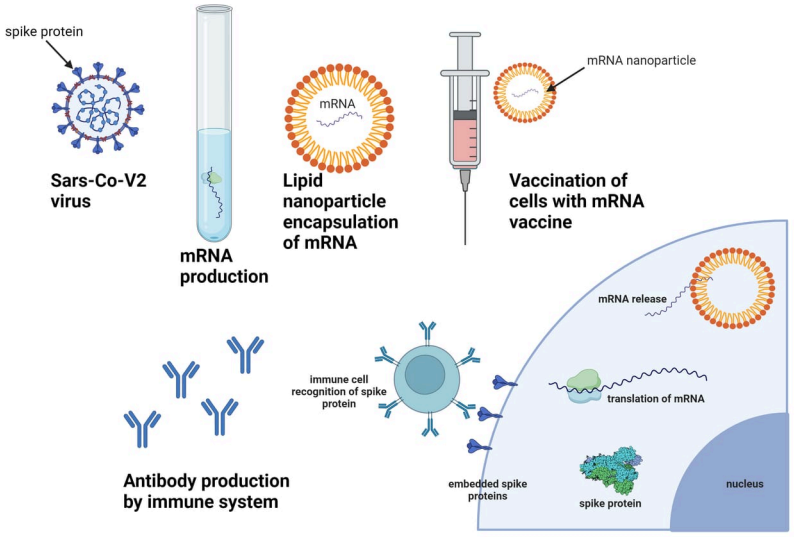


Figure 14.3.4: mRNA vaccine production. Vaccines against the spike protein of the Sars-CoV-2 virus can be made using mRNA. mRNA that codes for the spike protein is synthesized using a cell-free system (mRNA production). The mRNA particles are encapsulated into lipid nanoparticles (lipid nanoparticle 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

Summary

Therapeutic biotechnology applies living cells, microorganisms, and genetic engineering to develop treatments that prevent, manage, or cure disease. One of its greatest successes was the development of recombinant human insulin. In 1978, scientists inserted the human insulin gene into *Escherichia coli* (*E. coli*), allowing bacteria to produce human insulin. Approved in 1982, this became the first genetically engineered drug for human use, replacing insulin isolated from animal pancreases and improving safety and availability for people with type 1 diabetes.

A major focus of therapeutic biotechnology is gene therapy, which aims to treat disease by replacing, repairing, silencing, or editing faulty genes. Modern approaches include the use of viral vectors or lipid nanoparticles to deliver genetic material into cells, while CRISPR technology offers a more precise method for correcting disease-causing mutations. Another important application is CAR-T cell therapy, in which a patient's own T cells are genetically modified to recognize and destroy certain cancer cells.

Therapeutic biotechnology also produces many important medicines known as therapeutic proteins. These include hormones

such as insulin and growth hormone, clotting factors for hemophilia, monoclonal antibodies used to treat cancer and autoimmune diseases, and immune-modulating proteins. Most are produced by genetically engineered bacteria, yeast, or mammalian cells.

Vaccines are another key application. Traditional vaccines use purified proteins or weakened pathogens to stimulate immunity, while mRNA vaccines deliver genetic instructions that allow the body's cells to temporarily produce a harmless antigen, triggering a protective immune response. Together, these technologies have transformed modern medicine by providing safer, more targeted treatments and improving the prevention and management of infectious diseases, genetic disorders, cancer, and other chronic conditions.

Key Terms

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

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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14.4: 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.

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

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.

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** 14.4.1). 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.

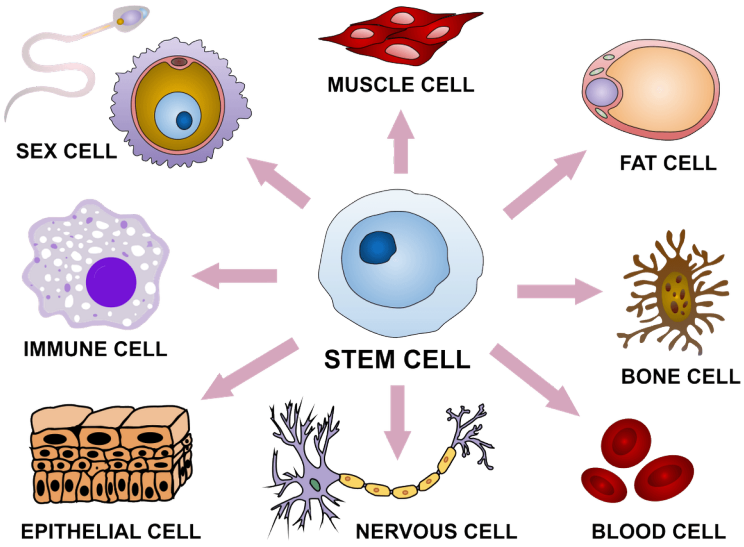


Figure 14.4.1: Stem cell differentiation. The stem cell differentiates in specific cell types such as epithelial, immune, sex, muscle, fat, bone, blood, and nervous cells (i.e., neurons) (Stem Cell by Haileyfournier, CC BY-SA 4.0)

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** 14.4.2):

- 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)

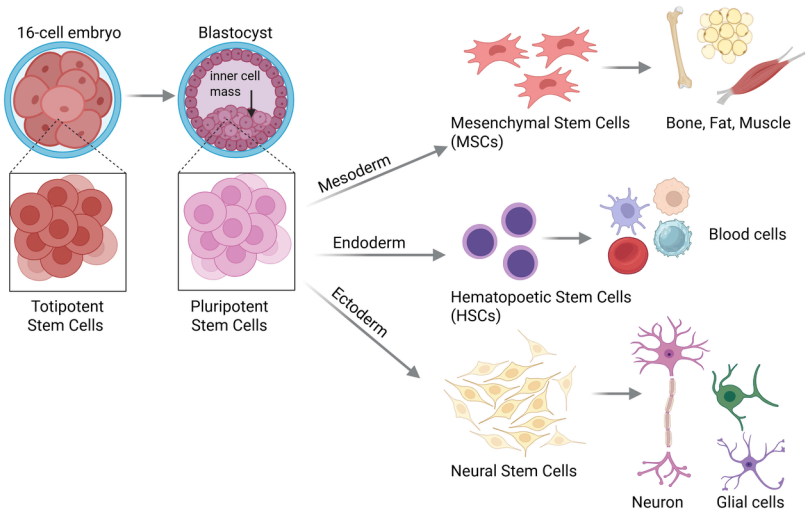


Figure 14.4.2: Stem cells have specific potency. The 16-cell embryo is made of cells called totipotent stem cells. Over time the embryo develops into a blastocyst. The inner cell mass of the blastocyst is a population of pluripotent stem cells. These stem cells develop into the mesoderm, endoderm, and 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** 14.4.1).

Table 14.4.1: *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** 14.4.2). These materials have the advantages of being bioactive and biocompatible. However, they can be variable in composition and have lower mechanical strength.

Table 14.4.2*: 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
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** 14.4.3) and tricalcium phosphate (**Table** 14.4.3).

Table: 14.4.3*: 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

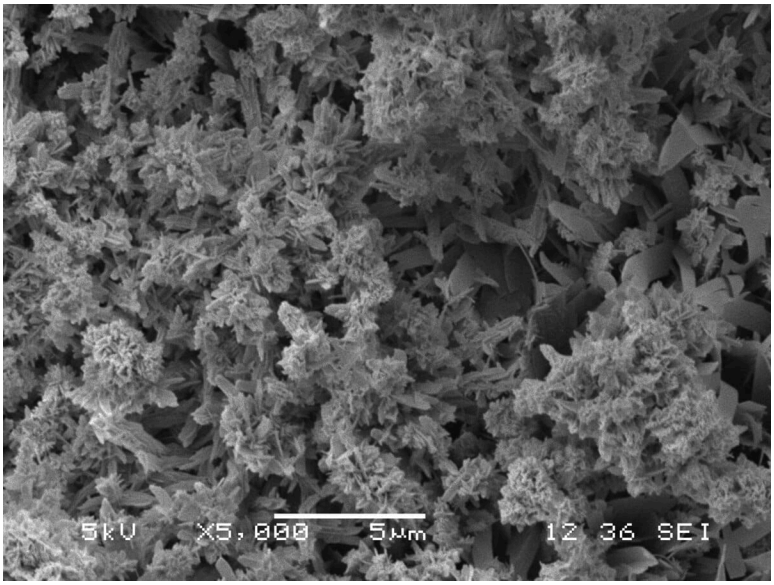


Figure 14.4.3: Hydroxyapatite crystals. This electron micrograph shows an extensive network of hydroxyapatite crystals that closely mimics the matrix of bone tissue. (Crystals of Hydroxylapatite by Nabilah, CC BY-SA 4.0)

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:** 14.4.4 below outlines some common bioactive molecules used in tissue engineering.

Table: 14.4.4: ***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.

Summary

Regenerative medicine is an interdisciplinary field that aims to repair, replace, or regenerate damaged cells, tissues, and organs by combining biology, engineering, and biotechnology. A key component is stem cell technology, which uses undifferentiated cells capable of self-renewal and differentiation into specialized cell types. Stem cells are classified by their potency: totipotent (can form all body and extraembryonic tissues), pluripotent (can form nearly all body cell types), and multipotent (can form a limited range of related cell types). Adult stem cells, especially hematopoietic stem cells from bone marrow, are widely used to treat blood disorders and cancers.

Another major area is tissue engineering, which combines living cells, three-dimensional biomaterial scaffolds, and bioactive molecules such as growth factors to create or repair tissues. Biomaterials may be natural, synthetic, or composite and must support cell attachment, growth, and integration with the body. Current applications include engineered skin, cartilage, bone, and blood vessels, while emerging technologies such as 3D printing and decellularized tissue show promise for creating functional organs. Although challenges remain, including immune responses, vascularization, and large-scale manufacturing, regenerative medicine has the potential to transform treatment for conditions such as burns, diabetes, heart disease, spinal cord injuries, and organ failure.

Key Terms

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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15: AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY – “GREEN” AND "GRAY" BIOTECHNOLOGY

15.1: "Green" vs. "Gray" Biotechnology
15.2: Applications of "Green" Biotechnology
15.3: Applications of "Gray" Biotechnology
15.4: Benefits of "Green" Biotechnology

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15.1: "Green" vs. "Gray" Biotechnology

Biotech Focus

Brassica juncea is a type of Indian mustard, a fast-growing plant species in the mustard family (*Brassicaceae*). Indian mustard is native to South Asia (India, China). It grows quickly and easily in a variety of soils, even under poor or stressful conditions. Upon harvesting, it is used as a leafy vegetable and produces mustard seeds for cooking oil and spices. Industrial processes in south Asia has resulted in the deposition of many types of heavy metals, like selenium, into the soil. Using both "green" and "gray" biotechnology, scientists have engineered Indian mustard to absorb more selenium from contaminated soils through a process called phytoremediation. In phytoremediation, native plants or plants that have been genetically-engineered can be used to clean up heavy metal contamination from selenium, cadmium, lead, and arsenic. Through phytoremediation, plants can improve the environment (gray biotech) and can be harvested for safe disposal or metal recovery, making the land usable for agriculture again (green biotech).

Learning Objectives

"Green" biotechnology uses science and engineering to improve agriculture and the environment. "Gray" biotechnology focuses on the environment. As such, these two biotech areas overlap significantly in their goals and techniques. At the end of this page, you will be able to:

- Define "green" biotechnology
- Define "gray" biotechnology
- Compare these two areas of biotechnology

Introduction

"Green" biotechnology, often called agricultural biotechnology, is the use of science and engineering on agriculture and the environment. As such, it overlaps with "gray" biotechnology, which is also known as environmental biotechnology. Gray biotechnology is a branch of biotechnology that focuses on solving environmental problems using living organisms. While these two areas share numerous areas in common, they can have distinct approaches and focuses. **Table** 15.1.1 compares these two areas of biotechnology.

Table 15.1.1**: Green vs. Gray Biotechnology**

	Green Biotechnology	Gray Biotechnology
Purpose	Improve farming and crop production	Clean and restore the environment
Main Tools	Genetic engineering of plants, biofertilizers, biopesticides	Bioremediation, engineered microbes and plants
Focus Areas	Agriculture, food safety	Pollution control, waste management

Green vs. Gray

"Green" biotechnology focuses on agriculture and food production. Its main goal is to improve crops, farming, and the environment using plant-based biotechnology techniques. "Gray" biotechnology uses plants, microbes, or enzymes to clean up pollution and waster. Its main goal is to restore ecosystems, reduce pollution, and make waste management more sustainable and eco-friendly. A quick way to distinguish these two biotech fields is to remember that green biotechnology grows better crops, while gray biotechnology cleans up a polluted world. While they may focus on distinct areas, both fields can use genetically-modified organisms created through biotechnology techniques to minimize environmental harms (**Figure** 15.1.1).

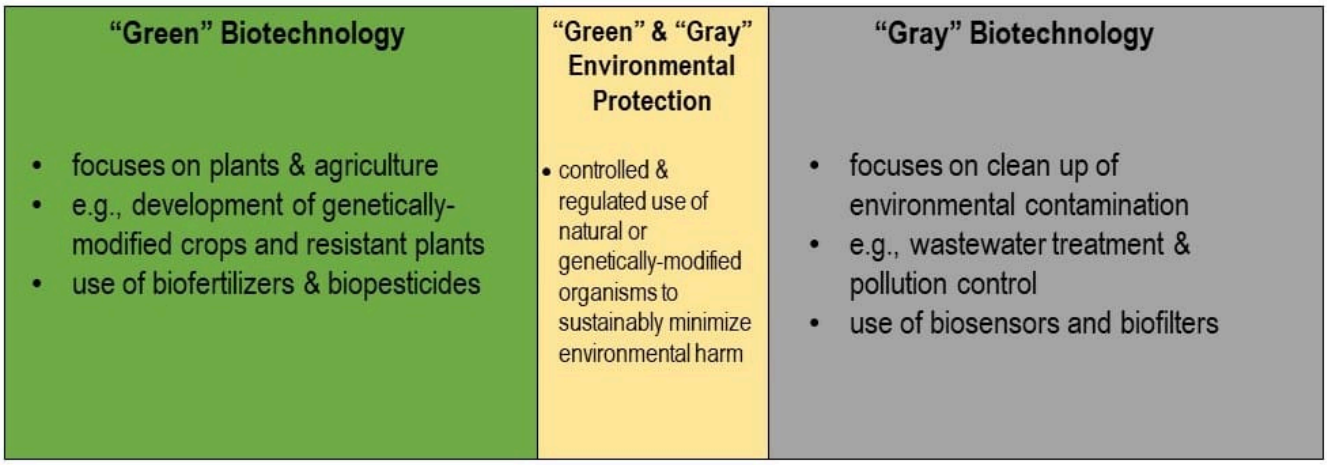


Figure 15.1.1: "Green" vs. "Gray" biotechnology. "Green" biotechnology focuses on plants and agriculture practices and can involve the development of genetically-modified crops and plants resistant to disease and pests. "Green" biotechnology uses biofertilizers and biopesticides to minimize environmental impact. "Gray" biotechnology focuses on the clean-up of environmental contamination. "Gray" biotechnology uses biosensors and biofilters in areas such as wastewater treatment and pollution control. Both biotechnology areas involved the controlled and regulated use of natural (i.e., unmodified) organisms or their genetically-modified counterparts to sustainably minimize harm to the environment. ("Green" vs. "Gray" Biotechnology by Patricia Zuk; CC BY 4.0)

Summary

Green and gray biotechnology both apply molecular biology and genetic engineering to address agricultural and environmental challenges. Green biotechnology focuses on improving crop production, food security, and sustainable farming through tools such as genetically modified plants, biofertilizers, and biopesticides. Its goal is to increase crop yields while reducing environmental impacts. Gray biotechnology, also known as environmental biotechnology, focuses on restoring ecosystems by using plants, microorganisms, and enzymes to remove pollutants, treat waste, and manage contaminated environments through processes such as bioremediation.

A good example of the overlap between these fields is phytoremediation, in which plants are used to remove toxic heavy metals from contaminated soil. Scientists have genetically engineered Indian mustard (Brassica juncea) to absorb higher levels of selenium and other metals, helping clean polluted land while restoring it for agricultural use. This demonstrates how molecular biology techniques can improve both crop performance and environmental health. Together, green and gray biotechnology show how genetic engineering and biotechnology can provide sustainable solutions for agriculture, pollution control, and ecosystem restoration.

Key Terms

Agriculture
: the science and practice of growing crops, raising animals, and producing food, fiber, and other products to support human life

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

Bioremediation
: the process of cleaning up a polluted environment using living organisms, such as microbes or plants, that break down harmful substances into less toxic or harmless forms

Fertilizer
: a substance that help plants grow by increasing the availability of nutrients (such as nitrogen or phosphorus)

Food safety
: practices and conditions that preserve the quality of food to prevent contamination and food-borne illnesses; ensured that food is safe to eat

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

"Green" Biotechnology
: a branch of biotechnology focused on developing genetically modified crops, biofertilizers, and biopesticides to promote sustainable farming

"Gray" Biotechnology
: a branch of biotechnology that uses using biological systems to protect and restore the environment; includes activities like bioremediation, pollution control, and wastewater treatment

Pesticide
: a synthetic or natural substance used to control pests

Pollution control
: efforts and technologies aimed at reducing or eliminating the release of harmful pollutants into the environment

Wastewater treatment
: the process of removing contaminants from used water (from homes, businesses, or industries) so that it can be safely returned to the environment or reused

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15.2: Applications of "Green" 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.

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

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

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 15.2.1: *Agrobacterium tumefaciens* and crown gall disease. Top image: *Agrobacterium tumefaciens* is a rod-shaped, Gram-neg bacteria that infects plant cells. (*Agrobacterium tumefaciens* by A. G. Matthysse, K. V. Holmes, R. H. G. Gurlitz; Public domain) Bottom i Infection with *Agrobacterium tumefaciens* leads to "crown gall" disease - the production of several small tumors (called "galls") on the (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** 15.2.2). *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.

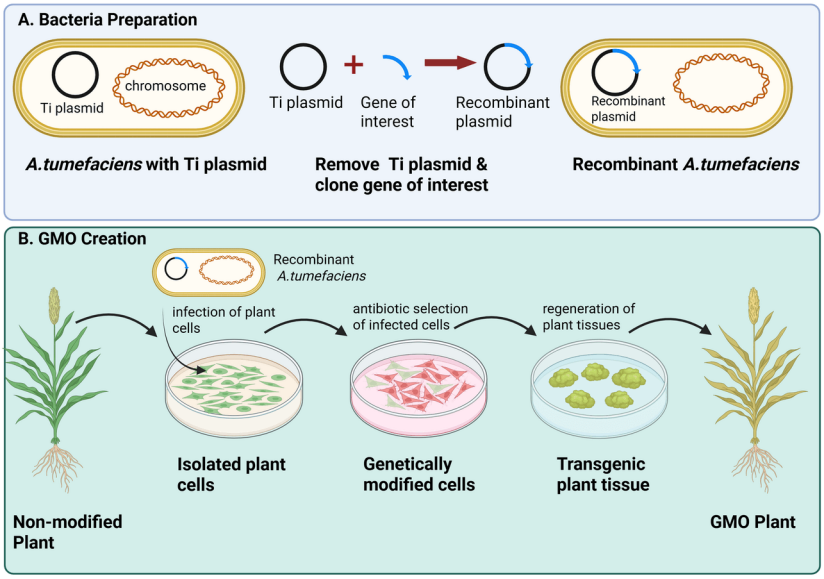


Figure 15.2.2: Production of a genetically-modified plant. (A) Bacteria Preparation (blue box at top of figure): *Agrobacterium tumefaciens* bacteria contain a plasmid called the Ti plasmid. The Ti plasmid is removed from *A.tumefaciens* bacterial cells (black circle) and the gene of interest (blue arrow) 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** 15.2.3). 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.

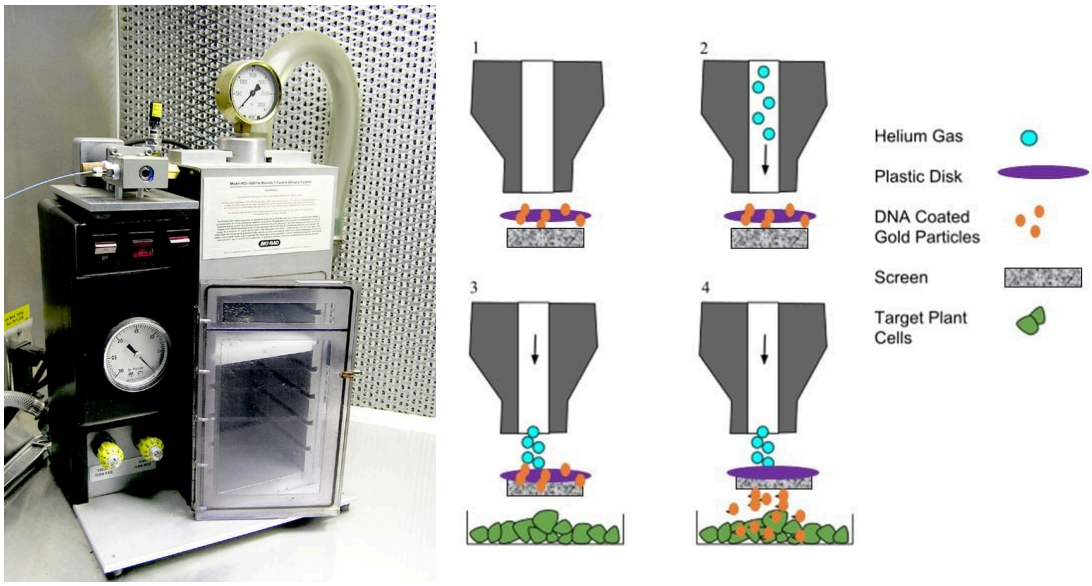


Figure 15.2.3: Gene delivery using a "gene gun". Top image: A picture of a gene gun with the chamber open to receive the cell sample. (Genegun by xmort; Public domain) Bottom image: 1. DNA-coated gold particles are loaded onto a plastic disk and placed in the gene gun on top of a screen. Cells are plated 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** 15.2.4). These nitrogenous compounds are used by the plants to synthesize proteins.

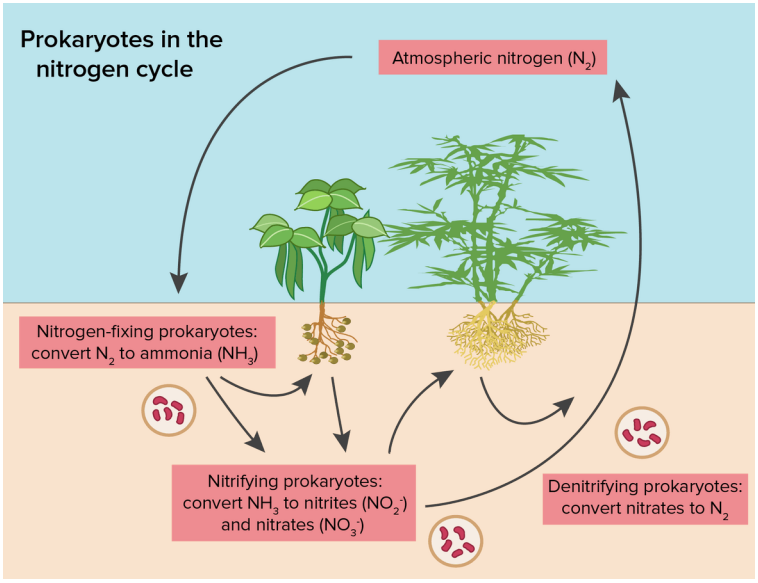


Figure 15.2.4: The nitrogen cycle. Nitrogen-fixing prokaryotes that live in soil and convert atmospheric N_2 intro ammonia (NH_3). In addition, nitrifying prokaryotics convert NH_3 into nitrites (NO_2^-) and nitrates (NO_3^-). Each of these compounds are used by plants. Denitrifying prokaryotes can convert 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** 15.2.1 lists some commonly used biofertilizers.

Table 15.2.1: *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** 15.2.5). 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.

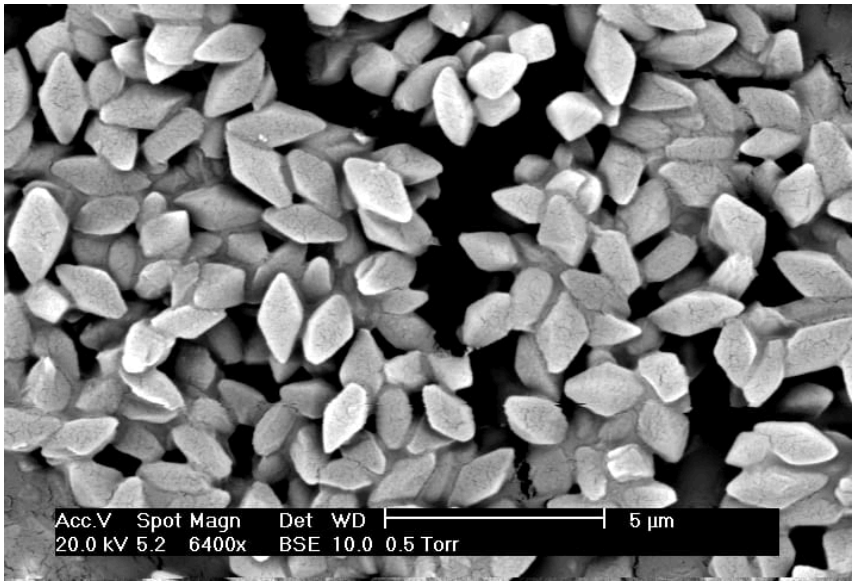


Figure 15.2.5: Bt-produced cry proteins are insecticidal towards certain insect pests. (Crystals of Bt Toxin by Jim Buckman; public domain)

Summary

Green biotechnology applies genetic engineering and other molecular techniques to improve agriculture while promoting environmental sustainability. Its goals include increasing crop yields, enhancing resistance to pests, diseases, and environmental stress, improving nutritional value, and reducing reliance on chemical fertilizers and pesticides. Genetically modified (GM) crops are created by introducing beneficial genes into plant cells, often using the soil bacterium *Agrobacterium tumefaciens* or a gene gun to deliver DNA. Green biotechnology also uses biofertilizers, which contain beneficial microorganisms that improve nutrient availability, including nitrogen-fixing bacteria that convert atmospheric nitrogen into forms plants can use. Biopesticides, such as the bacterium *Bacillus thuringiensis* (Bt), provide environmentally friendly pest control by producing proteins that target specific insect pests while minimizing harm to other organisms. These technologies can increase food production, lower farming costs, and reduce environmental impacts. However, genetically modified crops also raise important questions about biodiversity, food safety, environmental effects, and regulation, making green biotechnology both a powerful and carefully debated field of modern science.

Key Terms

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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15.3: Applications of "Gray" 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.

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

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

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** 15.3.1). 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 15.3.1: 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** 15.3.2).

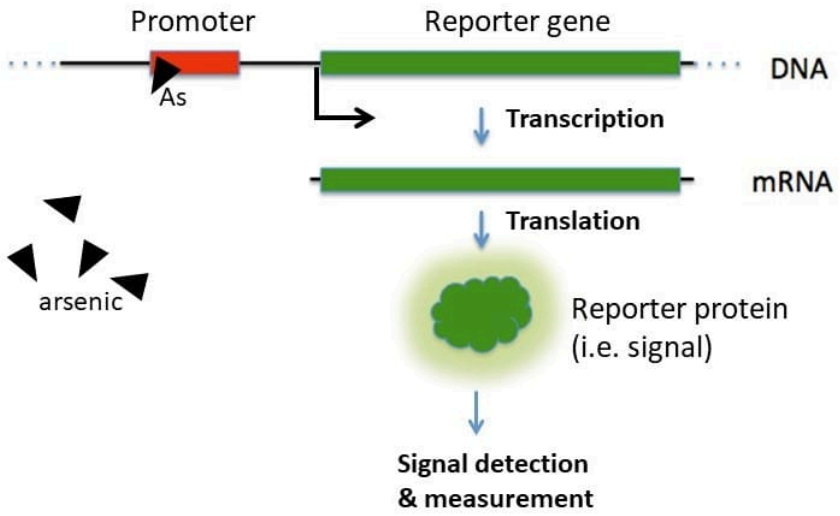


Figure 15.3.2: Biosensors can be built using reporter systems. A typical reporter system has a promoter (red rectangle) capable of being bound by the compound to be detected and measured (e.g., arsenic, As). Binding of this compound results in transcription of the reporter gene into mRNA and then translation 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.

Summary

Gray (environmental) biotechnology uses biological systems to reduce pollution, restore ecosystems, and improve waste management. One major application is bioremediation, which uses microorganisms, plants, or their enzymes to remove harmful pollutants from soil, water, and air. Scientists can enhance this process by genetically engineering bacteria to break down substances such as oil, industrial dyes, and toxic chemicals more efficiently. Phytoremediation uses plants to absorb, stabilize, or degrade contaminants, including heavy metals and pesticides. Biotechnology also improves wastewater treatment by engineering microbes that remove pollutants more rapidly and function under harsh conditions. In pollution control, biofilters and bioaugmentation use beneficial microorganisms to clean contaminated air and water. Another important application is environmental monitoring through biosensors, which combine biological molecules or genetically engineered bacteria with reporter proteins to detect pollutants and produce measurable signals, such as fluorescence or color changes. Together, these technologies provide environmentally friendly alternatives to traditional cleanup methods while helping protect ecosystems and human health, although the use of genetically modified organisms requires careful regulation to prevent unintended environmental impacts.

Key Terms

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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15.4: Benefits of "Green" Biotechnology

Biotech Focus

India has one of the highest rates of iron-deficient anemia globally, especially in women and children. In response, a public health initiative was undertaken in India to provide school-aged children in rural schools with biofortified "pearl millet" that was high in iron for 6 months. Children were served meals (like chapatis or porridge) made from either biofortified pearl millet or standard pearl millet with its normal iron level. Pearl millet is a staple food in many dry regions of India. It is a drought-resistant crop that is easy to improve using conventional breeding or biotechnology. Hemoglobin levels and iron stores in the students were measured before and after the study. At the end of the study, the children who ate the iron-biofortified pearl millet showed a significant improvement in iron status and a reduction in iron-deficiency anemia. Even small daily servings of pearl millet made a noticeable impact on the student's health. The iron-enriched pearl millet study in school children in India is a real-world example of biofortification in action—where science and nutrition come together to fight a nutritional deficiency in a vulnerable population.

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:

- Explain some of the benefits of green biotechnology

Introduction

Throughout history, farmers have selectively bred plants 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. Green biotechnology offers a more precise approach to developing desirable traits in plants. 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.

Benefits of "Green" Biotechnology

Plant biotechnology is part of the "Green" biotechnology field. 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. It is used to modify products, improve plants (or animals), or develop or engineer microbes for specific agricultural uses. Green biotechnology, in combination with traditional breeding techniques, can provide essential tools to farmers that can make agricultural production cheaper and more manageable.

There are many benefits of "Green" biotechnology with regards to plants and farming.

Improved crop production: Biotech crops can make farming more profitable by increasing crop quality and may in some cases increase yield. In addition, biotechnology can improve the health of crops. Some crops are engineered to tolerate specific herbicides without compromising their yields, making weed control simpler and more efficient. Other crops have been engineered to be resistant to specific plant diseases caused by certain microbes and insect pests, which can make pest control more reliable and effective, and/or can decrease the use of synthetic pesticides. Resistance to viruses, bacteria, fungi, insects or herbicides is known as biotic stress tolerance.

Biofortification: Biofortification is the process of increasing the nutritional value of crops through breeding, genetic engineering, or biotechnology. Biofortification focuses on the generation of micronutrient-dense crops through green biotechnology in order to improve the nutritional content in staple crops. Micronutrient-dense crops can effectively reduce vitamin and mineral deficiencies in populations who have limited access to diverse diets. Studies have shown its effectiveness, with iron-biofortified beans improving iron levels in Rwandan women and iron-biofortified pearl millet reversing iron deficiency in Indian schoolchildren.

Golden rice, modified through biotechnology techniques to produce beta-carotene, a precursor to vitamin A (**Figure** 15.4.1), was developed in 2000 to help combat vitamin A deficiency, a major cause of childhood blindness. Crops like canola, soybean, and corn have been improved with healthier oil compositions. Along with enhanced nutritional value, crops can also be generated to be resilient to environmental stresses, such as drought or pests. This dual benefit offers a promising strategy to address global malnutrition, improving the well-being of communities worldwide. However, biofortification comes with its challenges. Biofortified crops may be produced with heightened nutrient levels, but they need to be in a form the body can digest and absorb. These nutrients should also remain stable and retained throughout cooking, processing, and storage to ensure their full nutritional benefits are delivered. Another challenge lies in gaining acceptance and adoption of biofortified crops among both farmers and consumers, especially when traditional options are already well-established.



Figure 15.4.1: White rice and golden rice. Thanks to green biotechnology, golden rice (shown at the right) has significantly more vitamin A compared to white rice (shown at the left). The increased vitamin A content has been credited with preventing blindness in millions of people (Golden Rice by the International Rice Research Institute; CC BY 2.0)

Environmental benefits: Insect-resistant cotton has significantly reduced the use of persistent, synthetic pesticides that may contaminate groundwater and the environment. Herbicide-tolerant crops are compatible with no-till or reduced tillage agriculture systems that help preserve topsoil from erosion.

Pharmaceuticals: Green biotechnology has emerged has a promising tool for vaccine production through its development of an edible vaccine. In this approach, genes encoding bacterial or viral antigens - the compounds that trigger an immune response - are introduced into plants. One of the most common ways of introduction is the bacteria *Agrobacterium tumefaciens* (for more information see Chapter 9.1 Applications of Green Biotechnology). The plant then produces these antigens in its edible parts (e.g. seeds, leaves, roots). When a person eats the plant, their immune system will recognize the antigen as foreign and launch a small immune response, thus building immunity to the antigen. Bananas, potatoes, tomatoes, rice, and lettuce are all being explored as edible vaccine carriers. Pathogens being targeted include those that cause widespread diseases, like hepatitis B, cholera, measles, and even COVID-19. Although edible vaccines provide the advantage of convenient and needleless administration, they are not currently as effective as normal vaccines as they are consumed and not injected into the blood. This is because in order to elicit an significant enough immune response, the antigens must survive the digestive process and be absorbed into the blood.

Production of phytochemicals: Green biotechnology is being used to genetically-modify plants to enhance their production of phytochemicals or to allow them to make new phytochemicals not normally found in the plant. Phytochemicals (**Figure** 15.4.2) are naturally-occurring compounds found in plants that are not essential nutrients, like vitamins or minerals, but can have health-promoting properties. Phytochemicals are made by plants to protect themselves from pests, diseases, and environmental stress. In humans, phytochemicals act as antioxidants, anti-inflammatory agents and immune boosters. Common phytochemicals include the flavonoids and carotenoids. Using green biotechnology, tomatoes have been engineered to make higher levels of anthocyanins, a type of flavonoid, that can act as an antioxidant. The insertion of the stilbene synthase (STS) gene into the genomes of tomatoes and rice has resulted in the production of the antioxidant, resveratrol, in these plants. Outside of the farm, plant cells grown in tissue culture facilities can be used to produce specific phytochemicals in highly controlled environments, allowing for year-round production and avoidance of environmental variability.

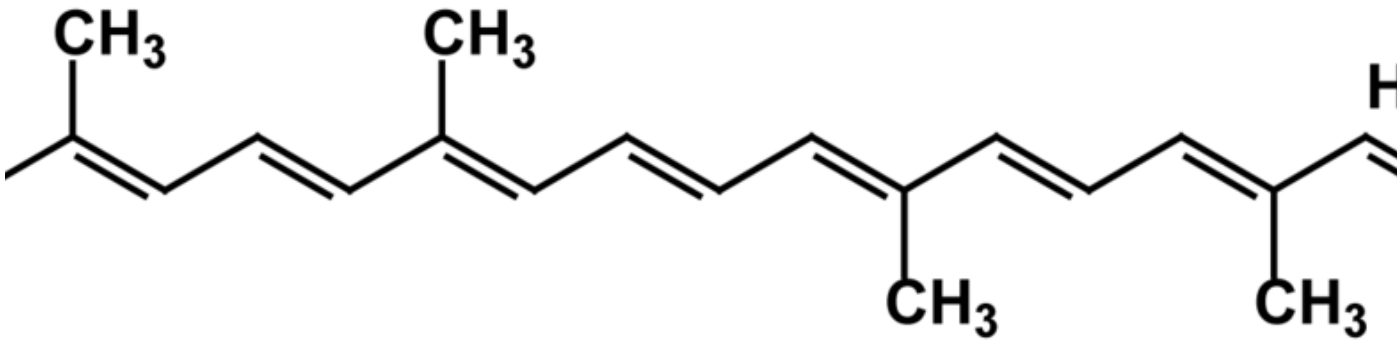


Figure 15.4.2: Phytochemicals. Top image: the chemical structure of the anthocyanin flavonoid, chrysanthemin chloride. (Chrysanthemin chloride by NiCrMo, CC BY-SA 4.0)
Center image: the chemical structure of the carotenoid, beta-carotene (Beta-carotene by Polimerek, CC BY-SA 3.0)
Bottom image: the chemical structure of resveratrol (Resveratrol by Fvasconcellos, Public Domain)

Green Biotechnology Concerns

One key concern of green biotechnology is the potential transfer of novel traits to wild relatives. However, this potential transfer is carefully evaluated during the review and approval of genetically-engineered crops. Environmental impacts of engineered plants are also assessed, particularly regarding their effects on birds, mammals, insects, and other organisms, especially in the case of engineering insect or disease resistance traits. For example, studies have been conducted to ensure that bees are not adversely affected by the crops engineering to be resistant to pests. Food safety is another critical focus; newly introduced traits in green biotechnology plants undergo rigorous testing to assess potential toxicity and the risk of allergic reactions. These comprehensive evaluations help ensure that genetically engineered crops are both safe and effective for consumers and the environment.

To provide regulatory oversight of organisms derived through genetic engineering, the federal government developed a Coordinated Framework for the Regulation of Biotechnology in 1986. This framework spells out the basic federal policy for regulating the development and introduction of products derived from biotechnology. The policy has three tenets:

1. U.S. policy focuses on the *product of genetic modification* (GM) techniques, not the process of genetic modification itself
2. only regulation grounded in verifiable scientific risks is tolerated
3. genetically-modified products are on a continuum with existing products and, therefore, existing statutes are sufficient to review these GM products

Products made through biotechnology are regulated according to their intended use, with some products being regulated under more than one agency. The three principal agencies that have provided primary guidance on the experimental testing, approval, and eventual commercial release of modified organisms to date are the:

1. Animal and Plant Health Inspection Service (APHIS) - under the USDA
2. Environmental Protection Agency (EPA)
3. Food and Drug Administration (FDA) - under the Department of Human and Health Services (HHS)

APHIS protects US agriculture from pests and diseases. APHIS regulations concentrate on genetically-modified plants in order to ensure that they don't harm other plants or the environment. The EPA protects human health and the environment. In the biotechnology field, the EPA regulates biopesticides, biofertilizers, and genetically-modified crops that produce pest-resistant compounds like the Bt toxin discussed in Chapter 9.1 Applications of Green Biotechnology. The FDA ensures the safety of food, drugs, and cosmetics. The FDA reviews genetically-modified foods for safety, proper labeling, and nutritional content.

The Future of Green Biotechnology

The future of green biotechnology is exciting and full of possibilities. Some examples include the development of climate-resistant plants that can survive droughts, floods, and extreme temperature by enhancing their abiotic stress tolerance, the continued creation of biofortified foods that are rich in specific vitamins, minerals, and antioxidants, and expansion of the use of plants in phytoremediation. However, some new areas are entering the field include the use of CRISPR-based systems to fine-tune desired plant traits (e.g. pest resistance, faster growth) with extreme accuracy. To read more about CRISPR, go to Chapter 8.1 Applications of Animal Biotechnology.

Summary

Green biotechnology uses genetic engineering and plant breeding to improve crop productivity, nutrition, and sustainability. One important application is biofortification, which increases the levels of essential vitamins and minerals in staple crops to help reduce nutrient deficiencies. For example, iron-enriched pearl millet has been shown to improve iron status and reduce anemia in schoolchildren, while Golden Rice was developed to produce beta-carotene, a precursor of vitamin A, to help prevent vitamin A deficiency. Green biotechnology also produces crops that are more resistant to pests, diseases, drought, and other environmental stresses, reducing the need for chemical pesticides and improving food security. Additional applications include developing edible vaccines and increasing beneficial plant compounds, called phytochemicals, with antioxidant and health-promoting properties. Before genetically modified crops are approved, they undergo extensive testing to evaluate food safety and environmental impacts. Government agencies regulate these products to ensure they are safe for consumers and ecosystems. Overall, green biotechnology offers promising tools to address global challenges in agriculture, nutrition, and environmental sustainability.

Key Terms

Abiotic
non-living physical or chemical factors in the environment, like temperature, sunlight, or soil quality

Agriculture
the science and practice of growing crops and raising animals for food, fiber, and other products to support human life

Agricultural biotechnology
the use of scientific tools and techniques, like genetic engineering, to improve crops, animals, and farming methods; often called
"green" biotechnology

Antigen
a substance (usually a protein or sugar) that triggers an immune response in the body; often used in vaccines

Anti-inflammatory
a substance that reduces swelling, pain, or irritation in an organism

Antioxidant
a compound that helps protect cells from DNA damage caused by free radicals

Biofortification
the process of increasing the nutritional value of crops through breeding and/or genetic engineering

Biotic
the living components of an ecosystem, such as plants, animals, fungi, and bacteria

Carotenoid
a natural pigment found in plants that gives yellow, orange, or red color; many act as antioxidants; examples include beta-carotene, lutein

Crop
a plant that is cultivated and harvested for food, fiber, or economic purposes

Flavonoid
: a type of phytochemical found in fruits, vegetables, and tea; known for antioxidant and anti-inflammatory properties

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

"Green" biotechnology
: a branch of biotechnology focused on developing genetically modified crops, biofertilizers, and biopesticides to promote sustainable farming

Pest
: any organism that damages or interferes with crops, livestock, food, or property—commonly includes insects, weeds, fungi, and rodent

Pesticide
: a chemical or biological agent used to kill or control pests, including insects (insecticides), weeds (herbicides), fungi (fungicides), and others

Phytochemical
: a naturally occurring compound in plants that is not essential for life but may offer health benefits; examples include antioxidants

Vaccine
: a biological preparation that stimulates the immune system to protect against a specific disease

Vitamin
: an essential nutrient that the body needs in small amounts to function properly; examples include vitamin C, vitamin D

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16: INDUSTRIAL BIOTECHNOLOGY – “WHITE” BIOTECHNOLOGY

16.1: Applications of Industrial Biotechnology
16.2: Bioprocess Engineering
16.3: Synthetic Biology

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16.1: Applications of Industrial Biotechnology

Biotech Focus

After the Deepwater Horizon oil spill in 2010, scientists began to study how bioremediation techniques could be used to speed up the natural process of breaking down oil in order to minimize the impact of oil spills on the environment. Their work was based on the fact that certain bacteria, like *Alcanivorax borkumensis*, can use the hydrocarbons in oil as a food source, breaking it down into simpler compounds. *A. borkumensis* was discovered near the island of Borkum (hence the epithet borkumensis) by scientists in 2006. It is classified as a halophile, an organism that thrives in salty environments, like the ocean. It can be found all across the world both in coastal environments and oceanic environments and has been found to be more common in oceanic areas containing petroleum - although whether this is from spills, natural fields, or other sources of oil, is not known.

The use of microbes in the clean-up of hazardous materials has since gained in popularity since the Deepwater Horizon spill. *Pseudomonas putida*, a versatile bacteria found in soil and water environments, can break down a variety of hydrocarbons, including benzene and other toxic oil-related compounds. Several species of *Mycobacterium* can degrade complex hydrocarbons, which are found in oils, and are often resistant to harsh environments. Other bacterials strains like those of the *Bacillus* and *Thalassolituus* genera have also become part of the ever expanding bioremediation "toolkit" available to industrial manufacturers.

Check out this video explaining how bacteria, like *A. borkumensis*, are used to "eat" oil spills.

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

Learning Objectives

Industrial biotechnology uses biological systems to develop products, processes, or services for industry. At the end of this page, you will be able to:

- Identify the key areas of industrial biotechnology
- Describe the types of bioplastics
- Describe the enzyme types made through industrial biotechnology
- Explain how industrial biotechnology can make enzymes
- Explain how the pharmaceutical industry and industrial biotechnology are related
- List some of the areas of environmental remediation
- List some of the advantages of industrial biotechnology

Introduction

Industrial biotechnology, also known as "white" biotechnology, is a branch of biotechnology that uses a biological system to develop bio-based products, processes, or services used in industry. These biological systems may be naturally occurring, modified slightly through genetic engineering, or can be redesigned from "scratch" to create synthetic systems. To learn about synthetic biology, go to Chapter 11.2 Synthetic Biology & Bioprocess Engineering.

One popular use of industrial biotechnology is the production of a "biologic", a therapeutic product extracted from a biological organism. The biological systems used in industrial biotechnology include the use of microbes (e.g. bacteria, yeast, other fungi), enzymes, and cell cultures. Industrial biotechnology integrates these biological systems with other areas, like engineering, to

create sustainable and efficient solutions for industries such as agriculture, energy, pharmaceuticals, chemicals, and textiles.

Some commonly used organisms used in industrial biotechnology are:

- *E.coli* - a bacterial strain used in recombinant DNA technology for protein production
- *Bacillus subtilis* – a bacterial strain that is used to produce industrially important enzymes like amylases and proteases
- *Saccharomyces cerevisiae* – a yeast strain used in fermentation processes for food (.e.g beer, wine), pharmaceuticals, and bioethanol production
- *Aspergillus niger* – a fungus used for citric acid production

Industrial biotechnology is sustainable, using renewable resources to replace fossil-based fuel processes. Its use of microbial systems, like bacteria and yeast, allows industries to manufacture products efficiently with relatively little energy input. The renewable nature of industrial biotechnology has a relatively small carbon footprint in comparison to conventional manufacturing processes, making this area of biotechnology eco-friendly and sustainable.

Key Areas of Industrial Biotechnology

Industrial biotechnology encompasses several key areas, including:

1. Bio-based chemicals and materials - the production of biodegradable plastics and bio-based solvents.
2. Biofuels and Bioenergy – the use of microbes, like bacteria and yeast, to produce renewable energy sources, such as bioethanol, biodiesel, and hydrogen
3. Enzyme Technologies – the production of enzymes used in food processing, detergents, textiles, and pharmaceutical industries
4. Biopharmaceuticals – the large-scale production of vaccines, antibodies, and other therapeutic compounds
5. Agricultural Biotechnology – the development of genetically-modified crops, biofertilizers, and biopesticides

Applications of Industrial Biotechnology

Bioplastics

Biodegradable plastics, also known as bioplastics, are a category of plastics that are derived from renewable biological sources like plant starches, plant oils, organic waste, and microbes. Unlike conventional plastics, which are made from petroleum products, bioplastics are either biodegradable, compostable, or bio-based and non-degradable, making them eco-friendly alternatives.The uses of bioplastics are wide and varied and can include medical implants and devices, food packaging and agricultural films.

Types of bioplastics include:

- bio-based, non-degradable - made from renewable resources but do not degrade easily
  - e.g. bio-based polyethylene (PE) - derived from sugar cane; chemically identical to fossil-fuel based PE
- biodegradable - break down naturally in the environment through microbial action
  - cellulose-based (e.g., cellulose acetate, cellophane) - produced through the microbial fermentation of cellulose; source material includes wood pulp, cotton, and agricultural waste; used in food packaging, textiles and fibers, coatings and films, filters and electronics
  - starch-based (e.g. thermoplastic starch) - produced through the microbial fermentation of cellulose;
- compostable - break down into water, CO_2, and other biological compounds under industrial composting conditions

Production of bioplastics typically begins with a biopolymer, a natural polymer made by living organisms. Polysaccharides and proteins are well-known examples of biopolymers, along with more complex compounds that come from microbial sources. Many bioplastics are produced through the fermentation of biopolymers like starch and cellulose. Advances in genetic engineering have led to more efficient microbial strains capable of producing high yields of bioplastics.

Examples of biopolymers used in bioplastic production are:

- polylactic acid (PLA) - produced through the microbial fermentation of corn starch or sugar cane
- polyhydroxyalkanoates (PHA) - produced from bacteria; used in medical implants, food packaging and agricultural films

Biofuel Production

With the rising demand for sustainable energy, biofuels such as ethanol, biodiesel, and biogas have gained significance. Industrial biotechnology facilitates the conversion of biomass (e.g., sugarcane, corn, and algae) into renewable fuels through microbial fermentation. Algae-based biofuels, in particular, hold promise due to their high lipid content and rapid growth. To read more about biofuel production, go to Chapter 7.4 Industrial Applications of Microbial Biotechnology.

Industrial Enzyme Production

Industrial enzyme production is the large-scale process of culturing microbes, like bacteria or yeast to produce enzymes for commercial applications. These microbes may be genetically-modified or used unaltered for this production. The enzymes made can be used in a wide variety of industries, such as the food, textile, pharmaceutical, and energy sectors.

Examples of industrial enzymes produced, include:

- Amylases: break down starches into sugars
  - used in the food industry (e.g., baking, brewing) and in the production of biofuels
- Proteases: degrade proteins
  - used in the food industry (e.g., dairy production), consumer product industry (e.g., detergent production), and textile industry (e.g., processing of leather)
- Lipases: break down fats
  - used in the food industry (e.g., dairy production), consumer product industry (e.g., detergent production) and production of biofuels
- Cellulases: degrade cellulases into simple sugars
  - used in the textile industry (e.g., fabric softening) and production of biofuels
- Lactases: used to break down lactose
  - used in the food industry (e.g. dairy production)

While done at a large-scale, industrial enzyme production is relatively straightforward. The basic steps are:

1. Microbe selection
   - common microbes are bacteria (e.g. Bacillus), yeast or other fungal species (e.g. Aspergillus)
   - microbes can be genetically-modified to increase their yield of a specific enzyme or to produce a non-native enzyme
2. Fermentation
   - microbes are fermented in either a liquid medium (i.e., submerged fermentation) or grown on solid materials like agricultural or food waste (i.e., solid-state fermentation)
   - solid-state fermentation mimics natural environments and is used for the production of specific enzymes like amylases and cellulases
3. Enzyme extraction and purification
   - enzymes are harvested and purified using techniques like filtration, centrifugation, and chromatography
4. Formulation and stabilization
   - enzymes are formed into liquids, powders, or granules
   - compounds like calcium ions or glycerol can be added to stabilize the product for commercial use

Because it reduces chemical waste (and even uses it in some cases) and requires low amounts of energy, industrial enzyme production is eco-friendly. It is also highly specific and efficient as enzymes target specific reactions in industrial processes. Lastly, industrially-produced enzymes can lower production costs in many industries, including food, pharmaceutical, and energy production.

Pharmaceutical Industry

Industrial biotechnology plays a vital role in drug development, including the production of antibiotics, the development of monoclonal antibodies for treating diseases, the production of vaccines through the use of recombinant DNA technology, and the synthesis of therapeutic compounds (e.g. hormones, vitamins, enzymes) using genetically-modified bacteria. For example, human insulin is now produced at scale through the insertion of the human insulin gene into bacteria. Industrial biotechnology has resulted in the development of customized therapies based on an individual's genetic makeup, including CAR-T cell therapy for treating certain cancers. Like other applications, industrial biotechnology practices in the pharmaceutical industry reduces

pollution, greenhouse gas emissions, and saves energy and resources. It can also reduce the production of toxic waste products that result from drug manufacturing. Engineered yeast strains can now produce the compound artemisinin, an anti-malarial molecule that is traditionally extracted from plants. As such, the production of this compound using biotechnology approaches is significantly reducing the impact of this drug on the environment.

Waste Treatment and Environmental Bioremediation

Industrial biotechnology offers numerous solutions for waste management and environmental remediation - the process of using bacteria, fungi, or plants to naturally breakdown and remove pollutants, contaminants, or toxins from the environment in order to restore ecosystems and protect human health. Microbes are becoming a key part of remediation approaches that degrade pollutants, including oil spills, heavy metals, and plastic waste (**Figure** 16.1.1). Bacteria and their enzymes are also used in wastewater treatment to break down organic waste and treat industrial effluents. Organic waste products are also the starting point for conversion into methane-rich biogas for energy generation.

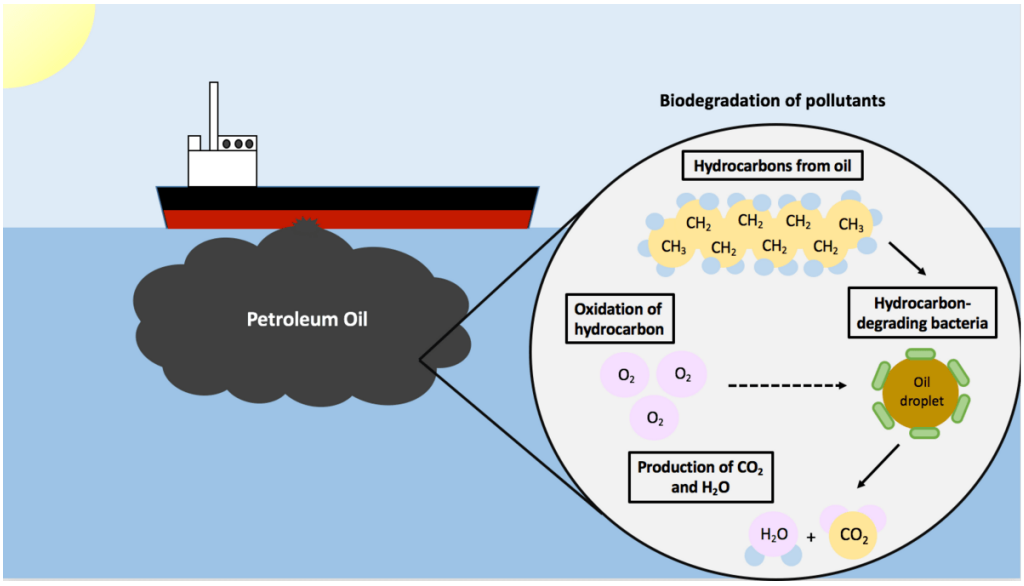


Figure 16.1.1: Bioremediation. Petroleum oil spills can be treated through the biodegradation of pollutants. Hydrocarbons from oil can be converted into H20 and CO2 by hydrocarbon-degrading bacteria that surround the oil droplets. (Biodegradation of Pollutants by Timmer26, CC BY-SA 4.0)

There are numerous areas of environmental bioremediation that utilize industrial biotechnology:

1. Soil Remediation – the removal of pollutants like heavy metals, petroleum, or pesticides from soil
2. Water Remediation – the treatment of contaminated groundwater, rivers, or oceans by removing toxins, oil spills, or heavy metals
3. Air Remediation – the removal of air pollutants such as volatile organic compounds and greenhouse gases

Advantages and Challenges of Industrial Biotechnology

Industrial biotechnology offers manufacturers several advantages, including:

- Sustainability – Reduces reliance on fossil fuels and minimizes environmental impact
- Cost-Effectiveness – Uses renewable resources and reduces energy consumption
- Eco-Friendly – Produces biodegradable products and reduces carbon emissions
- Innovation – Facilitates the development of novel materials and drugs

Despite these numerous benefits, industrial biotechnology faces challenges in wide-spread implementation. The high initial cost of setting up a bioprocessing plant requires substantial investment that might discourage manufacturers from considering biotechnology as an option. Scaling up the required laboratory processes in order to optimize the system(s) can be complex. Strict regulations govern the use of genetically-modified organisms (GMOs) and bio-based products. However, continued improvements in fermentation technology, modifications in synthetic biology systems, the use of CRISPR-based techniques, and artificial

intelligence (AI) continue to drive the growth of industrial biotechnology and ensure a more sustainable industrial future.

Summary

Industrial (white) biotechnology uses microorganisms, enzymes, and cell cultures to produce sustainable products and improve industrial processes. Microbes such as bacteria and yeast are widely used to manufacture biofuels, biodegradable plastics, industrial enzymes, pharmaceuticals, and other valuable products. Enzymes produced by microorganisms, including amylases, proteases, and lipases, are essential in industries such as food production, textiles, detergents, and biofuel manufacturing. Industrial biotechnology also plays a major role in medicine by enabling large-scale production of therapeutic proteins, vaccines, and antibiotics using recombinant DNA technology. Another important application is environmental remediation, where bacteria naturally or through genetic engineering break down pollutants such as oil, toxic chemicals, and industrial waste. For example, hydrocarbon-degrading bacteria can help clean up oil spills by converting petroleum into less harmful substances. Compared with many traditional manufacturing methods, industrial biotechnology uses renewable resources, consumes less energy, reduces waste, and lowers greenhouse gas emissions. Although implementation can be expensive and highly regulated, advances in genetic engineering and fermentation continue to expand its applications in sustainable manufacturing and environmental protection.

Key Terms

Bio-based
: materials, products, or processes that are derived from living organisms, especially plants, animals, or microbes, rather than from fossil fuels or synthetic chemicals

Biocatalyst
: a natural substance, like an enzyme or a whole microbe, that speeds up (catalyzes) chemical reactions in biological or industrial processes

Biodegradable
: a material that can be broken down naturally by biological processes in the body into harmless byproducts over time

Biologic
: a product extracted from a living organism

Bioplastic
: a type of plastic that is made from a renewable biological source; may or may not be biodegradable

Biopolymer
: a natural polymer produced by living organisms, or a synthetic polymer made from biological materials

Biological system
: a group of parts in a living organism that work together to perform a specific function and keep the organism alive and healthy

Compostable
: refers to a material that can break down naturally into nutrient-rich compost; leaves no harmful residue behind

Industrial biotechnology
: the use of living organisms, enzymes, or biological systems to create industrial products and processes; also known as white biotechnology

Industrial enzyme
: an enzyme used in large-scale industrial processes to speed up chemical reactions in a cost-effective, efficient, and eco-friendly way

Remediation
: the process of fixing, correcting, or cleaning up a problem, especially when it comes to the environment or contaminated areas

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16.2: Bioprocess Engineering

Biotech Focus

You can't ship a pharmacy to Mars. Pills expire, conditions change, and space is limited. As a result, bioprocess engineers, working with NASA, are designing mini, portable "biofactories" that can make critical compounds on demand using engineering microbes. One application being studied today is the use of engineering yeast to produce essential nutrients, like vitamin B12, which degrades quickly in stored food. Enter the bioprocess engineer.....

Bioprocess engineers are now designing a sealed, automated system comprised of a bioreactor that can provide precise control of culture temperature, oxygen levels, and pH, together with the systems needed to harvest and purify the vitamin. The resulting system will be autonomous, requiring no human operator, would work in microgravity and come equipped with fail-safes and sensors that can adapt the system in real-time to environmental changes.T he whole set-up is being designed to be compact enough to run in a space station—or eventually a base on Mars.

Learning Objectives

Bioprocess engineering takes the biological techniques used in biotechnology and scales them up for use in a biomanufacturing plant. At the end of this page, you will be able to:

- Distinguish between industrial biotechnology and bioprocess engineering
- Understand the differences between bioprocess engineering and other "bio"-based terms like bioengineering, biochemical engineering, and biomedical engineering
- Explain some of the key areas used in bioprocess engineering
- Explain biomanufacturing
- Define and explain the upstream and downstream processes used in biomanufacturing
- List some areas critical to upstream processing
- List some downstream processing steps

Introduction

Bioprocess engineering uses engineering principles to design industrial biotechnology processes at a scale large enough to make products for commercial sale. In simple terms, bioprocess engineering tries to figure out how to use living cells or biological molecules to make things like medicines, vaccines, food products, and eco-friendly materials like bioplastics. While bioprocess engineering is closely related to industrial biotechnology (**Table** 16.2.1), it is not the same thing. Industrial biotechnology *focuses on uses biological systems* like microbes, enzymes, or other biological materials, in order to develop biologic products for commercial use. To learn more about industrial biotechnology, go to Chapter 11.1 Applications of Industrial Biotechnology.

Table 16.2.1*: Industrial Biotech vs. Bioprocess Engineering*

Industrial Biotechnology	Bioprocess Engineering
Develops biological tools, like cells and enzymes, for creation of a product	Designs systems to use biological tools at scale
Focuses on innovative biological techniques that are sustainable and eco-friendly	Focuses on implementation and optimization of biological techniques
Often starts in the research & development laboratory	Bridges the gap between the laboratory and industry

In contrast, bioprocess engineering *focuses on engineering principles* in order to make these biologic products at a high-enough quality and quantity that they be sold commercially (**Figure** 16.2.1). Bioprocess engineering designs, optimizes, and scales-up the processes used in industrial biotechnology in order to make these products. Like industrial biotechnology, the processes created by bioprocess engineering aim to be be sustainable and eco-friendly.

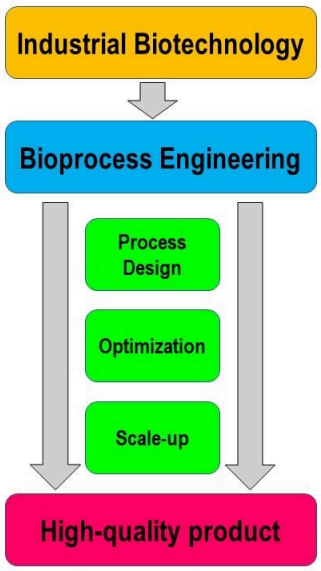


Figure 16.2.1: Industrial biotechnology and bioprocess engineering. Industrial biotechnology uses bioprocess engineering principles for the design of a manufacturing process (process design), its optimization and then application of this process at a large enough scale for commercial use (scale-up). The end result of bioprocess engineering is a high-quality product. (Industrial Biotechnology and Bioprocess Engineering by Patricia Zuk; CC BY-SA 4.0(opens in new window))

Without bioprocess engineering, the principles and techniques that form the foundation of industrial biotechnology could not be applied at a large enough scale. The ultimate goal of bioprocess engineering is to make biology easier to engineer—more predictable, reliable, and scalable.

"Bio" Engineering Terminology

Many words have been used to describe engineers that work with biotechnology. For example, the term bioprocess engineering is often used interchangeably with other terms like biomedical engineering, biochemical engineering, bioengineering, and biomolecular engineering. However, each of these terms are different and distinct and are often misunderstood.

The following is a list of some of the commonly used "bio" engineering fields:

- Bioengineering: a broad title that includes work in medical and agricultural systems
  - applies engineering principles to biological systems for the development of technologies and products
  - practitioners include many types of engineers, including agricultural, electrical, mechanical, industrial, and chemical
- Biological engineering: similar to bioengineering but emphasizes applications to plants and animals
- Biochemical engineering: the extension of chemical engineering principles to biological systems in order to bring about a specific chemical transformation
  - subdivided into bioreaction engineering and bioseparation engineering
- Biomedical engineering: the application of engineering techniques and design concepts to medicine and biology for healthcare purposes, such as diagnosis, treatment, and rehabilitation
- Biomolecular engineering: deals with the interface of biology and chemical engineering with a focus on the molecular level
- Bioprocess engineering: applies engineering principles to processes based on living cells or biological components (e.g. organelles, enzymes)
  - includes the work of mechanical, chemical, and industrial engineers

Key Areas in Bioprocess Engineering

Bioprocess engineering is a highly interdisciplinary field of engineering, combining the principles of chemical engineering, biology, and biotechnology. Key areas of bioprocess engineering include:

- Fermentation Technology: focuses on the use of microbes to convert raw materials into products
  - optimizes the fermentation process, including the design of bioreactors and the control of fermentation at a large scale

- Bioreactor Design: focuses on the design and optimization of bioreactors for efficient cell growth and product formation
  - includes bioreactors for the fermentation of microbes (e.g. bacteria, yeast, fungus) or the growth of plant and animal cells
- Downstream Processing: focuses on the development of methods for separating and purifying products produced from a bioprocess
  - includes the field of bioseparation - the development of methods for separating and purifying biological molecules
- Cell Culture Engineering: focuses on understanding and optimizing cell culture conditions for specific industrial biotechnology applications
- Bioprocess Modeling: uses mathematical models to understand and predict bioprocess behavior
- Biomanufacturing: focuses on the design and implementation of processes for the large-scale production of bioproducts
  - critical for moving the bioprocess from the lab to industrial production

Biomanufacturing

Bioprocess engineering is a key aspect of biomanufacturing. Biomanufacturing facilities use bioprocess engineering principles to commercially produce materials made through synthetic biology (e.g. therapeutics like antibodies, vaccines, biofuels, and food ingredients) at very large scales. The process of biomanufacturing begins with research and development within a laboratory. The lab is responsible for determining the biological system that will be used to make the product and creating the workflow using this system. Once the workflow has been created, bioprocess engineering focuses on how to design and run the process at a large enough scale. A biomanufacturing facility (**Figure** 16.2.2) then runs these scaled-up processes.



Figure 16.2.2: A biomanufacturing facility. The biomanufacturing facility in this picture contains numerous large bioreactors connected to one another(Copyright; author via source)

Biomanufacturing involves both upstream and downstream processes (**Table** 16.2.2).

"Upstream" is the growth and production phase and refers to the steps involved in growing and preparing the microbes, plant or animal cells that will produce the desired product. Upstream processes happen before the downstream extraction and purification steps. Because of this, the design of the upstream steps can have a significant effect on what takes place downstream.

The following are critical to upstream processes:

1. Cell Line Development
   - the appropriate cells are chosen and/or engineering to make the product
   - cells include prokaryotic (bacteria) or eukaryotic (yeast, plant, animal)
2. Media Preparation & Optimization
   - precise mixtures of nutrients (e.g. amino acids, sugars, vitamins, salts) that are critical for cell growth
   - dependent upon the cell chosen for production
   - compositions are optimized for cell viability, growth, and expression of new genes

3. Bioreactors
    - where cells grow and make the product
    - provide cells with controlled external conditions - temperature, pH, oxygen (i.e., aeration), agitation/mixing
    - designed for fermentation of microbes or culture of plant of animal cells
4. Monitoring & Control Systems
    - sensors and systems track the process in real-time to ensure optimal growth and product formation

"Downstream" in biomanufacturing are the steps that follow production. In general, downstream processes focus on extraction, purification, and packaging of the product. The design of downstream processes are critical to ensuring that the product meets purity, quality, and safety standards.

Depending on the product made and the upstream processes used, downstream steps might include:

1. Cell Harvesting: separates cells from the culture medium using techniques like centrifugation or filtration
2. Cell Disruption/Lysis: produces a cell lysate containing the product; required if the product is located within the cell
3. Filtration: removes large particles or debris from the culture medium or the cell lysate
4. Chromatography: purifies the compound of interest from a mixture
5. Ultrafiltration/Diafiltration: concentrates and purifies the product; used to improve the yield of the product
6. Sterilization: ensures the product is microbe-free
7. Formulation and Testing: designs and prepares the product so that the active ingredient(s) can be delivered in a safe, stable, and effective way; mixes the product with stabilizers, preservatives, etc...; tests for stability
8. Quality Control: ensures product meets all specifications, including formulation and safety
9. Filling and Packaging: transfers the product into the appropriate package for distribution (e.g. vials, syringes, ampules, bottles)

Table 16.2.2 below summarizes the differences between upstream and downstream processing.

Table 16.2.2*: Upstream vs. Downstream Processing*

	Upstream Processing	Downstream Processing
Definition	• **First part of the bioprocess.** • **Results in the crude production of a product**	• **Second part of the bioprocess** • **Involves extraction and purification of the product**
Major Event(s)	• **Creation of the product in bioreactors**	• **Harvesting, purification, and formulation of the product**
Steps Involved	• **Selection of microbes or cell line** • **Development of media conditions** • **Growth of cells in bioreactors**	• **Centrifugation, filtration, chromatography, and concentration steps** • **Quality control** • **Formulation** • **Packaging**
Challenges	• **Sterility** • **Optimal cell growth** • **Scalability**	• **Separation techniques** • **Purity standards** • **Quality control**

Biomanufacturing facilities must contend with government regulations, production logistics, and quality control. Products that are manufactured for medical or food use must be produced in facilities designed and operated according to Good Manufacturing Practice (GMP) regulations. Cleanrooms, that use sterilization and aseptic techniques, are a key component of both upstream and downstream processes. To read more GMP and quality control, go to Chapter 12.1 Quality Assurance and Quality Control in Biotechnology. To learn more about sterilization and aseptic technique, go to Chapter 6.2 Principles of Aseptic Techniques.

Today, thousands of products, including medicines like cytokines, fusion proteins, growth factors, monoclonal antibodies, and vaccines, are made in biomanufacturing facilities across the world. Numerous food and beverages products like amino acids, enzymes, and proteins supplements are also made. Finally, biomanufacturing has played a key role in industrial biotechnology applications such as bioremediation, and the production of detergents and bioplastics.

Summary

Bioprocess engineering applies engineering principles to biotechnology so that biological products can be manufactured efficiently, safely, and at large scale. Unlike industrial biotechnology, which develops useful cells, enzymes, or microbes, bioprocess engineering focuses on designing and optimizing the systems that produce these products. A modern example is NASA's development of compact "biofactories" that use engineered yeast to produce nutrients like vitamin B12 on demand during long-duration space missions. These automated systems rely on bioreactors that carefully control temperature, oxygen, pH, and other conditions while also purify the final product. Bioprocess engineering is essential for producing medicines, vaccines, enzymes, biofuels, and food ingredients. Manufacturing involves upstream processing, where cells are selected, grown, and monitored in bioreactors, followed by downstream processing, where products are harvested, purified, tested, and packaged. By combining biology with engineering, bioprocess engineering makes biotechnology more reliable, scalable, and sustainable, enabling applications ranging from healthcare and agriculture to environmental protection and future space exploration.

Key Terms

Biomanufacturing
: the production of biological products, such as vaccines, therapeutic proteins, or enzymes using living cells or organisms in industrial settings

Bioprocess
: any process that uses living cells to create a product

Bioprocess engineering
: an engineering field that designs and develops the processes that use biologic systems to make a product

Bioreactor
: a container or vessel used to grow cells, microorganisms, or tissues under controlled conditions

Downstream
: refers to the steps in a bioprocess after the cells have made the desired product

Engineering
: the science of designing and building complex processes or machines

Fermentation
: a biological process where microbes (like bacteria and yeast) convert sugars into other products like alcohol, gases, or acids

Upstream
: refers to all the steps involved in preparing and growing the biological system that will produce the desired product

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16.3: Synthetic Biology

Biotech Focus

Can you synthesize an organism's entire genome?

The answer to this question is yes. In 2002, scientists in the US synthesized an "artificial" viral genome for the first time when they recreated the polio virus genome from scratch. Their work brought attention to the risk that synthetic biology could be used to develop biological weapons.The first synthetic bacterial genome was completed 6 years later with the synthesis of the *Mycoplasm genitalium* genome, a bacterium that causes urinary and genital tract infections in humans. In 2017, another group of scientists partially synthesized the genome of *Saccharomyces cerevisiae*, the yeast that is used to make bread and brew wine and beer. Today, researchers are continuing to push the limits of synthetic biology and DNA-synthesis technology in order to help understand how genomes work. Researchers

One group of researchers, called the "Genome Project-Write" (GP-Write)", is seeking to synthesize, or "write" the human genome, in addition to the genomes of other plants and animals important to agriculture and public health. However, like the work on the polio virus, ethical concerns have been raised in association with GP-Write. What is the exact purpose of artificially creating a human genome? Will the work be restricted to cell lines or be used to alter human embryos. How synthetic biology and artificial genomes will continue to develop will definitely be an area to keep an eye on.

To learn more about synthetic biology and what it can do, check out the National Institute of Health (NIH) webpage on synthetic biology.

Learning Objectives

Synthetic biology combines the techniques of biology and engineering to create new biological systems. At the end of this page, you will be able to:

- Explain synthetic biology and its importance
- Explain BioBricks and list some of the common parts used

Introduction

Synthetic biology is a field of science that combines biology and engineering to design and build new biological parts, systems, or organisms. In synthetic biology, organisms are often completely "redesigned" to give them new abilities, like produce a produce a new therapeutic compound, detect a toxin, or make enzymes that turn sugars into biofuels. Alternatively, entirely new organisms can be built from the "ground up". Synthetic biology is analogous to computer programming, but instead of writing code, scientists use DNA, RNA, and proteins to write new genetic "software" that makes "old" cells behave in "new" ways.

Synthetic biology is often confused with genetic engineering. Genetic engineering is the *direct modification of an organism*'s *DNA using biotechnology*. It focuses on using genes that are already found in nature and involves the insertion of new genes into an organism's genome, or the editing of the genome by either deleting a gene or changing its sequence. Based on this, genetic engineering is part of synthetic biology. However, synthetic biology is a more advanced and design-oriented field that builds new biological parts, systems, or even organisms that may not exist in nature at all.

Synthetic Biology Applications

The applications of synthetic biology are wide and varied. Examples include building synthetic circuits in bacteria to make them behave like computers or creating entirely new synthetic genomes like the Synthetic Yeast Project. However, one of the most well-known examples of synthetic biology is the production of lab grown "meat". Since the introduction of the first cultured beef hamburger in 2013, snthetic biology is allowing researchers to rethink meat production by growing meat without animals. To do this, a sample of animal cells (e.g. cow or chicken muscle cells) is collected without harming the donor animal. The cells are placed

into a bioreactor containing a nutrient-rich culture medium that mimics what they would be exposed to in the animal's body. The cells are induced to grow rapidly, differentiate into muscle and fat tissue, and then formed into something that resembles meat. Improvements in this technology have allowed for improved taste and texture, enhanced nutritional content, and cell growth using synthetic alternatives to animal serum. Today the global lab-grown meat "market" has increased, with companies like Upside Foods (USA) and GOOD Meat (Singapore) being among the first companies to sale lab-grown "chicken" to customers.



Figure 16.3.1: The Cultivated Hamburger. Lab-grown meat can be used to make delicious hamburgers. (First cultivated hamburger by Mosa Meat; CC BY 4.0)

Lab-grown meat is a more conventional application of synthetic biology, but there are others that may seem more in the realm of science fiction than science. Today, researchers are exploring the use of synthetic biology in order to produce self-healing materials like concrete, metal, and plastic. For this, scientists engineer microbes or biological components (like proteins or enzymes) and embed them in a material. When damage happens, these biological agents activate and “heal” the material. Engineered bacteria, like *Bacillus subtilis*, have been mixed into concrete to product a "bio-concrete". This bio-concrete, when cracked, allows the entrance of water and activates the bacteria to begin to produce limestone, thus sealing the crack. Synthetic biology and bio-concrete have been proposed as a way of extending the life of roads, bridges, and buildings. Similarly, scientists have embedded engineered yeast or *E. coli* into hydrogels and plastics, enabling them to detect stress or damage and respond by producing repair proteins that mend the material. This approach could be used to make materials more sustainable rather than disposable and could even be used on spacecraft or space stations to repair damage on their own.

BioBricks

BioBricks are standardized DNA sequences (i.e. "parts) that encode biological functions and can be easily assembled together physically to build new biological "circuits". These synthetic biological circuits can then be incorporated into living cells to construct new biological systems.

Examples of BioBrick parts include the:

1. promoter which directs gene expression
2. ribosome binding site (RBS) that helps start protein production via translation
3. gene or coding sequence
4. terminator which tells the cell when to stop translating the coding sequence

BioBrick parts are used according to a specific hierarchy (**Figure** 16.3.2). Specifically, the BioBrick parts (e.g. promoter, RBS etc...) are combined to form a "device" - a genetic circuit to perform a defined function. A complementary set of devices can then be combined as a biological system to perform a high-level task.

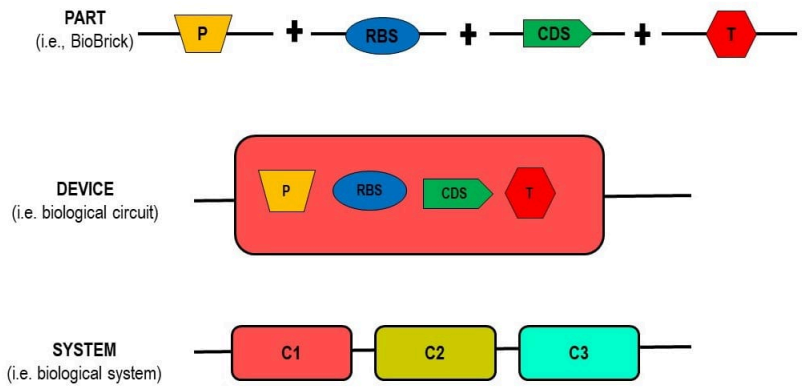


Figure 16.3.2: Biobricks are used in synthetic biology. Each BioBrick, known as a "part", is a DNA sequence. The BioBrick parts of promoter (P), ribosome binding site (RBS), coding sequence (CDS), and terminator sequence (T) are ligated together in a plasmid to form a "device" or biological circuit. Several circuits are combined to form a "system" or biological system (BioBricks by Patricia Zuk, CC BY 4.0; modified from Abstraction Hierarchy by Shammie23, CC BY-SA 3.0)

To create a synthetic system using BioBricks, each part is cloned into a plasmid. The sequences flanking each part are specific restriction sites that allow the BioBrick parts to be digested out of their plasmid and then ligated into another plasmid in a specific order - not unlike snapping several Lego bricks together to make a bigger toy. These restriction sites are standardized across all BioBricks. The "prefix" restriction site at the start of the part's sequence is either EcoRI, NotI, or XbaI. The "suffix" restriction sites found at the end of the sequence are either SpeI, NotI, or PstI. Ultimately, when these parts are ligated to one another in their desired order, the majority of the restriction sites are eliminated to prevent the accidental removal of a key BioBrick part. However, the flanking sites at the extreme ends are still intact, allowing for the addition of more parts or the transfer of the assembled parts. To learn more about restriction sites and ligation, go to Chapter 4.1 Principles of Genetic Engineering and Chapter 4.2 Creating Recombinant DNA.

Concept in Action

BioBrick Assembly

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

Testing of BioBrick systems are often done using cell-free systems or a "chassis" - a host organism (like *E.coli* or yeast) that is used to "run" synthetic genetic circuits. A chassis in synthetic biology is not unlike the hardware of the computer that runs the newly written software. Choosing the right chassis is important in synthetic biology. This is because different chassis have different metabolisms, growth rates, and tolerances. Others are better for industrial-scale production, drug delivery, or biosensoring.

Common chassis organisms used in synthetic biology include:

- *E. coli* - fast-growing, well-studied, and easy to modify
- *Saccharomyces cerevisiae* - a eukaryotic chassis used for more complex tasks, like producing drugs of biofuels
- Cyanobacteria - used as photosynthetic chassis to produce energy
- Mammalian cells - used for advanced medical or therapeutic applications

Numerous applications of BioBricks are being proposed each and every day. One way could be in the creation of biosensors within cells capable of detecting biotoxins like heavy metals in cells (**Figure** 16.3.3). Others include detection systems for pests in agriculture, systems for carbon capture, and the creation of chemical "factories" that would produce things like complex flavors, fragrances, and materials.

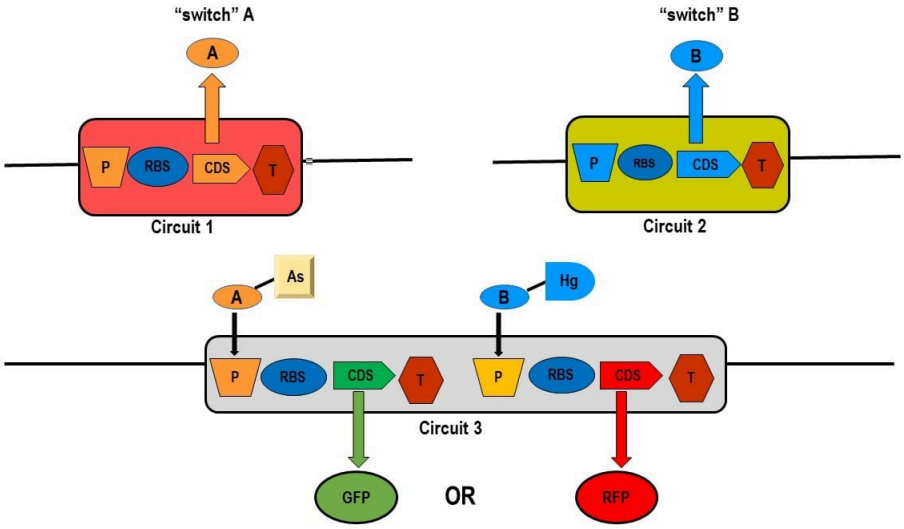


Figure 16.3.3: Heavy metal detection using BioBricks. BioBricks are assembled in Circuit 1 for the expression of a DNA-binding protein that act as a molecular "switch" (Switch A) for the detection of arsenic (As). A similar switch protein (Switch B) that detects mercury (Hg) is expressed using Circuit 2. These switch proteins control gene expression from Circuit 3. Switch protein A binds arsenic (A-As) which then binds to its promoter, driving expression of green fluorescent protein (GFP) from its coding sequence. Switch protein B binds mercury (B-Hg). Binding of this switch to its promoter drives expression of red fluorescent protein (RFP). (BioBrick Detection of Heavy Metals by Patricia Zuk; CC BY 4.0)

Summary

Synthetic biology combines biology and engineering to design new biological systems or even build entire genomes from scratch. Unlike traditional genetic engineering, which modifies existing genes, synthetic biology creates new genetic circuits and biological functions that may not exist in nature. Scientists have synthesized the genomes of viruses, bacteria, and parts of the yeast genome to better understand how genomes work, although these advances raise important ethical and safety questions. A key tool in synthetic biology is the use of BioBricks—standardized DNA parts such as promoters, coding sequences, and terminators that can be assembled into genetic circuits. These circuits are often tested in organisms like *E. coli* or yeast, known as chassis organisms. Synthetic biology has many potential applications, including producing medicines, biofuels, biosensors, and lab-grown meat, as well as creating self-healing materials. As DNA synthesis technologies continue to advance, synthetic biology is expected to play an increasingly important role in medicine, agriculture, industry, and environmental sustainability.

Key Terms

Bioengineering
: the application of engineering principles to biological systems for the development of technologies and products

BioBricks
: standardized DNA sequences called "parts" with defined functions that can be combined to build synthetic genes and circuits; includes parts like promoters, ribosomal binding sequences, coding sequences, and terminator sequence

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)

Cell-free system
: a synthetic environment outside living cells where biological reactions can occur using extracted cellular

components like enzymes and ribosomes

Coding sequence (CDS)
: the sequence of DNA that is ultimately translated into a polypeptide by ribosomes

Genetic circuit
: a designed combination of genes and regulatory elements that control how a cell behaves, similar to electronic circuits

Genome
: the totality of genetic information found within a cell

Promoter sequence (promoter)
: a sequence of DNA where the RNA polymerase binds; determines the site of transcription initiation

Restriction enzyme
: an endonuclease that cuts the DNA at a specific sequence called a restriction sequence

Restriction sequence
: the sequence of DNA cut by a restriction enzyme

Restriction site
: the physical location of a restriction sequence in a DNA sequence

Ribosomal Binding Site (RBS)
: a short sequence found in messenger RNA (mRNA) that signals the ribosome where to start translating the mRNA into a protein

Synthetic genome
: a genome that has been entirely built from synthetic DNA rather than copied from nature

Terminator sequence
: a short sequence found in mRNA that signals where the ribosome stops its translation

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