Biology Part I

BIOLOGY PART I

JUNG CHOI; MARY ANN CLARK; AND MATTHEW DOUGLAS

CHRISTIAN CLEMENT; HOPE CLAY; ILLYA TIETZEL; JENNIFER BLANCHARD; PETER YAUKEY; RUBY BROADWAY; SARAH HUNTER; STEPHANIE AAMODT; AND WANEENE DORSEY

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The Creation of This Book

This textbook was created as part of the Interactive OER for Dual Enrollment project, facilitated by LOUIS: The Louisiana Library Network and funded by a $2 million Open Textbooks Pilot Program grant from the Department of Education.

This project supports the extension of access to high-quality post-secondary opportunities to high school students across Louisiana and beyond by creating materials that can be adopted for dual enrollment environments. Dual enrollment is the opportunity for a student to be enrolled in high school and college at the same time.

The cohort-developed OER course materials are released under a license that permits their free use, reuse, modification and sharing with others. This includes a corresponding course available in MoodleNet and Canvas Commons with labs that corresponds to the lab manual, and in MoodleNet and Canvas Commons without labs. These can be imported to other Learning Management System platforms. For access/questions, contact Affordable Learning Louisiana.

If you are adopting this textbook, we would be glad to know of your use via this brief survey.

Adaptation Statement

"Biology Part I" has been revised and remixed for the LOUIS: The Louisiana Library Network and Open Textbooks Pilot Program grant from the Department of Education by Stephanie Aamodt, Jennifer Blanchard, Ruby Broadway, Hope Clay, Christian Clement, Waneene Dorsey, Sarah Hunter, Illya Tietzel, and Peter Yaukey, with editors Emily Frank, Elizabeth Joan Kelly, and Maletta Payne. This work retains the original CC BY 4.0 license set forth by the original authors, unless otherwise noted in the text.

This book is an adaptation of the OpenStax "Biology 2e" textbook (originally published by OpenStax, downloaded for adaptation here from BC Campus) and was originally authored by Mary Ann Clark, Texas Wesleyan University, Matthew Douglas, Grand Rapids Community College, Jung Choi, Georgia Institute of Technology and last updated 2018. This text is licensed as CC BY 4.0. We would like to wholeheartedly thank these authors as well as Rice University's OpenStax for their tremendous work in this area of open educational resources.

The following changes were made to this book as a whole:

- Some chapters were removed and all chapter numbers were updated to make this a concise resource to

align with Biology I Lecture and Lab for Science Majors.
- H5P activities/multimedia were inserted into sections to reinforce concepts.
- Minor text edits for grammar, punctuation, and clarification were made throughout
- Minor edits involving fonts, spacing, and other formatting
- Updated figure and table numbers to match added/deleted figures and captions
- Added additional key terms and bolded in the text

The following chapter sections were updated to address specific topics:

- Chapter 1 The Study of Life: added section on levels of taxonomy (Hope Clay)
- Chapter 2 The Chemical Foundation of Life: replaced some critical thinking questions (Peter Yaukey)
- Chapter 3 Biological Macromolecules: replaced some critical thinking questions; replaced figure 3.32 (Peter Yaukey)
- Chapter 4 Cell Structure: added information about cell walls, microorganisms (Sarah Hunter)
- Chapter 5 Structure and Function of Plasma Membranes: added additional examples; replaced diffusion figure; replaced some critical thinking questions (Waneene Dorsey)
- Chapter 6 Metabolism: replaced energy figure; replaced metabolic pathways figure; added enzyme figure (Waneene Dorsey)
- Chapter 7 Cellular Respiration: removed some parts of Glycolysis section; added image captions; renumbered figures; resized some figures; removed some review questions; replaced some critical thinking questions; renumbered review and critical thinking questions (Stephanie Aamodt)
- Chapter 8: added "photosynthesis" definition (Jennifer Blanchard)
- Chapter 10 Cell Reproduction: added introductory paragraph; added section on Mitotic Spindle; added content to Karyokinesis (Mitosis) (Christian Clement)
- Chapter 11 Meiosis and Sexual Reproduction: replaced image Figure 5.12.1 Family resemblance (Christian Clement)
- Chapter 12 Mendel's Experiments and Heredity: replaced and renumbered some critical thinking questions; replaced figure in 12.3, 12.7, 12.7B, 12.15 image (Illya Tietzel)
- Chapter 13 Modern Understandings of Inheritance: reorganized sections (Hope Clay)
- Chapter 14 DNA Structure and Function: replaced critical thinking questions (Ruby Broadway)
- Chapter 15 Genes and Proteins: replaced critical thinking questions (Ruby Broadway)
- Chapter 17 Ethics & Societal Responsibility: authored entirely new content (Illya Tietzel)

Review Statement

This textbook and its accompanying course materials went through at least two review processes:

- Peer reviewers, coordinated by Jared Eusea, River Parish Community College, used an online course development standard rubric for assessing the quality and content of each course to ensure that the courses developed through Interactive OER for Dual Enrollment support online learners in that environment. The evaluation framework reflects a commitment to accessibility and usability for all learners.
    - Reviewers
        - Andrea Leonard
        - Francesca Mellieon-Williams
        - Esperanza Zenon
        - Iris Henry
- The Institute for the Study of Knowledge Management in Education (ISKME) collaborated with LOUIS to review course materials and ensure their appropriateness for dual enrollment audiences. Review criteria were drawn from factors that apply across dual enrollment courses and subject areas, such as determining appropriate reading levels, assessing the fit of topics and examples for high school DE students; applying high-level principles for quality curriculum design, including designing for accessibility, appropriate student knowledge checks, and effective scaffolding of student tasks and prior knowledge requirements, addressing adaptability and open educational practices, and principles related to inclusion and representational social justice.
    - Reviewers
        - Jennifer Simon
        - Latoya T Paul
        - Emily Jackson-Osagie

PART I

THE STUDY OF LIFE

1.

INTRODUCTION

Figure 1.1 This NASA image is a composite of several satellite-based views of Earth. To make the whole-Earth image, NASA scientists combine observations of different parts of the planet. (credit: NASA/GSFC/NOAA/USGS)

Viewed from space, Earth offers no clues about the diversity of life forms that reside there. Scientists believe that the first forms of life on Earth were microorganisms that existed for billions of years in the ocean before plants and animals appeared. The mammals, birds, and flowers so familiar to us are all relatively recent, originating 130 to 250 million years ago. The earliest representatives of the genus *Homo*, to which we belong, have inhabited this planet for only the last 2.5 million years, and only in the last 300,000 years have humans started looking like we do today.

2.

THE SCIENCE OF BIOLOGY

Learning Objectives

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

- Identify the shared characteristics of the natural sciences
- Summarize the steps of the scientific method
- Compare inductive reasoning with deductive reasoning
- Describe the goals of basic science and applied science

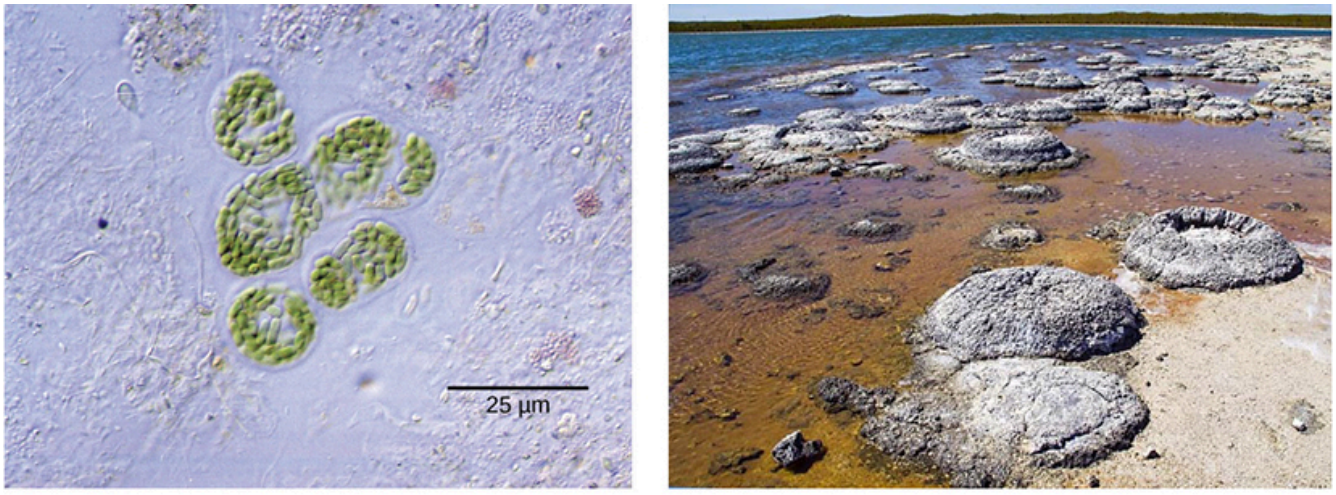


Figure 1.2 Formerly called blue-green algae, these (a) cyanobacteria, magnified 300x under a light microscope, are some of Earth's oldest life forms. These (b) stromatolites along the shores of Lake Thetis in Western Australia are ancient structures formed by layering cyanobacteria in shallow waters. (credit a: modification of work by NASA; credit b: modification of work by Ruth Ellison; scale-bar data from Matt Russell)

What is biology? In simple terms, **biology** is the study of life. This is a very broad definition because the scope of biology is vast. Biologists may study anything from the microscopic or submicroscopic view of a cell to ecosystems and the whole living planet (Figure 1.2). Listening to the daily news, you will quickly realize how many aspects of biology we discuss every day. For example, newsworthy items in recent years have included *Escherichia coli* (Figure 1.3) outbreaks in spinach and *Salmonella* contamination in peanut butter. Other biological subjects often in the media include efforts toward finding cures for AIDS, Alzheimer's disease, and cancer. On a global scale, many researchers are committed to finding ways to protect the planet, solve environmental issues, and reduce the effects of climate change. All of these diverse endeavors are related to different facets of the discipline of biology.

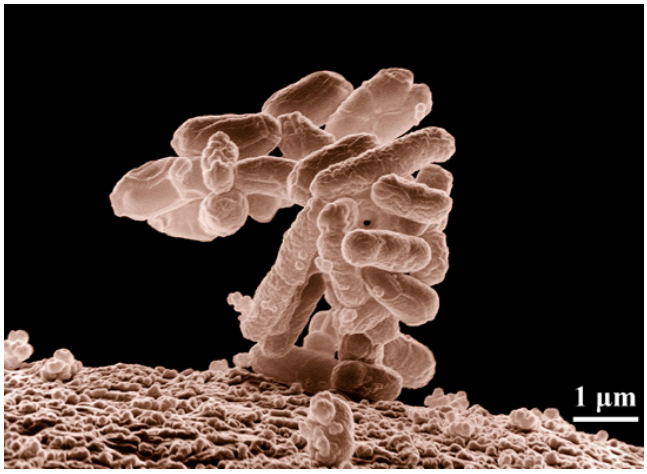


Figure 1.3 Escherichia coli (E. coli) bacteria, in this scanning electron micrograph, are normal residents of our digestive tracts that aid in absorbing vitamin K and other nutrients. However, virulent strains are sometimes responsible for disease outbreaks. (credit: Eric Erbe, digital colorization by Christopher Pooley, both of USDA, ARS, EMU)

The Process of Science

Biology is a science, but what exactly is science? What does the study of biology share with other scientific disciplines? We can define **science** (from the Latin *scientia*, meaning "knowledge") as knowledge that covers general truths or the operation of general laws, especially when acquired and tested by the scientific method. It becomes clear from this definition that applying **scientific method** plays a major role in science. The scientific method is a method of research with defined steps that include experiments and careful observation.

We will examine scientific method steps in detail later, but one of the most important aspects of this method is the testing of hypotheses by means of repeatable experiments. A **hypothesis** is a suggested explanation for an event, which one can test. Although using the scientific method is inherent to science, it is inadequate in determining what science is. This is because it is relatively easy to apply the scientific method to disciplines such as physics and chemistry, but when it comes to disciplines like archaeology, psychology, and geology, the scientific method becomes less applicable as repeating experiments becomes more difficult.

These areas of study are still sciences, however. Consider archaeology—even though one cannot perform repeatable experiments, hypotheses may still be supported. For instance, archaeologists can hypothesize that an ancient culture existed based on finding a piece of pottery. They could make further hypotheses about various characteristics of this culture, which could be correct or false through continued support or contradictions from other findings. A hypothesis may become a verified theory. A **theory** is a tested and confirmed explanation for observations or phenomena. Therefore, we may be better off to define science as fields of study that attempt to comprehend the nature of the universe.

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? Maybe all of the above? Science includes such diverse fields as astronomy, biology, computer sciences, geology, logic, physics, chemistry, and mathematics (Figure 1.4). However, scientists consider those fields of science related to the physical world and its phenomena and processes **natural sciences**. Thus, a museum of natural sciences might contain any of the items listed above.

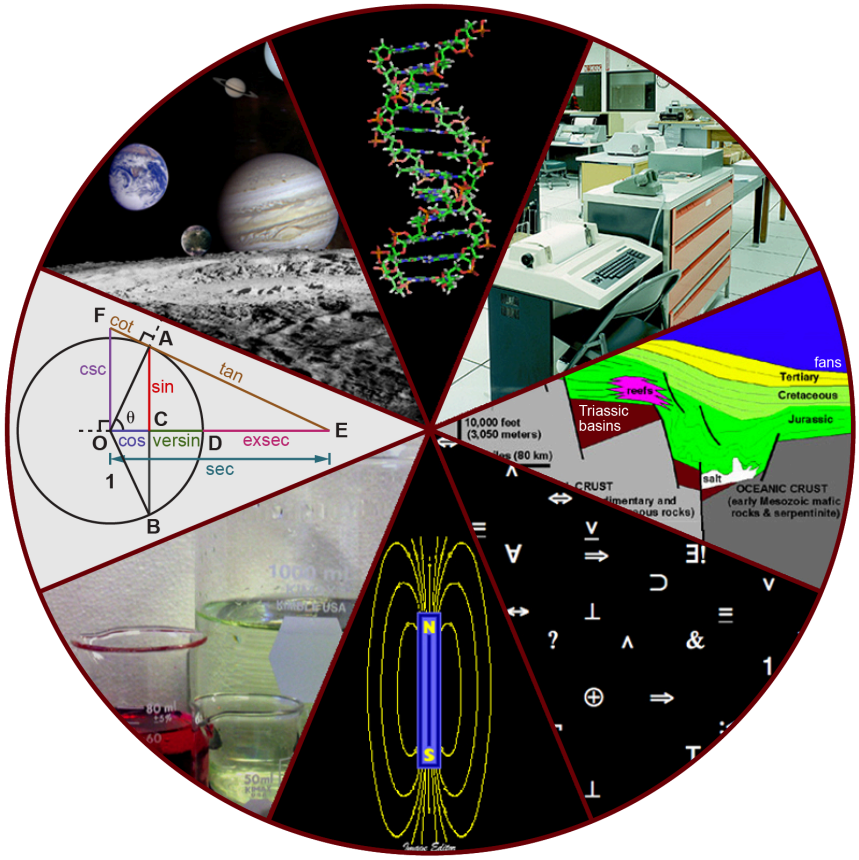


Figure 1.4 The diversity of scientific fields includes astronomy, biology, computer science, geology, logic, physics, chemistry, mathematics, and many other fields. (credit: "Image Editor"/Flickr)

There is no complete agreement when it comes to defining what the natural sciences include, however. 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, geology, physics, and chemistry. Some disciplines such as biophysics and biochemistry build on both life and physical sciences and are interdisciplinary. Some refer to natural sciences as "hard science" because they rely on the use of quantitative data. Social sciences that study society and human behavior are more likely to use qualitative assessments to

drive investigations and findings.

Not surprisingly, the natural science of biology has many branches or subdisciplines. Cell biologists study cell structure and function, while biologists who study anatomy investigate the structure of an entire organism. Those biologists studying physiology, however, focus on the internal functioning of an organism. Some areas of biology focus on only particular types of living things. For example, botanists explore plants, while zoologists specialize in animals.

Scientific Reasoning

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. To do this, they use two methods of logical thinking: 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 or quantitative, and one can supplement the raw data 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 analyzing a large amount of data. Brain studies provide an example. In this type of research, scientists observe many live brains while people are engaged in a specific activity, such as viewing images of food. The scientist then predicts the part of the brain that "lights up" during this activity to be the part controlling the response to the selected stimulus, in this case, images of food. Excess absorption of radioactive sugar derivatives by active areas of the brain causes the various areas to "light up". Scientists use a scanner to observe the resultant increase in radioactivity. Then, researchers can stimulate that part of the brain to see if similar responses result.

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 predict specific results. From those general principles, a scientist can deduce and predict the specific results that would be valid as long as the general principles are valid. Studies in climate change can illustrate this type of reasoning. For example, scientists may predict that if the climate becomes warmer in a particular region, then the distribution of plants and animals should change.

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**, which is usually inductive, aims to observe, explore, and discover, while **hypothesis-based science**, which is usually deductive, begins with a specific question or problem and a potential answer or solution that one can test. The boundary between these two forms of study is often blurred, and most scientific endeavors combine both approaches. The fuzzy boundary becomes apparent when thinking about how easily observation can lead to specific questions. For example, a

gentleman in the 1940s observed that the burr seeds that stuck to his clothes and his dog's fur had a tiny hook structure. On closer inspection, he discovered that the burrs' gripping device was more reliable than a zipper. He eventually experimented to find the best material that acted similarly, and produced the hook-and-loop fastener popularly known today as Velcro. Descriptive science and hypothesis-based science are in continuous dialogue.

The Scientific Method

Biologists study the living world by posing questions about it and seeking science-based responses. Known as scientific method, this approach is common to other sciences as well. The scientific method was used even in ancient times, but England's Sir Francis Bacon (1561–1626) first documented it (Figure 1.5). He set up inductive methods for scientific inquiry. The scientific method is not used only by biologists; researchers from almost all fields of study can apply it as a logical, rational problem-solving method.

Figure 1.5 Historians credit Sir Francis Bacon (1561–1626) as the first to define the scientific method. (credit: Paul van Somer)

The scientific process typically starts with an observation (often a problem to solve) 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?"

Proposing a Hypothesis

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

Once one has selected a hypothesis, the student can make a prediction. 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."

Testing a Hypothesis

A valid hypothesis must be testable. It should also be **falsifiable**, meaning that experimental results can disprove it. Importantly, science does not claim to "prove" anything because scientific understandings are always subject to modification with further information. This step—openness to disproving ideas—is what distinguishes sciences from non-sciences. The presence of the supernatural, for instance, is neither testable nor falsifiable. To test a hypothesis, a researcher will conduct one or more experiments designed to eliminate one or more of the hypotheses. 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.

In an experiment, the variables used can be classed as either dependent or independent variables. The **dependent variable** is the possible outcome of the experiment; the effect. The **independent variable** is what you have control over; what you can choose and manipulate. It is usually what you think will affect the dependent variable. For example, if you want to know how much light a plant needs to grow, the amount of growth is the dependent variable. If you wanted to see if a plant would grow better in hot or cold areas, your independent variable would be the temperature.

The **control group** contains every feature of the experimental group except it is not given the manipulation that the researcher hypothesizes. Therefore, if the experimental group's results differ from the control group, the difference must be due to the hypothesized manipulation, rather than some outside factor. To test the first hypothesis in the warm classroom scenario from above, the student would find out if the air conditioning is on. If the air conditioning is turned on but does not work, there should be another reason, and the student should reject this hypothesis. To test the second hypothesis, the student could check if the lights in the classroom are functional. If so, there is no power failure, and the student should reject this hypothesis. The students should test each hypothesis by carrying out appropriate experiments. Be aware that rejecting one hypothesis does not determine whether or not one can accept the other hypotheses. It simply

eliminates one hypothesis that is not valid (Figure 1.6). Using the scientific method, the student rejects the hypotheses that are inconsistent with experimental data.

While this "warm classroom" example is based on observational results, other hypotheses and experiments might have clearer controls. For instance, a student might attend class on Monday and realize she had difficulty concentrating on the lecture. One observation to explain this occurrence might be, "When I eat breakfast before class, I am better able to pay attention." The student could then design an experiment with a control to test this hypothesis.

In hypothesis-based science, researchers predict specific results from a general premise. We call this type of reasoning deductive reasoning: deduction proceeds from the general to the particular. However, the reverse of the process is also possible: sometimes, scientists reach a general conclusion from a number of specific observations. We call this type of reasoning inductive reasoning, and it proceeds from the particular to the general. Researchers often use inductive and deductive reasoning in tandem to advance scientific knowledge (Figure 1.7).

Visual Connection

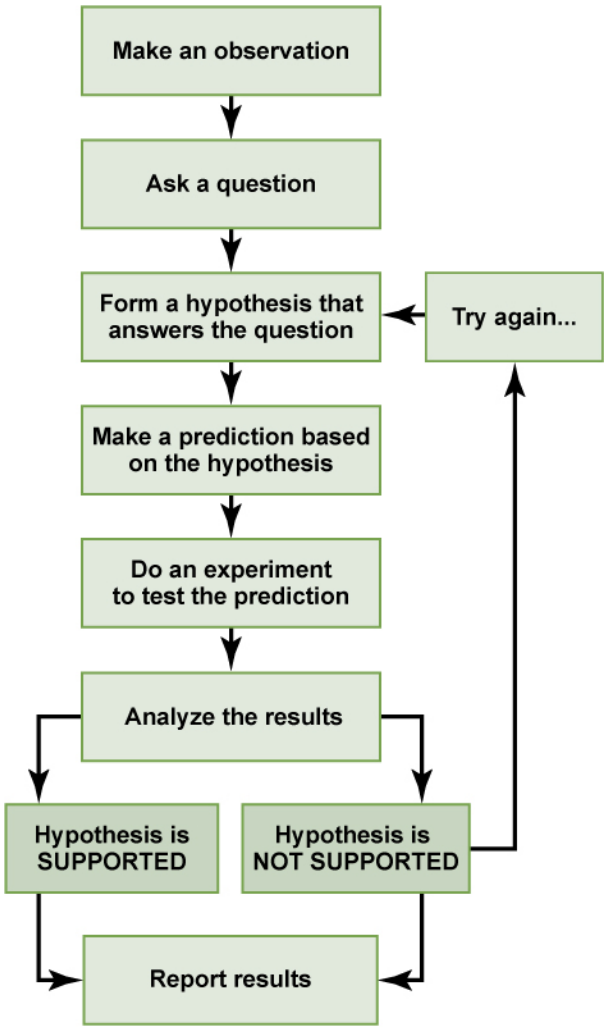


Figure 1.6 The scientific method consists of a series of well-defined steps. If a hypothesis is not supported by experimental data, one can propose a new hypothesis.

In the example below, the scientific method is used to solve an everyday problem. **Match the scientific method steps (numbered items) with the process of solving the everyday problem (lettered items).** Based on the results of the experiment, is the hypothesis correct? If it is incorrect, propose some alternative hypotheses.

1. Observation	a. There is something wrong with the electrical outlet.
2. Question	b. If something is wrong with the outlet, my coffeemaker also won't work when plugged into it.
3. Hypothesis (answer)	c. My toaster doesn't toast my bread.
4. Prediction	d. I plug my coffee maker into the outlet.
5. Experiment	e. My coffeemaker works.
6. Result	f. Why doesn't my toaster work?

Visual Connection

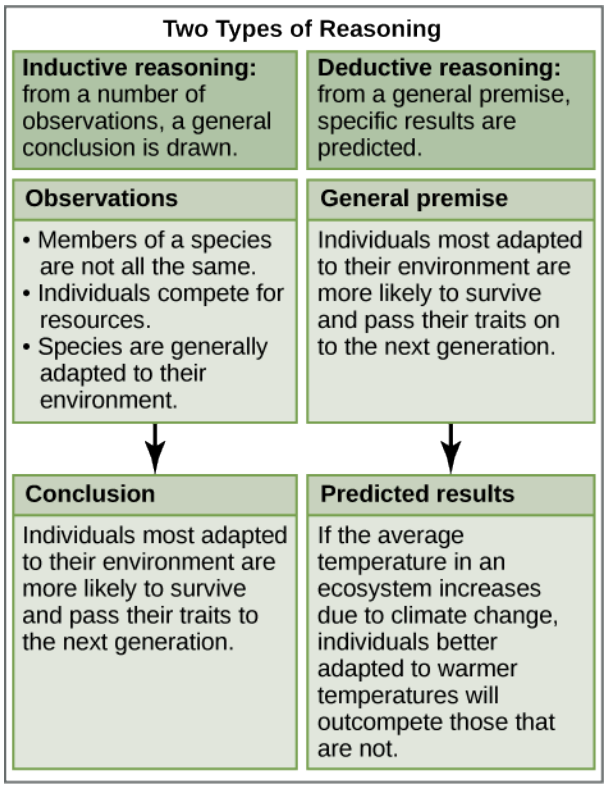


Figure 1.7 Scientists use two types of reasoning, inductive and deductive reasoning, to advance scientific knowledge. As is the case in this example, the conclusion from inductive reasoning can often become the premise for deductive reasoning.

Decide if each of the following is an example of inductive or deductive reasoning.

1. All flying birds and insects have wings. Birds and insects flap their wings as they move through the air. Therefore, wings enable flight.
2. Insects generally survive mild winters better than harsh ones. Therefore, insect pests will become more problematic if global temperatures increase.
3. Chromosomes, the carriers of DNA, are distributed evenly between the daughter cells during cell division. Therefore, each daughter cell will have the same chromosome set as the mother cell.
4. Animals as diverse as humans, insects, and wolves all exhibit social behavior. Therefore,

social behavior must have an evolutionary advantage.

The scientific method may seem too rigid and structured. It is important to keep in mind that, although scientists often follow this sequence, there is flexibility. 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. Notice, too, that we can apply the scientific method to solving problems that aren't necessarily scientific in nature.

Two Types of Science: Basic Science 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 to 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, although this does not mean that, in the end, it may not result in a practical application.

In contrast, **applied science** 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 (Figure 1.8). In applied science, the problem is usually defined for the researcher.

Figure 1.8 After Hurricane Irma struck the Caribbean and Florida in 2017, thousands of baby squirrels like this one were thrown from their nests. Thanks to applied science, scientists knew how to rehabilitate the squirrel. (credit: audreyjm529, Flickr)

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?" However, a careful look at the history of science 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 researchers develop an application; therefore, applied science relies on the results that researchers generate through basic science. Other scientists think that it is time to move on from basic science in order to find solutions to actual problems. Both approaches are valid. It is true that there are problems that demand immediate attention; however, scientists would find few solutions without the help of the wide knowledge foundation that basic science generates.

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. DNA strands, unique in every human, are in our cells, where they provide the instructions necessary for life. When DNA replicates, it produces new copies of itself, shortly before a cell divides. Understanding DNA replication mechanisms enabled scientists to develop laboratory techniques that researchers now use to identify genetic diseases, pinpoint individuals who were at a crime scene, and determine paternity. Without basic science, it is unlikely that applied science could exist.

Another example of the link between basic and applied research is the Human Genome Project, a study in which researchers analyzed and mapped each human chromosome to determine the precise sequence of DNA subunits and each gene's exact location. (The gene is the basic unit of heredity represented by a specific DNA segment that codes for a functional molecule. An individual's complete collection of genes is their

genome.) Researchers have studied other less complex organisms as part of this project in order to gain a better understanding of human chromosomes. The Human Genome Project (Figure 1.9) relied on basic research with simple organisms and, later, with the human genome. An important end goal eventually became using the data for applied research, seeking cures and early diagnoses for genetically related diseases.

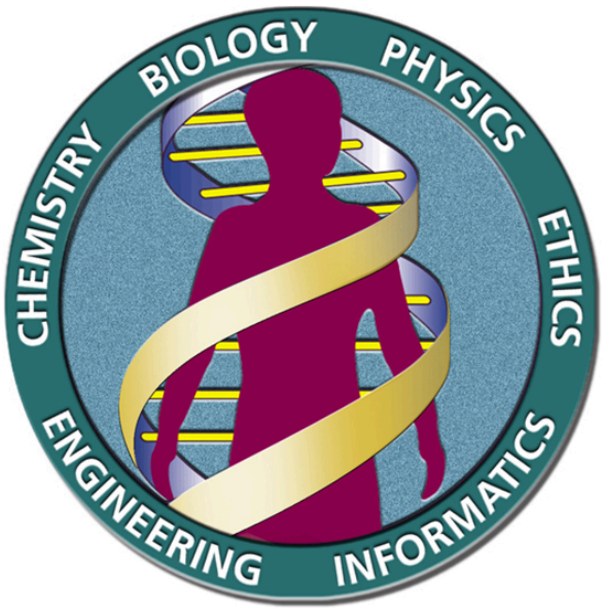


Figure 1.9 The Human Genome Project was a 13-year collaborative effort among researchers working in several different science fields. Researchers completed the project, which sequenced the entire human genome, in 2003. (credit: the U.S. Department of Energy Genome Programs (http://genomics.energy.gov))

While scientists usually carefully plan research efforts in both basic science and applied science, note that some discoveries are made by **serendipity**, that is, by means of a fortunate accident or a lucky surprise. Scottish biologist Alexander Fleming discovered penicillin when he accidentally left a petri dish of *Staphylococcus* bacteria open. An unwanted mold grew on the dish, killing the bacteria. Fleming's curiosity to investigate the reason behind the bacterial death, followed by his experiments, led to the discovery of the antibiotic penicillin, which is produced by the fungus *Penicillium*. 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 in order for other researchers to expand and build upon their discoveries. Collaboration with other scientists—when planning, conducting, and analyzing results—is important for scientific research. For this reason, important

aspects of a scientist's work are communicating with peers and disseminating results to peers. Scientists can share results by presenting them at a scientific meeting or conference, but this approach can reach only the select few who are present. Instead, most scientists present their results in peer-reviewed manuscripts that are published in scientific journals. **Peer-reviewed manuscripts** are scientific papers that a scientist's colleagues or peers review. 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 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.

A scientific paper is very different from creative writing. Although creativity is required to design experiments, there are fixed guidelines when it comes to presenting scientific results. First, scientific writing must be brief, concise, and accurate. A scientific paper needs to be succinct but detailed enough to allow peers to reproduce the experiments.

The scientific paper consists of several specific sections—introduction, materials and methods, results, and discussion. This structure is sometimes called the "IMRaD" format. There are usually acknowledgment and reference sections as well as an **abstract** (a concise summary) at the beginning of the paper. There might be additional sections depending on the type of paper and the journal where it will be published. For example, some review papers require an outline.

The **introduction** starts with brief, but broad, background information about what is known in the field. A good introduction also gives the rationale of the work. It justifies the work carried out and also presents the hypothesis or research question driving the research. The introduction refers to the published scientific work of others and therefore requires citations following the style of the journal. Using the work or ideas of others without proper citation is **plagiarism**.

The **materials and methods** section includes a complete and accurate description of the substances the researchers use, and the method and techniques they use to gather data. The description should be thorough enough to allow another researcher to repeat the experiment and obtain similar results, but it does not have to be verbose. This section will also include information on how the researchers made measurements and the types of calculations and statistical analyses they used to examine raw data. Although the materials and methods section gives an accurate description of the experiments, it does not discuss them.

Some journals require a results section followed by a discussion section, but it is more common to combine both. If the journal does not allow combining both sections, the **results** section simply narrates the findings without any further interpretation. The researchers present results with tables or graphs, but they do not present duplicate information. In the **discussion** section, the researchers will interpret the results, describe how variables may be related, and attempt to explain the observations. It is indispensable to conduct an

extensive literature search to put the results in the context of previously published scientific research. Therefore, researchers include proper citations in this section as well.

Finally, the **conclusion** section summarizes the importance of the experimental findings. While the scientific paper almost certainly answers one or more scientific questions that the researchers stated, any good research should lead to more questions. Therefore, a well-done scientific paper allows the researchers and others to continue and expand on the findings.

Review articles do not follow the IMRAD format because they do not present original scientific findings, or primary literature. Instead, they summarize and comment on findings that were published as primary literature and typically include extensive reference sections.

3.

THEMES AND CONCEPTS OF BIOLOGY

Learning Objectives

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

- Identify and describe the properties of life
- Describe the levels of organization among living things
- Recognize and interpret a phylogenetic tree
- List examples of different subdisciplines in biology

Biology is the science that studies life, but what exactly is life? This may sound like a silly question with an obvious response, but it is not always 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. Although viruses can attack living organisms, cause diseases, and even reproduce, they do not meet the criteria that biologists use to define life. Consequently, virologists are not biologists, strictly speaking. Similarly, some biologists study the early molecular evolution that gave rise to life. Since the events that preceded life are not biological events, these scientists are also excluded from biology in the strict sense of the term.

From its earliest beginnings, biology has wrestled with three questions: What are the shared properties that make something "alive"? Once we know something is alive, how do we find meaningful levels of organization in its structure? Finally, when faced with the remarkable diversity of life, how do we organize the different kinds of organisms so that we can better understand them? As scientists discover new organisms every day, biologists continue to seek answers to these and other questions.

Properties of Life

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

Order

Figure 1.10 A toad represents a highly organized structure consisting of cells, tissues, organs, and organ systems. (credit: "Ivengo"/Wikimedia Commons)

Organisms are highly organized, coordinated structures that consist of one or more cells. Even very simple, single-celled organisms are remarkably complex: inside each cell, atoms compose molecules. These in turn compose cell organelles and other cellular inclusions. In multicellular organisms (Figure 1.10), similar cells form tissues. Tissues, in turn, collaborate to create organs (body structures with a distinct function). Organs work together to form organ systems.

Sensitivity or Response to Stimuli

Figure 1.11 The leaves of this sensitive plant (Mimosa pudica) will instantly droop and fold when touched. After a few minutes, the plant returns to normal. (credit: Alex Lomas)

Organisms respond to diverse stimuli. For example, plants can bend toward a source of light, climb on fences and walls, or respond to touch (Figure 1.11). Even tiny bacteria can move toward or away from chemicals (a process called *chemotaxis*) or light (*phototaxis*). Movement toward a stimulus is a positive response, while movement away from a stimulus is a negative response.

Link to Learning

Watch this video to see how plants respond to a stimulus—from opening to light, to wrapping a tendril around a branch, to capturing prey.

Reproduction

Single-celled organisms reproduce by first duplicating their DNA, and then dividing it equally as the cell prepares to divide to form two new cells. Multicellular organisms often produce specialized reproductive cells—gametes and oocyte and sperm cells. After fertilization (the fusion of an oocyte and a sperm cell), a new individual develops. When reproduction occurs, DNA-containing genes are passed along to an organism's

offspring. These genes ensure that the offspring will belong to the same species and will have similar characteristics, such as size and shape.

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. Adaptations enhance the reproductive potential of the individuals 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 as a result of genes providing specific instructions that will direct cellular growth and development. This ensures that a species' young (Figure 1.12) will grow up to exhibit many of the same characteristics as its parents.

Figure 1.12 Although no two look alike, these kittens have inherited genes from both parents and share many of the same characteristics. (credit: Rocky Mountain Feline Rescue)

Regulation/Homeostasis

Even the smallest organisms are complex and require multiple regulatory mechanisms to coordinate internal functions, respond to stimuli, and cope with environmental stresses. **Homeostasis** (literally, "steady state") refers to the relatively stable internal environment required to maintain life. Two examples of internal functions regulated in an organism are nutrient transport and blood flow. Organs (groups of tissues working

together) perform specific functions, such as carrying oxygen throughout the body, removing wastes, delivering nutrients to every cell, and cooling the body.

Figure 1.13 Polar bears (Ursus maritimus) 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)

In order to function properly, cells require appropriate conditions such as proper temperature, pH, and appropriate concentration of diverse chemicals. These conditions may, however, change from one moment to the next. Organisms are able to maintain homeostatic internal conditions within a narrow range almost constantly, despite environmental changes, by activation of regulatory mechanisms. For example, an organism needs to regulate body temperature through the thermoregulation process. Organisms that live in cold climates, such as the polar bear (Figure 1.13), have body structures that help them withstand low temperatures and conserve body heat. Structures that aid in this type of insulation include fur, feathers, blubber, and fat. In hot climates, organisms have methods (such as perspiration in humans or panting in dogs) that help them to shed excess body heat.

Energy Processing

Figure 1.14 The California condor (Gymnogyps californianus) uses chemical energy derived from food to power flight. California condors are an endangered species. This bird has a wing tag that helps biologists identify the individual. (credit: Pacific Southwest Region U.S. Fish and Wildlife Service)

All organisms 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 in molecules they take in as food (Figure 1.14).

Evolution

The diversity of life on Earth is a result of mutations, or random changes in hereditary material over time. These mutations allow the possibility for organisms to adapt to a changing environment. An organism that evolves characteristics fit for the environment will have greater reproductive success, subject to the forces of natural selection.

Levels of Organization of Living Things

Living things are highly organized and structured, following a hierarchy that we can examine on a scale from small to large. The **atom** is the smallest and most fundamental unit of matter that retains the properties of an

element. 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 one or more chemical bonds. Many molecules that are biologically important are **macromolecules**, large molecules that are typically formed by polymerization (a polymer is a large molecule that is made by combining smaller units called monomers, which are simpler than macromolecules). An example of a macromolecule is deoxyribonucleic acid (DNA) (Figure 1.15), which contains the instructions for the structure and functioning of all living organisms.

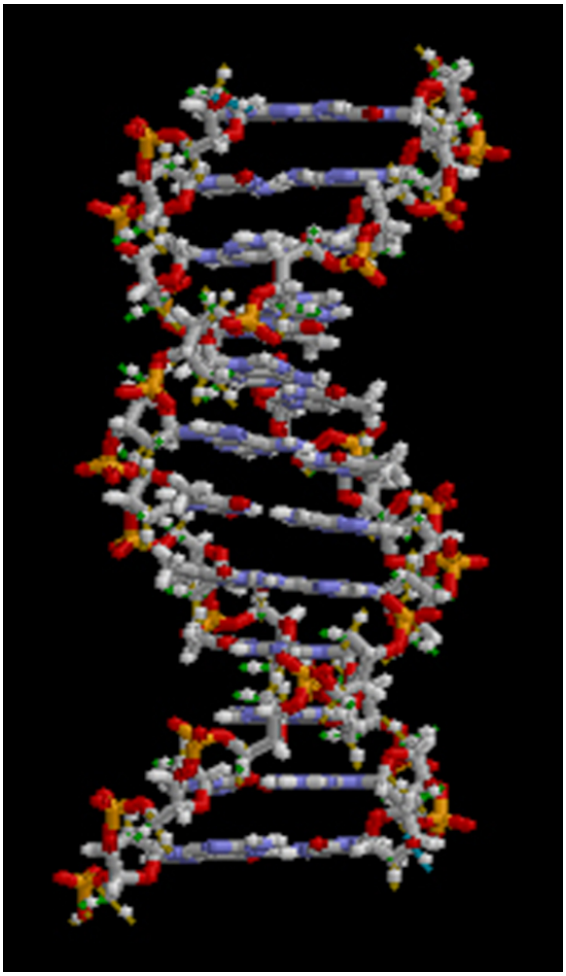


Figure 1.15 All molecules, including this DNA molecule, are composed of atoms. (credit: "brian0918"/Wikimedia Commons)

Link to Learning

Watch this video that animates the three-dimensional structure of the DNA molecule in Figure 1.15.

Some cells contain aggregates of macromolecules surrounded by membranes. We call these organelles. **Organelles** are small structures that exist within cells. Examples of organelles include mitochondria and chloroplasts, which carry out indispensable functions: mitochondria produce energy to power the cell, while chloroplasts enable green plants to utilize the energy in sunlight to make sugars. 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 scientists do not consider viruses living: they are not made of cells. To make new viruses, they have to invade and hijack the reproductive mechanism of 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. Scientists classify cells as prokaryotic or eukaryotic. **Prokaryotes** are single-celled or colonial organisms that do not have membrane-bound nuclei. In contrast, the cells of **eukaryotes** do have membrane-bound organelles and a membrane-bound nucleus.

In larger organisms, cells combine to make **tissues**, which are groups of similar cells carrying out similar or related functions. Organs are collections of tissues grouped together performing 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. Mammals have many organ systems. For instance, the circulatory system transports blood through 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 organisms, which biologists typically call microorganisms.

Biologists collectively call all the individuals of a species living within a specific area a **population**. For example, a forest may include many pine trees, which represent the population of 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, insects, and microbial populations. A **community** is the sum 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, nonliving parts of that environment such as nitrogen in the soil or rain water. At the highest level of organization (Figure 1.16), the **biosphere** is the collection of all ecosystems, and it represents the zones of life on Earth. It includes land, water, and even the atmosphere to a certain extent.

Visual Connection

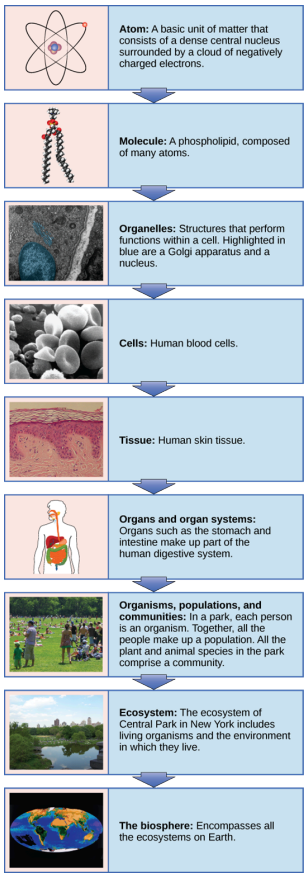


Figure 1.16 From an atom to the entire Earth, biology examines all aspects of life. (credit "molecule": modification of work by Jane Whitney; credit "organelles": modification of work by Louisa Howard; credit "cells": modification of work by Bruce Wetzel, Harry Schaefer, National Cancer Institute; credit "tissue": modification of work by "Kilbad"/Wikimedia Commons; credit "organs": modification of work by Mariana Ruiz Villareal, Joaquim Alves Gaspar; credit "organisms": modification of work by Peter Dutton; credit "ecosystem": modification of work by "gigi4791"/Flickr; credit "biosphere": modification of work by NASA)

Which of the following statements is false?

a. Tissues exist within organs, which exist within organ systems.
b. Communities exist within populations, which exist within ecosystems.
c. Organelles exist within cells, which exist within tissues.

d. Communities exist within ecosystems, which exist in the biosphere.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=101#h5p-41

The Diversity of Life

The fact that biology, as a science, has such a broad scope has to do with the tremendous diversity of life on earth. The source of this diversity is evolution, the process of gradual change in a population or species over time. Evolutionary biologists study the evolution of living things in everything from the microscopic world to ecosystems.

A phylogenetic tree (Figure 1.17) can summarize the evolution of various life forms on Earth. It is a diagram showing the evolutionary relationships among biological species based on similarities and differences in genetic or physical traits or both. Nodes and branches compose a phylogenetic tree. The internal nodes represent ancestors and are points in evolution when, based on scientific evidence, researchers believe an ancestor has diverged to form two new species. The length of each branch is proportional to the time elapsed since the split.

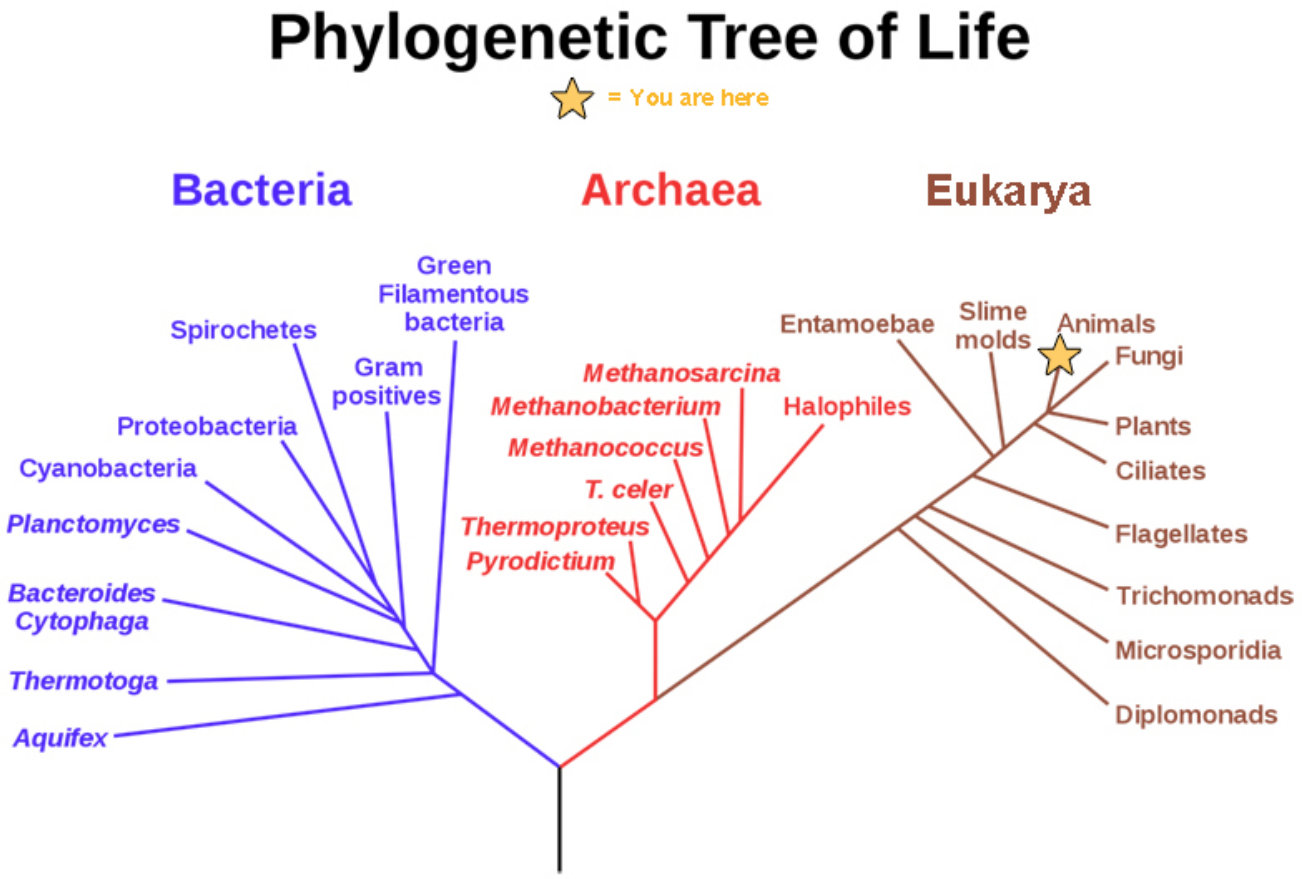


Figure 1.17 Microbiologist Carl Woese constructed this phylogenetic tree using data that he obtained from sequencing ribosomal RNA genes. The tree shows the separation of living organisms into three domains: Bacteria, Archaea, and Eukarya. Bacteria and Archaea are prokaryotes, single-celled organisms lacking intracellular organelles. (credit: Eric Gaba; NASA Astrobiology Institute)

EVOLUTION CONNECTION

Evolution Connection

Carl Woese and the Phylogenetic Tree

In the past, biologists grouped living organisms into five kingdoms: animals, plants, fungi, protists, and bacteria. They based the organizational scheme mainly on physical features, as opposed to physiology, biochemistry, or molecular biology, all of which modern systematics use. American microbiologist Carl Woese's pioneering work in the early 1970s has shown, however, that life on Earth has evolved along three lineages, now called domains—Bacteria, Archaea, and Eukarya. The first two are prokaryotic cells with microbes that lack membrane-enclosed nuclei and organelles. The third domain contains the eukaryotes and includes unicellular microorganisms (protists), together with the three remaining kingdoms (fungi, plants, and animals). Woese defined Archaea as a new domain, and this resulted in a new taxonomic tree (Figure 1.17).

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).

Woese constructed his tree from universally distributed comparative gene sequencing that is present in every organism and conserved (meaning that these genes have remained essentially unchanged throughout evolution). Woese's approach was revolutionary because comparing physical features is insufficient to differentiate between the prokaryotes that appear fairly similar in spite of their tremendous biochemical diversity and genetic variability (Figure 1.18). Comparing rRNA sequences provided Woese with a sensitive device that revealed the extensive variability of prokaryotes, and which justified separating the prokaryotes into two domains: bacteria and archaea.

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 1.17)

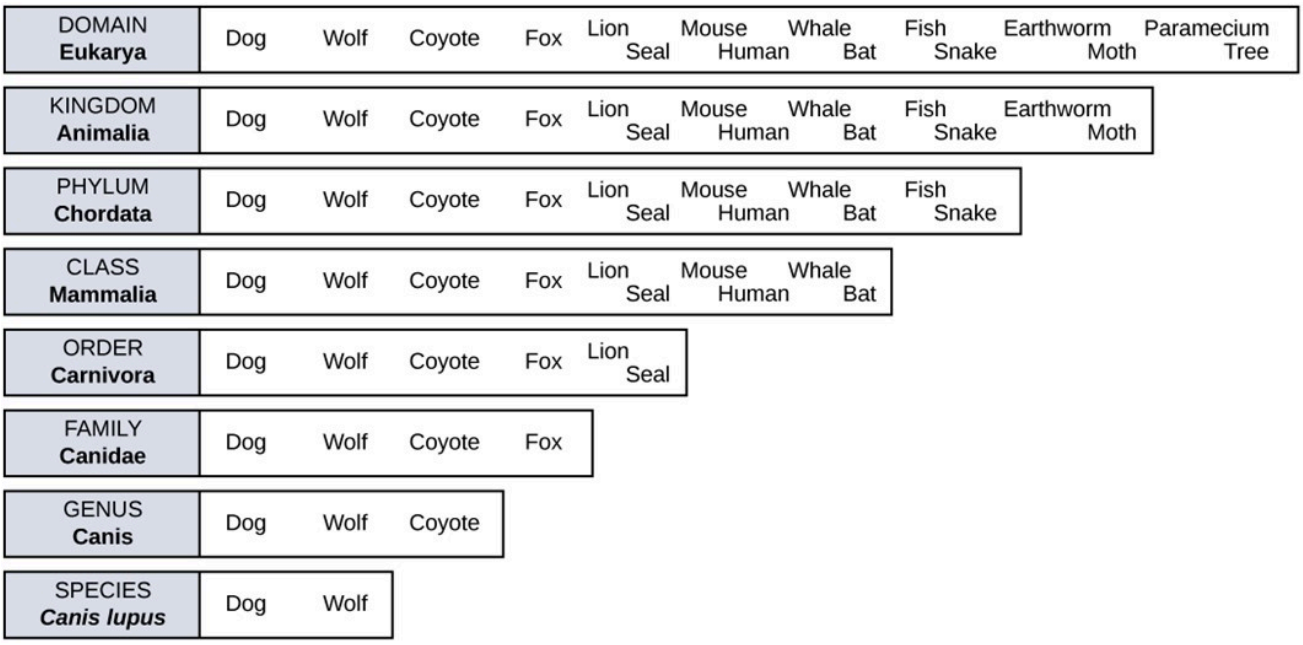


Figure 1.17 This diagram shows the levels of taxonomic hierarchy for a dog, from the broadest category—domain—to the most specific—species. Dogs are domesticated wolves, and thus the same species.

The highest level, domain, is a relatively new addition to the system since the 1970s. 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, and animals and several kingdoms of single-celled 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 1.18). Both the Archaea and the Bacteria are prokaryotes, an informal name for cells without nuclei. The recognition in the 1970s 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 1.18 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)

Branches of Biological Study

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

Career Connection

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 materials 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, developing molecular techniques and establishing DNA databases have expanded 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 1.20) found in many different environments and materials. Forensic scientists also analyze other biological evidence left at crime scenes, such as insect larvae or pollen grains. Students who want to pursue careers in forensic science will most likely have to take chemistry and biology courses as well as some intensive math courses.

Figure 1.20 This forensic scientist works in a DNA extraction room at the U.S. Army Criminal Investigation Laboratory at Fort Gillem, GA. (credit: United States Army CID Command Public Affairs)

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

Figure 1.21 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 1.20)(Figure 1.21). **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. This is just a small sample of the many fields that biologists can pursue.

Biology is the culmination of the achievements of the natural sciences from their inception to today. Excitingly, it is the cradle of emerging sciences, such as the biology of brain activity, genetic engineering of custom organisms, and the biology of evolution that uses the laboratory tools of molecular biology to retrace the earliest stages of life on Earth. A scan of news headlines—whether reporting on immunizations, a newly discovered species, sports doping, or a genetically modified food—demonstrates the way biology is active in and important to our everyday world.

4.

KEY TERMS

abstract
: opening section of a scientific paper that summarizes the research and conclusions

applied science
: form of science that aims to solve real-world problems

atom
: smallest and most fundamental unit of matter that retains the properties of an element

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

biochemistry
: study of the chemistry of biological organisms

biology
: the study of life

biosphere
: collection of all the ecosystems on Earth

botany
: study of plants

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

community
: set of populations inhabiting a particular area

conclusion
: section of a scientific paper that summarizes the importance of the experimental findings

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

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

dependent variable
the possible outcome of the experiment; the effect

descriptive science
(also, discovery science) form of science that aims to observe, explore, and investigate

discussion
section of a scientific paper in which the author interprets experimental results, describes how variables may be related, and attempts to explain the phenomenon in question

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

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

evolution
the process of gradual change in a population or species over time

falsifiable
able to be disproven by experimental results

homeostasis
ability of an organism to maintain constant internal conditions

hypothesis
suggested explanation for an observation, which one can test

hypothesis-based science
form of science that begins with a specific question and potential testable answers

independent variable
what you have control over; what you can choose and manipulate

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

introduction
opening section of a scientific paper, which provides background information about what was known in the field prior to the research reported in the paper

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

macromolecule
large molecule, typically formed by the joining of smaller molecules

materials and methods
section of a scientific paper that includes a complete description of the substances, methods, and techniques that the researchers used to gather data

microbiology
study of the structure and function of microorganisms

molecular biology
: study of biological processes and their regulation at the molecular level, including interactions among molecules such as DNA, RNA, and proteins

molecule
: chemical structure consisting of at least two atoms held together by one or more chemical bonds

natural science
: field of science that is related to the physical world and its phenomena and processes

neurobiology
: study of the biology of the nervous system

organ
: collection of related tissues grouped together performing a common function

organ system
: level of organization that consists of functionally related interacting organs

organelle
: small structures that exist within cells and carry out cellular functions

organism
: individual living entity

paleontology
: study of life's history by means of fossils

peer-reviewed manuscript
: scientific paper that a scientist's colleagues review who are experts in the field of study

phylogenetic tree
: diagram showing the evolutionary relationships among various biological species based on similarities and differences in genetic or physical traits or both; in essence, a hypothesis concerning evolutionary connections

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

plagiarism
: using other people's work or ideas without proper citation, creating the false impression that those are the author's original ideas

population
: all of the individuals of a species living within a specific area

prokaryote
: single-celled organism that lacks organelles and does not have nuclei surrounded by a nuclear membrane

results
: section of a scientific paper in which the author narrates the experimental findings and presents relevant figures, pictures, diagrams, graphs, and tables, without any further interpretation

review article
: paper that summarizes and comments on findings that were published as primary literature

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

scientific method
: method of research with defined steps that include observation, formulation of a hypothesis, testing, and confirming or falsifying the hypothesis

serendipity
: fortunate accident or a lucky surprise

theory
: tested and confirmed explanation for observations or phenomena

tissue
: group of similar cells carrying out related functions

variable
: part of an experiment that the experimenter can vary or change

zoology
: study of animals

5.

CHAPTER SUMMARY

1.1 The Science of Biology

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 by rational means. Science has many fields. Those fields related to the physical world and its phenomena are natural sciences.

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.

Science uses two types of logical reasoning. Inductive reasoning uses particular 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 using the scientific method, a step-based process that consists of making observations, defining a problem, posing hypotheses, testing these hypotheses, and drawing one or more conclusions. The testing uses proper controls. Scientists present their results in peer-reviewed scientific papers published in scientific journals. A scientific research paper consists of several well-defined sections: introduction, materials and methods, results, and finally, a concluding discussion. Review papers summarize the conducted research in a particular field over a period of time.

1.2 Themes and Concepts of Biology

Biology is the science of life. All living organisms share several key properties such as order, sensitivity or response to stimuli, reproduction, growth and development, regulation, homeostasis, and energy processing. Living things are highly organized parts of a hierarchy that includes atoms, molecules, organelles, cells, tissues, organs, and organ systems. In turn, biologists group organisms as populations, communities, ecosystems, and the biosphere. The great diversity of life today evolved from less-diverse ancestral organisms over billions of years. We can use a phylogenetic tree to show evolutionary relationships among organisms.

Biology is very broad and includes many branches and subdisciplines. Examples include molecular biology, microbiology, neurobiology, zoology, and botany, among others.

6.

VISUAL CONNECTION QUESTIONS

1. Figure 1.6 In the example below, the scientific method is used to solve an everyday problem. Match the scientific method steps (numbered items) with the process of solving the everyday problem (lettered items). Based on the results of the experiment, is the hypothesis correct? If it is incorrect, propose some alternative hypotheses.

1. Observation	a. There is something wrong with the electrical outlet.
2. Question	b. If something is wrong with the outlet, my coffeemaker also won't work when plugged into it.
3. Hypothesis (answer)	c. My toaster doesn't toast my bread.
4. Prediction	d. I plug my coffee maker into the outlet.
5. Experiment	e. My coffeemaker works.
6. Result	f. Why doesn't my toaster work?

2. Figure 1.7 Decide if each of the following is an example of inductive or deductive reasoning.

a. All flying birds and insects have wings. Birds and insects flap their wings as they move through the air. Therefore, wings enable flight.
b. Insects generally survive mild winters better than harsh ones. Therefore, insect pests will become more problematic if global temperatures increase.
c. Chromosomes, the carriers of DNA, separate into daughter cells during cell division. Therefore, each daughter cell will have the same chromosome set as the mother cell.
d. Animals as diverse as humans, insects, and wolves all exhibit social behavior. Therefore, social behavior must have an evolutionary advantage.

3. Figure 1.16 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.

7.

REVIEW QUESTIONS

4. The first forms of life on Earth were _____.

a. plants
b. microorganisms
c. birds
d. dinosaurs

5. A suggested and testable explanation for an event is called a _____.

a. hypothesis
b. variable
c. theory
d. control

6. Which of the following sciences is not considered a natural science?

a. biology
b. astronomy
c. physics
d. computer science

7. The type of logical thinking that uses related observations to arrive at a general conclusion is called _____.

a. deductive reasoning
b. the scientific method
c. hypothesis-based science
d. inductive reasoning

8. The process of _____ helps to ensure that a scientist's research is original, significant, logical, and thorough.

a. publication
b. public speaking
c. peer review
d. the scientific method

9 . A person notices that her houseplants that are regularly exposed to music seem to grow more quickly than those in rooms with no music. As a result, she determines that plants grow better when exposed to music. This example most closely resembles which type of reasoning?

a. inductive reasoning
b. deductive reasoning
c. neither, because no hypothesis was made
d. both inductive and deductive reasoning

10 . The smallest unit of biological structure that meets the functional requirements of "living" is the _____.

a. organ
b. organelle
c. cell
d. macromolecule

11 . Viruses are not considered living because they _____.

a. are not made of cells
b. lack cell nuclei
c. do not contain DNA or RNA
d. cannot reproduce

12 . The presence of a membrane-enclosed nucleus is a characteristic of _____.

a. prokaryotic cells
b. eukaryotic cells
c. living organisms
d. bacteria

13 . A group of individuals of the same species living in the same area is called a(n) _____.

a. family
b. community
c. population
d. ecosystem

14 . Which of the following sequences represents the hierarchy of biological organization from the most inclusive to the least complex level?

a. organelle, tissue, biosphere, ecosystem, population
b. organ, organism, tissue, organelle, molecule
c. organism, community, biosphere, molecule, tissue, organ
d. biosphere, ecosystem, community, population, organism

15 . Where in a phylogenetic tree would you expect to find the organism that had evolved most recently?

a. at the base
b. within the branches
c. at the nodes
d. at the branch tips

8.

CRITICAL THINKING QUESTIONS

16 . Although the scientific method is used by most of the sciences, it can also be applied to everyday situations. Think about a problem that you may have at home, at school, or with your car, and apply the scientific method to solve it.

17 . Give an example of how applied science has had a direct effect on your daily life.

18 . Name two topics that are likely to be studied by biologists, and two areas of scientific study that would fall outside the realm of biology.

19 . Thinking about the topic of cancer, write a basic science question and an applied science question that a researcher interested in this topic might ask.

20 . Select two items that biologists agree are necessary in order to consider an organism "alive." For each, give an example of a nonliving object that otherwise fits the definition of "alive."

21 . Consider the levels of organization of the biological world, and place each of these items in order from smallest level of organization to most encompassing: skin cell, elephant, water molecule, planet Earth, tropical rainforest, hydrogen atom, wolf pack, liver.

22 . You go for a long walk on a hot day. Give an example of a way in which homeostasis keeps your body healthy.

23 . Using examples, explain how biology can be studied from a microscopic approach to a global approach.

PART II

THE CHEMICAL FOUNDATION OF LIFE

9.

THE CHEMICAL FOUNDATION OF LIFE: INTRODUCTION

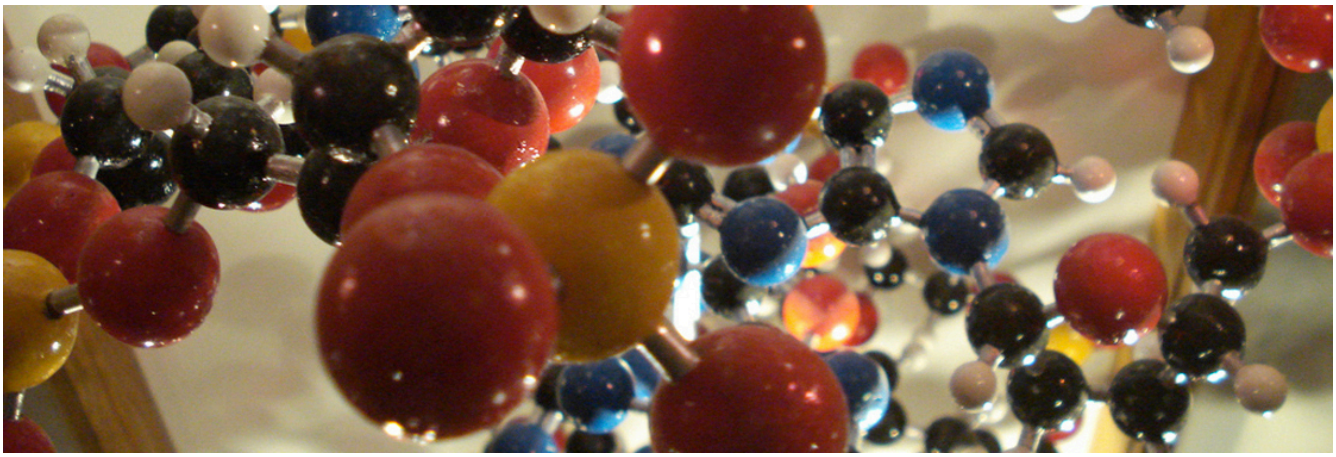


Figure 2.1 Atoms are the building blocks of molecules in the universe—air, soil, water, rocks . . . and also the cells of all living organisms. In this model of an organic molecule, the atoms of carbon (black), hydrogen (white), nitrogen (blue), oxygen (red), and phosphorus (yellow) are in proportional atomic size. The silver rods indicate chemical bonds. (credit: modification of work by Christian Guthier)

Elements in various combinations compose all matter, including living things. Some of the most abundant elements in living organisms include carbon, hydrogen, nitrogen, oxygen, sulfur, and phosphorus. These form the nucleic acids, proteins, carbohydrates, and lipids that are the fundamental components of living matter. Biologists must understand these important building blocks and the unique structures of the atoms that compose molecules, allowing for cells, tissues, organ systems, and entire organisms to form.

All biological processes follow the laws of physics and chemistry, so in order to understand how biological systems work, it is important to understand the underlying physics and chemistry. For example, the flow of blood within the circulatory system follows the laws of physics that regulate the modes of fluid flow. The breakdown of the large, complex molecules of food into smaller molecules—and the conversion of these to release energy to be stored in adenosine triphosphate (ATP)—is a series of chemical reactions that follow chemical laws. The properties of water and the formation of hydrogen bonds are key to understanding living processes. Recognizing the properties of acids and bases is important, for example, to our understanding of the digestive process. Therefore, the fundamentals of physics and chemistry are important for gaining insight into biological processes.

10.

ATOMS, ISOTOPES, IONS, AND MOLECULES: THE BUILDING BLOCKS

Learning Objectives

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

- Define matter and elements
- Describe the interrelationship between protons, neutrons, and electrons
- Compare the ways in which electrons can be donated or shared between atoms
- Explain the ways in which naturally occurring elements combine to create molecules, cells, tissues, organ systems, and organisms

At its most fundamental level, life is made up of matter. **Matter** is any substance that occupies space and has mass. **Elements** are unique forms of matter with specific chemical and physical properties that cannot break down into smaller substances by ordinary chemical reactions. There are 118 elements, but only 98 occur naturally. The remaining elements are unstable and require scientists to synthesize them in laboratories.

Each element is designated by its chemical symbol, which is a single capital letter or, when the first letter is already "taken" by another element, a combination of two letters. Some elements follow the English term for the element, such as C for carbon and Ca for calcium. Other elements' chemical symbols derive from their Latin names. For example, the symbol for sodium is Na, referring to *natrium*, the Latin word for sodium.

Four elements common to all living organisms are oxygen (O), carbon (C), hydrogen (H), and nitrogen (N). These are found in greatest quantity, but some others (e.g. phosphorous) can be critically important. In the nonliving world, elements are found in different proportions, and some elements common to living organisms are relatively rare on the earth as a whole, as Table 2.1 shows. For example, the atmosphere is rich in nitrogen and oxygen but contains little carbon and hydrogen, while the earth's crust, although it contains oxygen and

a small amount of hydrogen, has little nitrogen and carbon. In spite of their differences in abundance, all elements and the chemical reactions between them obey the same chemical and physical laws regardless of whether they are a part of the living or nonliving world.

Approximate Percentage of Elements in Living Organisms (Humans) Compared to the Nonliving World

Element	Life (Humans)	Atmosphere	Earth's Crust
Oxygen (O)	65%	21%	46%
Carbon (C)	18%	trace	trace
Hydrogen (H)	10%	trace	0.1%
Nitrogen (N)	3%	78%	trace

Table 2.1

The Structure of the Atom

To understand how elements come together, we must first discuss the element's smallest component or building block, the atom. An **atom** is the smallest unit of matter that retains all of the element's chemical properties. For example, one gold atom has all of the properties of gold, like its chemical reactivity. A gold coin is simply a very large number of gold atoms molded into the shape of a coin and contains small amounts of other elements known as impurities. We cannot break down gold atoms into anything smaller while still retaining the properties of gold.

An atom is composed of two regions. The **nucleus** in the atom's center contains protons and neutrons. The atom's outermost region holds its electrons in orbit around the nucleus, as Figure 2.2 illustrates. All atoms contain protons, electrons, and neutrons, except hydrogen (H), which is made of one proton and one electron with no neutrons.

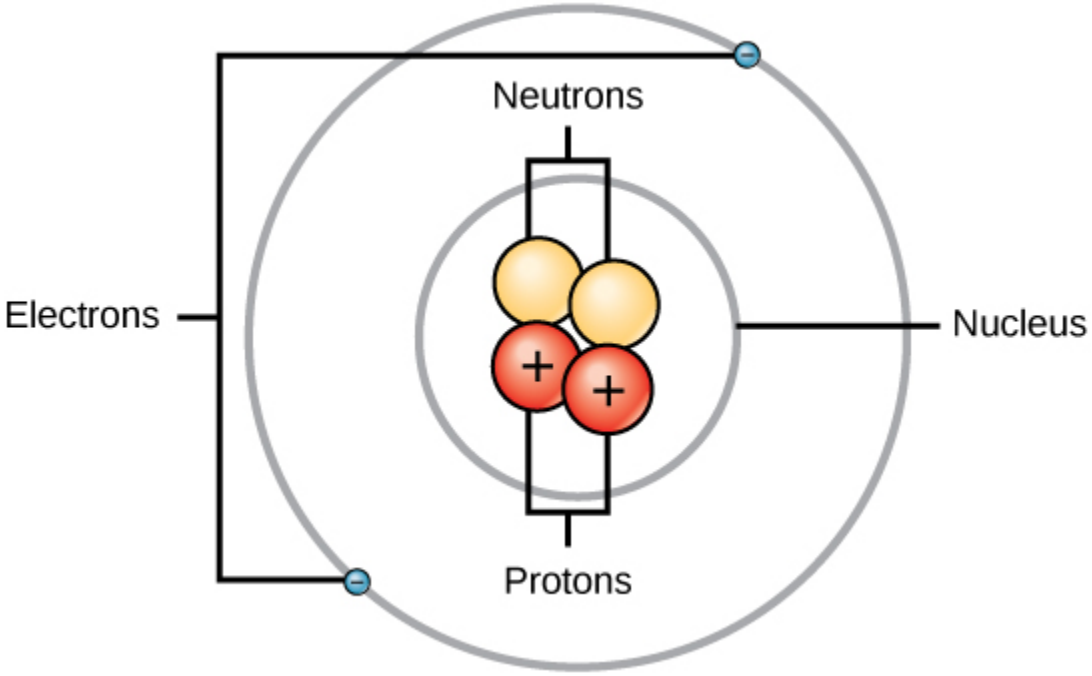


Figure 2.2 Elements, such as helium, depicted here, are made up of atoms. Atoms are made up of protons and neutrons located within the nucleus, with electrons in orbitals surrounding the nucleus.

Protons and neutrons have approximately the same mass, about 1.67×10^{-24} grams. Scientists arbitrarily define this amount of mass as one atomic mass unit (amu) or one Dalton, as Table 2.2 shows. Although similar in mass, protons and neutrons differ in their electric charge. A **proton** is positively charged, whereas a **neutron** is uncharged. Therefore, the number of neutrons in an atom contributes significantly to its mass, but not to its charge. **Electrons** are much smaller in mass than protons, weighing only 9.11×10^{-28} grams, or about 1/1800 of an atomic mass unit. Hence, they do not contribute much to an element's overall atomic mass. Although not significant contributors to mass, electrons do contribute greatly to the atom's charge, as each electron has a negative charge equal to the proton's positive charge. In uncharged, neutral atoms, the number of electrons orbiting the nucleus is equal to the number of protons inside the nucleus. In these atoms, the positive and negative charges cancel each other out, leading to an atom with no net charge.

Accounting for the sizes of protons, neutrons, and electrons, most of the atom's volume—greater than 99 percent—is empty space. With all this empty space, one might ask why so-called solid objects do not just pass through one another. The reason they do not is that the electrons that surround all atoms are negatively charged and negative charges repel each other.

Protons, Neutrons, and Electrons

	Charge	Mass (amu)	Location
Proton	+1	1	nucleus
Neutron	0	1	nucleus
Electron	−1	0	orbitals

Table 2.2

Atomic Number and Mass

Atoms of each element contain a characteristic number of protons and electrons. The number of protons determines an element's **atomic number**, which scientists use to distinguish one element from another. The number of neutrons is variable, resulting in isotopes, which are different forms of the same element that vary only in the number of neutrons they possess. Together, the number of protons and neutrons determine an element's **mass number**, as Figure 2.3 illustrates. Note that we disregard the small contribution of mass from electrons in calculating the mass number. We can use this approximation of mass to easily calculate how many neutrons an element has by simply subtracting the number of protons from the mass number. Since an element's isotopes will have slightly different mass numbers, scientists also determine the **atomic mass**, which is the calculated mean of the mass number for its naturally occurring isotopes. Often, the resulting number contains a fraction. For example, the atomic mass of chlorine (Cl) is 35.45 because chlorine is composed of several isotopes, some (the majority) with atomic mass 35 (17 protons and 18 neutrons) and some with atomic mass 37 (17 protons and 20 neutrons).

Visual Connection

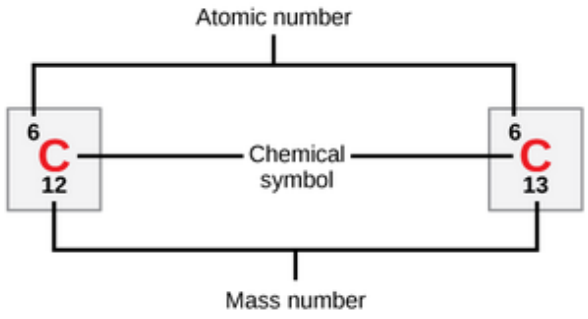


Figure 2.3 Carbon has an atomic number of six, and two stable isotopes with mass numbers of twelve and thirteen, respectively. Its relative atomic mass is 12.011.

How many neutrons do carbon-12 and carbon-13 have, respectively?

Isotopes

Isotopes are different forms of an 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 contains six protons, six neutrons, and six electrons; therefore, it has a mass number of 12 (six protons and six neutrons). Carbon-14 contains six protons, eight neutrons, and six electrons; its atomic mass is 14 (six protons and eight neutrons). These two alternate forms of carbon are isotopes. Some isotopes may emit neutrons, protons, and electrons, and attain a more stable atomic configuration (lower level of potential energy); these are radioactive isotopes, or **radioisotopes**. Radioactive decay (carbon-14 decaying to eventually become nitrogen-14) describes the energy loss that occurs when an unstable atom's nucleus undergoes this process.

Evolution Connection

Carbon Dating

Carbon is normally present in the atmosphere in the form of gaseous compounds like carbon dioxide and methane. Carbon-14 (^{14}C) is a naturally occurring radioisotope that is created in the atmosphere from atmospheric ^{14}N (nitrogen) by the addition of a neutron and the loss of a proton because of cosmic rays. This is a continuous process, so more ^{14}C is always being created. As a living organism incorporates ^{14}C initially as carbon dioxide fixed in the process of photosynthesis, the relative amount 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 between ^{14}C and ^{12}C will decline as ^{14}C decays gradually to ^{14}N by a process called beta decay—electrons or positrons emission. This decay emits energy in a slow process.

After approximately 5,730 years, half of the starting concentration of ^{14}C will convert back to ^{14}N. We call the time it takes for half of the original concentration of an isotope to decay back to its more stable form its half-life. Because the half-life of ^{14}C is long, scientists use it to date

formerly living objects such as old bones or wood. Comparing the ratio of the ^{14}C concentration in an object to the amount of ^{14}C in the atmosphere, scientists can determine the amount of the isotope that has not yet decayed. On the basis of this amount, Figure 2.4 shows that we can calculate the age of the material, such as the pygmy mammoth, with accuracy if it is not much older than about 50,000 years. Other elements have isotopes with different half-lives. For example, ^{40}K (potassium-40) has a half-life of 1.25 billion years, and ^{235}U (Uranium 235) has a half-life of about 700 million years. Through the use of radiometric dating, scientists can study the age of fossils or other remains of extinct organisms to understand how organisms have evolved from earlier species.

Figure 2.4 Scientists can determine the age of carbon-containing remains less than about 50,000 years old, such as this pygmy mammoth, using carbon dating. (credit: Bill Faulkner, NPS)

Link to Learning

To learn more about atoms, isotopes, and how to tell one isotope from another, click to view content.

The Periodic Table

The **periodic table** organizes and displays different elements. Devised by Russian chemist Dmitri Mendeleev (1834–1907) in 1869, the table groups elements that, although unique, share certain chemical properties with other elements. The properties of elements are responsible for their physical state at room temperature: they may be gases, solids, or liquids. Elements also have specific **chemical reactivity**, the ability to combine and to chemically bond with each other. In the periodic table in Figure 2.5, the elements are organized and displayed according to their atomic number and are arranged in a series of rows and columns based on shared chemical and physical properties. In addition to providing the atomic number for each element, the periodic table also displays the element's atomic mass. Looking at carbon, for example, its symbol (C) and name appear, as well as its atomic number of six (in the upper left-hand corner) and its atomic mass of 12.01.

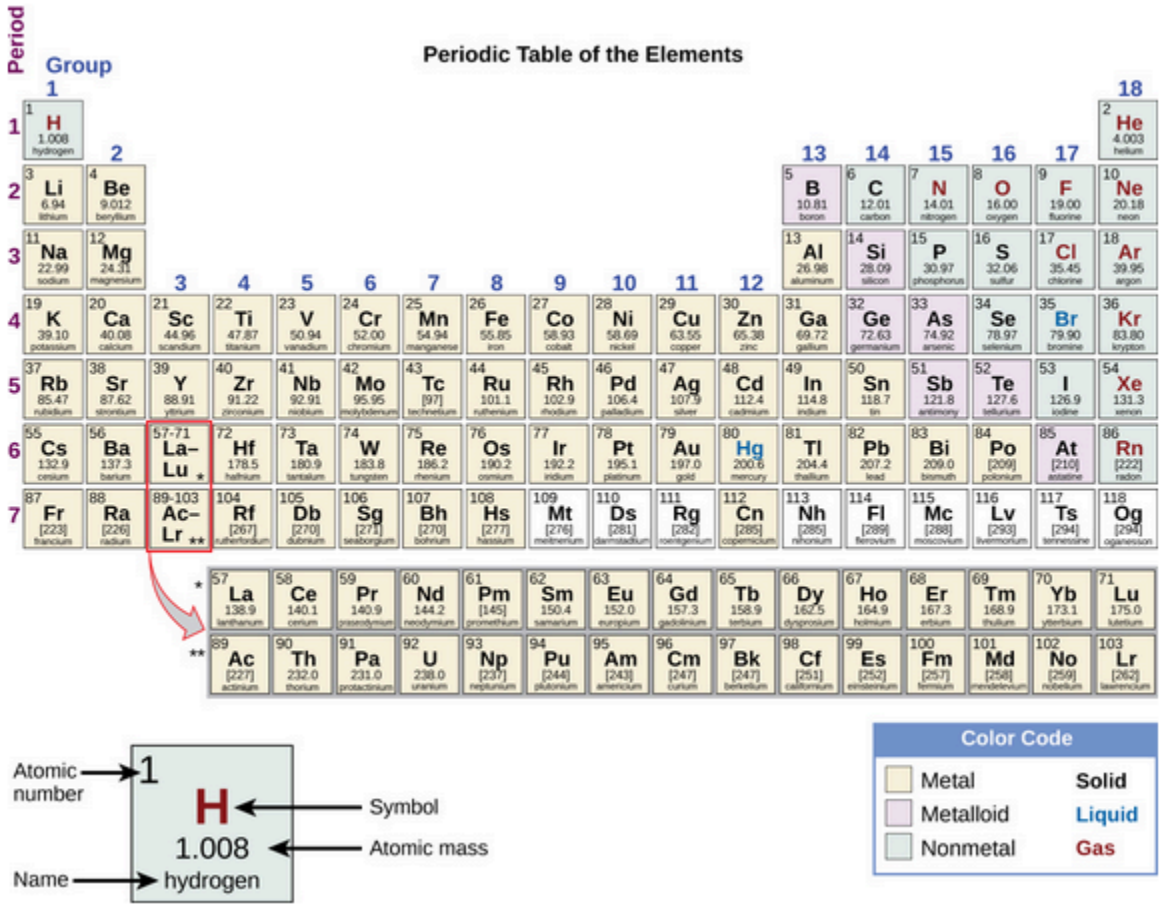


Figure 2.5 The periodic table shows each element's atomic mass and atomic number. The atomic number appears above the symbol for the element and the approximate atomic mass appears below it.

The periodic table groups elements according to chemical properties. Scientists base the differences in chemical reactivity between the elements on the number and spatial distribution of an atom's electrons.

Atoms that chemically react and bond to each other form molecules. **Molecules** are simply two or more atoms chemically bonded together. Logically, when two atoms chemically bond to form a molecule, their electrons, which form the outermost region of each atom, come together first as the atoms form a chemical bond.

Electron Shells and the Bohr Model

Note that there is a connection between the number of protons in an element, the atomic number that distinguishes one element from another, and the number of electrons it has. In all electrically neutral atoms, the number of electrons is the same as the number of protons. Thus, each element, at least when electrically neutral, has a characteristic number of electrons equal to its atomic number.

In 1913, Danish scientist Niels Bohr (1885–1962) developed an early model of the atom. The Bohr model shows the atom as a central nucleus containing protons and neutrons, with the electrons in circular **orbitals** at specific distances from the nucleus, as Figure 2.6 illustrates. These orbits form electron shells or energy levels, which are a way of visualizing the number of electrons in the outermost shells. These energy levels are designated by a number and the symbol "n." For example, 1n represents the first energy level located closest to the nucleus.

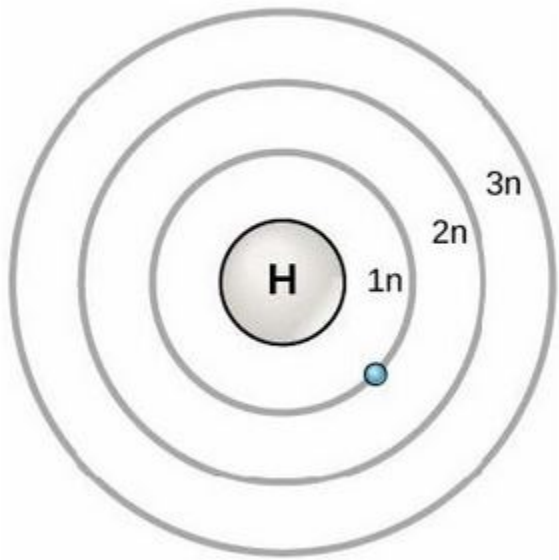


Figure 2.6 In 1913, Niels Bohr developed the Bohr model in which electrons exist within principal shells. An electron normally exists in the lowest energy shell available, which is the one closest to the nucleus. Energy from a photon of light can bump it up to a higher energy shell, but this situation is unstable, and the electron quickly decays back to the ground state. In the process, it releases a photon of light.

Electrons fill orbitals in a consistent order: they first fill the orbitals closest to the nucleus, then they continue to fill orbitals of increasing energy further from the nucleus. If there are multiple orbitals of equal energy, they fill with one electron in each energy level before adding a second electron. The electrons of the outermost energy level determine the atom's energetic stability and its tendency to form chemical bonds with other atoms to form molecules.

Under standard conditions, atoms fill the inner shells first, often resulting in a variable number of electrons in the outermost shell. The innermost shell has a maximum of two electrons, but the next two electron shells can each have a maximum of eight electrons. This is known as the **octet rule**, which states, with the exception of the innermost shell, that atoms are more stable energetically when they have eight electrons in their **valence shell**, the outermost electron shell. Figure 2.7 shows examples of some neutral atoms and their electron configurations. Notice that in Figure 2.7, helium has a complete outer electron shell, with two electrons filling its first and only shell. Similarly, neon has a complete outer 2n shell containing eight electrons. In contrast, chlorine and sodium have seven and one in their outer shells, respectively, but theoretically they would be more energetically stable if they followed the octet rule and had eight.

Visual Connection

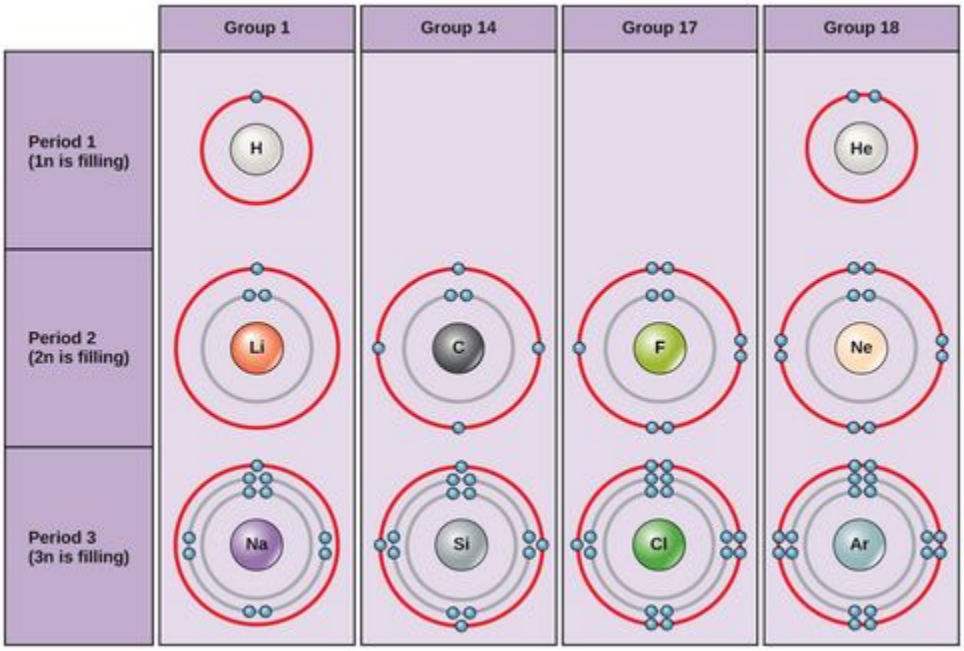


Figure 2.7 Bohr diagrams indicate how many electrons fill each principal shell in select rows (periods) and columns (groups) of the periodic table. Group 18 elements (helium, neon, and argon) have a full outer, or valence, shell. A full valence shell is the most stable electron configuration. Elements in other groups have partially filled valence shells and gain or lose electrons to achieve a stable electron configuration.

An atom may give, take, or share electrons with another atom to achieve a full valence shell, the most stable electron configuration. Looking at this figure, how many electrons do elements in group 1 need to lose in order to achieve a stable electron configuration? How many electrons do elements in groups 14 and 17 need to gain to achieve a stable configuration?

Understanding that the periodic table's organization is based on the total number of protons (and electrons) helps us know how electrons distribute themselves among the shells. The periodic table is arranged in columns and rows based on the number of electrons and their location. Examine more closely some of the elements in the table's far right column in Figure 2.5. The group 18 atoms helium (He), neon (Ne), and argon (Ar) all have filled outer electron shells, making it unnecessary for them to share electrons with other atoms to attain stability. They are highly stable as single atoms. Because they are non reactive, scientists coin them **inert** (or **noble gases**). Compare this to the group 1 elements in the left-hand column. These elements, including hydrogen (H), lithium (Li), and sodium (Na), all have one electron in their outermost shells. That means that they can achieve a stable configuration and a filled outer shell by donating or sharing one electron

with another atom or a molecule such as water. Hydrogen will donate or share its electron to achieve this configuration, while lithium and sodium will donate their electron to become stable. As a result of losing a negatively charged electron, they become positively charged **ions**. Group 17 elements, including fluorine and chlorine, have seven electrons in their outmost shells, so they tend to fill this shell with an electron from other atoms or molecules, making them negatively charged ions. Group 14 elements, of which carbon is the most important to living systems, have four electrons in their outer shell, allowing them to make several covalent bonds (discussed below) with other atoms. Thus, the periodic table's columns represent the potential shared state of these elements' outer electron shells that is responsible for their similar chemical characteristics.

Electron Orbitals

Although useful to explain the reactivity and chemical bonding of certain elements, the Bohr model does not accurately reflect how electrons spatially distribute themselves around the nucleus. They do not circle the nucleus like the earth orbits the sun, but we find them in **electron orbitals**. These relatively complex shapes result from the fact that electrons behave not just like particles, but also like waves. Mathematical equations from quantum mechanics, which scientists call wave functions, can predict within a certain level of probability where an electron might be at any given time. Scientists call the area where an electron is most likely to be found its orbital.

Recall that the Bohr model depicts an atom's electron shell configuration. Within each electron shell are subshells, and each subshell has a specified number of orbitals containing electrons. While it is impossible to calculate exactly an electron's location, scientists know that it is most probably located within its orbital path. The letters *s*, *p*, *d*, and *f* designate the subshells. The *s* subshell is spherical in shape and has one orbital. Principal shell 1n has only a single *s* orbital, which can hold two electrons. Principal shell 2n has one *s* and one *p* subshell, and can hold a total of eight electrons. The *p* subshell has three dumbbell-shaped orbitals, as Figure 2.8 illustrates. Subshells *d* and *f* have more complex shapes and contain five and seven orbitals, respectively. We do not show these in the illustration. Principal shell 3n has *s*, *p*, and *d* subshells and can hold 18 electrons. Principal shell 4n has *s*, *p*, *d* and *f* orbitals and can hold 32 electrons. Moving away from the nucleus, the number of electrons and orbitals in the energy levels increases. Progressing from one atom to the next in the periodic table, we can determine the electron structure by fitting an extra electron into the next available orbital.

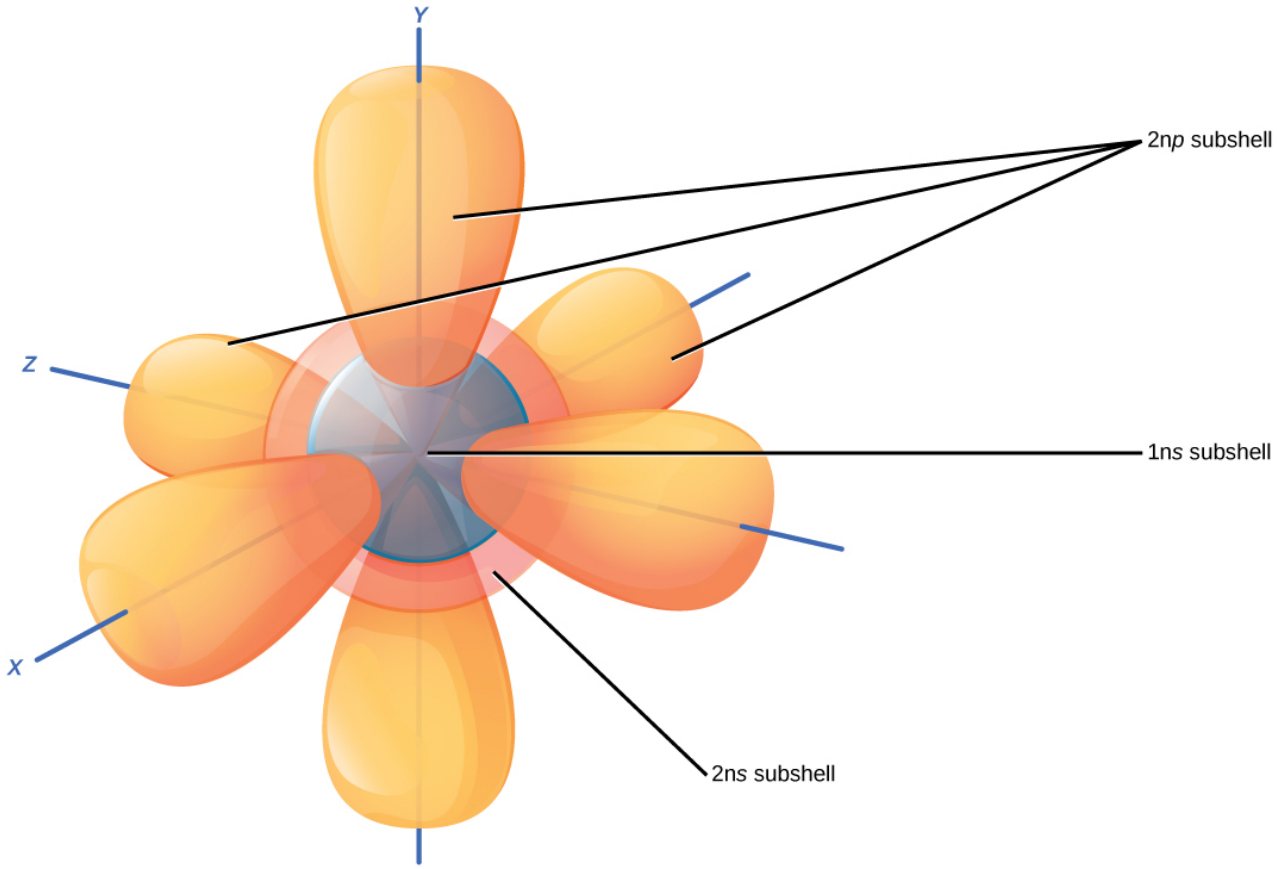


Figure 2.8 The s subshells are shaped like spheres. Both the 1n and 2n principal shells have an s orbital, but the size of the sphere is larger in the 2n orbital. Each sphere is a single orbital. Three dumbbell-shaped orbitals comprise p subshells. Principal shell 2n has a p subshell, but shell 1 does not.

The closest orbital to the nucleus, the 1s orbital, can hold up to two electrons. This orbital is equivalent to the Bohr model's innermost electron shell. Scientists call it the 1*s* orbital because it is spherical around the nucleus. The 1*s* orbital is the closest orbital to the nucleus, and it is always filled first, before any other orbital fills. Hydrogen has one electron; therefore, it occupies only one spot within the 1*s* orbital. We designate this as $1s^1$, where the superscripted 1 refers to the one electron within the 1*s* orbital. Helium has two electrons; therefore, it can completely fill the 1*s* orbital with its two electrons. We designate this as $1s^2$, referring to the two electrons of helium in the 1*s* orbital. On the periodic table in Figure 2.5, hydrogen and helium are the only two elements in the first row (period). This is because they only have electrons in their first shell, the 1*s* orbital. Hydrogen and helium are the only two elements that have the 1*s* and no other electron orbitals in the electrically neutral state.

The second electron shell may contain eight electrons. This shell contains another spherical *s* orbital and three "dumbbell" shaped *p* orbitals, each of which can hold two electrons, as Figure 2.8 shows. After the 1*s* orbital fills, the second electron shell fills, first filling its 2*s* orbital and then its three *p* orbitals. When filling the *p* orbitals, each takes a single electron. Once each *p* orbital has an electron, it may add a second. Lithium (Li) contains three electrons that occupy the first and second shells. Two electrons fill the 1*s* orbital, and the

third electron then occupies the 2*s* orbital. Its **electron configuration** is $1s^2 2s^1$. Neon (Ne), alternatively, has a total of ten electrons: two are in its innermost 1*s* orbital, and eight fill its second shell (two each in the 2*s* and three *p* orbitals). Thus it is an inert gas and energetically stable as a single atom that will rarely form a chemical bond with other atoms. Larger elements have additional orbitals, comprising the third electron shell. While the concepts of electron shells and orbitals are closely related, orbitals provide a more accurate depiction of an atom's electron configuration because the orbital model specifies the different shapes and special orientations of all the places that electrons may occupy.

Chemical Reactions and Molecules

All elements are most stable when their outermost shell is filled with electrons, according to the octet rule. This is because it is energetically favorable for atoms to be in that configuration and it makes them stable. However, since not all elements have enough electrons to fill their outermost shells, atoms form **chemical bonds** with other atoms, thereby obtaining the electrons they need to attain a stable electron configuration. When two or more atoms chemically bond with each other, the resultant chemical structure is a molecule. The familiar water molecule, H_2O, consists of two hydrogen atoms and one oxygen atom. These bond together to form water, as Figure 2.9 illustrates. Atoms can form molecules by donating, accepting, or sharing electrons to fill their outer shells.

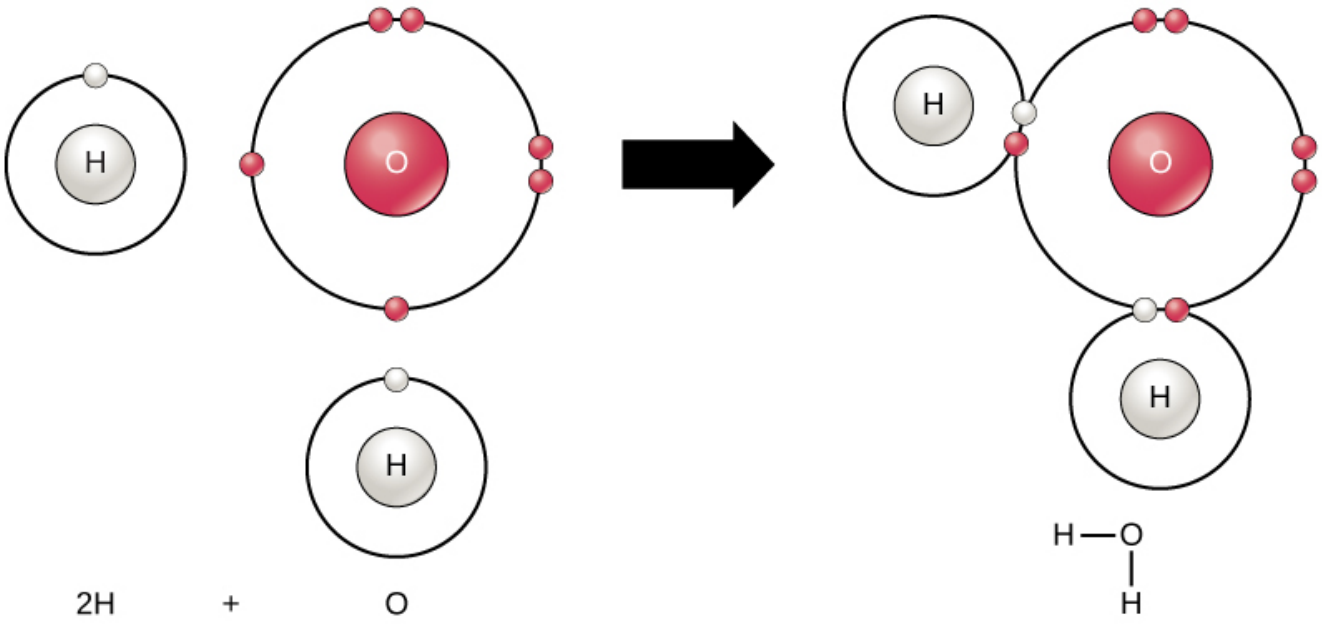


Figure 2.9 Two or more atoms may bond with each other to form a molecule. When two hydrogens and an oxygen share electrons via covalent bonds, it forms a water molecule.

Chemical reactions occur when two or more atoms bond together to form molecules or when bonded atoms break apart. Scientists call the substances used in the beginning of a chemical reaction **reactants** (usually on

the left side of a chemical equation), and we call the substances at the end of the reaction **products** (usually on the right side of a chemical equation). We typically draw an arrow between the reactants and products to indicate the chemical reaction's direction. This direction is not always a "one-way street." To create the water molecule above, the chemical equation would be:

$2H + O \rightarrow H_2O$

An example of a simple chemical reaction is breaking down hydrogen peroxide molecules, each of which consists of two hydrogen atoms bonded to two oxygen atoms (H_2O_2). The reactant hydrogen peroxide breaks down into water, containing one oxygen atom bound to two hydrogen atoms (H_2O), and oxygen, which consists of two bonded oxygen atoms (O_2). In the equation below, the reaction includes two hydrogen peroxide molecules and two water molecules. This is an example of a **balanced chemical equation**, wherein each element's number of atoms is the same on each side of the equation. According to the law of conservation of matter, the number of atoms before and after a chemical reaction should be equal, such that no atoms are, under normal circumstances, created or destroyed.

$2H_2O_2$ (hydrogen peroxide) $\rightarrow 2H_2O$ (water) + O_2 (oxygen)

Even though all of the reactants and products of this reaction are molecules (each atom remains bonded to at least one other atom), in this reaction only hydrogen peroxide and water are representatives of **compounds**: they contain atoms of more than one type of element. Molecular oxygen, alternatively, as Figure 2.10 shows, consists of two doubly bonded oxygen atoms and is not classified as a compound but as a homonuclear molecule.

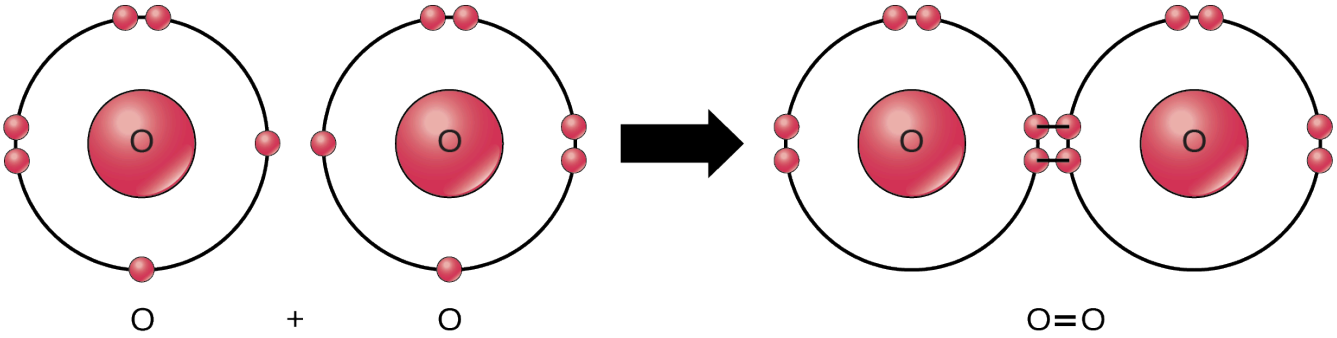


Figure 2.10 A double bond joins the oxygen atoms in an O2 molecule.

Some chemical reactions, such as the one above, can proceed in one direction until they expend all the reactants. The equations that describe these reactions contain a unidirectional arrow and are **irreversible**. **Reversible reactions** are those that can go in either direction. In reversible reactions, reactants turn into products, but when the product's concentration goes beyond a certain threshold (characteristic of the particular reaction), some of these products convert back into reactants. At this point, product and reactant designations reverse. This back and forth continues until a certain relative balance between reactants and products occurs—a state called **equilibrium**. A chemical equation with a

double-headed arrow pointing towards both the reactants and products often denotes these reversible reaction situations.

For example, in human blood, excess hydrogen ions (H^+) bind to bicarbonate ions (HCO_3^-) forming an equilibrium state with carbonic acid (H_2CO_3). If we added carbonic acid to this system, some of it would convert to bicarbonate and hydrogen ions.

$HCO_3^- + H^+ \leftrightarrow H_2CO_3$

However, biological reactions rarely obtain equilibrium because the concentrations of the reactants or products or both are constantly changing, often with one reaction's product a reactant for another. To return to the example of excess hydrogen ions in the blood, forming carbonic acid will be the reaction's major direction. However, the carbonic acid can also leave the body as carbon dioxide gas (via exhalation) instead of converting back to bicarbonate ion, thus driving the reaction to the right by the **law of mass action**. These reactions are important for maintaining homeostasis in our blood.

$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O$

Ions and Ionic Bonds

Some atoms are more stable when they gain or lose an electron (or possibly two) and form ions. This fills their outermost electron shell and makes them energetically more stable. Because the number of electrons does not equal the number of protons, each ion has a net charge. **Cations** are positive ions that form by losing electrons. Negative ions form by gaining electrons, which we call anions. We designate **anions** by their elemental name and change the ending to "-ide", thus the anion of chlorine is chloride, and the anion of sulfur is sulfide.

Scientists refer to this movement of electrons from one element to another as **electron transfer**. As Figure 2.11 illustrates, sodium (Na) only has one electron in its outer electron 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, 11 neutrons, and only 10 electrons, leaving it with an overall charge of +1. We now refer to it as a sodium ion. Chlorine (Cl) in its lowest energy state (called the ground state) 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, 17 neutrons, and 18 electrons, giving it a net negative (−1) charge. We now refer to it as a chloride ion. In this example, sodium will donate its one electron to empty its shell, and chlorine will accept that electron to fill its shell. 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 cation) or −1 (chloride anion) charge. Note that these transactions can normally only take place simultaneously: in order for a sodium atom to lose an electron, it must be in the presence of a suitable recipient like a chlorine atom.

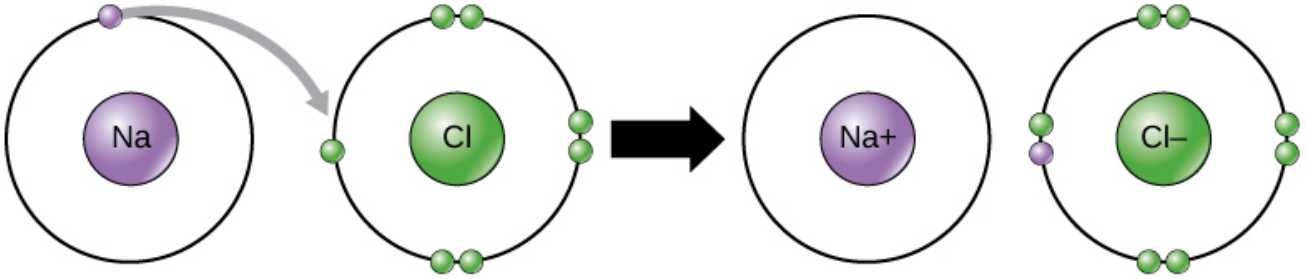


Figure 2.11 In the formation of an ionic compound, atoms lose electrons and others gain electrons.

Ionic bonds form between ions with opposite charges. For instance, positively charged sodium ions and negatively charged chloride ions bond together to make crystals of sodium chloride, or table salt, creating a crystalline molecule with zero net charge.

Physiologists refer to certain salts as **electrolytes** (including sodium, potassium, and calcium), ions necessary for nerve impulse conduction, muscle contractions, and water balance. Many sports drinks and dietary supplements provide these ions to replace those lost from the body via sweating during exercise.

Covalent Bonds and Other Bonds and Interactions

Another way to satisfy the octet rule is by sharing electrons between atoms to form **covalent bonds**. These bonds are stronger and much more common than ionic bonds in the molecules of living organisms. We commonly find covalent bonds in carbon-based organic molecules, such as our DNA and proteins. We also find covalent bonds in inorganic molecules like H_2O, CO_2, and O_2. The bonds may share one, two, or three pairs of electrons, making single, double, and triple bonds, respectively. The more covalent bonds between two atoms, the stronger their connection. Thus, triple bonds are the strongest.

The strength of different levels of covalent bonding is one of the main reasons living organisms have a difficult time acquiring nitrogen for use in constructing their molecules, even though molecular nitrogen, N_2, is the most abundant gas in the atmosphere. Molecular nitrogen consists of two nitrogen atoms triple bonded to each other and, as with all molecules, sharing these three pairs of electrons between the two nitrogen atoms allows for filling their outer electron shells, making the molecule more stable than the individual nitrogen atoms. This strong triple bond makes it difficult for living systems to break apart this nitrogen in order to use it as constituents of proteins and DNA.

Forming water molecules provides an example of covalent bonding. Covalent bonds bind the hydrogen and oxygen atoms that combine to form water molecules, as Figure 2.9 shows. The electron from the hydrogen splits its time between the hydrogen atoms' incomplete outer shell and the oxygen atoms' incomplete outer shell. To completely fill the oxygen's outer shell, which has six electrons but which would be more stable with eight, two electrons (one from each hydrogen atom) are needed: hence, the well-known formula H_2O. The two elements share the electrons to fill the outer shell of each, making both elements more stable.

Polar Covalent Bonds

There are two types of covalent bonds: polar and nonpolar. In a **polar covalent bond**, Figure 2.12 shows atoms unequally share the electrons, which are attracted more to one nucleus than the other. Because of the unequal electron distribution between the atoms of different elements, a slightly positive ($\delta+$) or slightly negative ($\delta-$) charge develops. This partial charge is an important property of water and accounts for many of its characteristics.

Water is a polar molecule, with the hydrogen atoms acquiring a partial positive charge and the oxygen a partial negative charge. This occurs because the oxygen atom's nucleus is more attractive to the hydrogen atoms' electrons than the hydrogen nucleus is to the oxygen's electrons. Thus, oxygen has a higher **electronegativity** than hydrogen and the shared electrons spend more time near the oxygen nucleus than the hydrogen atoms' nucleus, giving the oxygen and hydrogen atoms slightly negative and positive charges, respectively. Another way of stating this is that the probability of finding a shared electron near an oxygen nucleus is more likely than finding it near a hydrogen nucleus. Either way, the atom's relative electronegativity contributes to developing partial charges whenever one element is significantly more electronegative than the other, and the charges that these polar bonds generate may then be used to form hydrogen bonds based on the attraction of opposite partial charges. (Hydrogen bonds, which we discuss in detail below, are weak bonds between slightly positively charged hydrogen atoms to slightly negatively charged atoms in other molecules.) Since macromolecules often have atoms within them that differ in electronegativity, polar bonds are often present in organic molecules.

Nonpolar Covalent Bonds

Nonpolar covalent bonds form between two atoms of the same element or between different elements that share electrons equally. For example, molecular oxygen (O_2) is nonpolar because the electrons distribute equally between the two oxygen atoms.

Figure 2.12 also shows another example of a nonpolar covalent bond—methane (CH_4). Carbon has four electrons in its outermost shell and needs four more to fill it. It obtains these four from four hydrogen atoms, each atom providing one, making a stable outer shell of eight electrons. Carbon and hydrogen do not have the same electronegativity but are similar; thus, nonpolar bonds form. The hydrogen atoms each need one electron for their outermost shell, which is filled when it contains two electrons. These elements share the electrons equally among the carbons and the hydrogen atoms, creating a nonpolar covalent molecule.

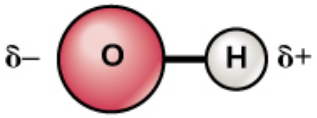
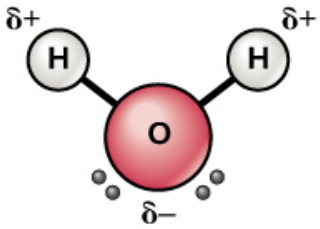
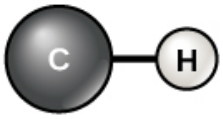
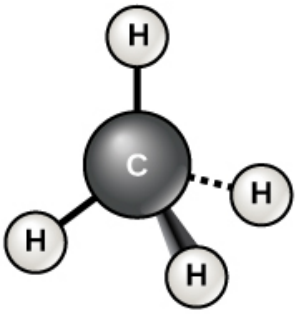
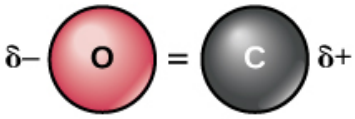
	Bond type	Molecular shape	Molecular type
Water	 Polar covalent	 Bent	Polar
Methane	 Nonpolar covalent	 Tetrahedral	Nonpolar
Carbon dioxide	 Polar covalent	 Linear	Nonpolar

Figure 2.12 Whether a molecule is polar or nonpolar depends both on bond type and molecular shape. Both water and carbon dioxide have polar covalent bonds, but carbon dioxide is linear, so the partial charges on the molecule cancel each other out.

Hydrogen Bonds and Van Der Waals Interactions

Ionic and covalent bonds between elements require energy to break. Ionic bonds are not as strong as covalent, which determines their behavior in biological systems. However, not all bonds are ionic or covalent bonds. Weaker bonds can also form between molecules. Two weak bonds that occur frequently are hydrogen bonds and van der Waals interactions. Without these two types of bonds, life as we know it would not exist. Hydrogen bonds provide many of the critical, life-sustaining properties of water and also stabilize the structures of proteins and DNA, the building block of cells.

When polar covalent bonds containing hydrogen form, the hydrogen in that bond has a slightly positive charge because hydrogen's electron is pulled more strongly toward the other element and away from the

hydrogen. Because the hydrogen is slightly positive, it will be attracted to neighboring negative charges. When this happens, a weak interaction occurs between the hydrogen's δ^+ from one molecule and the molecule's δ^- charge on another molecule with the more electronegative atoms, usually oxygen. Scientists call this interaction a **hydrogen bond**. This type of bond is common and occurs regularly between water molecules. Individual hydrogen bonds are weak and easily broken; however, they occur in very large numbers in water and in organic polymers, creating a major force in combination. Hydrogen bonds are also responsible for zipping together the DNA double helix.

Like hydrogen bonds, **van der Waals interactions** are weak attractions or interactions between molecules. Van der Waals attractions can occur between any two or more molecules and are dependent on slight fluctuations of the electron densities, which are not always symmetrical around an atom. For these attractions to happen, the molecules need to be very close to one another. These bonds—along with ionic, covalent, and hydrogen bonds—contribute to the proteins' three-dimensional structure in our cells that is necessary for their proper function.

Career Connection

Pharmaceutical Chemist

Pharmaceutical chemists are responsible for developing new drugs and trying to determine the mode of action of both old and new drugs. They are involved in every step of the drug development process. We can find drugs in the natural environment or we can synthesize them in the laboratory. In many cases, chemists change potential drugs from nature chemically in the laboratory to make them safer and more effective, and sometimes synthetic versions of drugs substitute for the version we find in nature.

After a drug's initial discovery or synthesis, the chemist then develops the drug, perhaps chemically altering it, testing it to see if it is toxic, and then designing methods for efficient large-scale production. Then, the process of approving the drug for human use begins. In the United States, the Food and Drug Administration (FDA) handles drug approval. This involves a series of large-scale experiments using human subjects to ensure the drug is not harmful and effectively treats the condition for which it is intended. This process often takes several years and requires the participation of physicians and scientists, including chemists, to complete testing and gain approval.

An example of a drug that was originally discovered in a living organism is Paclitaxel (Taxol), an anti-cancer drug used to treat breast cancer. This drug was discovered in the bark of the pacific yew tree. Another example is aspirin, originally isolated from willow tree bark. Finding drugs

often means testing hundreds of samples of plants, fungi, and other forms of life to see if they contain any biologically active compounds. Sometimes, traditional medicine can give modern medicine clues as to where to find an active compound. For example, humans have used willow bark to make medicine for thousands of years, dating back to ancient Egypt. However, it was not until the late 1800s that scientists and pharmaceutical companies purified and marketed the aspirin molecule, acetylsalicylic acid, for human use.

Occasionally, drugs developed for one use have unforeseen effects that allow usage in other, unrelated ways. For example, scientists originally developed the drug minoxidil (Rogaine) to treat high blood pressure. When tested on humans, researchers noticed that individuals taking the drug would grow new hair. Eventually the pharmaceutical company marketed the drug to people with baldness to restore lost hair.

Finally, a pharmaceutical chemist may discover negative effects or even lack of effects. In the early 1960s, inventors, doctors, and even a U.S. senator claimed anti-cancer properties of a new drug, Krebiozen, and began to market and sell it aggressively. Through the process of infrared spectrometry, FDA chemist Alma Levant Hayden and her team discovered that the "miracle drug" was nothing more than a common compound called creatine. A pharmaceutical chemist's career may involve detective work, experimentation, and drug development, all with the goal of making human beings healthier.

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11.

WATER

Learning Objectives

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

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

Why do scientists spend time looking for water on other planets? Why is water so important? It is because water is essential to life as we know it. Water is one of the more abundant molecules and the one most critical to life on Earth. Water comprises approximately 60–70 percent of the human body. Without it, life as we know it simply would not exist.

The polarity of the water molecule and its resulting hydrogen bonding make water a unique substance with special properties that are intimately tied to the processes of life. Life originally evolved in a watery environment, and most of an organism's cellular chemistry and metabolism occur inside the watery contents of the cell's cytoplasm. Special properties of water are its high heat capacity and heat of vaporization, its ability to dissolve polar molecules, its cohesive and adhesive properties, and its dissociation into ions that leads to generating pH. Understanding these characteristics of water helps to elucidate its importance in maintaining life.

Water's Polarity

One of water's important properties is that it is composed of polar molecules: the hydrogen and oxygen within

water molecules (H_2O) form polar covalent bonds. While there is no net charge to a water molecule, water's polarity creates a slightly positive charge on hydrogen and a slightly negative charge on oxygen, contributing to water's properties of attraction. Water generates charges because oxygen is more electronegative than hydrogen, making it more likely that a shared electron would be near the oxygen nucleus than the hydrogen nucleus, thus generating the partial negative charge near the oxygen.

As a result of water's polarity, each water molecule attracts other water molecules because of the opposite charges between water molecules, forming hydrogen bonds. Water also attracts or is attracted to other polar molecules and ions. We call a polar substance that interacts readily with or dissolves in water **hydrophilic** (hydro- = "water"; -philic = "loving"). In contrast, nonpolar molecules such as oils and fats do not interact well with water, as Figure 2.13 shows. A good example of this is vinegar and oil salad dressing (an acidic water solution). We call such nonpolar compounds **hydrophobic** (hydro- = "water"; -phobic = "fearing").

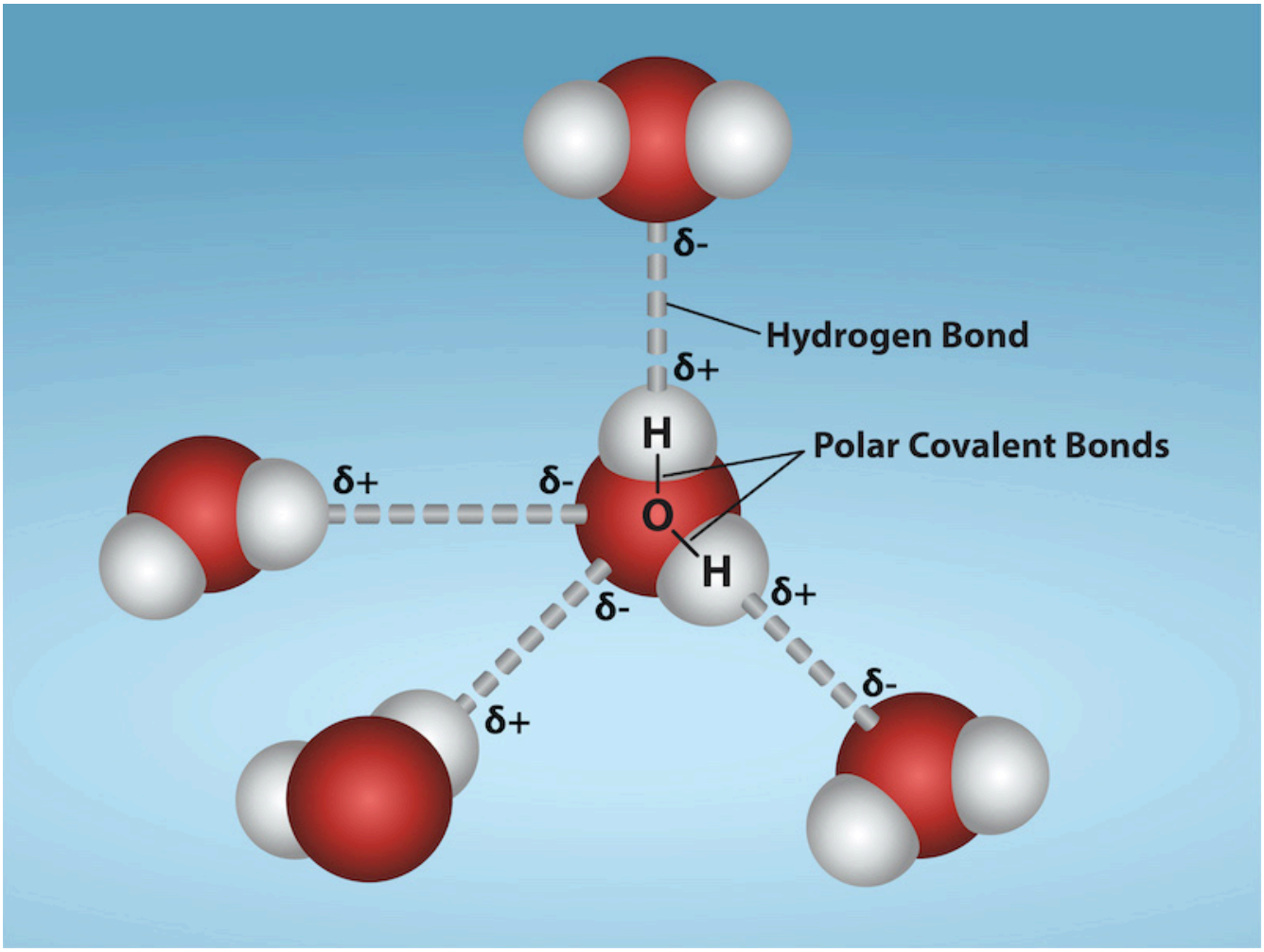

Figure 2.13 The polarity of water. The polarity of water is due to the differing electronegativities of hydrogen and oxygen. As a consequence, hydrogen bonds are formed when the slightly negative oxygen on one water molecule is attracted to the slightly positive hydrogen of another water molecule. Credit: Rao, A., Fletcher, S., Ryan, K., Tag, A. and Hawkins, A. Department of Biology, Texas A&M University

Water's States: Gas, Liquid, and Solid

The formation of hydrogen bonds is an important quality of liquid water that is crucial to life as we know it. As water molecules make hydrogen bonds with each other, water takes on some unique chemical characteristics compared to other liquids and, since living things have a high water content, understanding these chemical features is key to understanding life. In liquid water, hydrogen bonds constantly form and break as the water molecules slide past each other. The water molecules' motion (kinetic energy) causes the bonds to break due to the heat contained in the system. When the heat rises as water boils, the water molecules' higher kinetic energy causes the hydrogen bonds to break completely and allows water molecules to escape into the air as gas (steam or water vapor). Alternatively, when water temperature reduces and water freezes, the water molecules form a crystalline structure maintained by hydrogen bonding (there is not enough energy to break the hydrogen bonds) that makes ice less dense than liquid water, a phenomenon that we do not see when other liquids solidify.

Water's lower density in its solid form is due to the way hydrogen bonds orient as they freeze: the water molecules push farther apart compared to liquid water. With most other liquids, solidification when the temperature drops includes lowering kinetic energy between molecules, allowing them to pack even more tightly than in liquid form and giving the solid a greater density than the liquid.

The lower density of ice, as Figure 2.14 depicts, is an anomaly that causes it to float at the surface of liquid water, such as in an iceberg or ice cubes in a glass of water. In lakes and ponds, ice will form on the water's surface, creating an insulating barrier that protects the animals and plant life in the pond from freezing. Without this insulating ice layer, plants and animals living in the pond would freeze in the solid block of ice and could not survive. The expansion of ice relative to liquid water causes the detrimental effect of freezing on living organisms. The ice crystals that form upon freezing rupture the delicate membranes essential for living cells to function, irreversibly damaging them. Cells can only survive freezing if another liquid like glycerol temporarily replaces the water in them.

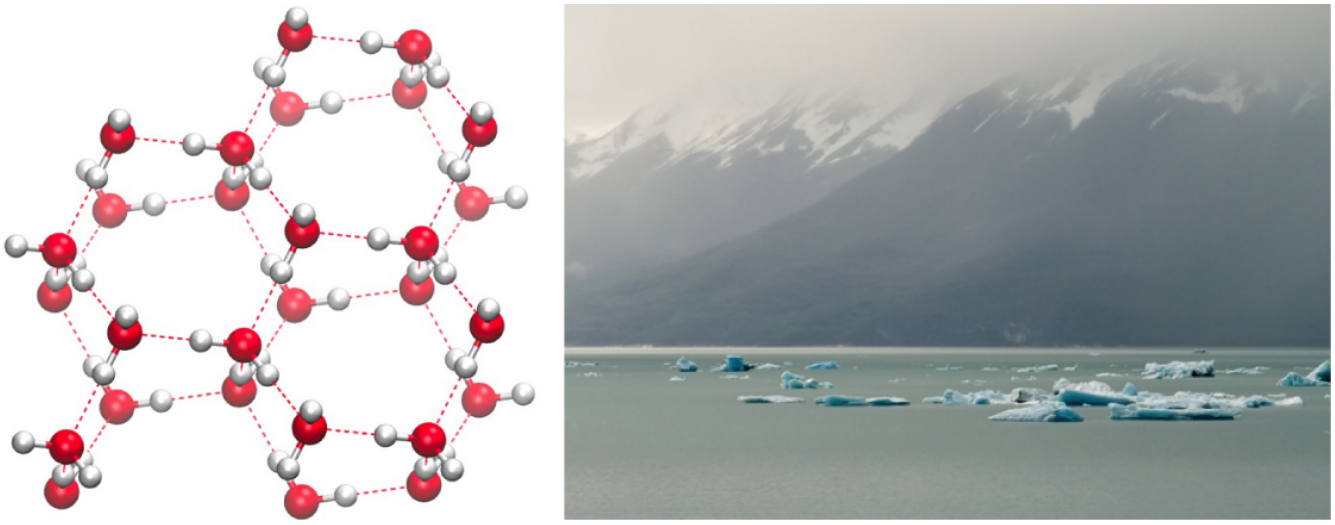

Figure 2.14 Hydrogen bonding makes ice less dense than liquid water. The (a) lattice structure of ice makes it less dense than the liquid water's freely flowing molecules, enabling it to (b) float on water. (credit a: modification of work by Jane Whitney, image created using Visual Molecular Dynamics (VMD) software1; credit b: modification of work by Carlos Ponte)

Link to Learning

Click here to see a 3-D animation of an ice lattice structure.

Water's High Heat Capacity

Water's high heat capacity is a property that hydrogen bonding among water molecules causes. Water has the highest **specific heat capacity** of any liquid. We define specific heat as the amount of heat one gram of a substance must absorb or lose to change its temperature by one degree Celsius. For water, this amount is one **calorie**. It therefore takes water a long time to heat and a long time to cool. In fact, water's specific heat capacity is about five times more than that of sand. This explains why the land cools faster than the sea. Due to its high heat capacity, warm-blooded animals use water to more evenly disperse heat in their bodies: it acts in a similar manner to a car's cooling system, transporting heat from warm places to cool places, causing the body to maintain a more even temperature.

Water's Heat of Vaporization

Water also has a high **heat of vaporization**, the amount of energy required to change one gram of a liquid substance to a gas. A considerable amount of heat energy (586 cal) is required to accomplish this change in water. This process occurs on the water's surface. As liquid water heats up, hydrogen bonding makes it difficult to separate the liquid water molecules from each other, which is required for it to enter its gaseous phase (steam). As a result, water acts as a heat sink or heat reservoir and requires much more heat to boil than does a liquid such as ethanol (grain alcohol), whose hydrogen bonding with other ethanol molecules is weaker than water's hydrogen bonding. Eventually, as water reaches its boiling point of 100° Celsius (212° Fahrenheit), the heat is able to break the hydrogen bonds between the water molecules, and the kinetic energy (motion) between the water molecules allows them to escape from the liquid as a gas. Even when below its boiling point, water's individual molecules acquire enough energy from other water molecules such that some surface water molecules can escape and vaporize: we call this process **evaporation**.

The fact that hydrogen bonds need to be broken for water to evaporate means that bonds use a substantial amount of energy in the process. As the water evaporates, energy is taken up by the process, cooling the environment where the evaporation is taking place. In many living organisms, including in humans, the evaporation of sweat, which is 90 percent water, allows the organism to cool so that it can maintain homeostasis of body temperature.

Water's Solvent Properties

Since water is a polar molecule with slightly positive and slightly negative charges, ions and polar molecules can readily dissolve in it. Therefore, we refer to water as a **solvent**, a substance capable of dissolving other polar molecules and ionic compounds. The charges associated with these molecules will form hydrogen bonds with water, surrounding the particle with water molecules. We refer to this as a **sphere of hydration**, or a hydration shell, as Figure 2.15 illustrates, which serves to keep the particles separated or dispersed in the water.

When we add ionic compounds to water, the individual ions react with the water molecules' polar regions, and their ionic bonds are disrupted in the process of **dissociation**. Dissociation occurs when atoms or groups of atoms break off from molecules and form ions. Consider table salt (NaCl, or sodium chloride): when we add NaCl crystals to water, the NaCl molecules dissociate into Na^+ and Cl^- ions, and spheres of hydration form around the ions, as Figure 2.15 illustrates. The partially negative charge of the water molecule's oxygen surrounds the positively charged sodium ion. The hydrogen's partially positive charge on the water molecule surrounds the negatively charged chloride ion.

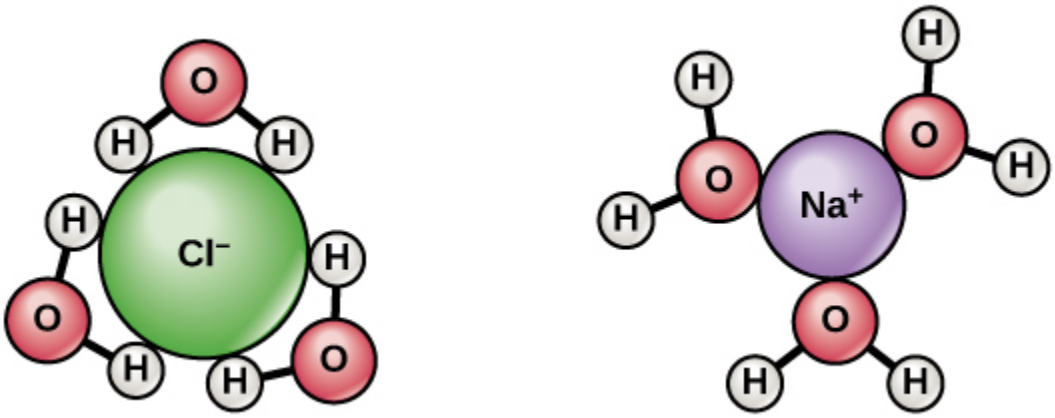

Figure 2.15 When we mix table salt (NaCl) in water, it forms spheres of hydration around the ions.

Water's Cohesive and Adhesive Properties

Have you ever filled a glass of water to the very top and then slowly added a few more drops? Before it overflows, the water 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-gas (water-air) interface, although there is no more room in the glass.

Cohesion allows for **surface tension**, the capacity of a substance to withstand rupturing when placed under tension or stress. This is also why water forms droplets when on a dry surface rather than flattening by gravity. When we place a small scrap of paper onto a water droplet, the paper floats on top even though paper is denser (heavier) than the water. Cohesion and surface tension keep the water molecules' hydrogen bonds intact and support the item floating on the top. It's even possible to "float" a needle on top of a glass of water if you place it gently without breaking the surface tension, as Figure 2.16 shows.

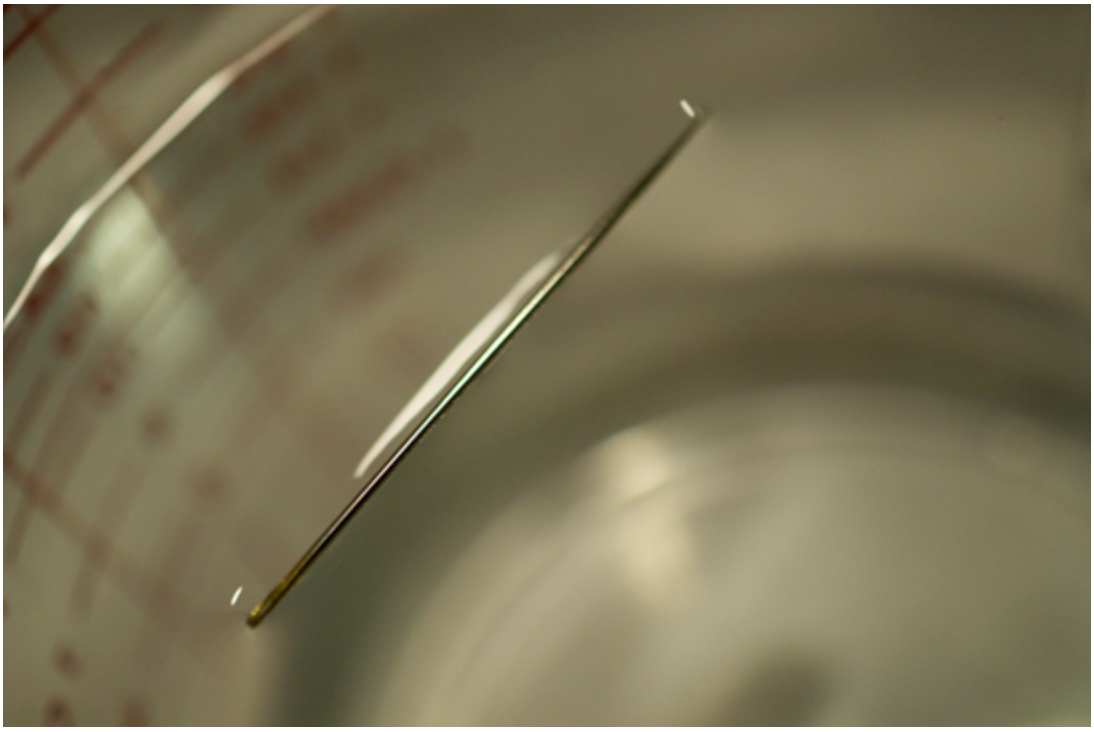

Figure 2.16 A needle's weight pulls the surface downward. At the same time, the surface tension pulls it up, suspending it on the water's surface and preventing it from sinking. Notice the indentation in the water around the needle. (credit: Cory Zanker)

These cohesive forces are related to water's property of **adhesion**, or the attraction between water molecules and other molecules. This attraction is sometimes stronger than water's cohesive forces, especially when the water is exposed to charged surfaces such as those on the inside of thin glass tubes known as capillary tubes. We observe adhesion when water "climbs" up the tube placed in a glass of water: notice that the water appears to be higher on the tube's sides than in the middle. This is because the water molecules are attracted to the capillary's charged glass walls more than they are to each other and therefore adhere to it. We call this type of adhesion **capillary action**, as Figure 2.17 illustrates.

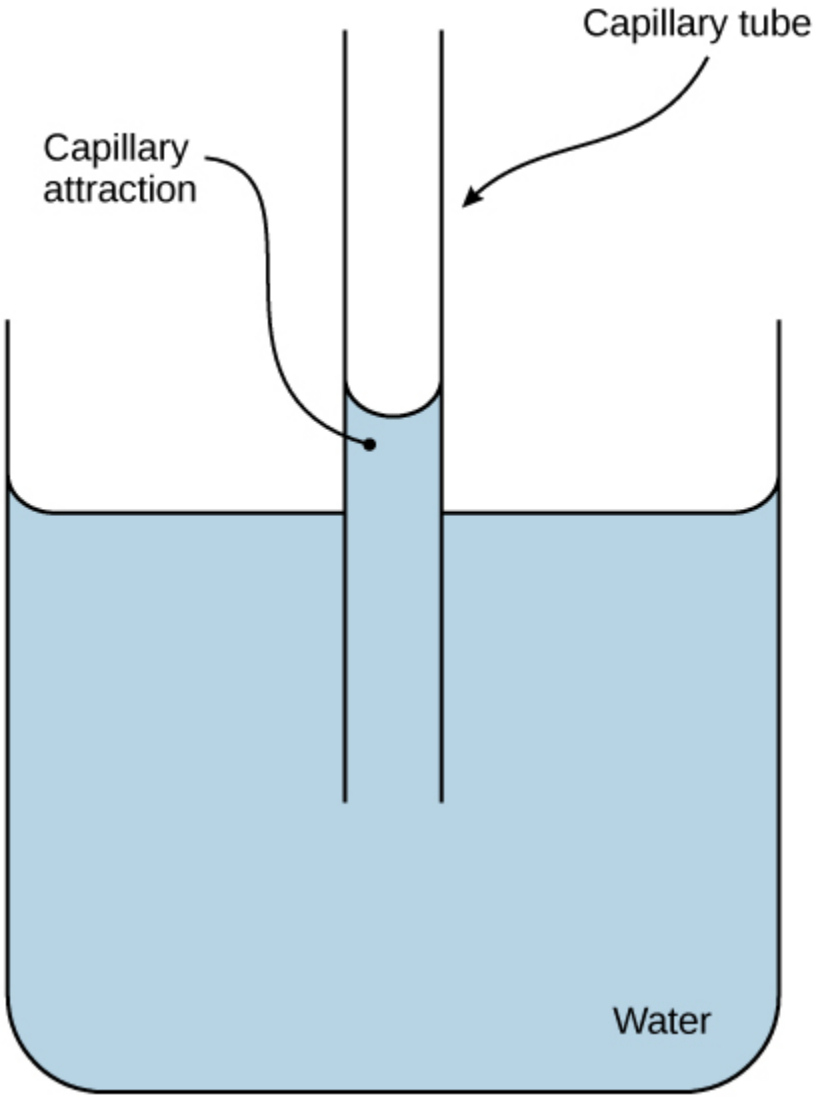

Figure 2.17 The adhesive forces exerted by the glass's internal surface exceeding the cohesive forces between the water molecules themselves causes capillary action in a glass tube. (credit: modification of work by Pearson-Scott Foresman, donated to the Wikimedia Foundation)

Why are cohesive and adhesive forces important for life? Cohesive and adhesive forces are important for transporting water from the roots to the leaves in plants. These forces create a "pull" on the water column. This pull results from the tendency of water molecules evaporating on the plant's surface to stay connected to water molecules below them, and so they are pulled along. Plants use this natural phenomenon to help transport water from their roots to their leaves. Without these properties of water, plants would be unable to receive the water and the dissolved minerals they require. In another example, insects such as the water strider, as Figure 2.18 shows, use the water's surface tension to stay afloat on the water's surface layer and even mate there.

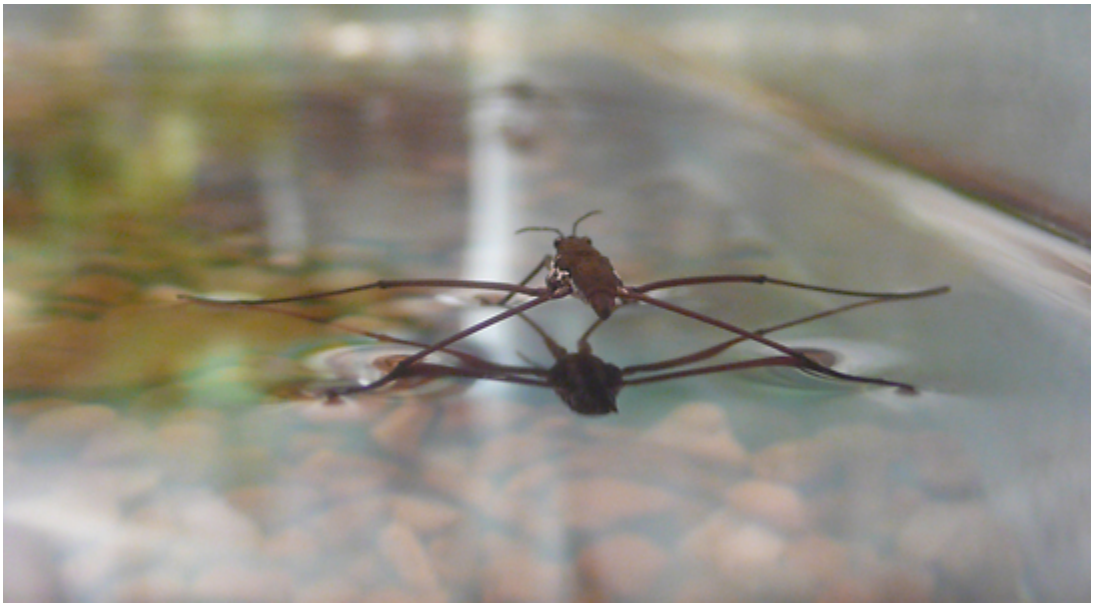

Figure 2.18 Water's cohesive and adhesive properties allow this water strider (Gerris sp.) to stay afloat. (credit: Tim Vickers)

pH, Buffers, Acids, and Bases

The pH of a solution indicates its acidity or basicity.

$H_2O(I) \leftrightarrow H^+(aq) + OH^-(aq)$

You may have used **litmus** or **pH paper**, filter paper treated with a natural water-soluble dye for use as a pH indicator, which tests how much acid (acidity) or base (basicity) exists in a solution. You might have even used some to test whether the water in a swimming pool is properly treated. In both cases, the pH test measures hydrogen ions' concentration in a given solution.

Hydrogen ions spontaneously generate in pure water by the dissociation (ionization) of a small percentage of water molecules into equal numbers of hydrogen (H^+) ions and hydroxide (OH^-) ions. While the hydroxide ions are kept in solution by their hydrogen bonding with other water molecules, the hydrogen ions, consisting of naked protons, immediately attract to un-ionized water molecules, forming hydronium ions (H_3O^+). Still, by convention, scientists refer to hydrogen ions and their concentration as if they were free in this state in liquid water.

The concentration of hydrogen ions dissociating from pure water is 1×10^{-7} moles H^+ ions per liter of water. Moles (mol) are a way to express the amount of a substance (which can be atoms, molecules, ions, etc.). Mathematically, one mole is equal to 6.02×10^{23} particles of the substance. Therefore, 1 mole of water is equal to 6.02×10^{23} water molecules. The pH inside of human cells and blood are examples of two body areas where near-neutral pH is maintained.

High concentrations of hydrogen ions yield a low pH number, whereas low levels of hydrogen ions result in

a high pH. An **acid** is a substance that increases hydrogen ions' (H^+) concentration in a solution, usually by having one of its hydrogen atoms dissociate. A **base** provides either hydroxide ions (OH^-) or other negatively charged ions that combine with hydrogen ions, reducing their concentration in the solution and thereby raising the pH. In cases where the base releases hydroxide ions, these ions bind to free hydrogen ions, generating new water molecules.

The stronger the acid, the more readily it donates H^+. For example, hydrochloric acid (HCl) completely dissociates into hydrogen and chloride ions and is highly acidic, whereas the acids in tomato juice or vinegar do not completely dissociate and are weak acids. Conversely, strong bases are those substances that readily donate OH^- or take up hydrogen ions. Sodium hydroxide (NaOH) and many household cleaners are highly alkaline and give up OH^- rapidly when we place them in water, thereby raising the pH. An example of a weak basic solution is seawater, which has a pH near 8.0 This is close enough to a neutral pH that marine organisms have adapted in order to live and thrive in a saline environment.

The **pH scale** ranges from 0 to 14 with each change of 1 representing a ten-fold change in the abundance of H+ions. (Figure 2.19). 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**. Extremes in pH in either direction from 7.0 are usually inhospitable to life. The pH inside cells (6.8) and the pH in the blood (7.4) are both very close to neutral. However, the environment in the stomach is highly acidic, with a pH of 1 to 2. As a result, how do stomach cells survive in such an acidic environment? How do they homeostatically maintain the near neutral pH inside them? The answer is that they cannot do it and are constantly dying. The stomach constantly produces new cells to replace dead ones, which stomach acids digest. Scientists estimate that the human body completely replaces the stomach lining every seven to ten days.

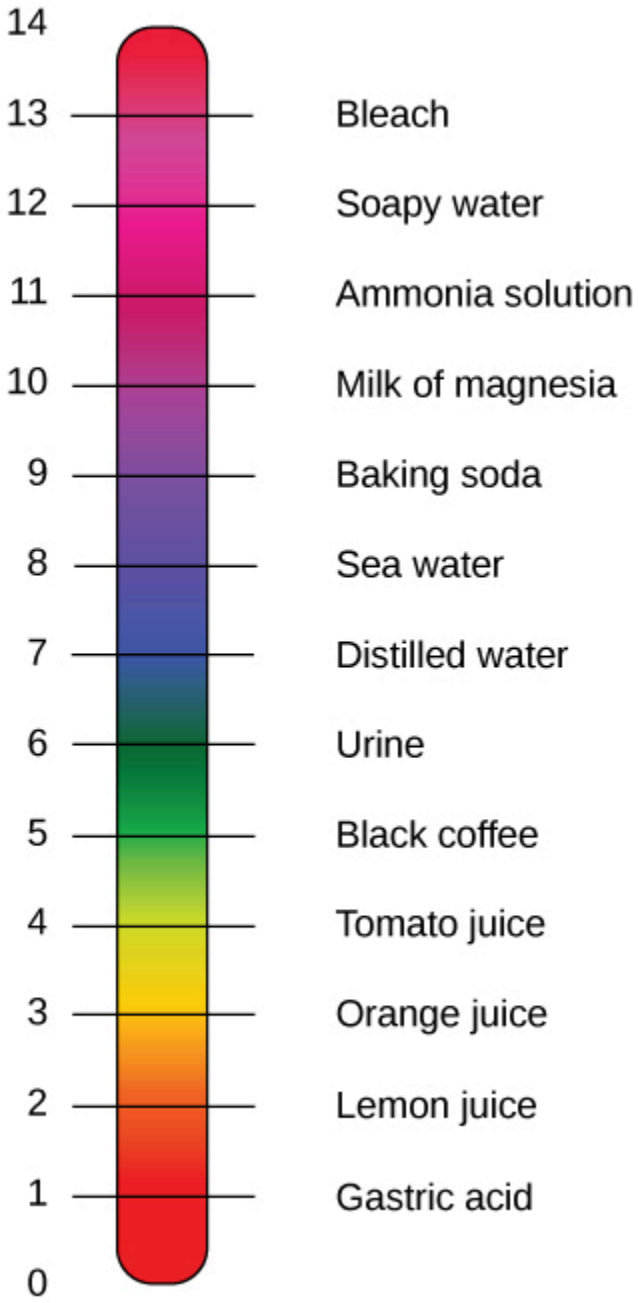

Figure 2.19 The pH scale measures hydrogen ions' (H+) concentration in a solution. (credit: modification of work by Edward Stevens)

Link to Learning

Watch this video for a straightforward explanation of pH and its logarithmic scale. Click to view content

How can organisms whose bodies require a near-neutral pH ingest acidic and basic substances (a human drinking orange juice, for example) and survive? Buffers are the key. **Buffers** readily absorb excess H^+ or OH^-, keeping the body's pH carefully maintained in the narrow range required for survival. Maintaining a constant blood pH is critical to a person's well-being. The buffer maintaining the pH of human blood involves carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-), and carbon dioxide (CO_2). When bicarbonate ions combine with free hydrogen ions and become carbonic acid, it removes hydrogen ions and moderates pH changes. Similarly, as Figure 2.20 shows, excess carbonic acid can convert to carbon dioxide gas which we exhale through the lungs. This prevents too many free hydrogen ions from building up in the blood and dangerously reducing the blood's pH. Likewise, if too much OH^- enters into the system, carbonic acid will combine with it to create bicarbonate, lowering the pH. Without this buffer system, the body's pH would fluctuate enough to put survival in jeopardy.

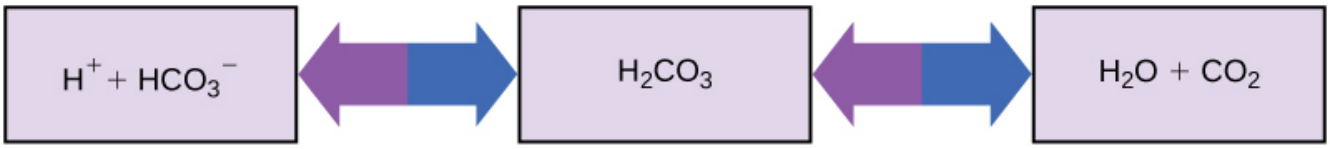

Figure 2.20 This diagram shows the body's buffering of blood pH levels. The blue arrows show the process of raising pH as more CO2 is made. The purple arrows indicate the reverse process: the lowering of pH as more bicarbonate is created.

Other examples of buffers are antacids that some people use to combat excess stomach acid. Many of these over-the-counter medications work in the same way as blood buffers, usually with at least one ion capable of absorbing hydrogen and moderating pH, bringing relief to those who suffer "heartburn" after eating. Water's unique properties that contribute to this capacity to balance pH—as well as water's other characteristics—are essential to sustaining life on Earth.

Link to Learning

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

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https://louis.pressbooks.pub/generalbiology1leclab/?p=141#h5p-13

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=141#h5p-14

Footnotes

- 1 W. Humphrey W., A. Dalke, and K. Schulten, "VMD—Visual Molecular Dynamics," *Journal of Molecular Graphics* 14 (1996): 33-38.

12.

CARBON

Learning Objectives

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

- Explain why carbon is important for life
- Describe the role of functional groups in biological molecules

Many complex molecules called **macromolecules**, such as proteins, nucleic acids (RNA and DNA), carbohydrates, and lipids comprise cells. The macromolecules are a subset of **organic molecules** (carbon-containing molecules) that are especially important for life. The fundamental component for all of these macromolecules is carbon. The carbon atom has unique properties that allow it to form covalent bonds to as many as four different atoms, making this versatile element ideal to serve as the basic structural component, or "backbone," of the macromolecules.

Individual carbon atoms have an incomplete outermost electron shell. With an atomic number of 6 (six electrons and six protons), the first two electrons fill the inner shell, leaving four in the second shell. Therefore, carbon atoms can form up to four covalent bonds with other atoms to satisfy the octet rule. The methane molecule provides an example: it has the chemical formula CH_4. Each of its four hydrogen atoms forms a single covalent bond with the carbon atom by sharing a pair of electrons. This results in a filled outermost shell.

Hydrocarbons

Hydrocarbons are organic molecules consisting entirely of carbon and hydrogen, such as methane (CH_4) described above. We often use hydrocarbons in our daily lives as fuels—like the propane in a gas grill or the butane in a lighter. The many covalent bonds between the atoms in hydrocarbons store a great amount

of energy, which releases when these molecules burn (oxidize). Methane, an excellent fuel, is the simplest hydrocarbon molecule, with a central carbon atom bonded to four different hydrogen atoms, as Figure 2.21 illustrates. The shape of its electron orbitals determines the shape of the methane molecule's geometry, where the atoms reside in three dimensions.

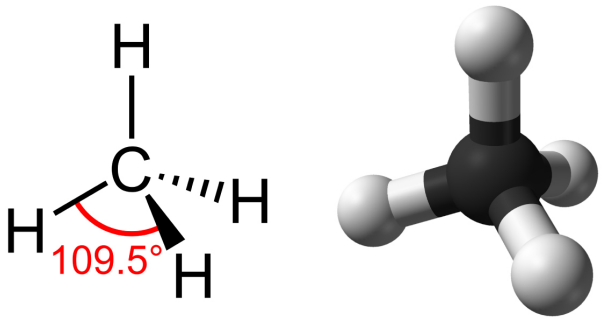


Figure 2.21 Methane has four hydrogen atoms spaced 109.5° apart.

As the backbone of the large molecules of living things, hydrocarbons may exist as linear carbon chains, carbon rings, or combinations of both. Furthermore, individual carbon-to-carbon bonds may be single, double, or triple covalent bonds, and each type of bond affects the molecule's geometry in a specific way. This three-dimensional shape or conformation of the large molecules of life (macromolecules) is critical to how they function.

Hydrocarbon Chains

Successive bonds between carbon atoms form hydrocarbon chains. These may be branched or unbranched. Furthermore, a molecule's different geometries of single, double, and triple covalent bonds alter the overall molecule's geometry, as Figure 2.22 illustrates. The hydrocarbons ethane, ethene, and ethyne serve as examples of how different carbon-to-carbon bonds affect the molecule's geometry. The names of all three molecules start with the prefix "eth-," which is the prefix for two carbon hydrocarbons. The suffixes "-ane," "-ene," and "-yne" refer to the presence of single, double, or triple carbon-carbon bonds, respectively. Thus, propane, propene, and propyne follow the same pattern with three carbon molecules, butane, butene, and butyne for four carbon molecules, and so on. Double and triple bonds change the molecule's geometry: single bonds allow rotation along the bond's axis, whereas double bonds lead to a planar configuration and triple bonds to a linear one. These geometries have a significant impact on the shape a particular molecule can assume.

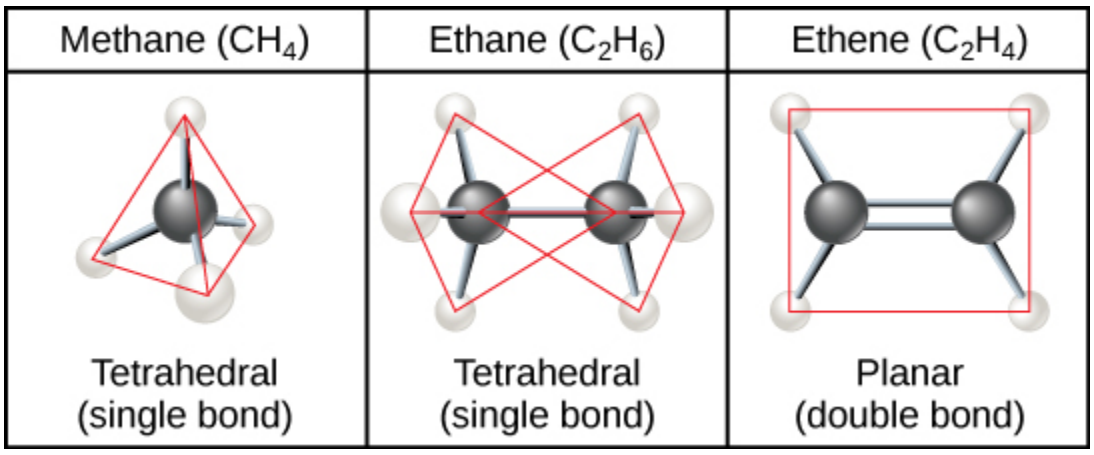


Figure 2.22 When carbon forms single bonds with other atoms, the shape is tetrahedral. When two carbon atoms form a double bond, the shape is planar, or flat. Single bonds, like those in ethane, are able to rotate. Double bonds, like those in ethene, cannot rotate, so the atoms on either side are locked in place.

Hydrocarbon Rings

So far, the hydrocarbons we have discussed have been **aliphatic hydrocarbons.** Another special type of hydrocarbon, the **aromatic hydrocarbon**, consists of closed rings of carbon atoms with alternating single and double bonds. We also find ring structures in aliphatic hydrocarbons, which we can see by comparing cyclohexane's structure to benzene in Figure 2.23. Examples of biological molecules that incorporate the benzene ring include some amino acids and cholesterol and its derivatives, including the hormones estrogen and testosterone. We also find the benzene ring in the herbicide 2,4-D. Benzene is a natural component of crude oil and has been classified as a carcinogen. Some hydrocarbons have both aliphatic and aromatic portions. Beta-carotene is an example of such a hydrocarbon.

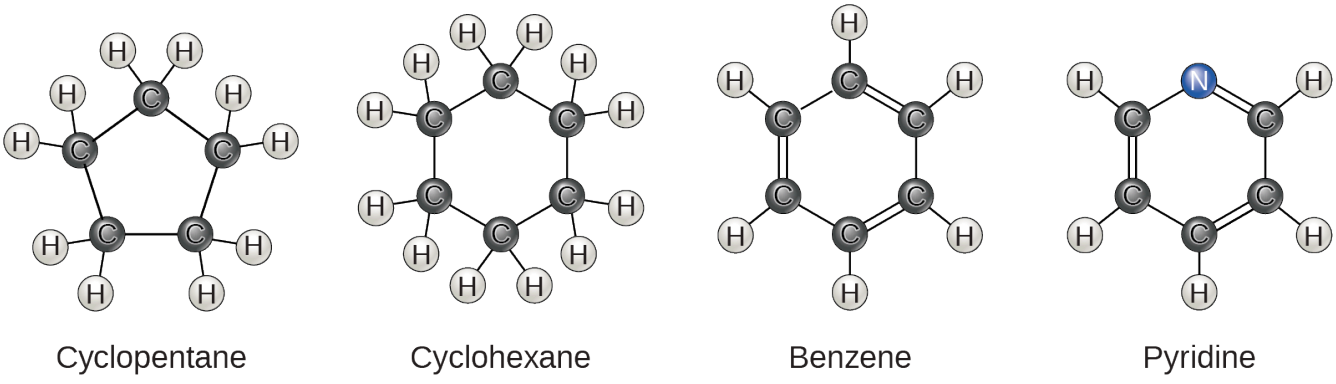


Figure 2.23 Carbon can form five- and six-membered rings. Single or double bonds may connect the carbons in the ring, and nitrogen may be substituted for carbon.

Isomers

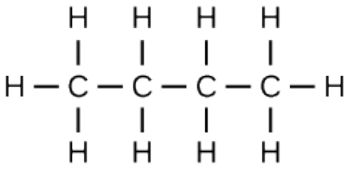
The three-dimensional placement of atoms and chemical bonds within organic molecules is central to understanding their chemistry. We call molecules that share the same chemical formula but differ in the placement (structure) of their atoms and/or chemical bonds **isomers**. **Structural isomers** (like butane and isobutane in Figure 2.24a) differ in the placement of their covalent bonds: both molecules have four carbons and ten hydrogens (C_4H_{10}), but the different atom arrangement within the molecules leads to differences in their chemical properties. For example, butane is suited for use as a fuel for cigarette lighters and torches, whereas isobutane is suited for use as a refrigerant and a propellant in spray cans.

Geometric isomers, alternatively, have similar placements of their covalent bonds but differ in how these bonds are made to the surrounding atoms, especially in carbon-to-carbon double bonds. In the simple molecule butene (C_4H_8), the two methyl groups (CH_3) can be on either side of the double covalent bond central to the molecule, as Figure 2.24b illustrates. When the carbons are bound on the same side of the double bond, this is the *cis* configuration. If they are on opposite sides of the double bond, it is a *trans* configuration. In the *trans* configuration, the carbons form a more or less linear structure, whereas the carbons in the *cis* configuration make a bend (change in direction) of the carbon backbone.

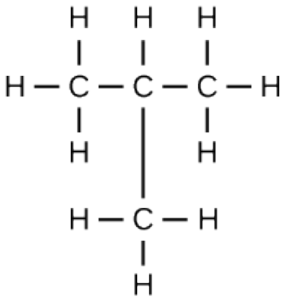
Visual Connection

(a) Structural isomers

Butane

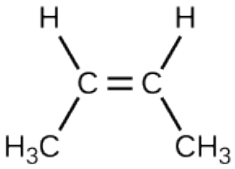


Isobutane



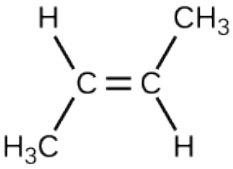
(b) Geometric isomers

cis-2-butene



methyl groups on
same side of double bond

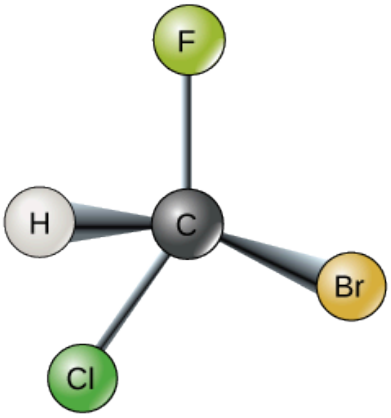
trans-2-butene



methyl groups on opposite
sides of double bond

(c) Enantiomers

L-isomer



D-isomer

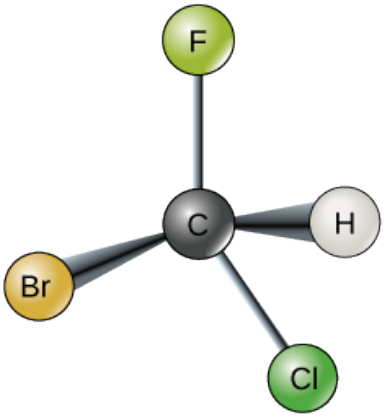


Figure 2.24 We call molecules that have the same number and type of atoms arranged differently isomers. (a) Structural isomers have a different covalent arrangement of atoms. (b) Geometric isomers have a different arrangement of atoms around a double bond. (c) Enantiomers are mirror images of each other.

Which of the following statements is false?

a. Molecules with the formulas CH_3CH_2COOH and $C_3H_6O_2$ could be structural isomers.
b. Molecules must have a double bond to be *cis–trans* isomers.
c. To be enantiomers, a molecule must have at least three different atoms or groups connected to a central carbon.
d. To be enantiomers, a molecule must have at least four different atoms or groups connected to a central carbon.

In triglycerides (fats and oils), long carbon chains known as fatty acids may contain double bonds, which can be in either the *cis* or *trans* configuration, as Figure 2.25 illustrates. Fats with at least one double bond between carbon atoms are unsaturated fats. When some of these bonds are in the *cis* configuration, the resulting bend in the chain's carbon backbone means that triglyceride molecules cannot pack tightly, so they remain liquid (oil) at room temperature. Alternatively, triglycerides with *trans* double bonds (popularly called trans fats) have relatively linear fatty acids that are able to pack tightly together at room temperature and form solid fats. In the human diet, trans fats are linked to an increased risk of cardiovascular disease, so many food manufacturers have reduced or eliminated their use in recent years. **Polyunsaturated** fats contain more than one double bond. In contrast to unsaturated fats, we call triglycerides without double bonds between carbon atoms saturated fats, meaning that they contain all the hydrogen atoms that a carbon chain of their length possibly could. Saturated fats are a solid at room temperature and usually of animal origin.

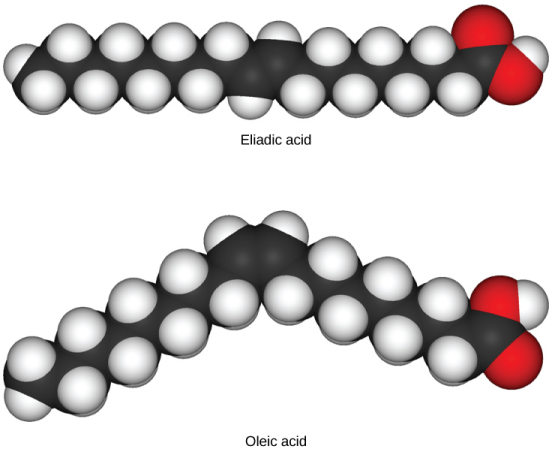


Figure 2.25 These space-filling models show a cis (oleic acid) and a trans (eliadic acid) fatty acid. Notice the bend in the molecule caused by the cis configuration.

Enantiomers

Enantiomers are molecules that share the same chemical structure and chemical bonds but differ in the three-dimensional placement of atoms so that they are non-superimposable mirror images. Figure 2.26 shows an amino acid alanine example, where the two structures are nonsuperimposable. In nature, the L-forms of amino acids are predominant in proteins. Some D forms of amino acids are seen in the cell walls of bacteria and polypeptides in other organisms. Similarly, the D-form of glucose is the main product of photosynthesis and we rarely see the molecule's L-form in nature.

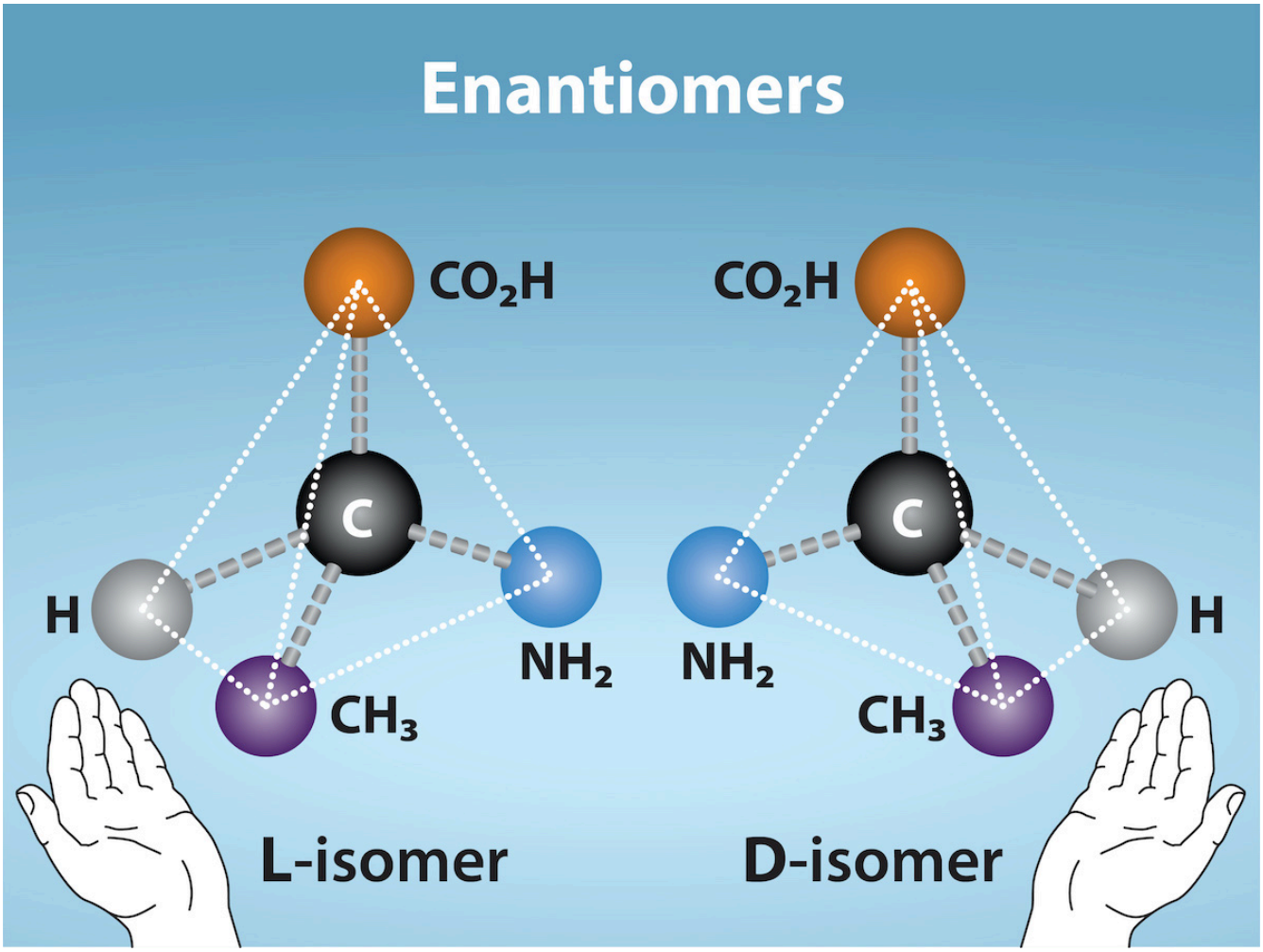


Figure 2.26 Enantiomers are molecules that are mirror images of each other and are non-superimposable. The L/D naming system is from the Latin words for left and right: laevus and dexter, respectively. This example shows the L and D isomers of the amino acid alanine. Credit: Rao, A., Hawkins, A., Fletcher, S. and Ryan K. Department of Biology, Texas A&M University.

Functional Groups

Functional groups are groups of atoms that occur within molecules and confer specific chemical properties to those molecules. We find them along the "carbon backbone" of macromolecules. Chains and/or rings of carbon atoms with the occasional substitution of an element such as nitrogen or oxygen form this carbon backbone. Molecules with such other elements in their carbon backbone are **substituted hydrocarbons**.

The functional groups in a macromolecule are usually attached to the carbon backbone at one or several different places along its chain and/or ring structure. Each of the four types of macromolecules—proteins, lipids, carbohydrates, and nucleic acids—has its own characteristic set of functional groups that contributes greatly to its differing chemical properties and its function in living organisms.

A functional group can participate in specific chemical reactions. Figure 2.27 shows some of the important

functional groups in biological molecules. They include: hydroxyl, methyl, carbonyl, carboxyl, amino, phosphate, and sulfhydryl. These groups play an important role in forming molecules like DNA, proteins, carbohydrates, and lipids. We usually classify functional groups as hydrophobic or hydrophilic depending on their charge or polarity characteristics. An example of a hydrophobic group is the nonpolar methyl molecule. Among the hydrophilic functional groups is the carboxyl group in amino acids, some amino acid side chains, and the fatty acids that form triglycerides and phospholipids. This carboxyl group ionizes to release hydrogen ions (H^+) from the COOH group, resulting in the negatively charged COO^- group. This contributes to the hydrophilic nature of whatever molecule bears it. Other functional groups, such as the carbonyl group, have a partially negatively charged oxygen atom that may form hydrogen bonds with water molecules, again making the molecule more hydrophilic.

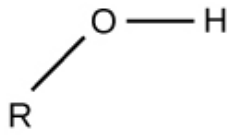
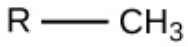
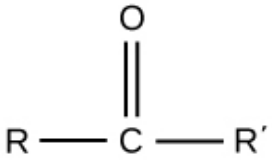
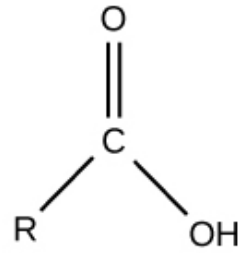
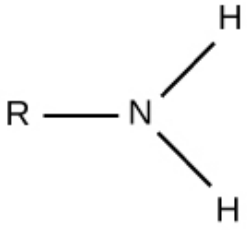
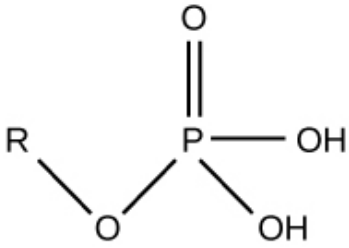
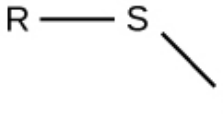
Functional Group	Structure	Properties
Hydroxyl		Polar
Methyl		Nonpolar
Carbonyl		Polar
Carboxyl		Charged, ionizes to release H^+. Since carboxyl groups can release H^+ ions into solution, they are considered acidic.
Amino		Charged, accepts H^+ to form NH_3^+. Since amino groups can remove H^+ from solution, they are considered basic.
Phosphate		Charged, ionizes to release H^+. Since phosphate groups can release H^+ ions into solution, they are considered acidic.
Sulfhydryl		Polar

Figure 2.27 These functional groups are in many different biological molecules. R, also known as R-group, is an abbreviation for the structure to which the functional group is attached.

Hydrogen bonds between functional groups (within the same molecule or between different molecules) are important to the function of many macromolecules and help them to fold properly into and maintain the appropriate shape for functioning. Hydrogen bonds are also involved in various recognition processes, such as DNA complementary base pairing and the binding of an enzyme to its substrate, as Figure 2.28 illustrates.

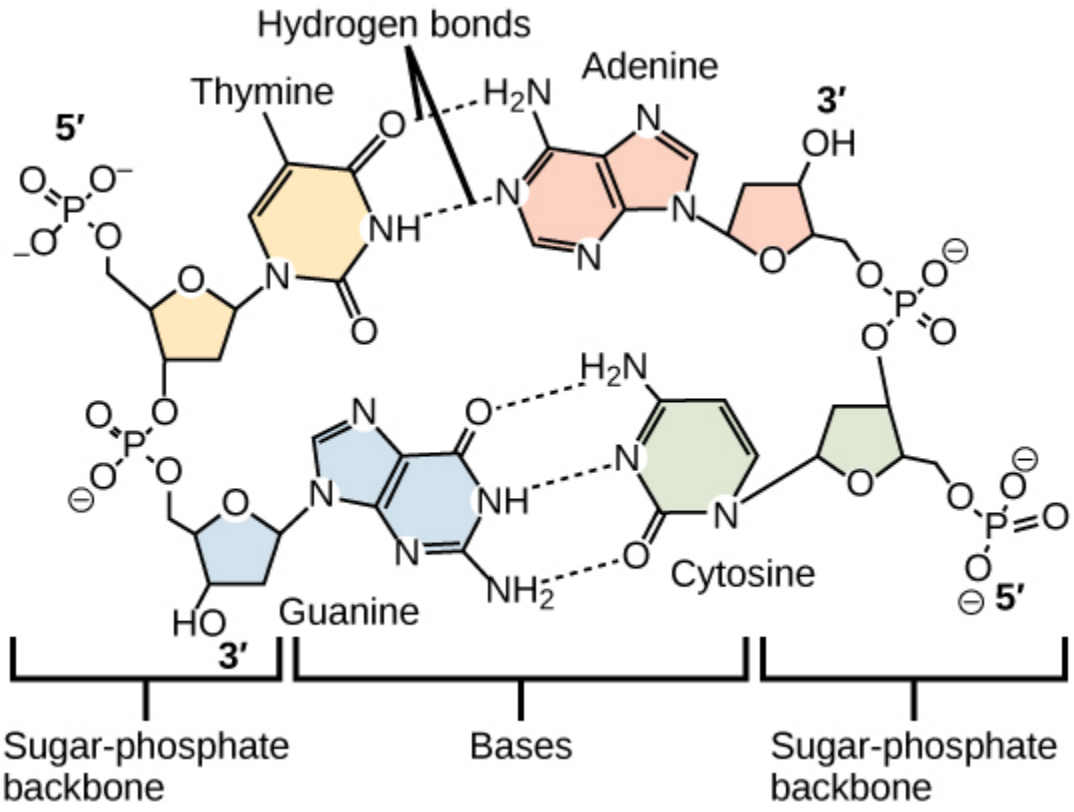


Figure 2.28 Hydrogen bonds zip two strands of DNA together. This figure focuses in on a small segment of the two DNA strands that combine to create the double-helix structure.

13.

KEY TERMS

acid
: molecule that donates hydrogen ions and increases the concentration of hydrogen ions in a solution

acidic
: possessing a pH < 7.0

adhesion
: attraction between water molecules and other molecules

alkaline
: possessing a pH > 7.0

aliphatic hydrocarbon
: hydrocarbon consisting of a linear chain, branched chain, or non-aromatic ring of carbon atoms

anion
: negative ion that is formed by an atom gaining one or more electrons

aromatic hydrocarbon
: hydrocarbon consisting of closed rings of carbon atoms with alternating single and double bonds

atom
: the smallest unit of matter that retains all of the chemical properties of an element

atomic mass
: calculated mean of the mass number for an element's isotopes

atomic number
: total number of protons in an atom

balanced chemical equation
: statement of a chemical reaction with the number of each type of atom equalized for both the products and reactants

base
: molecule that donates hydroxide ions or otherwise binds excess hydrogen ions and decreases the hydrogen ions' concentration in a solution

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

calorie
: amount of heat required to change the temperature of one gram of water by one degree Celsius

capillary action
occurs because water molecules are attracted to charges on the inner surfaces of narrow tubular structures such as glass tubes, drawing the water molecules to the tubes' sides

cation
positive ion that is formed by an atom losing one or more electrons

chemical bond
interaction between two or more of the same or different atoms that results in forming molecules

chemical reaction
process leading to rearranging atoms among molecules

chemical reactivity
the ability to combine and to chemically bond with each other

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

compound
substance composed of molecules consisting of atoms of at least two different elements

covalent bond
type of strong bond formed between two atoms of the same or different elements; forms when electrons are shared between atoms

dissociation
release of an ion from a molecule such that the original molecule now consists of an ion and the charged remains of the original, such as when water dissociates into H^+ and OH^-

electrolyte
ion necessary for nerve impulse conduction, muscle contractions, and water balance

electron
subatomic particle of inconsequential mass that resides outside of the nucleus in an electron orbital; has −1 charge

electron configuration
arrangement of electrons in an atom's electron shell (for example, $1s^2 2s^2 2p^6$)

electron orbital
how electrons are spatially distributed surrounding the nucleus; the area where we are most likely to find an electron

electron transfer
movement of electrons from one element to another; important in creating ionic bonds

electronegativity
ability of some elements to attract electrons (often of hydrogen atoms), acquiring partial negative charges in molecules and creating partial positive charges on the hydrogen atoms

element
: one of 118 unique substances that cannot break down into smaller substances; each element has unique properties and a specified number of protons

enantiomers
: molecules that share overall structure and bonding patterns, but differ in how the atoms are three dimensionally placed such that they are mirror images of each other

equilibrium
: steady state condition of balanced forward and backward progress in reversible chemical reactions

evaporation
: change of water from liquid to gaseous state, often removing heat from a wet surface

functional group
: group of atoms that provides or imparts a specific function to a molecule with a carbon skeleton

geometric isomer
: isomer with similar bonding patterns differing in the placement of atoms alongside a double covalent bond

heat of vaporization of water
: high amount of energy required for liquid water to turn into water vapor

hydrocarbon
: molecule that consists only of carbon and hydrogen

hydrogen bond
: weak bond between slightly positively charged hydrogen atoms and slightly negatively charged atoms in other molecules

hydrophilic
: describes ions or polar molecules that interact well with other polar molecules such as water

hydrophobic
: describes uncharged nonpolar molecules that do not interact well with polar molecules such as water

inert gas
: (also, noble gas) element with filled outer electron shell that is unreactive with other atoms

ion
: atom or chemical group that does not contain equal numbers of protons and electrons and so bears a net charge

ionic bond
: chemical bond that forms between ions with opposite charges (cations and anions)

irreversible chemical reaction
: chemical reaction where reactants proceed unidirectionally to form products

isomers
: molecules that differ from one another even though they share the same chemical formula

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

law of mass action
: chemical law stating that the rate of a reaction is proportional to the concentration of the reacting substances

litmus paper
: (also, pH paper) filter paper treated with a natural water-soluble dye that changes its color as the pH of the environment changes in order to use it as a pH indicator

macromolecule
: very large molecule, biologically important

mass number
: total number of protons and neutrons in an atom

matter
: anything that has mass and occupies space

molecule
: two or more atoms chemically bonded together

neutron
: uncharged particle that resides in an atom's nucleus; has a mass of one amu

noble gas
: see inert gas

nonpolar covalent bond
: type of covalent bond that forms between atoms when electrons are shared equally between them

nucleus
: core of an atom; contains protons and neutrons

octet rule
: rule that atoms are most stable when they hold eight electrons in their outermost shells

orbital
: electron orbital

organic molecule
: any molecule containing carbon (except carbon dioxide)

periodic table
: organizational chart of elements indicating each element's atomic number and atomic mass; provides key information about the elements' properties

pH paper
: see litmus paper

pH scale
: scale ranging from zero to 14 that is inversely proportional to the hydrogen ions' concentration in a

solution

polar covalent bond
: type of covalent bond that forms as a result of unequal electron sharing, resulting in creating slightly positive and negative charged molecule regions

polyunsaturated fat
: fats that have more than one carbon-carbon double bond

product
: molecule that is result of chemical reaction

proton
: positively charged particle that resides in the atom's nucleus; has a mass of one amu and a charge of +1

radioisotope
: isotope that emits radiation comprised of subatomic particles to form more stable elements

reactant
: molecule that takes part in a chemical reaction

reversible chemical reaction
: chemical reaction that functions bidirectionally, where products may turn into reactants if their concentration is great enough

solvent
: substance capable of dissolving another substance

specific heat capacity
: the amount of heat one gram of a substance must absorb or lose to change its temperature by one degree Celsius

sphere of hydration
: when a polar water molecule surrounds charged or polar molecules thus keeping them dissolved and in solution

structural isomers
: molecules that share a chemical formula but differ in the placement of their chemical bonds

substituted hydrocarbon
: hydrocarbon chain or ring containing an atom of another element in place of one of the backbone carbons

surface tension
: tension at the surface of a body of liquid that prevents the molecules from separating; created by the attractive cohesive forces between the liquid's molecules

valence shell
: outermost shell of an atom

van der Waals interaction
: very weak interaction between molecules due to temporary charges attracting atoms that are very close

together

14.

CHAPTER SUMMARY

2.1 Atoms, Isotopes, Ions, and Molecules: The Building Blocks

Matter is anything that occupies space and has mass. It is comprised of elements. All of the 98 elements that occur naturally have unique qualities that allow them to combine in various ways to create molecules, which in turn combine to form cells, tissues, organ systems, and organisms. 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 transfer, be shared between, or cause charge disparities between atoms to create bonds, including ionic, covalent, and hydrogen bonds, as well as van der Waals interactions.

2.2 Water

Water has many properties that are critical to maintaining life. It is a polar molecule, allowing for forming hydrogen bonds. Hydrogen bonds allow ions and other polar molecules to dissolve in water. Therefore, water is an excellent solvent. The hydrogen bonds between water molecules cause the water to have a high specific heat capacity, meaning it takes considerable added heat to raise its temperature. As the temperature rises, energy is diverted to liberating water molecules from hydrogen bonds. This allows for the overall temperature to remain stable, although energy is added to the system. Water also exhibits a high heat of vaporization, which is key to how organisms cool themselves by evaporating sweat. Water's cohesive forces allow for the property of surface tension, whereas we see its adhesive properties as water rises inside capillary tubes. The pH value is a measure of hydrogen ion concentration in a solution and is one of many chemical characteristics that is highly regulated in living organisms through homeostasis. Acids and bases can change pH values, but buffers tend to moderate the changes they cause. These properties of water are intimately connected to the biochemical and physical processes performed by living organisms, and life would be very different if these properties were altered, if it could exist at all.

2.3 Carbon

The unique properties of carbon make it a central part of biological molecules. Carbon binds to oxygen,

hydrogen, and nitrogen covalently to form the many molecules important for cellular function. Carbon has four electrons in its outermost shell and can form four bonds. Carbon and hydrogen can form hydrocarbon chains or rings. Functional groups are groups of atoms that confer specific properties to hydrocarbon (or substituted hydrocarbon) chains or rings that define their overall chemical characteristics and function.

15.

VISUAL CONNECTION QUESTIONS

1 . Figure 2.3 How many neutrons do carbon-12 and carbon-13 have, respectively?

2 . Figure 2.7 An atom may give, take, or share electrons with another atom to achieve a full valence shell, the most stable electron configuration. Looking at this figure, how many electrons do elements in group 1 need to lose in order to achieve a stable electron configuration? How many electrons do elements in groups 14 and 17 need to gain to achieve a stable configuration?

3 . Figure 2.24 Which of the following statements is false?

a. Molecules with the formulas CH_3CH_2COOH and $C_3H_6O_2$ could be structural isomers.
b. Molecules must have a double bond to be *cis–trans* isomers.
c. To be enantiomers, a molecule must have at least three different atoms or groups connected to a central carbon.
d. To be enantiomers, a molecule must have at least four different atoms or groups connected to a central carbon.

16.

REVIEW QUESTIONS

4. If xenon has an atomic number of 54 and a mass number of 108, how many neutrons does it have?

a. 54
b. 27
c. 100
d. 108

5. Atoms that vary in the number of neutrons found in their nuclei are called _____.

a. ions
b. neutrons
c. neutral atoms
d. isotopes

6. Potassium has an atomic number of 19. What is its electron configuration?

a. shells 1 and 2 are full, and shell 3 has nine electrons
b. shells 1, 2, and 3 are full and shell 4 has three electrons
c. shells 1, 2, and 3 are full and shell 4 has one electron
d. shells 1, 2, and 3 are full and no other electrons are present

7. Which type of bond represents a weak chemical bond?

a. hydrogen bond
b. atomic bond
c. covalent bond
d. nonpolar covalent bond

8. 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 molecule in the Earth's atmosphere.

9. When acids are added to a solution, the pH should _____.

a. decrease
b. increase
c. stay the same
d. cannot tell without testing

10. We call a molecule that binds up excess hydrogen ions in a solution a(n) _____.

a. acid
b. isotope
c. base
d. donator

11. Which of the following statements is true?

a. Acids and bases cannot mix together.
b. Acids and bases will neutralize each other.
c. Acids, but not bases, can change the pH of a solution.
d. Acids donate hydroxide ions (OH−); bases donate hydrogen ions (H+).

12. Each carbon molecule can bond with as many as_____ other atom(s) or molecule(s).

a. one
b. two
c. six
d. four

13. Which of the following is not a functional group that can bond with carbon?

a. sodium
b. hydroxyl
c. phosphate
d. carbonyl

17.

CRITICAL THINKING QUESTIONS

14. What makes ionic bonds different from covalent bonds?

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

16. While we have discussed acidity within the internal fluids of organisms, a significant impact of acidity on the planet comes from artificial acidification of fish habitats from industrial emissions of sulfur and nitrogen compounds, causing fish to die off. Speculate how these emissions, which are discharged into the air, get into ponds and lakes where the fish live.

17. Why can some insects walk on water?

18. What property of carbon makes it essential for organic life?

19. American diets commonly include both saturated and unsaturated triglycerides. Make an educated guess as to what food items in your own diet are the greatest sources of each of these.

PART III

BIOLOGICAL MACROMOLECULES

18.

INTRODUCTION

Figure 3.1 Foods such as bread, fruit, and cheese are rich sources of biological macromolecules. (credit: modification of work by Bengt Nyman)

Food provides the body with the nutrients it needs to survive. Many of these critical nutrients are biological macromolecules, or large molecules, necessary for life. Different smaller organic molecule (monomer) combinations build these macromolecules (polymers). What specific biological macromolecules do living things require? How do these molecules form? What functions do they serve? We explore these questions in this chapter.

19.

SYNTHESIS OF BIOLOGICAL MACROMOLECULES

Learning Objectives

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

- Understand macromolecule synthesis
- Explain dehydration (or condensation) and hydrolysis reactions

As you've learned, **biological macromolecules** are large molecules, necessary for life, that are built from smaller organic molecules. There are four major biological macromolecule classes (carbohydrates, lipids, proteins, and nucleic acids). Each is an important cell component and performs a wide array of functions. Combined, these molecules make up the majority of a cell's dry mass (recall that water makes up the majority of its complete mass). Biological macromolecules are organic, meaning they contain carbon. In addition, they may contain hydrogen, oxygen, nitrogen, and other minor elements.

Dehydration Synthesis

Most macromolecules are made from single subunits, or building blocks, called **monomers**. The monomers combine with each other using covalent bonds to form larger molecules known as **polymers**. In doing so, monomers release water molecules as byproducts. This type of reaction is **dehydration synthesis**, which means "to put together while losing water."

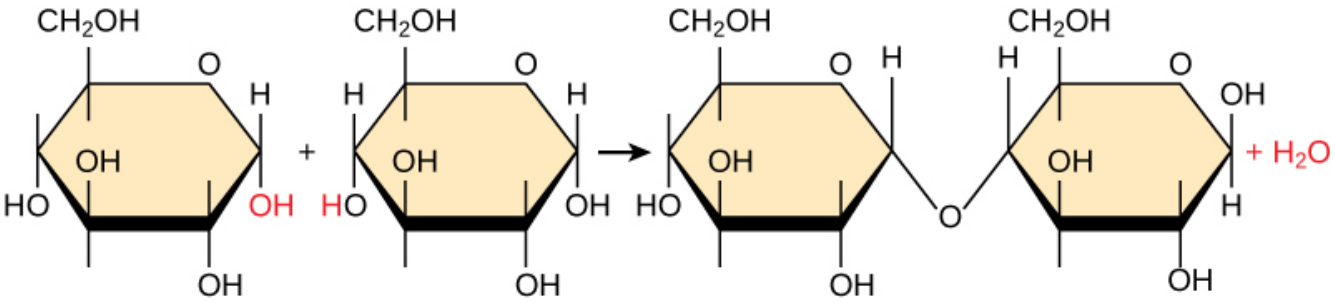


Figure 3.2 In the dehydration synthesis reaction above, two glucose molecules link to form the disaccharide maltose. In the process, it forms a water molecule.

In a dehydration synthesis reaction (Figure 3.2), the hydrogen of one monomer combines with the hydroxyl group of another monomer, releasing a water molecule. At the same time, the monomers share electrons and form covalent bonds. As additional monomers join, this chain of repeating monomers forms a polymer. Different monomer types can combine in many configurations, giving rise to a diverse group of macromolecules. Even one kind of monomer can combine in a variety of ways to form several different polymers. For example, glucose monomers are the constituents of starch, glycogen, and cellulose.

Hydrolysis

Polymers break down into monomers by a process called **hydrolysis**. A chemical reaction occurs that involves splitting a water molecule (Figure 3.3). During these reactions, the polymer breaks into two components: one part gains a hydrogen atom (H+) and the other gains a hydroxyl molecule (OH–) from a split water molecule.

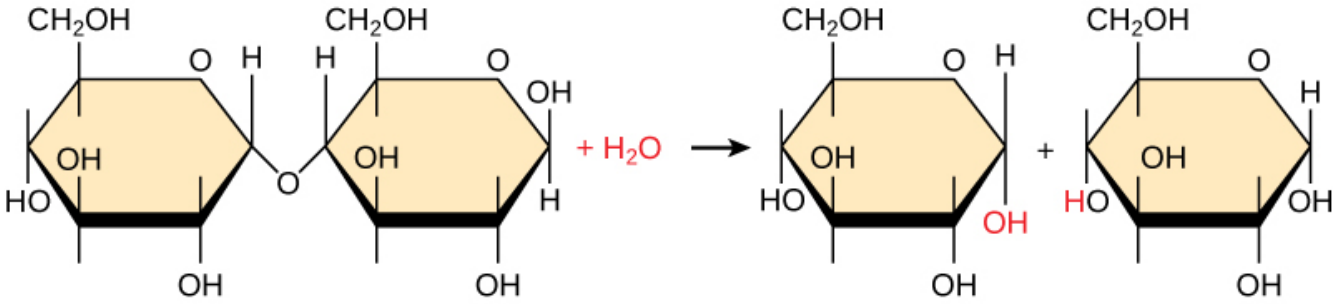


Figure 3.3 In the hydrolysis reaction here, the disaccharide maltose breaks down to form two glucose monomers by adding a water molecule. Note that this reaction is the reverse of the synthesis reaction in Figure 3.2.

Dehydration and **hydrolysis reactions** are catalyzed, or "sped up," by specific enzymes; dehydration reactions involve the formation of new bonds, requiring energy, while hydrolysis reactions break bonds and release energy. These reactions are similar for most macromolecules, but each monomer and polymer reaction is

specific for its class. For example, catalytic enzymes in the digestive system hydrolyze or break down the food we ingest into smaller molecules. This allows cells in our body to easily absorb nutrients in the intestine. A specific enzyme breaks down each macromolecule. For instance, amylase, sucrase, lactase, or maltase break down carbohydrates. Enzymes called proteases, such as pepsin and peptidase, and hydrochloric acid break down proteins. Lipases break down lipids. These broken-down macromolecules provide energy for cellular activities.

Link to Learning

Visit this site to see visual representations of dehydration synthesis and hydrolysis.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=165#h5p-15

20.

CARBOHYDRATES

Learning Objectives

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

- Discuss the role of carbohydrates in cells and in the extracellular materials of animals and plants
- Explain carbohydrate classifications
- List common monosaccharides, disaccharides, and polysaccharides

Most people are familiar with carbohydrates, one type of macromolecule, especially when it comes to what we eat. To lose weight, some individuals adhere to "low-carb" diets. Athletes, in contrast, often "carb-load" before important competitions to ensure that they have enough energy to compete at a high level. Carbohydrates are, in fact, an essential part of our diet. Grains, fruits, and vegetables are all natural carbohydrate sources that provide energy to the body, particularly through glucose, a simple sugar that is a component of **starch** and an ingredient in many staple foods. Carbohydrates also have other important functions in humans, animals, and plants.

Molecular Structures

The ratio of carbon to hydrogen to oxygen is 1:2:1 in **carbohydrate** molecules. This formula also explains the origin of the term "carbohydrate": the components are carbon ("carbo") and the components of water (hence, "hydrate"). Scientists classify carbohydrates into three subtypes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides

Monosaccharides (mono- = "one"; sacchar- = "sweet") are simple sugars, the most common of which is glucose. In monosaccharides, the number of carbons usually ranges from three to seven. Most monosaccharide names end with the suffix -ose. If the sugar has an aldehyde group (the functional group with a C=O double bond on an end carbon), it is an aldose, and if it has a ketone group (C=O on an internal carbon), it is a ketose. Depending on the number of carbons in the sugar, they can be trioses (three carbons), pentoses (five carbons), and/or hexoses (six carbons). Figure 3.4 illustrates monosaccharides.

MONOSACCHARIDES

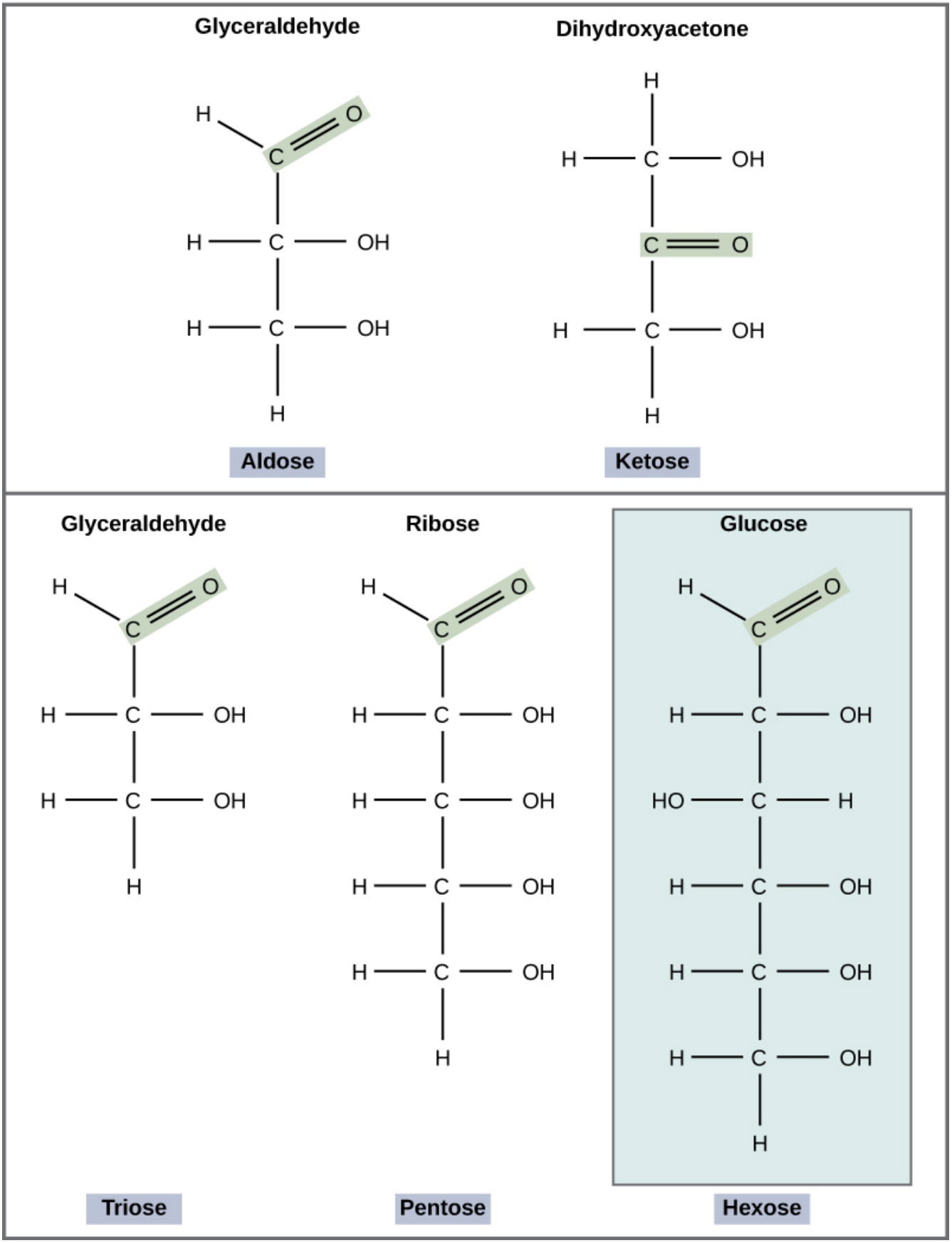


Figure 3.4 Scientists classify monosaccharides based on the position of their carbonyl group and the number of carbons in the backbone. Aldoses have a carbonyl group (indicated in green) at the end of the carbon chain, and ketoses have a carbonyl group in the middle of the carbon chain. Trioses, pentoses, and hexoses have three-, five-, and six-carbon backbones, respectively.

The chemical formula for glucose is $C_6H_{12}O_6$. In humans, glucose is an important source of energy. During cellular respiration, energy released from glucose helps make adenosine triphosphate (ATP). Plants synthesize glucose using carbon dioxide and water, and glucose in turn provides energy requirements for the plant.

Galactose (part of lactose, or milk sugar) and fructose (found in sucrose, 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 therefore isomers) because of the different arrangement of functional groups (Figure 3.5).

Visual Connection

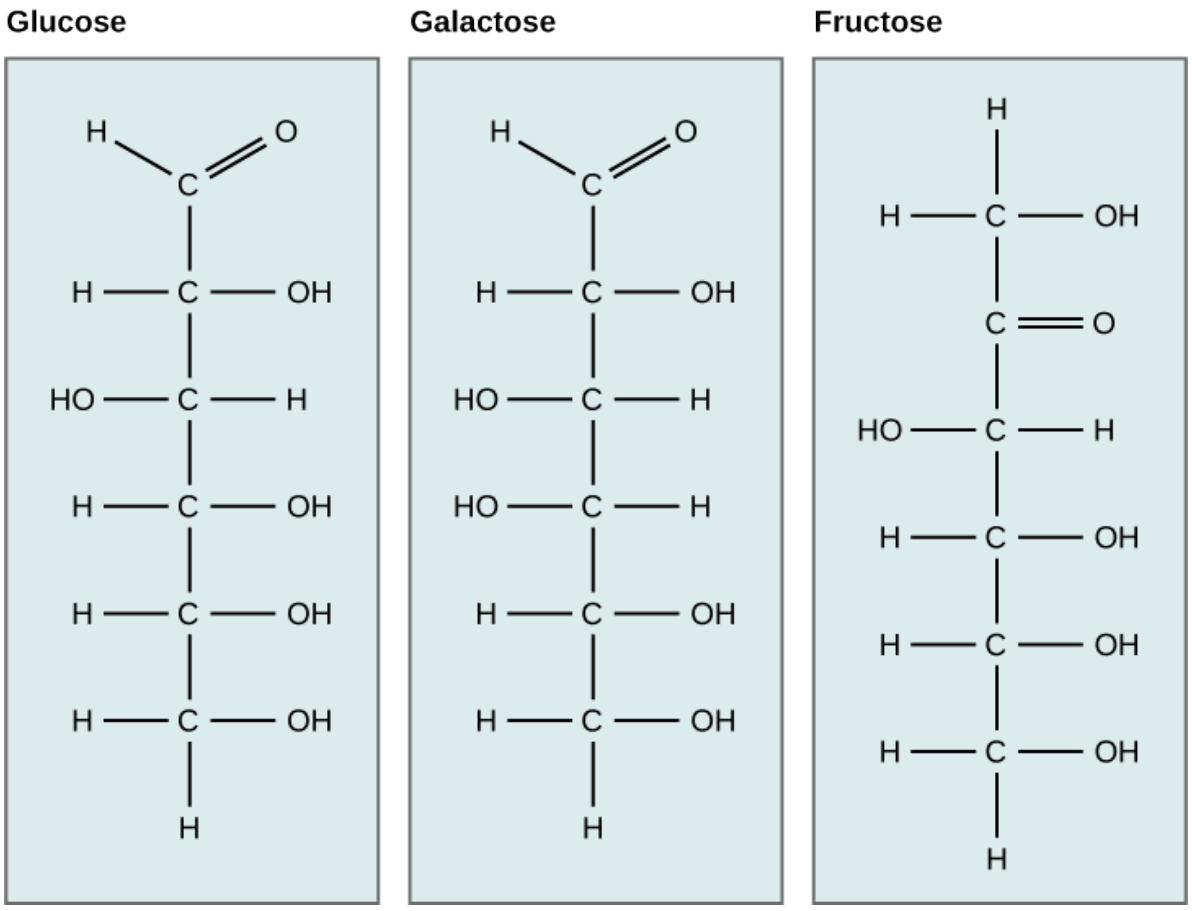


Figure 3.5 Glucose, galactose, and fructose are all hexoses. They are structural isomers, meaning they have the same chemical formula ($C_6H_{12}O_6$) but a different atom arrangement.

What kind of sugars are these, aldose or ketose?

Glucose, galactose, and fructose are isomeric monosaccharides (hexoses), meaning they have the same chemical formula but have slightly different structures. Glucose and galactose are aldoses, and fructose is a ketose.

Monosaccharides can exist as a linear chain or as ring-shaped molecules. In aqueous solutions, they are usually in ring forms (Figure 3.6). Glucose in a ring form can have two different hydroxyl group arrangements (OH) around the anomeric carbon (carbon 1 that becomes asymmetric in the ring formation process). If the hydroxyl group is below carbon number 1 in the sugar, it is in the alpha (α) position, and if it is above the plane, it is in the beta (β) position.

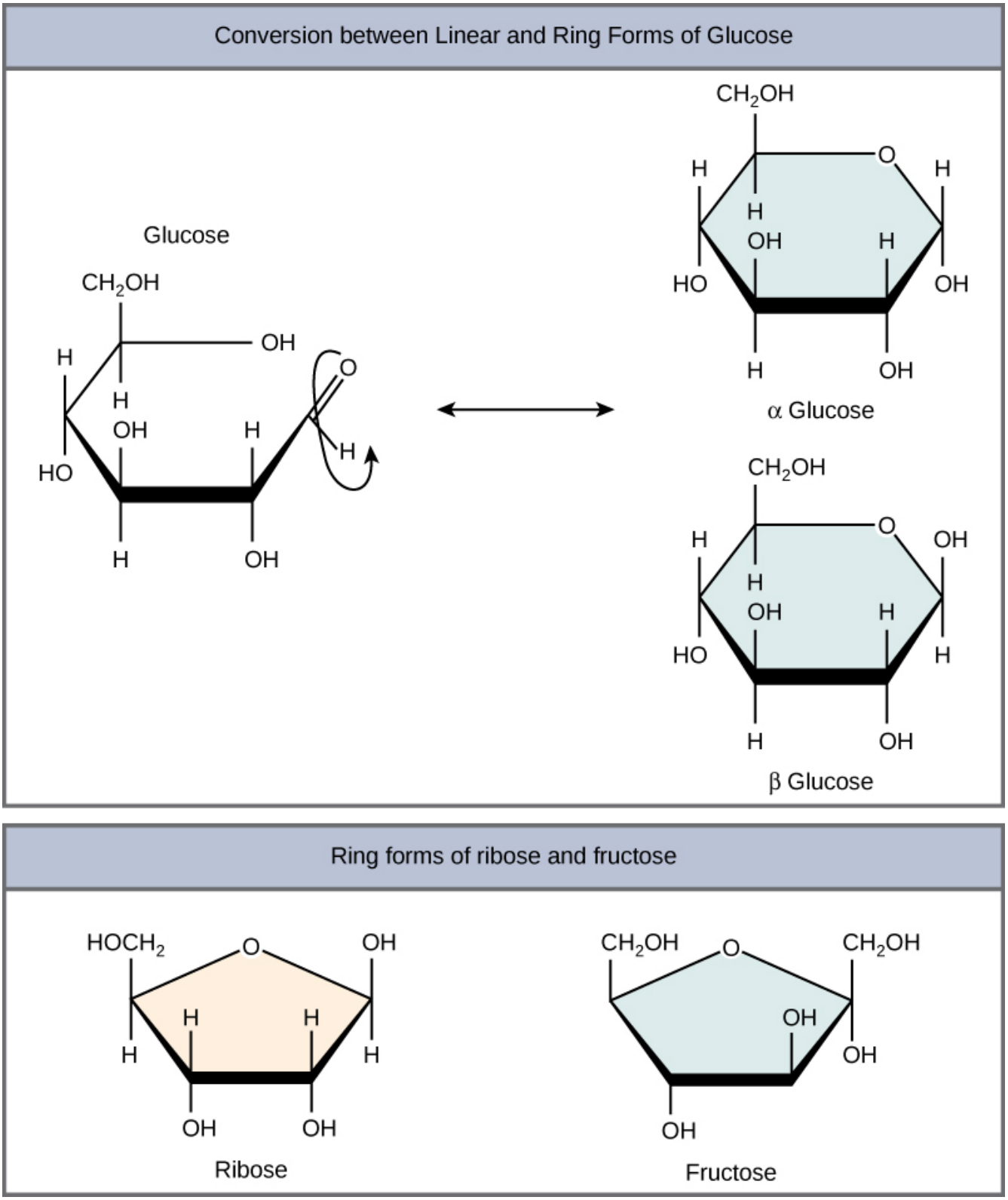


Figure 3.6 Five and six carbon monosaccharides exist in equilibrium between linear and ring forms. When the ring forms, the side chain it closes on locks into an α or β position. Fructose and ribose also form rings, although they form five-membered rings as opposed to the six-membered ring of glucose.

Disaccharides

Disaccharides (di- = "two") form when two monosaccharides undergo dehydration synthesis. During this process, one monosaccharide's hydroxyl group combines with another monosaccharide's hydrogen, releasing a water molecule and forming a covalent bond. A covalent bond forms between a carbohydrate molecule and another molecule (in this case, between two monosaccharides). Scientists call this a **glycosidic bond** (Figure 3.7). Glycosidic bonds (or glycosidic linkages) can be an alpha or beta type. An alpha bond is formed when the OH group on the carbon-1 of the first glucose is below the ring plane, and a beta bond is formed when the OH group on the carbon-1 is above the ring plane.

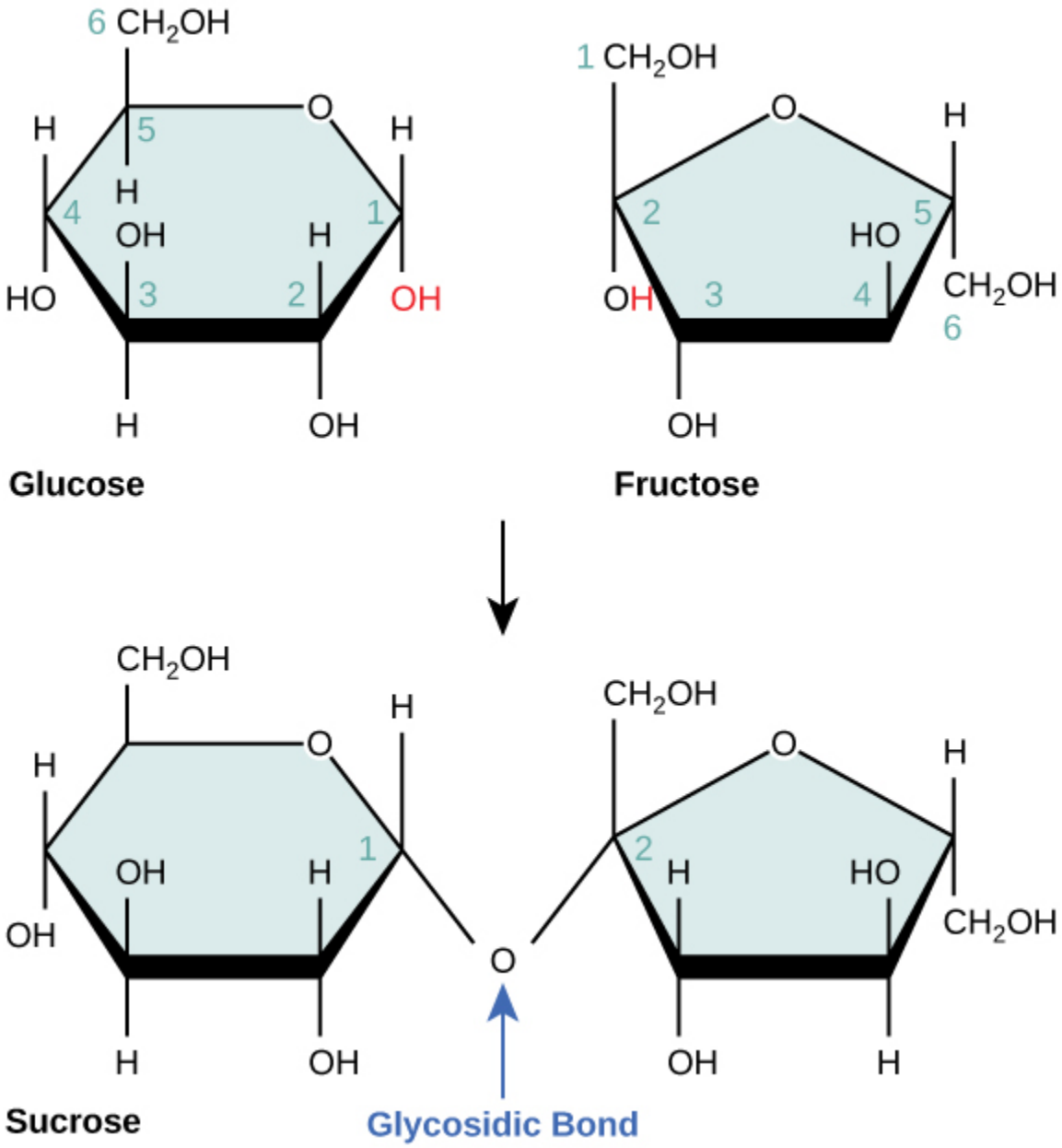
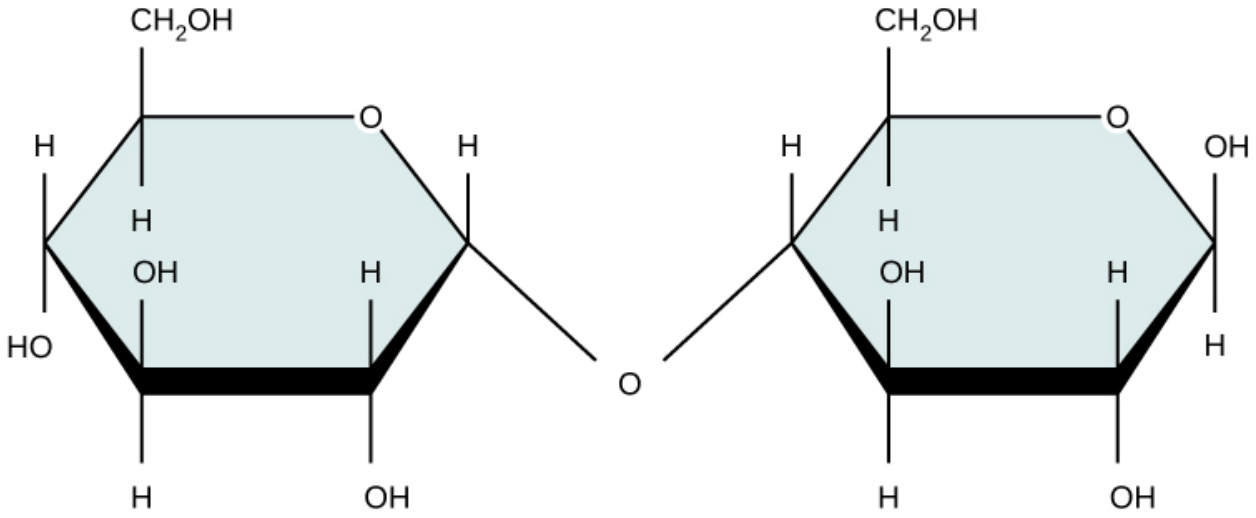
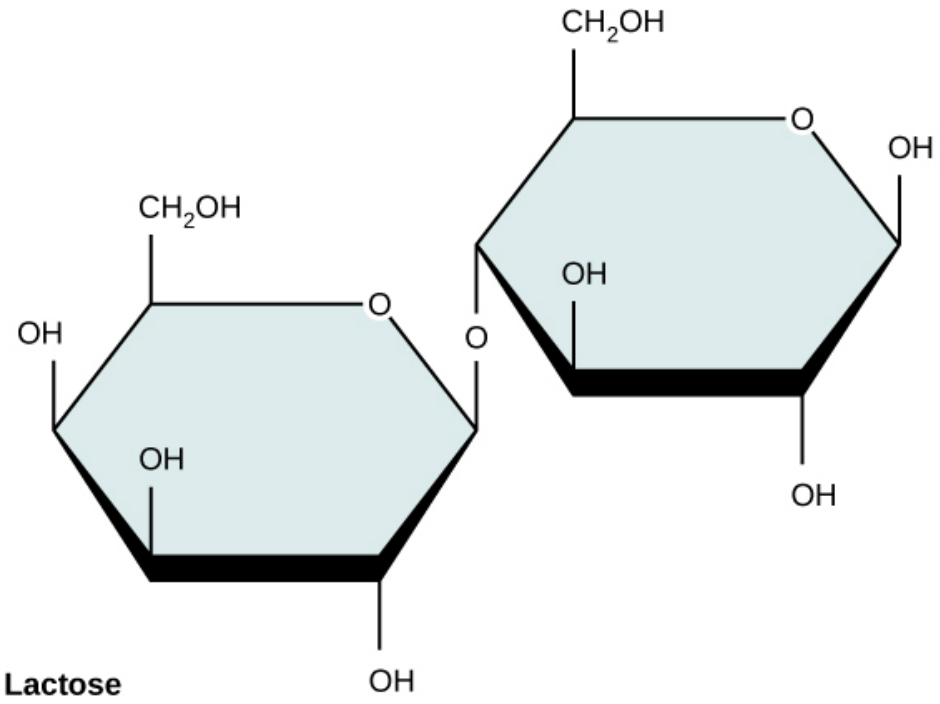


Figure 3.7 Sucrose forms when a glucose monomer and a fructose monomer join in a dehydration reaction to form a glycosidic bond. In the process, a water molecule is lost. By convention, the carbon atoms in a monosaccharide are numbered from the terminal carbon closest to the carbonyl group. In sucrose, a glycosidic linkage forms between carbon 1 in glucose and carbon 2 in fructose.

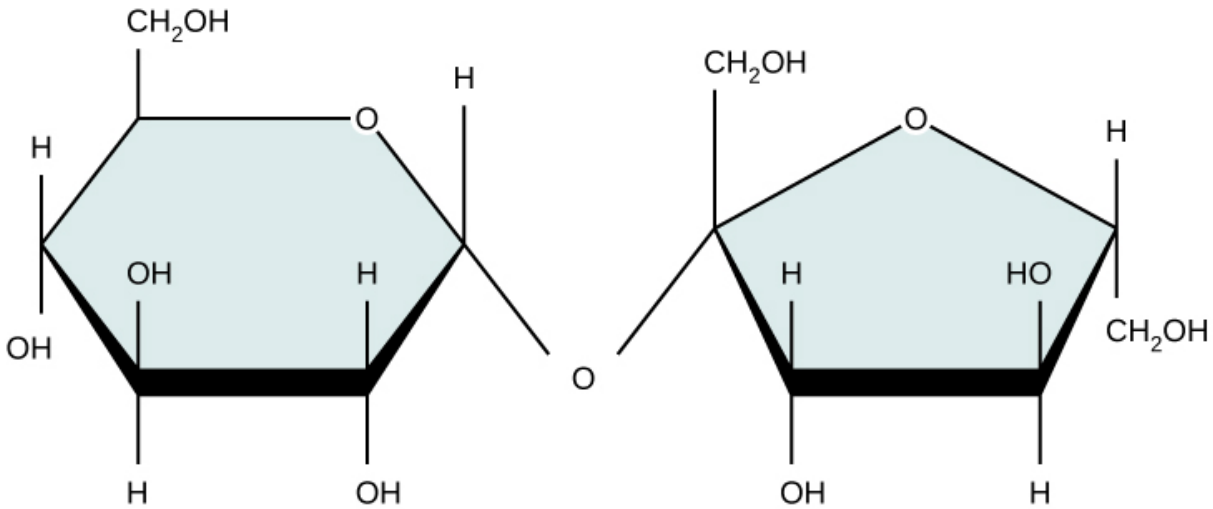
Common disaccharides include lactose, maltose, and sucrose (Figure 3.8). Lactose is a disaccharide consisting of the monomers glucose and galactose. It is naturally in milk. Maltose, or malt sugar, is a disaccharide formed by a dehydration reaction between two glucose molecules. The most common disaccharide is sucrose, or table sugar, which is comprised of glucose and fructose monomers.



Maltose



Lactose



Sucrose

Figure 3.8 Common disaccharides include maltose (grain sugar), lactose (milk sugar), and sucrose (table sugar).

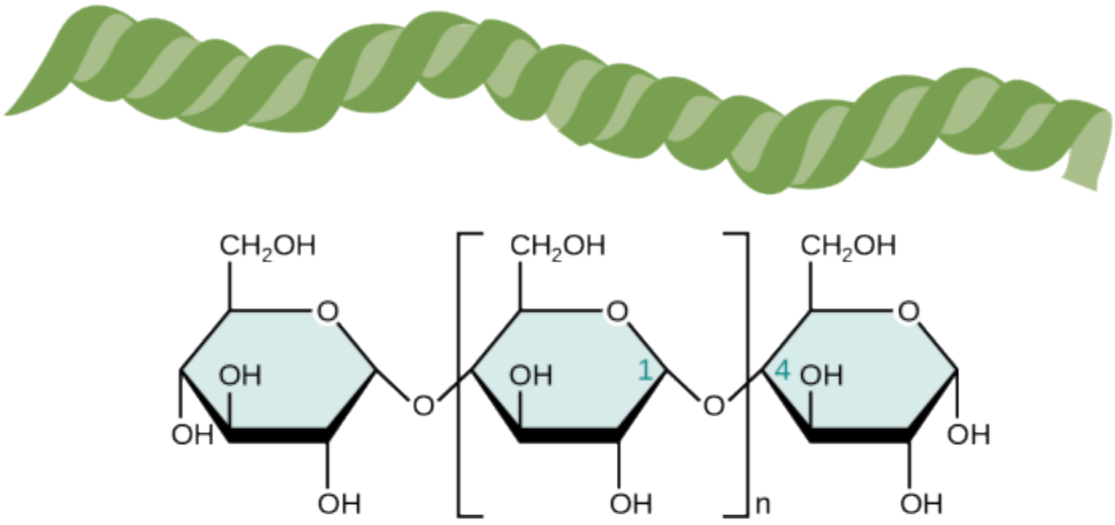
Polysaccharides

A long chain of monosaccharides linked by glycosidic bonds is a **polysaccharide** (poly- = "many"). The chain may be branched or unbranched, and it may contain different types of monosaccharides. The molecular weight may be 100,000 daltons or more depending on the number of joined monomers. Starch, glycogen, cellulose, and chitin are primary examples of polysaccharides.

Plants store sugars in the form of starch. In plants, starch consists of an amylose and amylopectin mixture (both glucose polymers). Plants are able to synthesize glucose, and they store the excess glucose, beyond their immediate energy needs, as starch in different plant parts, including roots and seeds. The starch in the seeds provides food for the embryo as it germinates and can also act as a food source for humans and animals. Enzymes break down the starch that humans consume. For example, an amylase present in saliva catalyzes, or breaks down this starch into smaller molecules, such as maltose and glucose. The cells can then absorb the glucose.

Glucose starch comprises monomers that are joined by α 1-4 or α 1-6 glycosidic bonds. The numbers 1-4 and 1-6 refer to the carbon number of the two residues that have joined to form the bond. As Figure 3.9 illustrates, unbranched glucose monomer chains (only α 1-4 linkages) form the starch, whereas amylopectin is a branched polysaccharide (α 1-6 linkages at the branch points).

Amylose



Amylopectin

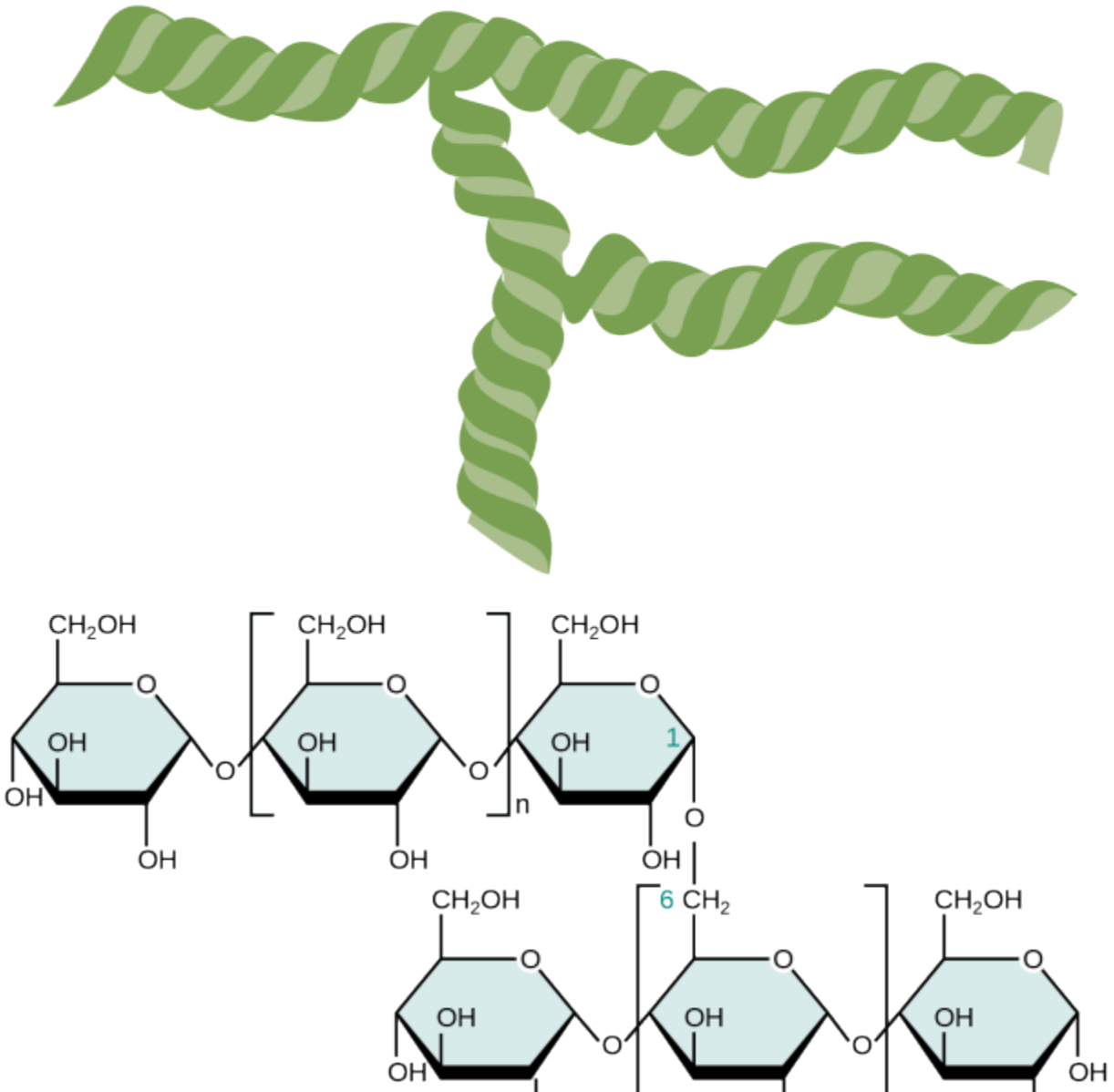


Figure 3.9 Amylose and amylopectin are two different starch forms. Unbranched glucose monomer chains comprise amylose by α 1-4 glycosidic linkages. Amylopectin is a branched polymer of glucose monomers, utilizing α 1-4 and α 1-6 glycosidic linkages. Because of the way the subunits are joined, the glucose chains have a helical structure. Glycogen (not shown) is similar in structure to amylopectin but more highly branched.

Glycogen is the storage form of glucose in humans and other vertebrates and is comprised 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 blood glucose levels decrease, glycogen breaks down to release glucose in a process scientists call glycogenolysis.

Cellulose is the most abundant natural biopolymer and is all around in our daily experience in paper, cardboard, trees, grass, or any plant we see. Cellulose comprises most of a plant's cell wall. This provides the cell structural support. Cellulose is composed of glucose monomers linked by β 1-4 gylcosidic bonds (Figure 3.10).

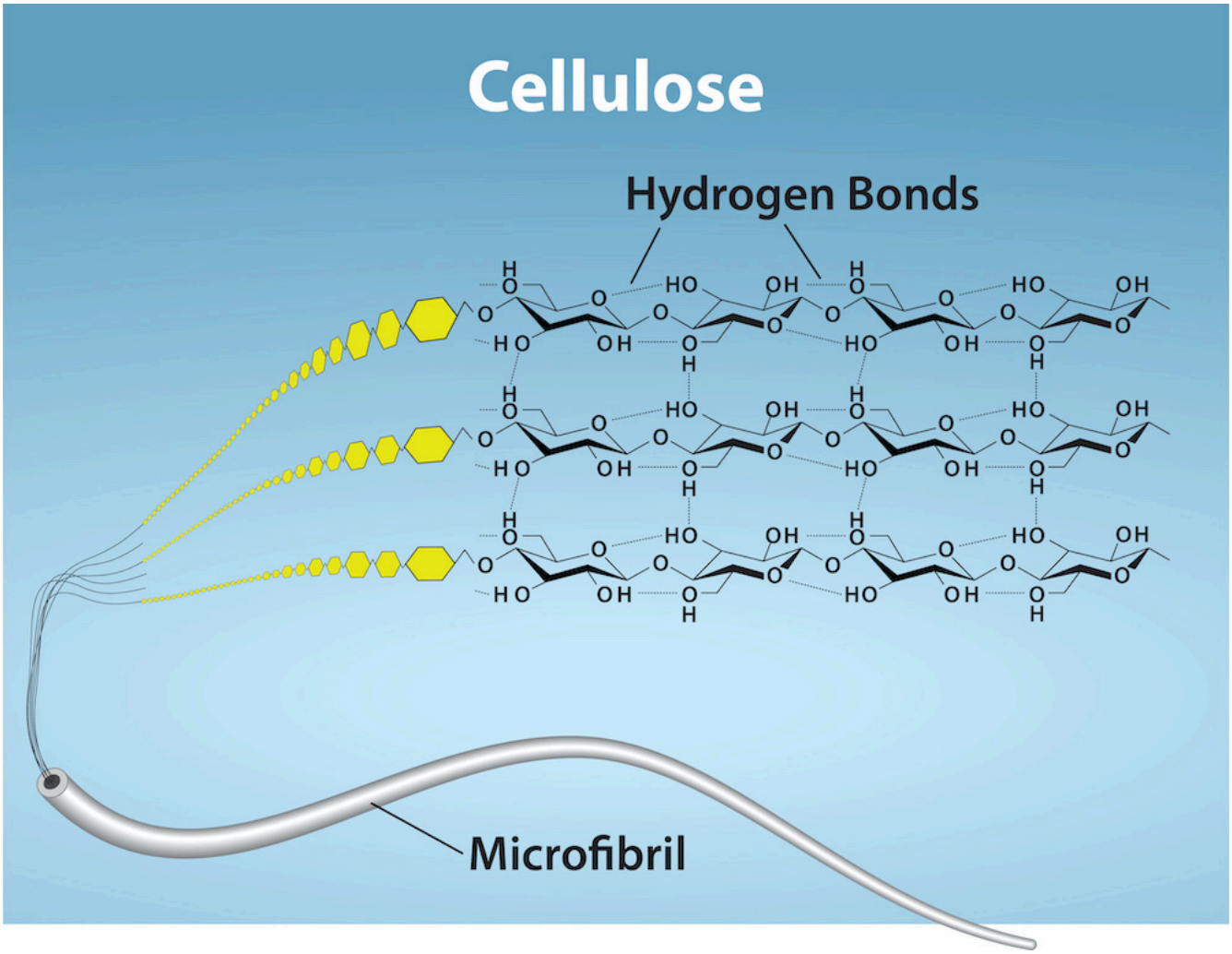


Figure 3.10 Cellulose is an organic compound composed of linear chains of hundreds to thousands of linked glucose molecules. The glucose monomers form hydrogen bonds, holding the chains firmly together side-by-side, and form strong microfibrils. This rigidity is an important structural component of the cell walls found in plants. Credit: Ryan, K. Rao, A. and Hawkins, A. Department of Biology, Texas A&M University.

As Figure 3.10 shows, every other glucose monomer in cellulose is flipped over, and the monomers are packed tightly as extended long chains. This gives cellulose its rigidity and high tensile strength—which is so important to plant cells. While human digestive enzymes cannot break down the β 1-4 linkage, herbivores such as cows, koalas, and buffalos are able, with the help of the specialized flora in their stomach, to digest plant material that is rich in cellulose and use it as a food source. In some of these animals, certain species of bacteria and protists reside in the rumen (part of the herbivore's digestive system) and secrete the enzyme cellulase. The appendix of grazing animals also contains bacteria that digest cellulose, giving it an important role in ruminants' digestive systems. Cellulases can break down cellulose into glucose monomers that animals use as an energy source. Termites are also able to break down cellulose because of the presence of other organisms in their bodies that secrete cellulases.

Carbohydrates serve various functions in different animals. Arthropods (insects, crustaceans, and others)

have an outer skeleton, the exoskeleton, which protects their internal body parts (as we see in the bee in Figure 3.11). This exoskeleton is made of the biological macromolecule **chitin**, which is a nitrogen-containing polysaccharide. It is made of repeating N-acetyl-β-d-glucosamine units, which are a modified sugar. Chitin is also a major component of fungal cell walls. Fungi are neither animals nor plants and form a kingdom of their own in the domain Eukarya.

Figure 3.11 Insects have a hard outer exoskeleton made of chitin, a type of polysaccharide. (credit: Louise Docker)

Career Connections

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 people increasingly seek out registered dietitians for advice. Registered dietitians help plan nutrition programs for individuals in various settings. They often work with patients in health care facilities, designing nutrition plans to treat and prevent 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 physiology (biological functions) of food (proteins, carbohydrates, and fats).

Benefits of Carbohydrates

Are carbohydrates good for you? Some people believe that carbohydrates are bad and they should avoid them. Some diets completely forbid carbohydrate consumption, claiming that a low-carbohydrate diet helps people to lose weight faster. However, carbohydrates have been an important part of the human diet for thousands of years. Artifacts from ancient civilizations show the presence of wheat, rice, and corn in our ancestors' storage areas.

As part of a well-balanced diet, we should supplement carbohydrates with proteins, vitamins, and fats. Calorie-wise, a gram of carbohydrate provides 4.3 Kcal. For comparison, fats provide 9 Kcal/g, a less desirable ratio. Carbohydrates contain soluble and insoluble elements. The insoluble part, fiber, is mostly cellulose. Fiber has many uses. It promotes regular bowel movement by adding bulk, and it regulates the blood glucose consumption rate. Fiber also helps to remove excess cholesterol from the body. Fiber binds to the cholesterol in the small intestine, and prevents the cholesterol particles from entering the bloodstream. Cholesterol then exits the body via the feces. Fiber-rich diets also have a protective role in reducing the occurrence of colon cancer. In addition, a meal containing whole grains and vegetables gives a feeling of fullness. As an immediate source of energy, glucose breaks down during the cellular respiration process, which produces ATP, the cell's energy currency. Without consuming carbohydrates, we reduce the availability of "instant energy." Eliminating carbohydrates from the diet may be necessary for some people, but such a step may not be healthy for everyone.

Link to Learning

For an additional perspective on carbohydrates, explore "Biomolecules: the Carbohydrates" through this interactive animation.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=174#h5p-16

21.

LIPIDS

Learning Objectives

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

- Describe the four major types of lipids
- Explain the role of fats in storing energy
- Differentiate between saturated and unsaturated fatty acids
- Describe phospholipids and their role in cells
- Define the basic structure of a steroid and some steroid functions

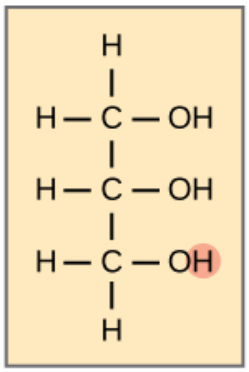
Lipids include a diverse group of compounds that are largely nonpolar in nature. This is because they are hydrocarbons that include mostly nonpolar carbon–carbon or carbon–hydrogen bonds. Nonpolar molecules are hydrophobic ("water fearing"), or insoluble in water. Lipids perform many different functions in a cell. Cells store energy for long-term use in the form of fats. Lipids also provide insulation from the environment for plants and animals (Figure 3.12). For example, they help keep aquatic birds and mammals dry when forming a protective layer over fur or feathers because of their water-repellant hydrophobic nature. Lipids are also the building blocks of many hormones and are an important constituent of all cellular membranes. Lipids include fats, oils, waxes, phospholipids, and steroids.

Figure 3.12 Hydrophobic lipids in aquatic mammals' fur, such as this river otter, protect them from the elements. (credit: Ken Bosma)

Fats and Oils

A fat molecule consists of two main components—glycerol and fatty acids. Glycerol is an organic compound (alcohol) with three carbons, five hydrogens, and three hydroxyl (OH) groups. Fatty acids have a long chain of hydrocarbons to which a carboxyl acid group (also called carboxyl group) is attached, hence the name "fatty acid." The number of carbons in the fatty acid may range from 4 to 36. The most common are those containing 12–18 carbons. In a fat molecule, the fatty acids attach to each of the glycerol molecule's three carbons via **ester** bonds (bonds of an oxygen to a carbon that is double-bonded to another oxygen) (Figure 3.13).

Glycerol



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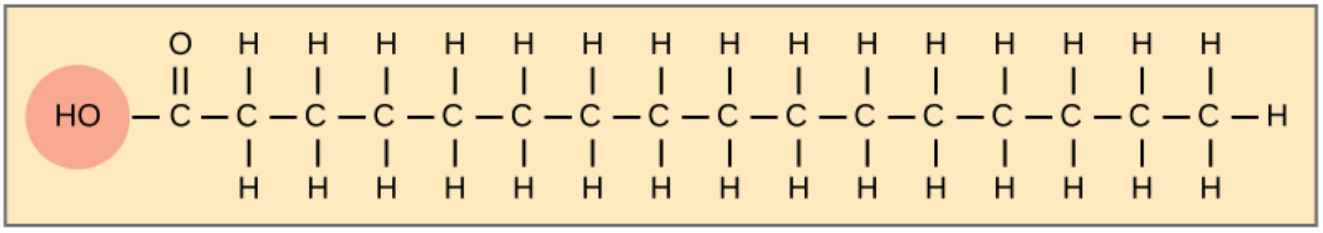
Fatty Acid



Triacylglycerol

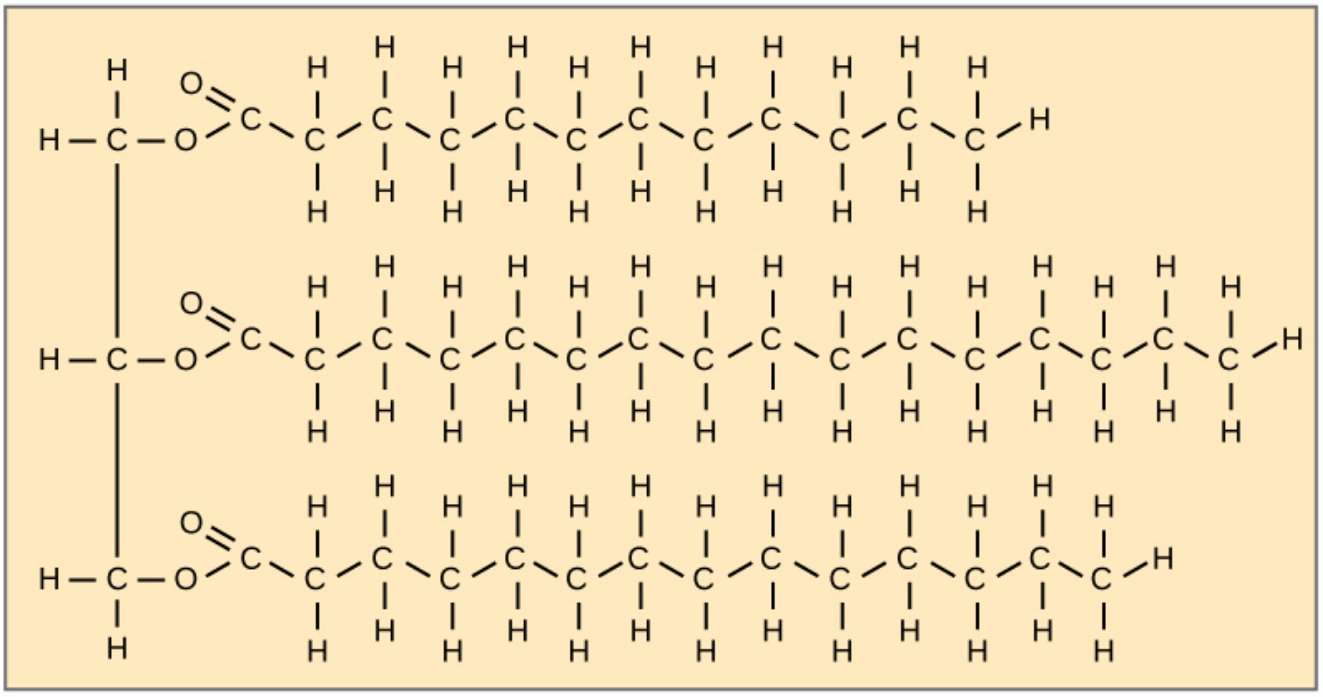


Figure 3.13 Joining three fatty acids to a glycerol backbone in a dehydration reaction forms triacylglycerol. Three water molecules release in the process.

During this ester bond formation, three water molecules are released. The three fatty acids in the triacylglycerol may be similar or dissimilar. We also call fats **triacylglycerols** or **triglycerides** because of their chemical structure. 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 hypogea,* the scientific name for groundnuts or 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. Stearic acid is an example of a saturated fatty acid (Figure 3.14).

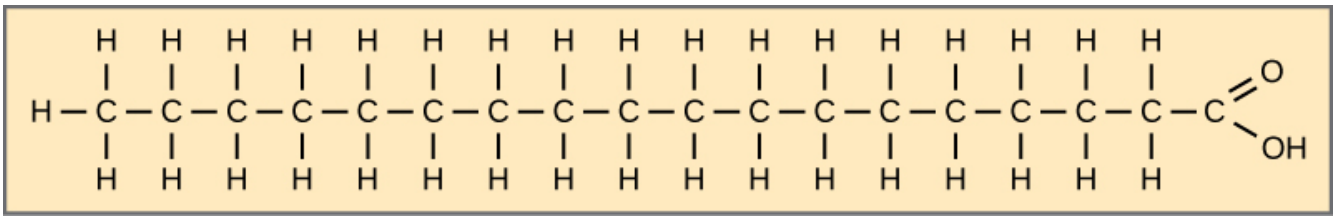


Figure 3.14 Stearic acid is a common saturated fatty acid.

When the hydrocarbon chain contains a double bond, the fatty acid is unsaturated. Oleic acid is an example of an **unsaturated** fatty acid (Figure 3.15).

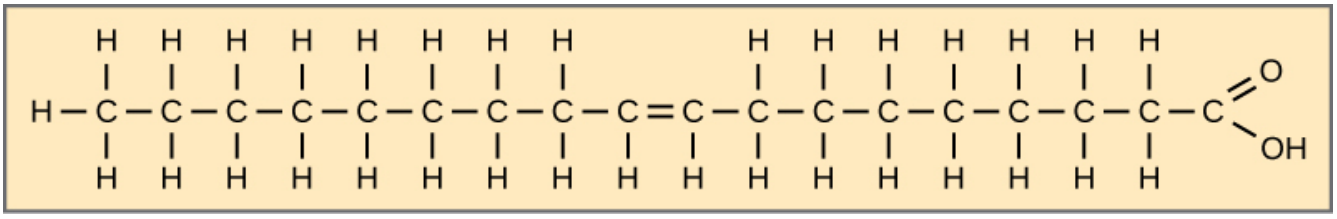


Figure 3.15 Oleic acid is a common unsaturated fatty acid.

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

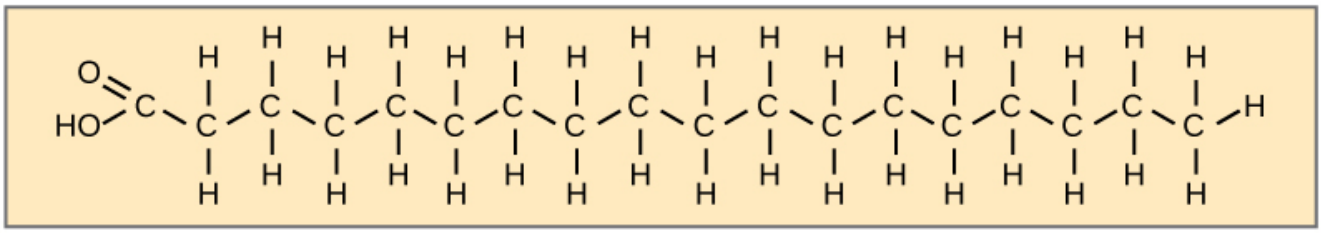
When a fatty acid has no double bonds, it is a saturated fatty acid because it is not possible to add more hydrogen to the chain's carbon atoms. A fat may contain similar or different fatty acids attached to glycerol. Long straight fatty acids with single bonds generally pack tightly and are solid at room temperature. Animal

fats with stearic acid and palmitic acid (common in meat) and the fat with butyric acid (common in butter) are examples of saturated fats. Mammals store fats in specialized cells, or adipocytes, where fat globules occupy most of the cell's volume. Plants store fat or oil in many seeds and use them as a source of energy during seedling development. Unsaturated fats or oils are usually of plant origin and contain *cis* unsaturated fatty acids. *Cis* and *trans* indicate the configuration of the molecule around the double bond. If hydrogens are present on the same side of the double bond, it is a cis fat. If the hydrogen atoms are on two different sides, it is a **trans fat**. The *cis* double bond causes a bend or a "kink" that prevents the fatty acids from packing tightly, keeping them liquid at room temperature (Figure 3.16). Olive oil, corn oil, canola oil, and cod liver oil are examples of unsaturated fats. Unsaturated fats help to lower blood cholesterol levels, whereas saturated fats contribute to plaque formation in the arteries.

Figure 3.16 Saturated fatty acids have hydrocarbon chains connected by single bonds only. Unsaturated fatty acids have one or more double bonds. Each double bond may be in a *cis* or *trans* configuration. In the *cis* configuration, both hydrogens are on the same side of the hydrocarbon chain. In the *trans* configuration, the hydrogens are on opposite sides. A *cis* double bond causes a kink in the chain.

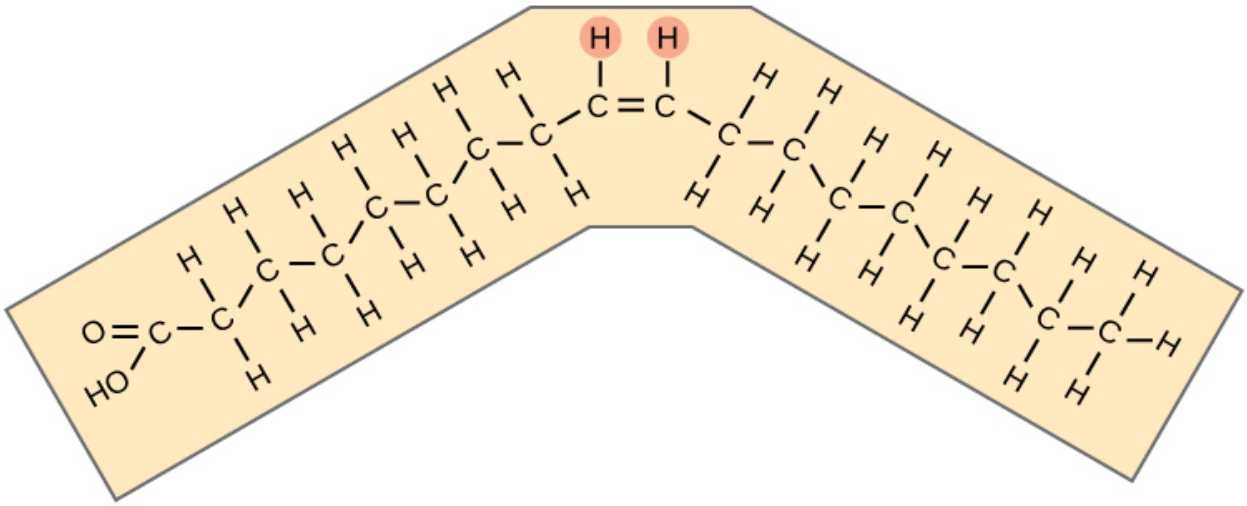
Saturated fatty acid

Stearic acid



Unsaturated fatty acids

Cis oleic acid



Trans oleic acid

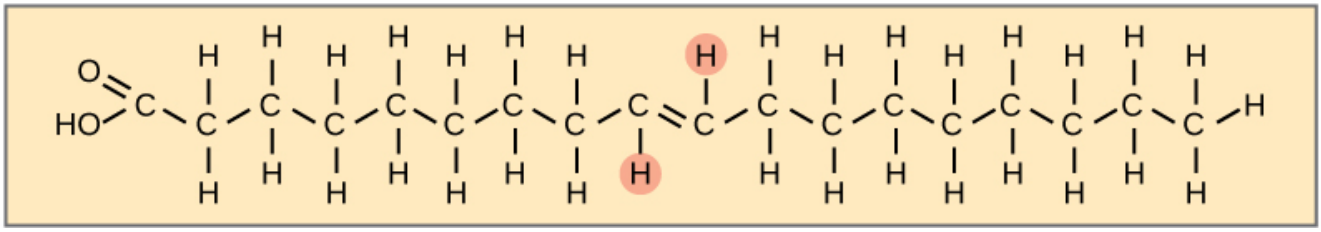


Figure 3.16 Saturated fatty acids have hydrocarbon chains connected by single bonds only. Unsaturated fatty acids have one or more double bonds. Each double bond may be in a cis or trans configuration. In the cis configuration, both hydrogens are on the same side of the hydrocarbon chain. In the trans configuration, the hydrogens are on opposite sides. A cis double bond causes a kink in the chain.

Trans Fats

The food industry artificially hydrogenates oils to make them semi-solid and of a consistency desirable for many processed food products. 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 convert to double bonds in the *trans*– conformation.

Margarine, some types of peanut butter, and shortening are examples of artificially hydrogenated trans fats. Recent studies have shown that an increase in trans fats in the human diet may lead to higher levels of low-density lipoproteins (LDL), or "bad" cholesterol, which in turn may lead to plaque deposition in the arteries, resulting in heart disease. Many fast food restaurants have recently banned using trans fats, and food labels are required to display the trans fat content.

Omega Fatty Acids

Essential fatty acids are those that the human body requires but does not synthesize. Consequently, they have to be supplemented through ingestion via the diet. **Omega**-3 fatty acids (like those in Figure 3.17) fall into this category and are one of only two known for humans (the other is omega-6 fatty acid). These are polyunsaturated fatty acids and are omega-3 because a double bond connects the third carbon from the hydrocarbon chain's end to its neighboring carbon.

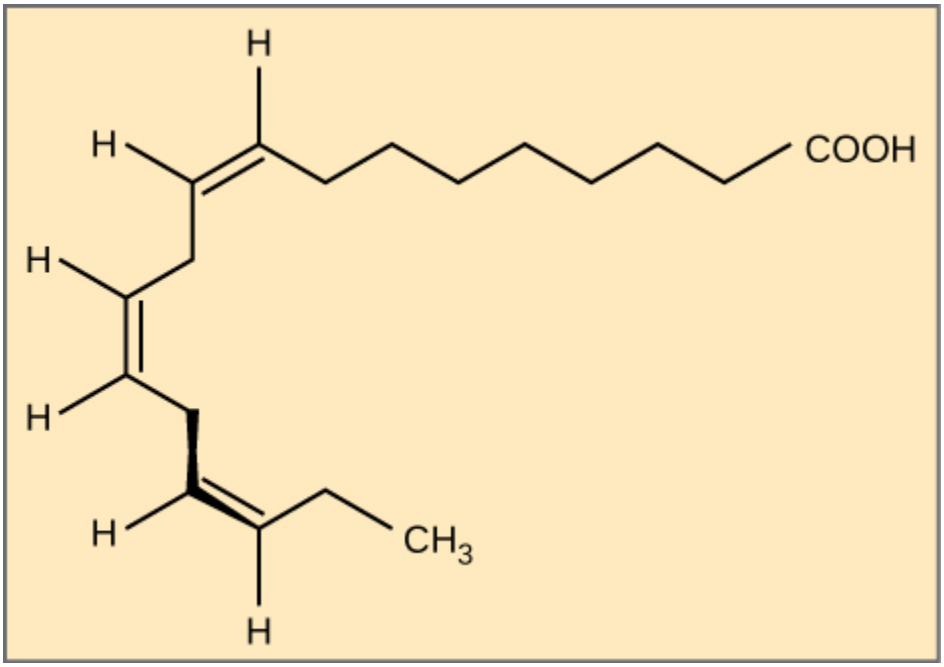


Figure 3.17 Alpha-linolenic acid is an example of an omega-3 fatty acid. It has three cis double bonds and, as a result, a curved shape. For clarity, the diagram does not show the carbons. Each singly bonded carbon has two hydrogens associated with it, which the diagram also does not show.

The farthest carbon away from the carboxyl group is numbered as the omega (ω) carbon, and if the double bond is between the third and fourth carbon from that end, it is an omega-3 fatty acid. Nutritionally important

because the body does not make them, omega-3 fatty acids include alpha-linoleic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), all of which are polyunsaturated. Salmon, trout, and tuna are good sources of omega-3 fatty acids. Research indicates that omega-3 fatty acids reduce the risk of sudden death from heart attacks, lower triglycerides in the blood, decrease blood pressure, and prevent thrombosis by inhibiting blood clotting. They also reduce inflammation, and may help lower the risk of some cancers in animals.

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

Waxes

Wax covers some aquatic birds' feathers and some plants' leaf surfaces. Because of waxes' hydrophobic nature, they prevent water from sticking on the surface (Figure 3.18). Long fatty acid chains linked to long-chain alcohols via ester bonds comprise waxes.

Figure 3.18 Lipids comprise waxy coverings on some leaves. (credit: Roger Griffith)

Phospholipids

Phospholipids are major plasma membrane constituents that comprise cells' outermost layer. Like fats, they are comprised of fatty acid chains attached to a glycerol backbone (or in some cases to another molecule called sphingosine).

However, instead of three fatty acids attached as in triglycerides, there are two fatty acids forming diacylglycerol, and a modified phosphate group occupies the glycerol backbone's third carbon (Figure 3.19).

A phosphate group alone attached to a diacylglycerol does not qualify as a phospholipid. It is phosphatidate (diacylglycerol 3-phosphate), the precursor of phospholipids. An alcohol modifies the phosphate group. Phosphatidylcholine and phosphatidylserine are two important phospholipids that are in plasma membranes.

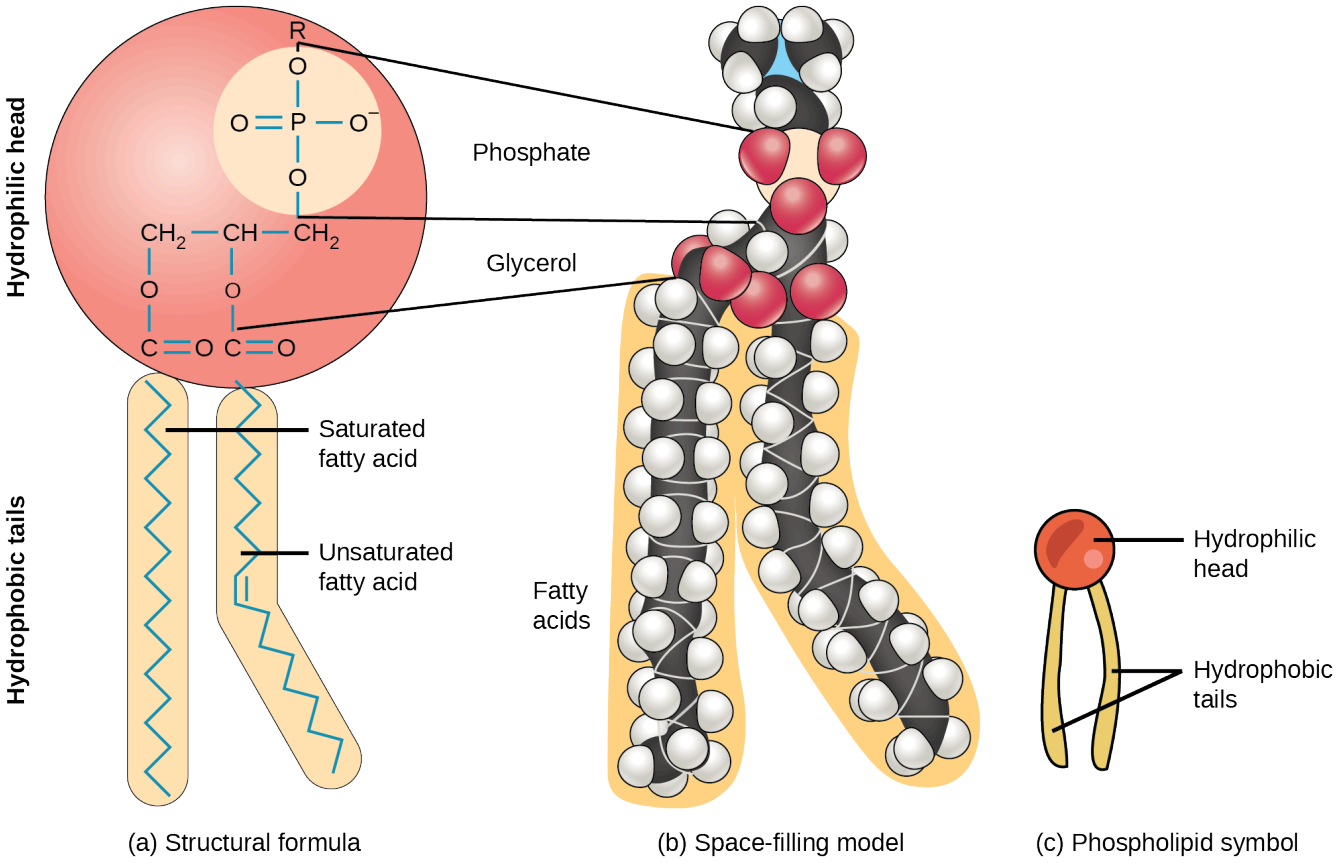


Figure 3.19 A phospholipid is a molecule with two fatty acids and a modified phosphate group attached to a glycerol backbone. Adding a charged or polar chemical group may modify the phosphate.

A phospholipid is an amphipathic molecule, meaning it has a hydrophobic and a hydrophilic part. The fatty acid chains are hydrophobic and cannot interact with water; whereas, the phosphate-containing group is hydrophilic and interacts with water (Figure 3.20).

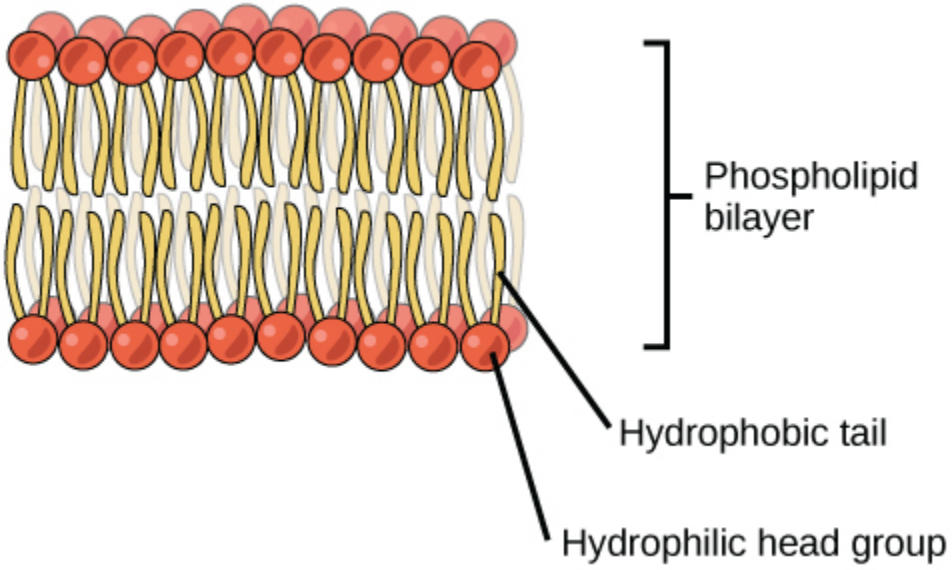


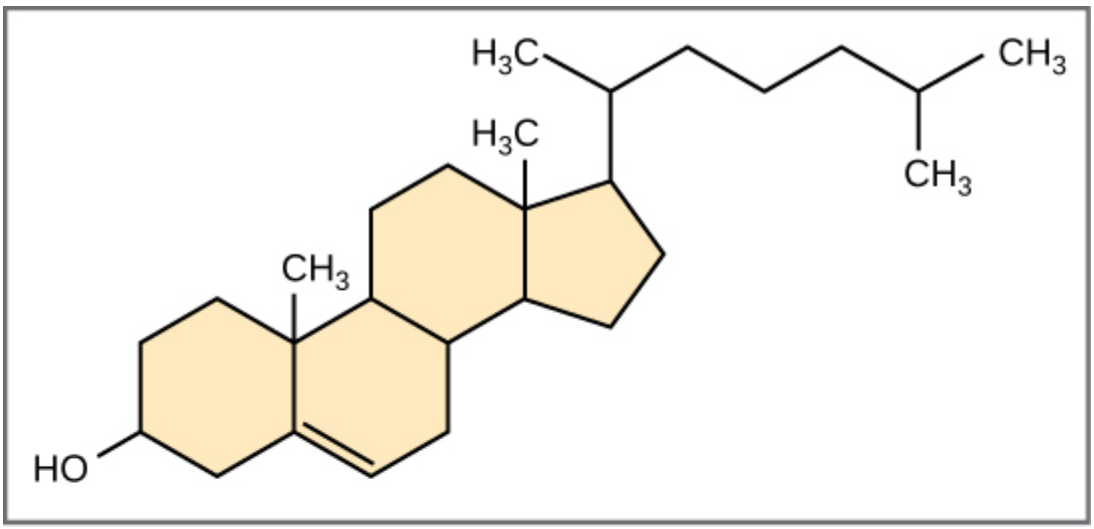
Figure 3.20 The phospholipid bilayer is the major component of all cellular membranes. The hydrophilic head groups of the phospholipids face the aqueous solution. The hydrophobic tails are sequestered in the middle of the bilayer.

The head is the hydrophilic part, and the tail contains the hydrophobic fatty acids. In a membrane, a bilayer of phospholipids forms the structure's matrix, phospholipids' fatty acid tails face inside, away from water, whereas the phosphate group faces the outer, aqueous side (Figure 3.20).

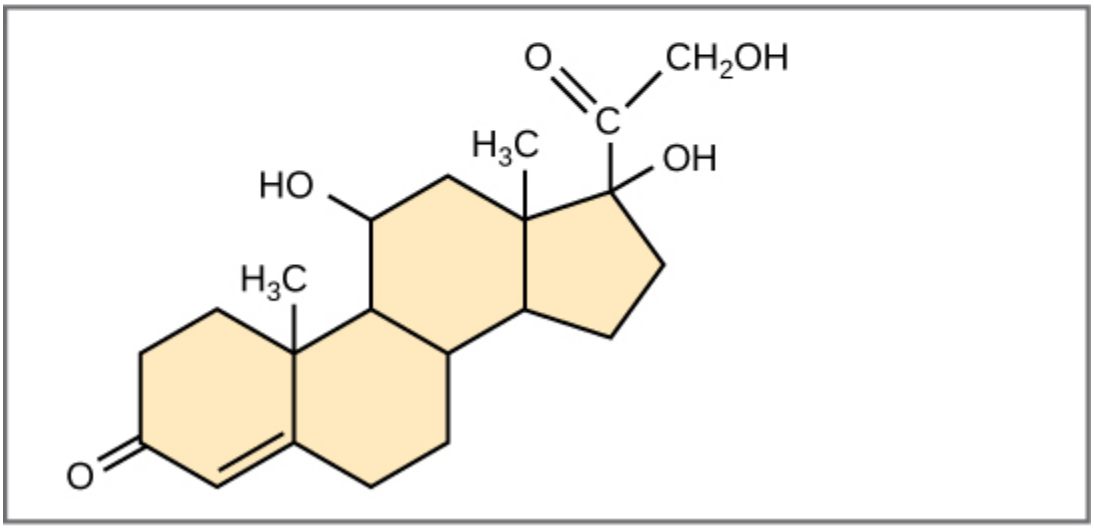
Phospholipids are responsible for the plasma membrane's dynamic nature. If a drop of phospholipids is placed in water, it spontaneously forms a structure that scientists call a micelle, where the hydrophilic phosphate heads face the outside and the fatty acids face the structure's interior.

Steroids

Unlike the phospholipids and fats that we discussed earlier, **steroids** have a fused ring structure. Although they do not resemble the other lipids, scientists group them with them because they are also hydrophobic and insoluble in water. All steroids have four linked carbon rings and several of them, like cholesterol, have a short tail (Figure 3.21). Many steroids also have the –OH functional group, which puts them in the alcohol classification (sterols).



Cholesterol



Cortisol

Figure 3.21 Four fused hydrocarbon rings comprise steroids such as cholesterol and cortisol.

Cholesterol is the most common steroid. The liver synthesizes cholesterol, which is the precursor to many steroid hormones such as testosterone and estradiol, which gonads and endocrine glands secrete. It is also the precursor to Vitamin D. Cholesterol is also the precursor of bile salts, which help emulsifying fats and their subsequent absorption by cells. Although lay people often speak negatively about cholesterol, it is necessary for the body's proper functioning. Sterols (cholesterol in animal cells, phytosterol in plants) are components of the plasma membrane of cells and are found within the phospholipid bilayer.

Link to Learning

For an additional perspective on lipids, explore the interactive animation “Biomolecules: The Lipids”.

22.

PROTEINS

Learning Objectives

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

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

Proteins are one of the most abundant organic molecules in living systems and have the most diverse range of functions of all macromolecules. Proteins may be structural, regulatory, contractile, or protective. They may serve in transport, storage, or membranes, or they may be toxins or enzymes. Each cell in a living system may contain thousands of proteins, each with a unique function. Their structures, like their functions, vary greatly. They are all, however, amino acid polymers arranged in a linear sequence.

Types and Functions of Proteins

Enzymes, which living cells produce, are catalysts in biochemical reactions (like digestion) and are usually complex or conjugated proteins (proteins combined with something else). Each enzyme is specific for the substrate (a reactant that binds to an enzyme) upon which it acts. The enzyme may help in breakdown, rearrangement, or synthesis reactions. We call enzymes that break down their substrates catabolic enzymes. Those that build more complex molecules from their substrates are anabolic enzymes, and enzymes that affect the rate of reaction are catalytic enzymes. Note that all enzymes increase the reaction rate and, therefore,

are organic catalysts. An example of an enzyme is salivary amylase, which hydrolyzes its substrate amylose, a component of starch.

Hormones are chemical-signaling molecules, usually small proteins or steroids, secreted by endocrine cells that act to control or regulate specific physiological processes, including growth, development, metabolism, and reproduction. For example, insulin is a protein hormone that helps regulate the blood glucose level. Table 3.1 lists the primary types and functions of proteins.

Protein Types and Functions

Type	Examples	Functions
Digestive Enzymes	Amylase, lipase, pepsin, trypsin	Help in food by catabolizing (breaking down) nutrients into monomeric units
Transport	Hemoglobin, albumin	Carry substances in the blood or lymph throughout the body
Structural	Actin, tubulin, keratin	Constitute different structures, like the cytoskeleton
Hormones	Insulin, thyroxine	Coordinate different body systems' activity
Defense	Immunoglobulins	Protect the body from foreign pathogens
Contractile	Actin, myosin	Effect muscle contraction
Storage	Legume storage proteins, egg white (albumin)	Provide nourishment in early embryo development and the seedling

Table 3.1

Proteins have different shapes and molecular weights. Some proteins are globular in shape, whereas others are fibrous in nature. For example, hemoglobin is a globular protein, but collagen, located in our skin, is a fibrous protein. Protein shape is critical to its function, and many different types of chemical bonds maintain this shape. Changes in temperature, pH, and exposure to chemicals may lead to permanent changes in the protein's shape, leading to loss of function, or **denaturation**. Different arrangements of the same 20 types of amino acids comprise all proteins. Two rare new amino acids were discovered recently (selenocystein and pirrolysine), and additional new discoveries may be added to the list.

Amino Acids

Amino acids are the monomers that comprise proteins. Each amino acid has the same fundamental structure, which consists of a central carbon atom, or the alpha (α) carbon, bonded to an amino group (NH_2), a carboxyl group (COOH), and to a hydrogen atom. Every amino acid also has another atom or group of atoms bonded to the central atom known as the R group (Figure 3.22).

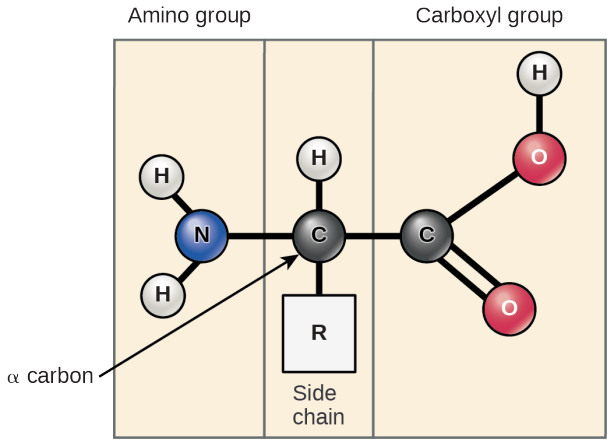


Figure 3.22 Amino acids have a central asymmetric carbon to which an amino group, a carboxyl group, a hydrogen atom, and a side chain (R group) are attached.

Scientists use the name "amino acid" because these acids contain both amino group and carboxyl-acid-group in their basic structure. 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 3.23).

Visual Connection

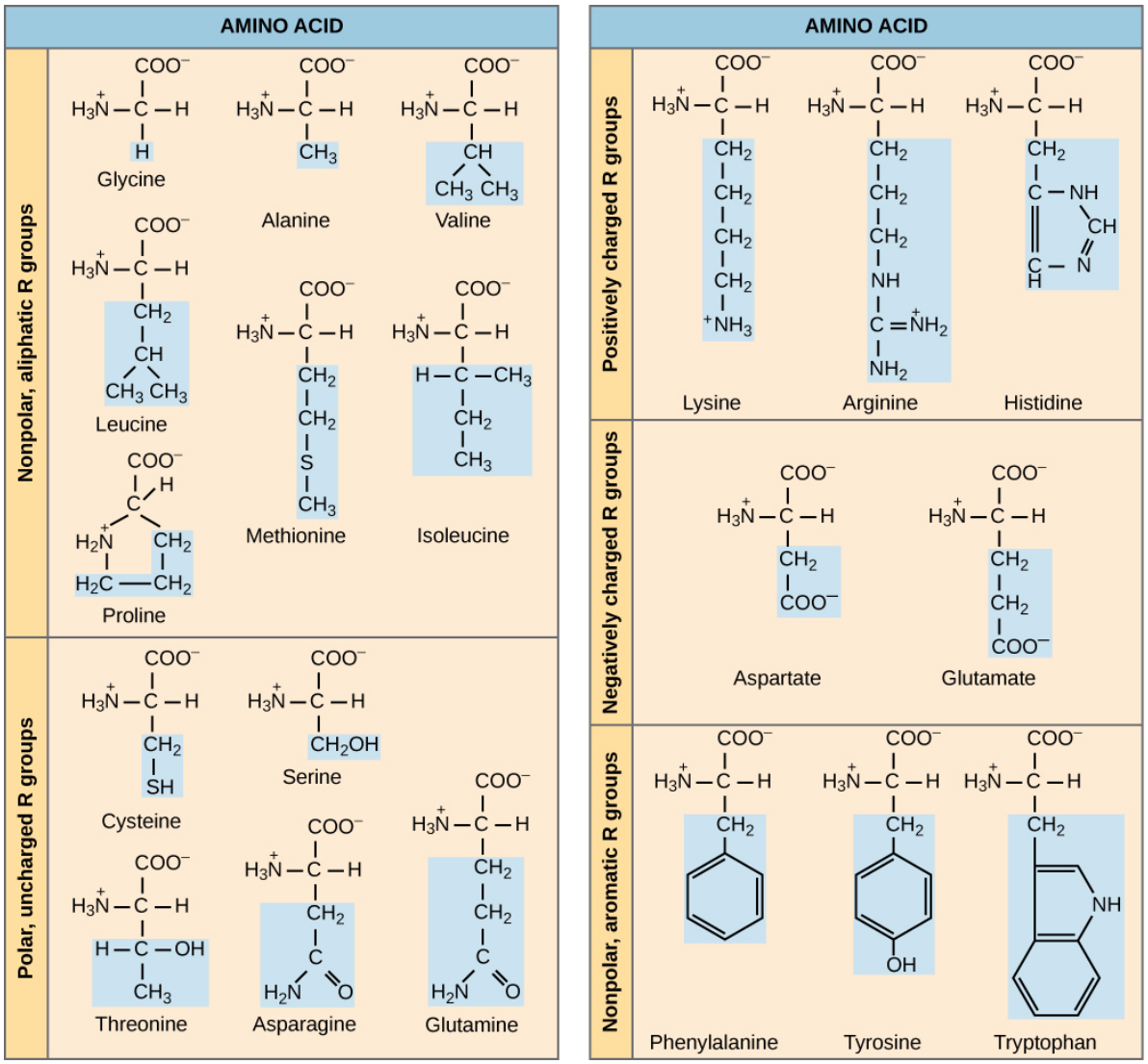


Figure 3.23 There are 20 common amino acids commonly found in proteins, each with a different R group (variant group) that determines its chemical nature.

Which categories of amino acid would you expect to find on a soluble protein's surface and which would you expect to find in the interior? What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?

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. 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 3.23).

A single upper case letter or a three-letter abbreviation represents amino acids. For example, the letter V or the three-letter symbol val represents valine. Just as some fatty acids are essential to a diet, some amino acids also are necessary. These essential amino acids in humans include isoleucine, leucine, cysteine, and others. Essential amino acids refer to those necessary to build proteins in the body, but which the body does not produce and must be obtained from food. If a body doesn't get the essential amino acids, malnutrition can develop. During malnutrition the body cannot function properly and the organism suffers. Which amino acids are essential varies from organism to organism.

The sequence and the number of amino acids ultimately determine the protein's shape, size, and function. Amino acids link when a covalent **peptide bond** forms by dehydration, between one amino acid's carboxyl group and the next amino acid's amino group (Figure 3.24).

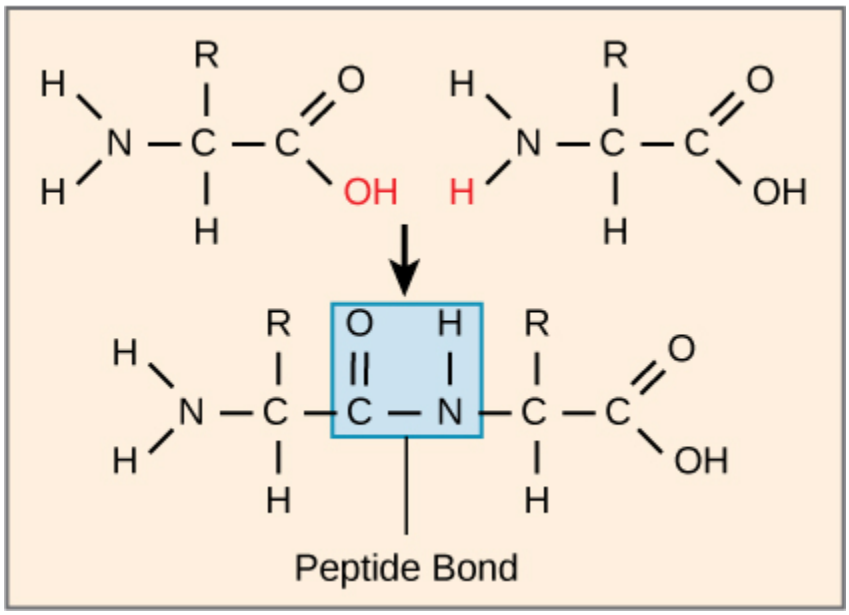


Figure 3.24 Peptide bond formation is a dehydration synthesis reaction. The carboxyl group of one amino acid is linked to the incoming amino acid's amino group. In the process, it releases a water molecule.

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 has 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 the steps of protein synthesis in this interactive tutorial.

Evolution Connection

The Evolutionary Significance of Cytochrome c

Cytochrome c is an important component of the electron transport chain, a part of cellular respiration (energy metabolism), and it is normally located in the cellular organelle the mitochondrion. This protein has a heme prosthetic group. A prosthetic group is a non-protein part bound tightly to the protein part of an enzyme. Prosthetic groups can be simple metal ions or more complicated molecules containing carbon atoms. The heme's central ion alternately reduces and oxidizes (receives and donates electrons) during electron transfer. Because this essential protein's role in producing cellular energy is crucial, it has changed very little over millions of years. Protein sequencing has shown that there is a considerable amount of cytochrome c amino acid sequence equivalence among different species. In other words, we can assess evolutionary kinship by measuring the similarities or differences among various species' DNA or protein sequences.

Scientists have determined that human cytochrome c contains 104 amino acids. For each cytochrome c molecule from different organisms that scientists have sequenced to date, 37 of these amino acids appear in the same position in all cytochrome c samples. This indicates that there may have been a common ancestor. On comparing the human and chimpanzee protein sequences, scientists did not find a sequence difference. When researchers compared human and rhesus monkey sequences, the single difference was in one amino acid. In another comparison, human to yeast sequencing shows a difference in the 44th position.

Protein Structure

As we discussed earlier, a protein's shape is critical to its function. For example, an enzyme can bind to a specific substrate at an **active site** (location on the protein configured to bind the substrate). If this active site is altered because of local changes or changes in overall protein structure, the enzyme may be unable to bind to the substrate. 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.

Primary Structure

Amino acids' unique sequence in a polypeptide chain is a protein's **primary structure**. For example, the pancreatic hormone insulin is a protein that has two polypeptide chains, A and B, and they are linked together by disulfide bonds. The N terminal amino acid of the A chain is glycine, whereas the C terminal amino acid is asparagine (Figure 3.25). The amino acid sequences in the A and B chains are unique to insulin.

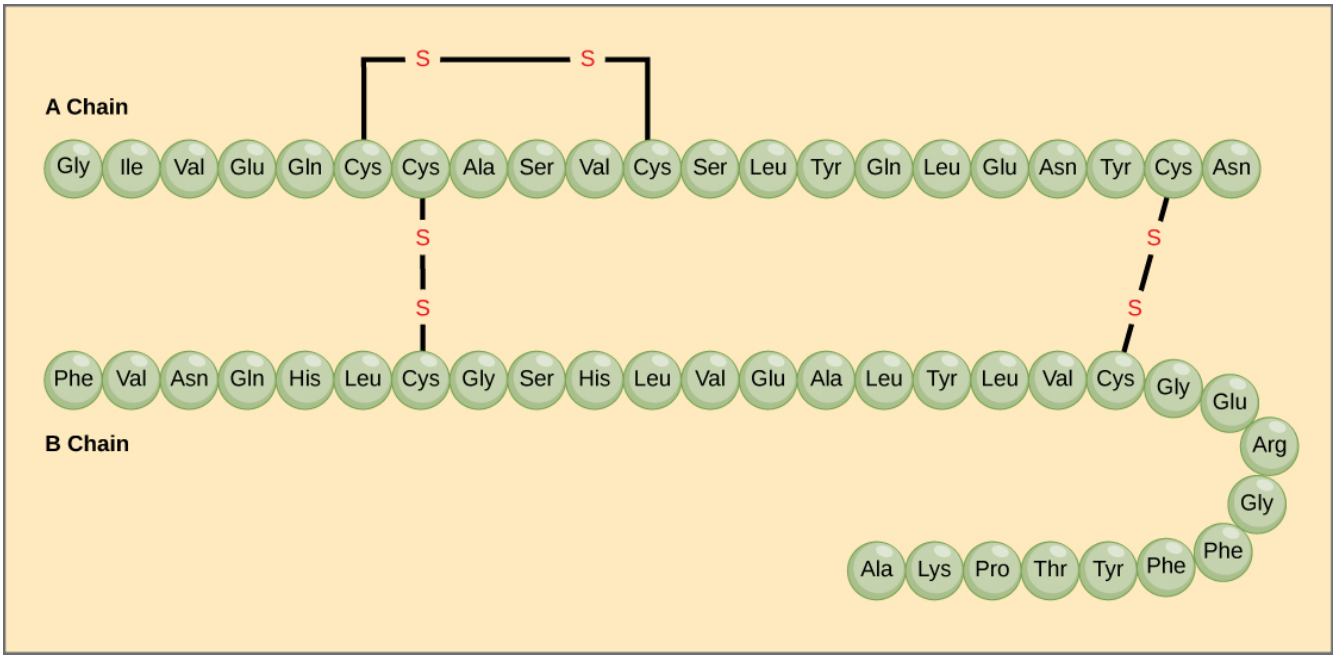


Figure 3.25 Bovine serum insulin is a protein hormone comprised of two peptide chains, A (21 amino acids long) and B (30 amino acids long). In each chain, three-letter abbreviations that represent the amino acids' names in the order they are present indicate primary structure. The amino acid cysteine (cys) has a sulfhydryl (SH) group as a side chain. Two sulfhydryl groups can react in the presence of oxygen to form a disulfide (S-S) bond. Two disulfide bonds connect the A and B chains together, and a third helps the A chain fold into the correct shape. Note that all disulfide bonds are the same length, but we have drawn them different sizes for clarity.

The gene encoding the protein ultimately determines the unique sequence for every protein. A change in nucleotide sequence of the gene's coding region may lead to adding a different amino acid to the growing

polypeptide chain, causing a change in protein structure and function. In sickle cell anemia, the hemoglobin β chain (a small portion of which we show in Figure 3.26) has a single amino acid substitution, causing a change in protein structure and function. Specifically, valine in the β chain substitutes the amino acid glutamic. What is most remarkable to consider is that a hemoglobin molecule is comprised of two alpha 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—which dramatically decreases life expectancy—is a single amino acid of the 600. What is even more remarkable is that three nucleotides (monomers on DNA) encode each of those 600 amino acids, and a single base change (point mutation), 1 in 1800 bases, causes the mutation.

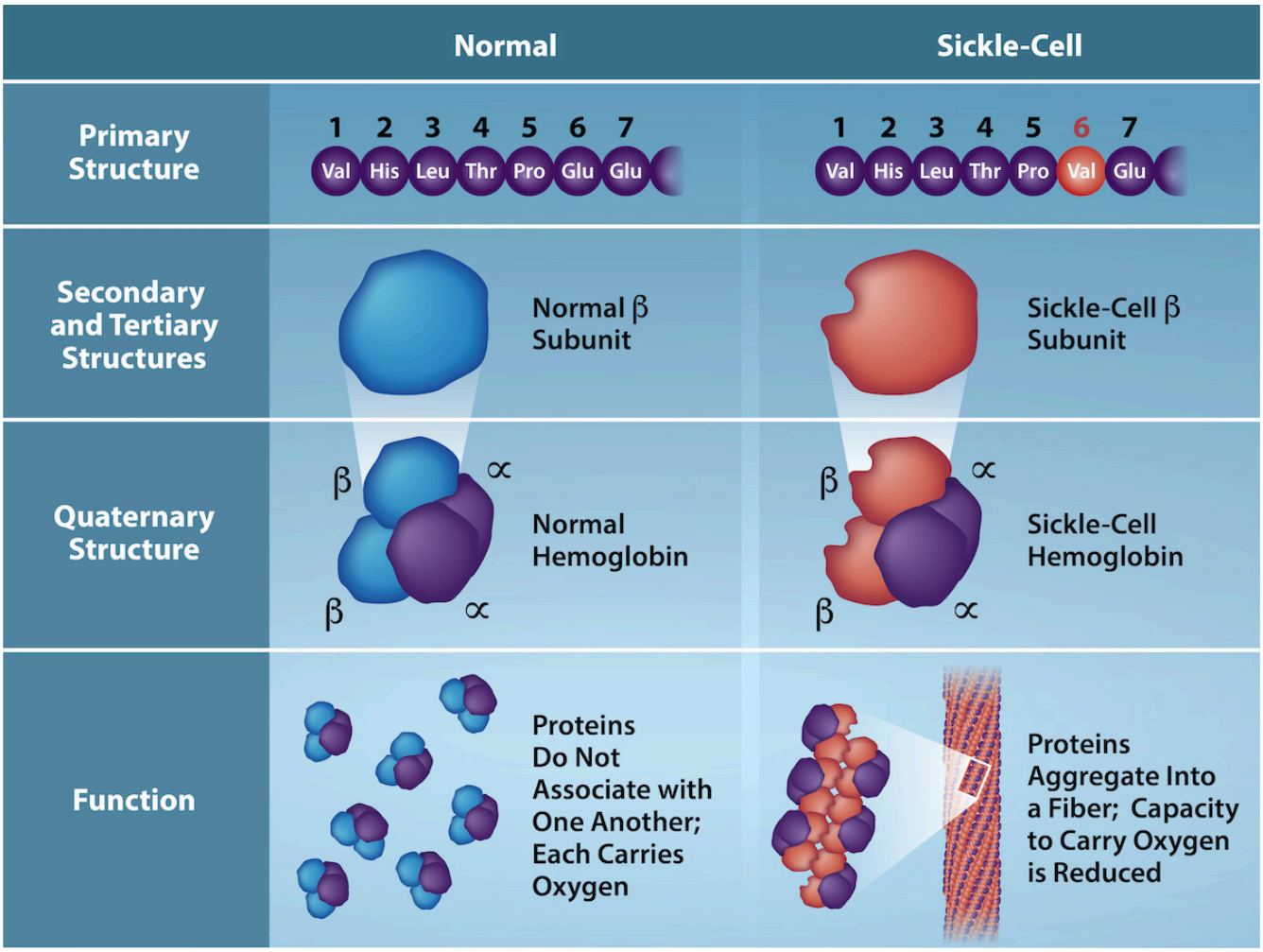


Figure 3.26 Because of this change of one amino acid in the chain, hemoglobin molecules form long fibers that distort the biconcave, or disc-shaped, red blood cells and cause them to assume a crescent or "sickle" shape, which clogs blood vessels (Figure 3.27). The beta (β)- chain of hemoglobin is 147 amino acids in length, yet a single amino acid substitution in the primary sequence leads to changes in secondary, tertiary, and quaternary structures and sickle cell anemia. In normal hemoglobin, the amino acid at position six is glutamate. In sickle cell hemoglobin, glutamate is replaced by valine. Credit: Rao, A., Tag, A. Ryan, K. and Fletcher, S. Department of Biology, Texas A&M University.

Because of this change of one amino acid in the chain, hemoglobin molecules form long fibers that distort the biconcave, or disc-shaped, red blood cells and cause them to assume a crescent or "sickle" shape, which clogs blood vessels (Figure 3.27). This can lead to myriad serious health problems such as breathlessness, dizziness, headaches, and abdominal pain for those affected by this disease. William Warrick Cardozo showed that sickle-cell anemia is an inherited disorder, meaning that the difference in the specific gene's encoding region is passed down from parents to children. As you will learn in the genetics unit, the inheritance of such traits is determined by a combination of genes from both parents, and these very small differences can have significant impacts on organisms.

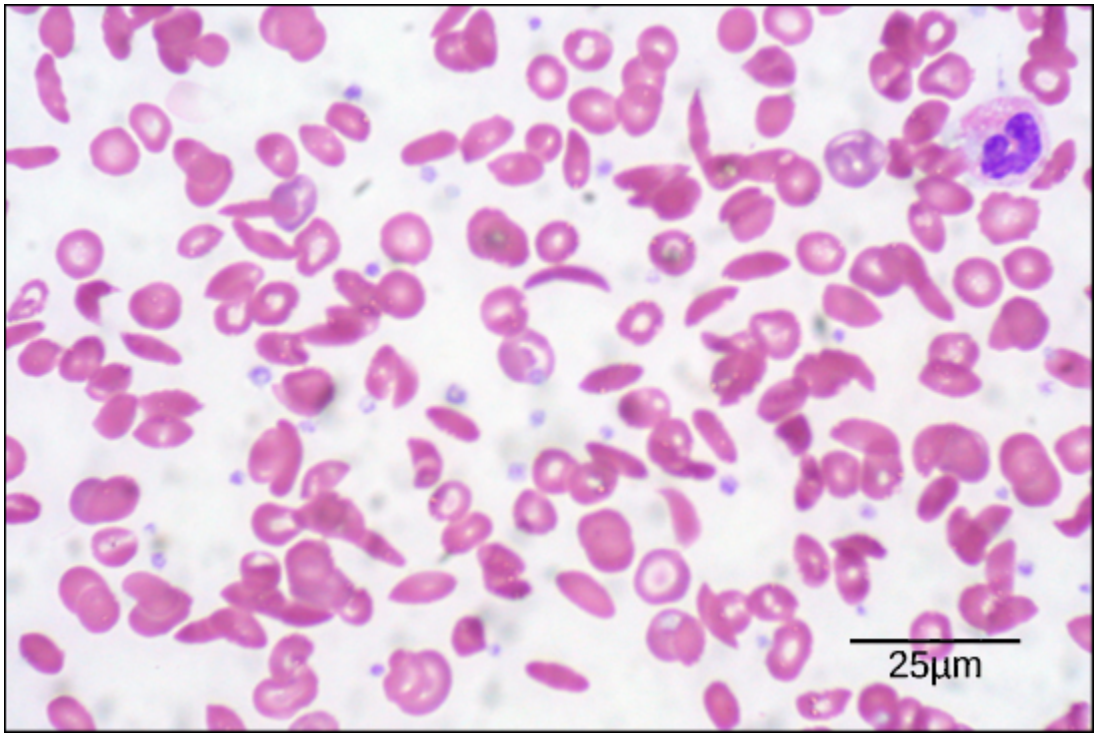


Figure 3.27 In this blood smear, visualized at 535x magnification using bright field microscopy, sickle cells are crescent shaped, while normal cells are disc-shaped. (credit: modification of work by Ed Uthman; scale-bar data from Matt Russell)

Secondary Structure

The local folding of the polypeptide in some of its regions gives rise to the **secondary structure** of the protein. The most common are the ***α*-helix** and ***β*-pleated sheet** structures (Figure 3.28). Both structures are held in shape by hydrogen bonds. The hydrogen bonds form between the oxygen atom in the carbonyl group in one amino acid and another amino acid that is four amino acids farther along the chain.

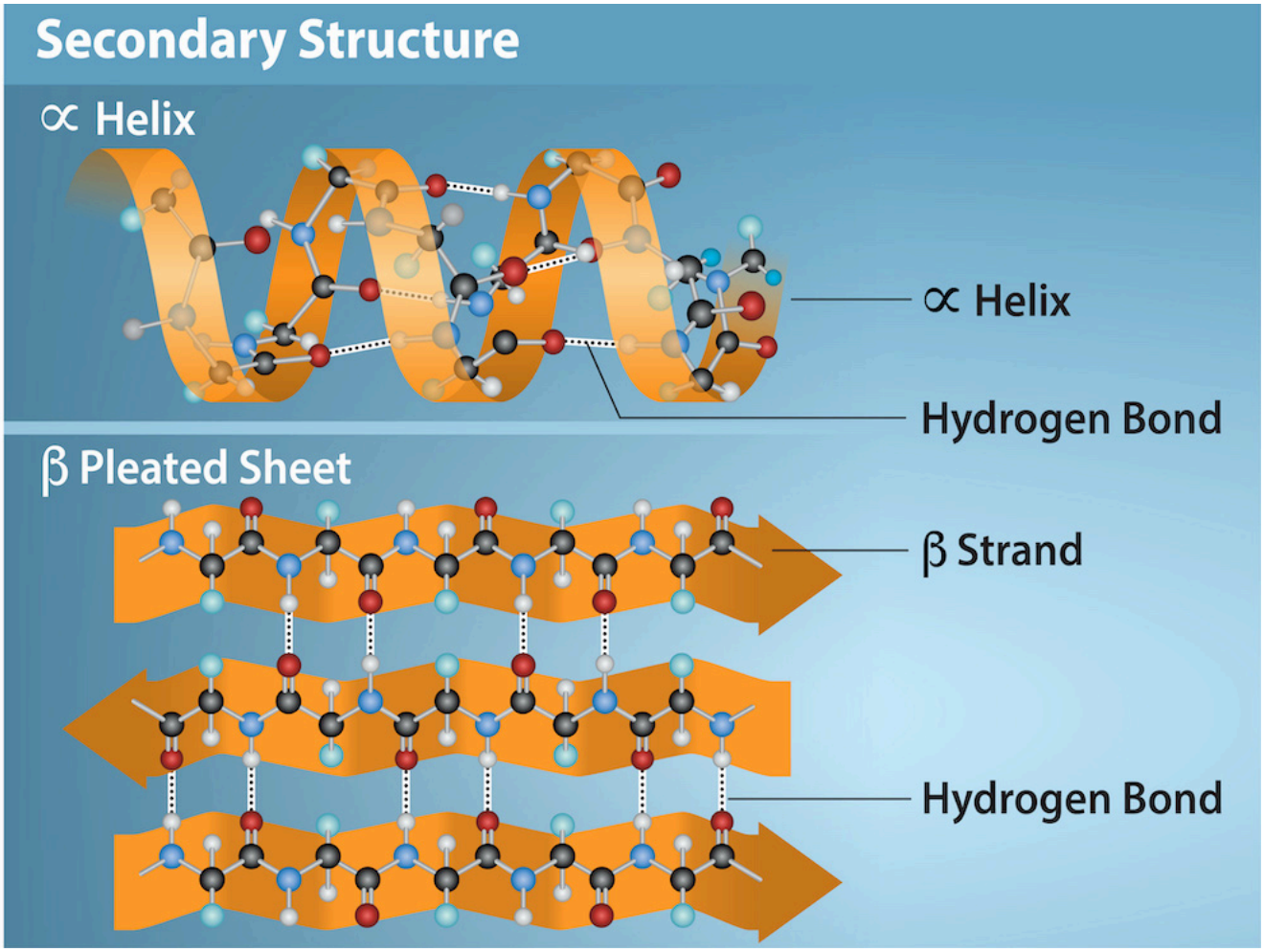


Figure 3.28 The α-helix and β-pleated sheet are secondary protein structures formed when hydrogen bonds form between the carbonyl oxygen and the amino hydrogen in the peptide backbone. Certain amino acids have a propensity to form an α-helix while others favor β-pleated sheet formation. Black = carbon, White = hydrogen, Blue = nitrogen, and Red = oxygen. Credit: Rao, A., Ryan, K. Fletcher, S. and Tag, A. Department of Biology, Texas A&M University.

Every helical turn in an alpha helix has 3.6 amino acids. The polypeptide's R groups (the variant groups) protrude out from the α-helix chain. In the β-pleated sheet, hydrogen bonding between atoms on the polypeptide chain's backbone form the "pleats". The R groups are attached to the carbons and extend above and below the pleat's folds. The pleated segments align parallel or antiparallel (in reversed chain direction) to each other, and hydrogen bonds form between the partially positive nitrogen atom in the amino group and the partially negative oxygen atom in the peptide backbone's carbonyl group. The α-helix and β-pleated sheet structures are in most globular and fibrous proteins, and they play an important structural role.

Tertiary Structure

The overall polypeptide's unique three-dimensional structure is its **tertiary structure** (Figure 3.29). This

structure is in part due to chemical interactions at work on the polypeptide chain. Primarily, the interactions among R groups create the protein's complex three-dimensional tertiary structure. For example, R groups with like charges repel each other and those with unlike charges are attracted to each other (ionic bonds). When protein folding takes place, the nonpolar amino acids' hydrophobic R groups lie in the protein's interior, whereas the hydrophilic R groups lie on the outside. Scientists also call the former interaction types hydrophobic interactions. Interaction between cysteine side chains forms disulfide linkages in the presence of oxygen, the only covalent bond that forms during protein folding.

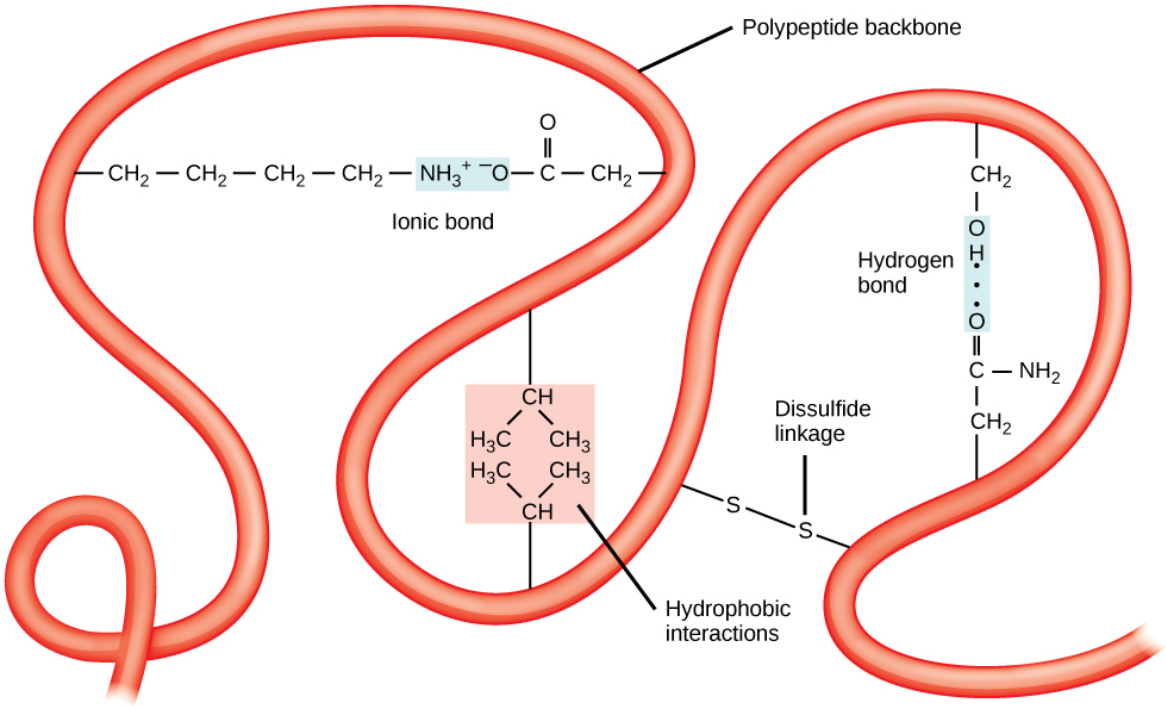


Figure 3.29 A variety of chemical interactions determine the proteins' tertiary structure. These include hydrophobic interactions, ionic bonding, hydrogen bonding, and disulfide linkages.

All of these interactions, weak and strong, determine the protein's final three-dimensional shape. When a protein loses its three-dimensional shape, it may no longer be functional.

Quaternary Structure

In nature, some proteins form from several polypeptides, or subunits, and the interaction of these subunits forms the **quaternary structure**. Weak interactions between the subunits help to stabilize the overall structure. For example, insulin (a globular protein) has a combination of hydrogen and disulfide bonds that cause it to mostly clump into a ball shape. Insulin starts out as a single polypeptide and loses some internal sequences in the presence of post-translational modification after forming the disulfide linkages that hold the

remaining chains together. Silk (a fibrous protein), however, has a β-pleated sheet structure that is the result of hydrogen bonding between different chains.

Figure 3.30 illustrates the four levels of protein structure (primary, secondary, tertiary, and quaternary).

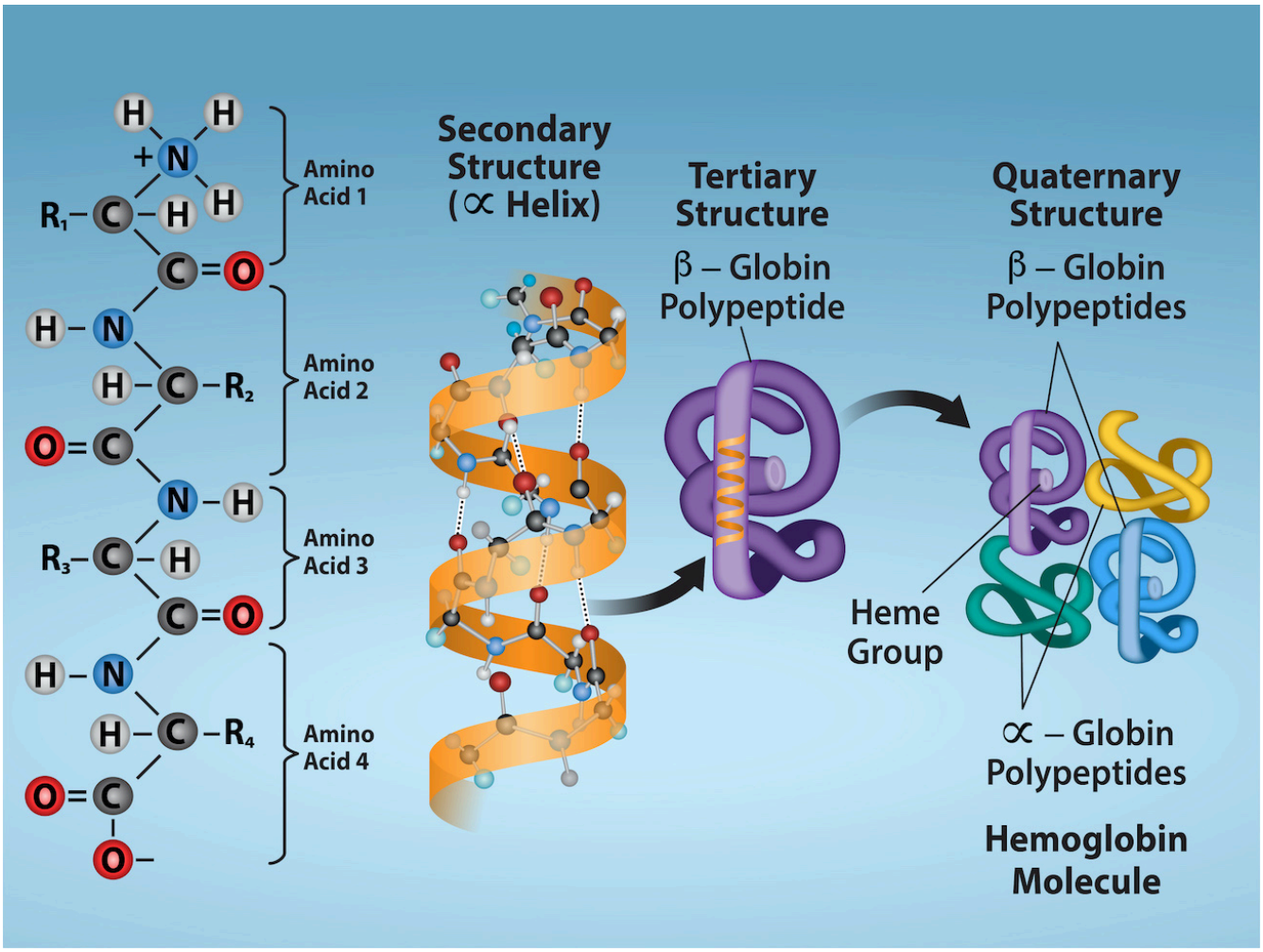


Figure 3.30 Observe the four levels of protein structure in these illustrations. Credit: Rao, A. Ryan, K. and Tag, A. Department of Biology, Texas A&M University.

Denaturation and Protein Folding

Each protein has its own unique sequence and shape that chemical interactions hold together. If the protein is subject to changes in temperature, pH, or exposure to chemicals, the protein structure may change, losing its shape without losing its primary sequence in what scientists call denaturation. Denaturation is often reversible because the polypeptide's primary structure is conserved in the process, allowing the protein to resume its function if the denaturing agent is removed. Sometimes denaturation is irreversible, leading to loss of function. One example of irreversible protein denaturation is frying an egg. The albumin protein in the liquid egg white

denatures when placed in a hot pan. Not all proteins denature at high temperatures. For instance, bacteria that survive in hot springs have proteins that function at temperatures close to boiling. The stomach is also very acidic, has a low pH, and denatures proteins as part of the digestion process; however, the stomach's digestive enzymes retain their activity under these conditions.

Protein folding is critical to its function. Scientists originally thought that the proteins themselves were responsible for the folding process. Only recently researchers discovered that often they receive assistance in the folding process from protein helpers, or **chaperones** (or chaperonins) that associate with the target protein during the folding process. They act by preventing polypeptide aggregation that comprises the complete protein structure, and they disassociate from the protein once the target protein is folded.

Link to Learning

For an additional perspective on proteins, view this animation called "Biomolecules: The Proteins."

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=179#h5p-17

23.

NUCLEIC ACIDS

Learning Objectives

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

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

Nucleic acids are the most important macromolecules for the continuity of life. They carry the cell's genetic blueprint and carry instructions for its functioning.

DNA and RNA

The two main types of nucleic acids are **deoxyribonucleic acid (DNA)** and **ribonucleic acid (RNA)**. DNA is the genetic material in all living organisms, ranging from single-celled bacteria to multicellular mammals. It is in the nucleus of eukaryotes and in their chloroplasts and mitochondria (organelles). In prokaryotes, the DNA is not enclosed in a membranous envelope.

The cell's entire genetic content is its **genome**, and the study of genomes is genomics. In eukaryotic cells but not in prokaryotes, DNA forms a complex with histone proteins to form **chromatin**, the substance of eukaryotic **chromosomes**. A chromosome may contain tens of thousands of genes. Many genes contain the information to make protein products. Other genes code for RNA products. DNA controls all of the cellular activities by turning the genes "on" or "off."

The other type of nucleic acid, RNA, is mostly involved in protein synthesis. The DNA molecules never leave the nucleus but instead use an intermediary to communicate with the rest of the cell. This intermediary

is the **messenger RNA (mRNA)**. Other types of RNA—like rRNA, tRNA, and microRNA—are involved in protein synthesis and its regulation.

DNA and RNA are comprised of monomers that scientists call **nucleotides**. The nucleotides combine with each other to form a **polynucleotide**, DNA or RNA. Three components comprise each nucleotide: a nitrogenous base, a pentose (five-carbon) sugar, and a phosphate group (Figure 3.31). Each nitrogenous base in a nucleotide is attached to a sugar molecule, which is attached to one or more phosphate groups.

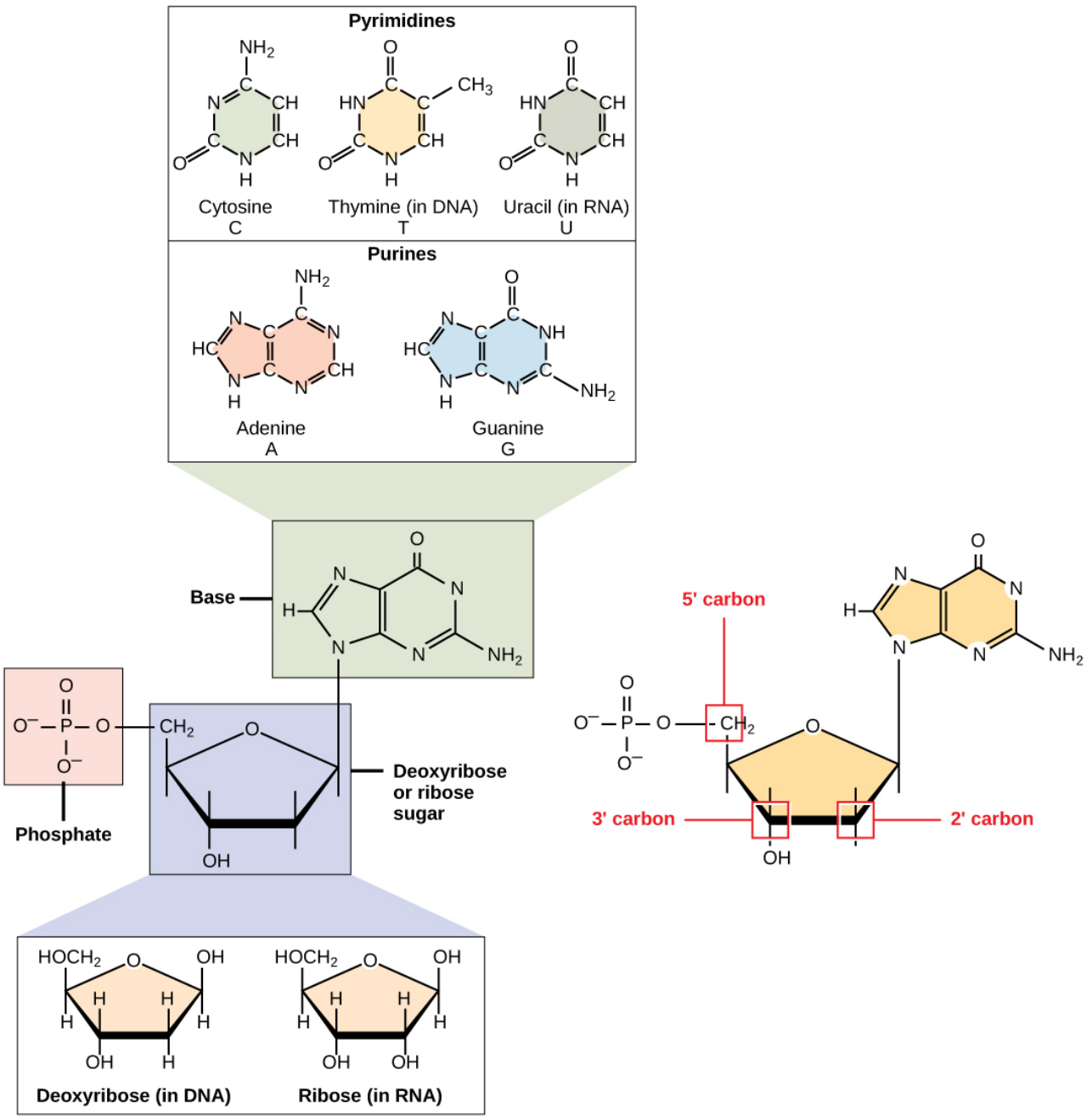


Figure 3.31 Three components comprise a nucleotide: a nitrogenous base, a pentose sugar, and one or more phosphate groups. Carbons in the pentose are numbered 1' through 5' (the prime distinguishes these from those in the base, which are numbered without using a prime notation). The base is attached to the ribose's 1' position, and the phosphate is attached to the 5' position. When a polynucleotide forms, the incoming nucleotide's 5' phosphate attaches to the 3' hydroxyl group at the end of the growing chain. Two types of pentose are in nucleotides, deoxyribose (found in DNA) and ribose (found in RNA). Deoxyribose is similar in structure to ribose, but it has an H instead of an OH at the 2' position. We can divide bases into two categories: purines and pyrimidines. Purines have a double ring structure, and pyrimidines have a single ring.

The nitrogenous bases, important components of nucleotides, are organic molecules and are so named because

they contain carbon and nitrogen. They are bases because they contain an amino group that has the potential of binding an extra hydrogen, and thus decreasing the hydrogen ion concentration in its environment, making it more basic. Each nucleotide in DNA contains one of four possible nitrogenous bases: adenine (A), guanine (G) cytosine (C), and thymine (T).

Scientists classify adenine and guanine as **purines**. The purine's primary structure is two carbon-nitrogen rings. Scientists classify cytosine, thymine, and uracil as **pyrimidines** which have a single carbon-nitrogen ring as their primary structure (Figure 3.31). Each of these basic carbon-nitrogen rings has different functional groups attached to it. In molecular biology shorthand, we know the nitrogenous bases by their symbols A, T, G, C, and U. DNA contains A, T, G, and C, whereas RNA contains A, U, G, and C.

The pentose sugar in DNA is deoxyribose, and in RNA, the sugar is ribose (Figure 3.31). The difference between the sugars is the presence of the hydroxyl group on the ribose's second carbon and hydrogen on the deoxyribose's second carbon. The carbon atoms of the sugar molecule are numbered as 1′, 2′, 3′, 4′, and 5′ (1′ is read as "one prime"). The phosphate attaches to the hydroxyl group of the 5′ carbon of one sugar and the hydroxyl group of the 3′ carbon of the sugar of the next nucleotide, which forms a 5′–3′ **phosphodiester** linkage. A simple dehydration reaction like the other linkages connecting monomers in macromolecules does not form the phosphodiester linkage. Nucleotides approaching linkage actually arrive with two extra phosphates attached to the first, which are released in the process. A polynucleotide may have thousands of such phosphodiester linkages.

DNA Double-Helix Structure

DNA has a double-helix structure (Figure 3.32). The sugar and phosphate lie on the outside of the helix, forming the DNA's backbone. The nitrogenous bases are stacked in the interior, like a set of staircase steps. Hydrogen bonds bind two bases to each other to form each step. Every base pair in the double helix is separated from the next base pair by 0.34 nm. The helix's two strands run in opposite directions, meaning that the 5′ carbon end of one strand will face the 3′ carbon end of its matching strand. (Scientists call this an antiparallel orientation and is important to DNA replication and in many nucleic acid interactions.)

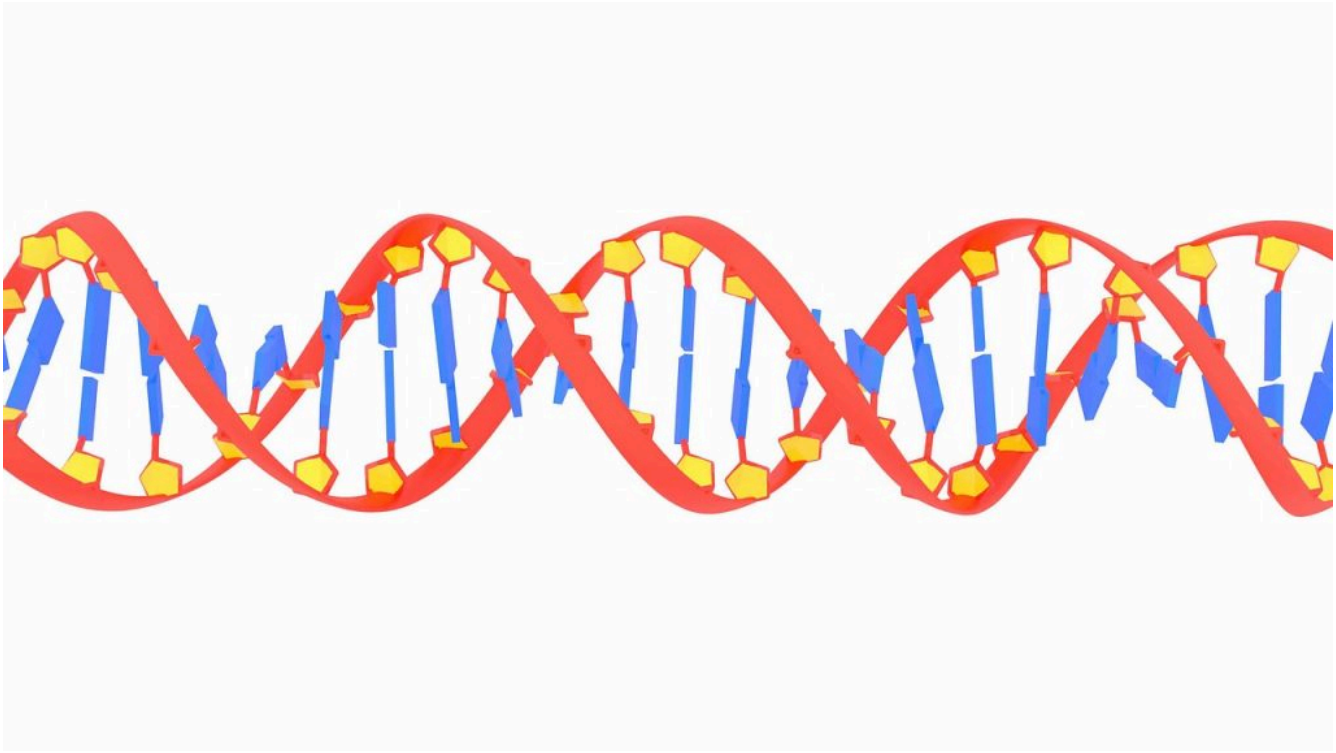


Figure 3.32 Native DNA is an antiparallel double helix. The phosphate backbone (indicated by the curvy red lines) of nucleotides is on the outside, and the bases (blue) are on the inside. Pentose sugars are in yellow. Each base from one strand interacts via hydrogen bonding with a base from the opposing strand.

Only certain types of base pairing are allowed. For example, a certain purine can only pair with a certain pyrimidine. This means A can pair with T, and G can pair with C, as Figure 3.33 shows. This is the base complementary rule. In other words, the DNA strands are complementary to each other. If the sequence of one strand is AATTGGCC, the complementary strand would have the sequence TTAACCGG. During DNA replication, each strand copies itself, resulting in a daughter DNA double helix containing one parental DNA strand and a newly synthesized strand.

Visual Connection

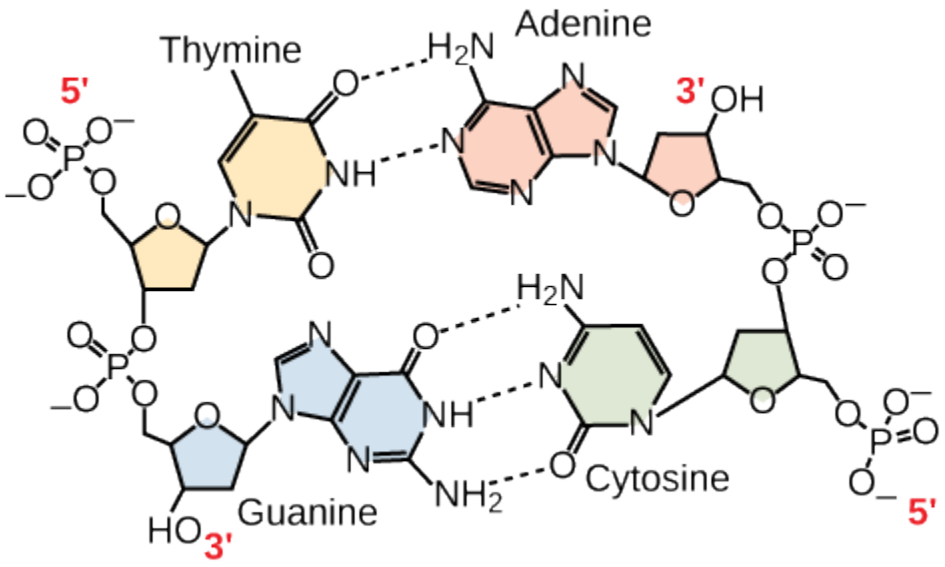


Figure 3.33 In a double stranded DNA molecule, the two strands run antiparallel to one another so that one strand runs 5' to 3' and the other 3' to 5'. The phosphate backbone is located on the outside, and the bases are in the middle. Adenine forms hydrogen bonds (or base pairs) with thymine, and guanine base pairs with cytosine.

A mutation occurs, and adenine replaces cytosine. What impact do you think this will have on the DNA structure?

RNA

Ribonucleic acid, or RNA, is mainly involved in the process of protein synthesis under the direction of DNA. RNA is usually single-stranded and is comprised of ribonucleotides that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and the phosphate group.

There are four major types of RNA: messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and microRNA (miRNA). The first, mRNA, carries the message from DNA, which controls all of the cellular activities in a cell. If a cell requires synthesizing a certain protein, the gene for this product turns "on" and the messenger RNA is synthesized in the nucleus. The RNA base sequence is complementary to the DNA's coding sequence from which it has been copied. However, in RNA, the base T is absent and U is present instead. If the DNA strand has a sequence AATTGCGC, the sequence of the complementary RNA is UUAACGCG. In the cytoplasm, the mRNA interacts with ribosomes and other cellular machinery

(Figure 3.34).

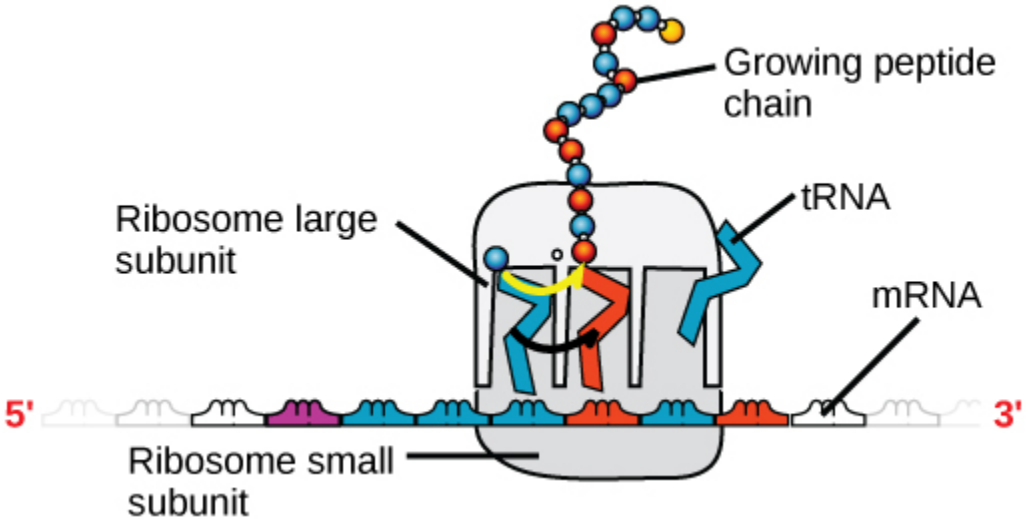


Figure 3.34 A ribosome has two parts: a large subunit and a small subunit. The mRNA sits in between the two subunits. A tRNA molecule recognizes a codon on the mRNA, binds to it by complementary base pairing, and adds the correct amino acid to the growing peptide chain.

The mRNA is read in sets of three bases known as codons. Each codon codes for a single amino acid. In this way, the mRNA is read and the protein product is made. **Ribosomal RNA (rRNA)** is a major constituent of ribosomes on which the mRNA binds. The rRNA ensures the proper alignment of the mRNA and the Ribosomes. The ribosome's rRNA also has an enzymatic activity (peptidyl transferase) and catalyzes peptide bond formation between two aligned amino acids. **Transfer RNA (tRNA)** is one of the smallest of the four types of RNA, usually 70–90 nucleotides long. It carries the correct amino acid to the protein synthesis site. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to insert itself in the polypeptide chain. MicroRNAs are the smallest RNA molecules and their role involves regulating gene expression by interfering with the expression of certain mRNA messages. Table 3.2 summarizes DNA and RNA features.

DNA and RNA Features

	DNA	RNA
Function	Carries genetic information	Involved in protein synthesis
Location	Remains in the nucleus	Leaves the nucleus
Structure	Double helix	Usually single-stranded
Sugar	Deoxyribose	Ribose
Pyrimidines	Cytosine, thymine	Cytosine, uracil
Purines	Adenine, guanine	Adenine, guanine

Table 3.2

Even though the RNA is single stranded, most RNA types show extensive intramolecular base pairing between complementary sequences, creating a predictable three-dimensional structure essential for their function.

As you have learned, information flow in an organism takes place from DNA to RNA to protein. DNA dictates the structure of mRNA in a process scientists call **transcription**, and RNA dictates the protein's structure in a process scientists call **translation**. This is the Central Dogma of Life, which holds true for all organisms; however, exceptions to the rule occur in connection with viral infections.

Link to Learning

To learn more about DNA, explore the Howard Hughes Medical Institute BioInteractive animations on the topic of DNA.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=181#h5p-18

24.

KEY TERMS

active site
: location on an enzyme configured to bind a substrate

alpha-helix structure (*α*-helix)
: type of secondary protein structure formed by folding the polypeptide into a helix shape with hydrogen bonds stabilizing the structure

amino acid
: a protein's monomer; has a central carbon or alpha carbon to which an amino group, a carboxyl group, a hydrogen, and an R group or side chain are attached; the R group is different for all 20 common amino acids

beta-pleated sheet (*β*-pleated)
: secondary structure in proteins in which hydrogen bonding forms "pleats" between atoms on the polypeptide chain's backbone

biological macromolecule
: large molecule necessary for life that is built from smaller organic molecules

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

cellulose
: polysaccharide that comprises the plant's cell wall; provides structural support to the cell

chaperone
: (also, chaperonin) protein that helps nascent protein in the folding process

chitin
: type of carbohydrate that forms the outer skeleton of all arthropods including crustaceans and insects; it also forms fungi cell walls

chromatin
: complex of histone proteins and DNA, the substance of eukaryotic chromosomes

chromosome
: structure composed of DNA and histone proteins, containing genetic information

dehydration synthesis
: (also, condensation) reaction that links monomer molecules, releasing a water molecule for each bond

formed

denaturation

loss of shape in a protein as a result of changes in temperature, pH, or chemical exposure

deoxyribonucleic acid (DNA)

double-helical molecule that carries the cell's hereditary information

disaccharide

two sugar monomers that a glycosidic bond links

enzyme

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

genome

a cell or organism's entire genetic content

glycogen

storage carbohydrate in animals

glycosidic bond

bond formed by a dehydration reaction between two monosaccharides, involving eliminating a water molecule

hormone

chemical signaling molecule, usually protein or steroid, secreted by endocrine cells that act to control or regulate specific physiological processes

hydrolysis

reaction that causes breakdown of larger molecules into smaller molecules by utilizing water

hydrolysis reactions

breaking a large molecule into smaller molecules by adding and splitting a water molecule

lipid

macromolecule that is nonpolar and insoluble in water

messenger RNA (mRNA)

RNA that carries information from DNA to ribosomes during protein synthesis

monomer

smallest unit of larger molecules that are polymers

monosaccharide

single unit or monomer of carbohydrates

nucleic acid

biological macromolecule that carries the cell's genetic blueprint and carries instructions for the cell's functioning

nucleotide

monomer of nucleic acids; contains a pentose sugar, one or more phosphate groups, and a nitrogenous base

omega fat
type of polyunsaturated fat that the body requires; numbering the carbon, omega starts from the methyl end, or the end that is farthest from the carboxylic end

peptide bond
bond formed between two amino acids by a dehydration reaction

phosphodiester
linkage covalent chemical bond that holds together the polynucleotide chains with a phosphate group linking neighboring nucleotides' two pentose sugars

phospholipid
membranes' major constituent; comprised of two fatty acids and a phosphate-containing group attached to a glycerol backbone

polymer
chain of monomers that covalent bonds link; polymerization is the process of polymer formation from monomers by condensation

polynucleotide
long chain of nucleotides

polypeptide
long chain of amino acids that peptide bonds link

polysaccharide
long chain of monosaccharides; may be branched or unbranched

primary structure
linear sequence of amino acids in a protein

protein
biological macromolecule comprised of one or more amino acid chains

purine
type of nitrogenous base in DNA and RNA; adenine and guanine are purines

pyrimidine
type of nitrogenous base in DNA and RNA; cytosine, thymine, and uracil are pyrimidines

quaternary structure
arrangement of polypeptide subunits within a protein that has multiple subunits

ribonucleic acid (RNA)
single-stranded, often internally base paired, molecule that is involved in protein synthesis

ribosomal RNA (rRNA)
RNA that ensures the proper alignment of mRNA and ribosomes during protein synthesis and catalyzes formation of the peptide linkage

saturated fatty acid
long-chain hydrocarbon with single covalent bonds in the carbon chain; the number of hydrogen atoms

attached to the carbon skeleton is maximized

secondary structure

regular structure that proteins form by intramolecular hydrogen bonding between the oxygen atom of one amino acid and the hydrogen attached to the nitrogen atom of another

starch

storage carbohydrate in plants

steroid

type of lipid comprised of four fused hydrocarbon rings forming a planar structure

tertiary structure

a protein's three-dimensional conformation, including interactions between secondary structural elements; formed from interactions between amino acid side chains

trans fat

fat formed artificially by hydrogenating oils, leading to a different arrangement of double bond(s) than those in naturally occurring lipids

transcription

process through which messenger RNA forms on a template of DNA

transfer RNA (tRNA)

RNA that carries activated amino acids to the site of protein synthesis on the ribosome

translation

process through which RNA directs the protein's formation

triacylglycerol (also, triglyceride)

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

unsaturated fatty acid

long-chain hydrocarbon that has one or more double bonds in the hydrocarbon chain

wax

lipid comprised of a long-chain fatty acid that is esterified to a long-chain alcohol; serves as a protective coating on some feathers, aquatic mammal fur, and leaves

25.

CHAPTER SUMMARY

3.1 Synthesis of Biological Macromolecules

Proteins, carbohydrates, nucleic acids, and lipids are the four major classes of biological macromolecules—large molecules necessary for life that are built from smaller organic molecules. Macromolecules are comprised of single units scientists call monomers that are joined by covalent bonds to form larger polymers. A monomer joins with another monomer in a process that generates a water molecule and forms a covalent bond linking the monomers. Scientists call these dehydration or condensation reactions. When polymers break down into smaller units (monomers), they consume a water molecule in each breaking of a bond by these reactions. Such reactions are hydrolysis reactions. Dehydration and hydrolysis reactions are similar for all macromolecules, but each monomer and polymer reaction is specific to its class. Dehydration reactions typically require an investment of energy for new bond formation, while hydrolysis reactions typically release energy by breaking bonds.

3.2 Carbohydrates

Carbohydrates are a group of macromolecules that are a vital energy source for the cell and provide structural support to plant cells, fungi, and all of the arthropods, including lobsters, crabs, shrimp, insects, and spiders. Scientists classify carbohydrates as monosaccharides, disaccharides, and polysaccharides depending on the number of monomers in the molecule. Monosaccharides are linked by glycosidic bonds that form as a result of dehydration reactions, forming disaccharides and polysaccharides while forming a water molecule for each bond formed. Glucose, galactose, and fructose are common monosaccharides, whereas common disaccharides include lactose, maltose, and sucrose. Starch and glycogen, examples of polysaccharides, are the storage forms of glucose in plants and animals, respectively. The long polysaccharide chains may be branched or unbranched. Cellulose is an example of an unbranched polysaccharide, whereas amylopectin, a constituent of starch, is a highly branched molecule. Glucose storage, in the form of polymers like starch or glycogen, makes it slightly less accessible for metabolism; however, this prevents it from leaking out of the cell.

3.3 Lipids

Lipids are a class of macromolecules that are nonpolar and hydrophobic in nature. Major types include fats and oils, waxes, phospholipids, and steroids. Fats are a stored form of energy and are also known as triacylglycerols or triglycerides. Fats are comprised of fatty acids and either glycerol or sphingosine. Fatty acids may be unsaturated or saturated, depending on the presence or absence of double bonds in the hydrocarbon chain. If only single bonds are present, they are saturated fatty acids. Unsaturated fatty acids have one or more double bonds in the hydrocarbon chain. Phospholipids comprise any cellular membrane's matrix. They have a glycerol or sphingosine backbone to which two fatty acid chains and a phosphate-containing group are attached. Steroids are another class of lipids. Their basic structure has four fused carbon rings. Cholesterol is a type of steroid and is an important constituent of the plasma membrane, where it helps to maintain the membrane's fluid nature. It is also the precursor of steroid hormones such as testosterone.

3.4 Proteins

Proteins are a class of macromolecules that perform a diverse range of functions for the cell. They help in metabolism by acting as enzymes, carriers, or hormones, and provide structural support. The building blocks of proteins (monomers) are amino acids. Each amino acid has a central carbon that bonds to an amino group, a carboxyl group, a hydrogen atom, and an R group or side chain. There are 20 commonly occurring amino acids, each of which differs in the R group. A peptide bond links each amino acid to its neighbors. A long amino acid chain is a polypeptide.

Proteins are organized at four levels: primary, secondary, tertiary, and (sometimes) quaternary. The primary structure is the unique sequence of amino acids in the polypeptide. The polypeptide's local folding to form structures such as the α-helix and β-pleated sheet constitutes the secondary structure. The overall three-dimensional structure is the tertiary structure. When two or more polypeptides combine to form the complete protein structure, the configuration is the protein's quaternary structure. Protein shape and function are intricately linked. Any change in shape caused by changes in temperature or pH may lead to protein denaturation and a loss in function.

3.5 Nucleic Acids

Nucleic acids are molecules comprised of nucleotides that encode the information of amino acid sequence for protein synthesis. A pentose sugar, a nitrogenous base, and a phosphate group comprise each nucleotide. There are two types of nucleic acids: DNA and RNA. DNA carries the cell's genetic blueprint and passes it on from parents to offspring (in the form of chromosomes). It has a double-helical structure with the two strands running in opposite directions, connected by hydrogen bonds, and complementary to each other.

RNA is a single-stranded polymer composed of linked nucleotides made up of a pentose sugar (ribose), a nitrogenous base, and a phosphate group. RNA is involved in protein synthesis and its regulation. Messenger RNA (mRNA) copies from the DNA, exports itself from the nucleus to the cytoplasm, and contains information for constructing proteins. Ribosomal RNA (rRNA) is a part of the ribosomes at the site of protein synthesis, whereas transfer RNA (tRNA) carries the amino acid to the site of protein synthesis. MicroRNA regulates translation when mRNA is used for protein synthesis.

26.

VISUAL CONNECTION QUESTIONS

1. Figure 3.5 What kind of sugars are these, aldose or ketose?

2. Figure 3.23 Which categories of amino acid would you expect to find on the surface of a soluble protein, and which would you expect to find in the interior? What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?

3. Figure 3.33 A mutation occurs, and cytosine is replaced with adenine. What impact do you think this will have on the DNA structure?

27.

REVIEW QUESTIONS

4. Dehydration synthesis leads to formation of

a. monomers
b. polymers
c. water and polymers
d. none of the above

5. During the breakdown of polymers, which of the following reactions takes place?

a. hydrolysis
b. dehydration
c. condensation
d. covalent bond

6. The following chemical reactants produce the ester ethyl ethanoate ($C_4H_8O_2$):
$C_2H_6O + CH_3COOH$
What type of reaction occurs to make ethyl ethanoate?

a. condensation
b. hydrolysis
c. combustion
d. acid-base reaction

7. An example of a monosaccharide is _____.

a. fructose
b. glucose
c. galactose
d. all of the above

8. Cellulose and starch are examples of:

a. monosaccharides
b. disaccharides
c. lipids
d. polysaccharides

9. Plant cell walls contain which of the following in abundance?

a. starch
b. cellulose
c. glycogen
d. lactose

10. Lactose is a disaccharide formed by the formation of a _____ bond between glucose and _____.

a. glycosidic; lactose
b. glycosidic; galactose
c. hydrogen; sucrose
d. hydrogen; fructose

11. Which of the following is not a role of carbohydrates?

a. protect an insect's internal organs from external trauma
b. prevent plant cells from lysing after the plant is watered
c. maintain the shape of an animal cell, such as a fish or cat
d. provide energy for muscle movement

12. Saturated fats have all of the following characteristics except:

a. they are solid at room temperature
b. they have single bonds within the carbon chain
c. they are usually obtained from animal sources
d. they tend to dissolve in water easily

13. Phospholipids are important components of _____.

a. the plasma membrane of cells
b. the ring structure of steroids
c. the waxy covering on leaves

d. the double bond in hydrocarbon chains

14. Cholesterol is an integral part of plasma membranes. Based on its structure, where is it found in the membrane?

a. on the extracellular surface
b. embedded with the phospholipid heads
c. within the tail bilayer
d. attached to the intracellular surface

15. The monomers that make up proteins are called _____.

a. nucleotides
b. disaccharides
c. amino acids
d. chaperones

16. The α-helix and the β-pleated sheet are part of which protein structure?

a. primary
b. secondary
c. tertiary
d. quaternary

17. Mad cow disease is an infectious disease where one misfolded protein causes all other copies of the protein to begin misfolding. This is an example of a disease impacting ____ structure.

a. primary
b. secondary
c. tertiary
d. quaternary

18. A nucleotide of DNA may contain _____.

a. ribose, uracil, and a phosphate group
b. deoxyribose, uracil, and a phosphate group
c. deoxyribose, thymine, and a phosphate group
d. ribose, thymine, and a phosphate group

19. The building blocks of nucleic acids are _____.

a. sugar
b. nitrogenous bases
c. peptides
d. nucleotides

20. How does the double helix structure of DNA support its role in encoding the genome?

a. The sugar-phosphate backbone provides a template for DNA replication.
b. tRNA pairing with the template strand creates proteins encoded by the genome.
c. Complementary base pairing creates a very stable structure.
d. Complementary base pairing allows for easy editing of both strands of DNA.

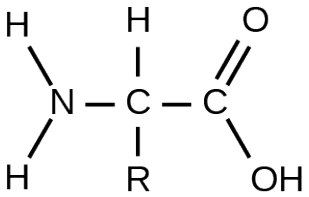
28.

CRITICAL THINKING QUESTIONS

21. Why are biological macromolecules considered organic?

22. Soil covers a vast proportion of earth's land surface, and is composed of both organic and inorganic components. By what process might it acquire its organic content? Propose an answer, describing this process in as much detail as you can imagine.

23. Amino acids have the generic structure seen below, where R represents different carbon-based side chains.



Describe how the structure of amino acids allows them to be linked into long peptide chains to form proteins.

24. Describe the similarities and differences between glycogen and starch.

25. Why is it impossible for humans to digest food that contains cellulose?

26. Draw the ketose and aldose forms of a monosaccharide with the chemical formula $C_3H_6O_3$. How is the structure of the monosaccharide changed from one form to the other in the human body?

27. Explain at least three functions that lipids serve in plants and/or animals.

28. Why have trans fats been banned from some restaurants? How are they created?

29. Why are fatty acids better than glycogen for storing large amounts of chemical energy?

30. Part of cortisol's role in the body involves passing through the plasma membrane to initiate signaling inside a cell. Describe how the structures of cortisol and the plasma membrane allow this to occur.

31. Explain what happens if even one amino acid is substituted for another in a polypeptide chain. Provide a specific example.

32. Describe the differences in the four protein structures.

33. Aquaporins are proteins embedded in the plasma membrane that allow water molecules to move between the extracellular matrix and the intracellular space. Based on its function and location, describe the key features of the protein's shape and the chemical characteristics of its amino acids.

34. The information coding strategy employed by DNA is analogous to that used by a written alphabet. DNA varies the order of 4 bases (which we symbolize ATGC) to record information. How many symbols

would you say are used by the English alphabet to record information? How many are used in mathematics to code numerical information? How many are used to code information within computer circuits?

35. What are the four types of RNA and how do they function?

PART IV
CELL STRUCTURE

29.

INTRODUCTION

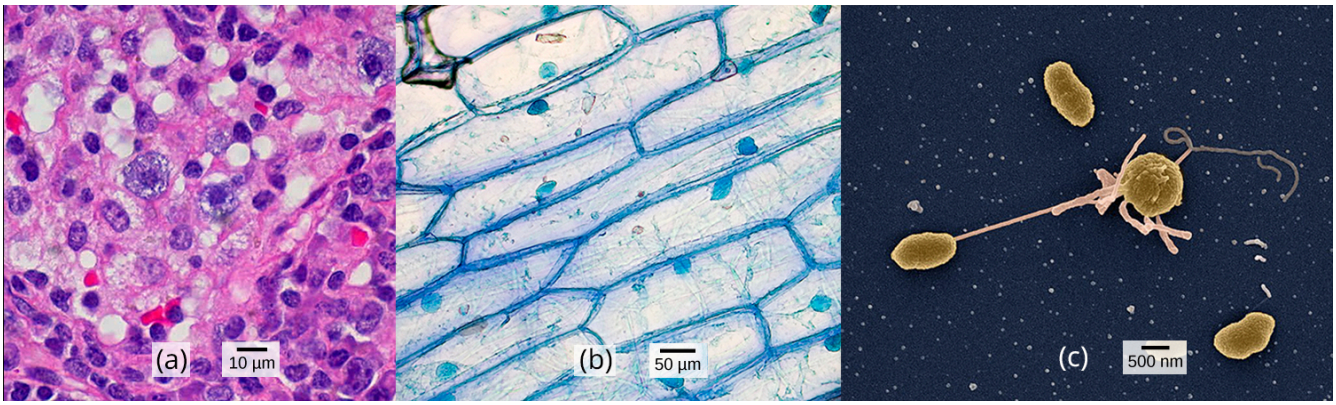


Figure 4.1 (a) Nasal sinus cells (viewed with a light microscope), (b) onion cells (viewed with a light microscope), and (c) Vibrio tasmaniensis bacterial cells (seen through a scanning electron microscope) are from very different organisms, yet all share certain basic cell structure characteristics. (credit a: modification of work by Ed Uthman, MD; credit b: modification of work by Umberto Salvagnin; credit c: modification of work by Anthony D'Onofrio, William H. Fowle, Eric J. Stewart, and Kim Lewis of the Lewis Lab at Northeastern University; scale-bar data from Matt Russell)

Close your eyes and picture a brick wall. What is the wall's basic building block? It is a single brick. Like a brick wall, cells are the building blocks that make up your body.

Your body has many kinds of cells, each specialized for a specific purpose. Just as we use a variety of materials to build a home, the human body is constructed from many cell types. For example, epithelial cells protect the body's surface and cover the organs and body cavities within. Bone cells help to support and protect the body. Immune system cells fight invading bacteria. Additionally, blood and blood cells carry nutrients and oxygen throughout the body while removing carbon dioxide. Each of these cell types plays a vital role during the body's growth, development, and day-to-day maintenance. In spite of their enormous variety, however, cells from all organisms—even ones as diverse as bacteria, onion, and human—share certain fundamental characteristics.

30.

STUDYING CELLS

Learning Objectives

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

- Describe the role of cells in organisms
- Compare and contrast light microscopy and electron microscopy
- Summarize cell theory

A cell is the smallest unit of a living thing. Whether comprised of one cell (like bacteria) or many cells (like a human), we call it an organism. Thus, cells are the basic building blocks of all organisms.

Several cells of one kind that interconnect with each other and perform a shared function form tissues. These tissues combine to form an organ (your stomach, heart, or brain), and several organs comprise an organ system (such as the digestive system, circulatory system, or nervous system). Several systems that function together form an organism (like a human being). Here, we will examine the structure and function of cells.

There are many types of cells, which scientists group into one of two broad categories: prokaryotic and eukaryotic. For example, we classify both animal and plant cells as eukaryotic cells, whereas we classify bacterial cells as prokaryotic. Before discussing the criteria for determining whether a cell is prokaryotic or eukaryotic, we will first examine how biologists study cells.

Microscopy

Cells vary in size. With few exceptions, we cannot see individual cells with the naked eye, so scientists use microscopes (micro- = "small"; -scope = "to look at") to study them. A **microscope** is an instrument that magnifies an object. We photograph most cells with a microscope, so we can call these images micrographs.

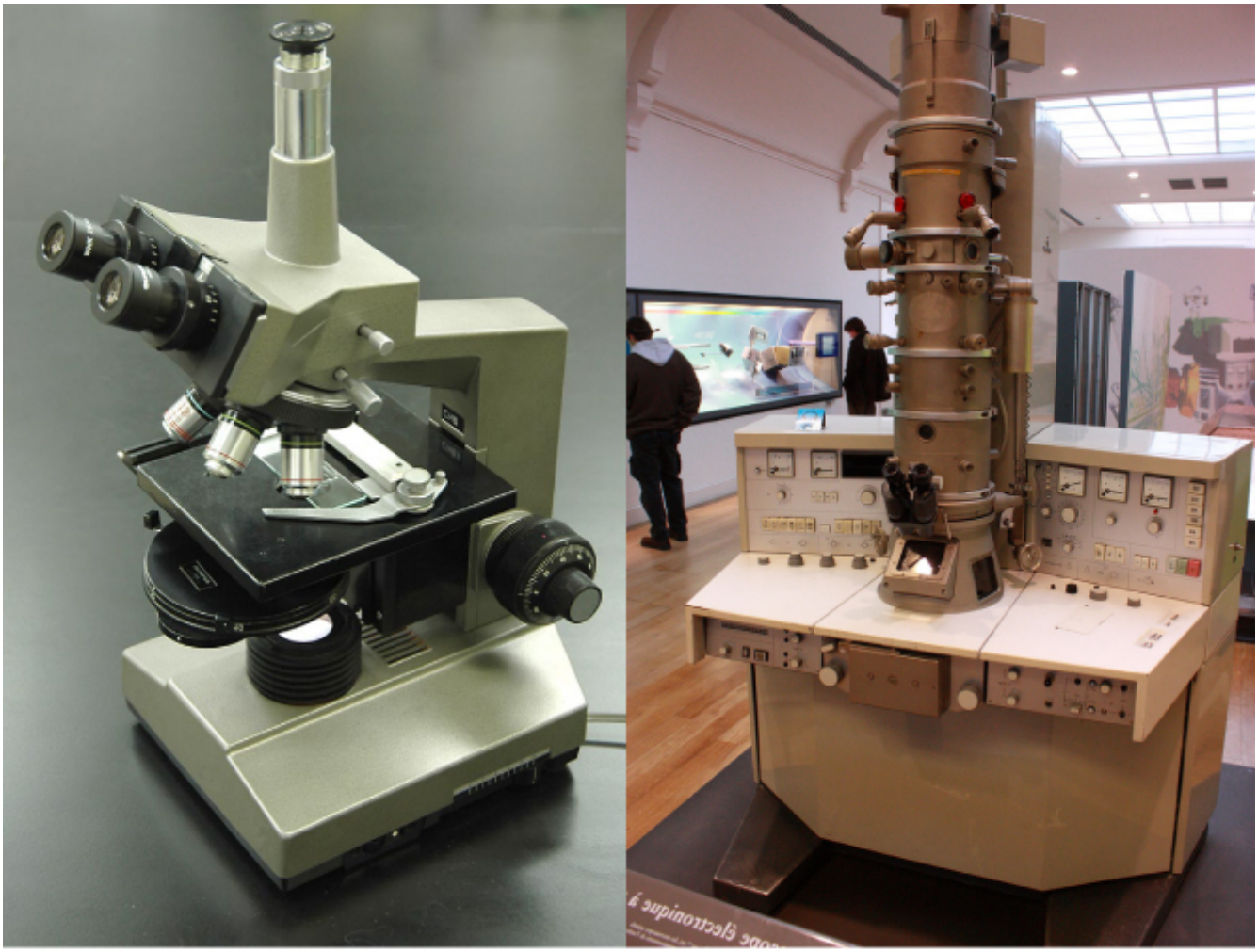
The optics of a microscope's lenses change the image orientation that the user sees. A specimen that is right-side up and facing right on the microscope slide will appear upside-down and facing left when one views through a microscope, and vice versa. Similarly, if one moves the slide left while looking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

Light Microscopes

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

Most student microscopes are **light microscopes** (Figure 4.2a). Visible light passes and bends through the lens system to enable the user to see the specimen. Light microscopes are advantageous for viewing living organisms, but since individual cells are generally transparent, their components are not distinguishable unless they are colored with special stains. Staining, however, usually kills the cells.

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



(a) (b)

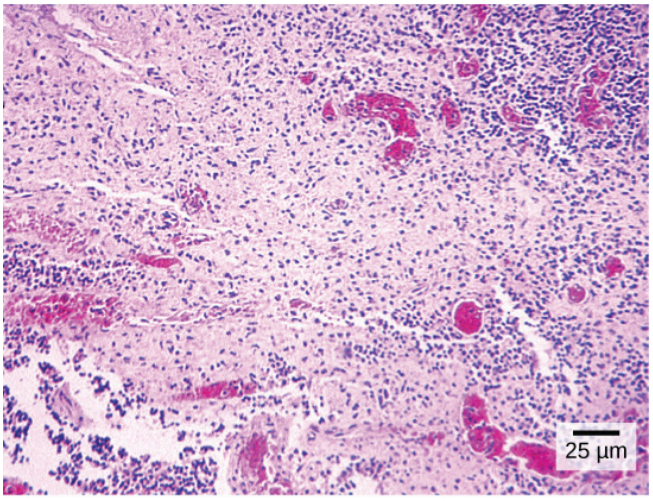
Figure 4.2 (a) Most light microscopes in a college biology lab can magnify cells up to approximately 400 times and have a resolution of about 200 nanometers. (b) Electron microscopes provide a much higher magnification, 100,000x, and have a resolution of 50 picometers. (credit a: modification of work by "GcG"/Wikimedia Commons; credit b: modification of work by Evan Bench)

Electron Microscopes

In contrast to light microscopes, **electron microscopes** (Figure 4.2b) use a beam of electrons instead of a beam of light. Not only does this allow for higher magnification and, thus, more detail (Figure 4.3), it also provides higher resolving power. The method to prepare the specimen for viewing with an electron microscope kills the specimen. Electrons have short wavelengths (shorter than photons) that move best in a vacuum, so we cannot view living cells with an electron microscope.

In a scanning electron microscope, a beam of electrons moves back and forth across a cell's surface, creating details of cell surface characteristics. In a transmission electron microscope, the electron beam penetrates

the cell and provides details of a cell's internal structures. As you might imagine, electron microscopes are significantly more bulky and expensive than light microscopes.



(a)

(b)

Figure 4.3 (a) These Salmonella bacteria appear as tiny purple dots when viewed with a light microscope. (b) This scanning electron microscope micrograph shows Salmonella bacteria (in red) invading human cells (yellow). Even though subfigure (b) shows a different Salmonella specimen than subfigure (a), you can still observe the comparative increase in magnification and detail. (credit a: modification of work by CDC/Armed Forces Institute of Pathology, Charles N. Farmer, Rocky Mountain Laboratories; credit b: modification of work by NIAID, NIH; scale-bar data from Matt Russell)

Link to Learning

For another perspective on cell size, try the HowBig interactive at this site.

Cell Theory

The microscopes we use today are far more complex than those that Dutch shopkeeper Antony van Leeuwenhoek used in the 1600s. Skilled in crafting lenses, van Leeuwenhoek observed the movements of single-celled organisms, which he collectively termed "animalcules."

In the 1665 publication *Micrographia*, experimental scientist Robert Hooke coined the term "cell" for the box-like structures he observed when viewing cork tissue through a lens. In the 1670s, van Leeuwenhoek

discovered bacteria and protozoa. Later advances in lenses, microscope construction, and staining techniques enabled other scientists to see some components inside cells.

By the late 1830s, botanist Matthias Schleiden and zoologist Theodor Schwann were studying tissues and proposed the **unified cell theory**, which states that one or more cells comprise all living things, the cell is the basic unit of life, and new cells arise from existing cells. Rudolf Virchow later made important contributions to this theory.

Career Connection

Cytotechnologist

Have you ever heard of a medical test called a Pap smear (Figure 4.4)? In this test, a doctor takes a small sample of cells from the patient's uterine cervix and sends it to a medical lab where a cytotechnologist stains the cells and examines them for any changes that could indicate cervical cancer or a microbial infection.

Cytotechnologists (cyto- = "cell") are professionals who study cells via microscopic examinations and other laboratory tests. They are trained to determine which cellular changes are within normal limits and which are abnormal. Their focus is not limited to cervical cells. They study cellular specimens that come from all organs. When they notice abnormalities, they consult a pathologist, a medical doctor who interprets and diagnoses changes that disease in body tissue and fluids cause.

Cytotechnologists play a vital role in saving people's lives. When doctors discover abnormalities early, a patient's treatment can begin sooner, which usually increases the chances of a successful outcome.

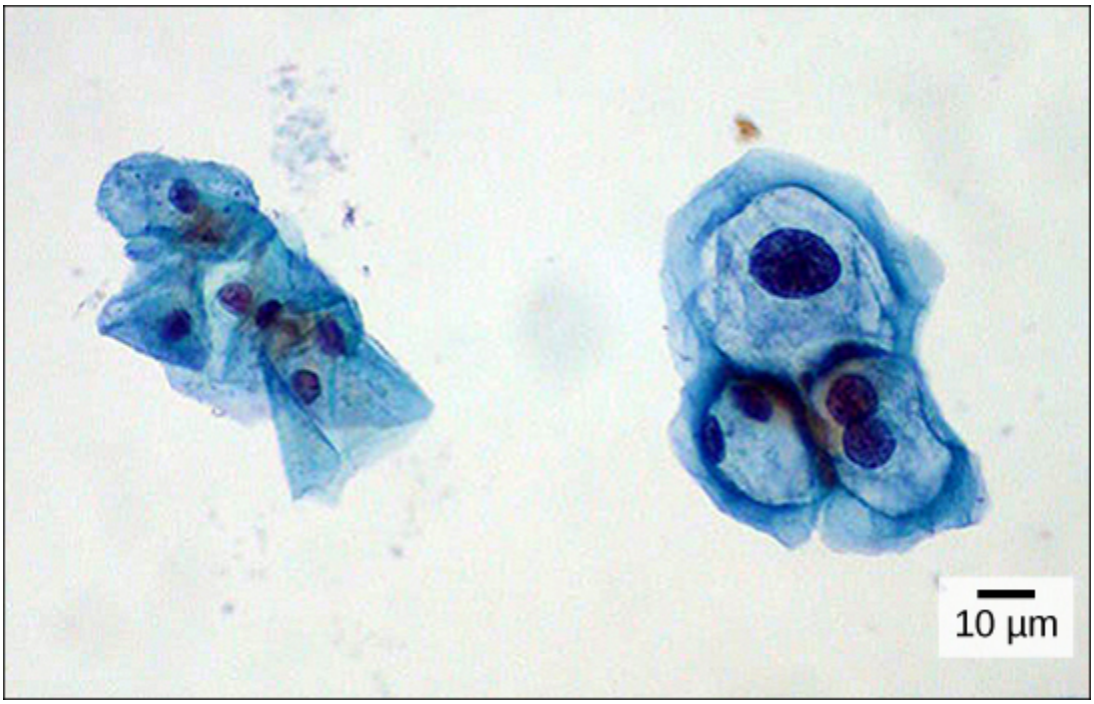


Figure 4.4 These uterine cervix cells, viewed through a light microscope, are from a Pap smear. Normal cells are on the left. The cells on the right are infected with human papillomavirus (HPV). Notice that the infected cells are larger. Also, two of these cells each have two nuclei instead of one, the normal number. (credit: modification of work by Ed Uthman, MD; scale-bar data from Matt Russell)

31.

PROKARYOTIC CELLS

Learning Objectives

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

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

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

Components of Prokaryotic Cells

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

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

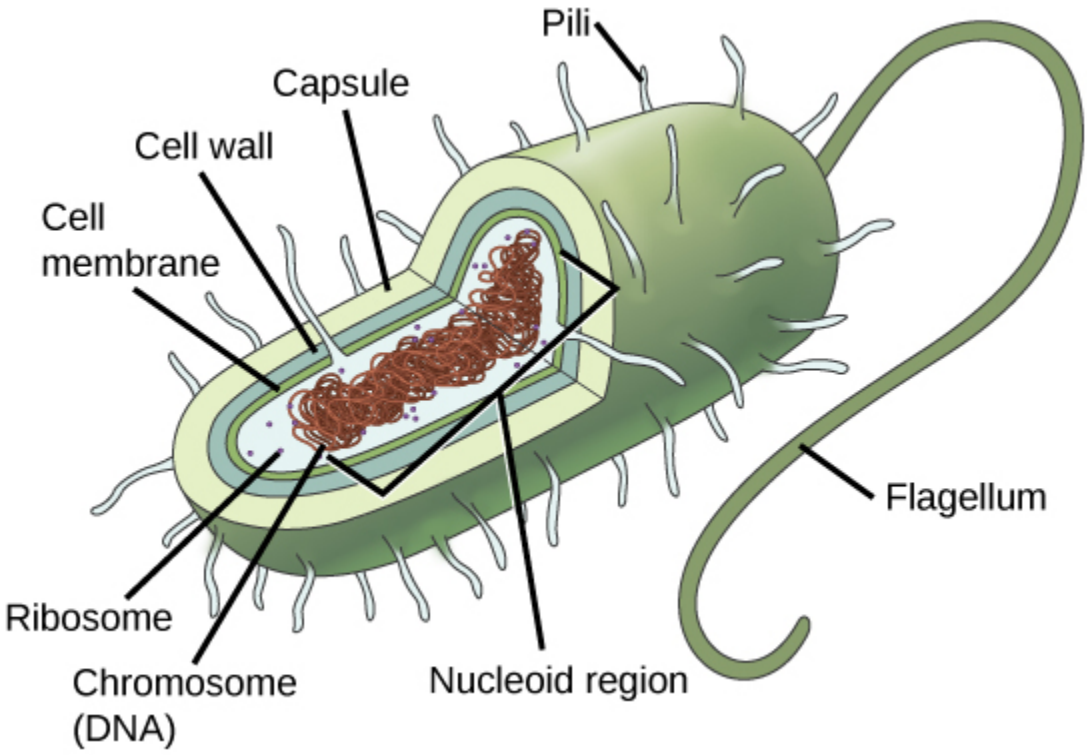


Figure 4.5 This figure shows the generalized structure of a prokaryotic cell. All prokaryotes have chromosomal DNA localized in a nucleoid, ribosomes, a cell membrane, and a cell wall. The other structures shown are present in some, but not all, bacteria.

There are two groups of organisms that are composed of prokaryotic cells: Bacteria and Archaea. Bacteria are diverse, but all have a cell wall composed of a carbohydrate and protein polymer called peptidoglycan and many have a polysaccharide capsule (Figure 4.5). Archaeans also have a cell wall, but it is composed of different molecules and does not contain peptidoglycan. The cell wall acts as a protective layer, 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. A flagellum is a single strand of protein that spins like a propeller and is used for locomotion. Prokaryotes may have a single or multiple flagella. Pili allow prokaryotes to adhere to each other to allow for the exchange of genetic material during conjugation, the process by which one bacterium transfers genetic material to another through direct contact. Bacteria use fimbriae to attach to a host cell.

The DNA of a prokaryote is arranged in a single circular chromosome that is found in the middle of the cell in a region called the nucleoid. Prokaryotes also all possess a cell membrane, cytoplasm and ribosomes; these will be considered in more detail in the next section.

Career Connection

Microbiologist

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

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

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

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

Cell Size

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

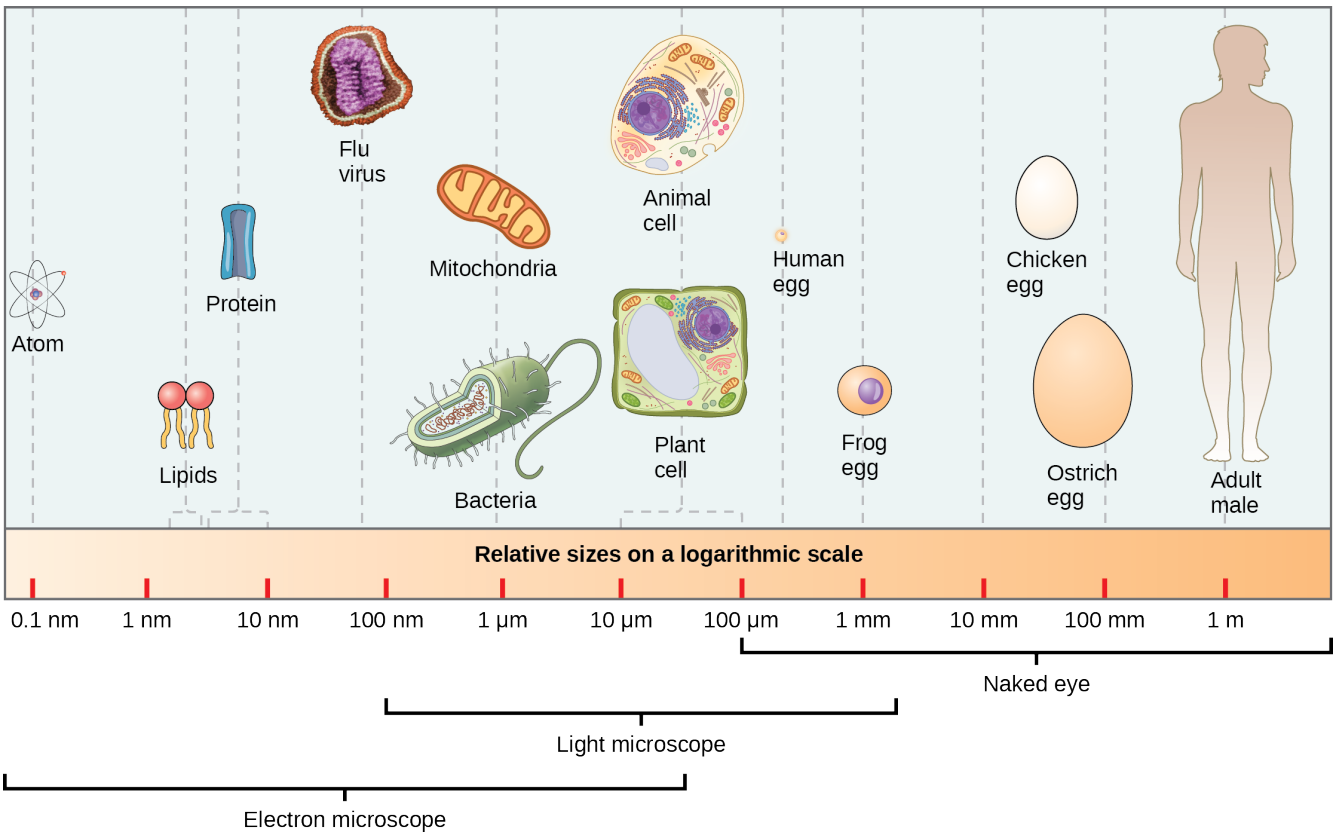


Figure 4.6 This figure shows relative sizes of microbes on a logarithmic scale (recall that each unit of increase in a logarithmic scale represents a 10-fold increase in the quantity measured).

Small size, in general, is necessary for all cells, whether prokaryotic or eukaryotic. Let's examine why that is so. First, we'll consider the area and volume of a typical cell. Not all cells are spherical in shape, but most tend to approximate a sphere. You may remember from your high school geometry course that the formula for the surface area of a sphere is $4\pi r^2$, while the formula for its volume is $4\pi r^3/3$. Thus, as the radius of a cell increases, its surface area increases as the square of its radius, but its volume increases as the cube of its radius (much more rapidly). Therefore, as a cell increases in size, its surface area-to-volume ratio decreases. This same principle would apply if the cell had a cube shape (Figure 4.7). If the cell grows too large, the plasma membrane will not have sufficient surface area to support the rate of diffusion required for the increased volume. In other words, as a cell grows, it becomes less efficient. One way to become more efficient is to divide. Other ways are to increase surface area by foldings of the cell membrane, become flat or thin and elongated, or develop organelles that perform specific tasks. These adaptations lead to developing more sophisticated cells, which we call eukaryotic cells.

Visual Connection

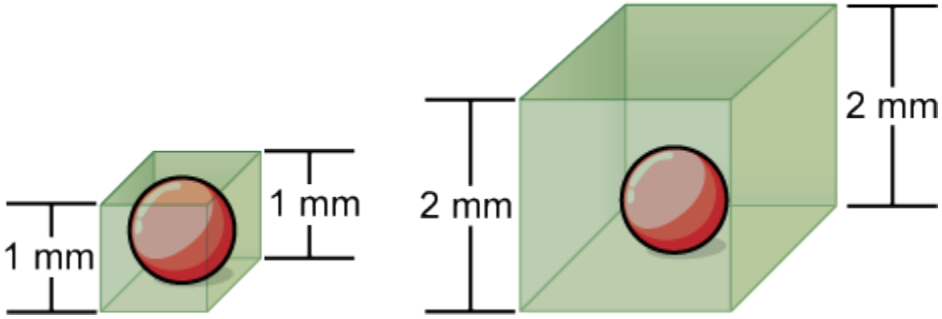


Figure 4.7 Notice that as a cell increases in size, its surface area-to-volume ratio decreases. When there is insufficient surface area to support a cell's increasing volume, a cell will either divide or die. The cell on the left has a volume of 1 mm3 and a surface area of 6 mm2, with a surface area-to-volume ratio of 6 to 1, whereas the cell on the right has a volume of 8 mm3 and a surface area of 24 mm2, with a surface area-to-volume ratio of 3 to 1.

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

32.

EUKARYOTIC CELLS

Learning Objectives

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

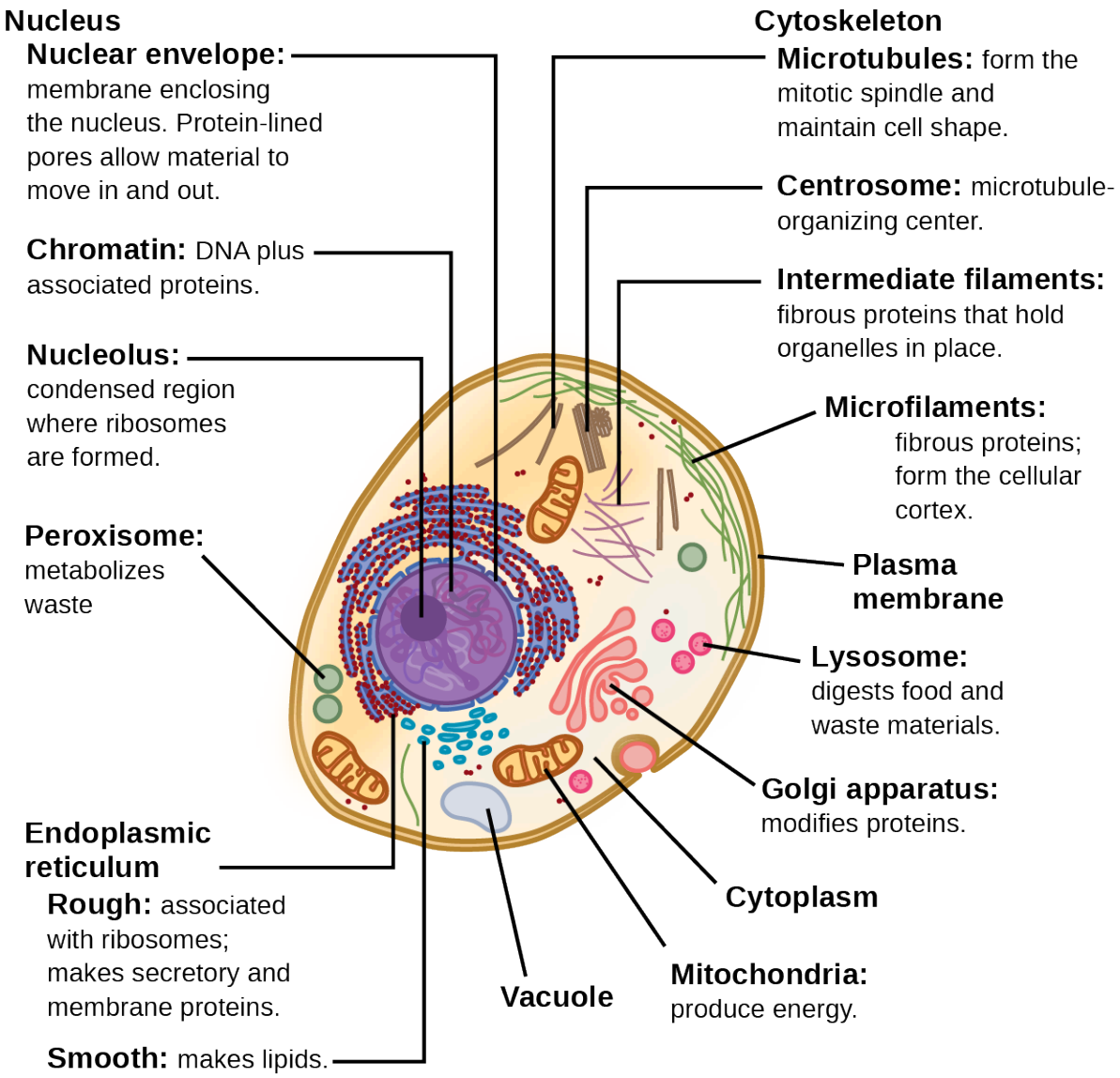
- Describe the structure of eukaryotic cells
- Compare animal cells with plant cells
- State the role of the plasma membrane
- Summarize the functions of the major cell organelles

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

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

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

Visual Connection



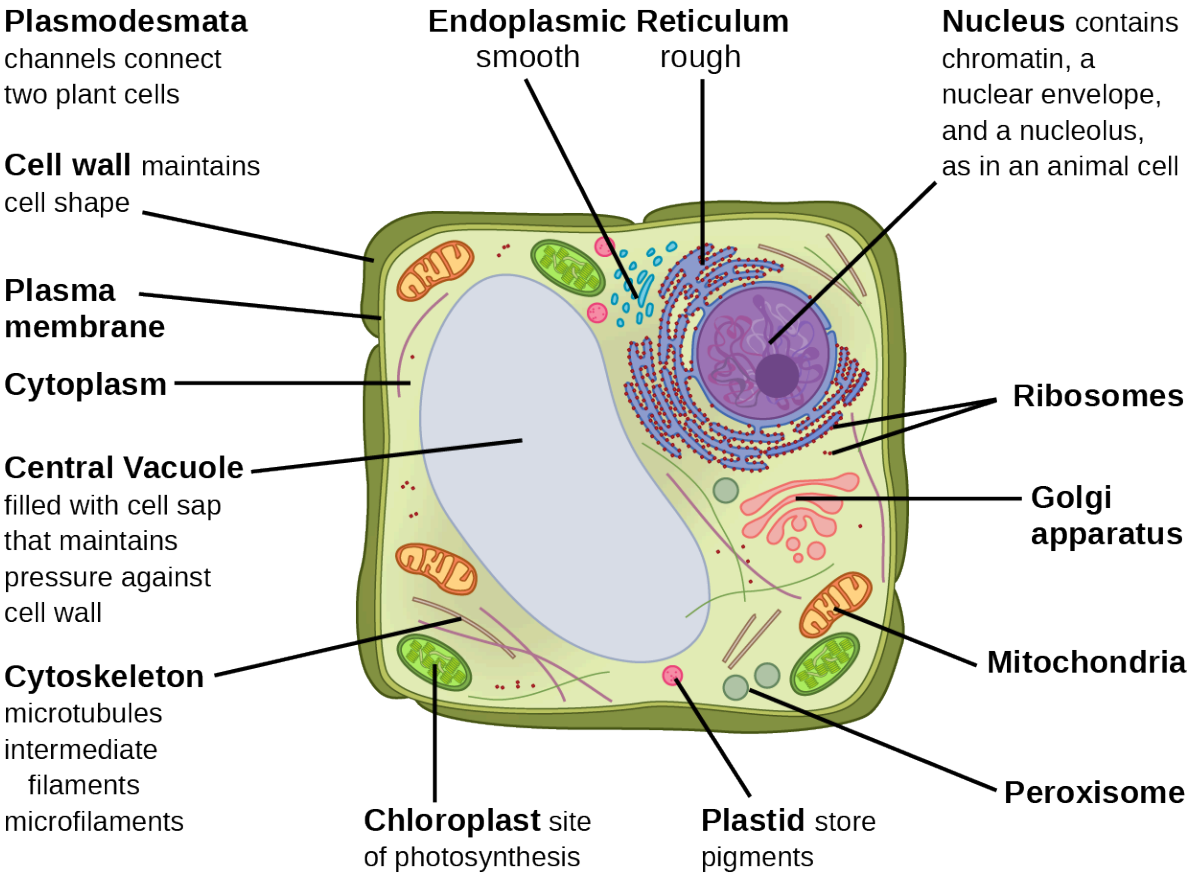


Figure 4.8 These figures show the major organelles and other cell components of (a) a typical animal cell and (b) a typical eukaryotic plant cell. The plant cell has a cell wall, chloroplasts, plastids, and a central vacuole—structures not in animal cells. Most cells do not have lysosomes or centrosomes.

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

The Plasma Membrane

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

(such as carbon dioxide and ammonia) also leave the cell by passing through the plasma membrane. The plasma membrane also allows the cell to interact with and respond to its environment.

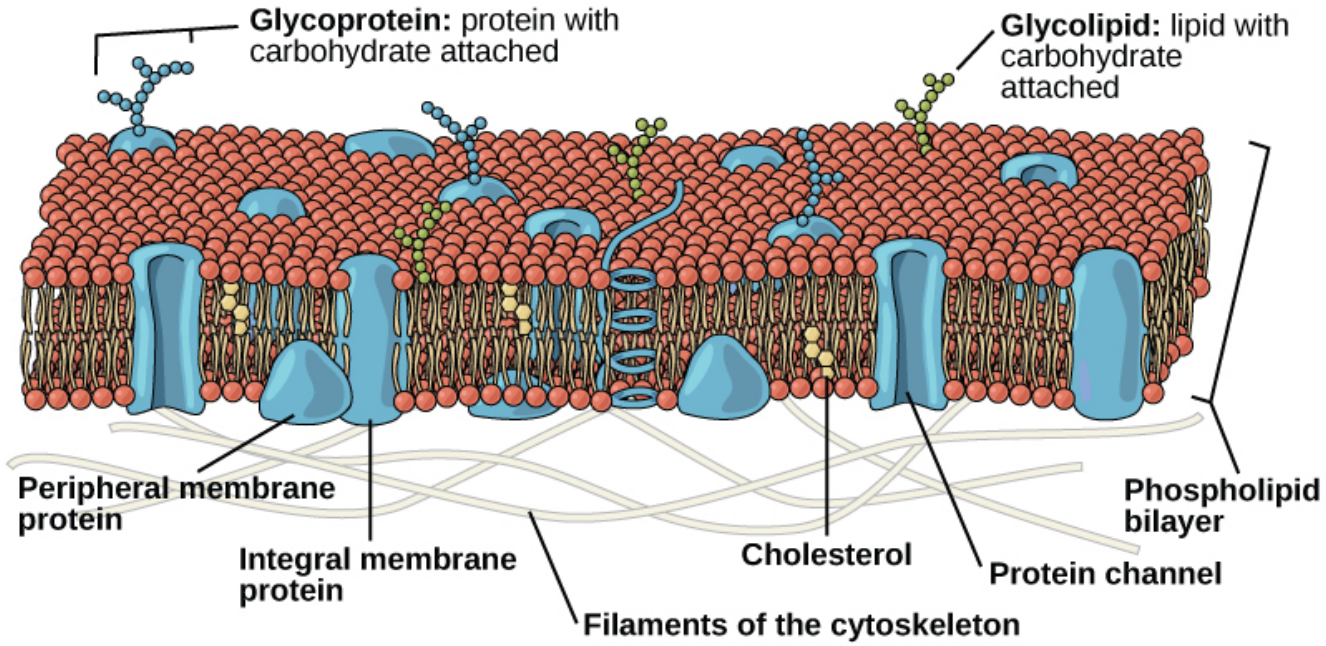


Figure 4.9 The eukaryotic plasma membrane is a phospholipid bilayer with proteins and cholesterol embedded in it. Proteins are used for various functions, such as transport across the membrane, cell signaling, and cell identification, while cholesterol maintains the fluidity of the membrane.

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

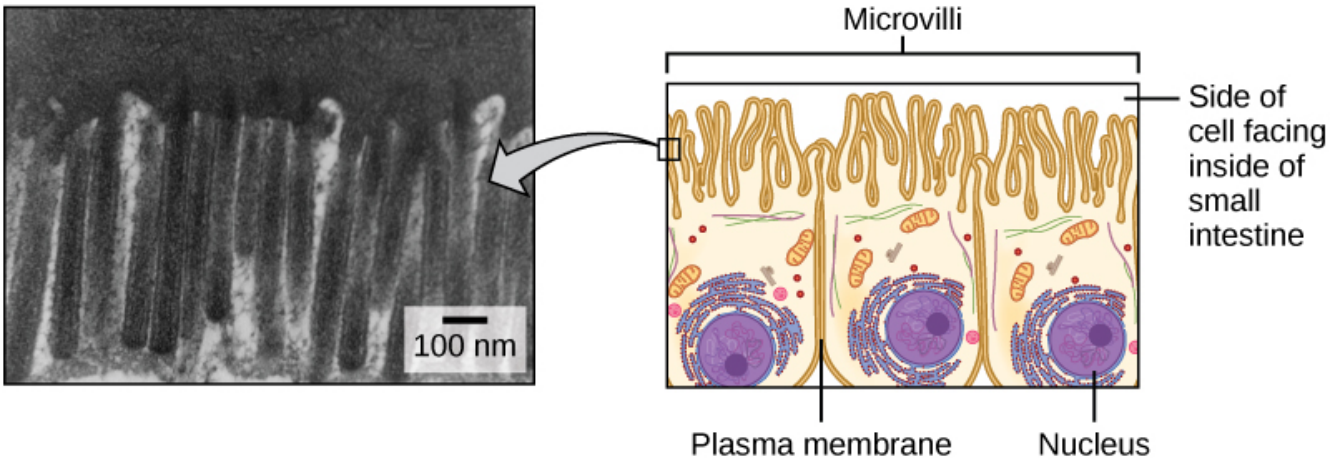


Figure 4.10 Microvilli, as they appear on cells lining the small intestine, increase the surface area available for absorption. These microvilli are only on the area of the plasma membrane that faces the cavity from which substances will be absorbed. (credit "micrograph": modification of work by Louisa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

The Cytoplasm

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

The Nucleus

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

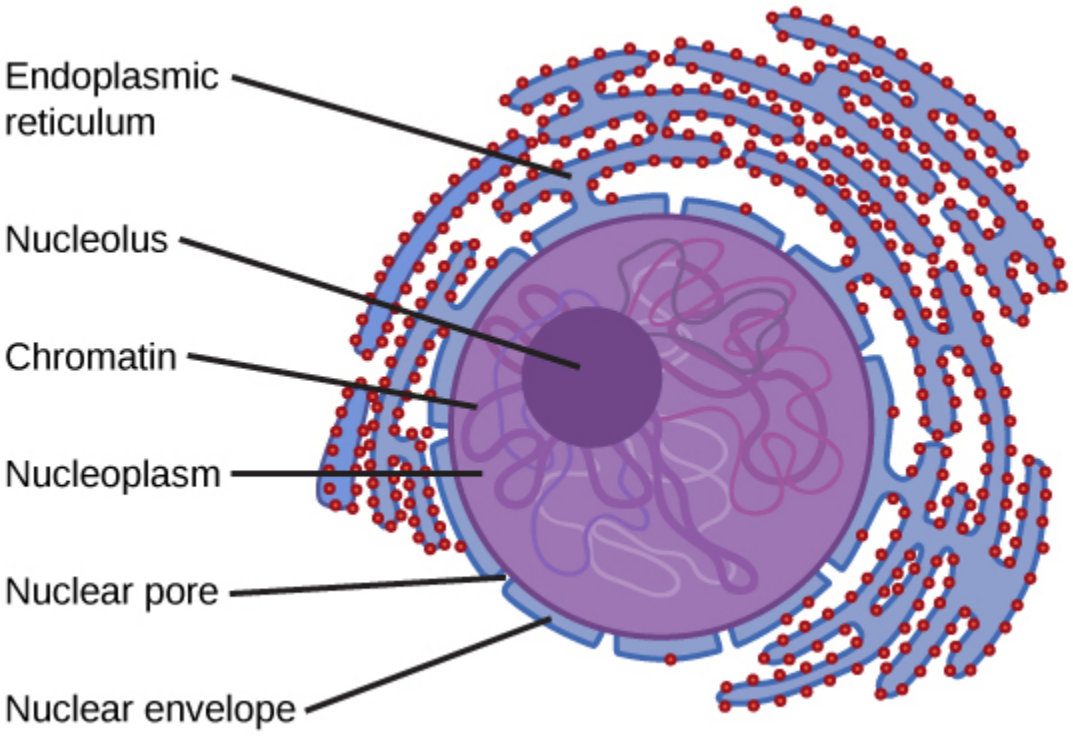


Figure 4.11 The nucleus stores chromatin (DNA plus proteins) in a gel-like substance called the nucleoplasm. The nucleolus is a condensed chromatin region where ribosome synthesis occurs. We call the nucleus's boundary the nuclear envelope. It consists of two phospholipid bilayers: an outer and an inner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

The Nuclear Envelope

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

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

Chromatin and Chromosomes

To understand chromatin, it is helpful to first explore **chromosomes**, structures within the nucleus that are made up of DNA, the hereditary material. You may remember that in prokaryotes, DNA is organized into a single circular chromosome. In eukaryotes, chromosomes are linear structures. Every eukaryotic species has a specific number of chromosomes in the nucleus of each cell. For example, in humans, the chromosome number is 46, while in fruit flies, it is eight. Chromosomes are only visible and distinguishable from one another when the cell is getting ready to divide. When the cell is in the growth and maintenance phases of its

life cycle, proteins attach to chromosomes, and they resemble an unwound, jumbled bunch of threads. We call these unwound protein-chromosome complexes **chromatin** (Figure 4.12). Chromatin describes the material that makes up the chromosomes both when condensed and decondensed.

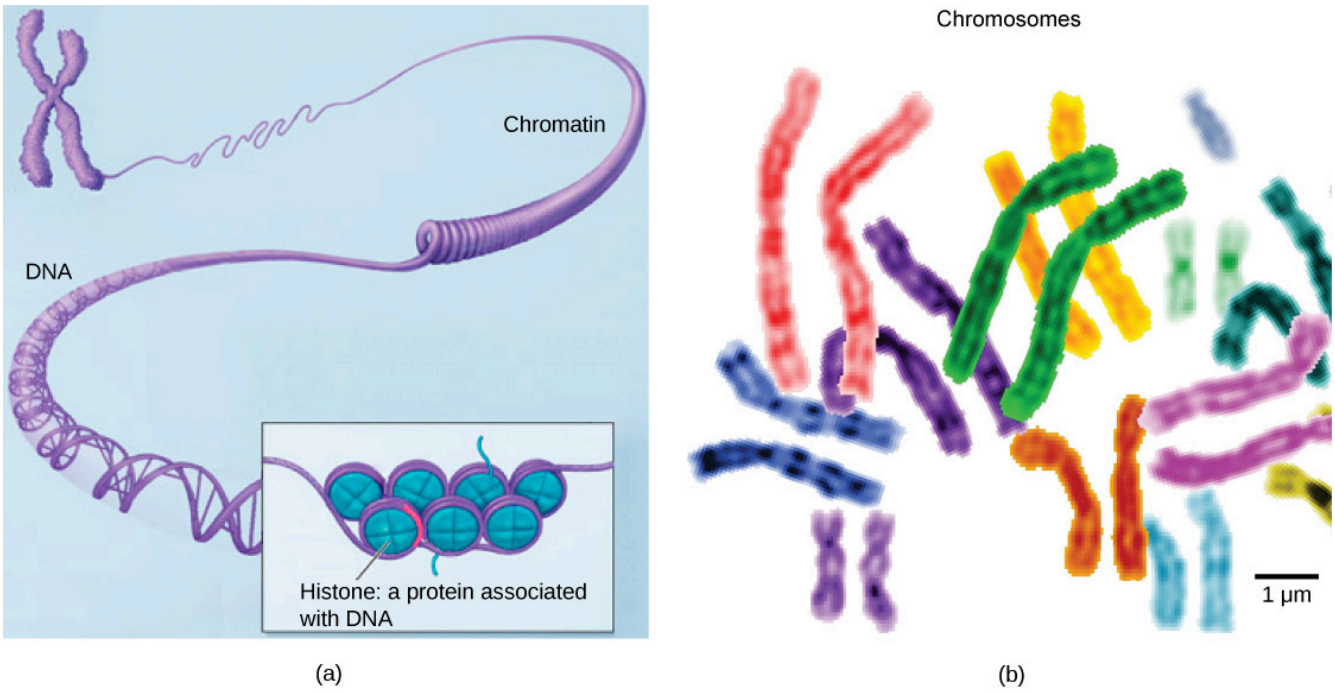


Figure 4.12 (a) This image shows various levels of chromatin's organization (DNA and protein). (b) This image shows paired chromosomes. (credit b: modification of work by NIH; scale-bar data from Matt Russell)

The Nucleolus

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

Ribosomes

Ribosomes are the cellular structures responsible for protein synthesis. When we view them through an electron microscope, ribosomes appear either as clusters (polyribosomes) or single tiny dots that float freely in the cytoplasm. They may be attached to the plasma membrane's cytoplasmic side or the endoplasmic reticulum's cytoplasmic side and the nuclear envelope's outer membrane (Figure 4.8). Electron microscopy shows us that ribosomes, which are large protein and RNA complexes, consist of two subunits, large and small (Figure 4.13). Ribosomes receive their "orders" for protein synthesis from the nucleus, where the DNA

transcribes into messenger RNA (mRNA). The mRNA travels to the ribosomes, which translate the code provided by the sequence of the nitrogenous bases in the mRNA into a specific order of amino acids in a protein. Amino acids are the building blocks of proteins.

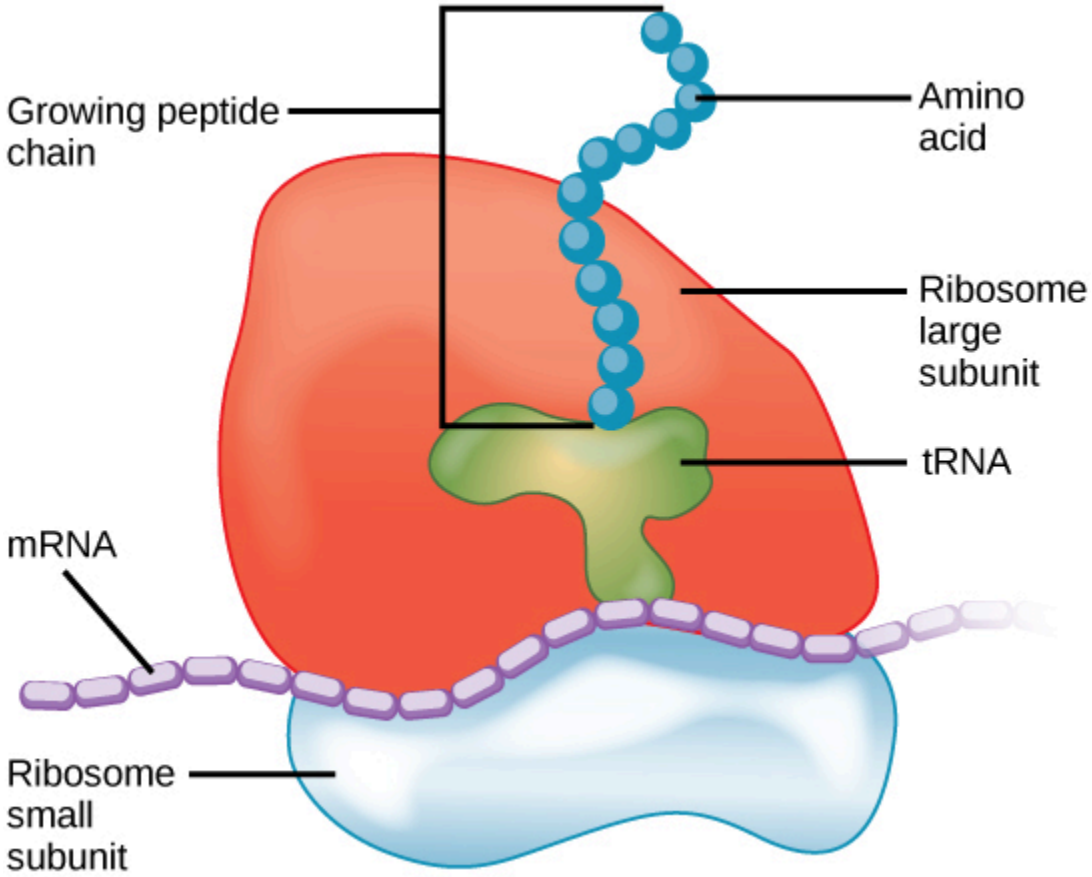


Figure 4.13 A large subunit (top) and a small subunit (bottom) comprise ribosomes. During protein synthesis, ribosomes assemble amino acids into proteins.

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

Mitochondria

Scientists often call **mitochondria** (singular = mitochondrion) "powerhouses" or "energy factories" of both plant and animal cells because they are responsible for making adenosine triphosphate (ATP), the cell's main energy-carrying molecule. ATP represents the cell's short-term stored energy. Cellular respiration is the process

of making ATP using the chemical energy in glucose and other nutrients. In mitochondria, this process uses oxygen and produces carbon dioxide as a waste product. In fact, the carbon dioxide that you exhale with every breath comes from the cellular reactions that produce carbon dioxide as a byproduct.

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

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

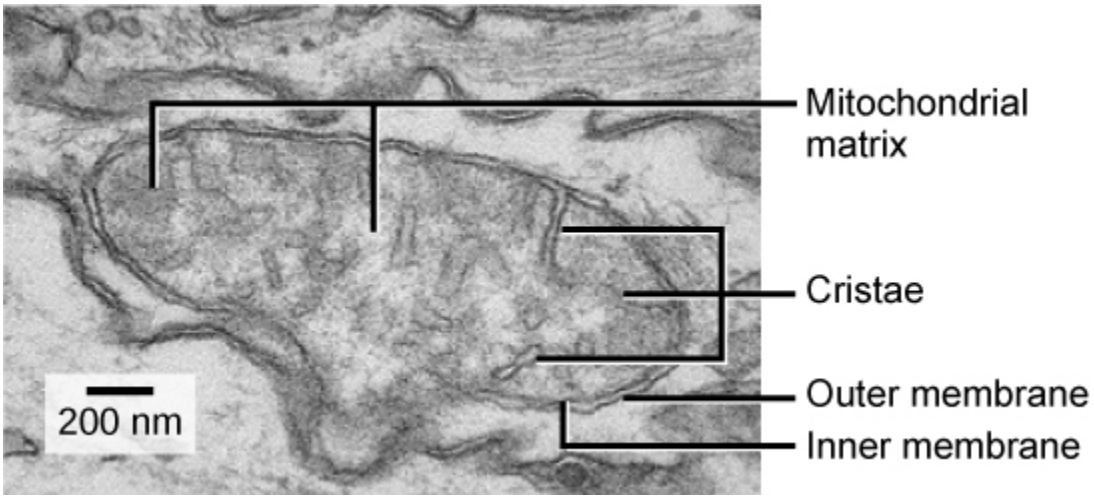


Figure 4.14 This electron micrograph shows a mitochondrion through an electron microscope. This organelle has an outer membrane and an inner membrane. The inner membrane contains folds, called cristae, which increase its surface area. We call the space between the two membranes the intermembrane space,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

Peroxisomes

Peroxisomes are small, round organelles enclosed by single membranes. They carry out oxidation reactions that break down fatty acids and amino acids. They also detoxify many poisons that may enter the body. (Many of these oxidation reactions release hydrogen peroxide, H_2O_2, which would be damaging to cells; however, when these reactions are confined to peroxisomes, enzymes safely break down the H_2O_2 into oxygen and water.) For example, peroxisomes in liver cells detoxify alcohol. Glyoxysomes, which are specialized

peroxisomes in plants, are responsible for converting stored fats into sugars. Plant cells contain many different types of peroxisomes that play a role in metabolism, pathogen defense, and stress response, to mention a few.

Vesicles and Vacuoles

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

Animal Cells versus Plant Cells

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

The Centrosome

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

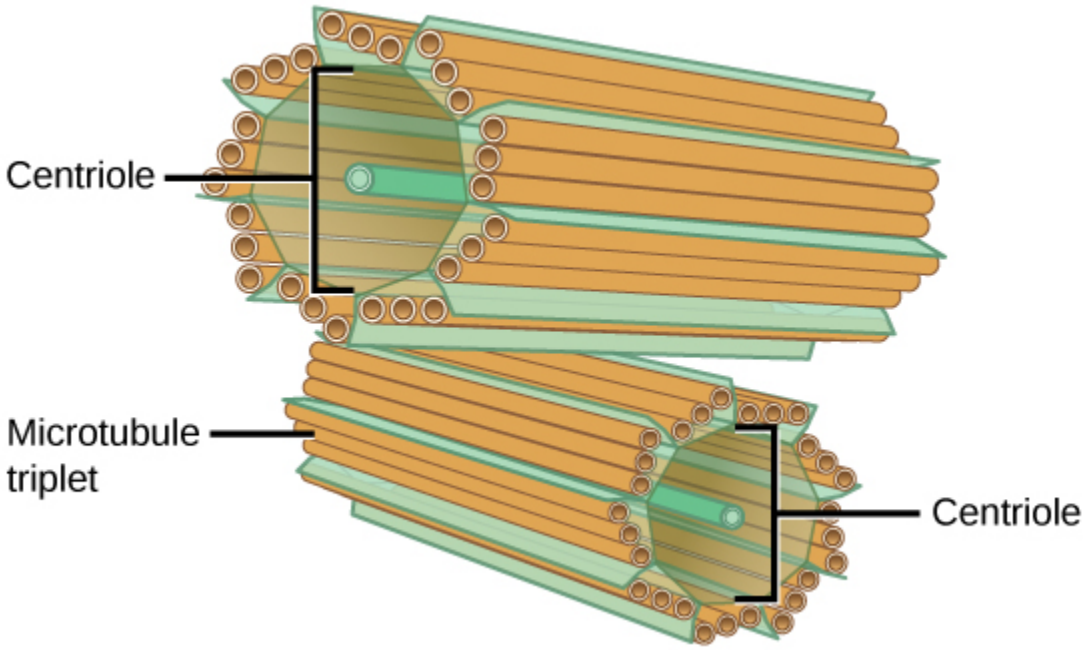


Figure 4.15 The centrosome consists of two centrioles that lie at right angles to each other. Each centriole is a cylinder comprised of nine triplets of microtubules. Nontubulin proteins (indicated by the green lines) hold the microtubule triplets together.

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

Lysosomes

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

The Cell Wall

If you examine Figure 4.8, the plant cell diagram, you will see a structure external to the plasma membrane. This is the **cell wall**, a rigid covering that protects the cell, provides structural support, and gives shape to the cell. Fungal and some protistan cells also have cell walls. While the prokaryotic cell walls' chief component is peptidoglycan, the major organic molecule in the plant (and some protists') cell wall is cellulose (Figure 4.16), a

polysaccharide comprised of glucose units. Have you ever noticed that when you bite into a raw vegetable, like celery, it crunches? That's because you are tearing the celery cells' rigid cell walls with your teeth. The cell walls of fungal cells are composed of the polysaccharide chitin, while protists have an array of materials that may be found in the cell wall.

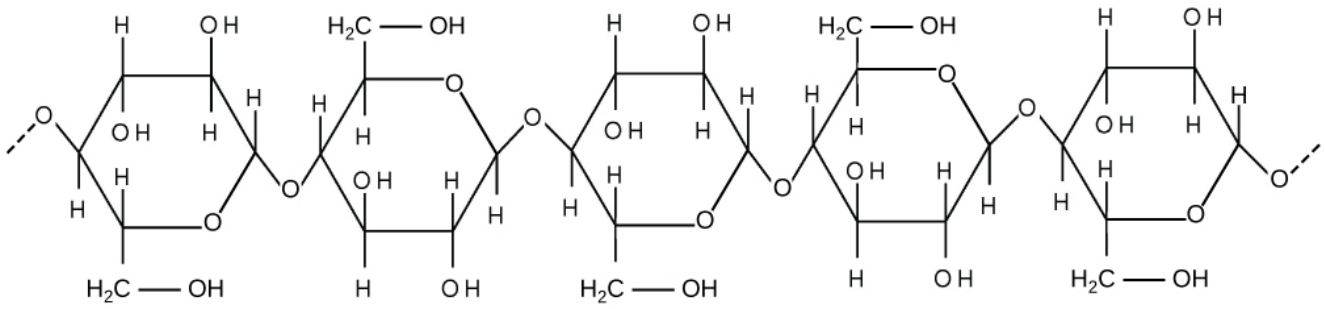


Figure 4.16 Cellulose is a long chain of β-glucose molecules connected by a 1-4 linkage. The dashed lines at each end of the figure indicate a series of many more glucose units. The size of the page makes it impossible to portray an entire cellulose molecule.

Chloroplasts

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

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

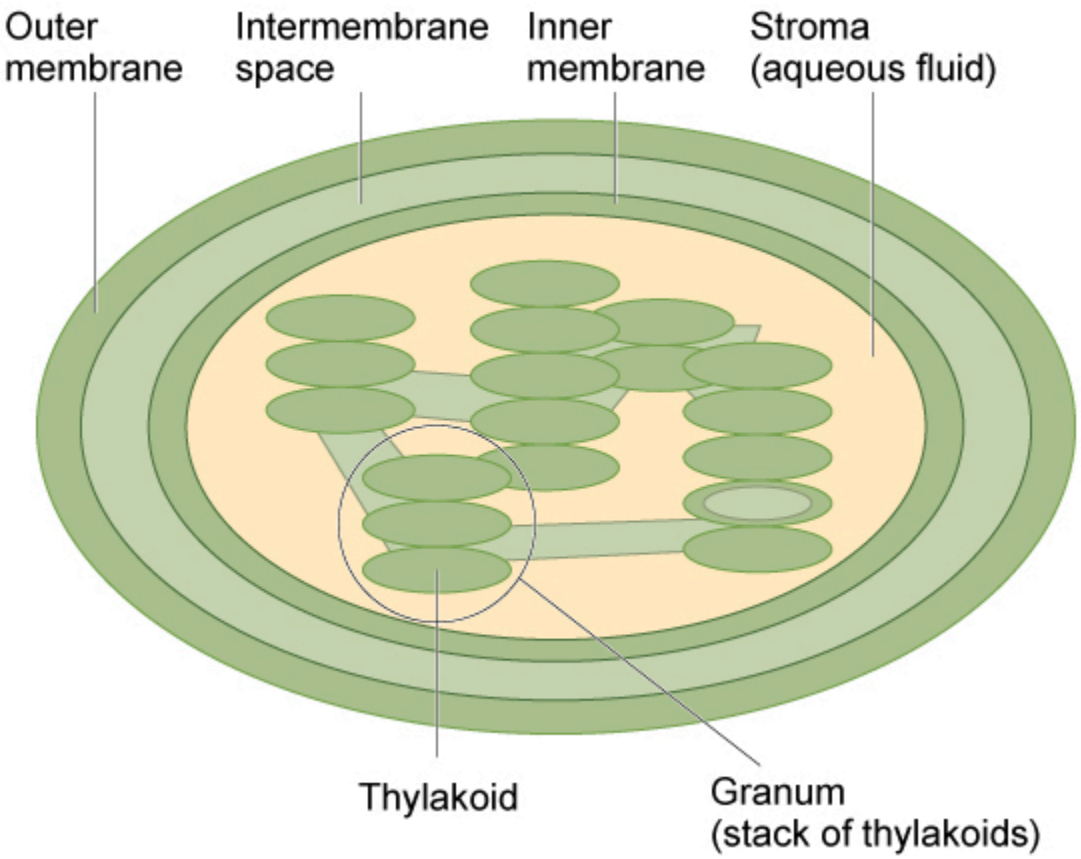


Figure 4.17 The chloroplast has an outer membrane, an inner membrane, and membrane structures – thylakoids that are stacked into grana. We call the space inside the thylakoid membranes the thylakoid space. The light harvesting reactions take place in the thylakoid membranes, and sugar synthesis takes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

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

Evolution Connection

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

Scientists have long noticed that bacteria, mitochondria, and chloroplasts are similar in size. We also know that bacteria have DNA and ribosomes that are similar to those found in mitochondria and chloroplasts. Scientists believe that host cells and bacteria formed an endosymbiotic relationship when the host cells ingested both aerobic and autotrophic bacteria (cyanobacteria) but did not destroy them. Through many millions of years of evolution, these ingested bacteria became more specialized in their functions, with the aerobic bacteria becoming mitochondria and the autotrophic bacteria becoming chloroplasts. Free living microorganisms often merge with larger organisms in this way. This theory was first proposed by Dr. Lynn Margulis in 1981, and while it was initially met with considerable criticism, it is now widely accepted.

The Central Vacuole

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

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

33.

THE ENDOMEMBRANE SYSTEM AND PROTEINS

Learning Objectives

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

- List the components of the endomembrane system
- Recognize the relationship between the endomembrane system and its functions

The endomembrane system (endo = "within") is a group of membranes and organelles (Figure 4.18) in eukaryotic cells that works together to modify, package, and transport lipids and proteins. It includes the nuclear envelope, lysosomes, and vesicles, which we have already mentioned, and 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 endomembrane system does not include either mitochondria or chloroplast membranes.

Visual Connection

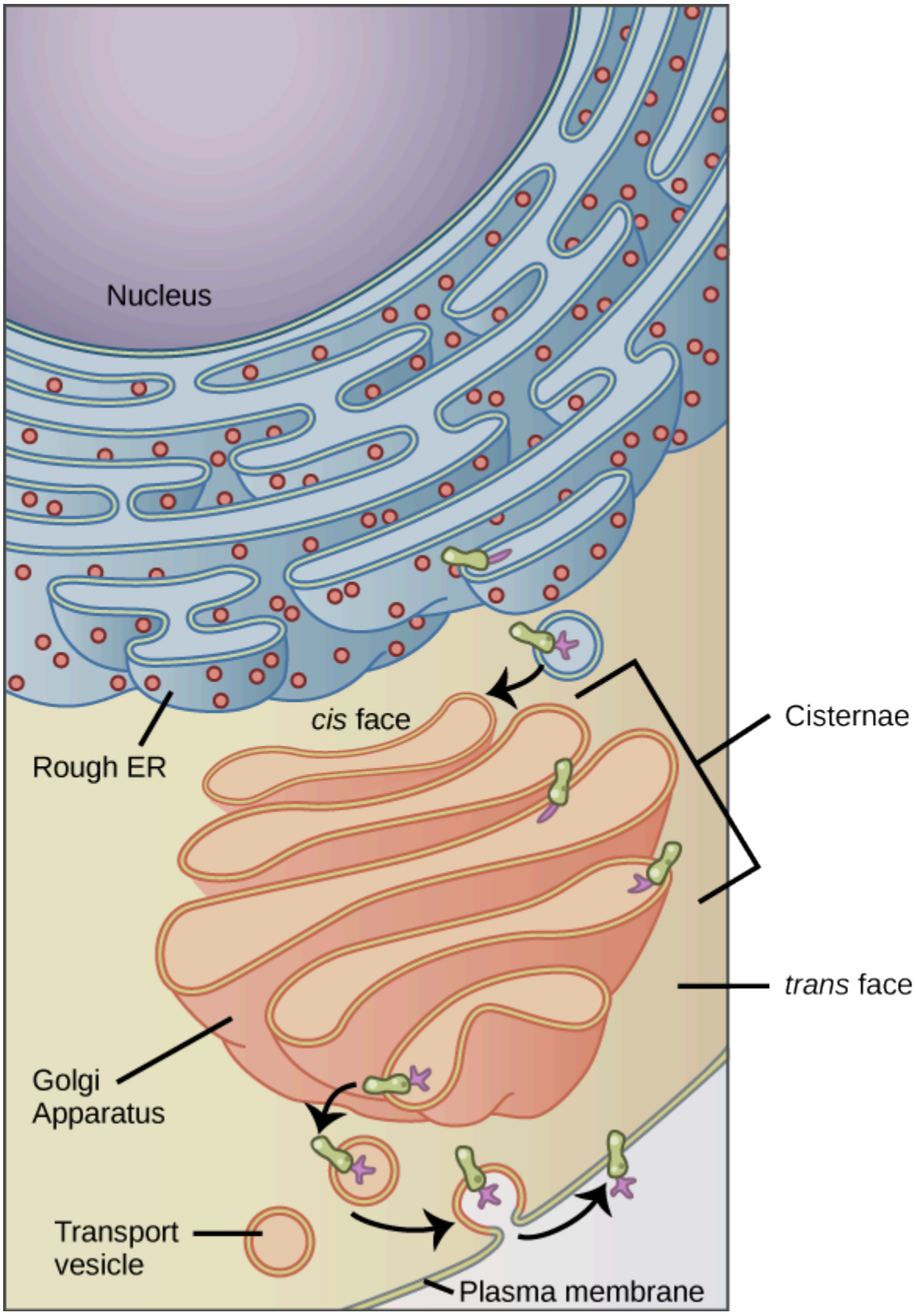

Nucleus
Rough ER
cis face
Cisternae
trans face
Golgi Apparatus
Transport vesicle
Plasma membrane

Figure 4.18 Membrane and secretory proteins are synthesized in the rough endoplasmic reticulum (RER). The RER also sometimes modifies proteins. In this illustration, a (green) integral membrane protein is modified by attachment of a (purple) carbohydrate in the ER. Vesicles with the integral protein bud from the ER and fuse with the Golgi apparatus' cis face. As the protein passes along the Golgi's cisternae, the addition of more carbohydrates further modifies it. After its synthesis is complete, it exits as an integral membrane protein of the vesicle that buds from the Golgi's trans face. When the vesicle fuses with the cell membrane, the protein becomes an integral portion of that cell membrane. (credit: modification of work by Magnus Manske)

If a peripheral membrane protein were synthesized in the lumen (inside) of the ER, would it end up on the inside or outside of the plasma membrane?

The Endoplasmic Reticulum

The **endoplasmic reticulum (ER)** (Figure 4.18) is a series of interconnected membranous sacs and tubules that collectively modifies proteins and synthesizes lipids. However, these two functions take place in separate areas of the ER: the rough ER and the smooth ER, respectively.

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

Rough ER

Scientists have named the **rough endoplasmic reticulum (RER)** as such because the ribosomes attached to its cytoplasmic surface give it a studded appearance when viewing it through an electron microscope (Figure 4.19).

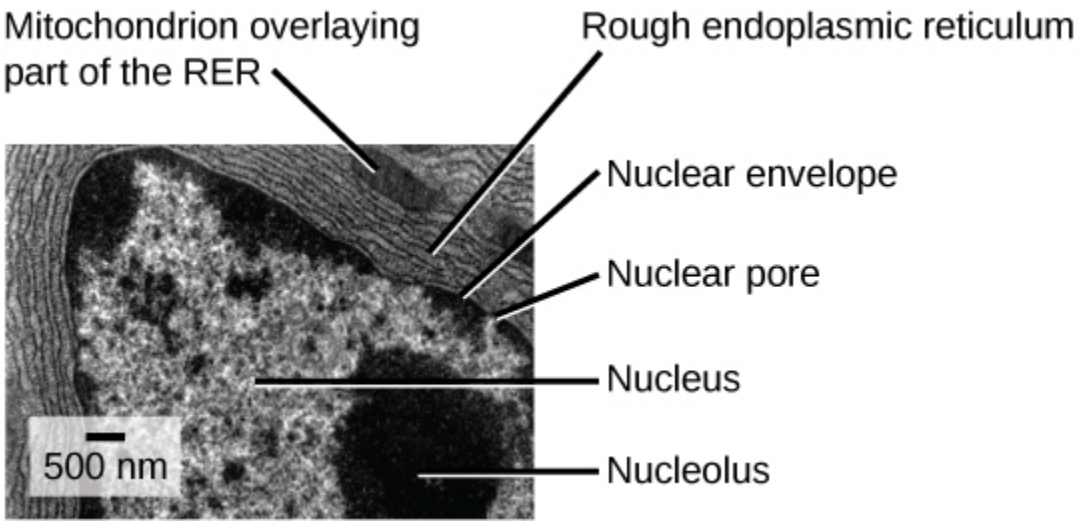


Figure 4.19 This transmission electron micrograph shows the rough endoplasmic reticulum and other organelles in a pancreatic cell. (credit: modification of work by Louisa Howard)

Ribosomes transfer their newly synthesized proteins into the RER's lumen where they undergo structural modifications, such as folding or acquiring side chains. These modified proteins incorporate into cellular membranes—the ER or the ER's or other organelles' membranes. The proteins can also secrete from the cell (such as protein hormones, enzymes). The RER also makes phospholipids for cellular membranes.

If the phospholipids or modified proteins are not destined to stay in the RER, they will reach their destinations via transport vesicles that bud from the RER's membrane (Figure 4.18).

Since the RER is engaged in modifying proteins (such as enzymes, for example) that secrete from the cell, you would be correct in assuming that the RER is abundant in cells that secrete proteins. This is the case with liver cells, for example.

Smooth ER

The smooth endoplasmic reticulum (SER) is continuous with the RER but has few or no ribosomes on its cytoplasmic surface (Figure 4.18). SER functions include synthesis of carbohydrates, lipids, and steroid hormones; detoxification of medications and poisons; and storing calcium ions.

In muscle cells, a specialized SER, the sarcoplasmic reticulum, is responsible for storing calcium ions that are needed to trigger the muscle cells' coordinated contractions.

Link to Learning

You can watch an excellent animation of the endomembrane system here. At the end of the animation, there is a short self-assessment.

Career Connection

Cardiologist

Heart disease is the leading cause of death in the United States. This is primarily due to our sedentary lifestyle and our high trans-fat diets.

Heart failure is just one of many disabling heart conditions. Heart failure does not mean that the heart has stopped working. Rather, it means that the heart can't pump with sufficient force to transport oxygenated blood to all the vital organs. Left untreated, heart failure can lead to kidney failure and other organ failure.

Cardiac muscle tissue comprises the heart's wall. Heart failure occurs when cardiac muscle cells' endoplasmic reticula do not function properly. As a result, an insufficient number of calcium ions are available to trigger a sufficient contractile force.

Cardiologists (cardi- = "heart"; -ologist = "one who studies") are doctors who specialize in treating heart diseases, including heart failure. Cardiologists can diagnose heart failure via a physical examination, results from an electrocardiogram (ECG, a test that measures the heart's electrical activity), a chest X-ray to see whether the heart is enlarged, and other tests. If the cardiologist diagnoses heart failure, they will typically prescribe appropriate medications and recommend a reduced table salt intake and a supervised exercise program.

The Golgi Apparatus

We have already mentioned that vesicles can bud from the ER and transport their contents elsewhere, but where do the vesicles go? Before reaching their final destination, the lipids or proteins within the transport vesicles still need sorting, packaging, and tagging so that they end up in the right place. Sorting, tagging,

packaging, and distributing lipids and proteins takes place in the **Golgi apparatus** (also called the Golgi body), a series of flattened membranous sacs (Figure 4.20).

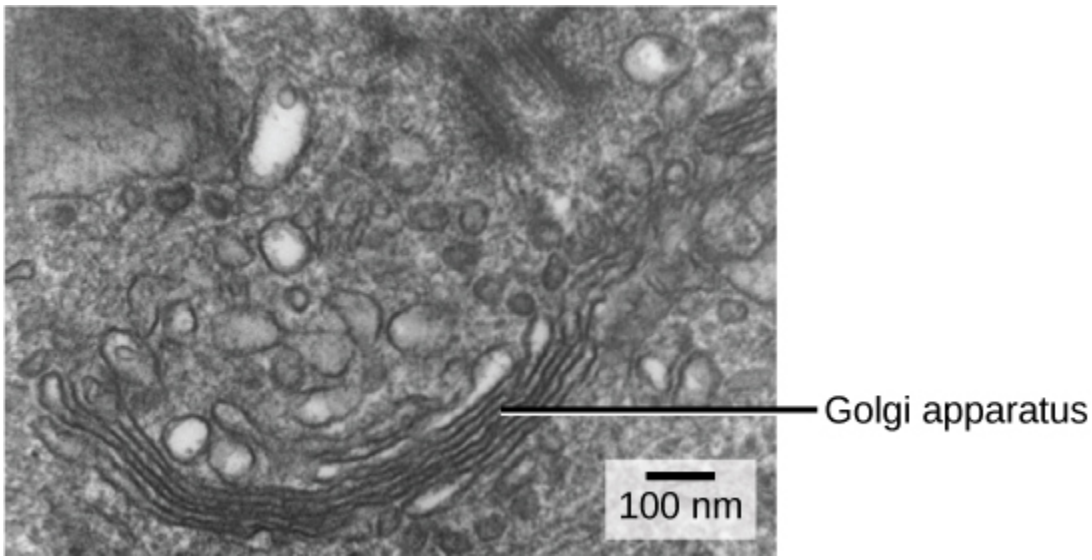


Figure 4.20 The Golgi apparatus in this white blood cell is visible as a stack of semicircular, flattened rings in the lower portion of the image. You can see several vesicles near the Golgi apparatus. (credit: modification of work by Louisa Howard)

The side of the Golgi apparatus that is closer to the ER is called the *cis* face. The opposite side is the *trans* face. The transport vesicles that formed from the ER travel to the *cis* face, fuse with it, and empty their contents into the Golgi apparatus' lumen. As the proteins and lipids travel through the Golgi, they undergo further modifications that allow them to be sorted. The most frequent modification is adding short sugar molecule chains. These newly modified proteins and lipids then tag with phosphate groups or other small molecules in order to travel to their proper destinations.

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

In another example of form following function, cells that engage in a great deal of secretory activity (such as salivary gland cells that secrete digestive enzymes or immune system cells that secrete antibodies) have an abundance of Golgi.

In plant cells, the Golgi apparatus has the additional role of synthesizing polysaccharides, some of which are incorporated into the cell wall and some of which other cell parts use.

Career Connection

Geneticist

Many diseases arise from genetic mutations that prevent synthesizing critical proteins. One such disease is Lowe disease (or oculocerebrorenal syndrome, because it affects the eyes, brain, and kidneys). In Lowe disease, there is a deficiency in an enzyme localized to the Golgi apparatus. Children with Lowe disease are born with cataracts, typically develop kidney disease after the first year of life, and may have intellectual disabilities.

A mutation on the X chromosome causes Lowe disease. The X chromosome is one of the two human sex chromosomes, as these chromosomes determine a person's sex. Females possess two X chromosomes, while males possess one X and one Y chromosome. In females, the genes on only one of the two X chromosomes are expressed. Females who carry the Lowe disease gene on one of their X chromosomes are carriers and do not show symptoms of the disease. However, males only have one X chromosome, and the genes on this chromosome are always expressed. Therefore, males will always have Lowe disease if their X chromosome carries the Lowe disease gene. Geneticists have identified the mutated gene's location, as well as many other mutation locations that cause genetic diseases. Through prenatal testing, a pregnant person can find out if the fetus they are carrying may be afflicted with one of several genetic diseases.

Geneticists analyze prenatal genetic test results and may counsel pregnant people on available options. They may also conduct genetic research that leads to new drugs or foods, or perform DNA analyses for forensic investigations.

Lysosomes

In addition to their role as the digestive component and organelle-recycling facility of animal cells, lysosomes are part of the endomembrane system. Lysosomes also use their hydrolytic enzymes to destroy pathogens (disease-causing organisms) that might enter the cell. A good example of this occurs in macrophages, a group of white blood cells that are part of your body's immune system. In a process that scientists call phagocytosis or endocytosis, a section of the macrophage's plasma membrane 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 4.21).

Phagocytosis

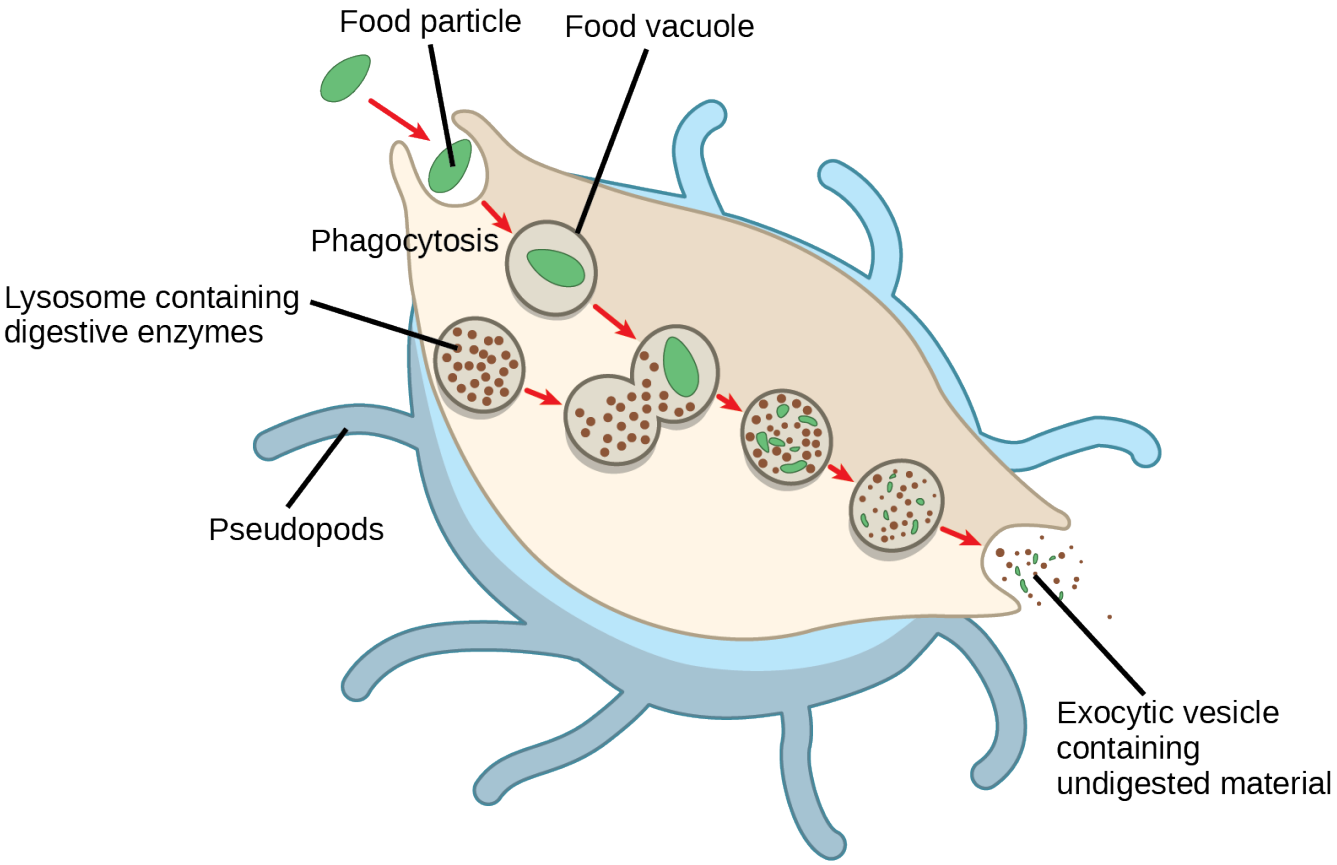


Figure 4.21 A macrophage has engulfed (phagocytized) a potentially pathogenic bacterium and then fuses with lysosomes within the cell to destroy the pathogen. Other organelles are present in the cell, but for simplicity we do not show them.

34.

THE CYTOSKELETON

Learning Objectives

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

- Describe the cytoskeleton
- Compare the roles of microfilaments, intermediate filaments, and microtubules
- Compare and contrast cilia and flagella
- Summarize the differences among the components of prokaryotic cells, animal cells, and plant cells

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 help maintain the cell's shape, secure some organelles in specific positions, allow cytoplasm and vesicles to move within the cell, and enable cells within multicellular organisms to move. Collectively, scientists call this network of protein fibers the **cytoskeleton**. There are three types of fibers within the cytoskeleton: microfilaments, intermediate filaments, and microtubules (Figure 4.22). Here, we will examine each.

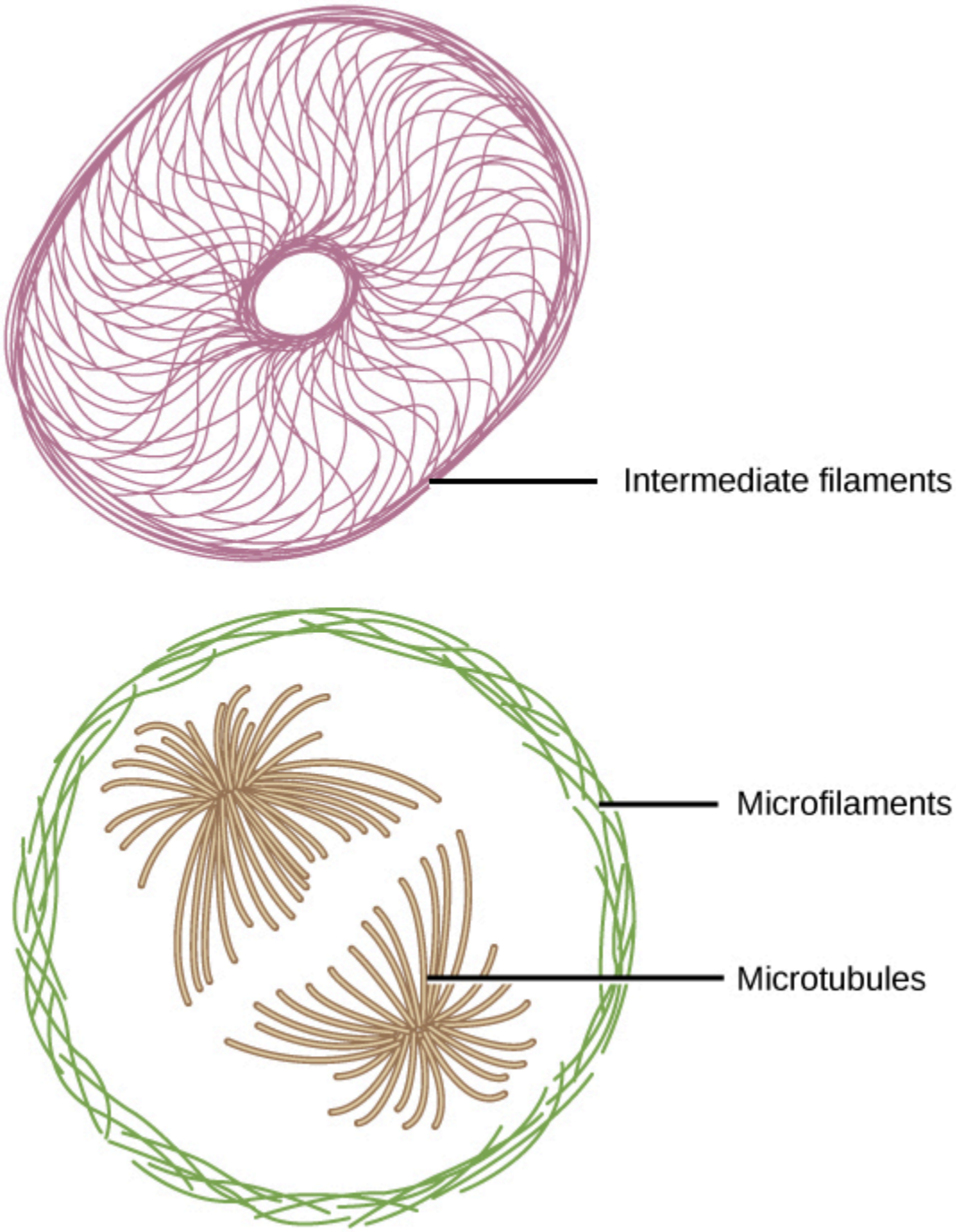


Figure 4.22 Microfilaments thicken the cortex around the cell's inner edge. Like rubber bands, they resist tension. There are microtubules in the cell's interior where they maintain their shape by resisting compressive forces. There are intermediate filaments throughout the cell that hold organelles in place.

Microfilaments

Of the three types of protein fibers in the cytoskeleton, **microfilaments** are the narrowest. They function in

cellular movement, have a diameter of about 7 nm, and are comprised of two globular protein intertwined strands, which we call actin (Figure 4.23). For this reason, we also call microfilaments actin filaments.

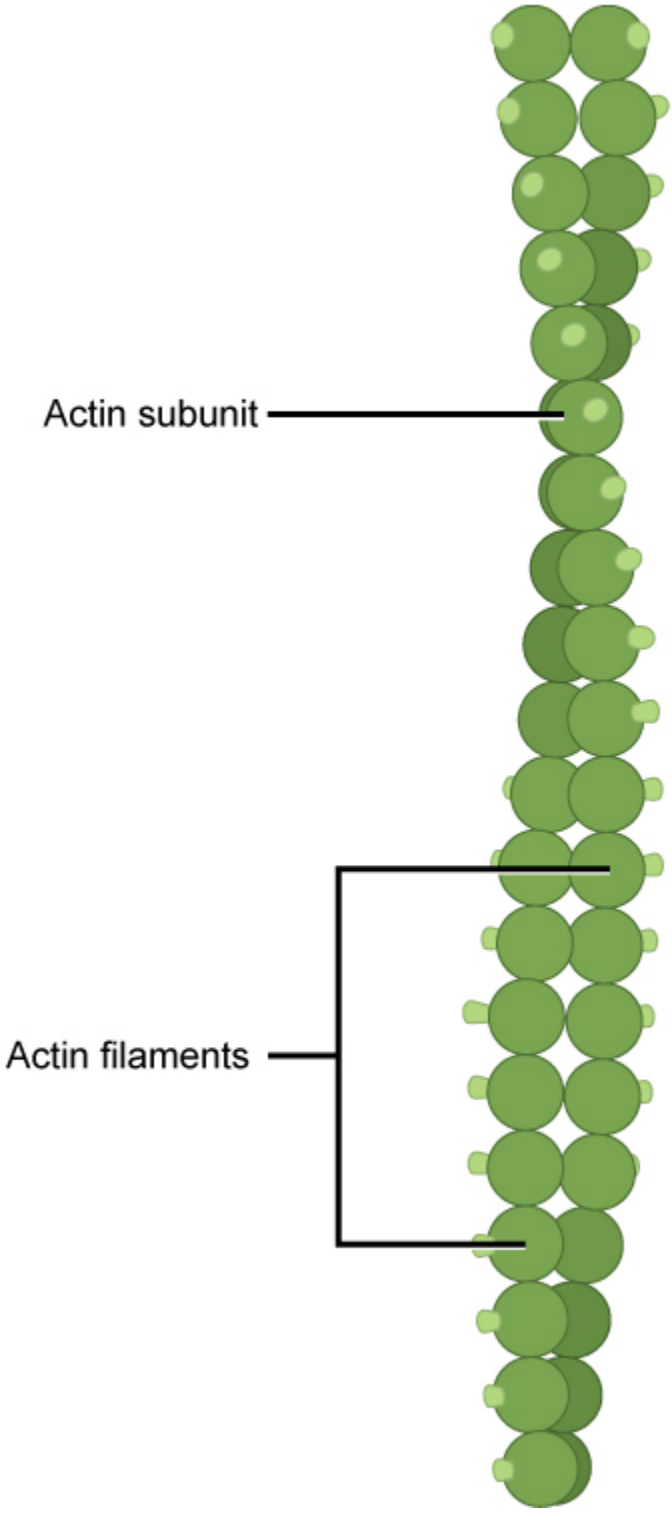


Figure 4.23 Two intertwined actin strands comprise microfilaments.

ATP powers actin to assemble its filamentous form, which serves as a track for the movement of a motor protein we call myosin. This enables actin to engage in cellular events requiring motion, such as cell division in eukaryotic cells and cytoplasmic streaming, which is the cell cytoplasm's circular movement in plant cells. Actin and myosin are plentiful in muscle cells. When your actin and myosin filaments slide past each other, your muscles contract.

Microfilaments also provide some rigidity and shape to the cell. They can depolymerize (disassemble) and reform quickly, thus enabling a cell to change its shape and move. White blood cells (your body's infection-fighting cells) make good use of this ability. They can move to an infection site and phagocytize the pathogen.

Link to Learning

To see an example of a white blood cell in action, watch a short time-lapse video of the cell capturing two bacteria. It engulfs one and then moves on to the other.

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One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1leclab/?p=211#oembed-1

Intermediate Filaments

Several strands of fibrous proteins that are wound together comprise intermediate filaments (Figure 4.24). Cytoskeleton elements get their name from the fact that their diameter, 8 to 10 nm, is between those of microfilaments and microtubules.

Figure 4.24 Intermediate filaments consist of several intertwined strands of fibrous proteins.

Intermediate filaments have no role in cell movement. Their function is purely structural. They bear tension, thus maintaining the cell's shape, and anchor the nucleus and other organelles in place. Figure 4.22 shows how intermediate filaments create a supportive scaffolding inside the cell.

The intermediate filaments are the most diverse group of cytoskeletal elements. Several fibrous protein types are in the intermediate filaments. You are probably most familiar with keratin, the fibrous protein that strengthens your hair, nails, and the skin's epidermis.

Microtubules

As their name implies, microtubules are small hollow tubes. Polymerized dimers of α-tubulin and β-tubulin, two globular proteins, comprise the microtubule's walls (Figure 4.25). With a diameter of about 25 nm, **microtubules** are cytoskeletons' widest components. They help the cell resist compression, provide a track along which vesicles move through the cell, and pull replicated chromosomes to opposite ends of a dividing cell. Like microfilaments, microtubules can disassemble and reform quickly.

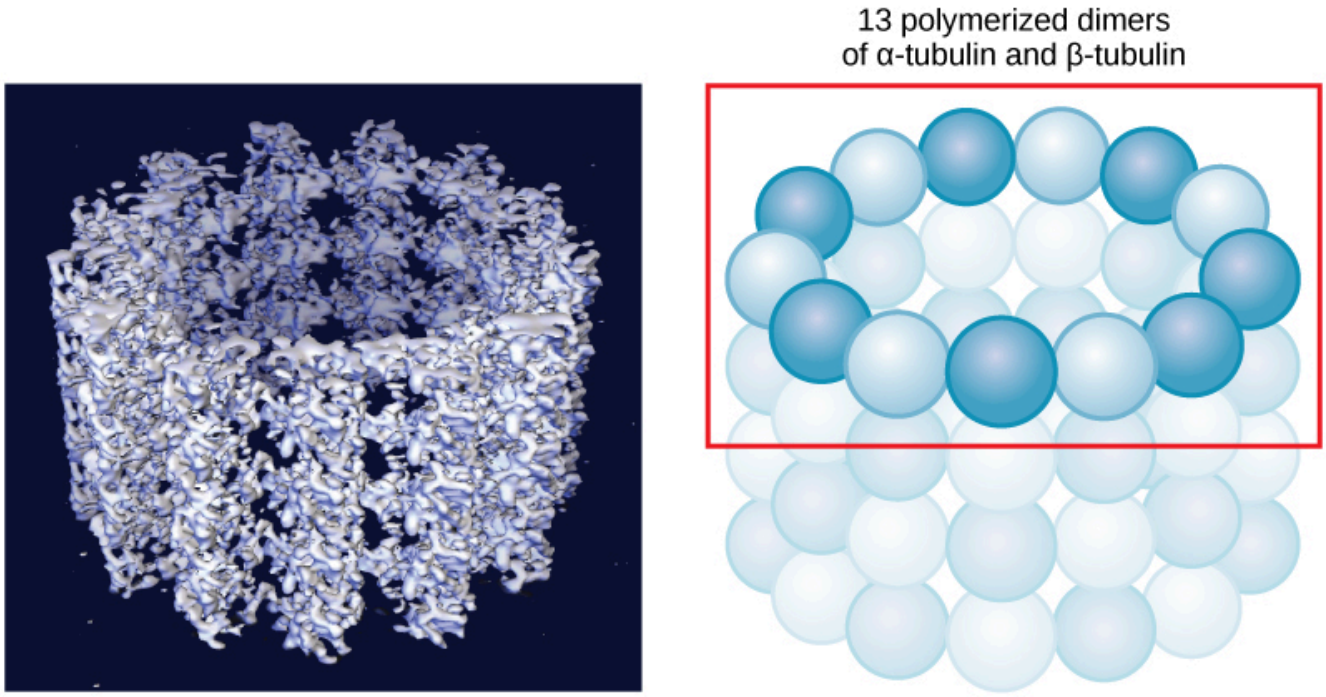

Figure 4.25 Microtubules are hollow. Their walls consist of 13 polymerized dimers of α-tubulin and β-tubulin (right image). The left image shows the tube's molecular structure.

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

Flagella and Cilia

The **flagella** (singular = flagellum) are long, hair-like structures composed of microtubules that extend from the plasma membrane and enable an entire cell to move with an undulating, wave-like motion (for example, sperm cells, and the protist *Euglena*). When present, the cell has just one flagellum or a few flagella. While prokaryotes may also have flagella, their structure and movement are distinct from those found in eukaryotes.

When **cilia** (singular = cilium) are present, many of them extend along the plasma membrane's entire surface. They are short, hair-like structures that move entire cells (such as protists called Paramecia) or substances along the cell's outer surface (for example, the cilia of cells lining the Fallopian tubes that move the ovum toward the uterus, or cilia lining the cells of the respiratory tract that trap particulate matter and move it toward your esophagus.)

Despite their differences in length and number, flagella and cilia share a common structural arrangement of microtubules called a "9 + 2 array." This is an appropriate name because a single flagellum or cilium is made of a ring of nine microtubule doublets, surrounding a single microtubule doublet in the center (Figure 4.26).

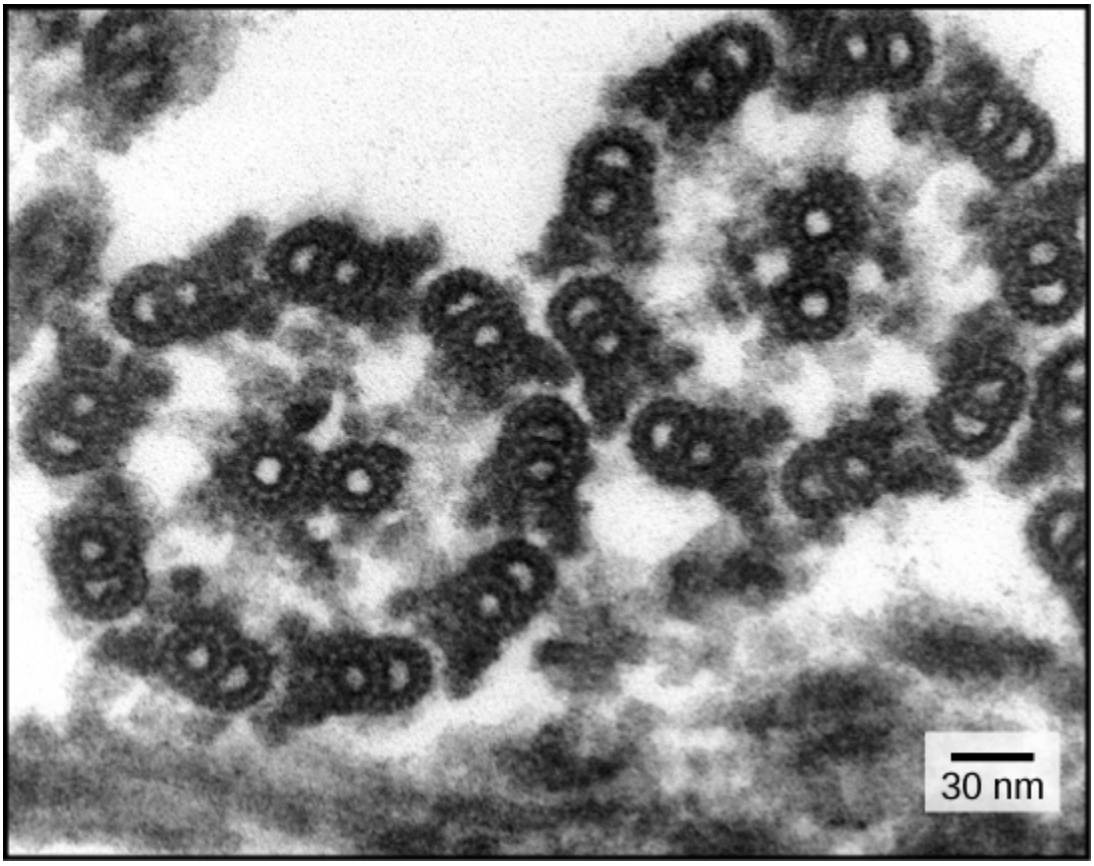

Figure 4.26 This transmission electron micrograph of two flagella shows the microtubules' 9 + 2 array: nine microtubule doublets surround a single microtubule doublet. (credit: modification of work by Dartmouth Electron Microscope Facility, Dartmouth College; scale-bar data from Matt Russell)

You have now completed a broad survey of prokaryotic and eukaryotic cell components. For a summary of cellular components in prokaryotic and eukaryotic cells, see Table 4.1.

Components of Prokaryotic and Eukaryotic Cells

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 cell	Yes	Yes	Yes
Cytoplasm	Provides turgor pressure to plant cells as fluid inside the central vacuole; site of many metabolic reactions; medium in which organelles are found	Yes	Yes	Yes
Nucleolus	Darkened area within the nucleus where ribosomal subunits are synthesized.	No	Yes	Yes
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	Oxidize and thus break down fatty acids and amino acids, and detoxify 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; microtubule source in animal cells	No	Yes	No
Lysosomes	Digestion of macromolecules; recycling of worn-out organelles	No	Yes	Some
Cell wall	Protection, structural support, and maintenance of cell shape	Yes, primarily peptidoglycan	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 cell, and enables unicellular organisms to move independently	Yes	Yes	Yes
Flagella	Cellular locomotion	Some	Some	No, except for some plant sperm cells
Cilia	Cellular locomotion, movement of particles along plasma membrane's extracellular surface, and filtration	Some	Some	No

Table 4.1

35.

CONNECTIONS BETWEEN CELLS AND CELLULAR ACTIVITIES

Learning Objectives

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

- Describe the extracellular matrix
- List examples of the ways that plant cells and animal cells communicate with adjacent cells
- Summarize the roles of tight junctions, desmosomes, gap junctions, and plasmodesmata

You already know that tissue is a group of similar cells working together. As you might expect, if cells are to work together, they must communicate with each other, just as you need to communicate with others if you work on a group project. Let's take a look at how cells communicate with each other.

Extracellular Matrix of Animal Cells

While cells in most multicellular organisms release materials into the extracellular space, animal cells will be discussed as an example. The primary components of these materials are proteins, and the most abundant protein is collagen. Collagen fibers are interwoven with proteoglycans, which are carbohydrate-containing protein molecules. Collectively, we call these materials the **extracellular matrix** (Figure 4.27). Not only does the extracellular matrix hold the cells together to form a tissue, but it also allows the cells within the tissue to communicate with each other. How can this happen?

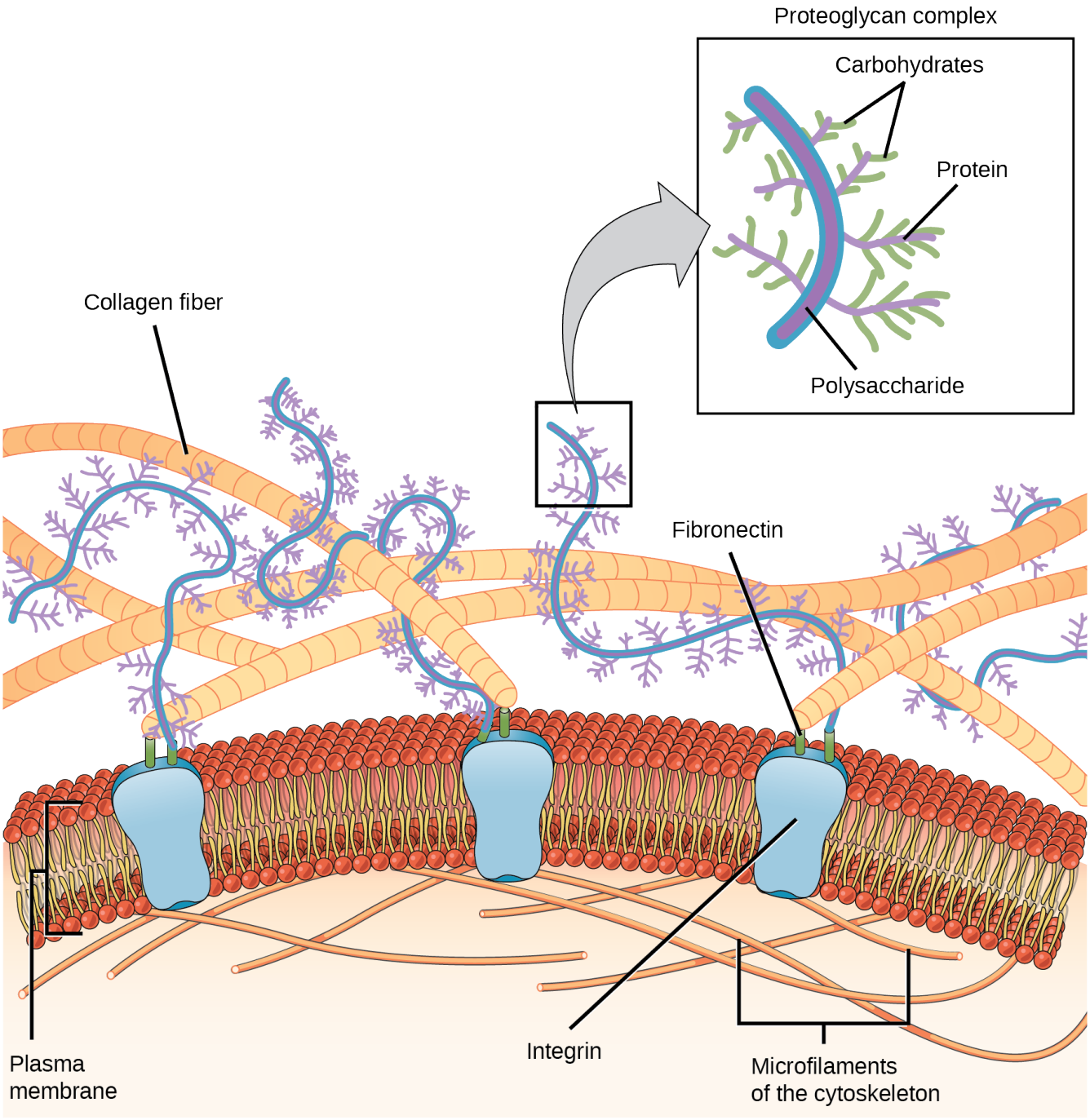

Figure 4.27 The extracellular matrix consists of a network of proteins and carbohydrates.

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

Blood clotting provides an example of the extracellular matrix's role in cell communication. When the cells

lining a blood vessel are damaged, they display a protein receptor, which we call tissue factor. When tissue factor binds with another factor in the extracellular matrix, it causes platelets to adhere to the damaged blood vessel's wall, stimulates the 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.

Intercellular Junctions

Cells can also communicate with each other via direct contact, or intercellular junctions. There are differences in the ways that plant and animal and fungal cells communicate. Plasmodesmata are junctions between plant cells, whereas animal cell contacts include tight junctions, gap junctions, and desmosomes.

Plasmodesmata

In general, long stretches of the plasma membranes of neighboring plant cells cannot touch one another because the cell wall that surrounds each cell separates them (Figure 4.8). How, then, can a plant transfer water and other soil nutrients from its roots, through its stems, and to its leaves? Such transport uses the vascular tissues (xylem and phloem) primarily. There also exist structural modifications, called **plasmodesmata** (singular = plasmodesma). These are numerous channels that pass between adjacent plant cells' cell walls, connect their cytoplasm, and enable transport of materials from cell to cell, and thus throughout the plant (Figure 4.28).

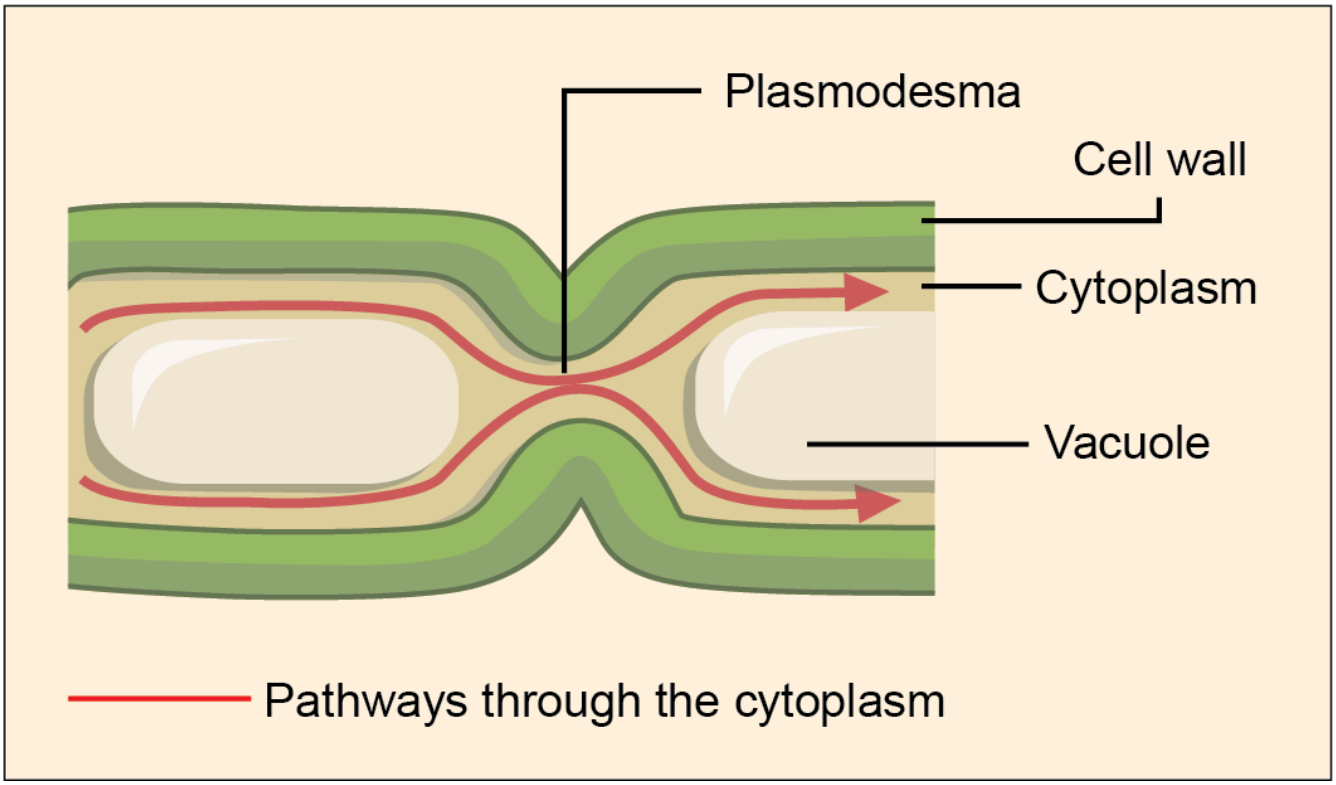

Figure 4.28 A plasmodesma is a channel between two adjacent plant cells' cell walls. Plasmodesmata allow materials to pass from one plant cell's cytoplasm to an adjacent cell's cytoplasm.

Tight Junctions

A **tight junction** is a watertight seal between two adjacent animal cells (Figure 4.29). Proteins (predominantly two proteins called claudins and occludins) tightly hold the cells against each other.

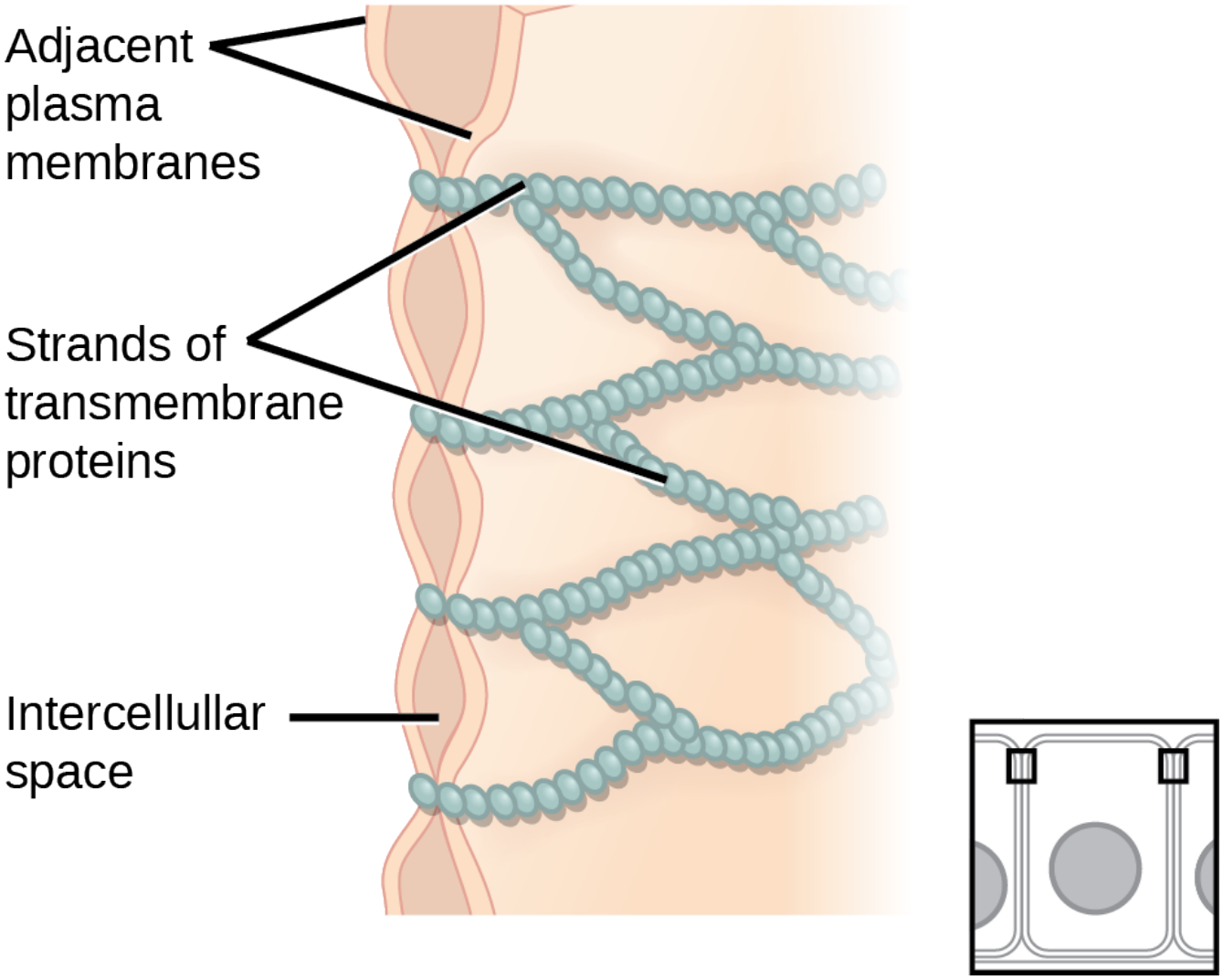

Figure 4.29 Tight junctions form watertight connections between adjacent animal cells. Proteins create tight junction adherence. (credit: modification of work by Mariana Ruiz Villareal)

This tight adherence prevents materials from leaking between the cells; tight junctions are typically found in epithelial tissues that line internal organs and cavities and comprise most of the skin. For example, the tight junctions of the epithelial cells lining your urinary bladder prevent urine from leaking out into the extracellular space.

Desmosomes

Also only in animal cells are **desmosomes**, which act like spot welds between adjacent epithelial cells (Figure 4.30). Cadherins, short proteins in the plasma membrane, connect to intermediate filaments to create

desmosomes. The cadherins connect two adjacent cells and maintain the cells in a sheet-like formation in organs and tissues that stretch, like the skin, heart, and muscles.

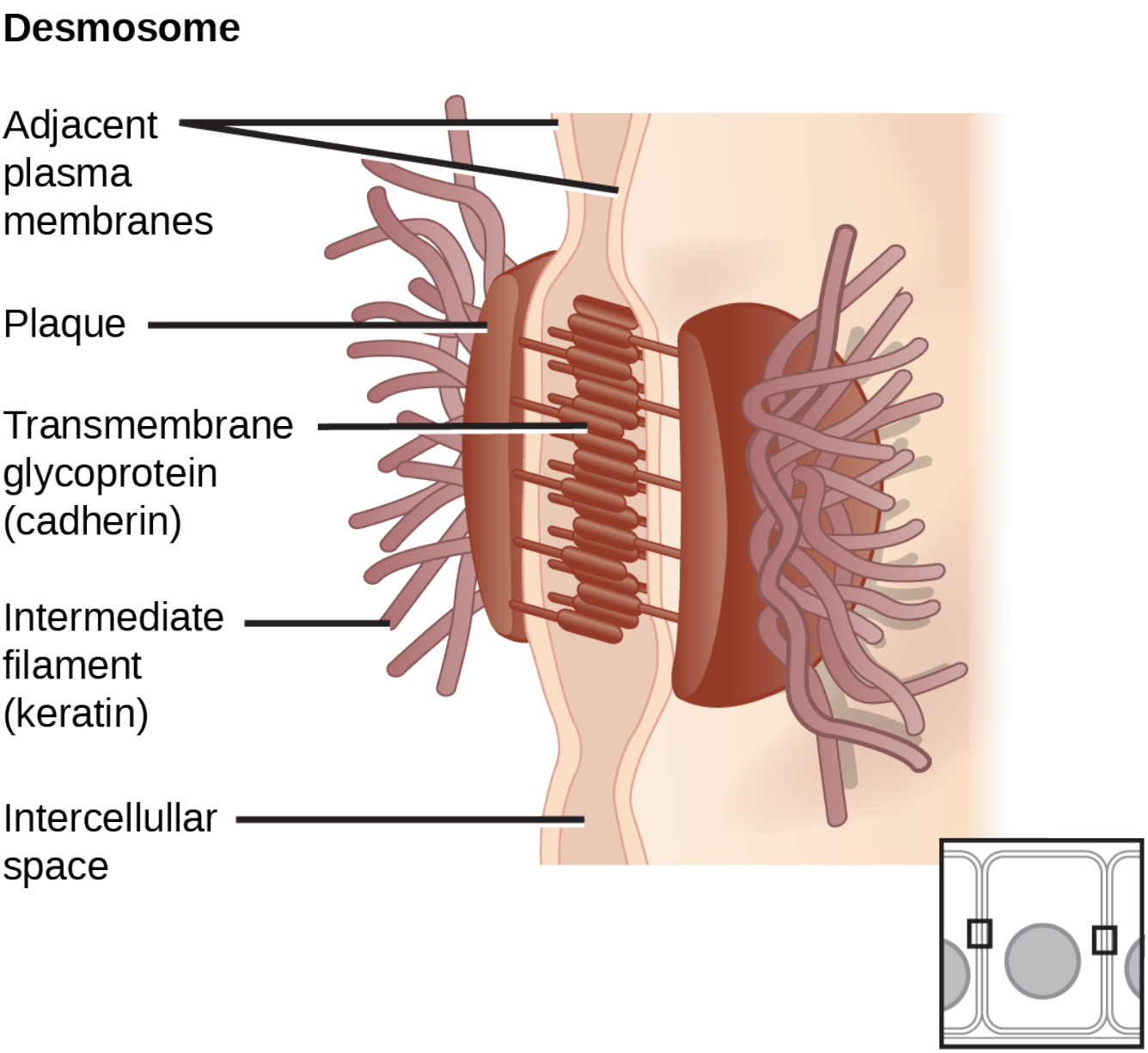

Figure 4.30 A desmosome forms a very strong spot weld between cells. Linking cadherins and intermediate filaments create it. (credit: modification of work by Mariana Ruiz Villareal)

Gap Junctions

Gap junctions in animal cells are like plasmodesmata in plant cells in that they are channels between adjacent cells that allow for transporting ions, nutrients, and other substances that enable cells to communicate (Figure 4.31). Structurally, however, gap junctions and plasmodesmata differ.

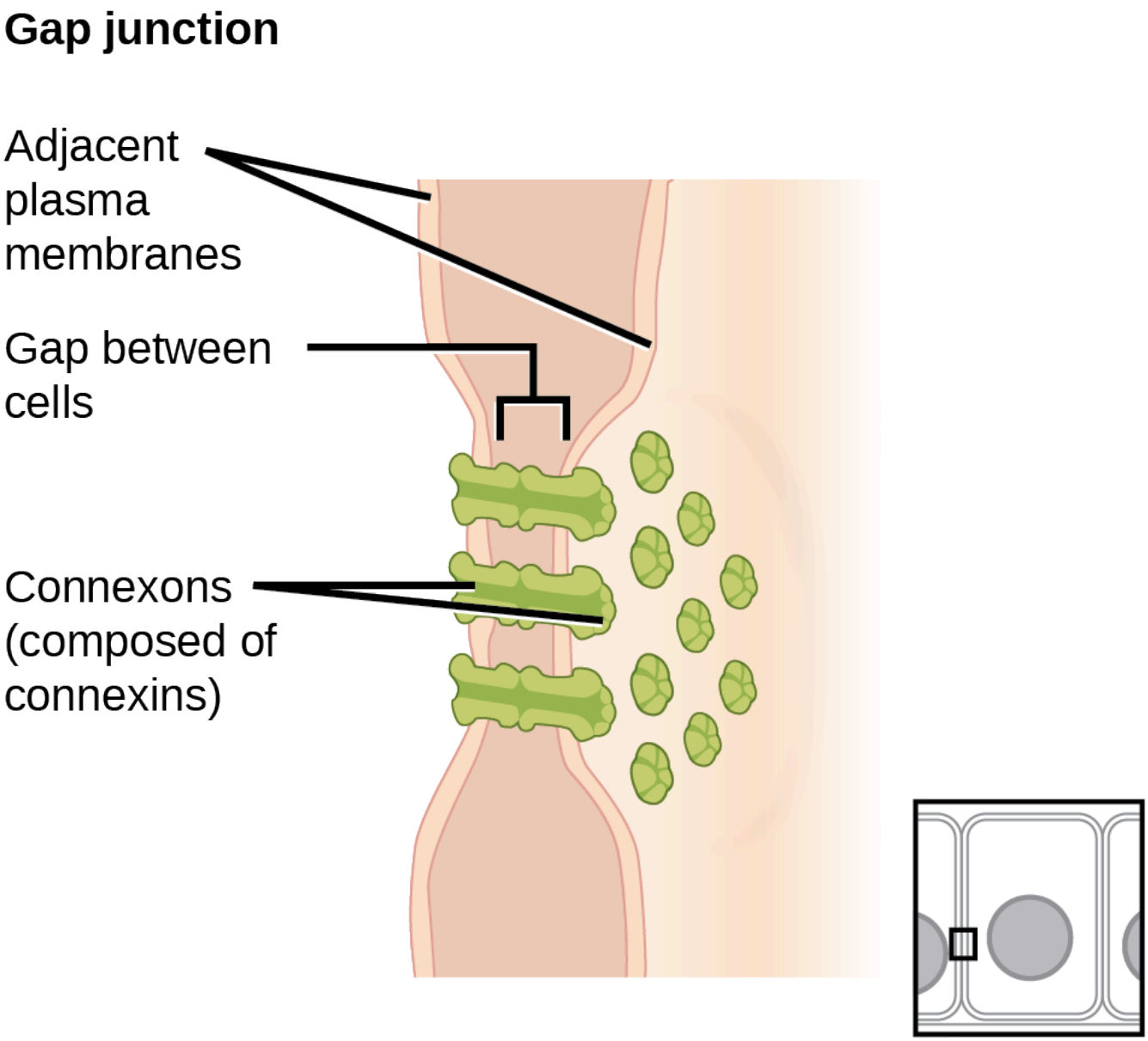

Figure 4.31 A gap junction is a protein-lined pore that allows water and small molecules to pass between adjacent animal cells. (credit: modification of work by Mariana Ruiz Villareal)

Gap junctions develop when a set of six proteins (connexins) in the plasma membrane arrange themselves in an elongated donut-like configuration – a connexon. When the connexon's pores ("donut holes") in adjacent animal cells align, a channel between the two cells forms. Gap junctions are particularly important in cardiac

muscle. The electrical signal for the muscle to contract passes efficiently through gap junctions, allowing the heart muscle cells to contract in tandem.

Link to Learning

To conduct a virtual microscopy lab and review the parts of a cell, work through the steps of this interactive assignment.

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36.

KEY TERMS

cell theory
: see unified cell theory

cell wall
: rigid cell covering comprised of various molecules that protects the cell, provides structural support, and gives shape to the cell

central vacuole
: large plant cell organelle that regulates the cell's storage compartment, holds water, and plays a significant role in cell growth as the site of macromolecule degradation

centrosome
: region in animal cells made of two centrioles that serves as an organizing center for microtubules

chlorophyll
: green pigment that captures the light energy that drives the light reactions of photosynthesis

chloroplast
: plant cell organelle that carries out photosynthesis

chromatin
: protein-DNA complex that serves as the chromosomes' building material

chromosome
: structure within the nucleus that comprises chromatin that contains DNA, the hereditary material

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

cytoplasm
: 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
: protein fiber network that collectively maintains the cell's shape, secures some organelles in specific positions, allows cytoplasm and vesicles to move within the cell, and enables unicellular organisms to move independently

cytosol
: the cytoplasm's gel-like material in which cell structures are suspended

desmosome
: linkages between adjacent epithelial cells that form when cadherins in the plasma membrane attach to intermediate filaments

electron microscope
: an instrument that magnifies an object using an electron beam that passes and bends through a lens system to visualize a specimen

endomembrane system
: group of organelles and membranes in eukaryotic cells that work together modifying, packaging, and transporting lipids and proteins

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

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

extracellular matrix
: material secreted from animal or fungal cells that provides mechanical protection and anchoring for the cells in the tissue

flagellum
: (plural = flagella) long, hair-like structure that extends from the plasma membrane and moves the cell

gap junction
: channel between two adjacent animal cells that allows ions, nutrients, and low molecular weight substances to pass between cells, enabling the cells to communicate

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

intermediate filament
: cytoskeletal component, comprised of several fibrous protein intertwined strands, that bears tension, supports cell-cell junctions, and anchors cells to extracellular structures

light microscope
: an instrument that magnifies an object using a beam of visible light that passes and bends through a lens system to visualize a specimen

lysosome
: 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
: the cytoskeleton system's narrowest element; it provides rigidity and shape to the cell and enables cellular movements

microscope
: an instrument that magnifies an object

microtubule
: the cytoskeleton system's widest element; it helps the cell resist compression, provides a track along which vesicles move through the cell, pulls replicated chromosomes to opposite ends of a dividing cell, and is the structural element of centrioles, flagella, and cilia

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

nuclear envelope
: double-membrane structure that constitutes the nucleus's outermost portion

nucleoid
: central part of a prokaryotic cell's central part where the chromosome is located

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

nucleoplasm
: semi-solid fluid inside the nucleus that contains the chromatin and nucleolus

nucleus
: cell organelle that houses the cell's DNA and directs ribosome and protein synthesis

organelle
: compartment or sac within a cell

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

plasma membrane
: phospholipid bilayer with embedded (integral) or attached (peripheral) proteins, and separates the cell's internal content from its surrounding environment

plasmodesma
: (plural = plasmodesmata) channel that passes between adjacent plant cells' cell walls, connects their cytoplasm, and allows transporting of materials from cell to cell

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

ribosome
: cellular structure that carries out protein synthesis

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

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

tight junction
: protein adherence that creates a firm seal between two adjacent animal cells

unified cell theory
: a biological concept that states that one or more cells comprise all organisms, the cell is the basic unit of life, and new cells arise from existing cells

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

vesicle
: 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

37.

CHAPTER SUMMARY

4.1 Studying Cells

A cell is the smallest unit of life. Most cells are so tiny that we cannot see them with the naked eye. Therefore, scientists use microscopes to study cells. Electron microscopes provide higher magnification, higher resolution, and more detail than light microscopes. The unified cell theory states that one or more cells comprise all organisms, the cell is the basic unit of life, and new cells arise from existing cells.

4.2 Prokaryotic Cells

Prokaryotes are single-celled organisms of the domains Bacteria and Archaea. All prokaryotes have plasma membranes, cytoplasm, ribosomes, and DNA that is not membrane-bound. The DNA is arranged in a single circular chromosome. Bacteria have peptidoglycan cell walls and many have polysaccharide capsules. Prokaryotic cells range in diameter from 0.1 to 5.0 μm.

As a cell increases in size, its surface area-to-volume ratio decreases. If the cell grows too large, the plasma membrane will not have sufficient surface area to support the rate of diffusion required for the increased volume.

4.3 Eukaryotic Cells

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 a membrane surrounds its DNA), and has other membrane-bound organelles that allow for compartmentalizing functions. The DNA of eukaryotes is arranged in multiple linear chromosomes. The plasma membrane is a phospholipid bilayer embedded with proteins. The nucleus's nucleolus is the site of ribosome assembly. We find ribosomes either in the cytoplasm or attached to the cytoplasmic side of the plasma membrane or endoplasmic reticulum. They perform protein synthesis. Mitochondria participate in cellular respiration. They are responsible for the majority of ATP produced in the cell. Peroxisomes hydrolyze 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 perpendicular to each

other, the centrioles, and has an unknown purpose in cell division. Lysosomes are the digestive organelles of animal cells.

Plant cells and plant-like cells each 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 the cell shape. Photosynthesis takes place in chloroplasts. The central vacuole can expand without having to produce more cytoplasm.

4.4 The Endomembrane System and Proteins

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

The RER modifies proteins and synthesizes phospholipids in cell membranes. The SER synthesizes carbohydrates, lipids, and steroid hormones; engages in the detoxification of medications and poisons; and stores calcium ions. Sorting, tagging, packaging, and distributing lipids and proteins take place in the Golgi apparatus. Budding RER and Golgi membranes create lysosomes. Lysosomes digest macromolecules, recycle worn-out organelles, and destroy pathogens.

4.5 The Cytoskeleton

The cytoskeleton has three different protein element types. From narrowest to widest, they are the microfilaments (actin filaments), intermediate filaments, and microtubules. Biologists often associate microfilaments with myosin. They 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 element of centrioles, flagella, and cilia.

4.6 Connections between Cells and Cellular Activities

Animal cells communicate via their extracellular matrices and are connected to each other via tight junctions, desmosomes, and gap junctions. Plant cells are connected and communicate with each other via plasmodesmata.

When protein receptors on the plasma membrane's surface of an animal cell bind to a substance in the extracellular matrix, a chain of reactions begins that changes activities taking place within the cell. Plasmodesmata are channels between adjacent plant cells, while gap junctions are channels between adjacent

animal cells. However, their structures are quite different. A tight junction is a watertight seal between two adjacent cells, while a desmosome acts like a spot weld.

38.

VISUAL CONNECTION QUESTIONS

1. Figure 4.7 Prokaryotic cells are much smaller than eukaryotic cells. What advantages might small cell size confer on a cell? What advantages might large cell size have?

2. Figure 4.8 If the nucleolus were not able to carry out its function, what other cellular organelles would be affected?

3. Figure 4.18 If a peripheral membrane protein were synthesized in the lumen (inside) of the ER, would it end up on the inside or outside of the plasma membrane?

39.

REVIEW QUESTIONS

4. When viewing a specimen through a light microscope, scientists use _____ to distinguish the individual components of cells.

a. a beam of electrons
b. radioactive isotopes
c. special stains
d. high temperatures

5. The _____ is the basic unit of life.

a. organism
b. cell
c. tissue
d. organ

6. Prokaryotes depend on _____ to obtain some materials and to get rid of wastes.

a. ribosomes
b. flagella
c. cell division
d. diffusion

7. Bacteria that lack fimbriae are less likely to _____.

a. adhere to cell surfaces
b. swim through bodily fluids
c. synthesize proteins
d. retain the ability to divide

8. Which of the following organisms is a prokaryote?

a. amoeba
b. influenza A virus
c. charophyte algae
d. E. coli

9. Which of the following is surrounded by two phospholipid bilayers?

a. the ribosomes
b. the vesicles
c. the cytoplasm
d. the nucleoplasm

10. Peroxisomes got their name because hydrogen peroxide is:

a. used in their detoxification reactions
b. produced during their oxidation reactions
c. incorporated into their membranes
d. a cofactor for the organelles' enzymes

11. In plant cells, the function of the lysosomes is carried out by _____.

a. vacuoles
b. peroxisomes
c. ribosomes
d. nuclei

12. Which of the following is both in eukaryotic and prokaryotic cells?

a. nucleus
b. mitochondrion
c. vacuole
d. ribosomes

13. Tay-Sachs disease is a genetic disorder that results in the destruction of neurons due to a buildup of sphingolipids in the cells. Which organelle is malfunctioning in Tay-Sachs?

a. lysosome
b. endoplasmic reticulum

c. peroxisome
d. mitochondria

14. Which of the following is not a component of the endomembrane system?

a. mitochondrion
b. Golgi apparatus
c. endoplasmic reticulum
d. lysosome

15. The process by which a cell engulfs a foreign particle is known as:

a. endosymbiosis
b. phagocytosis
c. hydrolysis
d. membrane synthesis

16. Which of the following is most likely to have the greatest concentration of smooth endoplasmic reticulum?

a. a cell that secretes enzymes
b. a cell that destroys pathogens
c. a cell that makes steroid hormones
d. a cell that engages in photosynthesis

17. Which of the following sequences correctly lists in order the steps involved in the incorporation of a proteinaceous molecule within a cell?

a. protein synthesis of the protein on the ribosome; modification in the Golgi apparatus; packaging in the endoplasmic reticulum; tagging in the vesicle
b. synthesis of the protein on the lysosome; tagging in the Golgi; packaging in the vesicle; distribution in the endoplasmic reticulum
c. synthesis of the protein on the ribosome; modification in the endoplasmic reticulum; tagging in the Golgi; distribution via the vesicle
d. synthesis of the protein on the lysosome; packaging in the vesicle; distribution via the Golgi; tagging in the endoplasmic reticulum

18. Congenital disorders of glycosylation are a growing class of rare diseases. Which organelle would be most commonly involved in the glycoprotein disorder portion of the group?

a. RER
b. ribosomes
c. endosomes
d. Golgi apparatus

19. Which of the following have the ability to disassemble and reform quickly?

a. microfilaments and intermediate filaments
b. microfilaments and microtubules
c. intermediate filaments and microtubules
d. only intermediate filaments

20. Which of the following do not play a role in intracellular movement?

a. microfilaments and intermediate filaments
b. microfilaments and microtubules
c. intermediate filaments and microtubules
d. only intermediate filaments

21. In humans, _____ are used to move a cell within its environment, while _____ are used to move the environment relative to the cell.

a. cilia; pseudopodia
b. flagella; cilia
c. microtubules; flagella
d. microfilaments; microtubules

22. Which of the following are only in plant cells?

a. gap junctions
b. desmosomes
c. plasmodesmata
d. tight junctions

23. The key components of desmosomes are cadherins and _____.

a. actin
b. microfilaments

c. intermediate filaments
d. microtubules

24. Diseased animal cells may produce molecules that activate death cascades to kill the cells in a controlled manner. Why would neighboring healthy cells also die?

a. The death molecule is passed through desmosomes.
b. The death molecule is passed through plasmodesmata.
c. The death molecule disrupts the extracellular matrix.
d. The death molecule passes through gap junctions.

40.

CRITICAL THINKING QUESTIONS

25. In your everyday life, you have probably noticed that certain instruments are ideal for certain situations. For example, you would use a spoon rather than a fork to eat soup because a spoon is shaped for scooping, while soup would slip between the tines of a fork. The use of ideal instruments also applies in science. In what situation(s) would the use of a light microscope be ideal, and why?

26. In what situation(s) would the use of a scanning electron microscope be ideal, and why?

27. In what situation(s) would a transmission electron microscope be ideal, and why?

28. What are the advantages and disadvantages of each of these types of microscopes?

29. Explain how the formation of an adult human follows the cell theory.

30. Antibiotics are medicines that are used to fight bacterial infections. These medicines kill prokaryotic cells without harming human cells. What part or parts of the bacterial cell do you think antibiotics target? Why?

31. Explain why not all microbes are harmful.

32. You already know that ribosomes are abundant in red blood cells. In what other cells of the body would you find them in great abundance? Why?

33. What are the structural and functional similarities and differences between mitochondria and chloroplasts?

34. Why are plasma membranes arranged as a bilayer rather than a monolayer?

35. In the context of cell biology, what do we mean by form follows function? What are at least two examples of this concept?

36. In your opinion, is the nuclear membrane part of the endomembrane system? Why or why not? Defend your answer.

37. What are the similarities and differences between the structures of centrioles and flagella?

38. How do cilia and flagella differ?

39. Describe how microfilaments and microtubules are involved in the phagocytosis and destruction of a pathogen by a macrophage.

40. Compare and contrast the boundaries that plant, animal, and bacteria cells use to separate themselves from their surrounding environment.

41. How does the structure of a plasmodesma differ from that of a gap junction?

42. Explain how the extracellular matrix functions.

43. Pathogenic E. coli have recently been shown to degrade tight junction proteins during infection. How would this provide an advantage to the bacteria?

PART V

STRUCTURE AND FUNCTION OF PLASMA MEMBRANES

41.

INTRODUCTION

Figure 5.1 Despite its seeming hustle and bustle, Grand Central Station functions with a high level of organization: People and objects move from one location to another, they cross or are contained within certain boundaries, and they provide a constant flow as part of larger activity. Analogously, a plasma membrane's functions involve movement within the cell and across boundaries' activities. (credit: modification of work by Randy Le'Moine)

The plasma membrane, the cell membrane, has many functions, but the most basic one is to define the cell's borders and keep the cell functional. The plasma membrane is selectively permeable. This means that the membrane allows some materials to freely enter or leave the cell, while other materials cannot move freely but require a specialized structure and, occasionally, even energy investment for crossing.

42.

COMPONENTS AND STRUCTURE

Learning Objectives

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

- Understand the cell membrane fluid mosaic model
- Describe phospholipid, protein, and carbohydrate functions in membranes
- Discuss membrane fluidity

A cell's plasma membrane defines the cell, outlines its borders, and determines the nature of its interaction with its environment (see Table 5.1 for a summary). Cells exclude some substances, take in others, and excrete still others, all in controlled quantities. The plasma membrane must be very flexible to allow certain cells, such as red and white blood cells, to change shape as they pass through narrow capillaries. These are the more obvious plasma membrane functions. In addition, the plasma membrane's surface carries markers that allow cells to recognize one another, which is vital for tissue and organ formation during early development, and which later plays a role in the immune response's "self" versus "non-self" distinction.

The capacity of complex, integral proteins, receptors to transfer signals, is one of the most sophisticated plasma membrane activities. These proteins act both as extracellular input receivers and as intracellular processing activators. These membrane receptors provide extracellular attachment sites for effectors like hormones and growth factors, and they activate intracellular response cascades when their effectors are bound. Occasionally, viruses hijack receptors (HIV, human immunodeficiency virus, is one example) that use them to gain entry into cells, and at times, the genes encoding receptors become mutated, causing the signal transduction process to malfunction with disastrous consequences.

Fluid Mosaic Model

Scientists identified the plasma membrane in the 1890s, and its chemical components in 1915. The principal components they identified were lipids and proteins. In 1935, Hugh Davson and James Danielli proposed the plasma membrane's structure. This was the first model that others in the scientific community widely accepted. It was based on the plasma membrane's "railroad track" appearance in early electron micrographs. Davson and Danielli theorized that the plasma membrane's structure resembles a sandwich. They made the analogy of proteins to bread, and lipids to the filling. In the 1950s, advances in microscopy, notably transmission electron microscopy (TEM), allowed researchers to see that the plasma membrane's core consisted of a double, rather than a single, layer. In 1972, S.J. Singer and Garth L. Nicolson proposed a new model that provides microscopic observations and better explains plasma membrane function.

The explanation, the **fluid mosaic model**, has evolved somewhat over time, but it still best accounts for plasma membrane structure and function as we now understand them. The fluid mosaic model describes the plasma membrane structure as a mosaic of components—including phospholipids, cholesterol, proteins, and carbohydrates— that can move sideways in the lipid layer like ice cubes in a liquid or fluid. 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 about 1,000 times wider than a plasma membrane. The double-layered look of the membrane resembles a bit a sandwich structure (Figure 5.2).

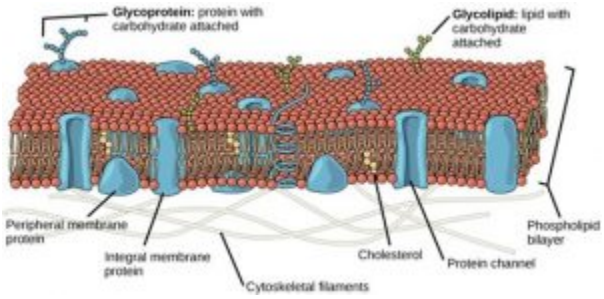


Figure 5.2 The plasma membrane fluid mosaic model describes the plasma membrane as a fluid combination of phospholipids, cholesterol, and proteins. Carbohydrates attached to lipids (glycolipids) and to proteins (glycoproteins) extend from the membrane's outward-facing surface.

A plasma membrane's principal components are lipids (phospholipids and cholesterol), proteins, and carbohydrates attached to some of the lipids and proteins. A phospholipid is a molecule consisting of glycerol, two fatty acids, and a phosphate-linked head group. Cholesterol, another lipid comprised of four fused carbon rings, is situated alongside the phospholipids in the membrane's core. The protein, lipid, and carbohydrate proportions in the plasma membrane vary with cell type, but for a typical human cell, protein accounts

for about 50 percent of the composition by mass, lipids (of all types) account for about 40 percent, and carbohydrates comprise the remaining 10 percent. However, protein and lipid concentration vary with different cell membranes. For example, myelin, an outgrowth of specialized cells' membrane that insulates the peripheral nerves' axons, contains only 18 percent protein and 76 percent lipid. The mitochondrial inner membrane contains 76 percent protein and only 24 percent lipid. The plasma membrane of human red blood cells is 30 percent lipid. Carbohydrates are present only on the plasma membrane's exterior surface and are attached to proteins, forming **glycoproteins**, or attached to lipids, forming glycolipids.

Phospholipids

The membrane's main fabric comprises amphiphilic, phospholipid molecules. Scientists call a molecule with a positively or negatively charged area and an uncharged, or non-polar, area **amphiphilic** ("dual-loving"). The **hydrophilic** ("water-loving") areas of these molecules (which look like a collection of balls in an artist's rendition of the model) (Figures 5.2, 5.3) are in contact with the aqueous fluid both inside and outside the cell. **Hydrophobic**, or water-fearing molecules, tend to be non-polar. 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 phospholipids' hydrophilic regions form hydrogen bonds with water and other polar molecules on both the cell's exterior and interior. Thus, the membrane surfaces that face the cell's interior and exterior are hydrophilic. In contrast, the cell membrane's interior (where the individual layers meet face to face) is hydrophobic and will not interact with water. Therefore, phospholipids form an excellent two-layer cell membrane that separates fluid within the cell from the fluid outside the cell.

A phospholipid molecule (Figure 5.3) 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 a head area (the phosphate-containing group), which has a polar character or negative charge, and a tail area (the fatty acids), which has no charge. The head can form hydrogen bonds, but the tail cannot.

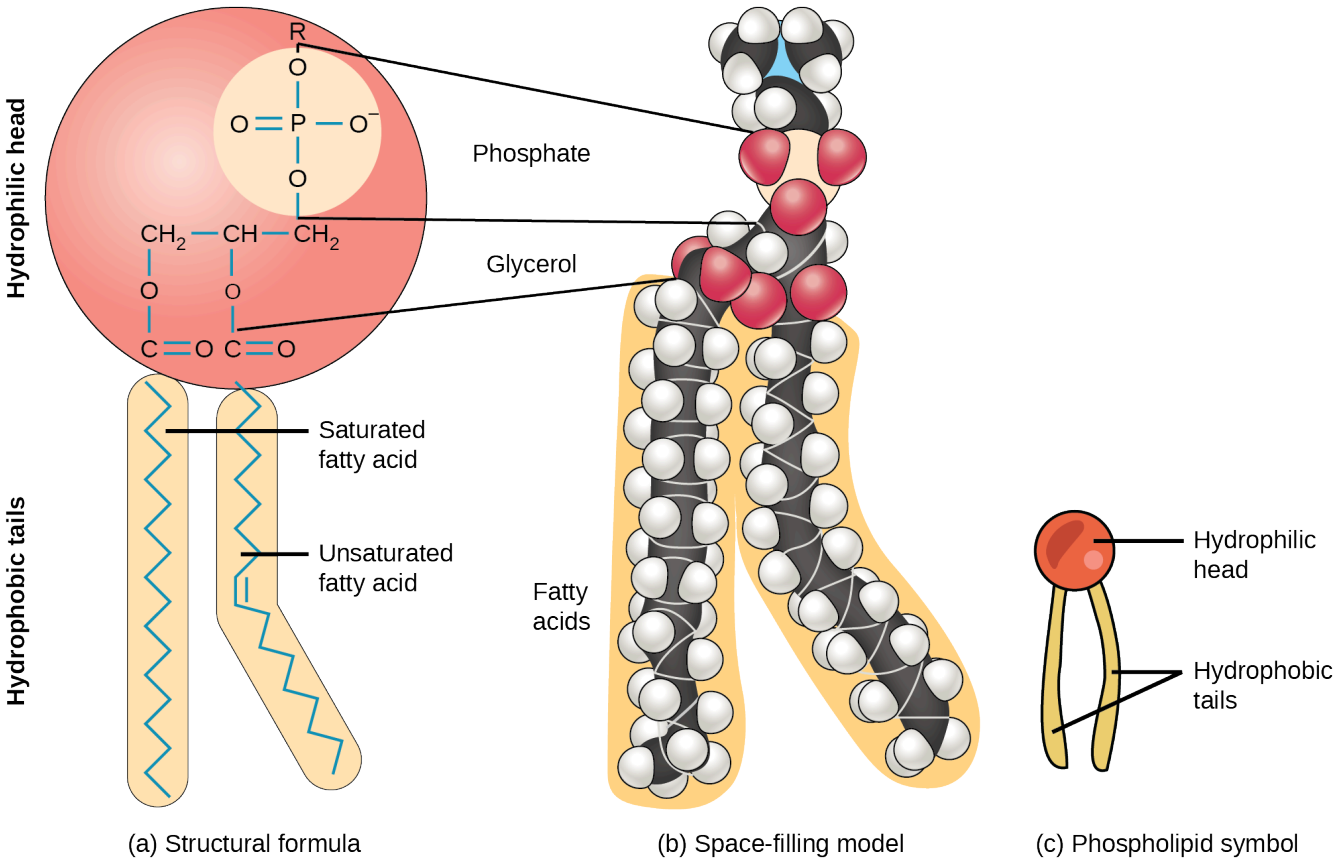
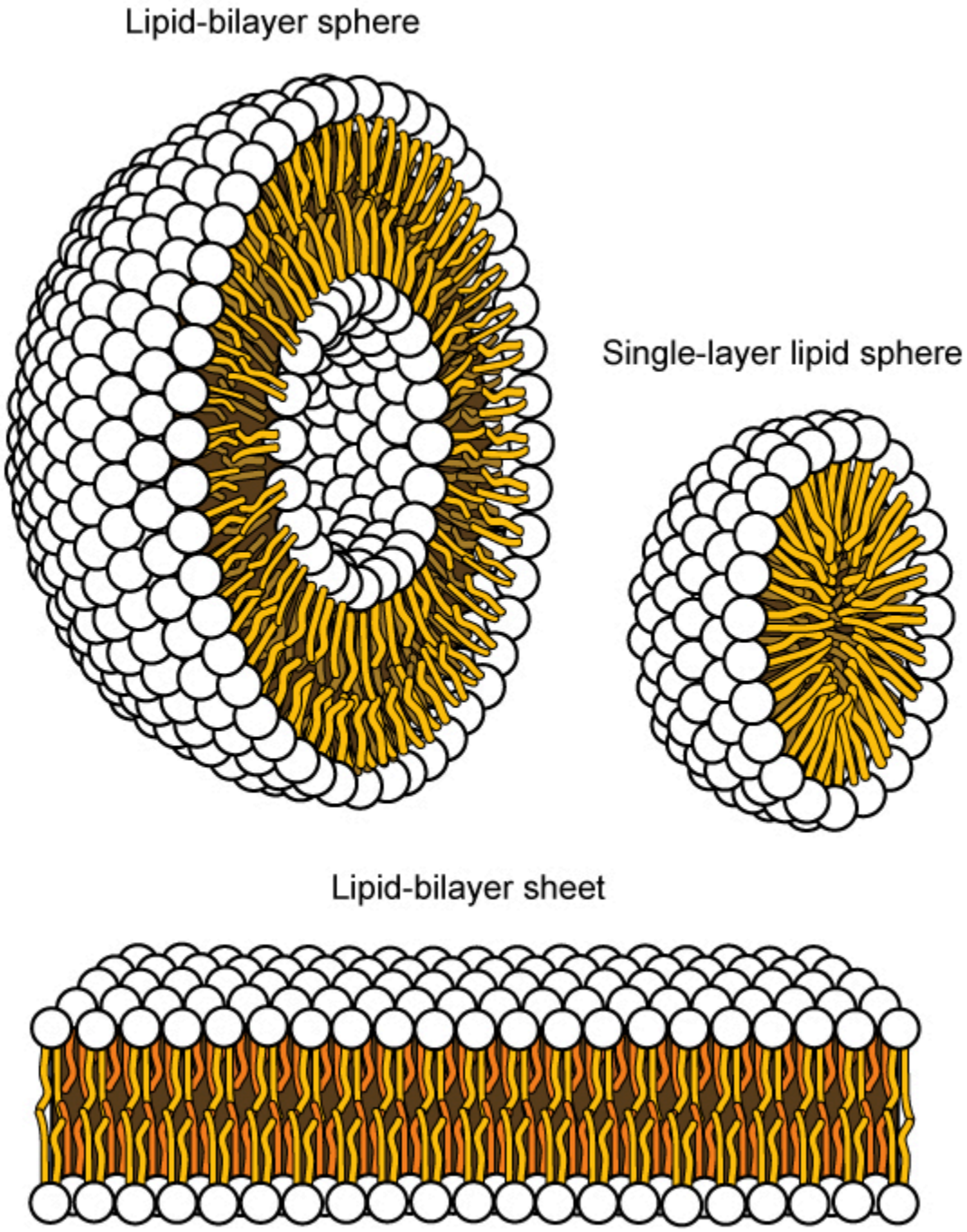


Figure 5.3 A hydrophilic head and two hydrophobic tails comprise this phospholipid molecule. The hydrophilic head group consists of a phosphate-containing group attached to a glycerol molecule. The hydrophobic tails, each containing either a saturated or an unsaturated fatty acid, are long hydrocarbon chains.

This characteristic is vital to the plasma membrane's structure because, in water, phospholipids arrange themselves with their hydrophobic tails facing each other and their hydrophilic heads facing out. In this way, they form a lipid bilayer—a double-layered phospholipid barrier that separates the water and other materials on one side from the water and other materials on the other side. **Phospholipids** heated in an aqueous solution usually spontaneously form small spheres or droplets (micelles or liposomes), with their hydrophilic heads forming the exterior and their hydrophobic tails on the inside (Figure 5.4).



In an aqueous solution, phospholipids usually arrange themselves with their polar heads facing outward and their hydrophobic tails facing inward. (credit: modification of work by Mariana Ruiz Villareal)

Integral Proteins

Proteins comprise the plasma membranes' second major component. **Integral proteins**, or integrins, as their name suggests, integrate completely into the membrane structure, and their hydrophobic membrane-spanning regions interact with the phospholipid bilayer's hydrophobic region (Figure 5.2). Single-pass integral

membrane proteins usually have a hydrophobic transmembrane segment that consists of 20–25 amino acids. Some span only part of the membrane—associating with a single layer—while others stretch from one side to the other and are exposed on either side. Up to 12 single protein segments comprise some complex proteins, which are extensively folded and embedded in the membrane (Figure 5.5). This protein type has a hydrophilic region or regions, and one or several mildly hydrophobic regions. This arrangement of protein regions orients the protein alongside the phospholipids, with the protein's hydrophobic region adjacent to the **phospholipids'** tails and the protein's hydrophilic region or regions protruding from the membrane and in contact with the cytosol or extracellular fluid.

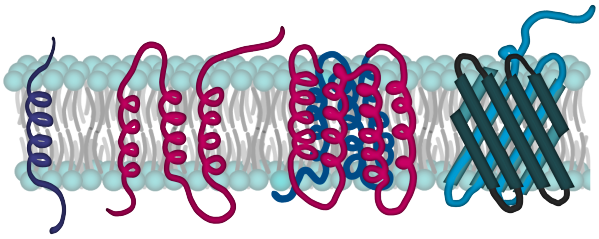


Figure 5.5 Integral membrane proteins may have one or more alpha-helices that span the membrane (examples 1 and 2), or they may have beta-sheets that span the membrane (example 3). (credit: "Foobar"/Wikimedia Commons)

Peripheral proteins are on the membranes' exterior and interior surfaces, attached either to integral proteins or to phospholipids. Peripheral proteins, along with integral proteins, may serve as enzymes, as structural attachments for the cytoskeleton's fibers, or as part of the cell's recognition sites. Scientists sometimes refer to these as "cell-specific" proteins. The body recognizes its own proteins and attacks foreign proteins associated with invasive pathogens.

Carbohydrates

Carbohydrates are the third major plasma membrane component. They are always on the cells' exterior surface and are bound either to proteins (forming glycoproteins) or to lipids (forming glycolipids) (Figure 5.2). 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. These sites have unique patterns that allow for cell recognition, much the way that the facial features unique to each person allow individuals to recognize him or her. This recognition function is very important to cells, as it allows the immune system to differentiate between body cells ("self") and foreign cells

or tissues ("non-self"). Similar glycoprotein and glycolipid types are on the surfaces of viruses and may change frequently, preventing immune cells from recognizing and attacking them.

We collectively refer to these carbohydrates on the cell's exterior surface—the carbohydrate components of both glycoproteins and glycolipids—as the glycocalyx (meaning "sugar coating"). The glycocalyx is highly hydrophilic and attracts large amounts of water to the cell's surface. This aids in the cell's interaction with its watery environment and in the cell's ability to obtain substances dissolved in the water. As we discussed above, the glycocalyx is also important for cell identification, self/non-self determination, and embryonic development and is used in cell-to-cell attachments to form tissues.

Evolution Connection

How Viruses Infect Specific Organs

Glycoprotein and glycolipid patterns on the cells' surfaces give many viruses an opportunity for infection. HIV and hepatitis viruses infect only specific organs or cells in the human body. Specific glycoproteins on HIV bind the molecule CD4 on human cells. Therefore, HIV is able to penetrate the plasma membranes of a subtype of lymphocytes called T-helper cells, as well as some monocytes and central nervous system cells that have CD4 (Figure 5.6). The hepatitis virus attacks liver cells.

These viruses are able to invade these cells because the cells have binding sites on their surfaces that are specific to and compatible with certain viruses (Figure 5.6). Other recognition sites on the virus's surface interact with the human immune system, prompting the body to produce antibodies. Antibodies are made in response to the antigens or proteins associated with invasive pathogens, or in response to foreign cells, such as might occur with an organ transplant. These same sites serve as places for antibodies to attach and either destroy or inhibit the virus's activity. Unfortunately, these recognition sites on HIV change at a rapid rate because of mutations, making an effective vaccine against the virus very difficult, as the virus evolves and adapts. A person infected with HIV will quickly develop different populations, or variants, of the virus that differences in these recognition sites distinguish. This rapid change of surface markers decreases the effectiveness of the person's immune system in attacking the virus because the antibodies will not recognize the surface patterns' new variations. In the case of HIV, the problem is compounded because the virus specifically infects and destroys cells involved in the immune response, further incapacitating the host.

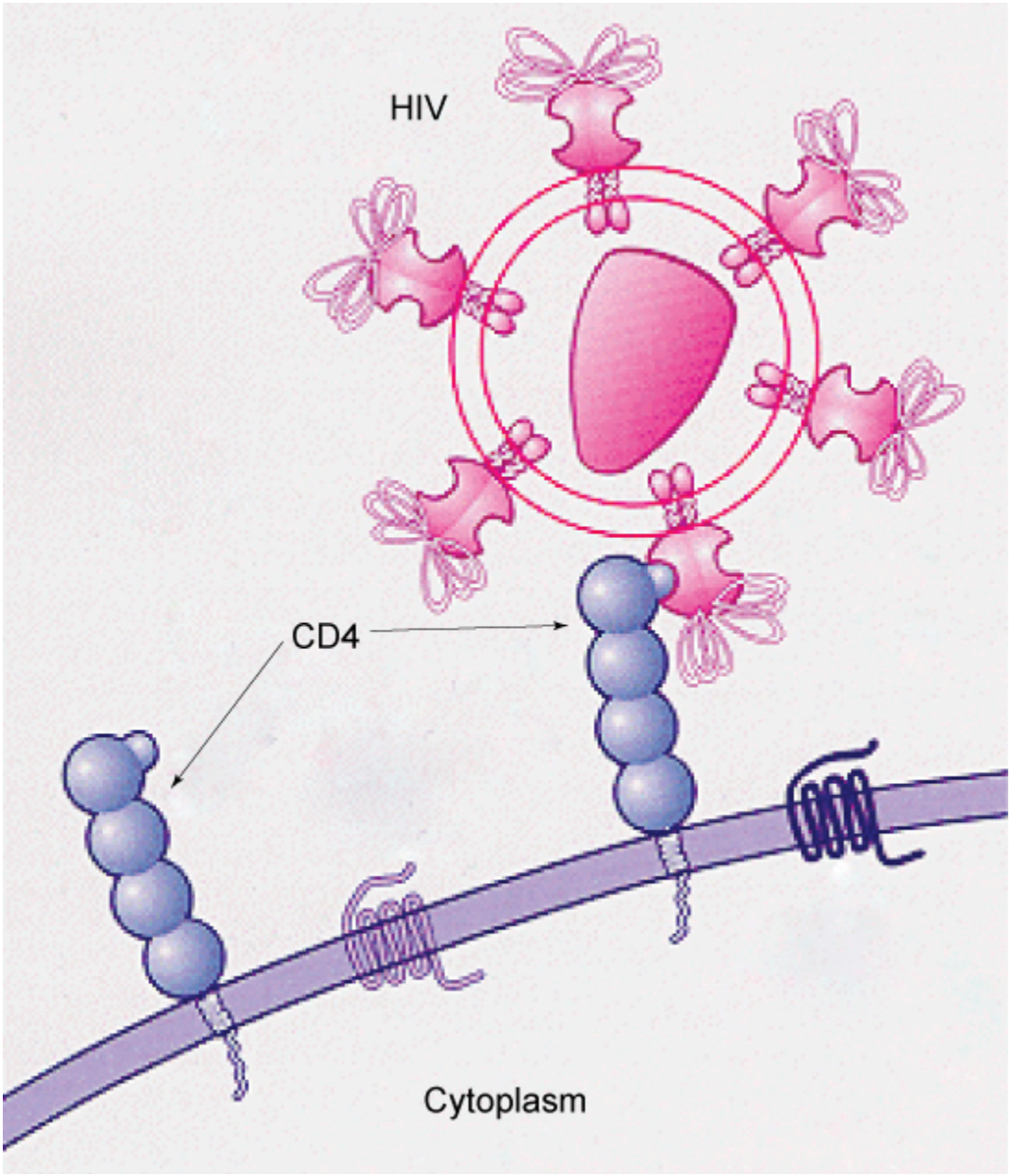


Figure 5.6 HIV binds to the CD4 receptor, a glycoprotein on T cell surfaces. (credit: modification of work by NIH, NIAID)

Membrane Fluidity

The membrane's mosaic characteristic helps to illustrate its nature. The integral proteins and lipids exist in

the membrane as separate but loosely attached molecules. These resemble the separate, multicolored tiles of a mosaic picture, and they float, moving somewhat with respect to one another. The membrane is not like a balloon, however, that can expand and contract; rather, it is fairly rigid and can burst if penetrated or if a cell takes in too much water. However, because of its mosaic nature, a very fine needle can easily penetrate a plasma membrane without causing it to burst, and the membrane will flow and self-seal when one extracts the needle.

The membrane's mosaic characteristics explain some but not all of its fluidity. There are two other factors that help maintain this fluid characteristic. One factor is the nature of the phospholipids themselves. In their saturated form, the fatty acids in phospholipid tails are saturated with bound hydrogen atoms. There are no double bonds between adjacent carbon atoms. This results in tails that are relatively straight. In contrast, unsaturated fatty acids do not contain a maximal number of hydrogen atoms, but they do contain some double bonds between adjacent carbon atoms. A double bond results in a bend in the carbon string of approximately 30 degrees (Figure 5.3).

Thus, if decreasing temperatures compress saturated fatty acids with their straight tails, they press in on each other, making a dense and fairly rigid membrane. If unsaturated fatty acids are compressed, the "kinks" in their tails elbow adjacent phospholipid molecules away, maintaining some space between the phospholipid molecules. This "elbow room" helps to maintain fluidity in the membrane at temperatures at which membranes with saturated fatty acid tails in their phospholipids would "freeze" or solidify. The membrane's relative fluidity is particularly important in a cold environment. A cold environment usually compresses membranes comprised largely of saturated fatty acids, making them less fluid and more susceptible to rupturing. Many organisms (fish are one example) are capable of adapting to cold environments by changing the proportion of unsaturated fatty acids in their membranes in response to lower temperature.

Link to Learning

Visit this site to see animations of the membranes' fluidity and mosaic quality.

Animals have an additional membrane constituent that assists in maintaining fluidity. Cholesterol, which lies alongside the phospholipids in the membrane, tends to dampen temperature effects on the membrane. Thus, this lipid functions as a buffer, preventing lower temperatures from inhibiting fluidity and preventing increased temperatures from increasing fluidity too much. Thus, cholesterol extends, in both directions, the temperature range in which the membrane is appropriately fluid and consequently functional. Cholesterol also serves other functions, such as organizing clusters of transmembrane proteins into lipid rafts.

Plasma Membrane Components and Functions

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

Table 5.1

Career Connection

Immunologist

The variations in peripheral proteins and carbohydrates that affect a cell's recognition sites are of prime interest in immunology. In developing vaccines, researchers have been able to conquer many infectious diseases, such as smallpox, polio, diphtheria, and tetanus.

Immunologists are the physicians and scientists who research and develop vaccines, as well as treat and study allergies or other immune problems. Some immunologists study and treat autoimmune problems (diseases in which a person's immune system attacks their own cells or tissues, such as lupus) and immunodeficiencies, whether acquired (such as acquired immunodeficiency syndrome, or AIDS) or hereditary (such as severe combined immunodeficiency, or SCID). Immunologists also help treat organ transplantation patients, who must have their immune systems suppressed so that their bodies will not reject a transplanted organ. Some immunologists work to understand natural immunity and the effects of a person's environment on it. Others work on questions about how the immune system affects diseases such as cancer. In the past, researchers did not understand the importance of having a healthy immune system in preventing cancer.

Immunologists who focus on certain types of diseases or pathogens can play an important role in saving lives during specific outbreaks and pandemics. Kizzmekia S. Corbett, for example, was a research fellow and scientific lead working specifically on

coronaviruses. When the COVID-19 pandemic occurred, Corbett's deep experience and knowledge were instrumental in developing one of the main vaccines (Moderna). She is now applying that experience to other respiratory diseases and vaccine development processes.

To work as an immunologist, one must have a PhD or MD. In addition, immunologists undertake at least two to three years of training in an accredited program and must pass the American Board of Allergy and Immunology exam. Immunologists must possess knowledge of the human body's functions as they relate to issues beyond immunization, and knowledge of pharmacology and medical technology, such as medications, therapies, test materials, and surgical procedures.

43.

PASSIVE TRANSPORT

Learning Objectives

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

- Explain why and how passive transport occurs
- Understand the osmosis and diffusion processes
- Define tonicity and its relevance to passive transport

Plasma membranes must allow certain substances to enter and leave a cell, and prevent some harmful materials from entering and some essential materials from leaving. In other words, plasma membranes are **selectively permeable** (semipermeable)—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. 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 doing just that. Most cells spend the majority of their energy on maintaining an imbalance of sodium and potassium ions between the cell's interior and exterior, as well as on protein synthesis.

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 single substance concentration range has a **concentration gradient**.

Selective Permeability

Plasma membranes are asymmetric: the membrane's interior is not identical to its exterior. There is a considerable difference between the array of phospholipids and proteins between the two leaflets that form a membrane. On the membrane's interior, some proteins serve to anchor the membrane to the cytoskeleton's fibers. There are peripheral proteins on the membrane's exterior that bind extracellular matrix elements. Carbohydrates, attached to lipids or proteins, are also on the plasma membrane's exterior surface. These carbohydrate complexes help the cell bind required substances in the extracellular fluid. This adds considerably to plasma membrane's selective nature (Figure 5.7).

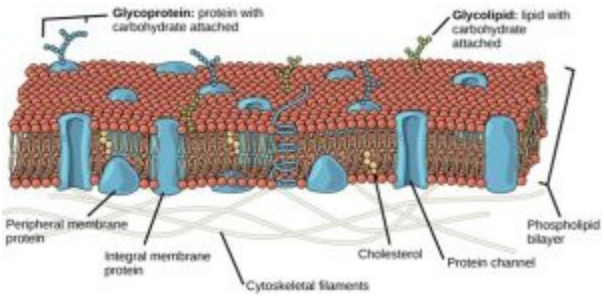


Figure 5.7 The plasma membrane's exterior surface is not identical to its interior surface.

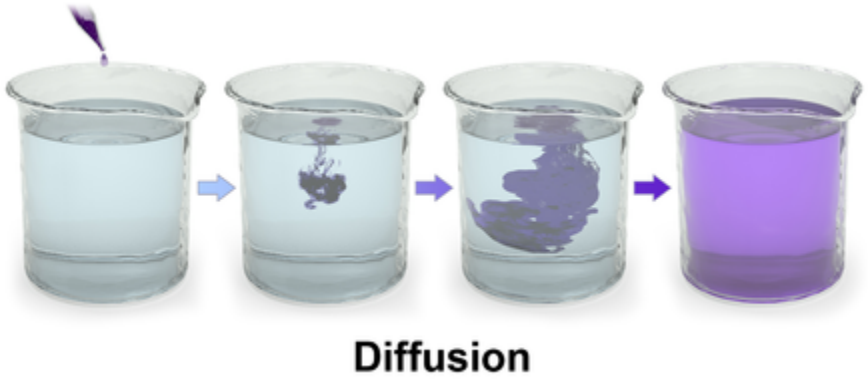
Recall that plasma membranes are amphiphilic: they have hydrophilic and hydrophobic regions. This characteristic helps move some materials through the membrane and hinders the movement of others. Non-polar and lipid-soluble material with a low molecular weight can easily slip through the membrane's hydrophobic lipid core. Substances such as the fat-soluble vitamins A, D, E, and K readily pass through the plasma membranes in the digestive tract and other tissues. Fat-soluble drugs and hormones also gain easy entry into cells and readily transport themselves into the body's tissues and organs. Oxygen and carbon dioxide molecules have no charge and pass through membranes by simple diffusion.

Polar substances present problems for the membrane. While some polar molecules connect easily with the cell's outside, they cannot readily pass through the plasma membrane's lipid core. Additionally, while small ions could easily slip through the spaces in the membrane's mosaic, their charge prevents them from doing so. Ions such as sodium, potassium, calcium, and chloride must have special means of penetrating plasma membranes. Ion channels and ion pumps are examples of these special means. Simple sugars and amino acids also need the help of various transmembrane proteins (channels) to transport themselves across plasma membranes.

Diffusion

Diffusion is a passive process of transport. A single substance moves from a high concentration to a low concentration area 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 room's edges. The ammonia vapor will diffuse, or spread away, from the bottle, and gradually, increasingly 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 (Figure 5.8). Diffusion expends no energy. On the contrary, concentration gradients are a form of potential energy, which dissipates as the gradient is eliminated.

(A)



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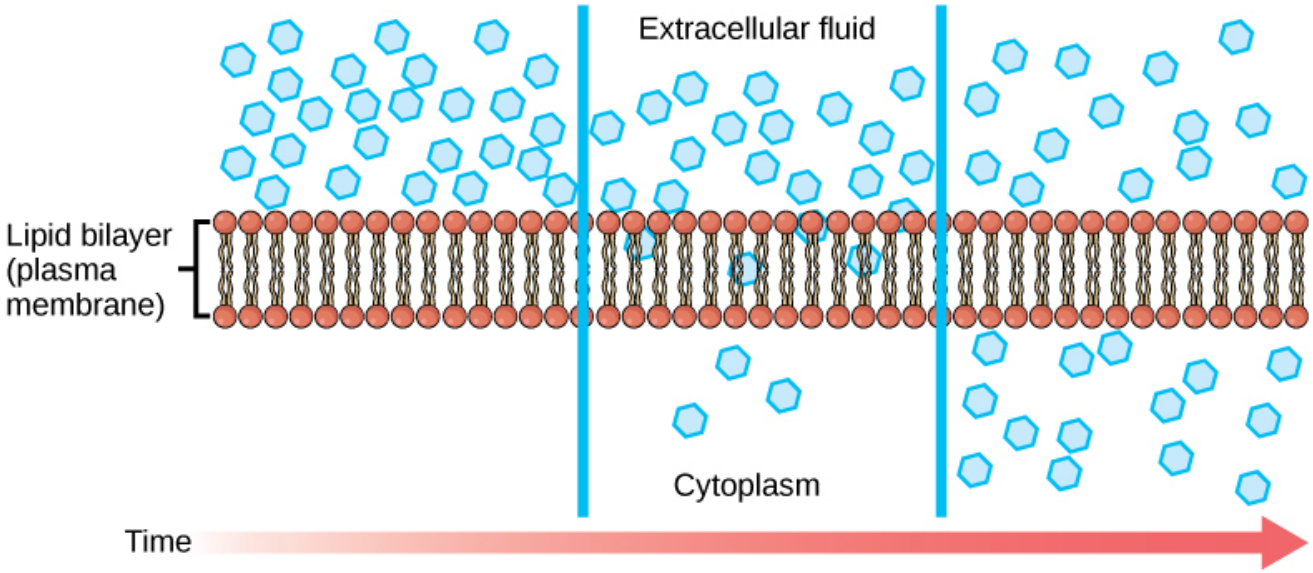

Figure 5.8 (a) Example of diffusion as substances move from a highly concentrated area to a lower concentrated area. **(b)** Diffusion through a permeable membrane moves a substance from a high concentration area (extracellular fluid, in this case) down its concentration gradient (into the cytoplasm). (credit: modification of work by Mariana Ruiz Villareal)

Each separate substance in a medium, such as the extracellular fluid, has its own concentration gradient, independent of other materials' concentration gradients. In addition, each substance will diffuse according to that gradient. Within a system, there will be different diffusion rates of various substances in the medium. For example, a sugar cube in a glass of tea has its own concentration. A drop of condensed milk added to the tea has its own independent concentration gradient. Over time, without using a spoon, the sugar would diffuse evenly within the cup of tea. Likewise, the milk would spread out by diffusion over time without using a spoon.

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 molecular diffusion through whatever medium in which they are localized. A substance moves into any space available to it until it evenly distributes itself throughout. 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. We call this lack of a concentration gradient in which the substance has no net movement dynamic equilibrium. While diffusion will go forward in the presence of a substance's concentration gradient, several factors affect the diffusion rate.

- 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 diffusion rate.
- 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 molecules' movement, increasing the diffusion rate. Lower temperatures decrease the molecules' energy, thus decreasing the diffusion rate.
- Solvent density: As the density of a solvent increases, the diffusion rate decreases. The molecules slow down because they have a more difficult time passing 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 diffusion rate 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 we discussed earlier, nonpolar or lipid-soluble materials pass through plasma membranes more easily than polar materials, allowing a faster diffusion rate.
- Surface area and plasma membrane thickness: Increased surface area increases the diffusion rate, whereas a thicker membrane reduces it.
- Distance traveled: The greater the distance that a substance must travel, the slower the diffusion rate. This places an upper limitation on cell size. A large, spherical cell will die because nutrients or waste cannot reach or leave the cell's center, respectively. Therefore, cells must either be small in size, as in the case of many prokaryotes, or be flattened, as with many single-celled eukaryotes.

A variation of diffusion is the process of filtration. In filtration, material moves according to its concentration gradient through a membrane. Sometimes pressure enhances the diffusion rate, 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 diffusion rate 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 abnormally high pressure "squeezes through."

Facilitated transport

In facilitated transport, or 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 polar molecule ions that the cell membrane's hydrophobic parts repel. Facilitated transport proteins shield these materials from the membrane's repulsive force, allowing them to diffuse into the cell.

The transported material first attaches to protein or glycoprotein receptors on the plasma membrane's exterior surface. This allows removal of material from the extracellular fluid that the cell needs. The substances then pass to specific integral proteins that facilitate their passage. Some of these integral proteins are collections of beta-pleated sheets that form a pore or channel through the phospholipid bilayer. Others are carrier proteins that bind with the substance and aid its diffusion through the membrane.

Channels

The integral proteins involved in facilitated transport are **transport proteins**, and they function as either channels for the material or carriers. In both cases, they are transmembrane proteins. Channels are specific for the transported substance. **Channel proteins** have hydrophilic domains exposed to the intracellular and extracellular fluids. In addition, they have a hydrophilic channel through their core that provides a hydrated opening through the membrane layers (Figure 5.9). Passage through the channel allows polar compounds to avoid the plasma membrane's nonpolar central layer that would otherwise slow or prevent their entry into the cell. **Aquaporins** are channel proteins that allow water to pass through the membrane at a very high rate.

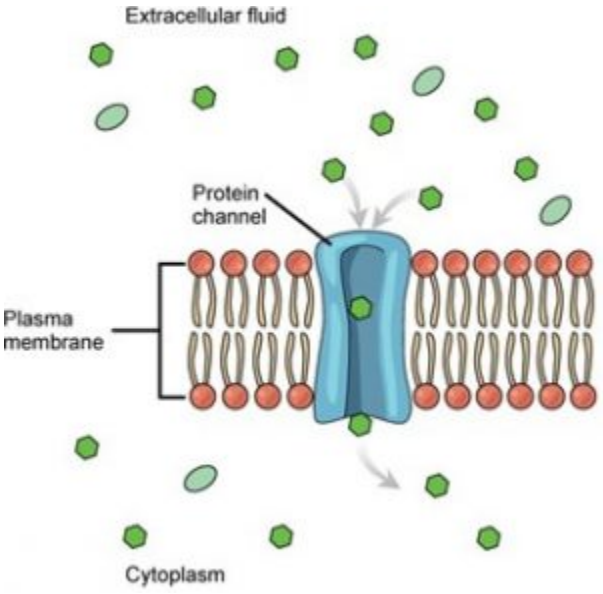

Figure 5.9 Ion Channel Proteins. Ion channel proteins are gated. When they are closed, no ions can pass through them. However, when a channel opens, select ions diffuse through the channel. Channel proteins are highly specific, letting only a specific ion or subset of ions pass. Credit: Rao, A., Ryan, K., Tag, A. and Fletcher, S. Department of Biology, Texas A&M University.

Channel proteins are either open at all times or they are "gated," which controls the channel's opening. When a particular ion attaches to the channel protein it may control the opening, or other mechanisms or substances may be involved. In some tissues, sodium and chloride ions pass freely through open channels, whereas in other tissues a gate must open to allow passage. An example of this occurs in the kidney, where there are both channel forms in different parts of the renal tubules. Cells involved in transmitting electrical impulses, such as nerve and muscle cells, have gated channels for sodium, potassium, and calcium in their membranes. Opening

and closing these channels changes the relative concentrations on opposing sides of the membrane of these ions, resulting in facilitating electrical transmission along membranes (in the case of nerve cells) or in muscle contraction (in the case of muscle cells).

Carrier Proteins

Another type of protein embedded in the plasma membrane is a **carrier protein**. This aptly named protein binds a substance and thus triggers a change of its own shape, moving the bound molecule from the cell's outside to its interior (Figure 5.10). Depending on the gradient, the material may move in the opposite direction. Carrier proteins are typically specific for a single substance. This selectivity adds to the plasma membrane's overall selectivity. Scientists poorly understand the exact mechanism for the change of shape. 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 material for the cell to function properly. When all of the proteins are bound to their ligands, they are saturated and the rate of transport is at its maximum. Increasing the concentration gradient at this point will not result in an increased transport rate.

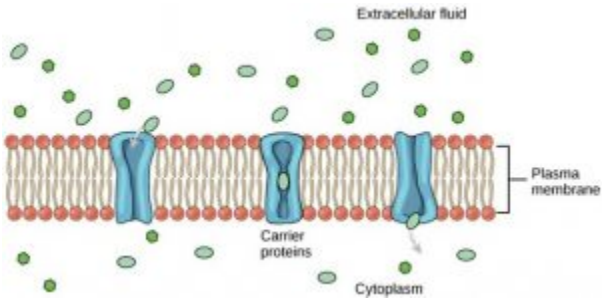

Figure 5.10 Some substances are able to move down their concentration gradient across the plasma membrane with the aid of carrier proteins. Carrier proteins change shape as they move molecules across the membrane. Credit: Rao, A., Tag, A. and Fletcher, S. Department of Biology, Texas A&M University.

An example of this process occurs in the kidney. In one part, the kidney filters glucose, water, salts, ions, and amino acids that the body requires. This filtrate, which includes glucose, then reabsorbs 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 and the body excretes this through urine. In a diabetic individual, the term is "spilling glucose into the urine." A different group of carrier proteins, glucose transport

proteins, or GLUTs, are involved in transporting glucose and other hexose sugars through plasma membranes within the body.

Channel and carrier proteins transport material at different rates. Channel proteins transport much more quickly than 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.

Osmosis

Osmosis is the movement of free water molecules through a semipermeable membrane according to the water's concentration gradient across the membrane, which is inversely proportional to the solutes' concentration. While diffusion transports material across membranes and within cells, osmosis transports *only water* across a membrane and the membrane limits the solutes' diffusion in the water. Not surprisingly, the aquaporins that facilitate water movement play a large role in osmosis, most prominently in red blood cells and the membranes of kidney tubules.

Mechanism

Osmosis is a special case of diffusion. Water, like other substances, moves from an area of high concentration of free water molecules to one of low free water molecule concentration. An obvious question is what makes water move at all? Imagine a beaker with a semipermeable membrane separating the two sides or halves (Figure 5.11). On both sides of the membrane the water level is the same, but there are different dissolved substance concentrations, or **solutes**, that cannot cross the membrane (otherwise the solute crossing the membrane would balance concentrations on each side). If the solution's volume on both sides of the membrane is the same, but the solute's concentrations are different, then there are different amounts of water, the solvent, on either side of the membrane.

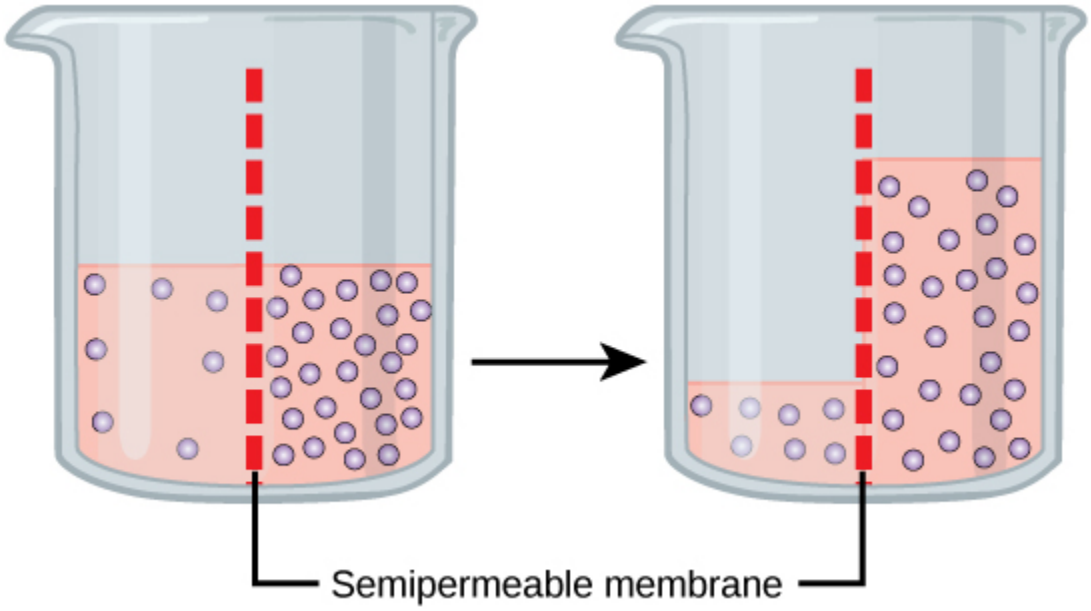

Figure 5.11 In osmosis, water always moves from an area of higher water concentration to one of lower concentration. In the diagram, the solute cannot pass through the selectively permeable membrane, but the water can. The solute is shown as round purple spheres and water is visualized as pink shading.

To illustrate this, imagine two full water glasses. 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 sugar amount 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.

Returning to the beaker example, recall that it has a solute mixture 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 getting 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 water's concentration gradient goes to zero or until the water's hydrostatic pressure balances the osmotic pressure. Osmosis proceeds constantly in living systems. Osmosis is a dynamic and continuous process in living organisms.

Tonicity

Tonicity describes how an extracellular solution can change a cell's volume by affecting osmosis. As a consequence, a cell will lose or gain water. A solution's tonicity often directly correlates with the solution's

osmolarity. **Osmolarity** describes the solution's total solute concentration. A solution with low osmolarity has a greater number of water molecules relative to the number of solute particles. A solution with high osmolarity has fewer water molecules with respect to solute particles. In a situation in which a membrane permeable to water, though not to the solute separates two different osmolarities, water will move from the membrane's side with lower osmolarity (and more water) to the side with higher osmolarity (and less water). This effect makes sense if you remember that the solute cannot move across the membrane, and thus the only component in the system that can move—the water—moves along its own concentration gradient. An important distinction that concerns living systems is that osmolarity measures the number of particles (which may be molecules) in a solution. Therefore, a solution that is cloudy with cells may have a lower osmolarity than a solution that is clear, if the second solution contains more dissolved molecules than there are cells.

Hypotonic Solutions

Scientists use three terms—hypotonic, isotonic, and hypertonic—to relate the cell's osmolarity to the extracellular fluid's osmolarity that contains the cells. In a **hypotonic** situation, the extracellular fluid has lower osmolarity than the fluid inside the cell, and water enters the cell. (In living systems, the point of reference is always the cytoplasm, so the prefix *hypo–* means that the extracellular fluid has a lower solute concentration, or a lower osmolarity, than the cell cytoplasm.) It also means that the extracellular fluid has a higher water concentration in the solution than does the cell. In this situation, water will follow its concentration gradient and enter the cell (Figure 5.12).

Hypertonic Solutions

As for a **hypertonic** solution, the prefix *hyper–* refers to the extracellular fluid having a higher osmolarity than the cell's cytoplasm; therefore, the fluid contains less water than the cell does. Because the cell has a relatively higher water concentration, water will leave the cell (Figure 5,12).

Isotonic Solutions

In an **isotonic** solution, the extracellular fluid has the same osmolarity as the cell. If the cell's osmolarity matches that of the extracellular fluid, there will be no net movement of water into or out of the cell, although water will still move in and out. Blood cells and plant cells in hypertonic, isotonic, and hypotonic solutions take on characteristic appearances (Figure 5.12).

Visual Connection

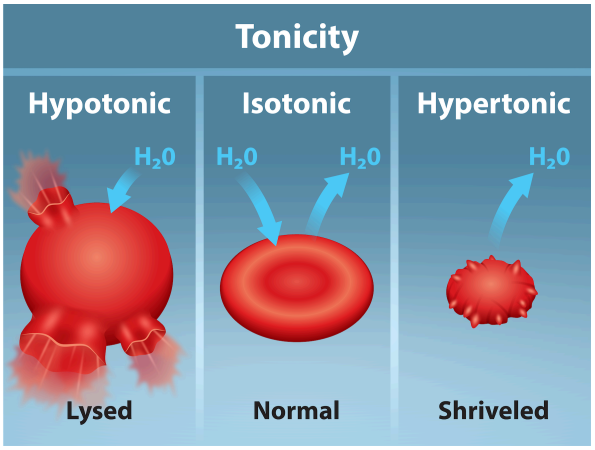

Figure 5.12 Three different scenarios involving red blood cells (RBC) are shown. Left: An RBC placed in a hypotonic solution, where the concentration of solutes in the surrounding fluid is lower than those in the cell, will cause water to rush into the RBC and lead to lysis of the cell. Middle: There is no net water movement into or out of the cell, as the concentration of the solutes inside the cell equal or is isotonic to that of the surrounding fluid. Right: An RBC placed in a hypertonic solution, where the concentration of solutes in the surrounding fluid is greater than that in the cell, will cause water to rush out of the cell and into the surrounding fluid. This will cause the RBC to shrivel. Credit: Tag, A., Rao, A., Hawkins, A and Fletcher, S. Department of Biology, Texas A&M University.

A doctor injects a patient with what the doctor thinks is an isotonic saline solution. The patient dies, and an autopsy reveals that many red blood cells have been destroyed. Do you think the solution the doctor injected was really isotonic?

Link to Learning

For a video illustrating the diffusion process in solutions, visit this site.

Tonicity in Living Systems

In a hypotonic environment, water enters a cell, and the cell swells. In an isotonic condition, the relative solute and solvent concentrations are equal on both membrane sides. There is no net water movement; therefore, there is no change in the cell's size. In a hypertonic solution, water leaves a cell and the cell shrinks. If either the hypo- or hyper-condition goes to excess, the cell's functions become compromised, and the cell may be destroyed.

A red blood cell will burst, or lyse, when it swells beyond the plasma membrane's capability to expand. Remember, the membrane resembles a mosaic, with discrete spaces between the molecules comprising it. If the cell swells, and the spaces between the lipids and proteins become too large, the cell will break apart. (**Figure 5.12**).

In contrast, when excessive water amounts leave a red blood cell, the cell shrinks, or crenates. This has the effect of concentrating the solutes left in the cell, making the cytosol denser and interfering with diffusion within the cell. The cell's ability to function will be compromised and may also result in the cell's death.

Various living things have ways of controlling the effects of osmosis—a mechanism we call osmoregulation. Some organisms, such as plants, fungi, bacteria, and some protists, have cell walls that surround the plasma membrane and prevent cell lysis in a hypotonic solution. The plasma membrane can only expand to the cell wall's limit, so the cell will not lyse. The cytoplasm in plants is always slightly hypertonic to the cellular environment, and water will always enter a cell if water is available. This water inflow produces turgor pressure, which stiffens the plant's cell walls (Figure 5.13). In nonwoody plants, turgor pressure supports the plant. Conversely, if you do not water the plant, the extracellular fluid will become hypertonic, causing water to leave the cell. In this condition, the cell does not shrink because the cell wall is not flexible. However, the cell membrane detaches from the wall and constricts the cytoplasm. We call this **plasmolysis**. Plants lose turgor pressure in this condition and wilt (Figure 5.14).

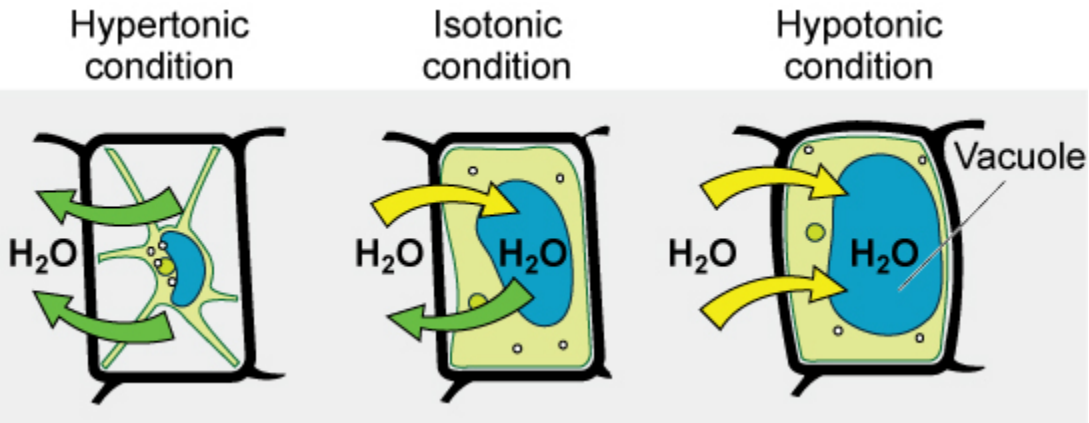

Figure 5.13 The turgor pressure within a plant cell depends on the solution's tonicity in which it is bathed. (credit: modification of work by Mariana Ruiz Villareal)

Figure 5.14 Without adequate water, the plant on the left has lost turgor pressure, visible in its wilting. Watering the plant (right) will restore the turgor pressure. (credit: Victor M. Vicente Selvas)

Tonicity is a concern for all living things. For example, paramecia and amoebas, which are protists that lack cell walls, have contractile vacuoles. This vesicle collects excess water from the cell and pumps it out, keeping the cell from lysing as it takes on water from its environment (Figure 5.15).

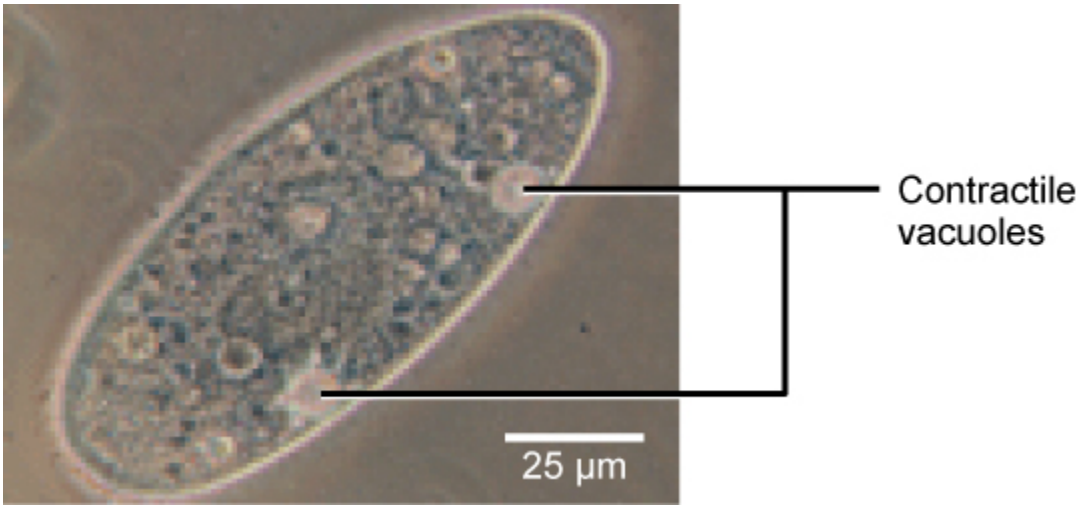

Figure 5.15 A paramecium's contractile vacuole, here visualized using bright field light microscopy at 480x magnification, continuously pumps water out of the organism's body to keep it from bursting in a hypotonic medium. (credit: modification of work by NIH; scale-bar data from Matt Russell)

Many marine invertebrates have internal salt levels matched to their environments, making them isotonic with the water in which they live. Fish, however, must spend approximately five percent of their metabolic energy maintaining osmotic homeostasis. Freshwater fish live in an environment that is hypotonic to their cells. These fish actively take in salt through their gills and excrete diluted urine to rid themselves of excess water. Saltwater fish live in the reverse environment, which is hypertonic to their cells, and they secrete salt through their gills and excrete highly concentrated urine.

In vertebrates, the kidneys regulate the water amount in the body. Osmoreceptors are specialized cells in the brain that monitor solute concentration in the blood. If the solute levels increase beyond a certain range, a hormone releases that slows water loss through the kidney and dilutes the blood to safer levels. Animals also have high albumin concentrations, which the liver produces, in their blood. This protein is too large to pass easily through plasma membranes and is a major factor in controlling the osmotic pressures applied to tissues.

44.

ACTIVE TRANSPORT

Learning Objectives

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

- Understand how electrochemical gradients affect ions
- Distinguish between primary active transport and secondary active transport

Active transport mechanisms require 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 substance's concentration inside the cell is greater than its concentration in the extracellular fluid (and vice versa)—the cell must use energy to move the substance. Some active transport mechanisms move small-molecular weight materials, such as ions, through the membrane. Other mechanisms transport much larger molecules.

Electrochemical Gradient

We have discussed simple concentration gradients—a substance's differential concentrations across a space or a membrane—but in living systems, gradients are more complex. Because ions move into and out of cells and because cells contain proteins that do not move across the membrane and are mostly negatively charged, there is also 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, and at the same time, cells have higher concentrations of potassium (K^+) and lower concentrations of sodium (Na^+) than the extracellular fluid. Thus in a living cell, the concentration gradient of Na^+ tends to drive it into the cell, and its electrical gradient (a positive ion) also drives it inward to the negatively charged interior. However, the situation is more complex for other elements such as potassium. The electrical gradient of K^+, a positive ion, also drives it into the cell, but the concentration gradient of K^+ drives K^+ *out* of the cell (Figure 5.16). We call the combined concentration gradient and electrical charge that affects an ion its **electrochemical gradient**.

Visual Connection

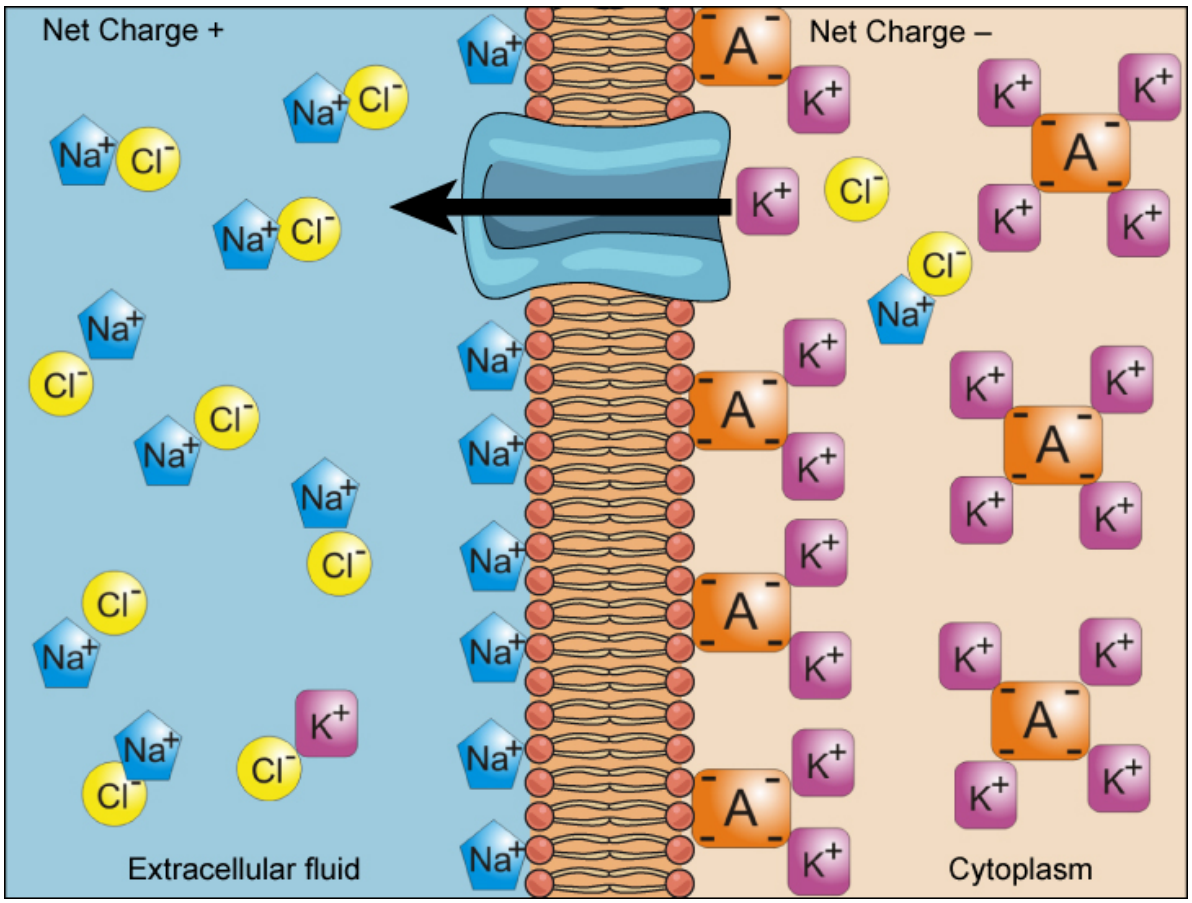

Figure 5.16 Electrochemical gradients arise from the combined effects of concentration gradients and electrical gradients. Structures labeled A represent proteins. (credit: "Synaptitude"/Wikimedia Commons)

Injecting a potassium solution into a person's blood is lethal. This is how capital punishment and euthanasia subjects die. Why do you think a potassium solution injection is lethal?

Moving Against a Gradient

To move substances against a concentration or electrochemical gradient, the cell must use energy. This energy comes from ATP generated through the cell's metabolism. Active transport mechanisms, or pumps, work

against electrochemical gradients. Small substances constantly pass through plasma membranes. Active transport maintains concentrations of ions and other substances that living cells require in the face of these passive movements. A cell may spend much of its metabolic energy supply maintaining these processes. (A red blood cell uses most of its metabolic energy to maintain the imbalance between exterior and interior sodium and potassium levels that the cell requires.) Because active transport mechanisms depend on a cell's metabolism for energy, they are sensitive to many metabolic poisons that interfere with the ATP supply.

Two mechanisms exist for transporting small-molecular weight material and small molecules. **Primary active transport** moves ions across a membrane and creates a difference in charge across that membrane, which is directly dependent on ATP. **Secondary active transport** does not directly require ATP: instead, it is the movement of material due to the electrochemical gradient established by primary active transport.

Carrier Proteins for Active Transport

An important membrane adaption for active transport is the presence of specific carrier proteins or pumps to facilitate movement: there are three protein types or **transporters** (Figure 5.17). A **uniporter** carries one specific ion or molecule. A **symporter** carries two different ions or molecules, both in the same direction. An **antiporter** also carries two different ions or molecules, but in different directions. All of these transporters can also transport small, uncharged organic molecules like glucose. These three types of carrier proteins are also in facilitated diffusion, but they do not require ATP to work in that process. Some examples of pumps for active transport are Na+-K+ ATPase, which carries sodium and potassium ions, and H+-K+ ATPase, which carries hydrogen and potassium ions. Both of these are antiporter carrier proteins. Two other carrier proteins are Ca2+ ATPase and H+ ATPase, which carry only calcium and only hydrogen ions, respectively. Both are pumps.

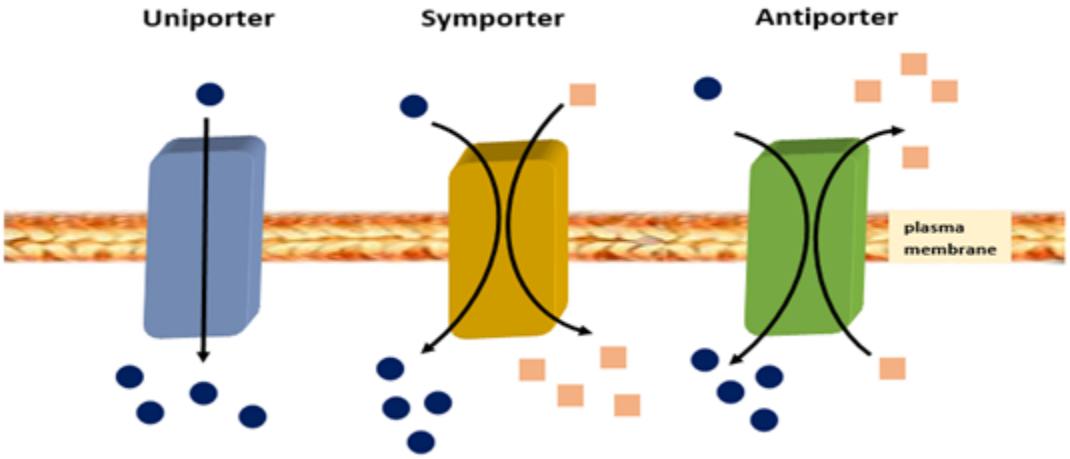

Figure 5.17 A uniporter carries one molecule or ion. A symporter carries two different molecules or ions, both in the same direction. An antiporter also carries two different molecules or ions, but in different directions. (Credit: Waneene C. Dorsey, Grambling State University. Adaption from OpenStax Lupask).

Primary Active Transport

The primary active transport that functions with the active transport of sodium and potassium allows secondary active transport to occur. The second transport method is still active because it depends on using energy as does primary transport (Figure 5.18).

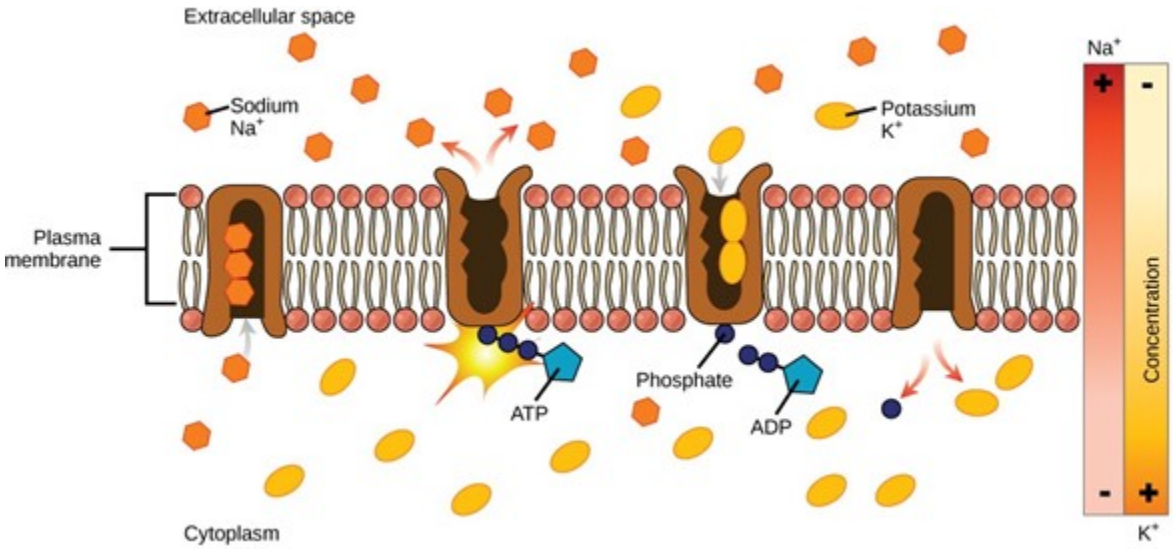

Figure 5.18 Primary active transport moves ions across a membrane, creating an electrochemical gradient (electrogenic transport). (credit: modification of work by Mariana Ruiz Villareal)

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

1. With the enzyme oriented toward the cell's interior, the carrier has a high affinity for sodium ions. Three ions bind to the protein.
2. The protein carrier hydrolyzes ATP and a low-energy phosphate group attaches to it.
3. As a result, the carrier changes shape and reorients itself toward the membrane's exterior. The protein's affinity for sodium decreases and the three sodium ions leave the carrier.
4. The shape change increases the carrier's affinity for potassium ions, and two such ions attach to the protein. Subsequently, the low-energy phosphate group detaches from the carrier.
5. With the phosphate group removed and potassium ions attached, the carrier protein repositions itself toward the cell's interior.
6. The carrier protein, in its new configuration, has a decreased affinity for potassium, and the two ions move into the cytoplasm. The protein now has a higher affinity for sodium ions, and the process starts again.

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

Link to Learning

Watch this video to see an active transport simulation in a sodium-potassium ATPase.

Secondary Active Transport (Co-transport)

Secondary active transport uses the kinetic energy of the sodium ions to bring other compounds, against their

concentration gradient into the cell. As sodium ion concentrations build outside of the plasma membrane because of the primary active transport process, this creates an electrochemical gradient. If a channel protein exists and is open, the sodium ions will move down its concentration gradient across the membrane. This movement transports other substances that must be attached to the same transport protein in order for the sodium ions to move across the membrane (Figure 5.20). Many amino acids, as well as glucose, enter a cell this way. This secondary process also stores high-energy hydrogen ions in the mitochondria of plant and animal cells in order to produce ATP. The potential energy that accumulates in the stored hydrogen ions translates into kinetic energy as the ions surge through the channel protein ATP synthase, and that energy then converts ADP into ATP.

Visual Connection

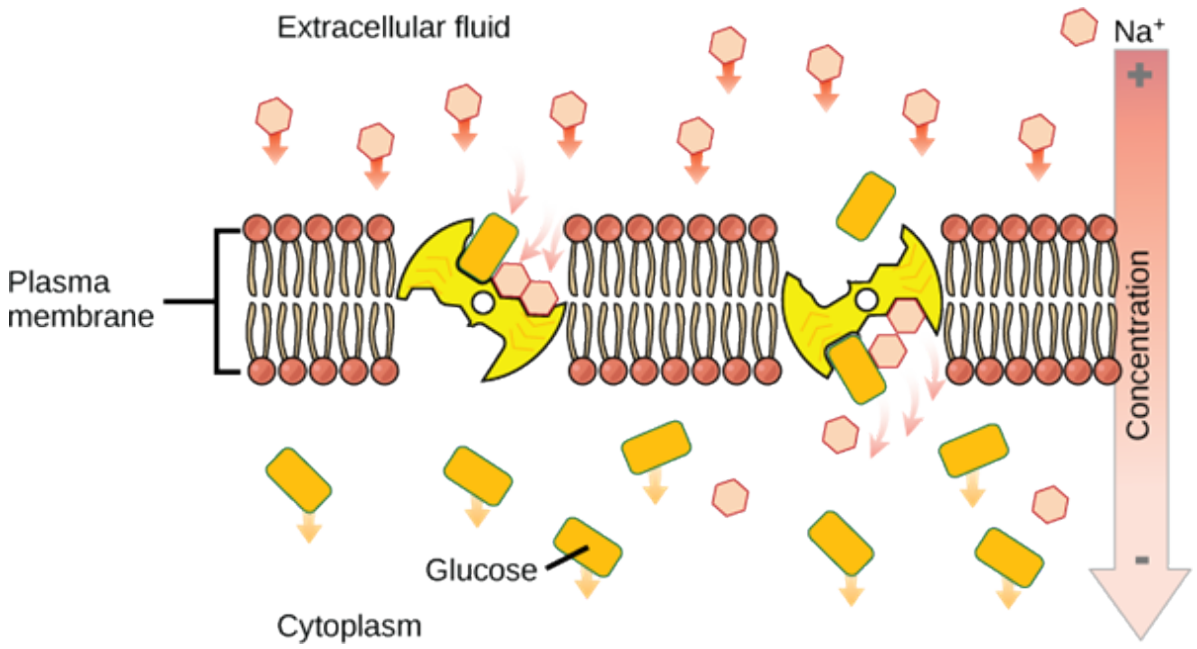

Figure 5.19 An electrochemical gradient, which primary active transport creates, can move other substances against their concentration gradients, a process scientists call co-transport or secondary active transport. (credit: modification of work by Mariana Ruiz Villareal)

If the pH outside the cell decreases, would you expect the amount of amino acids transported into the cell to increase or decrease?

45.

BULK TRANSPORT

Learning Objectives

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

- Describe endocytosis, including phagocytosis, pinocytosis, and receptor-mediated endocytosis
- Understand the process of exocytosis

In addition to moving small ions and molecules through the membrane, cells also need to remove and take in larger molecules and particles (see Table 5.2 for examples). Some cells are even capable of engulfing entire unicellular microorganisms. You might have correctly hypothesized that when a cell uptakes and releases large particles, it requires energy. A large particle, however, cannot pass through the membrane, even with energy that the cell supplies.

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 endocytosis variations, but all share a common characteristic: the cell's plasma membrane invaginates, forming a pocket around the target particle. The pocket pinches off, resulting in the particle containing itself in a newly created intracellular vesicle formed from the plasma membrane.

Phagocytosis

Phagocytosis (the condition of "cell eating") is the process by which a cell takes in large particles, such as

other cells or relatively large particles. For example, when microorganisms invade the human body, a type of white blood cell, a neutrophil, will remove the invaders through this process, surrounding and engulfing the microorganism, which the neutrophil then destroys (Figure 5.20).

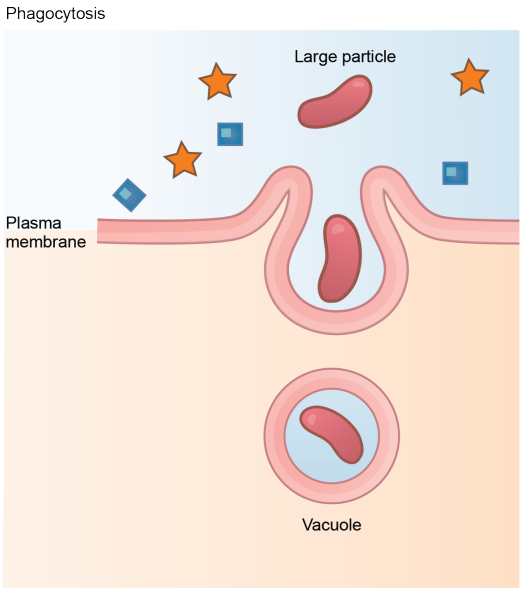

Figure 5.20 In phagocytosis, the cell membrane surrounds the particle and engulfs it. (credit: modification of work by Mariana Ruiz Villareal)

In preparation for phagocytosis, a portion of the plasma membrane's inward-facing surface becomes coated with the protein **clathrin**, which stabilizes this membrane's section. The membrane's coated portion then extends from the cell's body and surrounds the particle, eventually enclosing it. Once the vesicle containing the particle is enclosed within the cell, the clathrin disengages from the membrane and the vesicle merges with a lysosome for breaking down the material in the newly formed compartment (endosome). When accessible nutrients from the vesicular contents' degradation have been extracted, the newly formed endosome merges with the plasma membrane and releases its contents into the extracellular fluid. The endosomal membrane again becomes part of the plasma membrane.

Pinocytosis

A variation of endocytosis is **pinocytosis**. This literally means "cell drinking." Discovered by Warren Lewis in 1929, this American embryologist and cell biologist described a process whereby he assumed that the cell was purposefully taking in extracellular fluid. In reality, this is a process that takes in molecules, including water, which the cell needs from the extracellular fluid. Pinocytosis results in a much smaller vesicle than does phagocytosis, and the vesicle does not need to merge with a lysosome (Figure 5.21).

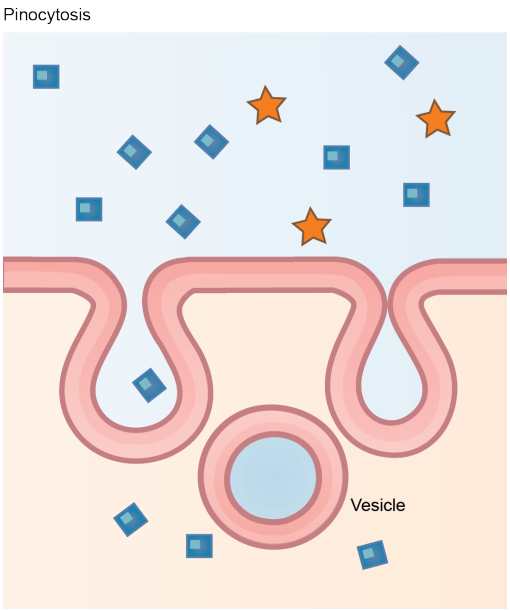

Figure 5.22 In pinocytosis, the cell membrane invaginates, surrounds a small volume of fluid, and pinches off. (credit: modification of work by Mariana Ruiz Villareal)

A variation of pinocytosis is **potocytosis**. This process uses a coating protein, **caveolin**, on the plasma membrane's cytoplasmic side, which performs a similar function to clathrin. The cavities in the plasma membrane that form the vacuoles have membrane receptors and lipid rafts in addition to caveolin. The vacuoles or vesicles formed in caveolae (singular caveola) are smaller than those in pinocytosis. Potocytosis brings small molecules into the cell and transports them through the cell for their release on the other side, a process we call transcytosis. In some cases, the caveolae deliver their cargo to membranous organelles like the ER.

Receptor-mediated Endocytosis

A targeted variation of endocytosis employs receptor proteins in the plasma membrane that have a specific binding affinity for certain substances (Figure 5.22).

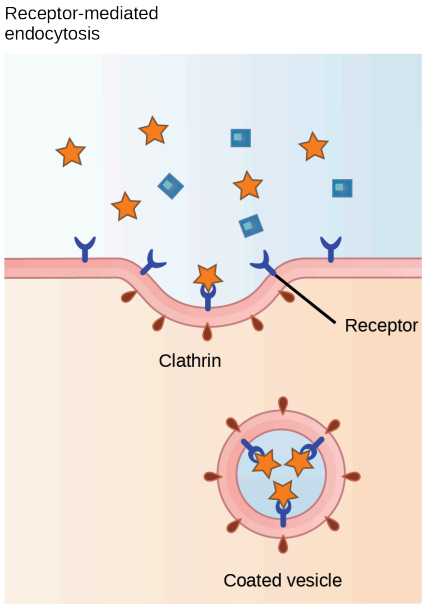

Figure 5.22 In receptor-mediated endocytosis, the cell's uptake of substances targets a single type of substance that binds to the receptor on the cell membrane's external surface. (credit: modification of work by Mariana Ruiz Villareal)

In **receptor-mediated endocytosis**, as in phagocytosis, clathrin attaches to the plasma membrane's cytoplasmic side. If a compound's uptake is dependent on receptor-mediated endocytosis and the process is ineffective, the material will not be removed from the tissue fluids or blood. Instead, it will stay in those fluids and increase in concentration. The failure of receptor-mediated endocytosis causes some human diseases. For example, receptor mediated endocytosis removes low density lipoprotein or LDL (or "bad" cholesterol) from the blood. In the human genetic disease familial hypercholesterolemia, the LDL receptors are defective or missing entirely. People with this condition have life-threatening levels of cholesterol in their blood because their cells cannot clear LDL particles.

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

Link to Learning

See receptor-mediated endocytosis in action, and click on different parts for a focused animation.

Exocytosis

The reverse process of moving material into a cell is the process of exocytosis. **Exocytosis** is the opposite of the processes we discussed above in that its purpose is to expel material from the cell into the extracellular fluid. Waste material is enveloped in a membrane and fuses with the plasma membrane's interior. This fusion opens the membranous envelope on the cell's exterior, and the waste material expels into the extracellular space (Figure 5.23). Other examples of cells releasing molecules via exocytosis include extracellular matrix protein secretion and neurotransmitter secretion into the synaptic cleft by synaptic vesicles.

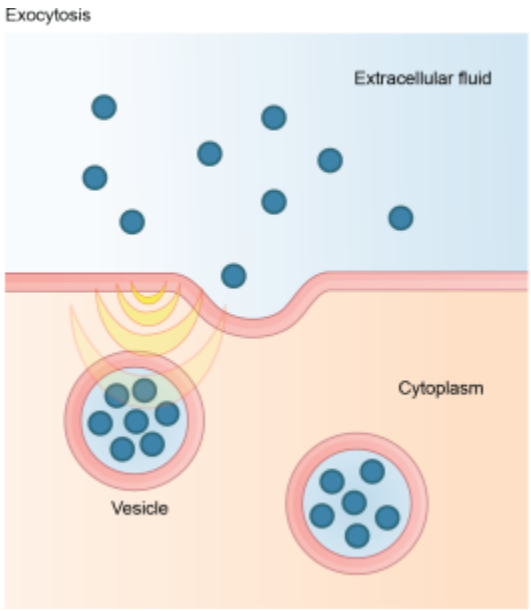

Figure 5.23 In exocytosis, vesicles containing substances fuse with the plasma membrane. The contents then release to the cell's exterior. (credit: modification of work by Mariana Ruiz Villareal)

Methods of Transport, Energy Requirements, and Types of Transported Material

Transport Method	Active/Passive	Material Transported
Diffusion	Passive	Small-molecular weight material
Osmosis	Passive	Water
Facilitated transport/diffusion	Passive	Sodium, potassium, calcium, glucose
Primary active transport	Active	Sodium, potassium, calcium
Secondary active transport	Active	Amino acids, lactose
Phagocytosis	Active	Large macromolecules, whole cells, or cellular structures
Pinocytosis and potocytosis	Active	Small molecules (liquids/water)
Receptor-mediated endocytosis	Active	Large quantities of macromolecules

Table 5.2

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46.

KEY TERMS

active transport
: method of transporting material that requires energy

amphiphilic
: molecule possessing a polar or charged area and a nonpolar or uncharged area capable of interacting with both hydrophilic and hydrophobic environments

antiporter
: transporter that carries two ions or small molecules in different directions

aquaporin
: channel protein that allows water through the membrane at a very high rate

carrier protein
: membrane protein that moves a substance across the plasma membrane by changing its own shape

caveolin
: protein that coats the plasma membrane's cytoplasmic side and participates in the liquid uptake process by potocytosis

channel protein
: membrane protein that allows a substance to pass through its hollow core across the plasma membrane

clathrin
: protein that coats the plasma membrane's inward-facing surface and assists in forming specialized structures, like coated pits, for phagocytosis

concentration gradient
: area of high concentration adjacent to an area of low concentration

diffusion
: passive transport process of low-molecular weight material according to its concentration gradient

electrochemical gradient
: a combined electrical and chemical force that produces a gradient

electrogenic pump
: pump that creates a charge imbalance

endocytosis
: type of active transport that moves substances, including fluids and particles, into a cell

exocytosis
: process of passing bulk material out of a cell

facilitated transport
: process by which material moves down a concentration gradient (from high to low concentration) using integral membrane proteins

fluid mosaic model
: describes the plasma membrane's structure as a mosaic of components including phospholipids, cholesterol, proteins, glycoproteins, and glycolipids (sugar chains attached to proteins or lipids, respectively), resulting in a fluid character (fluidity)

glycolipid
: combination of carbohydrates and lipids

glycoprotein
: combination of carbohydrates and proteins

hydrophilic
: molecule with the ability to bond with water; "water-loving"

hydrophobic
: molecule that does not have the ability to bond with water; "water-hating"

hypertonic
: situation in which extracellular fluid has a higher osmolarity than the fluid inside the cell, resulting in water moving out of the cell

hypotonic
: situation in which extracellular fluid has a lower osmolarity than the fluid inside the cell, resulting in water moving into the cell

integral protein
: protein integrated into the membrane structure that interacts extensively with the membrane lipids' hydrocarbon chains and often spans the membrane

isotonic
: situation in which the extracellular fluid has the same osmolarity as the fluid inside the cell, resulting in no net water movement into or out of the cell

osmolarity
: total amount of solutes dissolved in a specific amount of solution

osmosis
: transport of water through a semipermeable membrane according to the water's concentration gradient across the membrane that results from the presence of solute that cannot pass through the membrane

passive transport
: method of transporting material through a membrane that does not require energy

peripheral protein
: protein at the plasma membrane's surface either on its exterior or interior side

pinocytosis
: a variation of endocytosis that imports macromolecules that the cell needs from the extracellular fluid

plasmolysis
: detaching the cell membrane from the cell wall and constricting the cell membrane when a plant cell is in a hypertonic solution

potocytosis
: variation of pinocytosis that uses a different coating protein (caveolin) on the plasma membrane's cytoplasmic side

primary active transport
: active transport that moves ions or small molecules across a membrane and may create a difference in charge across that membrane

pump
: active transport mechanism that works against electrochemical gradients

receptor-mediated endocytosis
: variation of endocytosis that involves using specific binding proteins in the plasma membrane for specific molecules or particles, and clathrin-coated pits that become clathrin-coated vesicles

secondary active transport
: movement of material that results from primary active transport to the electrochemical gradient

selectively permeable
: membrane characteristic that allows some substances through (also known as semipermeable)

solute
: substance dissolved in a liquid to form a solution

symporter
: transporter that carries two different ions or small molecules, both in the same direction

tonicity
: amount of solute in a solution

transport protein
: membrane protein that facilitates a substance's passage across a membrane by binding it

transporter
: specific carrier proteins or pumps that facilitate movement

uniporter
: transporter that carries one specific ion or molecule

47.

CHAPTER SUMMARY

5.1 Components and Structure

Modern scientists refer to the plasma membrane as the fluid mosaic model. A phospholipid bilayer comprises the plasma membrane, with hydrophobic fatty acid tails in contact with each other. The membrane's landscape is studded with proteins, some which span the membrane. Some of these proteins serve to transport materials into or out of the cell. Carbohydrates are attached to some of the proteins and lipids on the membrane's outward-facing surface, forming complexes that function to identify the cell to other cells. The membrane's fluid nature is due to temperature, fatty acid tail configuration (some kinked by double bonds), cholesterol presence embedded in the membrane, and the mosaic nature of the proteins and protein-carbohydrate combinations, which are not firmly fixed in place. Plasma membranes enclose and define the cells' borders. Not static, they are dynamic and constantly in flux.

5.2 Passive Transport

The passive transport forms, diffusion and osmosis, move materials of small molecular weight across membranes. Substances diffuse from high to lower concentration areas, and this process continues until the substance evenly distributes itself in a system. In solutions containing more than one substance, each molecule type diffuses according to its own concentration gradient, independent of other substances diffusing. Many factors can affect the diffusion rate, such as concentration gradient, diffusing, particle sizes, and the system's temperature.

In living systems, the plasma membrane mediates substances diffusing in and out of cells. Some materials diffuse readily through the membrane, but others are hindered and only can pass through due to specialized proteins such as channels and transporters. The chemistry of living things occurs in aqueous solutions, and balancing the concentrations of those solutions is an ongoing problem. In living systems, diffusing some substances would be slow or difficult without membrane proteins that facilitate transport.

5.3 Active Transport

The combined gradient that affects an ion includes its concentration gradient and its electrical gradient. A

positive ion, for example, might diffuse into a new area, down its concentration gradient, but if it is diffusing into an area of net positive charge, its electrical gradient hampers its diffusion. When dealing with ions in aqueous solutions, one must consider electrochemical and concentration gradient combinations, rather than just the concentration gradient alone. Living cells need certain substances that exist inside the cell 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 this transport. Active transport of small molecular-sized materials uses integral proteins in the cell membrane to move the materials. These proteins are analogous to pumps. Some pumps, which carry out primary active transport, couple directly with ATP to drive their action. In co-transport (or secondary active transport), energy from primary transport can move another substance into the cell and up its concentration gradient.

5.4 Bulk Transport

Active transport methods require directly using ATP to fuel the transport. In a process scientists call phagocytosis, other cells can engulf large particles, such as macromolecules, cell parts, or whole cells. In phagocytosis, a portion of the membrane invaginates and flows around the particle, eventually pinching off and leaving the particle entirely enclosed by a plasma membrane's envelope. The cell breaks down vesicle contents, with the particles either used as food or dispatched. Pinocytosis is a similar process on a smaller scale. The plasma membrane invaginates and pinches off, producing a small envelope of fluid from outside the cell. Pinocytosis imports substances that the cell needs from the extracellular fluid. The cell expels waste in a similar but reverse manner. It pushes a membranous vacuole to the plasma membrane, allowing the vacuole to fuse with the membrane and incorporate itself into the membrane structure, releasing its contents to the exterior.

48.

VISUAL CONNECTION QUESTIONS

1. Figure 5.12 A doctor injects a patient with what the doctor thinks is an isotonic saline solution. The patient dies, and an autopsy reveals that many red blood cells have been destroyed. Do you think the solution the doctor injected was really isotonic?

2. Figure 5.16 Injecting a potassium solution into a person's blood is lethal. Capital punishment and euthanasia utilize this method in their subjects. Why do you think a potassium solution injection is lethal?

3. Figure 5.20 If the pH outside the cell decreases, would you expect the amount of amino acids transported into the cell to increase or decrease?

49.

REVIEW QUESTIONS

4. Which plasma membrane component can be either found on its surface or embedded in the membrane structure?

a. protein
b. cholesterol
c. carbohydrate
d. phospholipid

5. Which characteristic of a phospholipid contributes to the fluidity of the membrane?

a. its head
b. cholesterol
c. a saturated fatty acid tail
d. double bonds in the fatty acid tail

6. What is the primary function of carbohydrates attached to the exterior of cell membranes?

a. identification of the cell
b. flexibility of the membrane
c. strengthening the membrane
d. channels through membrane

7. A scientist compares the plasma membrane composition of an animal from the Mediterranean coast with one from the Mojave Desert. Which hypothesis is most likely to be correct?

a. The cells from the Mediterranean coast animal will have more fluid plasma membranes.
b. The cells from the Mojave Desert animal will have a higher cholesterol concentration in the plasma membranes.
c. The cells' plasma membranes will be indistinguishable.
d. The cells from the Mediterranean coast animal will have a higher glycoprotein content, while the cells from the Mojave Desert animal will have a higher lipoprotein content.

8. Water moves via osmosis _____.

a. throughout the cytoplasm
b. from an area with a high concentration of other solutes to a lower one
c. from an area with a high concentration of water to one of lower concentration
d. from an area with a low concentration of water to higher concentration

9. The principal force driving movement in diffusion is the _____.

a. temperature
b. particle size
c. concentration gradient
d. membrane surface area

10. What problem is faced by organisms that live in fresh water?

a. Their bodies tend to take in too much water.
b. They have no way of controlling their tonicity.
c. Only salt water poses problems for animals that live in it.
d. Their bodies tend to lose too much water to their environment.

11. In which situation would passive transport not use a transport protein for entry into a cell?

a. water flowing into a hypertonic environment
b. glucose being absorbed from the blood
c. an ion flowing into a nerve cell to create an electrical potential
d. oxygen moving into a cell after oxygen deprivation

12. Active transport must function continuously because _____.

a. plasma membranes wear out
b. not all membranes are amphiphilic
c. facilitated transport opposes active transport
d. diffusion is constantly moving solutes in opposite directions

13. How does the sodium-potassium pump make the interior of the cell negatively charged?

a. by expelling anions

b. by pulling in anions
c. by expelling more cations than are taken in
d. by taking in and expelling an equal number of cations

14. What is the combination of an electrical gradient and a concentration gradient called?

a. potential gradient
b. electrical potential
c. concentration potential
d. electrochemical gradient

15. What happens to the membrane of a vesicle after exocytosis?

a. It leaves the cell.
b. It is disassembled by the cell.
c. It fuses with and becomes part of the plasma membrane.
d. It is used again in another exocytosis event.

16. Which transport mechanism can bring whole cells into a cell?

a. pinocytosis
b. phagocytosis
c. facilitated transport
d. primary active transport

17. In what important way does receptor-mediated endocytosis differ from phagocytosis?

a. It transports only small amounts of fluid.
b. It does not involve the pinching off of membrane.
c. It brings in only a specifically targeted substance.
d. It brings substances into the cell, while phagocytosis removes substances.

18. Many viruses enter host cells through receptor-mediated endocytosis. What is an advantage of this entry strategy?

a. The virus directly enters the cytoplasm of the cell.
b. The virus is protected from recognition by white blood cells.
c. The virus only enters its target host cell type.

d. The virus can directly inject its genome into the cell's nucleus.

19. Which of the following organelles relies on exocytosis to complete its function?

a. Golgi apparatus
b. vacuole
c. mitochondria
d. endoplasmic reticulum

20. Imagine a cell can perform exocytosis, but only minimal endocytosis. What would happen to the cell?

a. The cell would secrete all its intracellular proteins.
b. The plasma membrane would increase in size over time.
c. The cell would stop expressing integral receptor proteins in its plasma membrane.
d. The cell would lyse.

50.

CRITICAL THINKING QUESTIONS

21. Why is it advantageous for the cell membrane to be fluid in nature?

22. Why do phospholipids tend to spontaneously orient themselves into something resembling a membrane?

23. How can a cell use an extracellular peripheral protein as the receptor to transmit a signal into the cell?

24. Discuss why the following affect the rate of diffusion: molecular size, temperature, solution density, and the distance that must be traveled.

25. Why does water move through a membrane?

26. Both of the regular intravenous solutions administered in medicine, normal saline and lactated Ringer's solution, are isotonic. Why is this important?

27. Describe two ways that decreasing temperature would affect the rate of diffusion of molecules across a cell's plasma membrane.

28. A cell develops a mutation in its potassium channels that prevents the ions from leaving the cell. If the cell's aquaporins are still active, what will happen to the cell? Be sure to describe the tonicity and osmolarity of the cell.

29. Where does the cell get energy for active transport processes?

30. How does the sodium-potassium pump contribute to the net negative charge of the interior of the cell?

31. Glucose from digested food enters intestinal epithelial cells by active transport. Why would intestinal cells use active transport when most body cells use facilitated diffusion?

32. The sodium/calcium exchanger (NCX) transports sodium into and calcium out of cardiac muscle cells. Describe why this transporter is classified as secondary active transport.

33. Why is it important that there are different types of proteins in plasma membranes for the transport of materials into and out of a cell?

34. Why are both chloroplasts and mitochondria found in plant cells? What are the purposes of both?

PART VI
METABOLISM

51.

INTRODUCTION

Figure 6.1 A hummingbird needs energy to maintain prolonged periods of flight. The bird obtains its energy from taking in food and transforming the nutrients into energy through a series of biochemical reactions. The flight muscles in birds are extremely efficient in energy production. (credit: modification of work by Cory Zanker)

Virtually every task performed by living organisms requires energy. Organisms require energy to perform heavy labor and exercise, but humans also use considerable energy while thinking, and even during sleep. Every organism's living cells constantly use energy. Organisms import nutrients and other molecules that are metabolized (break down) and possibly synthesize into new molecules. If necessary, molecules modify, move around the cell, and may distribute themselves to the entire organism. For example, the large proteins that make up muscles are actively built from smaller molecules. Complex carbohydrates break down into simple sugars that the cell uses for energy. Just as energy is required to both build and demolish a building, energy is required to synthesize and break down molecules. Additionally, signaling molecules such as hormones and neurotransmitters transport between cells. Cells ingest and break down bacteria and viruses. Cells must also export waste and toxins to stay healthy, and many cells must swim or move surrounding materials via the beating motion of cellular appendages like cilia and flagella.

The cellular processes that we listed above require a steady supply of energy. From where, and in what form, does this energy come? How do living cells obtain energy, and how do they use it? This chapter will discuss

different forms of energy and the physical laws that govern energy transfer. This chapter will also describe how cells use energy and replenish it, and how chemical reactions in the cell perform with great efficiency.

52.

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

Scientists use the term **bioenergetics** to discuss the concept of energy flow (Figure 6.2) through living systems 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 what they have used, cells must continually obtain more energy to replenish that which the many energy-requiring chemical reactions that constantly take place use. All of the chemical reactions that transpire inside cells, including those that use and release energy, are the cell's **metabolism**.

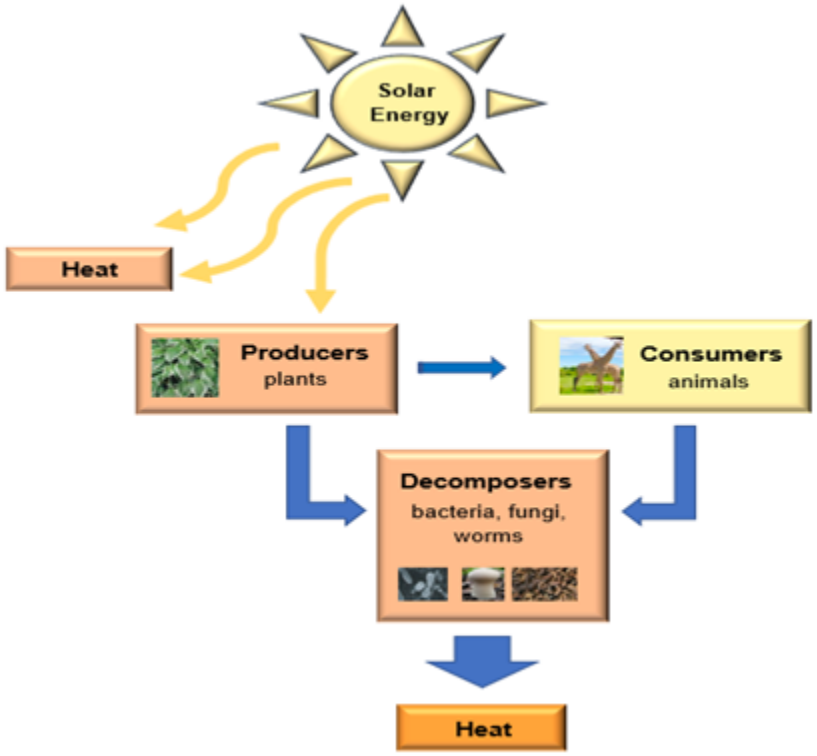


Figure 6.2 Most life forms on earth obtain their energy from the sun. Plants use photosynthesis to capture sunlight, and herbivores eat those plants to obtain energy. Carnivores eat the herbivores, and decomposers digest plant and animal matter. (Credit: Waneene C. Dorsey, Grambling State University, adopted from Figure 6.2 Openstax.)

Carbohydrate Metabolism

Sugar (a simple carbohydrate) metabolism (chemical reactions) is a classic example of the many cellular processes that use and produce energy. Living things consume sugar as a major energy source because sugar molecules have considerable energy stored within their bonds. The following equation describes the breakdown of glucose, a simple sugar:

$C_6H_{12}O_6+6O_2 \rightarrow 6CO_2+6H_2O+\text{energy}$

Consumed carbohydrates have their origins in photosynthesizing organisms like plants (Figure 6.3). During photosynthesis, plants use the energy of sunlight to convert carbon dioxide gas (CO_2) into sugar molecules, like glucose ($C_6H_{12}O_6$). Because this process involves synthesizing a larger, energy-storing molecule, it requires an energy input to proceed. The following equation (notice that it is the reverse of the previous equation) describes the synthesis of glucose:

$6CO_2+6H_2O+\text{energy} \rightarrow C_6H_{12}O_6+6O_2$

During photosynthesis chemical reactions, energy is in the form of a very high-energy molecule scientists call ATP, or adenosine triphosphate. This is the primary energy currency of all cells. Just as the dollar is the

currency we use to buy goods, cells use ATP molecules as energy currency to perform immediate work. The sugar (glucose) is stored as starch or glycogen. Energy-storing polymers like these break down into glucose to supply ATP molecules.

Solar energy is required to synthesize a glucose molecule during the photosynthesis reactions. In photosynthesis, light energy from the sun initially transforms into chemical energy that temporally stores itself in the energy carrier molecules ATP and NADPH (nicotinamide adenine dinucleotide phosphate). Photosynthesis later uses the stored energy in ATP and NADPH to build one glucose molecule from six molecules of CO_2. This process is analogous to eating breakfast in the morning to acquire energy for your body that you can use later in the day. Under ideal conditions, energy from 18 molecules of ATP is required to synthesize one glucose molecule during photosynthesis reactions. Glucose molecules can also combine with and convert into other sugar types. When an organism consumes sugars, glucose molecules eventually make their way into each organism's living cell. Inside the cell, each sugar molecule breaks down through a complex series of chemical reactions. The goal of these reactions is to harvest the energy stored inside the sugar molecules. The harvested energy makes high-energy ATP molecules, which perform work, powering many chemical reactions in the cell. The amount of energy needed to make one glucose molecule from six carbon dioxide molecules is 18 ATP molecules and 12 NADPH molecules (each one of which is energetically equivalent to three ATP molecules), or a total of 54 molecule equivalents required for synthesizing one glucose molecule. This process is a fundamental and efficient way for cells to generate the molecular energy that they require.

Figure 6.3 Plants, like this oak tree and acorn, use energy from sunlight to make sugar and other organic molecules. Both plants and animals (like this squirrel) use cellular respiration to derive energy from the organic molecules that plants originally produced. (credit "acorn": modification of work by Noel Reynolds; credit "squirrel": modification of work by Dawn Huczek)

Metabolic Pathways

The processes of making and breaking down sugar molecules illustrate two types of metabolic pathways. A metabolic pathway is a series of interconnected biochemical reactions that convert a substrate molecule or molecules, step-by-step, through a series of metabolic intermediates, eventually yielding a final product or products. In the case of sugar metabolism, the first metabolic pathway synthesized sugar from smaller molecules, and the other pathway broke sugar down into smaller molecules. Scientists call these two opposite processes—the first requiring energy and the second producing energy—anabolic (building) and catabolic (breaking down) pathways, respectively. Consequently, building (anabolism) and degradation (catabolism) comprise metabolism.

Evolution Connection

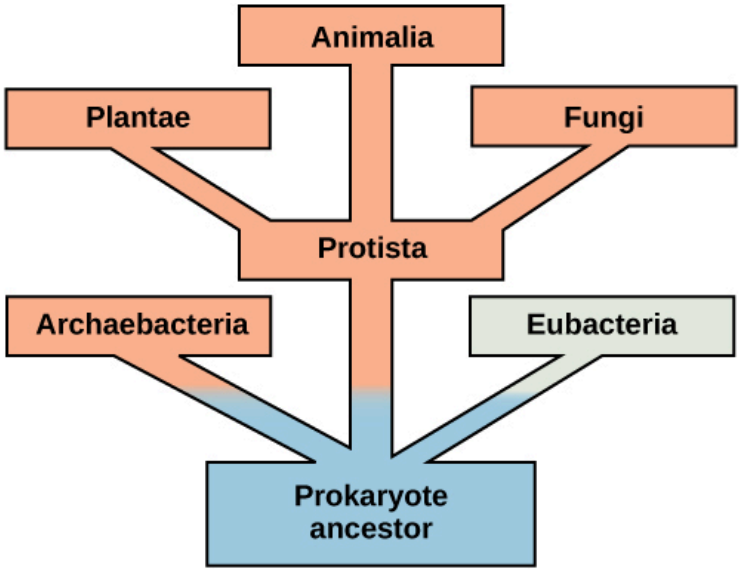


Figure 6.4 This tree shows the evolution of the various branches of life. The vertical dimension is time. Early life forms, in blue, used anaerobic metabolism to obtain energy from their surroundings.

There is more to the complexity of metabolism than understanding the metabolic pathways alone. Metabolic complexity varies from organism to organism. Photosynthesis is the primary pathway in which photosynthetic organisms like plants

(planktonic algae perform the majority of global photosynthesis) harvest the sun's energy and convert it into carbohydrates. The by-product of photosynthesis is oxygen, which some cells require to carry out cellular respiration. During cellular respiration, oxygen aids in the catabolic breakdown of carbon compounds, like carbohydrates. Among the products are CO2 and ATP. In addition, some eukaryotes perform catabolic processes without oxygen (fermentation); that is, they perform or use anaerobic metabolism.

Organisms probably evolved anaerobic metabolism to survive (living organisms came into existence about 3.8 billion years ago, when the atmosphere lacked oxygen). Despite the differences between organisms and the complexity of metabolism, researchers have found that all branches of life share some of the same metabolic pathways, suggesting that all organisms evolved from the same ancient common ancestor (Figure 6.4). Evidence indicates that over time, the pathways diverged, adding specialized enzymes to allow organisms to better adapt to their environment, thus increasing their chance to survive. However, the underlying principle remains that all organisms must harvest energy from their environment and convert it to ATP to carry out cellular functions.

Anabolic and Catabolic Pathways

Anabolic pathways require an input of energy to synthesize complex molecules from simpler ones. Synthesizing sugar from CO_2 is one example. Other examples are synthesizing large proteins from amino acid building blocks, and synthesizing new DNA strands from nucleic acid building blocks. These biosynthetic processes are critical to the cell's life, take place constantly, and demand energy that ATP and other high-energy molecules like NADH (nicotinamide adenine dinucleotide) and NADPH provide (Figure 6.5).

ATP is an important molecule for cells to have in sufficient supply at all times. The breakdown of sugars illustrates how a single glucose molecule can store enough energy to make a great deal of ATP, 36 to 38 molecules. This is a **catabolic** pathway. Catabolic pathways involve degrading (or breaking down) complex molecules into simpler ones. Molecular energy stored in complex molecule bonds is released in catabolic pathways and harvested in such a way that it can produce ATP. Other energy-storing molecules, such as fats, also break down through similar catabolic reactions to release energy and make ATP (Figure 6.5).

It is important to know that metabolic pathway chemical reactions do not take place spontaneously. A protein called an enzyme facilitates or catalyzes each reaction step. Enzymes are important for catalyzing all types of biological reactions—those that require energy as well as those that release energy.

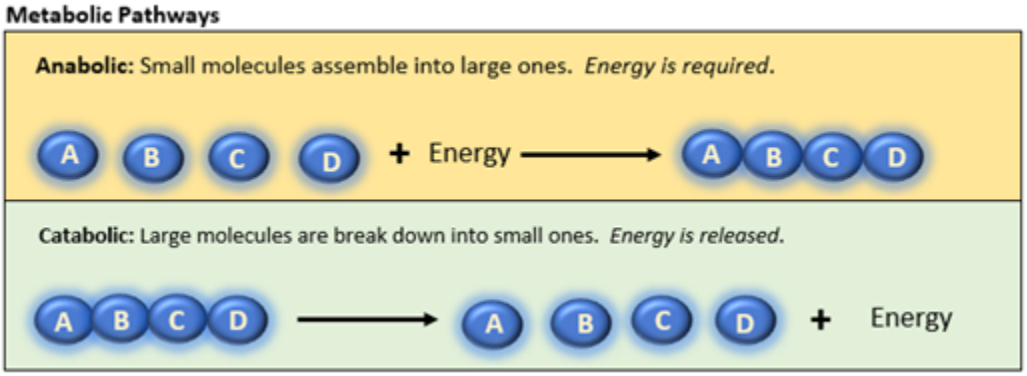


Figure 6.5 Anabolic pathways are those that require energy to synthesize larger molecules. Catabolic pathways are those that generate energy by breaking down larger molecules. Both types of pathways are required for maintaining the cell's energy balance. (Credit: Waneene C. Dorsey, Grambling State University, adapted from Figure 6.5 Openstax)

53.

POTENTIAL, KINETIC, FREE, AND ACTIVATION ENERGY

Learning Objectives

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

- Define "energy"
- Explain the difference between kinetic and potential energy
- Discuss the concepts of free energy and activation energy
- Describe endergonic and exergonic reactions

We define energy as the ability to do work. As you've learned, energy exists in different forms. For example, electrical energy, light energy, and heat energy are all different energy types. While these are all familiar energy types that one can see or feel, there is another energy type that is much less tangible. Scientists associate this energy with something as simple as an object above the ground. In order to appreciate the way energy flows into and out of biological systems, it is important to understand more about the different energy types that exist in the physical world.

Energy Types

When an object is in motion, there is energy. For example, an airplane in flight produces considerable energy. This is because moving objects are capable of enacting a change, or doing work. Think of a wrecking ball. Even a slow-moving wrecking ball can do considerable damage to other objects. However, a wrecking ball that is not in motion is incapable of performing work. Energy with objects in motion is **kinetic energy**. A speeding

bullet, a walking person, rapid molecule movement in the air (which produces heat), and electromagnetic radiation like light all have kinetic energy.

What if we lift that same motionless wrecking ball two stories above a car with a crane? If the suspended wrecking ball is unmoving, can we associate energy with it? The answer is yes. The suspended wrecking ball has associated energy that is fundamentally different from the kinetic energy of objects in motion. This energy form results from the *potential* for the wrecking ball to do work. If we release the ball it would do work. Because this energy type refers to the potential to do work, we call it **potential energy**. Objects transfer their energy between kinetic and potential in the following way: As the wrecking ball hangs motionless, it has 0 kinetic and 100 percent potential energy. Once it releases, its kinetic energy begins to increase because it builds speed due to gravity. Simultaneously, as it nears the ground, it loses potential energy. Somewhere mid-fall it has 50 percent kinetic and 50 percent potential energy. Just before it hits the ground, the ball has nearly lost its potential energy and has near-maximal kinetic energy. Other examples of potential energy include water's energy held behind a dam (Figure 6.6), or a person about to skydive from an airplane.

Figure 6.6 Water behind a dam has potential energy. Moving water, such as in a waterfall or a rapidly flowing river, has kinetic energy. (credit "dam": modification of work by "Pascal"/Flickr; credit "waterfall": modification of work by Frank Gualtieri)

We associate potential energy not only with the matter's location (such as a child sitting on a tree branch), but also with the matter's structure. A spring on the ground has potential energy if it is compressed; so does a tautly pulled rubber band. The very existence of living cells relies heavily on structural potential energy. On a chemical level, the bonds that hold the molecules' atoms together have potential energy. Remember that anabolic cellular pathways require energy to synthesize complex molecules from simpler ones, and catabolic pathways release energy when complex molecules break down. That certain chemical bonds' breakdown can

release energy 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 we eventually harness for use. This is because these bonds can release energy when broken. Scientists call the potential energy type that exists within chemical bonds and releases when those bonds break **chemical energy**(Figure 6.7). Chemical energy is responsible for providing living cells with energy from food. Breaking the molecular bonds within fuel molecules brings about the energy's release.

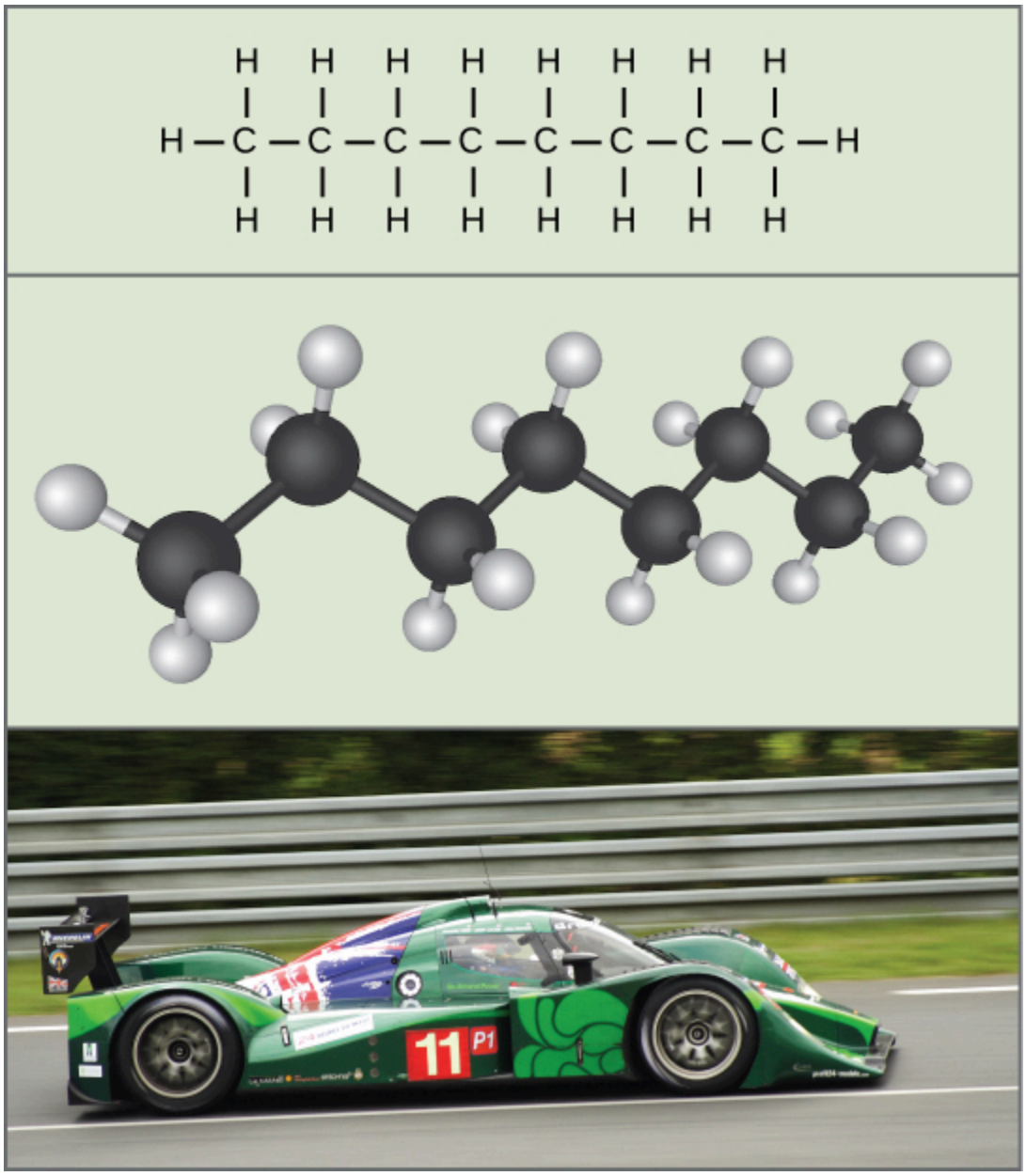


Figure 6.7 The molecules in gasoline contain chemical energy within the chemical bonds. This energy transforms into kinetic energy that allows a car to race on a racetrack. (credit "car": modification of work by Russell Trow)

Link to Learning

Visit this site and select "A simple pendulum" on the menu (under "Harmonic Motion") to see the shifting kinetic (K) and potential energy (U) of a pendulum in motion.

Free Energy

After learning that chemical reactions release energy when energy-storing bonds break, an important next question is how do we quantify and express the chemical reactions with the associated energy? How can we compare the energy that releases from one reaction to that of another reaction? We use a measurement of **free energy** to quantitate these energy transfers. Scientists call this free energy Gibbs free energy (abbreviated with the letter G) after Josiah Willard Gibbs, the scientist who developed the measurement. Recall that according to the second law of thermodynamics, all energy transfers involve losing some energy in an unusable form such as heat, resulting in entropy. Gibbs free energy specifically refers to the energy that takes place with a chemical reaction that is available after we account for entropy. In other words, Gibbs free energy is usable energy, or energy that is available to do work. Every chemical reaction involves a change in free energy, called delta G (ΔG). We can calculate the change in free energy for any system that undergoes such a change, such as a chemical reaction. To calculate ΔG, subtract the amount of energy lost to entropy (denoted as ΔS) from the system's total energy change. Scientists call this total energy change in the system **enthalpy** and we denote it as ΔH. The formula for calculating ΔG is as follows, where the symbol T refers to absolute temperature in Kelvin (degrees Celsius + 273): $\Delta G = \Delta H - T\Delta S$

We express a chemical reaction's standard free energy change as an amount of energy per mole of the reaction product (either in kilojoules or kilocalories, kJ/mol or kcal/mol; 1 kJ = 0.239 kcal) under standard pH, temperature, and pressure conditions. We generally calculate standard pH, temperature, and pressure conditions at pH 7.0 in biological systems, 25 degrees Celsius, and 100 kilopascals (1 atm pressure), respectively. Note that cellular conditions vary considerably from these standard conditions, and so standard calculated ΔG values for biological reactions will be different inside the cell.

Endergonic Reactions and Exergonic Reactions

If energy releases during a chemical reaction, then the resulting value from the above equation will be a negative number. In other words, reactions that release energy have a $\Delta G < 0$. A negative ΔG also means that the reaction's products have less free energy than the reactants, because they gave off some free energy during the reaction. Scientists call reactions that have a negative ΔG and consequently release free energy **exergonic**

reactions. Think: *ex*ergonic means energy is *ex*iting the system. We also refer to these reactions as spontaneous reactions, because they can occur without adding energy into the system. Understanding which chemical reactions are spontaneous and release free energy is extremely useful for biologists, because these reactions can be harnessed to perform work inside the cell. We must draw an important distinction between the term spontaneous and the idea of a chemical reaction that occurs immediately. Contrary to the everyday use of the term, a spontaneous reaction is not one that suddenly or quickly occurs. Rusting iron is an example of a spontaneous reaction that occurs slowly, little by little, over time.

If a chemical reaction requires an energy input rather than releasing energy, then the ΔG for that reaction will be a positive value. In this case, the products have more free energy than the reactants. Thus, we can think of the reactions' products as energy-storing molecules. We call these chemical reactions endergonic reactions, and they are non-spontaneous. An endergonic reaction will not take place on its own without adding free energy.

Let's revisit the example of the synthesis and breakdown of the food molecule, glucose. Remember that building complex molecules, such as sugars, from simpler ones is an anabolic process and requires energy. Therefore, the chemical reactions involved in anabolic processes are endergonic reactions. Alternatively the catabolic process of breaking sugar down into simpler molecules releases energy in a series of exergonic reactions. Like the rust example above, the sugar breakdown involves spontaneous reactions, but these reactions do not occur instantaneously. Figure 6.8 shows some other examples of endergonic and exergonic reactions. Later sections will provide more information about what else is required to make even spontaneous reactions happen more efficiently.

Visual Connection

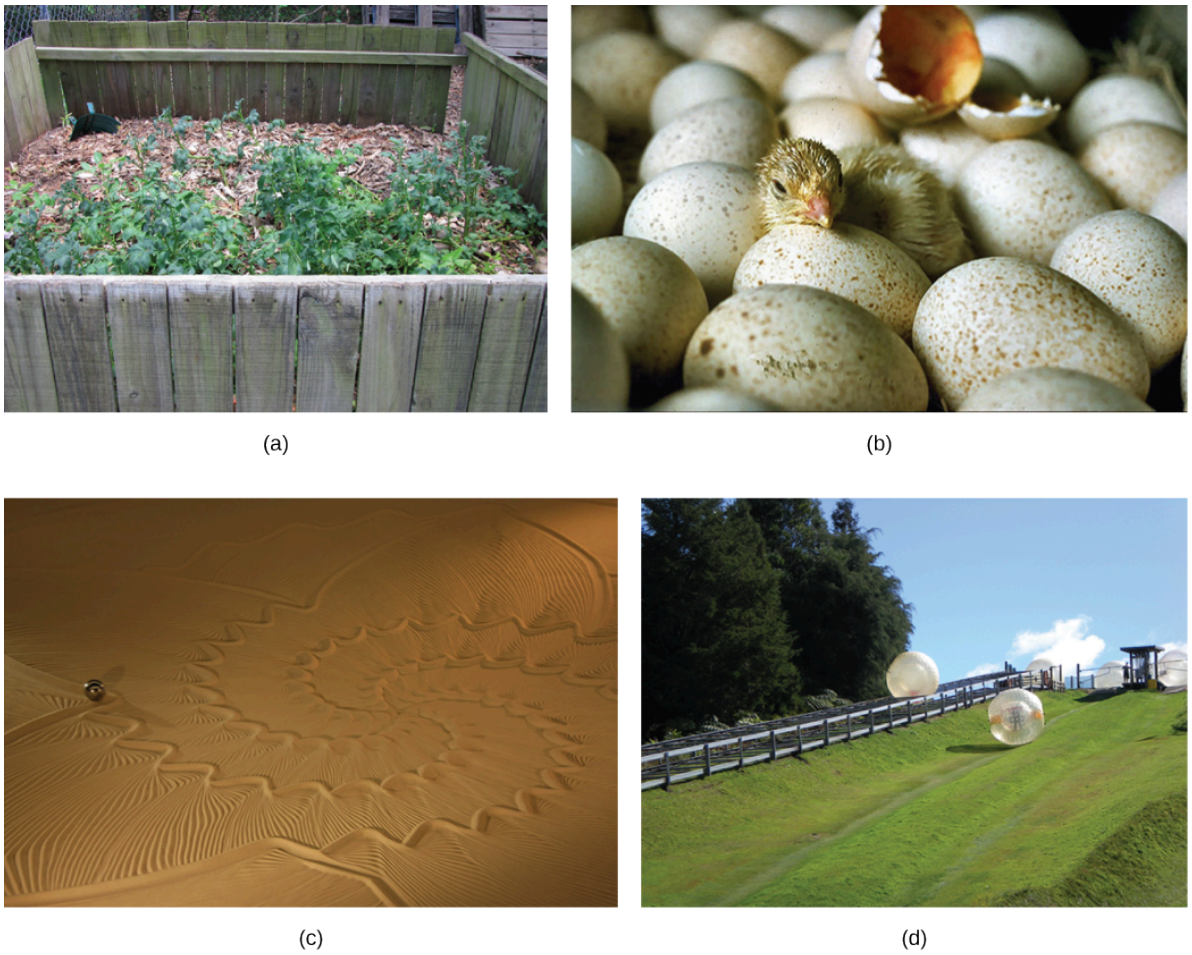


Figure 6.8 This figure shows some examples of endergonic processes (ones that require energy) and exergonic processes (ones that release energy). These include (a) a compost pile decomposing, (b) a chick developing from a fertilized egg, (c) sand art destruction, and (d) a ball rolling down a hill. (credit a: modification of work by Natalie Maynor; credit b: modification of work by USDA; credit c: modification of work by "Athlex"/Flickr; credit d: modification of work by Harry Malsch)

Look at each of the processes, and decide if it is endergonic or exergonic. In each case, does enthalpy increase or decrease, and does entropy increase or decrease?

An important concept in studying metabolism and energy is that of chemical equilibrium. Most chemical reactions are reversible. They can proceed in both directions, releasing energy into their environment in one direction, and absorbing it from the environment in the other direction (Figure 6.9). The same is true for the chemical reactions involved in cell metabolism, such as the breaking down and building up of proteins into and from individual amino acids, respectively. Reactants within a closed system will undergo chemical reactions in both directions until they reach a state of equilibrium, which is one of the lowest possible free energy and

a state of maximal entropy. To push the reactants and products away from a state of equilibrium requires energy. Either reactants or products must be added, removed, or changed. If a cell were a closed system, its chemical reactions would reach equilibrium, and it would die because there would be insufficient free energy left to perform the necessary work to maintain life. In a living cell, chemical reactions are constantly moving towards equilibrium, but never reach it. This is because a living cell is an open system. Materials pass in and out, the cell recycles the products of certain chemical reactions into other reactions, and there is never chemical equilibrium. In this way, living organisms are in a constant energy-requiring, uphill battle against equilibrium and entropy. This constant energy supply ultimately comes from sunlight, which produces nutrients in the photosynthesis process.

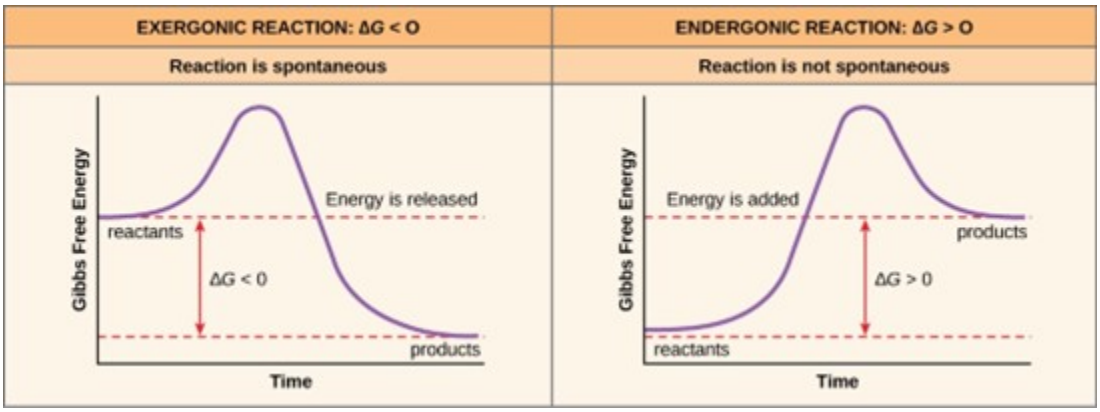


Figure 6.9 Exergonic and endergonic reactions result in changes in Gibbs free energy. Exergonic reactions release energy. Endergonic reactions require energy to proceed.

Link to Learning

Review Exergonic and Endergonic Reactions. (Closed captioning is not available for this video.) Click here.

One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1leclab/?p=284#oembed-1

Activation Energy

There is another important concept that we must consider regarding endergonic and exergonic reactions. Even exergonic reactions require a small amount of energy input before they can proceed with their energy-releasing steps. These reactions have a net release of energy, but still require some initial energy. Scientists call this small amount of energy input necessary for all chemical reactions to occur the **activation energy** (or free energy of activation) abbreviated as E_A (Figure 6.10).

Why would an energy-releasing, negative ΔG reaction actually require some energy to proceed? The reason lies in the steps that take place during a chemical reaction. During chemical reactions, certain chemical bonds break and new ones form. For example, when a glucose molecule breaks down, bonds between the molecule's carbon atoms break. Since these are energy-storing bonds, they release energy when broken. However, to get them into a state that allows the bonds to break, the molecule must be somewhat contorted. A small energy input is required to achieve this contorted state. This contorted state is the **transition state**, and it is a high-energy, unstable state. For this reason, reactant molecules do not last long in their transition state, but very quickly proceed to the chemical reaction's next steps. Free energy diagrams illustrate the energy profiles for a given reaction. Whether the reaction is exergonic or endergonic determines whether the products in the diagram will exist at a lower or higher energy state than both the reactants and the products. However, regardless of this measure, the transition state of the reaction exists at a higher energy

Link to Learning

Watch an animation of the move from free energy to transition state at this site.

From where does the activation energy that chemical reactants require come? The activation energy's required source to push reactions forward is typically heat energy from the surroundings. **Heat energy** (the total bond energy of reactants or products in a chemical reaction) speeds up the molecule's motion, increasing the frequency and force with which they collide. It also moves atoms and bonds within the molecule slightly, helping them reach their transition state. For this reason, heating a system will cause chemical reactants within that system to react more frequently. Increasing the pressure on a system has the same effect. Once reactants have absorbed enough heat energy from their surroundings to reach the transition state, the reaction will proceed.

The activation energy of a particular reaction determines the rate at which it will proceed. The higher the activation energy, the slower the chemical reaction. The example of iron rusting illustrates an inherently slow reaction. This reaction occurs slowly over time because of its high E_A. Additionally, burning many fuels,

which is strongly exergonic, will take place at a negligible rate unless sufficient heat from a spark overcomes their activation energy. However, once they begin to burn, the chemical reactions release enough heat to continue the burning process, supplying the activation energy for surrounding fuel molecules. Like these reactions outside of cells, the activation energy for most cellular reactions is too high for heat energy to overcome at efficient rates. In other words, in order for important cellular reactions to occur at appreciable rates (number of reactions per unit time), their activation energies must be lowered (Figure 6.10). Scientists refer to this as catalysis. This is a very good thing as far as living cells are concerned. Important macromolecules, such as proteins, DNA, and RNA, store considerable energy, and their breakdown is exergonic. If cellular temperatures alone provided enough heat energy for these exergonic reactions to overcome their activation barriers, the cell's essential components would disintegrate.

Visual Connection

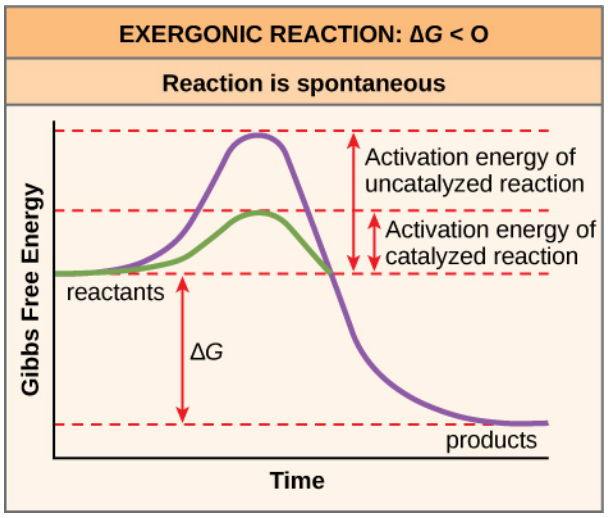


Figure 6.10 Activation energy is the energy required for a reaction to proceed, and it is lower if the reaction is catalyzed. This diagram's horizontal axis describes the sequence of events in time.

If no activation energy were required to break down sucrose (table sugar), would you be able to store it in a sugar bowl?

54.

THE LAWS OF THERMODYNAMICS

Learning Objectives

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

- Discuss the concept of entropy
- Explain the first and second laws of thermodynamics

Thermodynamics refers to the study of energy and energy transfer involving physical matter. The matter and its environment relevant to a particular case of energy transfer are classified as a system, and everything outside that system is the surroundings. For instance, when heating a pot of water on the stove, the system includes the stove, the pot, and the water. Energy transfers within the system (between the stove, pot, and water). There are two types of systems: open and closed. An open system is one in which energy and matter can transfer between the system and its surroundings. The stovetop system is open because it can lose heat into the air. A closed system is one that can transfer energy but not matter to its surroundings.

Biological organisms are open systems. Energy exchanges between them and their surroundings, as they consume energy-storing molecules and release energy to the environment by doing work. Like all things in the physical world, energy is subject to the laws of physics. The laws of thermodynamics govern the transfer of energy in and among all systems in the universe.

The First Law of Thermodynamics

The first law of thermodynamics deals with the total amount of energy in the universe. It states that this total amount of energy is constant. 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 transfer from place to place or transform 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 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 sunlight energy into the chemical energy stored within organic molecules (Figure 6.2). Figure 6.11 shows examples of energy transformations.

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 very well. Chemical energy stored within organic molecules such as sugars and fats transforms through a series of cellular chemical reactions into energy within ATP molecules. 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 beating motion of cilia or flagella, contracting muscle fibers to create movement, and reproduction.

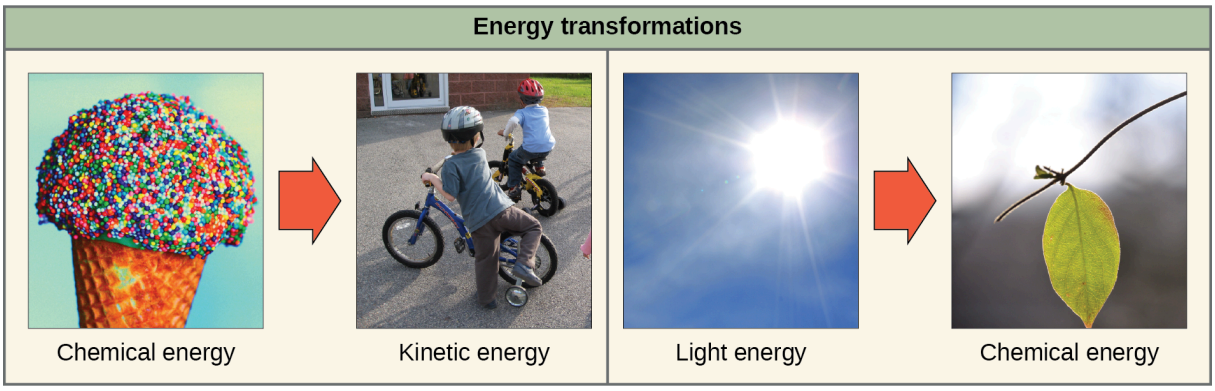


Figure 6.11 Here are two examples of energy transferring from one system to another and transformed from one form to another. Humans can convert the chemical energy in food, like this ice cream cone, into kinetic energy (the energy of movement to ride a bicycle). Plants can convert electromagnetic radiation (light energy) from the sun into chemical energy. (credit "ice cream": modification of work by D. Sharon Pruitt; credit "kids on bikes": modification of work by Michelle Riggen-Ransom; credit "leaf": modification of work by Cory Zanker)

The Second Law of Thermodynamics

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. None of the energy transfers that we have discussed, along with all energy transfers and transformations in the universe, is 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, scientists define **heat energy** as energy that transfers from one system to another that is not doing work. For example, when an airplane flies through the air, it

loses some of its energy as heat energy due to friction with the surrounding air. This friction actually heats the air by temporarily increasing air molecule speed. Likewise, some energy is lost as heat energy during cellular metabolic reactions. This is good for warm-blooded creatures like us, because heat energy helps to maintain our body temperature. Strictly speaking, no energy transfer is completely efficient, because some energy is lost in an unusable form.

An important concept in physical systems is that of order and disorder (or randomness). The more energy that a system loses to its surroundings, the less ordered and more random the system. Scientists refer to the measure of randomness or disorder within a system as **entropy**. High entropy means high disorder and low energy (Figure 6.12). To better understand entropy, think of a student's bedroom. If no energy or work were put into it, the room would quickly become messy. It would exist in a very disordered state, one of high entropy. Energy must be put into the system, in the form of the student doing work and putting everything away, in order to bring the room back to a state of cleanliness and order. This state is one of low entropy. Similarly, a car or house must be constantly maintained with work in order to keep it in an ordered state. Left alone, a house's or car's entropy gradually increases through rust and degradation. Molecules and chemical reactions have varying amounts of entropy as well. For example, as chemical reactions reach a state of equilibrium, entropy increases, and as molecules at a high concentration in one place diffuse and spread out, entropy also increases.

Link to Learning

Review the first law of thermodynamics at this site.

Review the second law of thermodynamics at this site.

Scientific Connection

Transfer of Energy and the Resulting Entropy

Set up a simple experiment to understand how energy transfers and how a change in entropy results.

1. Take a block of ice. This is water in solid form, so it has a high structural order. This means

that the molecules cannot move very much and are in a fixed position. The ice's temperature is 0°C. As a result, the system's entropy is low.

2. Allow the ice to melt at room temperature. What is the state of molecules in the liquid water now? How did the energy transfer take place? Is the system's entropy higher or lower? Why?
3. Heat the water to its boiling point. What happens to the system's entropy when the water is heated?

Think of all physical systems in this way: Living things are highly ordered, requiring constant energy input to maintain themselves in a state of low entropy. As living systems take in energy-storing molecules and transform them through chemical reactions, they lose some amount of usable energy in the process, because no reaction is completely efficient. They also produce waste and by-products that are not useful energy sources. This process increases the entropy of the system's surroundings. Since all energy transfers result in losing some usable energy, the second law of thermodynamics states that every energy transfer or transformation increases the universe's entropy. Even though living things are highly ordered and maintain a state of low entropy, the universe's entropy in total is constantly increasing due to losing usable energy with each energy transfer that occurs. Essentially, living things are in a continuous uphill battle against this constant increase in universal entropy.

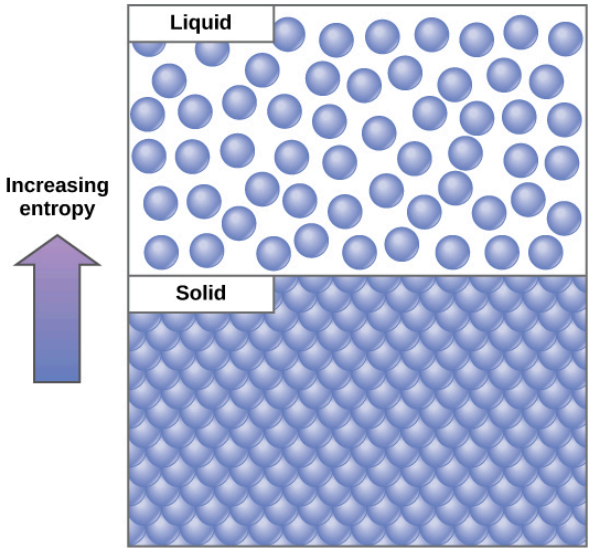


Figure 6.12 Entropy is a measure of randomness or disorder in a system. Gases have higher entropy than liquids, and liquids have higher entropy than solids.

55.

ATP: ADENOSINE TRIPHOSPHATE

Learning Objectives

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

- Explain ATP's role as the cellular energy currency
- Describe how energy releases through ATP hydrolysis

Even exergonic, energy-releasing reactions require a small amount of activation energy in order 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, from where does energy to power such reactions come? The answer lies with an energy-supplying molecule scientists call **adenosine triphosphate**, or **ATP**. This is a small, relatively simple molecule (Figure 6.13), but within some of its bonds, it contains the potential for a quick burst of energy that can be harnessed to perform cellular work. Think of this molecule as the cells' primary energy currency in much the same way that money is the currency that people exchange for things they need. ATP powers the majority of energy-requiring cellular reactions.

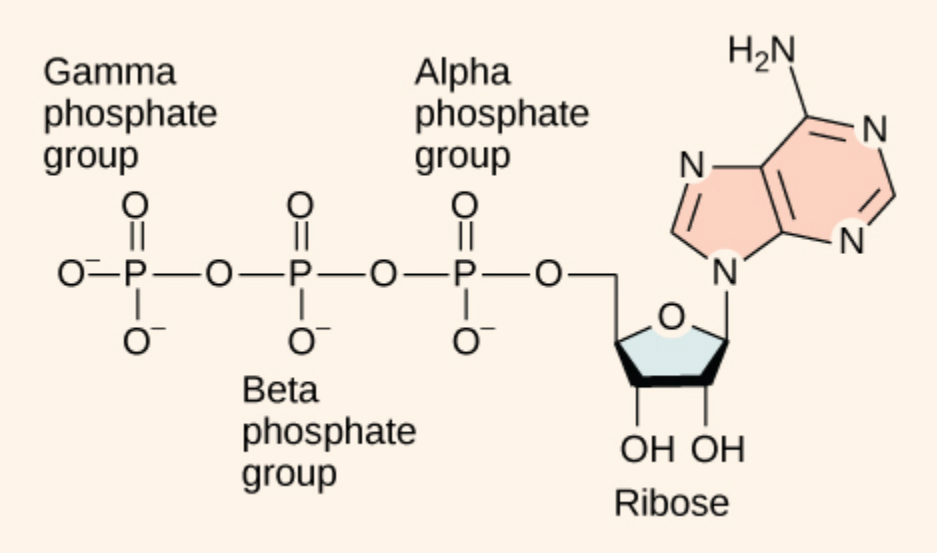


Figure 6.13 ATP is the cell's primary energy currency. It has an adenosine backbone with three phosphate groups attached.

As its name suggests, adenosine triphosphate is comprised of adenosine bound to three phosphate groups (Figure 6.13). Adenosine is a nucleoside consisting of the nitrogenous base adenine and a five-carbon sugar, ribose. The three phosphate groups, in order of closest to furthest from the ribose sugar, are alpha, beta, and gamma. Together, these chemical groups constitute an energy powerhouse. However, not all bonds within this molecule exist in a particularly high-energy state. Both bonds that link the phosphates are equally high-energy bonds (**phosphoanhydride bonds**) that, when broken, release sufficient energy to power a variety of cellular reactions and processes. These high-energy bonds are the bonds between the second and third (or beta and gamma) phosphate groups and between the first and second phosphate groups. These bonds are "high-energy" because the products of such bond breaking—adenosine diphosphate (ADP) and one inorganic phosphate group (P_i)—have considerably lower free energy than the reactants: ATP and a water molecule. Because this reaction takes place using a water molecule, it is a hydrolysis reaction. In other words, ATP hydrolyzes into ADP in the following reaction:

$ATP + H_2O \rightarrow ADP + P_i + \text{free energy}$

Like most chemical reactions, ATP to ADP hydrolysis is reversible. The reverse reaction regenerates ATP from ADP + P_i. Cells rely on ATP regeneration just as people rely on regenerating spent money through some sort of income. Since ATP hydrolysis releases energy, ATP regeneration must require an input of free energy. This equation expresses ATP formation:

$ADP + P_i + \text{free energy} \rightarrow ATP + H_2O$

Two prominent questions remain with regard to using ATP as an energy source. Exactly how much free energy releases with ATP hydrolysis, and how does that free energy do cellular work? The calculated ΔG for the hydrolysis of one ATP mole into ADP and P_i is −7.3 kcal/mole (−30.5 kJ/mol). Since this calculation is true under standard conditions, one would expect a different value exists under cellular conditions. In fact, the

ΔG for one ATP mole's hydrolysis in a living cell is almost double the value at standard conditions: −14 kcal/mol (−57 kJ/mol).

ATP is a highly unstable molecule. Unless quickly used to perform work, ATP spontaneously dissociates into ADP + P_i, and the free energy released during this process is lost as heat. The second question we posed above discusses how ATP hydrolysis energy release performs work inside the cell. This depends on a strategy scientists call **energy coupling**. Cells couple the ATP hydrolysis' exergonic reaction allowing them to proceed. One example of energy coupling using ATP involves a transmembrane ion pump that is extremely important for cellular function. This sodium-potassium pump (Na^+/K^+ pump) drives sodium out of the cell and potassium into the cell (Figure 6.14). A large percentage of a cell's ATP powers this pump, because cellular processes bring considerable sodium into the cell and potassium out of it. The pump works constantly to stabilize cellular concentrations of sodium and potassium. In order for the pump to turn one cycle (exporting three Na+ ions and importing two K^+ ions), one ATP molecule must hydrolyze. When ATP hydrolyzes, its gamma phosphate does not simply float away, but it actually transfers onto the pump protein. Scientists call this process of a phosphate group binding to a molecule phosphorylation. As with most ATP hydrolysis cases, a phosphate from ATP transfers onto another molecule. In a phosphorylated state, the Na^+/K^+ pump has more free energy and is triggered to undergo a conformational change. This change allows it to release Na^+ to the cell's outside. It then binds extracellular K^+, which, through another conformational change, causes the phosphate to detach from the pump. This phosphate release triggers the K^+ to release to the cell's inside. Essentially, the energy released from the ATP hydrolysis couples with the energy required to power the pump and transport Na^+ and K^+ ions. ATP performs cellular work using this basic form of energy coupling through phosphorylation.

Visual Connection

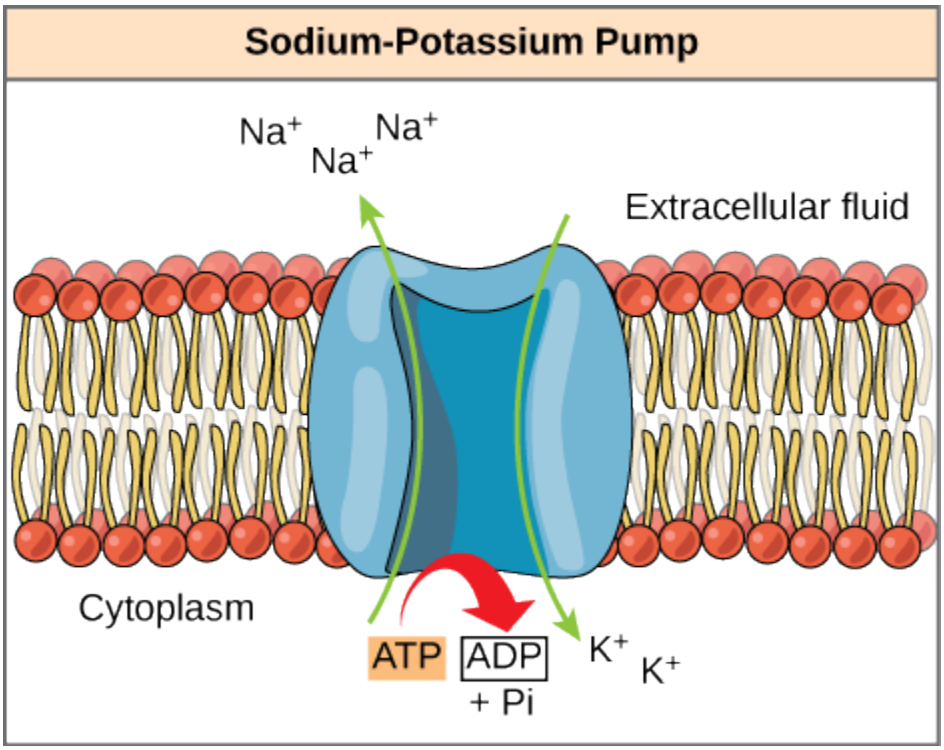


Figure 6.14 The sodium-potassium pump is an example of energy coupling. The energy derived from exergonic ATP hydrolysis pumps sodium and potassium ions across the cell membrane.

One ATP molecule's hydrolysis releases 7.3 kcal/mol of energy (ΔG = −7.3 kcal/mol of energy). If it takes 2.1 kcal/mol of energy to move one Na^+ across the membrane (ΔG = +2.1 kcal/mol of energy), how many sodium ions could one ATP molecule's hydrolysis move?

Often during cellular metabolic reactions, such as nutrient synthesis and breakdown, certain molecules must alter slightly in their conformation to become substrates for the next step in the reaction series. One example is during the very first steps of cellular respiration, when a sugar glucose molecule breaks down in the process of glycolysis. In the first step, ATP is required to phosphorylate glucose, creating a high-energy but unstable intermediate. This phosphorylation reaction powers a conformational change that allows the phosphorylated glucose molecule to convert to the phosphorylated sugar fructose. Fructose is a necessary intermediate for glycolysis to move forward. Here, ATP hydrolysis's exergonic reaction couples with the endergonic reaction of converting glucose into a phosphorylated intermediate in the pathway. Once again, the energy released by breaking a phosphate bond within ATP was used for phosphorylyzing another molecule, creating an unstable intermediate and powering an important conformational change.

Link to Learning

See an animation of the ATP-producing glycolysis process at this site.

Glycolysis: An overview at this site.

Steps of glycolysis reactions at this site.

Glycolysis explained (aerobic vs. anaerobic, pyruvate, gluconeogenesis) at this site.

Krebs Cycle at this site.

Cellular respiration: Glycolysis, Krebs Cycle, Electron Transport Chain at this site.

56.

ENZYMES

Learning Objectives

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

- Describe the role of enzymes in metabolic pathways
- Explain how enzymes function as molecular catalysts
- Discuss enzyme regulation by various factors

A substance that helps a chemical reaction to occur is a catalyst, and the special molecules that catalyze biochemical reactions are **enzymes**. Almost all enzymes are proteins, comprised of amino acid chains, and they perform the critical task of lowering the activation energies of chemical reactions inside the cell. Enzymes do this by binding to the reactant molecules, and holding them in such a way as to make the chemical bond-breaking and bond-forming processes take place more readily. It is important to remember that enzymes do not change the reaction's ΔG. In other words, they do not change whether a reaction is exergonic (spontaneous) or endergonic. This is because they do not change the reactants' or products' free energy. They only reduce the activation energy required to reach the transition state (Figure 6.15).

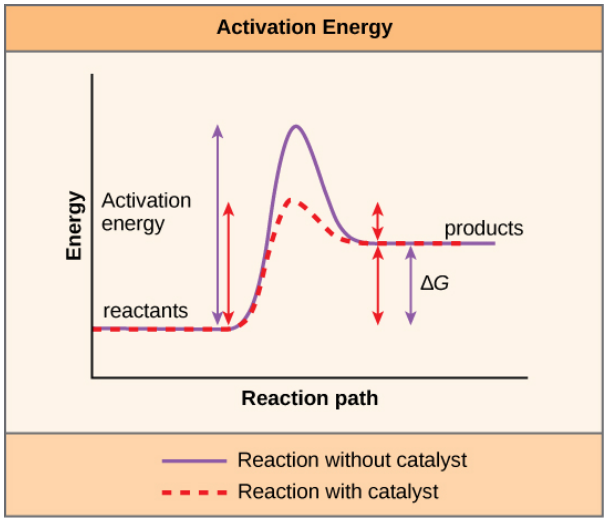


Figure 6.15 Enzymes lower the reaction's activation energy but do not change the reaction's free energy.

Enzyme Active Site and Substrate Specificity

The chemical reactants to which an enzyme binds are the enzyme's substrates. There may be one or more substrates, depending on the particular chemical reaction. In some reactions, a single-reactant substrate breaks down into multiple products. In others, two substrates may come together to create one larger molecule. Two reactants might also enter a reaction, both become modified, and leave the reaction as two products. The location within the enzyme where the substrate binds is the enzyme's **active site**. This is where the "action" happens. Since enzymes are proteins, there is a unique combination of amino acid residues (also side chains, or R groups) within the active site. Different properties characterize each residue. These can be large or small, weakly acidic or basic, hydrophilic or hydrophobic, positively or negatively charged, or neutral. The unique combination of amino acid residues, their positions, sequences, structures, and properties, creates a very specific chemical environment within the active site. This specific environment is suited to bind, albeit briefly, to a specific chemical substrate (or substrates). Due to this jigsaw puzzle-like match between an enzyme and its substrates (which adapts to find the best fit between the transition state and the active site), enzymes are known for their specificity. The "best fit" results from the shape and the amino acid functional group's attraction to the substrate. There is a specifically matched enzyme for each substrate and, thus, for each chemical reaction; however, there is flexibility as well.

The fact that active sites are so perfectly suited to provide specific environmental conditions also means that they are subject to local environmental influences. It is true that increasing the environmental temperature generally increases reaction rates, enzyme-catalyzed or otherwise. However, increasing or decreasing the temperature outside of an optimal range can affect chemical bonds within the active site in such a way that they are less well suited to bind substrates. High temperatures will eventually cause enzymes, like other

biological molecules, to **denature**, a process that changes the substance's natural properties. Likewise, the local environment's pH can also affect enzyme function. Active site amino acid residues have their own acidic or basic properties that are optimal for catalysis. These residues are sensitive to changes in pH that can impair the way substrate molecules bind. Enzymes are suited to function best within a certain pH range, and as with temperature, extreme environmental pH values (acidic or basic) can cause enzymes to denature.

Induced Fit and Enzyme Function

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 more refined view scientists call **induced fit** (Figure 6.16). This model expands upon the lock-and-key model by describing a more dynamic interaction between enzyme and substrate. As the enzyme and substrate come together, their interaction causes a mild shift in the enzyme's structure that confirms an ideal binding arrangement between the enzyme and the substrate's transition state. This ideal binding maximizes the enzyme's ability to catalyze its reaction.

Link to Learning

View an induced fit animation at this website.

Enzymes and How They Work: An Introduction

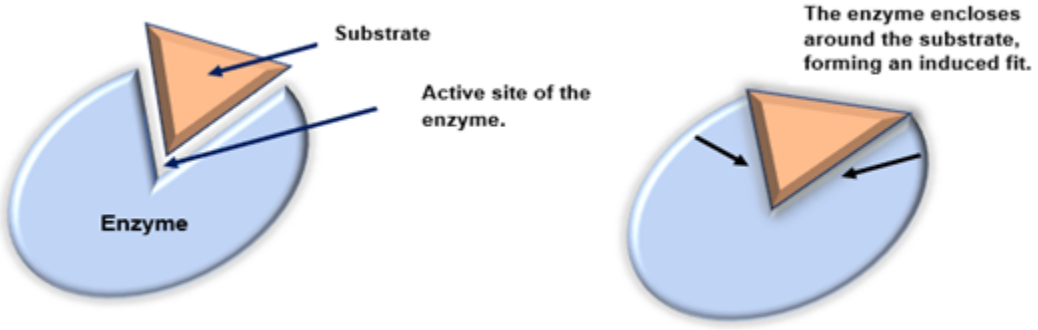
How Enzymes Work:

Function of Enzymes: Substrate, Active Site & Activation Energy

When an enzyme binds its substrate, it forms an enzyme-substrate complex. This complex lowers the reaction's activation energy and promotes its rapid progression in one of many ways. On a basic level, enzymes promote chemical reactions that involve more than one substrate by bringing the substrates together in an optimal orientation. The appropriate region (atoms and bonds) of one molecule is juxtaposed to the other molecule's appropriate region with which it must react. Another way in which enzymes promote substrate reaction is by creating an optimal environment within the active site for the reaction to occur. Certain chemical reactions might proceed best in a slightly acidic or non-polar environment. The chemical properties that emerge from the particular arrangement of amino acid residues within an active site create the perfect environment for an enzyme's specific substrates to react.

You have learned that the activation energy required for many reactions includes the energy involved in

manipulating or slightly contorting chemical bonds so that they can easily break and allow others to reform. Enzymatic action can aid this process. The enzyme-substrate complex can lower the activation energy by contorting substrate molecules in such a way as to facilitate bond-breaking, helping to reach the transition state. Finally, enzymes can also lower activation energies by taking part in the chemical reaction itself. The amino acid residues can provide certain ions or chemical groups that actually form covalent bonds with substrate molecules as a necessary step of the reaction process. In these cases, it is important to remember that the enzyme will always return to its original state at the reaction's completion. One of enzymes' hallmark properties is that they remain ultimately unchanged by the reactions they catalyze. After an enzyme catalyzes a reaction, it releases its product(s).



(Figure 6.16 (a) Courtesy: Waneene C. Dorsey, Grambling State University)

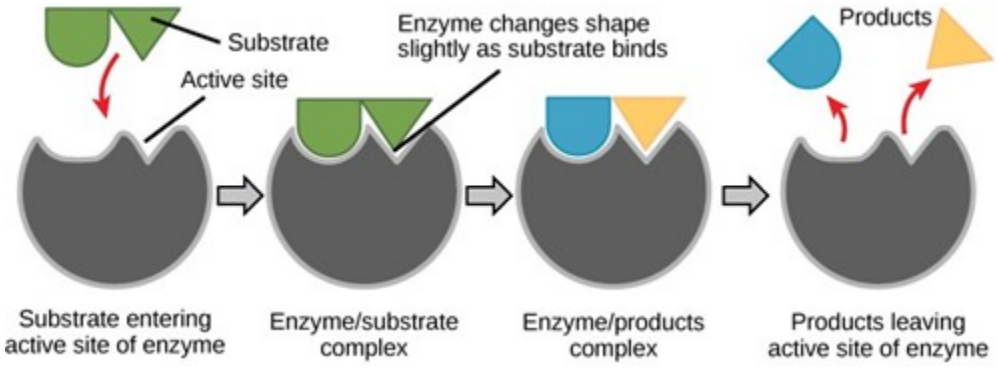


Figure 6.16 (b) According to the induced-fit model, both enzyme and substrate undergo dynamic conformational changes upon binding. The enzyme contorts the substrate into its transition state, thereby increasing the reaction's rate.

Metabolism Control Through Enzyme Regulation

It would seem ideal to have a scenario in which all the encoded enzymes in an organism's genome existed in abundant supply and functioned optimally under all cellular conditions, in all cells, at all times. In reality, this is far from the case. A variety of mechanisms ensure that this does not happen. Cellular needs and conditions vary from cell to cell and change within individual cells over time. The required enzymes and energetic demands of stomach cells are different from those of fat storage cells, skin cells, blood cells, and nerve cells. Furthermore, a digestive 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 do 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 which rates. This determination is tightly controlled. In certain cellular environments, environmental factors like pH and temperature partly control enzyme activity. There are other mechanisms through which cells control enzyme activity and determine the rates at which various biochemical reactions will occur.

Molecular Regulation of Enzymes

Enzymes can be regulated in ways that either promote or reduce their activity. There are many different kinds of molecules that inhibit or promote enzyme function, and various mechanisms exist for doing so. For example, 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 active site binding (Figure 6.17). 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 prevent 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 enzyme activity as it no longer effectively catalyzes the conversion of the substrate to product.

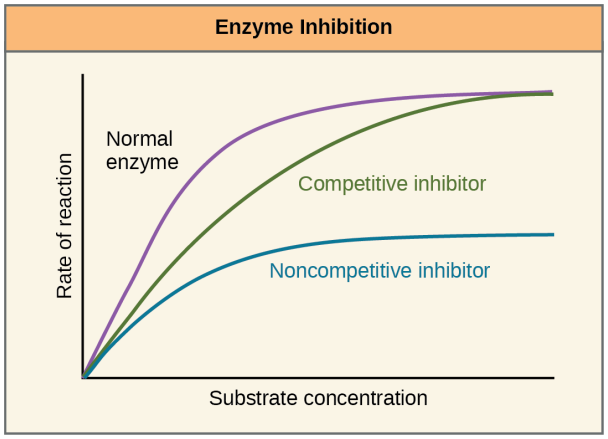


Figure 6.17 Competitive and noncompetitive inhibition affect the reaction's rate differently. Competitive inhibitors affect the initial rate but do not affect the maximal rate, whereas noncompetitive inhibitors affect the maximal rate.

Some inhibitor molecules bind to enzymes in a location where their binding induces a conformational change that reduces the enzyme's affinity for its substrate. This type of inhibition is an **allosteric inhibition** (Figure 6.18). More than one polypeptide comprise most allosterically regulated enzymes, meaning that they have more than one protein subunit. When an allosteric inhibitor binds to an enzyme, all active sites on the protein subunits change 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).

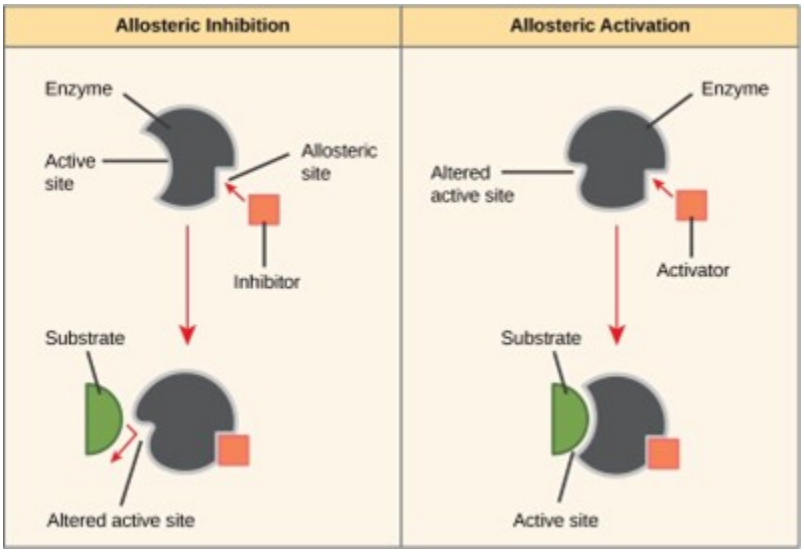


Figure 6.18 Allosteric inhibitors modify the enzyme's active site so that substrate binding is reduced or prevented. In contrast, allosteric activators modify the enzyme's active site so that the affinity for the substrate increases. Credit: Rao, A., Hawkins, A., Fletcher, S. and Tag, A. Department of Biology, Texas A&M University.

Everyday Connection

Figure 6.19 Have you ever wondered how pharmaceutical drugs are developed? (credit: Deborah Austin)

Drug Discovery by Looking for Inhibitors of Key Enzymes in Specific Pathways

Enzymes are key components of metabolic pathways. Understanding how enzymes work and how they can be regulated is a key principle behind developing many pharmaceutical drugs (Figure 6.19) on the market today. Biologists working in this field collaborate with other scientists, usually chemists, to design drugs.

For example, consider statins—a class of drugs that reduce cholesterol levels. These compounds are essentially inhibitors of the enzyme HMG-CoA reductase. HMG-CoA reductase is the enzyme that synthesizes cholesterol from lipids in the body. By inhibiting this enzyme, the drug reduces cholesterol levels synthesized in the body. Similarly, acetaminophen, popularly marketed under the brand name Tylenol, is an inhibitor of the enzyme cyclooxygenase. While it is effective in providing relief from fever and inflammation (pain), scientists still do not completely understand its mechanism of action.

How are drugs developed? One of the first challenges in drug development is identifying the specific molecule that the drug is intended to target. In the case of statins, HMG-CoA reductase is the drug target. Researchers identify targets through painstaking research in the laboratory. Identifying the target alone is not sufficient.

Scientists also need to know how the target acts inside the cell and which reactions go awry in the case of disease. Once researchers identify the target and the pathway, then the actual drug design process begins. During this stage, chemists and biologists work together to design and synthesize molecules that can either block or activate a particular reaction. However, this is only the beginning: both if and when a drug prototype is successful in performing its function, then it must undergo many tests from *in vitro* experiments to clinical trials before it can obtain FDA approval to be on the market.

Many enzymes don't work optimally, or even at all, unless bound to other specific non-protein helper molecules, either temporarily through ionic or hydrogen bonds or permanently through stronger covalent bonds. Two types of helper molecules are cofactors and coenzymes. Binding to these molecules promotes optimal conformation and function for their respective enzymes. Cofactors are inorganic ions such as iron (Fe++) and magnesium (Mg++). One example of an enzyme that requires a metal ion as a cofactor is the enzyme that builds DNA molecules, DNA polymerase, which requires a bound zinc ion (Zn++) to function. Coenzymes are organic helper molecules, with a basic atomic structure comprised of carbon and hydrogen, which are required for enzyme action. The most common sources of coenzymes are dietary vitamins (Figure 6.20). Some vitamins are precursors to coenzymes and others act directly as coenzymes. Vitamin C is a coenzyme for multiple enzymes that take part in building the important connective tissue component, collagen. An important step in breaking down glucose to yield energy is catalysis by a multi-enzyme complex scientists call pyruvate dehydrogenase. Pyruvate dehydrogenase is a complex of several enzymes that actually requires one cofactor (a magnesium ion) and five different organic coenzymes to catalyze its specific chemical reaction. Therefore, enzyme function is, in part, regulated by an abundance of various cofactors and coenzymes, which the diets of most organisms supply.

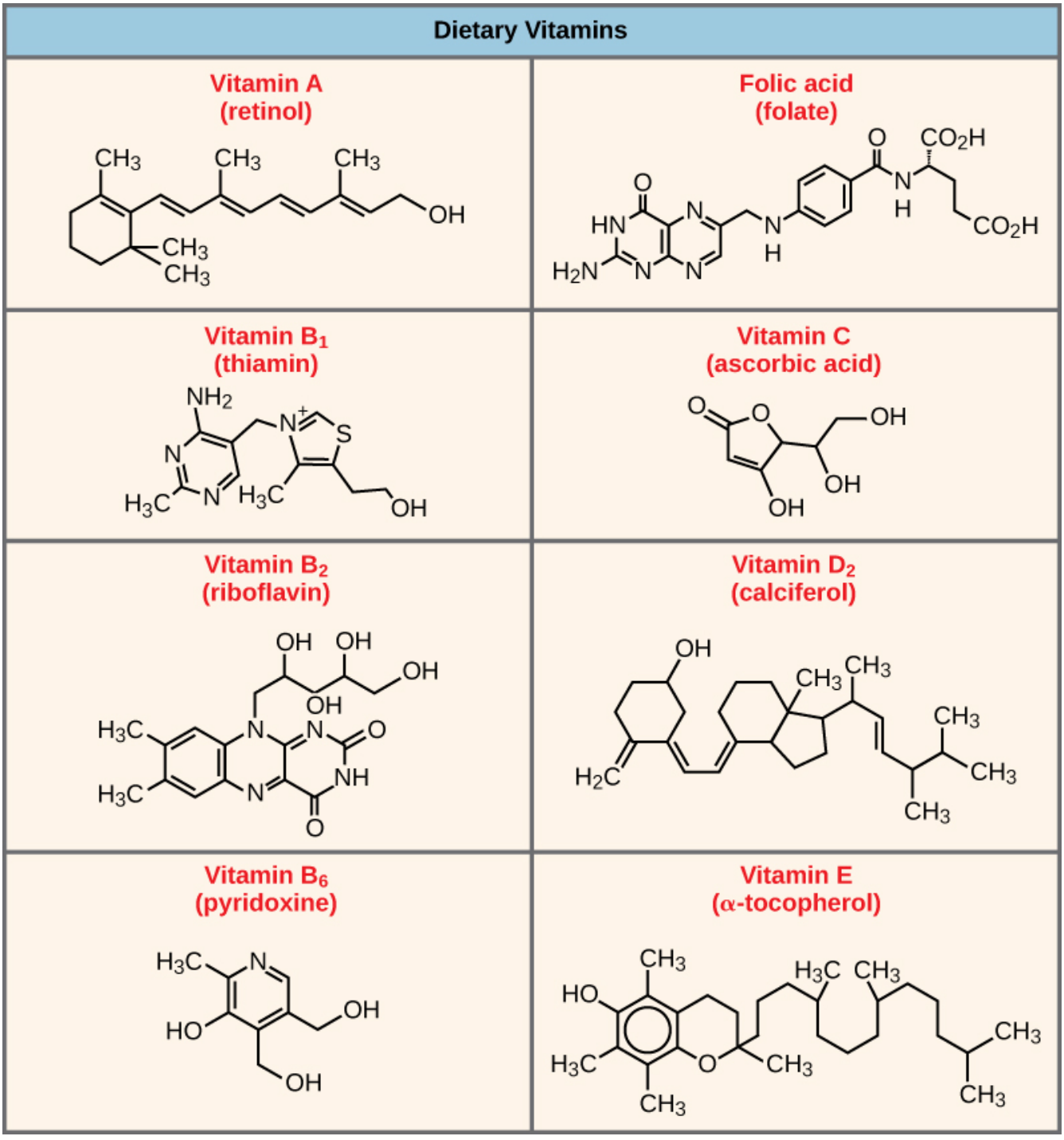


Figure 6.20 Vitamins are important coenzymes or precursors of coenzymes, and are required for enzymes to function properly. Multivitamin capsules usually contain mixtures of all the vitamins at different percentages.

Enzyme Compartmentalization

In eukaryotic cells, molecules such as enzymes are usually compartmentalized into different organelles. This allows for yet another level of regulation of enzyme activity. Enzymes required only for certain cellular

processes are sometimes housed separately along with their substrates, allowing for more efficient chemical reactions. Examples of this sort of enzyme regulation based on location and proximity include the enzymes involved in the latter stages of cellular respiration, which take place exclusively in the mitochondria, and the enzymes involved in digesting cellular debris and foreign materials, located within lysosomes.

Feedback Inhibition in Metabolic Pathways

Molecules can regulate enzyme function in many ways. However, a major question remains: What are these molecules and from where do they come? Some are cofactors and coenzymes, ions, and organic molecules, as you have learned. What other molecules in the cell provide enzymatic regulation, such as allosteric modulation, and competitive and noncompetitive inhibition? The answer is that a wide variety of molecules can perform these roles. Some include pharmaceutical and non-pharmaceutical drugs, toxins, and poisons from the environment. Perhaps the most relevant sources of enzyme regulatory molecules, with respect to cellular metabolism, are cellular metabolic reaction products themselves. In a most efficient and elegant way, cells have evolved to use their own reactions' products for feedback inhibition of enzyme activity. **Feedback inhibition** involves using a reaction product to regulate its own further production (Figure 6.21). The cell responds to the abundance of specific products by slowing down production during anabolic or catabolic reactions. Such reaction products may inhibit the enzymes that catalyzed their production through the mechanisms that we described above.

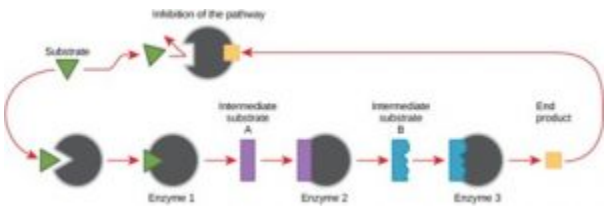


Figure 6.21 Metabolic pathways are a series of reactions that multiple enzymes catalyze. Feedback inhibition, where the pathway's end product inhibits an upstream step, is an important regulatory mechanism in cells.

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 sugar's catabolic breakdown, the process that produces ATP. In this way, when ATP is abundant, the cell can prevent its further production. Remember that ATP is an unstable molecule that can spontaneously dissociate into ADP and inorganic phosphate. If too much ATP were present in a cell, much of it would go to waste. Alternatively, ADP serves as a positive allosteric

regulator (an allosteric activator) for some of the same enzymes that ATP inhibits. Thus, when relative ADP levels are high compared to ATP, the cell is triggered to produce more ATP through sugar catabolism.

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57.

KEY TERMS

activation energy
: energy necessary for reactions to occur

active site
: enzyme's specific region to which the substrate binds

allosteric inhibition
: inhibition by a binding event at a site different from the active site, which induces a conformational change and reduces the enzyme's affinity for its substrate

anabolic
: (also, anabolism) pathways that require an energy input to synthesize complex molecules from simpler ones

ATP
: adenosine triphosphate, the cell's energy currency

bioenergetics
: study of energy flowing through living systems

catabolic
: (also, catabolism) pathways in which complex molecules break down into simpler ones

chemical energy
: potential energy in chemical bonds that releases when those bonds are broken

coenzyme
: small organic molecule, such as a vitamin or its derivative, which is required to enhance an enzyme's activity

cofactor
: inorganic ion, such as iron and magnesium ions, required for optimal enzyme activity regulation

competitive inhibition
: type of inhibition in which the inhibitor competes with the substrate molecule by binding to the enzyme's active site

denature
: process that changes a substance's natural properties

endergonic
: describes chemical reactions that require energy input

energy coupling
: process during which energy released by one reaction is used to drive another reaction

enthalpy
: a system's total energy

entropy (S)
: measure of randomness or disorder within a system

enzyme
: proteins, comprised of amino acid chains, that perform the critical task of lowering the activation energies of chemical reactions inside the cell

exergonic
: describes chemical reactions that release free energy

feedback inhibition
: a product's effect of a reaction sequence to decrease its further production by inhibiting the first enzyme's activity in the pathway that produces it

free energy
: Gibbs free energy is the usable energy, or energy that is available to do work

heat
: energy transferred from one system to another that is not work (energy of the molecules' motion or particles)

heat energy
: total bond energy of reactants or products in a chemical reaction

induced fit
: dynamic fit between the enzyme and its substrate, in which both components modify their structures to allow for ideal binding

kinetic energy
: energy type that takes place with objects or particles in motion

metabolism
: all the chemical reactions that take place inside cells, including anabolism and catabolism

phosphoanhydride bond
: bond that connects phosphates in an ATP molecule

potential energy
: energy type that has the potential to do work; stored energy

substrate
: molecule on which the enzyme acts

thermodynamics
: study of energy and energy transfer involving physical matter

transition state

high-energy, unstable state (an intermediate form between the substrate and the product) occurring during a chemical reaction

58.

CHAPTER SUMMARY

6.1 Energy and Metabolism

Cells perform the functions of life through various chemical reactions. A cell's metabolism refers to the chemical reactions that take place within it. There are metabolic reactions that involve breaking down complex chemicals into simpler ones, such as breaking down large macromolecules. Scientists refer to this process as catabolism, and we associate such reactions an energy release. On the other end of the spectrum, anabolism refers to metabolic processes that build complex molecules out of simpler ones, such as macromolecule synthesis. Anabolic processes require energy. Glucose synthesis and glucose breakdown are examples of anabolic and catabolic pathways, respectively.

6.2 Potential, Kinetic, Free, and Activation Energy

Energy comes in many different forms. Objects in motion do physical work, and kinetic energy is the energy of objects in motion. Objects that are not in motion may have the potential to do work, and thus have potential energy. Molecules also have potential energy because breaking molecular bonds has the potential to release energy. Living cells depend on harvesting potential energy from molecular bonds to perform work. Free energy is a measure of energy that is available to do work. A system's free energy changes during energy transfers such as chemical reactions, and scientists refer to this change as ΔG.

A reaction's ΔG can be negative or positive, meaning that the reaction releases energy or consumes energy, respectively. A reaction with a negative ΔG that gives off energy is an exergonic reaction. One with a positive ΔG that requires energy input is an endergonic reaction. Exergonic reactions are spontaneous because their products have less energy than their reactants. Endergonic reactions' products have a higher energy state than the reactants, and so these are nonspontaneous reactions. However, all reactions (including spontaneous $-\Delta G$ reactions) require an initial energy input in order to reach the transition state, at which they will proceed. This initial input of energy is the activation energy.

6.3 The Laws of Thermodynamics

In studying energy, scientists use the term "system" to refer to the matter and its environment involved in

energy transfers. Everything outside of the system is the surroundings. Single cells are biological systems. We can think of systems as having a certain amount of order. It takes energy to make a system more ordered. The more ordered a system, the lower its entropy. Entropy is a measure of a system's disorder. As a system becomes more disordered, the lower its energy and the higher its entropy.

The laws of thermodynamics are a series of laws that describe the properties and processes of energy transfer. The first law states that the total amount of energy in the universe is constant. This means that energy cannot be created or destroyed, only transferred or transformed. The second law of thermodynamics states that every energy transfer involves some loss of energy in an unusable form, such as heat energy, resulting in a more disordered system. In other words, no energy transfer is completely efficient, and all transfers trend toward disorder.

6.4 ATP: Adenosine Triphosphate

ATP is the primary energy-supplying molecule for living cells. ATP is comprised of a nucleotide, a five-carbon sugar, and three phosphate groups. The bonds that connect the phosphates (phosphoanhydride bonds) have high-energy content. The energy released from ATP hydrolysis into ADP + P_i performs cellular work. Cells use ATP to perform work by coupling ATP hydrolysis' exergonic reaction with endergonic reactions. ATP donates its phosphate group to another molecule via phosphorylation. The phosphorylated molecule is at a higher-energy state and is less stable than its unphosphorylated form, and this added energy from phosphate allows the molecule to undergo its endergonic reaction.

6.5 Enzymes

Enzymes are chemical catalysts that accelerate chemical reactions at physiological temperatures by lowering their activation energy. Enzymes are usually proteins consisting of one or more polypeptide chains. Enzymes have an active site that provides a unique chemical environment, comprised of certain amino acid R groups (residues). This unique environment is perfectly suited to convert particular chemical reactants for that enzyme, which scientists call substrates, into unstable intermediates that they call transition states. Enzymes and substrates bind with an induced fit, which means that enzymes undergo slight conformational adjustments upon substrate contact, leading to full, optimal binding. Enzymes bind to substrates and catalyze reactions in four different ways: bringing substrates together in an optimal orientation, compromising the bond structures of substrates so that bonds can break down more easily, providing optimal environmental conditions for a reaction to occur, or participating directly in their chemical reaction by forming transient covalent bonds with the substrates.

Enzyme action must be regulated so that in a given cell at a given time, the desired reactions catalyze and the undesired reactions are not. Enzymes are regulated by cellular conditions, such as temperature and pH.

They are also regulated through their location within a cell, sometimes compartmentalized so that they can only catalyze reactions under certain circumstances. Enzyme inhibition and activation via other molecules are other important ways that enzymes are regulated. Inhibitors can act competitively, noncompetitively, or allosterically. Noncompetitive inhibitors are usually allosteric. Activators can also enhance enzyme function allosterically. The most common method by which cells regulate the enzymes in metabolic pathways is through feedback inhibition. During feedback inhibition, metabolic pathway products serve as inhibitors (usually allosteric) of one or more of the enzymes (usually the first committed enzyme of the pathway) involved in the pathway that produces them.

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VISUAL CONNECTION QUESTIONS

1. Figure 6.8 Look at each of the processes, and decide if it is endergonic or exergonic. In each case, does enthalpy increase or decrease, and does entropy increase or decrease?

2. Figure 6.10 If no activation energy were required to break down sucrose (table sugar), would you be able to store it in a sugar bowl?

3. Figure 6.14 One ATP molecule's hydrolysis releases 7.3 kcal/mol of energy ($\Delta G = -7.3$ kcal/mol of energy). If it takes 2.1 kcal/mol of energy to move one Na^+ across the membrane ($\Delta G = +2.1$ kcal/mol of energy), how many sodium ions could one ATP molecule's hydrolysis move?

60.

REVIEW QUESTIONS

4. 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

5. DNA replication involves unwinding two strands of parent DNA, copying each strand to synthesize complementary strands, and releasing the parent and daughter DNA. Which of the following accurately describes this process?

a. This is an anabolic process.
b. This is a catabolic process.
c. This is both anabolic and catabolic.
d. This is a metabolic process but is neither anabolic nor catabolic.

6. Consider a pendulum swinging. Which type(s) of energy is/are associated with the pendulum in the following instances: i. the moment at which it completes one cycle, just before it begins to fall back towards the other end, ii. the moment that it is in the middle between the two ends, and iii. just before it reaches the end of one cycle (just before instant i.).

a. i. potential and kinetic, ii. potential and kinetic, iii. kinetic
b. i. potential, ii. potential and kinetic, iii. potential and kinetic
c. i. potential, ii. kinetic, iii. potential and kinetic
d. i. potential and kinetic, ii. kinetic iii. kinetic

7. Which of the following comparisons or contrasts between endergonic and exergonic reactions is false?

a. Endergonic reactions have a positive ΔG and exergonic reactions have a negative ΔG.
b. Endergonic reactions consume energy and exergonic reactions release energy.

c. Both endergonic and exergonic reactions require a small amount of energy to overcome an activation barrier.
d. Endergonic reactions take place slowly and exergonic reactions take place quickly.

8. Which of the following is the best way to judge the relative activation energies between two given chemical reactions?

a. Compare the ΔG values between the two reactions.
b. Compare their reaction rates.
c. Compare their ideal environmental conditions.
d. Compare the spontaneity between the two reactions.

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

a. turning on a light switch
b. solar panels at work
c. formation of static electricity
d. none of the above

10. In each of the three systems, determine the state of entropy (low or high) when comparing the first and second: i. the instant that a perfume bottle is sprayed compared with 30 seconds later, ii. an old 1950s car compared with a brand new car, and iii. a living cell compared with a dead cell.

a. i. low, ii. high, iii. low
b. i. low, ii. high, iii. high
c. i. high, ii. low, iii. high
d. i. high, ii. low, iii. low

11. The energy released by the hydrolysis of ATP is____

a. primarily stored between the alpha and beta phosphates
b. equal to −57 kcal/mol
c. harnessed as heat energy by the cell to perform work
d. providing energy to coupled reactions

12. Which of the following molecules is likely to have the most potential energy?

a. sucrose

b. ATP
c. glucose
d. ADP

13. Which of the following is not true about enzymes:

a. They increase ΔG of reactions.
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.

14. An allosteric inhibitor does which of the following?

a. Binds to an enzyme away from the active site and changes the conformation of the active site, increasing its affinity for substrate binding.
b. Binds to the active site and blocks it from binding substrate.
c. Binds to an enzyme away from the active site and changes the conformation of the active site, decreasing its affinity for the substrate.
d. Binds directly to the active site and mimics the substrate.

15. Which of the following analogies best describes the induced-fit model of enzyme-substrate binding?

a. a hug between two people
b. a key fitting into a lock
c. a square peg fitting through the square hole and a round peg fitting through the round hole of a children's toy
d. the fitting together of two jigsaw puzzle pieces

61.

CRITICAL THINKING QUESTIONS

16. Does physical exercise involve anabolic and/or catabolic processes? Give evidence for your answer.

17. Name two different cellular functions that require energy that parallel human energy-requiring functions.

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

19. Describe the position of the transition state on a vertical energy scale, from low to high, relative to the position of the reactants and products, for both endergonic and exergonic reactions.

20. Imagine an elaborate ant farm with tunnels and passageways through the sand where ants live in a large community. Now imagine that an earthquake shook the ground and demolished the ant farm. In which of these two scenarios, before or after the earthquake, was the ant farm system in a state of higher or lower entropy?

21. Energy transfers take place constantly in everyday activities. Think of two scenarios: cooking on a stove and driving. Explain how the second law of thermodynamics applies to these two scenarios.

22. Do you think that the EA for ATP hydrolysis is relatively low or high? Explain your reasoning.

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

24. Explain in your own words how enzyme feedback inhibition benefits a cell.

PART VII

CELLULAR RESPIRATION

62.

INTRODUCTION

Figure 7.1 This geothermal energy plant transforms thermal energy from deep in the ground into electrical energy, which can be easily used. (credit: modification of work by the U.S. Department of Defense)

The electrical energy plant in Figure 7.1 converts energy from one form to another form that can be more easily used. This type of generating plant starts with underground thermal energy (heat) and transforms it into electrical energy that will be transported to homes and factories. Like a generating plant, plants and animals also must take in energy from the environment and convert it into a form that their cells can use. Mass and its stored energy enter an organism's body in one form and are converted into another form that can fuel the organism's life functions. In the process of photosynthesis, plants and other photosynthetic producers take in energy in the form of light (solar energy) and convert it into chemical energy in the form of glucose, which stores this energy in its chemical bonds. Then, a series of metabolic pathways, collectively called cellular respiration, extracts the energy from the bonds in glucose and converts it into a form that all living things can use.

63.

ENERGY IN LIVING SYSTEMS

Learning Objectives

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

- Discuss the importance of electrons in the transfer of energy in living systems
- Explain how ATP is used by cells as an energy source

Energy production within a cell involves many coordinated chemical pathways. Most of these pathways are combinations of oxidation and reduction reactions, which occur at the same time. An oxidation reaction strips an electron from an atom in a compound, and the addition of this electron to another compound is a reduction reaction. Because oxidation and reduction usually occur together, these pairs of reactions are called oxidation reduction reactions, or **redox reactions**.

Electrons and Energy

The removal of an electron from a molecule (oxidizing it) results in a decrease in potential energy in the oxidized compound. However, the electron (sometimes as part of a hydrogen atom) does not remain unbonded in the cytoplasm of a cell. Rather, the electron is shifted to a second compound, reducing the second compound. *The shift of an electron from one compound to another removes some potential energy from the first compound (the oxidized compound) and increases the potential energy of the second compound (the reduced compound).* The transfer of electrons between molecules is important because most of the energy stored in atoms and used to fuel cell functions is in the form of high-energy electrons. The transfer of energy in the form of high-energy electrons allows the cell to transfer and use energy in an incremental fashion—in small packages rather than in a single, destructive burst. This chapter focuses on the extraction of energy from food; you will

see that as you track the path of the transfers, you are tracking the path of electrons moving through metabolic pathways.

Electron Carriers

In living systems, a small class of compounds functions as electron shuttles: they bind and carry high-energy electrons between compounds in biochemical pathways. The principal electron carriers we will consider are derived from the B vitamin group and are derivatives of nucleotides. These compounds can be easily reduced (that is, they accept electrons) or oxidized (they lose electrons). Nicotinamide adenine dinucleotide (NAD) (Figure 7.3) is derived from vitamin B_3, niacin. NAD^+ is the oxidized form of the molecule; NADH is the reduced form of the molecule after it has accepted two electrons and a proton (which together are the equivalent of a hydrogen atom with an extra electron). Note that if a compound has an "H" on it, it is generally reduced (e.g., NADH is the reduced form of NAD).

NAD^+ can accept electrons from an organic molecule according to the general equation:

$$\text{RH Reducing agent} + NAD^+ \text{ Oxidizing agent} \rightarrow \text{NADH Reduced} + \text{R Oxidized}$$

When electrons are added to a compound, *it is reduced*. A compound that reduces another is called a reducing agent. In the above equation, RH is a reducing agent, and NAD^+ is reduced to NADH. When electrons are removed from a compound, *it is oxidized*. A compound that oxidizes another is called an oxidizing agent. In the above equation, NAD^+ is an oxidizing agent, and RH is oxidized to R.

Similarly, flavin adenine dinucleotide (FAD^+) is derived from vitamin B_2, also called riboflavin. Its reduced form is $FADH_2$. A second variation of NAD, NADP, contains an extra phosphate group. Both NAD^+ and FAD^+ are extensively used in energy extraction from sugars, and NADP plays an important role in anabolic reactions and photosynthesis in plants.

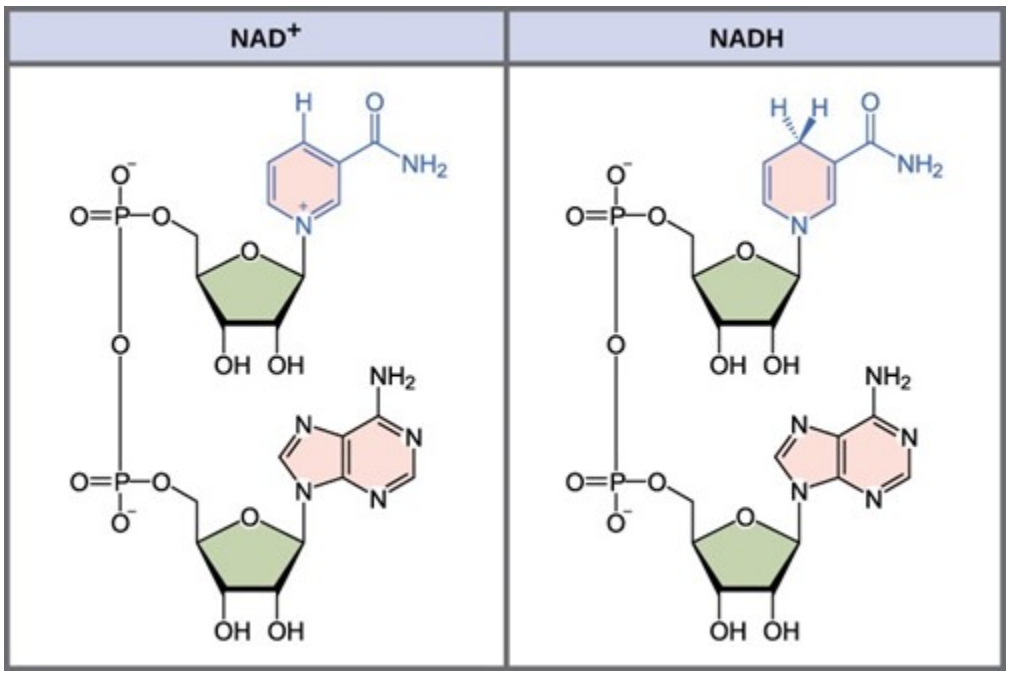

Figure 7.2 The oxidized form of the electron carrier (NAD+) is shown on the left, and the reduced form (NADH) is shown on the right. The nitrogenous base in NADH has one more hydrogen ion and two more electrons than in NAD+.

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 result in excessive thermal motion that could damage and then destroy the cell. Rather, a cell must be able to handle that energy in a way that enables the cell to store energy safely and release it for use only as needed. Living cells accomplish this by using the compound adenosine triphosphate (ATP). ATP is often called the "energy currency" of the cell, and, like currency, this versatile compound can be used to fill any energy need of the cell. How? It functions similarly to a rechargeable battery.

When ATP is broken down, usually by the removal of its terminal phosphate group, energy is released. The energy is used to do work by the cell, usually when the released phosphate binds to another molecule, thereby activating it. For example, in the mechanical work of muscle contraction, ATP supplies the energy to move the contractile muscle proteins. Recall the active transport work of the sodium-potassium pump in cell membranes. ATP alters the structure of the integral protein that functions as the pump, changing its affinity for sodium and potassium. In this way, the cell performs work, pumping ions against their electrochemical gradients.

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 a ribose molecule and to a single phosphate group (Figure 7.4). Ribose is a five-carbon sugar found in RNA, and AMP is one of the nucleotides in RNA. The addition of a second phosphate group to this core molecule results in the formation of adenosine diphosphate (ADP); the addition of a third phosphate group forms adenosine triphosphate (ATP).

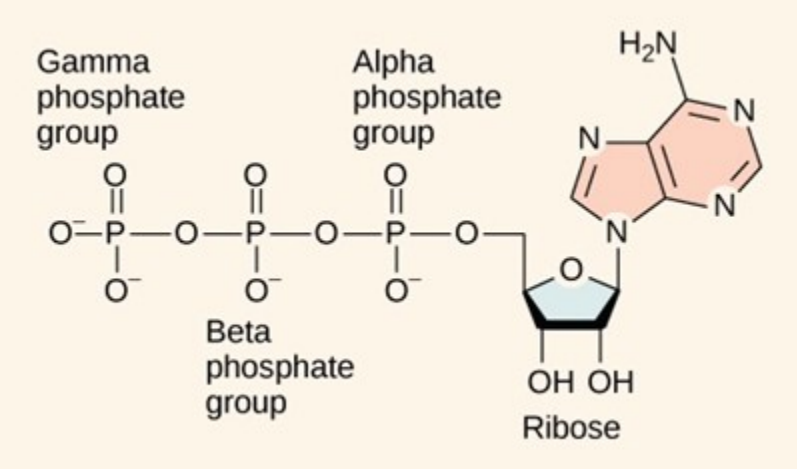

Figure 7.3 ATP (adenosine triphosphate) has three phosphate groups that can be removed by hydrolysis (addition of H2O) to form ADP (adenosine diphosphate) or AMP (adenosine monophosphate). The negative charges on the phosphate group naturally repel each other, requiring energy to bond them together and releasing energy when these bonds are broken.

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

Energy from ATP

Hydrolysis is the process of breaking complex macromolecules apart. During hydrolysis, water is split, or lysed, and the resulting hydrogen atom (H^+) and a hydroxyl group (OH^-), or *hydroxide,* are added to the larger molecule. The hydrolysis of ATP produces ADP, together with an inorganic phosphate ion (P_i), and the release of free energy. To carry out life processes, ATP is continuously broken down into ADP, and like a rechargeable battery, ADP is continuously regenerated into ATP by the reattachment of a third phosphate group. Water, which was broken down into its hydrogen atom and hydroxyl group (hydroxide) during ATP hydrolysis, is regenerated when a third phosphate is added to the ADP molecule, reforming ATP.

Obviously, energy must be infused into the system to regenerate ATP. Where does this energy come from? In nearly every living thing on Earth, the energy comes from the metabolism of glucose, fructose, or galactose,

all isomers with the chemical formula $C_6H_{12}O_6$ but different molecular configurations. In this way, ATP is a direct link between the limited set of exergonic pathways of glucose catabolism and the multitude of endergonic pathways that power living cells.

Phosphorylation

Recall that, in some chemical reactions, enzymes may bind to several substrates that react with each other on the enzyme, forming an intermediate complex. An intermediate complex is a temporary structure, and it allows one of the substrates (such as ATP) and reactants to more readily react with each other; in reactions involving ATP, ATP is one of the substrates and ADP is a product. During an endergonic chemical reaction, ATP forms an intermediate complex with the substrate and enzyme in the reaction. This intermediate complex allows the ATP to transfer its third phosphate group, with its energy, to the substrate, a process called phosphorylation. **Phosphorylation** refers to the addition of the phosphate (~P). This is illustrated by the following generic reaction, in which A and B represent two different substrates:

$$A + \text{enzyme} + ATP \rightarrow [A - \text{enzyme} - {\sim}P] \rightarrow B + \text{enzyme} + ADP + \text{phosphate ion}$$

When the intermediate complex breaks apart, the energy is used to modify the substrate and convert it into a product of the reaction. The ADP molecule and a free phosphate ion are released into the medium and are available for recycling through cell metabolism.

Substrate Phosphorylation

ATP is generated through two mechanisms during the breakdown of glucose. A few ATP molecules are generated (that is, regenerated from ADP) as a direct result of the chemical reactions that occur in the catabolic pathways. A phosphate group is removed from an intermediate reactant in the pathway, and the free energy of the reaction is used to add the third phosphate to an available ADP molecule, producing ATP (Figure 7.4). This very direct method of phosphorylation is called **substrate-level phosphorylation**.

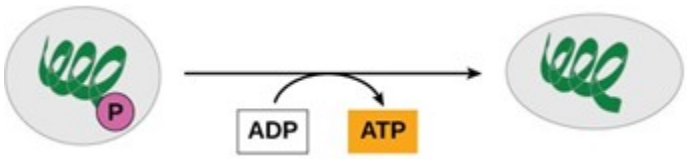

Figure 7.4 In substrate level phosphorylation, a phosphate group is transferred from a phosphorylated intermediate to ADP.

Oxidative Phosphorylation

Most of the ATP generated during glucose catabolism, however, is derived from a much more complex process, chemiosmosis, which takes place in mitochondria (Figure 7.5) within a eukaryotic cell or the plasma membrane of a prokaryotic cell. **Chemiosmosis**, a process of ATP production in cellular metabolism, is used to generate 90 percent of the ATP made during glucose catabolism and is also the method used in the light reactions of photosynthesis to harness the energy of sunlight. The production of ATP using the process of chemiosmosis is called **oxidative phosphorylation** because of the involvement of oxygen in the process.

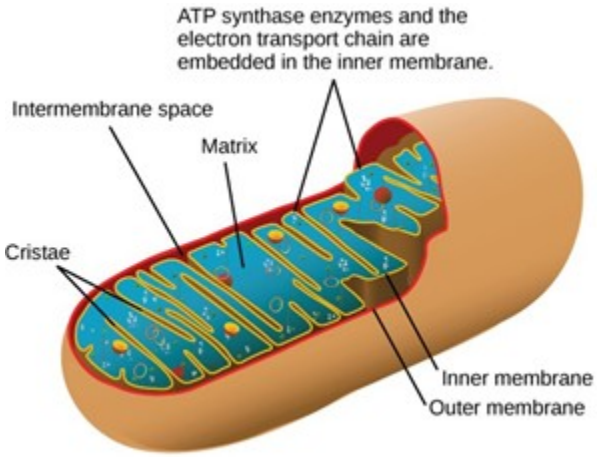

Figure 7.5 In eukaryotes, oxidative phosphorylation takes place in mitochondria. In prokaryotes, this process takes place in the plasma membrane. (Credit: modification of work by Mariana Ruiz Villareal)

Career Connections

Mitochondrial Disease Physician

What happens when the critical reactions of cellular respiration do not proceed correctly? This may happen in mitochondrial diseases, which 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. In type 2 diabetes, for instance, the oxidation efficiency of NADH is reduced, impacting oxidative phosphorylation but not the other steps of respiration. 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 diseases, such as the Mitochondrial Medicine Society and the Society for Inherited Metabolic Disorders.

64.

GLYCOLYSIS

Learning Objectives

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

- Identify the molecules produced from glucose in glycolysis
- State the number of ATP molecules and NADH molecules produced by glycolysis

Glycolysis is the first step in the breakdown of glucose to extract energy for cellular metabolism. In fact, nearly all living organisms carry out glycolysis as part of their metabolism. The process does not use oxygen directly and therefore is termed **anaerobic**. Glycolysis takes place in the cytoplasm of both prokaryotic and 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. The first part of the glycolysis pathway traps the glucose molecule in the cell and uses energy to modify it so that the six-carbon sugar molecule can be split evenly into the two three-carbon molecules. The second part of glycolysis extracts energy from the molecules and stores it in the form of ATP and NADH—remember: this is the reduced form of NAD.

First Half of Glycolysis (Energy-Requiring Steps)

Step 1. The first step in glycolysis (Figure 7.6) is catalyzed by hexokinase, an enzyme with broad specificity that catalyzes the phosphorylation of six-carbon sugars. Hexokinase phosphorylates glucose using ATP as the source of the phosphate, producing glucose-6-phosphate, a more reactive form of glucose. This reaction prevents the phosphorylated glucose molecule from continuing to interact with the GLUT proteins, and it

can no longer leave the cell because the negatively charged phosphate will not allow it to cross the hydrophobic interior of the plasma membrane.

Step 2. In the second step of glycolysis, an isomerase converts glucose-6-phosphate into one of its isomers, fructose-6-phosphate (this isomer has a phosphate attached at the location of the sixth carbon of the ring). An **isomerase** is an enzyme that catalyzes the conversion of a molecule into one of its isomers. (This change from phosphoglucose to phosphofructose allows the eventual split of the sugar into two three-carbon molecules.)

Step 3. The third step is the phosphorylation of fructose-6-phosphate, catalyzed by the enzyme phosphofructokinase. A second ATP molecule donates a high-energy phosphate to fructose-6-phosphate, producing fructose-1,6-bisphosphate. In this pathway, phosphofructokinase is a rate-limiting enzyme. It is active when the concentration of ADP is high; it is less active when ADP levels are low and the concentration of ATP is high. Thus, if there is "sufficient" ATP in the system, the pathway slows down. This is a type of end product inhibition, since ATP is the end product of glucose catabolism.

Step 4. The newly added high-energy phosphates further destabilize fructose-1,6-bisphosphate. The fourth step in glycolysis employs an enzyme, aldolase, to cleave fructose-1,6-bisphosphate into two three-carbon isomers: dihydroxyacetone phosphate and glyceraldehyde-3-phosphate.

Step 5. In the fifth step, an isomerase transforms the dihydroxyacetone-phosphate into its isomer, glyceraldehyde-3-phosphate. Thus, the pathway will continue with two molecules of a glyceraldehyde-3-phosphate. At this point in the pathway, there is a net investment of energy from two ATP molecules in the breakdown of one glucose molecule.

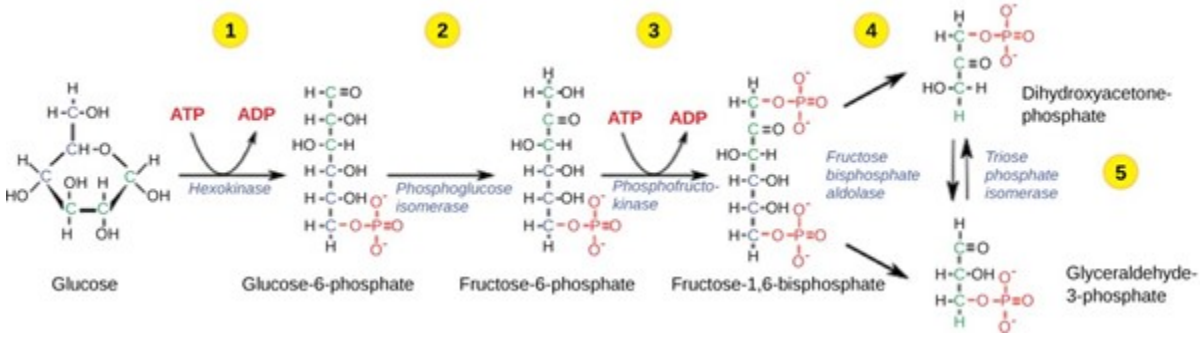

Figure 7.6 The first half of glycolysis uses two ATP molecules in the phosphorylation of glucose, which is then split into two three-carbon molecules.

Second Half of Glycolysis (Energy-Releasing Steps)

So far, glycolysis has cost the cell two ATP molecules and produced two small, three-carbon sugar molecules. Both of these molecules will proceed through the second half of the pathway, and sufficient energy will be

extracted to pay back the two ATP molecules used as an initial investment and produce a profit for the cell of two additional ATP molecules and two even higher-energy NADH molecules.

Step 6. The sixth step in glycolysis (Figure 7.7) oxidizes the sugar (glyceraldehyde-3-phosphate), extracting high-energy electrons, which are picked up by the electron carrier NAD^+, producing NADH. The sugar is then phosphorylated by the addition of a second phosphate group, producing 1,3-bisphosphoglycerate. Note that the second phosphate group does not require another ATP molecule.

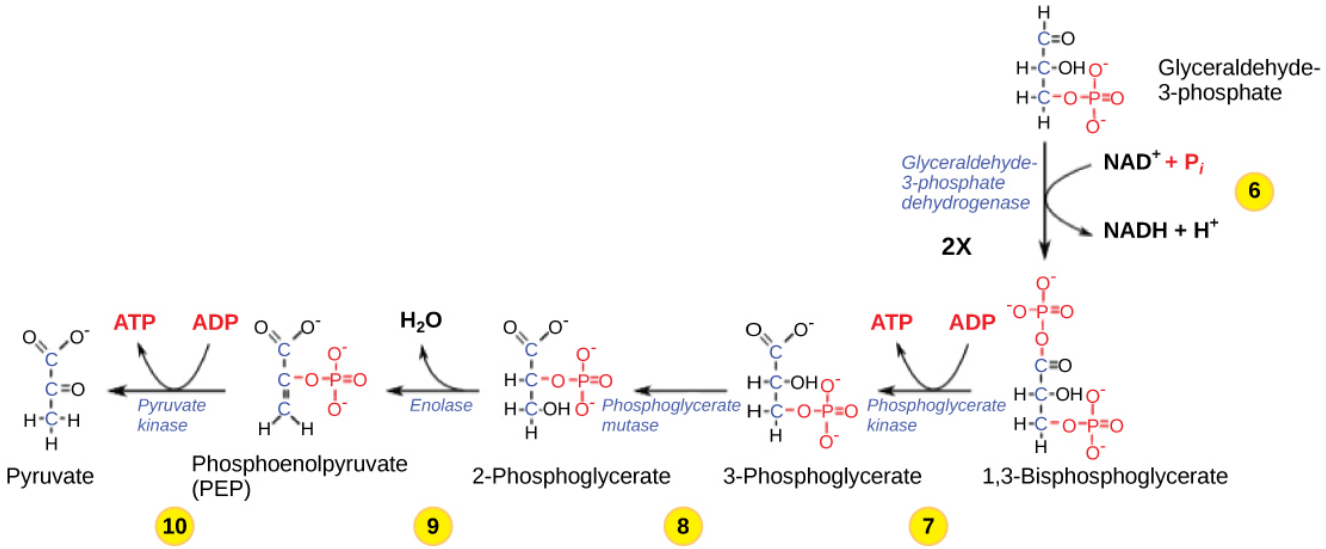

Figure 7.7 The second half of glycolysis involves phosphorylation without ATP investment (step 6) and produces two NADH and four ATP molecules per glucose.

Here again is a potential limiting factor for this pathway. The continuation of the reaction depends upon the availability of the oxidized form of the electron carrier, NAD^+. Thus, NADH must be continuously oxidized back into NAD^+ in order to keep this step going. If NAD^+ is not available, the second half of glycolysis slows down or stops. If oxygen is available in the system, the NADH will be oxidized readily, though indirectly, and the high-energy electrons from the hydrogen released in this process will be used to produce ATP. In an environment without oxygen, an alternate pathway (fermentation) can provide the oxidation of NADH to NAD^+.

Step 7. In the seventh step, catalyzed by phosphoglycerate kinase (an enzyme named for the reverse reaction), 1,3-bisphosphoglycerate donates a high-energy phosphate to ADP, forming one molecule of ATP. (This is an example of substrate-level phosphorylation.) A carbonyl group on the 1,3-bisphosphoglycerate is oxidized to a carboxyl group, and 3-phosphoglycerate is formed.

Step 8. In the eighth step, the remaining phosphate group in 3-phosphoglycerate moves from the third carbon to the second carbon, producing 2-phosphoglycerate (an isomer of 3-phosphoglycerate). The enzyme catalyzing this step is a mutase (isomerase).

Step 9. Enolase catalyzes the ninth step. This enzyme causes 2-phosphoglycerate to lose water from its

structure; this is a dehydration reaction, resulting in the formation of a double bond that increases the potential energy in the remaining phosphate bond and produces phosphoenolpyruvate (PEP).

Step 10. The last step in glycolysis is catalyzed by the enzyme pyruvate kinase (the enzyme in this case is named for the reverse reaction of pyruvate's conversion into PEP) and results in the production of a second ATP molecule by substrate-level phosphorylation and the compound pyruvic acid (or its salt form, pyruvate). Many enzymes in enzymatic pathways are named for the reverse reactions, since the enzyme can catalyze both forward and reverse reactions (these may have been described initially by the reverse reaction that takes place in vitro, under nonphysiological conditions).

Link to Learning

Gain a better understanding of the breakdown of glucose by glycolysis by visiting this site to see the process in action.

Outcomes of Glycolysis

Glycolysis begins with glucose and produces two pyruvate molecules, four new ATP molecules, and two molecules of NADH. (Note: two ATP molecules are used in the first half of the pathway to prepare the six-carbon ring for cleavage, so the cell has a *net gain of two ATP molecules* and two NADH molecules for its use). If the cell cannot catabolize the pyruvate molecules further, it will harvest only two ATP molecules from one molecule of glucose. Mature mammalian red blood cells do not have mitochondria and thus are not capable of **aerobic respiration**—the process in which organisms convert energy in the presence of oxygen—and glycolysis is their sole source of ATP. If glycolysis is interrupted, these cells lose their ability to maintain their sodium-potassium pumps, and eventually, they die.

The last step in glycolysis will not occur if pyruvate kinase, the enzyme that catalyzes the formation of pyruvate, is not available in sufficient quantities. In this situation, the entire glycolysis pathway will proceed, but only two ATP molecules will be made in the second half. Thus, pyruvate kinase is a rate-limiting enzyme for glycolysis.

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65.

OXIDATION OF PYRUVATE AND THE CITRIC ACID CYCLE

Learning Objectives

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

- Describe how pyruvate, the product of glycolysis, is prepared for entry into the citric acid cycle
- Explain how a circular pathway, such as the citric acid cycle, fundamentally differs from a linear biochemical pathway, such as glycolysis
- State the fate of the carbon atoms of pyruvate
- State the molecules produced by each turn of the citric acid cycle

If oxygen is available, aerobic respiration will go forward. In eukaryotic cells, the pyruvate molecules produced at the end of glycolysis are transported into the mitochondria, which are the sites of cellular respiration. There, pyruvate is transformed into an acetyl group that will be picked up and activated by a carrier compound called coenzyme A (CoA). The resulting compound is called **acetyl CoA**. CoA is derived from vitamin B5, pantothenic acid. 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 stage of the pathway in glucose catabolism.

Breakdown of Pyruvate

In order for pyruvate, the product of glycolysis, to enter the next pathway, it must undergo several changes. The conversion is a three-step process (Figure 7.8).

Step 1. A carboxyl group is removed from pyruvate, releasing a molecule of carbon dioxide into the

surrounding medium. This reaction creates a two-carbon hydroxyethyl group bound to the enzyme (pyruvate dehydrogenase). We should note that this is the first of the six carbons from the original glucose molecule to be removed. (This step proceeds twice because there are *two* pyruvate molecules produced at the end of glycolysis for every molecule of glucose metabolized anaerobically; thus, two of the six carbons will have been removed at the end of both steps.)

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

Step 3. The enzyme-bound acetyl group is transferred to CoA, producing a molecule of acetyl CoA.

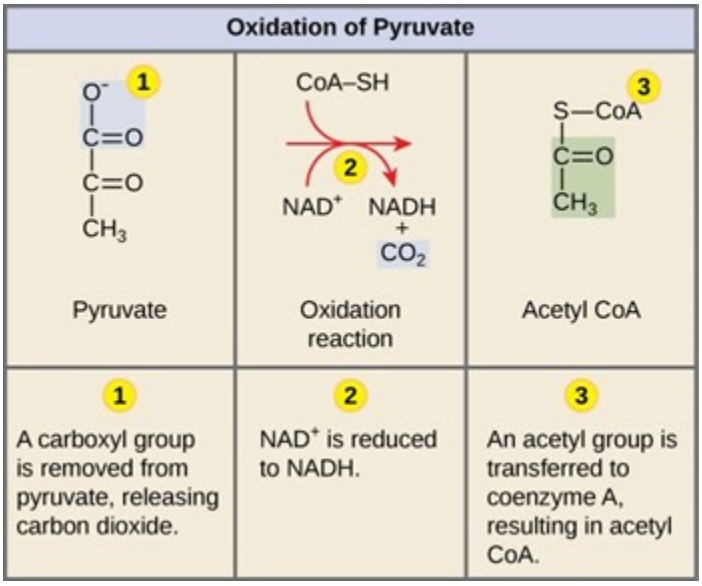

Figure 7.8 Upon entering the mitochondrial matrix, a multienzyme complex converts pyruvate into acetyl CoA. In the process, carbon dioxide is released, and one molecule of NADH is formed.

Note that during the second stage of glucose metabolism, whenever a carbon atom is removed, it is bound to two oxygen atoms, producing carbon dioxide, one of the major end products of cellular respiration.

Acetyl CoA to CO_2

CoA delivers its acetyl (2C) group to a four-carbon molecule, oxaloacetate, to form citrate, a six-carbon molecule with three carboxyl groups; this pathway will harvest the remainder of the extractable energy from what began as a glucose molecule and release the remaining four CO_2 molecules. This single pathway is called by different names: the **citric acid cycle** (for the first intermediate formed—citric acid, or citrate—when

acetate joins to the oxaloacetate), the **TCA cycle** (because citric acid or citrate and isocitrate are tricarboxylic acids), and the **Krebs cycle**, after Hans Krebs, who first identified the steps in the pathway in the 1930s in pigeon flight muscles.

Citric Acid Cycle

Like the conversion of pyruvate to acetyl CoA, the citric acid cycle takes place in the matrix of mitochondria. Almost all of the enzymes of the citric acid cycle are soluble, with the single exception of the enzyme succinate dehydrogenase, which is embedded in the inner membrane of the mitochondrion. Unlike glycolysis, the citric acid cycle is a closed loop: the last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of redox, dehydration, hydration, and decarboxylation reactions that produce two carbon dioxide molecules, one GTP/ATP, and the reduced carriers NADH and $FADH_2$ (Figure 7.9). *This is considered an aerobic pathway because the NADH and $FADH_2$ produced must transfer their electrons to the next pathway in the system, which will use oxygen.* If this transfer does not occur, the oxidation steps of the citric acid cycle also do not occur. Note that the citric acid cycle produces very little ATP directly and does not directly consume oxygen.

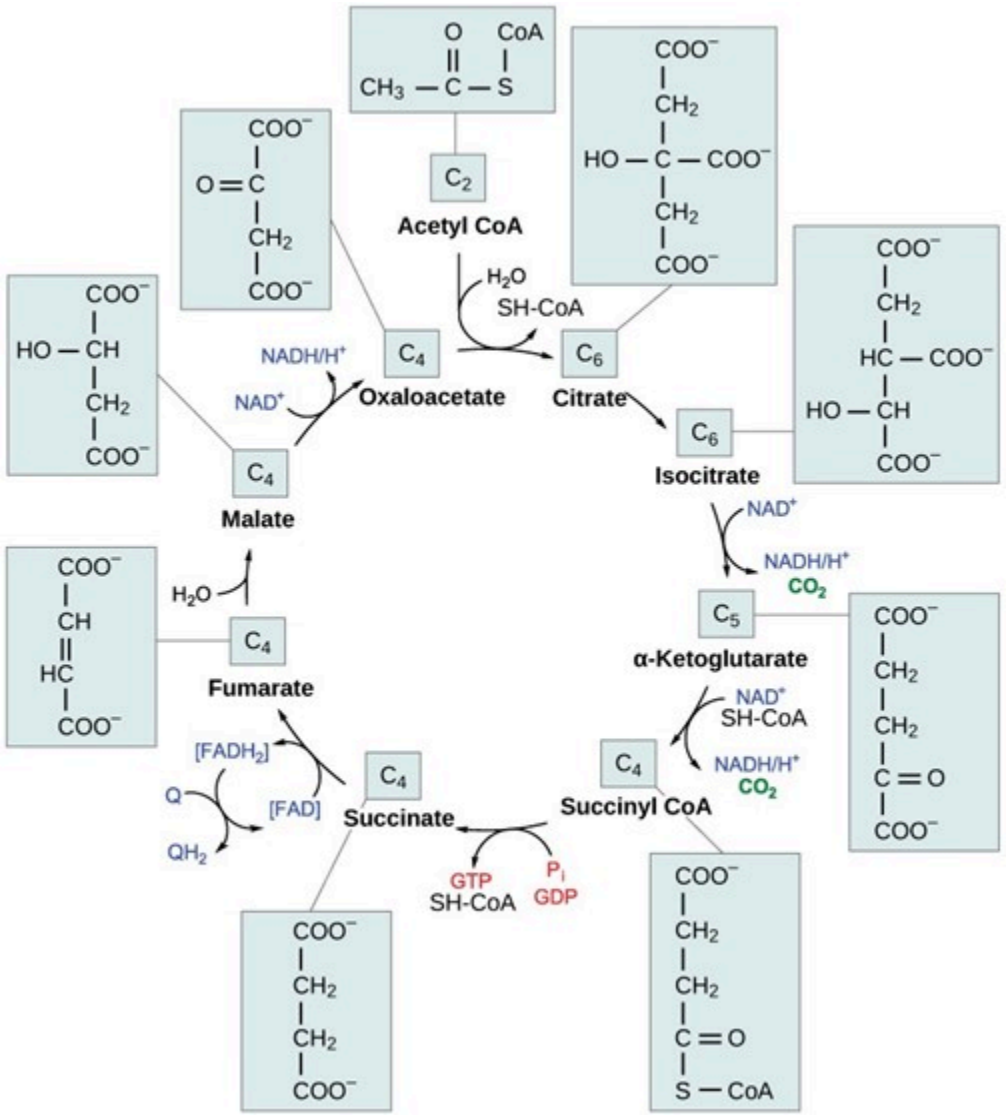

Figure 7.9 In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NAD+ molecules are reduced to NADH, one FAD molecule is reduced to FADH2, and one ATP or GTP (depending on the cell type) is produced (by substrate-level phosphorylation). Because the final product of the citric acid cycle is also the first reactant, the cycle runs continuously in the presence of sufficient reactants. (credit: modification of work by "Yikrazuul"/Wikimedia Commons)

Steps in the Citric Acid Cycle

Step 1. Prior to the first step, a transitional phase occurs during which pyruvic acid is converted to acetyl CoA. Then, the first step of the cycle begins: This condensation step combines the two-carbon acetyl group with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. CoA is bound to a sulfhydryl group (-SH) and diffuses away to eventually combine with another acetyl group. This step is irreversible

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

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

Step 3. In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, along with a molecule of CO_2 and two electrons, which reduce NAD^+ to NADH. This step is also regulated by negative feedback from ATP and NADH and a positive effect of ADP.

Step 4. Steps three and four are both oxidation and decarboxylation steps, which as we have seen, release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. Alpha-ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds with the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

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

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

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

Link to Learning

View an animation of the citric acid cycle here.

Products of the Citric Acid Cycle

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

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66.

OXIDATIVE PHOSPHORYLATION

Learning Objectives

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

- Describe how electrons move through the electron transport chain and explain what happens to their energy levels during this process
- Explain how a proton (H+) gradient is established and maintained by the electron transport chain

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. Instead, it is derived from a process that begins by moving electrons through a series of electron carriers that undergo redox reactions. This process causes hydrogen ions to accumulate within the intermembranous space. Therefore, a concentration gradient forms in which hydrogen ions diffuse out of the intermembranous space into the mitochondrial matrix by passing through ATP synthase. The current of hydrogen ions powers the catalytic action of ATP synthase, which phosphorylates ADP, producing ATP.

Electron Transport Chain

The **electron transport chain** (Figure 7.10) is the last component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Oxygen continuously diffuses into plant tissues (typically through stomata), as well as into fungi and bacteria; however, in animals, oxygen enters the body through a variety of respiratory systems. Electron transport is a series of redox reactions that resembles a relay race or bucket brigade, in that electrons are passed rapidly from one component to the next to the endpoint of the

chain, where the electrons reduce molecular oxygen and, along with associated protons, produce water. There are four complexes composed of proteins, labeled I through IV in Figure 7.10, 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 with multiple copies in the inner mitochondrial membrane of eukaryotes and within the plasma membrane of prokaryotes.

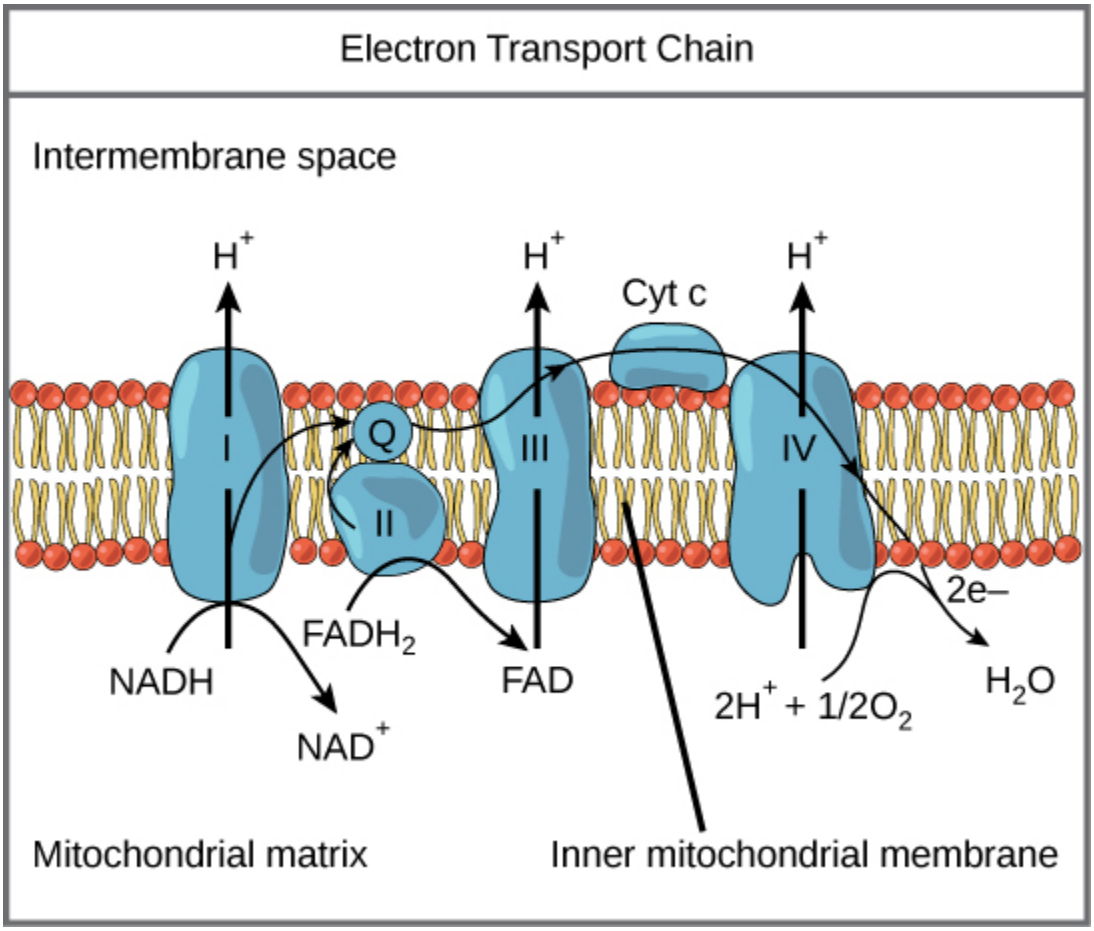


Figure 7.10 The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and FADH2 to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space, and oxygen is reduced to form water.

Complex I

First, two electrons are carried to the first complex via NADH. This complex, labeled **I**, is composed of flavin mononucleotide (FMN) and an iron-sulfur (Fe-S)-containing protein. FMN, which is derived from vitamin B_2 (also called riboflavin), is one of several prosthetic groups or cofactors in the electron transport chain. A **prosthetic group** is a nonprotein molecule required for the activity of a protein. Prosthetic groups

are organic or inorganic, nonpeptide molecules bound to a protein that facilitate its function. Prosthetic groups include coenzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase and is composed of 44 separate polypeptide chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space, and it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

Q and Complex II

Complex II directly receives $FADH_2$—which does not pass through complex I. The compound connecting the first and second complexes to the third is **ubiquinone** B. The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced (QH_2), ubiquinone delivers its electrons to the next complex in the electron transport chain. Q receives the electrons derived from NADH from complex I, and the electrons derived from $FADH_2$ from complex II. This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex. Since these electrons bypass and thus do not energize the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons. *The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.*

Complex III

The third complex is composed of cytochrome b—another Fe-S protein, a Rieske center (2Fe-2S center), and cytochrome c proteins. This complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic group of heme. The heme molecule is similar to the heme in hemoglobin, but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced and oxidized as it passes the electrons, fluctuating between different oxidation states: Fe^{++} (reduced) and Fe^{+++} (oxidized). The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding to them, giving slightly different characteristics to each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes. (Cytochrome c receives electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time.)

Complex IV

The fourth complex is composed of cytochrome proteins c, a, and a_3. This complex contains two heme groups (one in each of the two cytochromes, a, and a_3) and three copper ions (a pair of Cu_A and one Cu_B in cytochrome a_3). The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced by the gain of two electrons. The reduced oxygen then picks up two hydrogen

ions from the surrounding medium to make water (H_2O). The removal of the hydrogen ions from the system contributes to the ion gradient that forms the foundation for the process of chemiosmosis.

Chemiosmosis

In chemiosmosis, the free energy from the series of redox reactions just described is used to pump hydrogen ions (protons) across the mitochondrial membrane. The uneven distribution of H+ ions across the membrane establishes both concentration and electrical gradients (thus, an electrochemical gradient), owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane.

If the membrane were continuously open to simple diffusion by the hydrogen ions, the ions would tend to diffuse back across into the matrix, driven by the concentrations producing their electrochemical gradient. Recall that many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels. Similarly, hydrogen ions in the matrix space can only pass through the inner mitochondrial membrane by an integral membrane protein called **ATP synthase** (Figure 7.11). This complex protein (Figure 7.12) acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient. The turning of parts of this molecular machine facilitates the addition of a phosphate to ADP, forming ATP, *using the potential energy of the hydrogen ion gradient*.

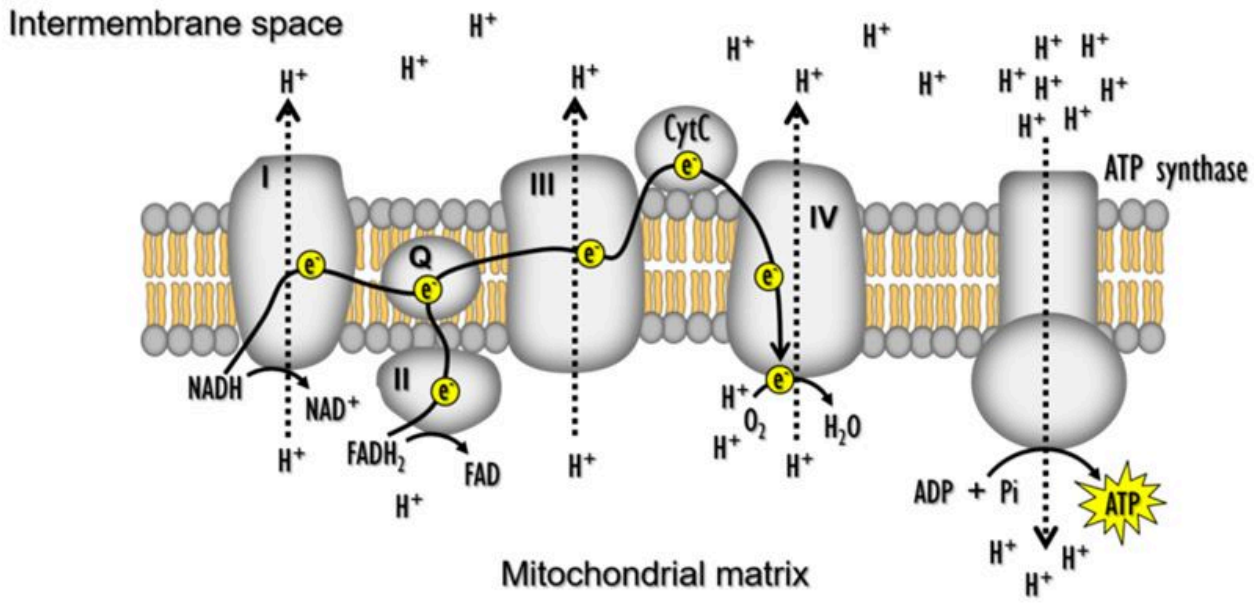
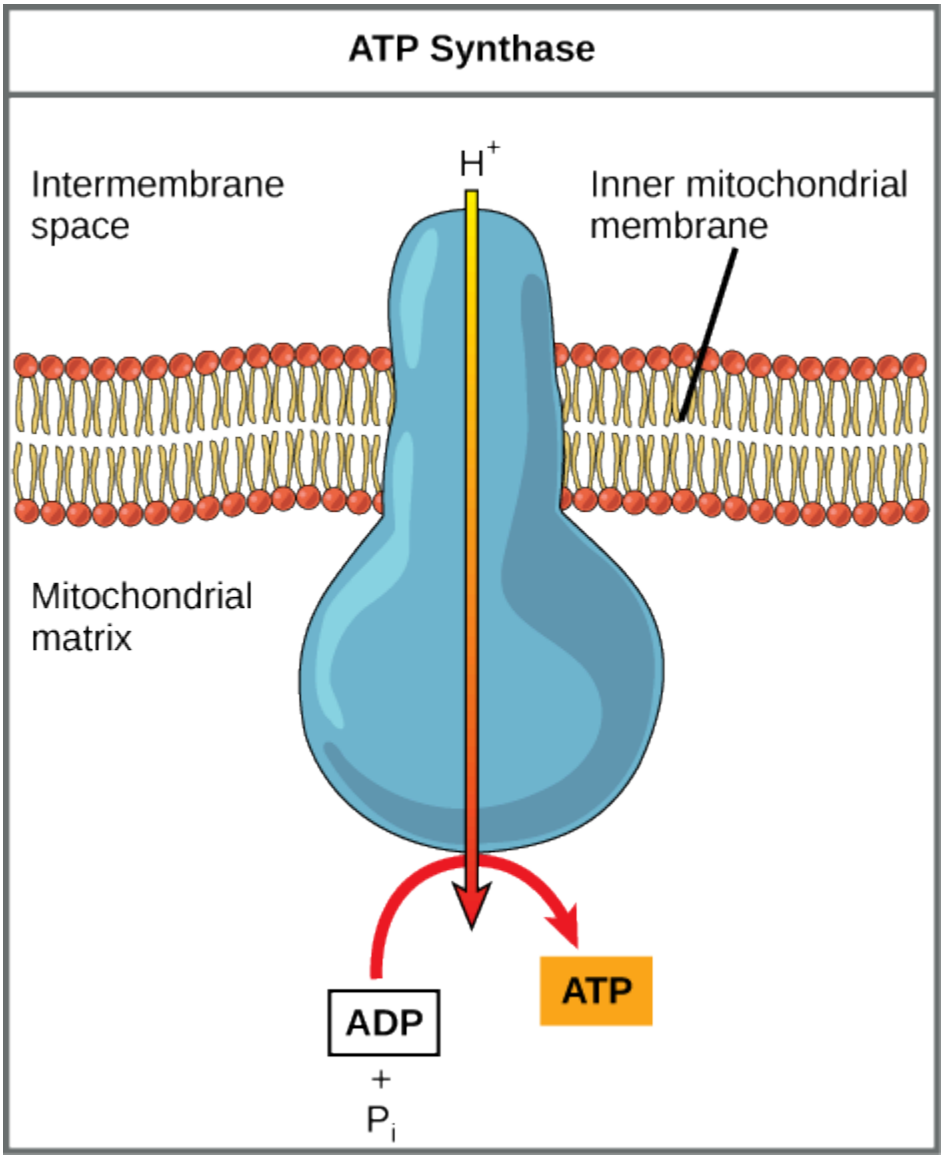


Figure 7.11 The electron transport chain generates a hydrogen ion gradient. This hydrogen ion gradient drives ATP synthesis by the ATP synthase complex.
(Eunice Laurent, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons)

Visual Connection

A.



B.

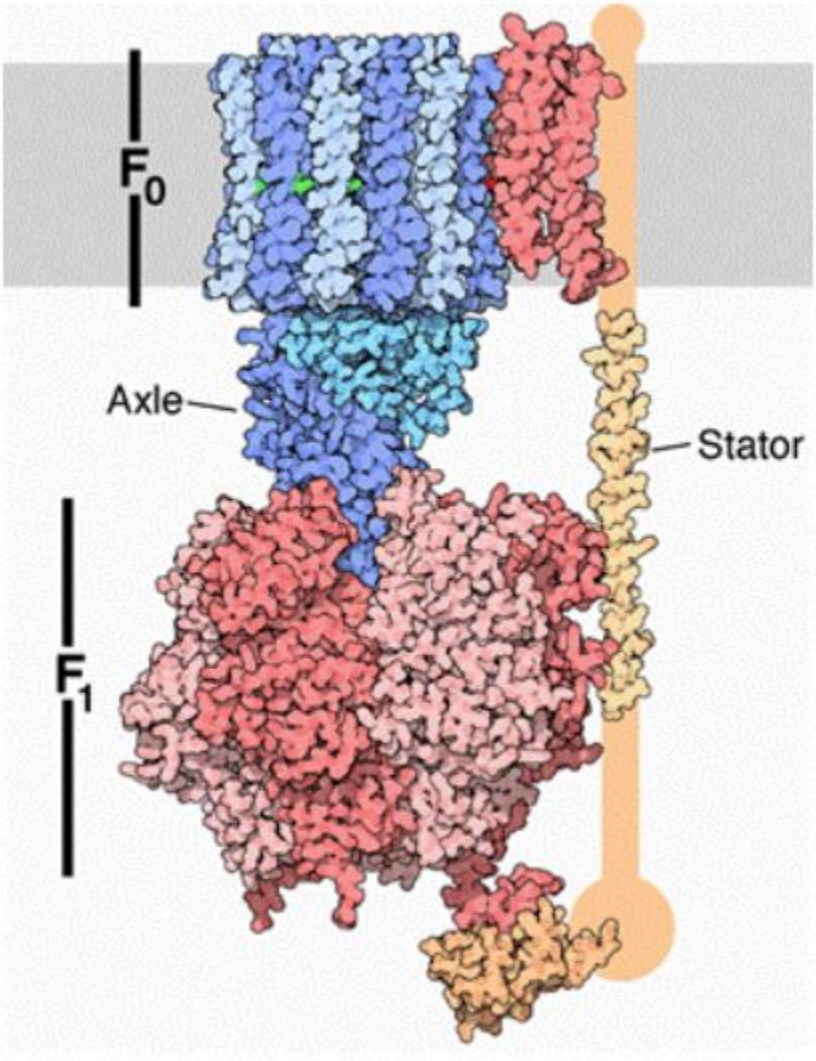


Figure 7.12 A. ATP synthase is a complex, molecular machine that uses a proton (H+) gradient to form ATP from ADP and inorganic phosphate (Pi). Notice that the electrons flow from the space between the inner and outer mitochondrial membranes through the ATP synthase complex into the matrix of the mitochondria. **B.** A model of the ATP synthase complex showing the F0, axle, stator, and F1 structures. (pdb101.rcsb.org/motm/72.)

Dinitrophenol (DNP) is an "uncoupler" that makes the inner mitochondrial membrane "leaky" to protons. It was used until 1938 as a weight-loss drug. What effect would you expect DNP to have on the change in pH across the inner mitochondrial membrane? Why do you think this might be an effective weight-loss drug?

Chemiosmosis (Figure 7.13) is used to generate 90 percent of the ATP made during aerobic glucose catabolism; it is also the method used in the light reactions of photosynthesis to harness the energy of sunlight in the process of photophosphorylation. Recall that the production of ATP using the process of chemiosmosis in mitochondria is called oxidative phosphorylation. The overall result of these 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 pathway, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen attract hydrogen ions (protons) from the surrounding medium, and water is formed. Thus, oxygen is the final electron acceptor in the electron transport chain.

Visual Connection

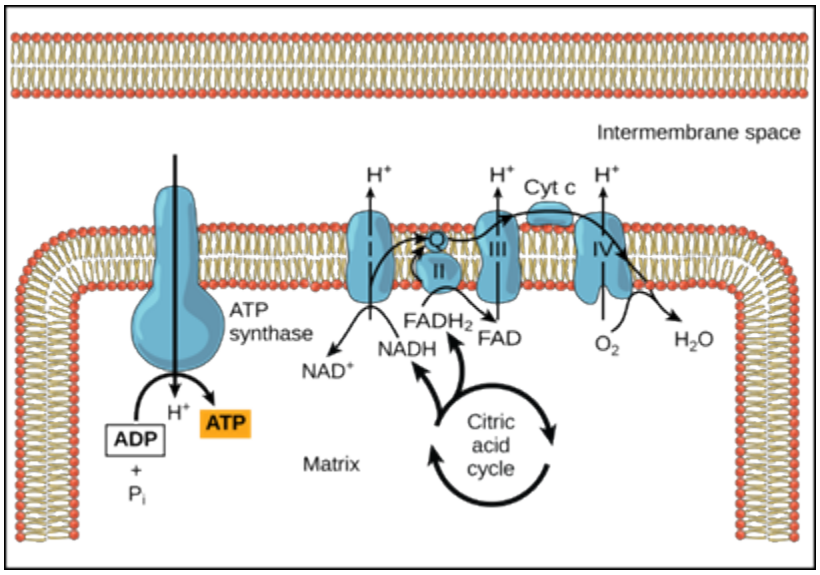


Figure 7.13 The difference in the concentration of H+ on opposite sides of the inner mitochondrial membrane drives chemiosmosis, the synthesis of ATP by the ATP synthase complex.

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 effect would cyanide have on ATP synthesis?

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 membranes of the mitochondria. (The NADH generated from glycolysis cannot easily enter mitochondria.) Thus, electrons are picked up on the inside of mitochondria by either NAD^+ or FAD^+. As you have learned earlier, these FAD^+ molecules can transport fewer ions; consequently, fewer ATP molecules are generated when FAD^+ acts as a carrier. NAD^+ is used as the electron transporter in the liver and FAD^+ acts in the brain.

Another factor that affects the yield of ATP molecules generated from glucose is the fact that intermediate compounds in these pathways are also 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. In addition, the five-carbon sugars that form nucleic acids are made from intermediates in glycolysis. Certain nonessential amino acids can be made from intermediates of both glycolysis and the citric acid cycle. Lipids, such as cholesterol and triglycerides, are also made from intermediates in these pathways, and both amino acids and triglycerides are broken down for energy through these pathways. Overall, in living systems, these pathways of glucose catabolism extract about 34 percent of the energy contained in glucose, with the remainder being released as heat.

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https://louis.pressbooks.pub/generalbiology1leclab/?p=319#h5p-10

67.

METABOLISM WITHOUT OXYGEN

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, O2. If aerobic respiration occurs, then ATP will be produced using the energy of high-energy electrons carried by NADH or FADH2 to the electron transport chain. If aerobic respiration does not occur, NADH must be reoxidized to NAD+ for reuse as an electron carrier for the glycolytic pathway 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 as a final electron acceptor. Both methods are called **anaerobic cellular respiration**, in which organisms convert energy for their use in the absence of oxygen.

Anaerobic Cellular Respiration

Certain prokaryotes, including some species in the domains Bacteria and Archaea, use anaerobic respiration. For example, a group of archaeans 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, most of which are anaerobic (Figure 7.14), reduce sulfate to hydrogen sulfide to regenerate NAD+ from NADH.

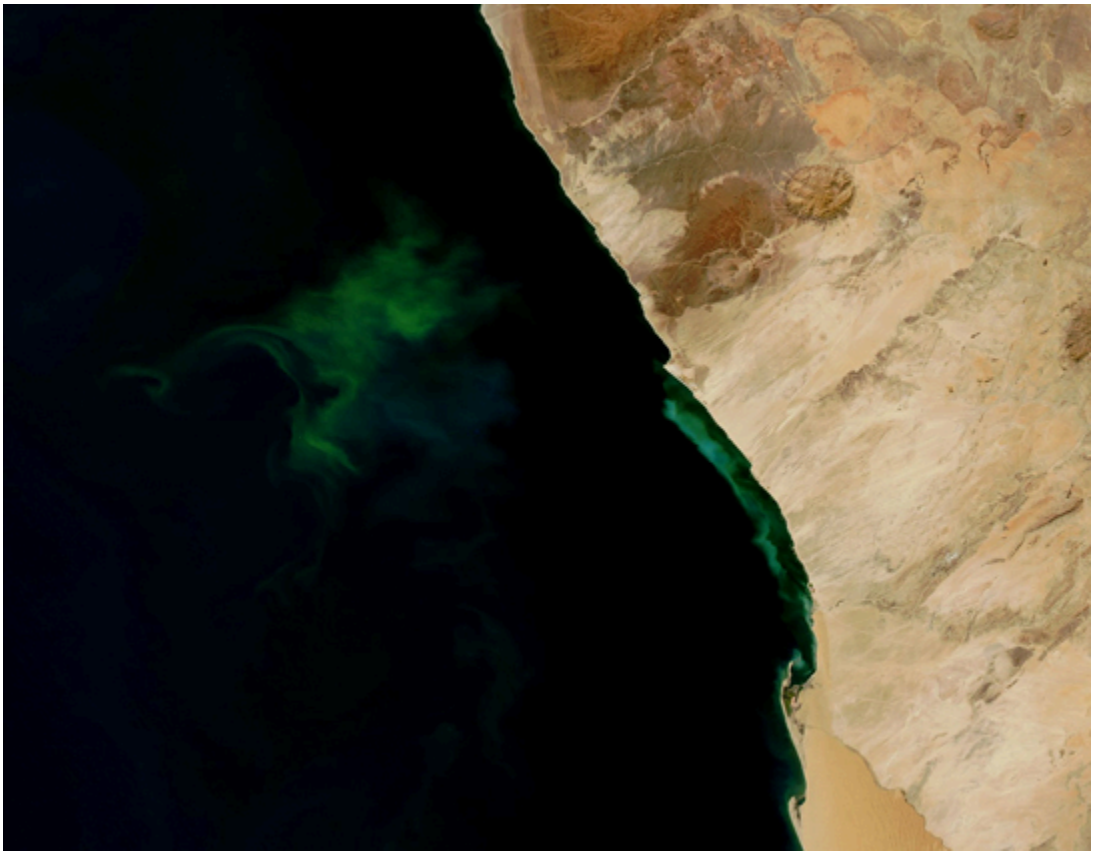

Figure 7.14 The green color seen in these coastal waters is from an eruption of hydrogen sulfide–producing bacteria. These anaerobic, sulfate-reducing bacteria release hydrogen sulfide gas as they decompose algae in the water. (credit: modification of work by NASA/Jeff Schmaltz, MODIS Land Rapid Response Team at NASA GSFC, Visible Earth Catalog of NASA images).

Link to Learning

Visit this site to see anaerobic cellular respiration in action.

Lactic Acid Fermentation

The fermentation method used by animals and certain bacteria, such as those in yogurt, is lactic acid fermentation (Figure 7.15). This type of fermentation is used routinely in mammalian red blood cells, which do not have mitochondria, and in skeletal muscle that has an insufficient oxygen supply to allow aerobic

respiration to continue (that is, in muscles used to the point of fatigue). In muscles, lactic acid accumulation must be removed by the blood circulation, and when the lactic acid loses a hydrogen, the resulting lactate is brought to the liver for further metabolism. The chemical reactions of lactic acid fermentation are the following:

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

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

Visual Connection

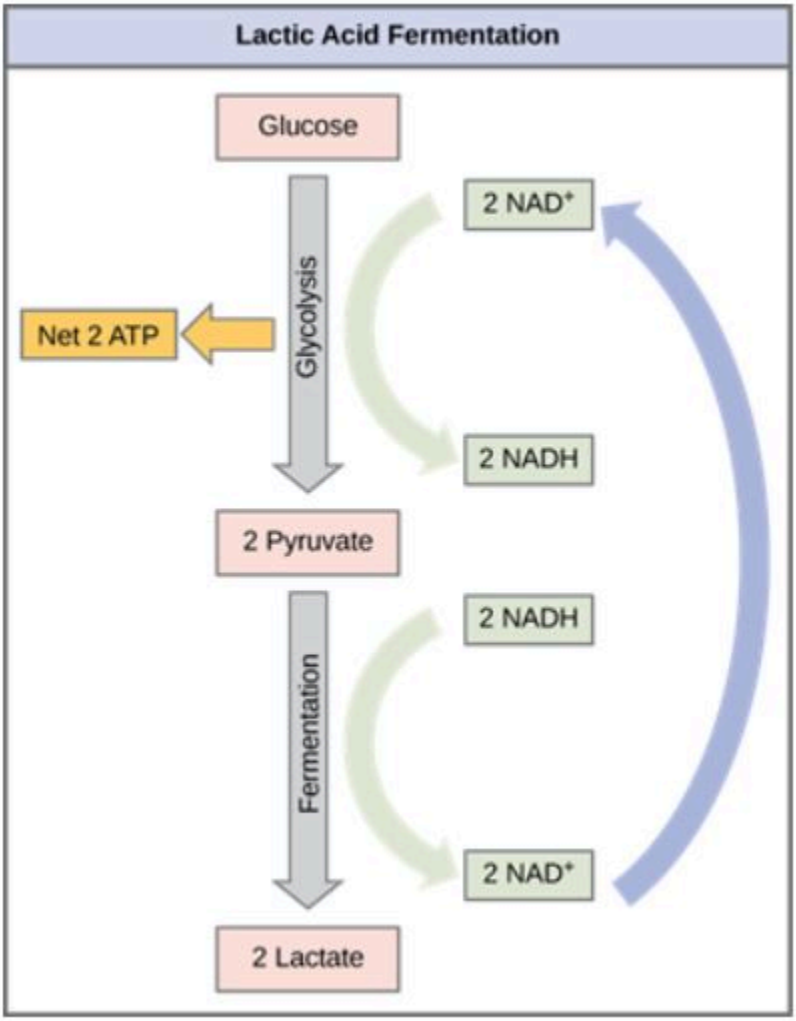

Figure 7.15 Lactic acid fermentation is common in muscle cells that have run out of oxygen.

Tremetol, a metabolic poison found in the white snakeroot plant, prevents the metabolism of lactate. When cows eat this plant, tremetol is concentrated in the milk they produce. Humans who consume the milk can become seriously 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 7.16**), which produces ethanol. The first chemical reaction of alcohol fermentation is the following (CO2 does not participate in the second reaction):

$$\text{pyruvic acid} + H^+ \rightarrow CO_2 + \text{acetaldehyde} + NADH + H^+ \rightarrow \text{ethanol} + NAD^+$$

The first reaction is catalyzed by pyruvate decarboxylase, a cytoplasmic enzyme, with a coenzyme of thiamine pyrophosphate (TPP, derived from vitamin B_1 and also called thiamine). A carboxyl group is removed from pyruvic acid, releasing carbon dioxide as a gas. The loss of carbon dioxide reduces the size of the molecule by one carbon, producing acetaldehyde. The second reaction is catalyzed by alcohol dehydrogenase to oxidize NADH to NAD^+ and reduce acetaldehyde to ethanol. The fermentation of pyruvic acid by yeast produces the ethanol found in alcoholic beverages. Ethanol tolerance of yeast is variable, ranging from about 5 percent to 21 percent, depending on the yeast strain and environmental conditions.

Figure 7.16 Fermentation of grape juice into wine produces CO2 as a byproduct. Fermentation tanks have valves so that the pressure inside the tanks created by the carbon dioxide produced can be released.

Other Types of Fermentation

Other fermentation methods take place in bacteria. We should note that many prokaryotes are *facultatively* anaerobic. This means that they can switch between aerobic respiration and fermentation,

depending on the availability of free oxygen. Certain prokaryotes, such as *Clostridia*, are obligate anaerobes. Obligate anaerobes live and grow in the absence of molecular oxygen. Oxygen is a poison to these microorganisms and kills them on exposure. We should also note 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. Various methods of fermentation are used by assorted organisms to ensure an adequate supply of NAD^+ for the sixth step in glycolysis. Without these pathways, this step would not occur, and ATP could not be harvested from the breakdown of glucose.

68.

CONNECTIONS OF CARBOHYDRATE, PROTEIN, AND LIPID METABOLIC PATHWAYS

Learning Objectives

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

- Discuss the ways in which carbohydrate metabolic pathways, glycolysis, and the citric acid cycle interrelate with protein and lipid metabolic pathways
- Explain why metabolic pathways are not considered closed systems

You have learned about the catabolism of glucose, which provides energy to living cells. But living things consume organic compounds other than glucose for food. How does a turkey sandwich end up as ATP in your cells? This happens because all of the catabolic pathways for carbohydrates, proteins, and lipids eventually connect into glycolysis and the citric acid cycle pathways (see Figure 7.17). Metabolic pathways should be thought of as porous and interconnecting—that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems! Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways.

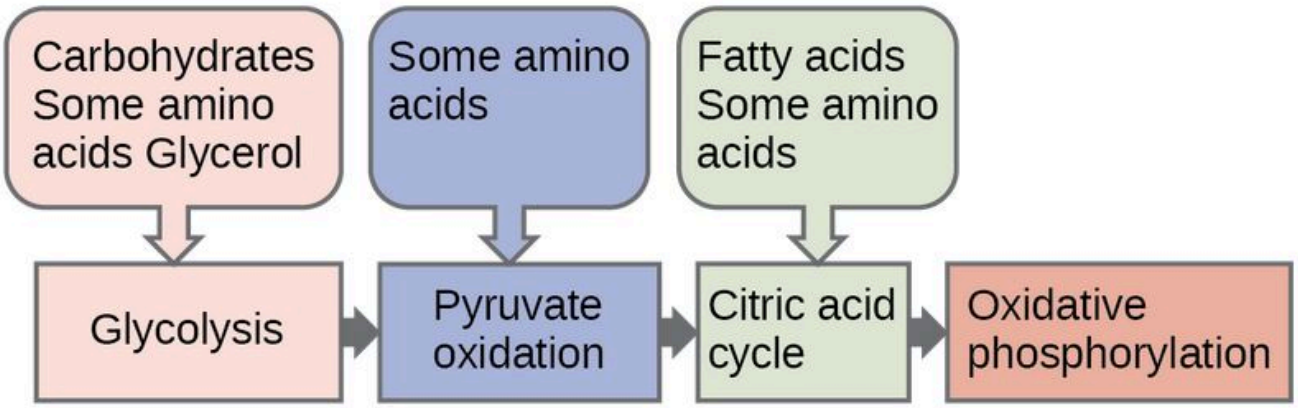

Figure 7.17 Glycogen from the liver and muscles, as well as other carbohydrates, hydrolyzed into glucose-1-phosphate, together with fats and proteins, can feed into the catabolic pathways for carbohydrates.

Connections of Other Sugars to Glucose Metabolism

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

Sucrose is a disaccharide with a molecule of glucose and a molecule of fructose bonded together with a glycosidic linkage. Fructose is one of the three "dietary" monosaccharides, along with glucose and galactose (part of the milk sugar disaccharide lactose), which are absorbed directly into the bloodstream during digestion. The catabolism of both fructose and galactose produces the same number of ATP molecules as glucose.

Connections of Proteins to Glucose Metabolism

Proteins are hydrolyzed by a variety of enzymes in cells. Most of the time, the amino acids are recycled into the synthesis of new proteins. If there are excess amino acids, however, or if the body is in a state of starvation, some amino acids will be shunted into the pathways of glucose catabolism (Figure 7.18). It is very important to note that each amino acid must have its amino group removed prior to entry into these pathways. The amino group is converted into ammonia. In mammals, the liver synthesizes urea from two ammonia molecules and a carbon dioxide molecule. Thus, urea is the principal waste product in mammals, produced from the nitrogen originating in amino acids, and it leaves the body in urine. It should be noted that amino acids can be synthesized from the intermediates and reactants in the cellular respiration cycle.

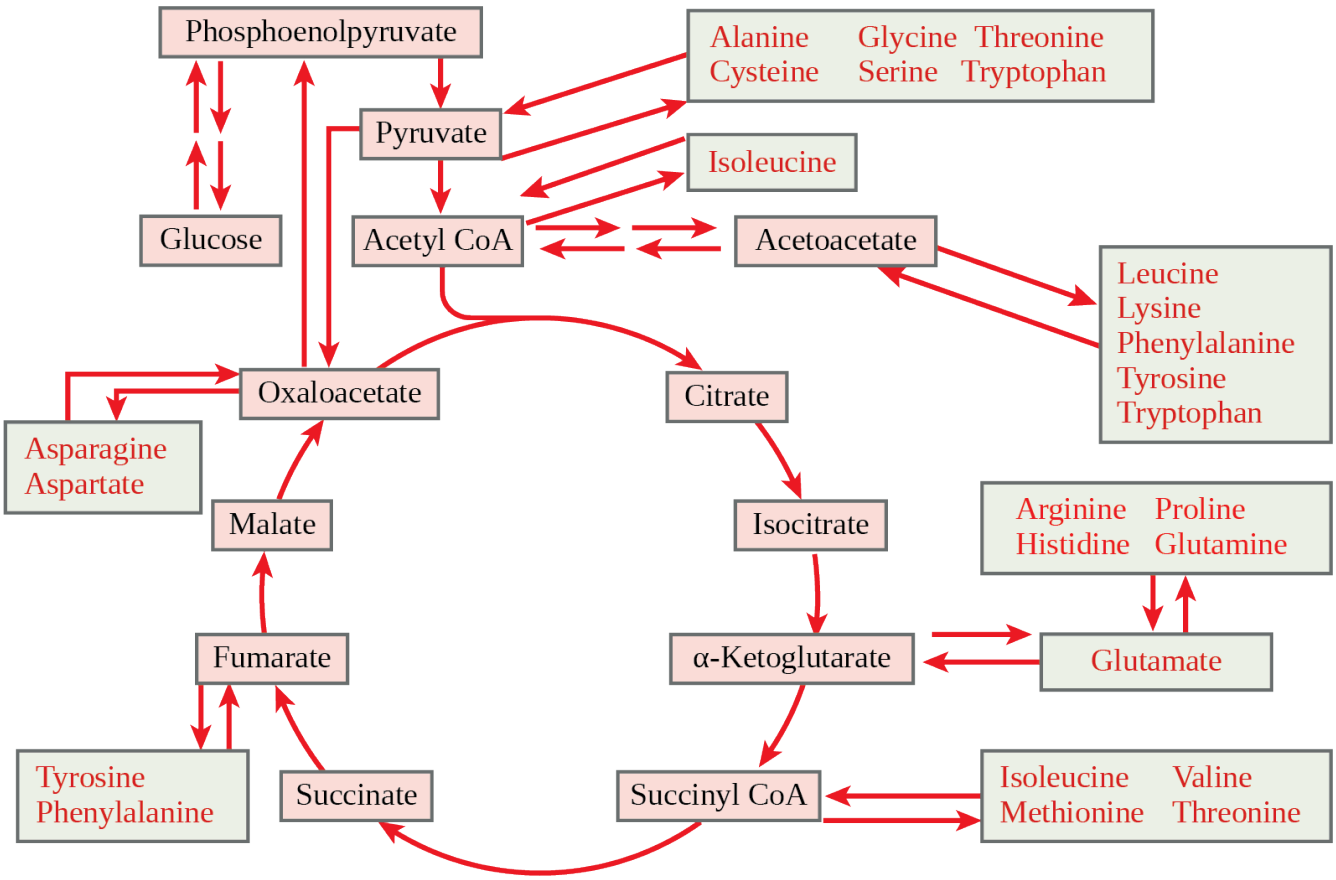

Figure 7.18 The carbon skeletons of certain amino acids (indicated in boxes) derived from proteins can feed into the citric acid cycle. (credit: modification of work by Mikael Häggström)

Connections of Lipid and Glucose Metabolisms

The lipids connected to the glucose pathway include cholesterol and triglycerides. Cholesterol is a lipid that contributes to cell membrane flexibility and is a precursor of steroid hormones. The synthesis of cholesterol starts with acetyl groups and proceeds in only one direction. The process cannot be reversed.

Triglycerides—made from the bonding of glycerol and three fatty acids—are a form of long-term energy storage in animals. Animals can make most of the fatty acids they need. Triglycerides can be both made and broken down through parts of the glucose catabolism pathways. Glycerol can be phosphorylated to glycerol-3-phosphate, which continues through glycolysis. Fatty acids are catabolized in a process called beta-oxidation, which takes place in the matrix of the mitochondria and converts their fatty acid chains into two-carbon units of acetyl groups. The acetyl groups are picked up by CoA to form acetyl CoA that proceeds into the citric acid cycle.

Evolution Connection

Pathways of Photosynthesis and Cellular Metabolism

The processes of photosynthesis and cellular metabolism consist of several very complex pathways. It is generally thought that the first cells arose in an aqueous environment—a "soup" of nutrients—possibly on the surface of some porous clays, perhaps in warm marine environments. If these cells reproduced successfully and their numbers climbed steadily, it follows that the cells would begin to deplete the nutrients from the medium in which they lived as they shifted the nutrients into the components of their own bodies. This hypothetical situation would have resulted in natural selection favoring those organisms that could exist by using the nutrients that remained in their environment and by manipulating these nutrients into materials upon which they could survive. Selection would favor those organisms that could extract maximal value from the nutrients to which they had access.

An early form of photosynthesis developed that harnessed the sun's energy using water as a source of hydrogen atoms, but this pathway did not produce free oxygen (anoxygenic photosynthesis). (Another type of anoxygenic photosynthesis did not produce free oxygen because it did not use water as the source of hydrogen ions; instead, it used materials such as hydrogen sulfide and consequently produced sulfur). It is thought that glycolysis developed at this time and could take advantage of the simple sugars being produced but that these reactions were unable to fully extract the energy stored in the carbohydrates. The development of glycolysis probably predated the evolution of photosynthesis, as it was well suited to extract energy from materials spontaneously accumulating in the "primeval soup." A later form of photosynthesis used water as a source of electrons and hydrogen and generated free oxygen. Over time, the atmosphere became oxygenated, but not before the oxygen released oxidized metals in the ocean and created a "rust" layer in the sediment, permitting the dating of the rise of the first oxygenic photosynthesizers. Living things adapted to exploit this new atmosphere that allowed aerobic respiration as we know it to evolve. When the full process of oxygenic photosynthesis developed and the atmosphere became oxygenated, cells were finally able to use the oxygen expelled by photosynthesis to extract considerably more energy from the sugar molecules using the citric acid cycle and oxidative phosphorylation.

69.

REGULATION OF CELLULAR RESPIRATION

Learning Objectives

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

- Describe how feedback inhibition would affect the production of an intermediate or product in a pathway
- Identify the mechanism that controls the rate of the transport of electrons through the electron transport chain

Cellular respiration must be regulated in order to provide balanced amounts of energy in the form of ATP. The cell also must generate a number of intermediate compounds that are used in the anabolism and catabolism of macromolecules. Without controls, metabolic reactions would quickly come to a standstill as the forward and backward reactions reached a state of equilibrium. Resources would be used inappropriately. A cell does not need the maximum amount of ATP that it can make all the time: At times, the cell needs to shunt some of the intermediates to pathways for amino acid, protein, glycogen, lipid, and nucleic acid production. In short, the cell needs to control its metabolism.

Regulatory Mechanisms

A variety of mechanisms is used to control cellular respiration. Some type of control exists at each stage of glucose metabolism. Access of glucose to the cell can be regulated using the **GLUT (glucose transporter) proteins** that transport glucose (Figure 7.19).

Different forms of the GLUT protein control passage of glucose into the cells of specific tissues.

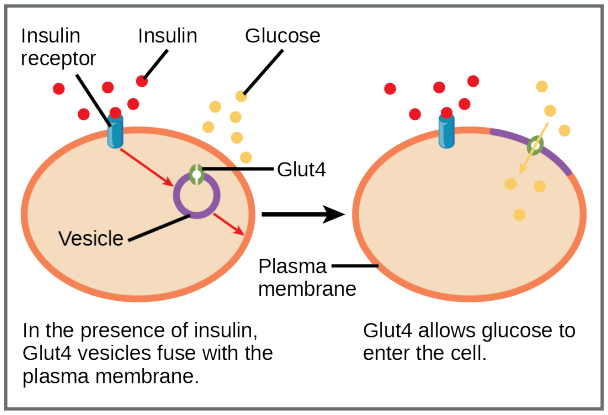

Figure 7.19 GLUT4 is a glucose transporter that is stored in vesicles. A cascade of events that occurs upon insulin binding to a receptor in the plasma membrane causes GLUT4-containing vesicles to fuse with the plasma membrane so that glucose may be transported into the cell.

Some reactions are controlled by having two different enzymes—one each for the two directions of a reversible reaction. Reactions that are catalyzed by only one enzyme can go to equilibrium, stalling the reaction. In contrast, if two different enzymes (each specific for a given direction) are necessary for a reversible reaction, the opportunity to control the rate of the reaction increases, and equilibrium is not reached.

A number of enzymes involved in each of the pathways—in particular, the enzyme catalyzing the first committed reaction of the pathway—are controlled by attachment of a molecule to an allosteric site on the protein. The molecules most commonly used in this capacity are the nucleotides ATP, ADP, AMP, NAD^+, and NADH. These regulators—allosteric effectors—may increase or decrease enzyme activity, depending on the prevailing conditions. The allosteric effector alters the steric structure of the enzyme, usually affecting the configuration of the active site. This alteration of the protein's (the enzyme's) structure either increases or decreases its affinity for its substrate, with the effect of increasing or decreasing the rate of the reaction. The attachment signals to the enzyme. This binding can increase or decrease the enzyme's activity, providing a feedback mechanism. This feedback type of control is effective as long as the chemical affecting it is attached to the enzyme. Once the overall concentration of the chemical decreases, it will diffuse away from the protein, and the control is relaxed.

Control of Catabolic Pathways

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

Glycolysis

The control of glycolysis begins with the first enzyme in the pathway, hexokinase (Figure 7.20). This enzyme catalyzes the phosphorylation of glucose, which helps to prepare the compound for cleavage in a later step. The presence of the negatively charged phosphate in the molecule also prevents the sugar from leaving the cell. When hexokinase is inhibited, glucose diffuses out of the cell and does not become a substrate for the respiration pathways in that tissue. The product of the hexokinase reaction is glucose-6-phosphate, which accumulates when a later enzyme, phosphofructokinase, is inhibited.

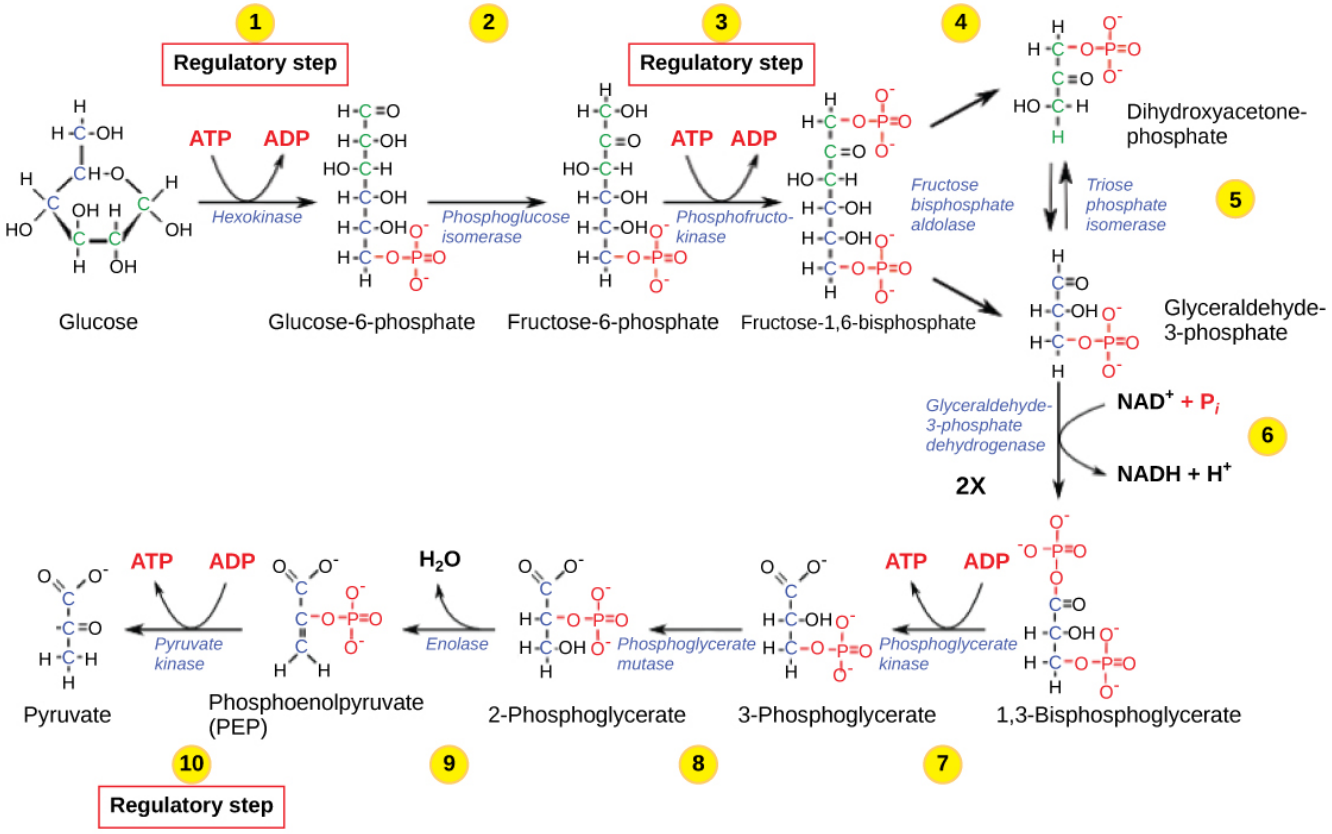

Figure 7.20 The glycolysis pathway is primarily regulated at the three key enzymatic steps (1, 3, and 10) as indicated. Note that the first two steps that are regulated occur early in the pathway and involve hydrolysis of ATP.

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

The last step in glycolysis is catalyzed by pyruvate kinase. The pyruvate produced can proceed to be

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

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

Citric Acid Cycle

The citric acid cycle is controlled through the enzymes that catalyze the reactions that make the first two molecules of NADH (Figure 7.9). These enzymes are isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. When adequate ATP and NADH levels are available, the rates of these reactions decrease. When more ATP is needed, as reflected in rising ADP levels, the rate increases. Alpha-ketoglutarate dehydrogenase will also be affected by the levels of succinyl CoA—a subsequent intermediate in the cycle—causing a decrease in activity. A decrease in the rate of operation of the pathway at this point is not necessarily negative, as the increased levels of the α-ketoglutarate not used by the citric acid cycle can be used by the cell for amino acid (glutamate) synthesis.

Electron Transport Chain

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

Link to Learning

Visit this site to see an animation of the electron transport chain and ATP synthesis.

For a summary of feedback controls in cellular respiration, see Table 7.1.

Summary of Feedback Controls in Cellular Respiration

Pathway	Enzyme affected	Elevated levels of effector	Effect on pathway activity
glycolysis	hexokinase	glucose-6-phosphate	decrease
	phosphofructokinase	low-energy charge (ATP, AMP), fructose-6-phosphate via fructose-2,6-bisphosphate	increase
		high-energy charge (ATP, AMP), citrate, acidic pH	decrease
	pyruvate kinase	fructose-1,6-bisphosphate	increase
		high-energy charge (ATP, AMP), alanine	decrease
pyruvate to acetyl CoA conversion	pyruvate dehydrogenase	ADP, pyruvate	increase
		acetyl CoA, ATP, NADH	decrease
citric acid cycle	isocitrate dehydrogenase	ADP	increase
		ATP, NADH	decrease
	α-ketoglutarate dehydrogenase	calcium ions, ADP	increase
		ATP, NADH, succinyl CoA	decrease
electron transport chain		ADP	increase
		ATP	decrease

Table 7.1

70.

KEY TERMS

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

aerobic respiration
: process in which organisms convert energy in the presence of oxygen

anaerobic
: process that does not use oxygen

anaerobic cellular respiration
: process in which organisms convert energy for their use in the absence of oxygen

ATP synthase
: (also F1F0 ATP synthase) membrane-embedded protein complex that adds a phosphate to ADP with energy from protons diffusing through it

chemiosmosis
: process in which there is a production of adenosine triphosphate (ATP) in cellular metabolism by the involvement of a proton gradient across a membrane

citric acid cycle
: (also Krebs cycle) series of enzyme-catalyzed chemical reactions of central importance in all living cells for extraction of energy from carbohydrates

dephosphorylation
: removal of a phosphate group from a molecule

fermentation
: process of regenerating NAD^+ with either an inorganic or organic compound serving as the final electron acceptor; occurs in the absence of oxygen

GLUT protein
: integral membrane protein that transports glucose

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

isomerase
: enzyme that converts a molecule into its isomer

Krebs cycle
: (also citric acid cycle) alternate name for the citric acid cycle, named after Hans Krebs, who first identified the steps in the pathway in the 1930s in pigeon flight muscles; see citric acid cycle

oxidative phosphorylation
: production of ATP using the process of chemiosmosis in the presence of oxygen

phosphorylation
: addition of a high-energy phosphate to a compound, usually a metabolic intermediate, a protein, or ADP

prosthetic group
: (also prosthetic cofactor) molecule bound to a protein that facilitates the function of the protein

pyruvate
: three-carbon sugar that can be decarboxylated and oxidized to make acetyl CoA, which enters the citric acid cycle under aerobic conditions; the end product of glycolysis

redox reaction
: chemical reaction that consists of the coupling of an oxidation reaction and a reduction reaction

substrate-level phosphorylation
: production of ATP from ADP using the excess energy from a chemical reaction and a phosphate group from a reactant

TCA cycle
: (also citric acid cycle) alternate name for the citric acid cycle, named after the group name for citric acid, tricarboxylic acid (TCA); see citric acid cycle

ubiquinone
: soluble electron transporter in the electron transport chain that connects the first or second complex to the third

71.

CHAPTER SUMMARY

7.1 Energy in Living Systems

ATP functions as the energy currency for cells. It allows the cell to store energy briefly and transport it within the cell to support endergonic chemical reactions. The structure of ATP is that of an RNA nucleotide with three phosphates attached. As ATP is used for energy, a phosphate group or two are detached, and either ADP or AMP is produced. Energy derived from glucose catabolism is used to convert ADP into ATP. When ATP is used in a reaction, the third phosphate is temporarily attached to a substrate in a process called phosphorylation. The two processes of ATP regeneration that are used in conjunction with glucose catabolism are substrate-level phosphorylation and oxidative phosphorylation through the process of chemiosmosis.

7.2 Glycolysis

Glycolysis is the first pathway within the cytoplasm used in the breakdown of glucose to extract energy. It was probably one of the earliest metabolic pathways to evolve and is used by nearly all of the organisms on Earth. Glycolysis consists of two parts: The first part prepares the six-carbon ring of glucose for cleavage into two three-carbon sugars. ATP is invested in the process during this half 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 by substrate phosphorylation during the second half. This produces a net gain of two ATP and two NADH molecules for the cell.

7.3 Oxidation of Pyruvate and the Citric Acid Cycle

In the presence of oxygen, pyruvate is transformed into an acetyl group attached to a carrier molecule of coenzyme A. The resulting acetyl CoA can enter several pathways, but most often, the acetyl group is delivered to the citric acid cycle for further catabolism. During the conversion of pyruvate into the acetyl group, a molecule of carbon dioxide and two high-energy electrons are removed. The carbon dioxide accounts for two (conversion of two pyruvate molecules) of the six carbons of the original glucose molecule. The electrons are picked up by NAD^+, and the NADH carries the electrons to a later pathway for ATP production. At this point, the glucose molecule that originally entered cellular respiration has been completely oxidized. Chemical

potential energy stored within the glucose molecule has been transferred to electron carriers or has been used to synthesize a few ATPs.

The citric acid cycle is a series of redox and decarboxylation reactions that removes high-energy electrons and carbon dioxide. The electrons, temporarily stored in molecules of NADH and $FADH_2$, are used to generate ATP in a subsequent pathway. One molecule of either GTP or ATP is produced by substrate-level phosphorylation on each turn of the cycle. There is no comparison of the cyclic pathway with a linear one.

7.4 Oxidative Phosphorylation

The electron transport chain is the portion of aerobic respiration that uses free oxygen as the final electron acceptor of the electrons removed from the intermediate compounds in glucose catabolism. The electron transport chain is composed of four large, multiprotein complexes embedded in the inner mitochondrial membrane and two small diffusible electron carriers shuttling electrons between them. The electrons are passed through a series of redox reactions, with a small amount of free energy used at three points to transport hydrogen ions across a membrane. This process contributes to the gradient used in chemiosmosis. The electrons passing through the electron transport chain gradually lose energy. High-energy electrons donated to the chain by either NADH or $FADH_2$ complete the chain, as low-energy electrons reduce oxygen molecules and form water. The level of free energy of the electrons drops from about 60 kcal/mol in NADH or 45 kcal/mol in $FADH_2$ to about 0 kcal/mol in water. The end products of the electron transport chain are water and ATP. A number of intermediate compounds of the citric acid cycle can be diverted into the anabolism of other biochemical molecules, such as nonessential amino acids, sugars, and lipids. These same molecules can serve as energy sources for the glucose pathways.

7.5 Metabolism without Oxygen

If NADH cannot be oxidized 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 of NADH to produce ATP using an electron transport chain is not utilized.

7.6 Connections of Carbohydrate, Protein, and Lipid Metabolic Pathways

The breakdown and synthesis of carbohydrates, proteins, and lipids connect with the pathways of glucose catabolism. The simple sugars are galactose, fructose, glycogen, and pentose. These are catabolized during glycolysis. The amino acids from proteins connect with glucose catabolism through pyruvate, acetyl CoA, and

components of the citric acid cycle. Cholesterol synthesis starts with acetyl groups, and the components of triglycerides come from glycerol-3-phosphate from glycolysis and acetyl groups produced in the mitochondria from pyruvate.

7.7 Regulation of Cellular Respiration

Cellular respiration is controlled by a variety of means. The entry of glucose into a cell is controlled by the transport proteins that aid glucose passage through the cell membrane. Most of the control of the respiration processes is accomplished through the control of specific enzymes in the pathways. This is a type of negative feedback mechanism, turning the enzymes off. The enzymes respond most often to the levels of the available nucleosides ATP, ADP, AMP, NAD^+, and FAD. Other intermediates of the pathway also affect certain enzymes in the systems.

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VISUAL CONNECTION QUESTIONS

1. Figure 7.13 Dinitrophenol (DNP) is an "uncoupler" that makes the inner mitochondrial membrane "leaky" to protons. It was used until 1938 as a weight-loss drug. What effect would you expect DNP to have on the change in pH across the inner mitochondrial membrane? Why do you think this might be an effective weight-loss drug?

2. Figure 7.14 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 effect would cyanide have on ATP synthesis?

3. Figure 7.16 Tremetol, a metabolic poison found in the white snake root plant, prevents the metabolism of lactate. When cows eat this plant, tremetol is concentrated in the milk they produce. Humans who consume the milk can become seriously ill. Symptoms of this disease, which include vomiting, abdominal pain, and tremors, become worse after exercise. Why do you think this is the case?

73.

REVIEW QUESTIONS

4. The energy currency used by cells is _____.

a. ATP
b. ADP
c. AMP
d. adenosine

5. A reducing chemical reaction _____.

a. reduces the compound to a simpler form
b. adds an electron to the substrate
c. removes a hydrogen atom from the substrate
d. is a catabolic reaction

6. During the second half of glycolysis, what occurs?

a. ATP is used up.
b. Fructose is split in two.
c. ATP is made.
d. Glucose becomes fructose.

7. What is removed from pyruvate during its conversion into an acetyl group?

a. oxygen
b. ATP
c. B vitamin
d. carbon dioxide

8. 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 to NADP.

9. GTP or ATP is produced during the conversion of _____.

a. isocitrate into α-ketoglutarate
b. succinyl CoA into succinate
c. fumarate into malate
d. malate into oxaloacetate

10. How many NADH molecules are produced on each turn of the citric acid cycle?

a. one
b. two
c. three
d. four

11. What compound receives electrons from NADH?

a. FMN
b. ubiquinone
c. cytochrome c1
d. oxygen

12. 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

13. 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

14. The effect of high levels of ADP is to _____ in cellular respiration.

a. increase the activity of specific enzymes
b. decrease the activity of specific enzymes
c. have no effect on the activity of specific enzymes
d. slow down the pathway

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CRITICAL THINKING QUESTIONS

15. Compare how you feel when you are in a crowded room with when you are on a walk in the woods. What might account for the differences? Relate the differences you identify to the process of cellular respiration.

16. Why is it beneficial for cells to use ATP rather than energy directly from the bonds of carbohydrates? What are the greatest drawbacks to harnessing energy directly from the bonds of several different compounds?

17. Nearly all organisms on Earth carry out some form of glycolysis. How does this fact support or not support the assertion that glycolysis is one of the oldest metabolic pathways?

18. Compare the numbers of ATP molecules produced per glucose molecule by glycolysis and by the mitochondria. Propose an explanation for how early eukaryotic cells benefitted from the acquisition of mitochondria through endosymbiosis.

19. Because they lose their mitochondria during development, red blood cells cannot perform aerobic respiration; however, they do perform glycolysis in the cytoplasm. Why do all cells need an energy source, and what would happen if glycolysis were blocked in a red blood cell?

20. What is the primary difference between a circular pathway and a linear pathway?

21. How do the roles of ubiquinone and cytochrome c differ from the roles of the other components of the electron transport chain?

22. What accounts for the different number of ATP molecules that are formed through cellular respiration?

23. What is the primary difference between fermentation and anaerobic respiration?

24. Would you describe metabolic pathways as inherently wasteful or inherently economical? Why?

PART VIII
PHOTOSYNTHESIS

75.

INTRODUCTION

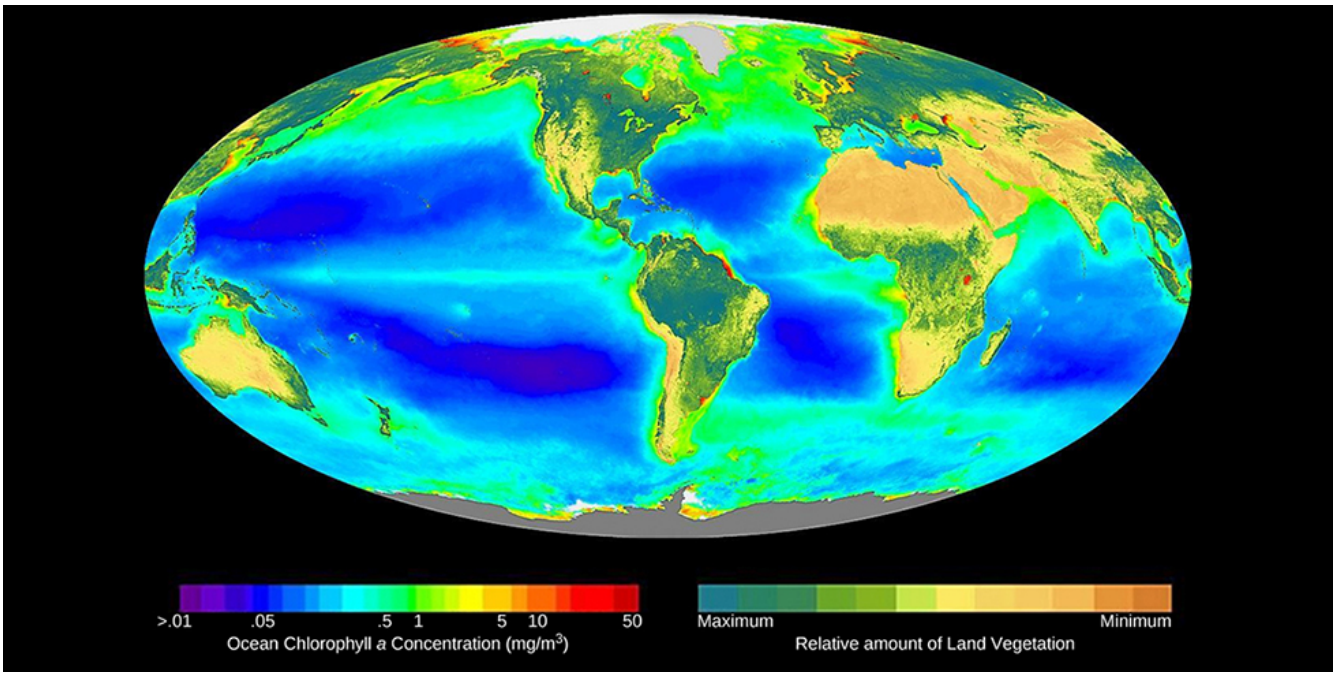

Figure 8.1 This world map shows Earth's distribution of photosynthetic activity determined by chlorophyll a concentrations. On land, chlorophyll is evident from terrestrial plants, and within oceanic zones, from chlorophyll from phytoplankton. (credit: modification of work by SeaWiFS Project, NASA/Goddard Space Flight Center and ORBIMAGE)

The metabolic processes in all organisms—from bacteria to humans—require energy. To get this energy, many organisms access stored energy by eating—that is, by ingesting other organisms. But where does the stored energy in food originate? All of this energy can be traced back to photosynthesis.

76.

OVERVIEW OF PHOTOSYNTHESIS

Learning Objectives

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

- Explain the significance of photosynthesis to other living organisms
- Describe the main structures involved in photosynthesis
- Identify the substrates and products of photosynthesis

Photosynthesis, or the process in which plants and some other living organisms transform light energy into chemical energy, is essential to all life on earth; both plants and animals depend on it. It is the only biological process that can capture energy that originates from sunlight and converts it into chemical compounds (carbohydrates) that every organism uses to power its metabolism. It is also a source of oxygen necessary for many living organisms. In brief, the energy of sunlight is "captured" to energize electrons, whose energy is then stored in the covalent bonds of sugar molecules. How long lasting and stable are those covalent bonds? The energy extracted today by the burning of coal and petroleum products represents sunlight energy captured and stored by photosynthesis 350 to 200 million years ago during the Carboniferous Period.

Plants, algae, and a group of bacteria called cyanobacteria are the only organisms capable of performing photosynthesis (Figure 8.2). Because they use light to manufacture their own food, they are called **photoautotrophs** (literally, "self-feeders using light"). Other organisms, such as animals, fungi, and most other bacteria, are termed **heterotrophs** ("other feeders"), because they must rely on the sugars produced by photosynthetic organisms for their energy needs. A third very interesting group of bacteria synthesize sugars, not by using sunlight's energy, but by extracting energy from inorganic chemical compounds. For this reason, they are referred to as **chemoautotrophs**.

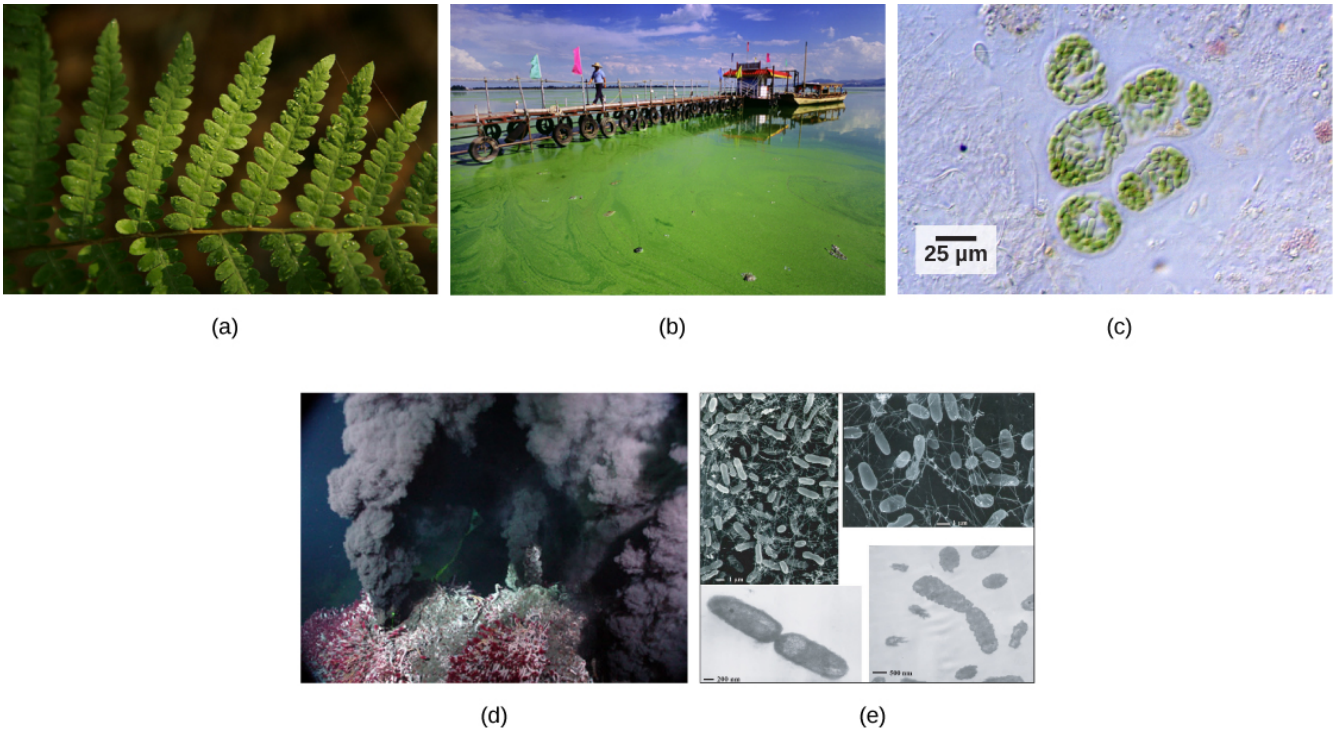

Figure 8.2 Photoautotrophs including (a) plants, (b) algae, and (c) cyanobacteria synthesize their organic compounds via photosynthesis using sunlight as an energy source. Cyanobacteria and planktonic algae can grow over enormous areas in water, at times completely covering the surface. In a (d) deep sea vent, chemoautotrophs, such as these (e) thermophilic bacteria, capture energy from inorganic compounds to produce organic compounds. The ecosystem surrounding the vents has a diverse array of animals, such as tubeworms, crustaceans, and octopuses that derive energy from the bacteria. (credit a: modification of work by Steve Hillebrand, U.S. Fish and Wildlife Service; credit b: modification of work by "eutrophication&hypoxia"/Flickr; credit c: modification of work by NASA; credit d: University of Washington, NOAA; credit e: modification of work by Mark Amend, West Coast and Polar Regions Undersea Research Center, UAF, NOAA)

The importance of photosynthesis is not just that it can capture sunlight's energy. After all, a lizard sunning itself on a cold day can use the sun's energy to warm up in a process called *behavioral thermoregulation*. In contrast, photosynthesis is vital because it evolved as a way to *store the energy from solar radiation (the "photo-" part) to energy in the carbon-carbon bonds of carbohydrate molecules (the "-synthesis" part).* Those carbohydrates are the energy source that heterotrophs use to power the synthesis of ATP via respiration. Therefore, photosynthesis powers 99 percent of Earth's ecosystems. When a top predator, such as a wolf, preys on a deer (Figure 8.3), the wolf is at the end of an energy path that went from nuclear reactions on the surface of the sun, to visible light, to photosynthesis, to vegetation, to deer, and finally to the wolf.

Figure 8.3 The energy stored in carbohydrate molecules from photosynthesis passes through the food chain. The predator that eats these deer receives a portion of the energy that originated in the photosynthetic vegetation that the deer consumed. (credit: modification of work by Steve VanRiper, U.S. Fish and Wildlife Service, licensed CC BY-ND Tambako 2012)

Main Structures and Summary of Photosynthesis

Photosynthesis is a multi-step process that requires specific wavelengths of visible sunlight, carbon dioxide (which is low in energy), and water as substrates (Figure 8.4). After the process is complete, it releases oxygen and produces glyceraldehyde-3-phosphate (G3P), as well as simple carbohydrate molecules (high in energy) that can then be converted into glucose, sucrose, or any of dozens of other sugar molecules. These sugar molecules contain energy and the energized carbon that all living things need to survive.

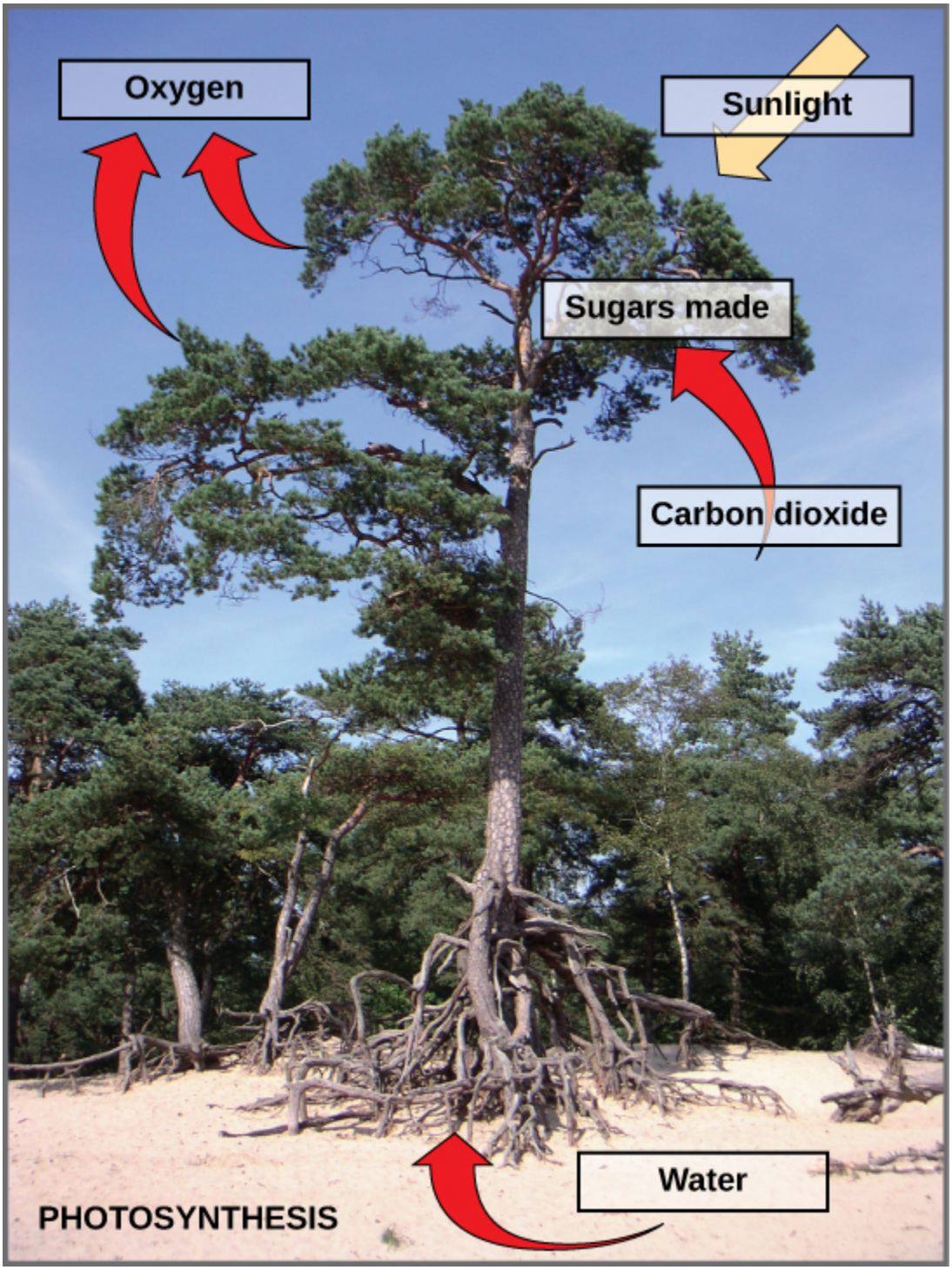

Figure 8.4 Photosynthesis uses solar energy, carbon dioxide, and water to produce energy-storing carbohydrates. Oxygen is generated as a waste product of photosynthesis.

The following is the chemical equation for photosynthesis (Figure 8.5):

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

Figure 8.5 The basic equation for photosynthesis is deceptively simple. In reality, the process takes place in many steps involving intermediate reactants and products. Glucose, the primary energy source in cells, is made from two three-carbon G3Ps.

Although the equation looks simple, the many steps that take place during photosynthesis are actually quite complex. Before learning the details of how photoautotrophs turn sunlight into food, it is important to become familiar with the structures involved.

Basic Photosynthetic Structures

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

In all autotrophic eukaryotes, photosynthesis takes place inside an organelle called a **chloroplast**. For plants, chloroplast-containing cells exist mostly in the mesophyll. Chloroplasts have a double membrane envelope (composed of an outer membrane and an inner membrane), and are ancestrally derived from ancient free-living cyanobacteria. Within the chloroplast are stacked, disc-shaped structures called **thylakoids**. Embedded in the thylakoid membrane is chlorophyll, a **pigment** (molecule that absorbs light) responsible for the initial interaction between light and plant material, and numerous proteins that make up the electron transport chain. The thylakoid membrane encloses an internal space called the **thylakoid lumen**. As shown in Figure 8.6, a stack of thylakoids is called a **granum**, and the liquid-filled space surrounding the granum is called **stroma** or "bed" (not to be confused with stoma or "mouth," an opening on the leaf epidermis).

Visual Connection

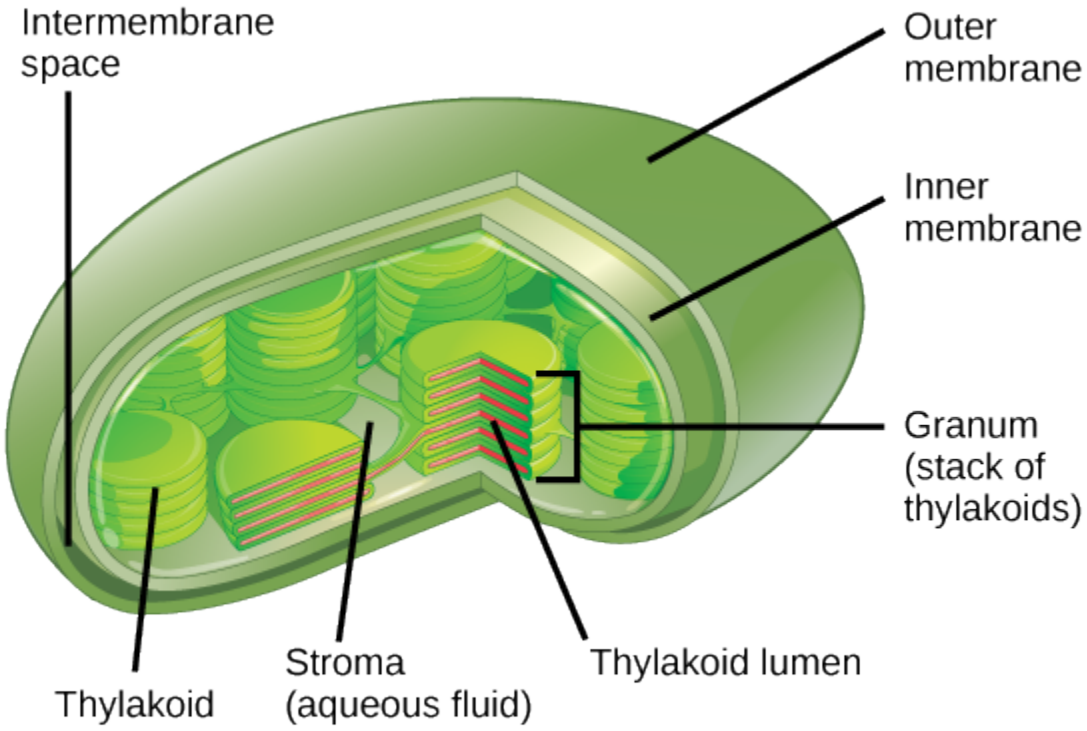

Figure 8.6 Photosynthesis takes place in chloroplasts, which have an outer membrane and an inner membrane. Stacks of thylakoids called grana form a third membrane layer.

On a hot, dry day, the guard cells of plants close their stomata to conserve water. What impact will this have on photosynthesis?

The Two Parts of Photosynthesis

Photosynthesis takes place in two sequential stages: the light-dependent reactions and the light-independent reactions. In the **light-dependent reactions**, energy from sunlight is absorbed by chlorophyll and that energy is converted into stored chemical energy. In the **light-independent reactions**, the chemical energy harvested during the light-dependent reactions drives the assembly of sugar molecules from carbon dioxide. Therefore, although the light-independent reactions do not use light as a reactant, they require the products of the light-dependent reactions to function. In addition, however, several enzymes of the light-independent reactions are activated by light. The light-dependent reactions utilize certain molecules to temporarily store

the energy: These are referred to as *energy carriers*. The energy carriers that move energy from light-dependent reactions to light-independent reactions can be thought of as "full" because they are rich in energy. After the energy is released, the "empty" energy carriers return to the light-dependent reaction to obtain more energy. Figure 8.7 illustrates the components inside the chloroplast where the light-dependent and light-independent reactions take place.

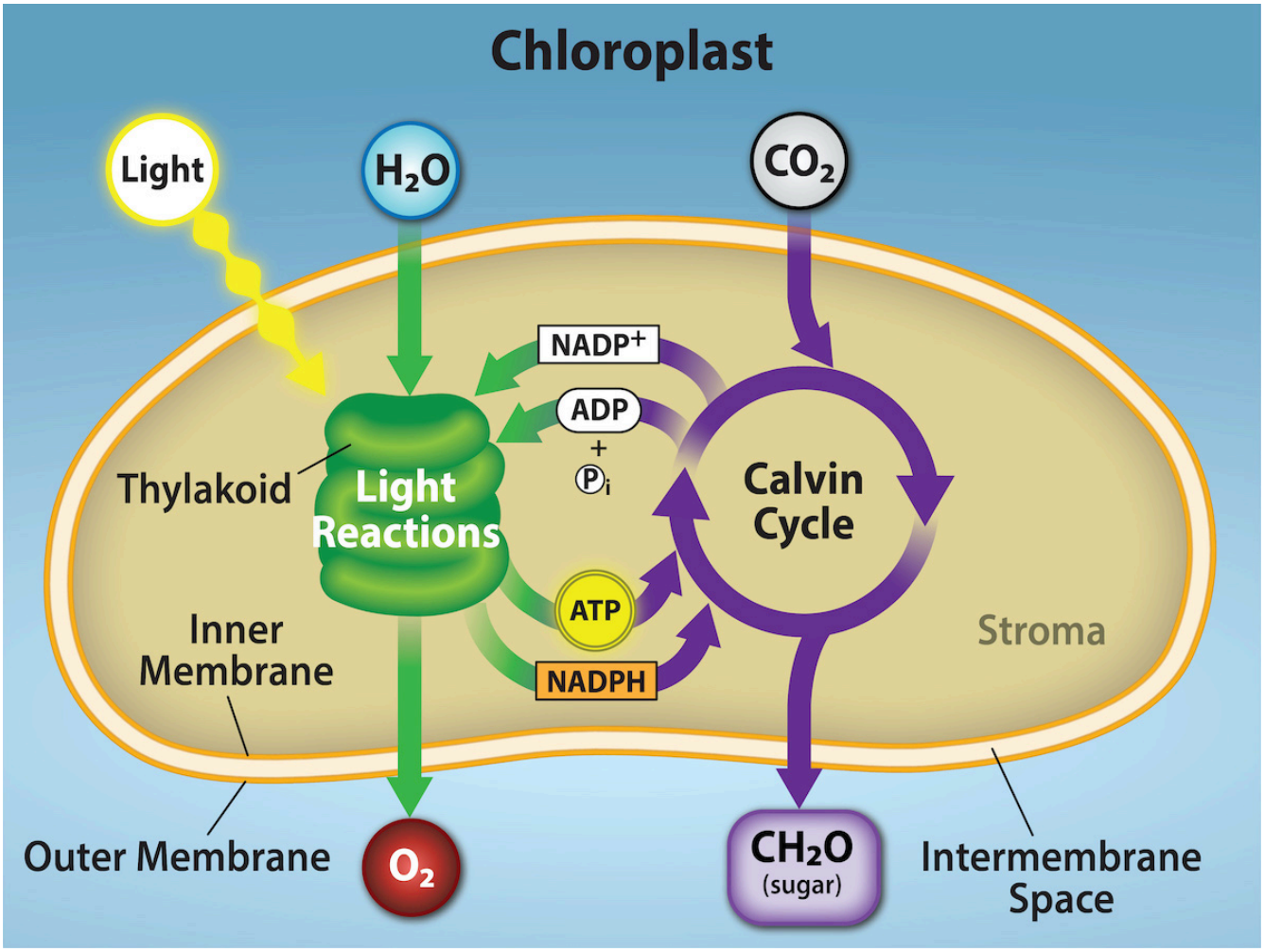

Figure 8.7 Photosynthesis takes place in two stages: light-dependent reactions and the Calvin cycle. Light-dependent reactions, which take place in the thylakoid membrane, use light energy to make ATP and NADPH. The Calvin cycle, which takes place in the stroma, uses energy derived from these compounds to make G3P from CO2. Credit: Rao, A., Ryan, K., Fletcher, S., Hawkins, A. and Tag, A. Texas A&M University.

Link to Learning

Click the link to learn more about photosynthesis.

Everyday Connection

Photosynthesis at the Grocery Store

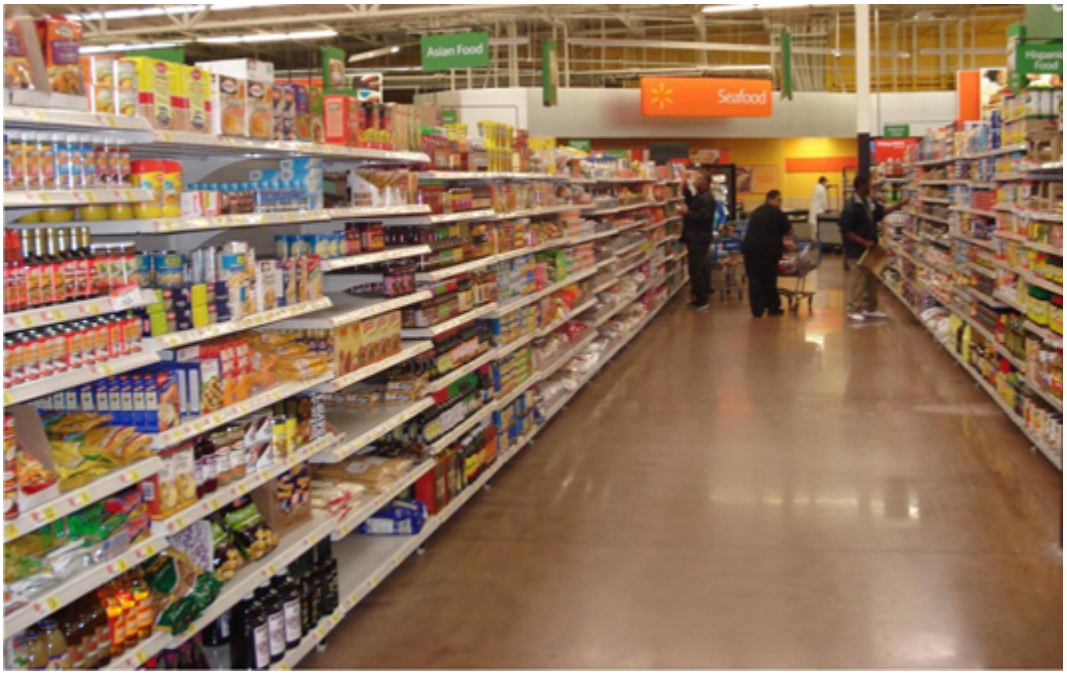

Figure 8.8 Foods that humans consume originate from photosynthesis. (credit: Associação Brasileira de Supermercados)

Major grocery stores in the United States are organized into departments, such as dairy, meats, produce, bread, cereals, and so forth. Each aisle (Figure 8.8) contains hundreds, if not thousands, of different products for customers to buy and consume. Although there is a large variety, each item ultimately can be linked back to photosynthesis. Meats and dairy link, because the animals were fed plant-based

foods. The breads, cereals, and pastas come largely from starchy grains, which are the seeds of photosynthesis-dependent plants. What about desserts and drinks? All of these products contain sugar—sucrose is a plant product, a disaccharide, a carbohydrate molecule, which is built directly from photosynthesis. Moreover, many items are less obviously derived from plants: For instance, paper goods are generally plant products, and many plastics (abundant as products and packaging) are derived from “algae” (unicellular plant-like organisms, and cyanobacteria). Virtually every spice and flavoring in the spice aisle was produced by a plant as a leaf, root, bark, flower, fruit, or stem. Ultimately, photosynthesis connects to every meal and every food a person consumes.

77.

THE LIGHT-DEPENDENT REACTIONS OF PHOTOSYNTHESIS

Learning Objectives

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

- Explain how plants absorb energy from sunlight
- Describe short and long wavelengths of light
- Describe how and where photosynthesis takes place within a plant

How can light energy be used to make food? When a person turns on a lamp, electrical energy becomes light energy. Like all other forms of kinetic energy, light can travel, change form, and be harnessed to do work. In the case of photosynthesis, light energy is converted into chemical energy, which photoautotrophs use to build basic carbohydrate molecules (**Figure 8.9**). However, autotrophs only use a few specific wavelengths of sunlight.

Figure 8.9 Photoautotrophs can capture visible light energy in specific wavelengths from the sun, converting it into the chemical energy used to build food molecules. (credit: Gerry Atwell)

What Is Light Energy?

The sun emits an enormous amount of electromagnetic radiation (solar energy in a spectrum from very short gamma rays to very long radio waves). Humans can see only a tiny fraction of this energy, which we refer to as "visible light." The manner in which solar energy travels is described as waves. Scientists can determine the amount of energy of a wave by measuring its **wavelength** (shorter wavelengths are more powerful than longer wavelengths)—the distance between consecutive crest points of a wave. Therefore, a single wave is measured from two consecutive points, such as from crest to crest or from trough to trough (Figure 8.10).

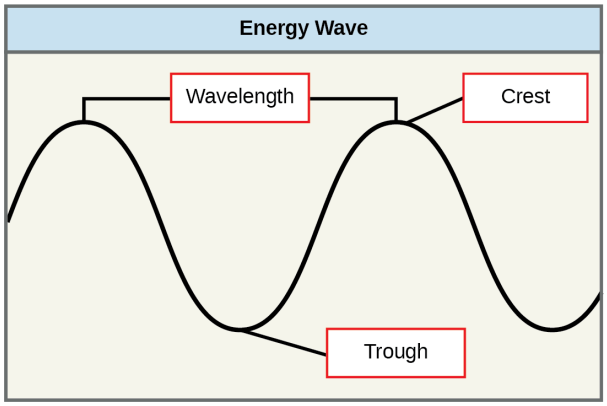

Figure 8.10 The wavelength of a single wave is the distance between two consecutive points of similar position (two crests or two troughs) along the wave.

Visible light constitutes only one of many types of electromagnetic radiation emitted from the sun and other stars. Scientists differentiate the various types of radiant energy from the sun within the electromagnetic spectrum. The **electromagnetic spectrum** is the range of all possible frequencies of radiation (**Figure 8.11**). The difference between wavelengths relates to the amount of energy carried by them.

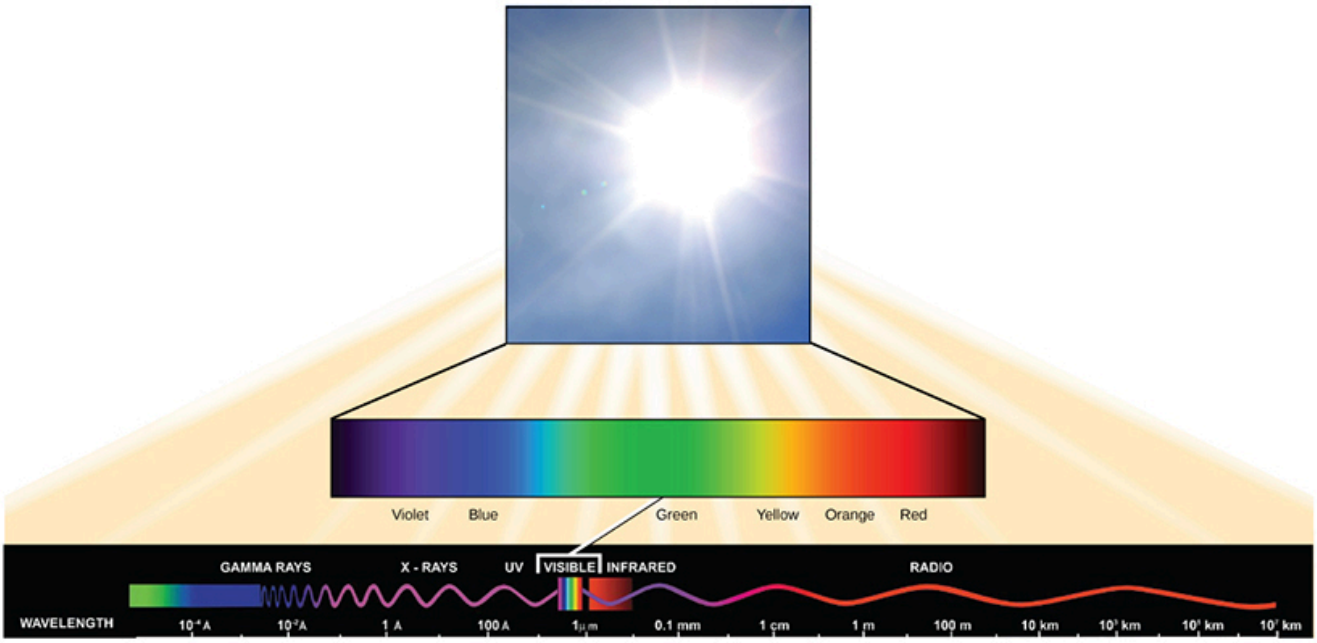

Figure 8.11 The sun emits energy in the form of electromagnetic radiation. This radiation exists at different wavelengths, each of which has its own characteristic energy. All electromagnetic radiation, including visible light, is characterized by its wavelength.

Each type of electromagnetic radiation travels at a particular wavelength. The longer the wavelength, the less

energy it carries. Short, tight waves carry the most energy. This may seem illogical, but think of it in terms of a piece of moving heavy rope. It takes little effort by a person to move a rope in long, wide waves. To make a rope move in short, tight waves, a person would need to apply significantly more energy.

The electromagnetic spectrum (Figure 8.11) shows several types of electromagnetic radiation originating from the sun, including X-rays and ultraviolet (UV) rays. The higher-energy waves can penetrate tissues and damage cells and DNA, which explains why both X-rays and UV rays can be harmful to living organisms.

Absorption of Light

Light energy initiates the process of photosynthesis when pigments absorb specific wavelengths of visible light. Organic pigments, whether in the human retina or the chloroplast thylakoid, have a narrow range of energy levels that they can absorb. Energy levels lower than those represented by red light are insufficient to raise an orbital electron to an excited (quantum) state. Energy levels higher than those in blue light will physically tear the molecules apart, in a process called bleaching. Our retinal pigments can only "see" (absorb) wavelengths between 700 nm and 400 nm of light, a spectrum that is therefore called visible light. For the same reasons, plants, pigment molecules absorb only light in the wavelength range of 700 nm to 400 nm; plant physiologists refer to this range for plants as photosynthetically active radiation.

The visible light seen by humans as white light actually exists in a rainbow of colors. Certain objects, such as a prism or a drop of water, disperse white light to reveal the colors to the human eye. The visible light portion of the electromagnetic spectrum shows the rainbow of colors, with violet and blue having shorter wavelengths, and therefore higher energy. At the other end of the spectrum toward red, the wavelengths are longer and have lower energy (Figure 8.13).

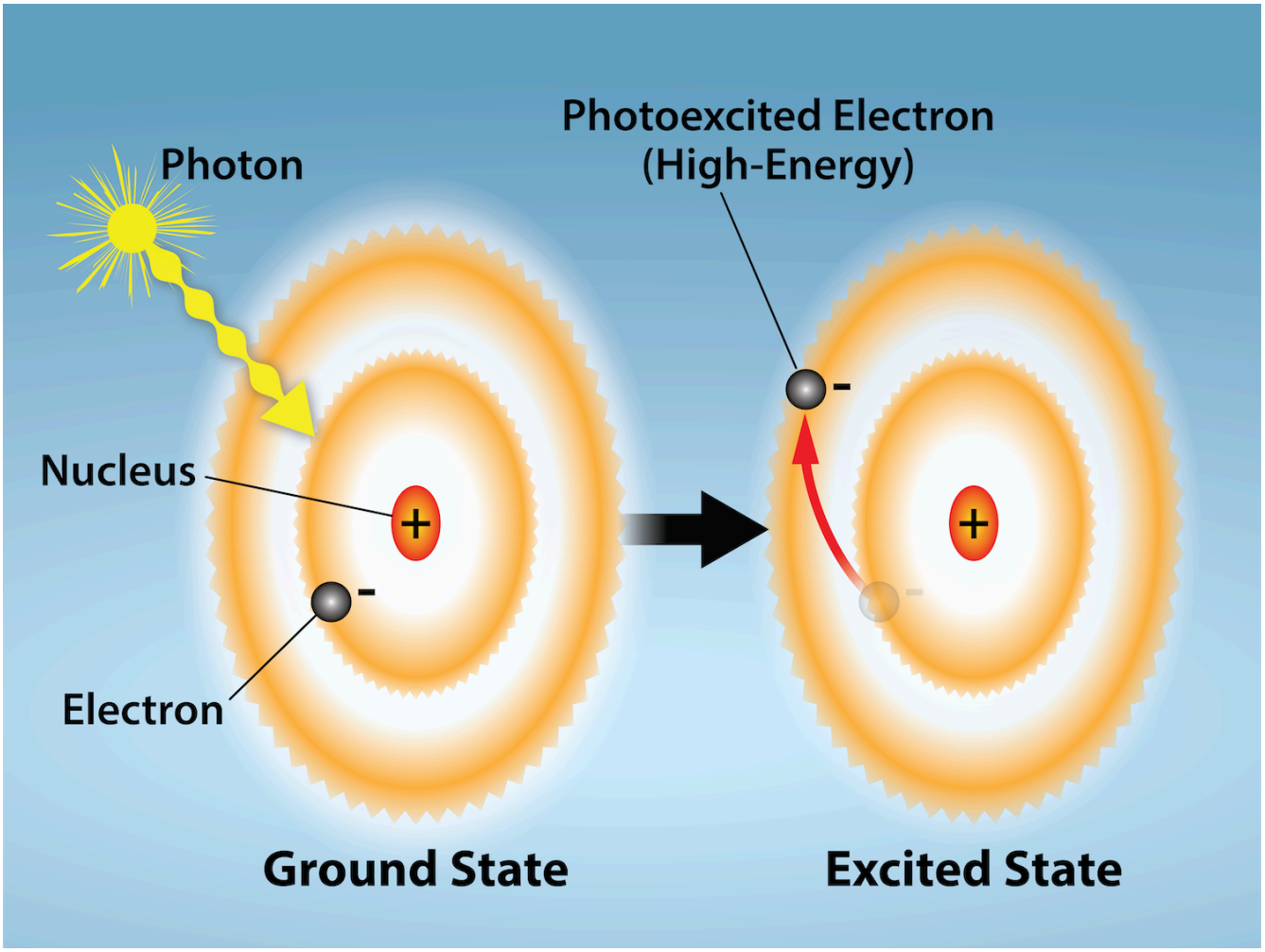

Figure 8.12 Light energy can excite electrons. When a photon of light energy interacts with an electron, the electron may absorb the energy and jump from its lowest energy ground state to an excited state. Credit: Rao, A. and Ryan, K. Department of Biology, Texas A&M University.

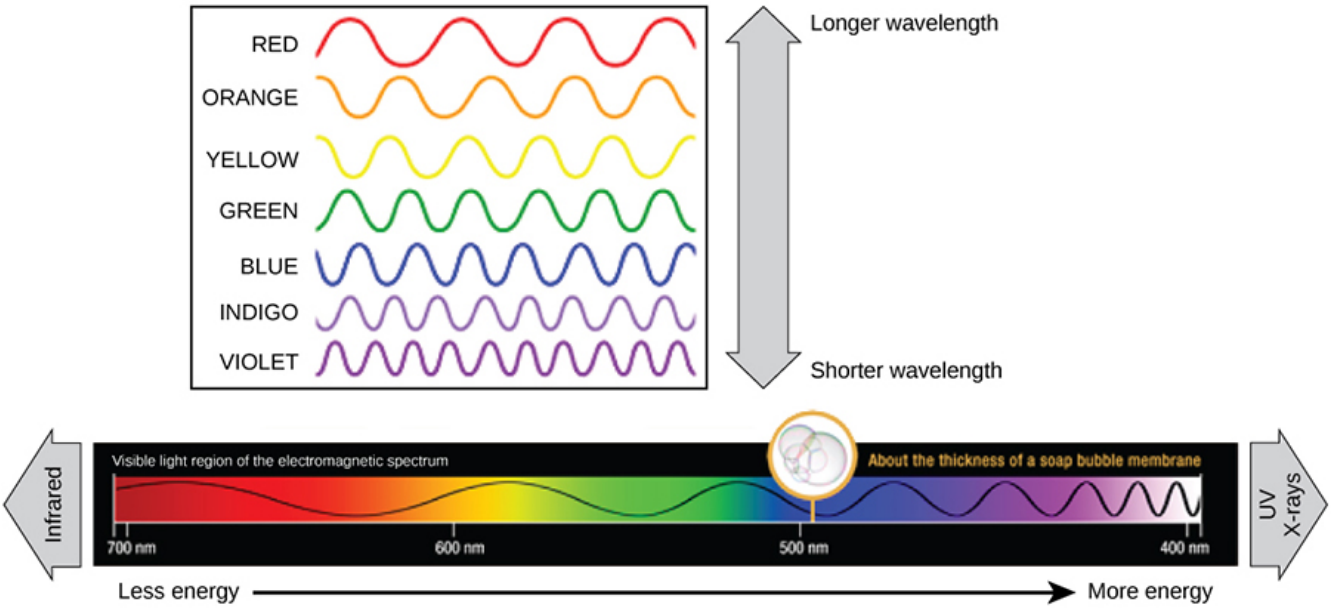

Figure 8.13 The colors of visible light do not carry the same amount of energy. Violet has the shortest wavelength and therefore carries the most energy, whereas red has the longest wavelength and carries the least amount of energy. (credit: modification of work by NASA)

Understanding Pigments

Different kinds of pigments exist, and each absorbs only specific wavelengths (colors) of visible light. Pigments reflect or transmit the wavelengths they cannot absorb, making them appear a mixture of the reflected or transmitted light colors.

Chlorophylls and carotenoids are the two major classes of photosynthetic pigments found in plants and algae; each class has multiple types of pigment molecules. There are five major chlorophylls: *a*, *b*, *c*, and *d* and a related molecule found in prokaryotes called *bacteriochlorophyll*. **Chlorophyll *a*** and **chlorophyll *b*** are found in higher plant chloroplasts and will be the focus of the following discussion.

With dozens of different forms, carotenoids are a much larger group of pigments. The carotenoids found in fruit—such as the red of tomato (lycopene), the yellow of corn seeds (zeaxanthin), or the orange of an orange peel (β-carotene)—are used as advertisements to attract seed dispersers. In photosynthesis, **carotenoids** function as photosynthetic pigments that are very efficient molecules for the disposal of excess energy. When a leaf is exposed to full sun, the light-dependent reactions are required to process an enormous amount of energy; if that energy is not handled properly, it can do significant damage. Therefore, many carotenoids reside in the thylakoid membrane, absorb excess energy, and safely dissipate that energy as heat.

Each type of pigment can be identified by the specific pattern of wavelengths it absorbs from visible light: This is termed the **absorption spectrum**. The graph in Figure 8.14 shows the absorption spectra for chlorophyll *a*, chlorophyll *b*, and a type of carotenoid pigment called β-carotene (which absorbs blue and green

light). Notice how each pigment has a distinct set of peaks and troughs, revealing a highly specific pattern of absorption. Chlorophyll *a* absorbs wavelengths from either end of the visible spectrum (blue and red), but not green. Because green is reflected or transmitted, chlorophyll appears green. Carotenoids absorb in the short-wavelength blue region, and reflect the longer yellow, red, and orange wavelengths.

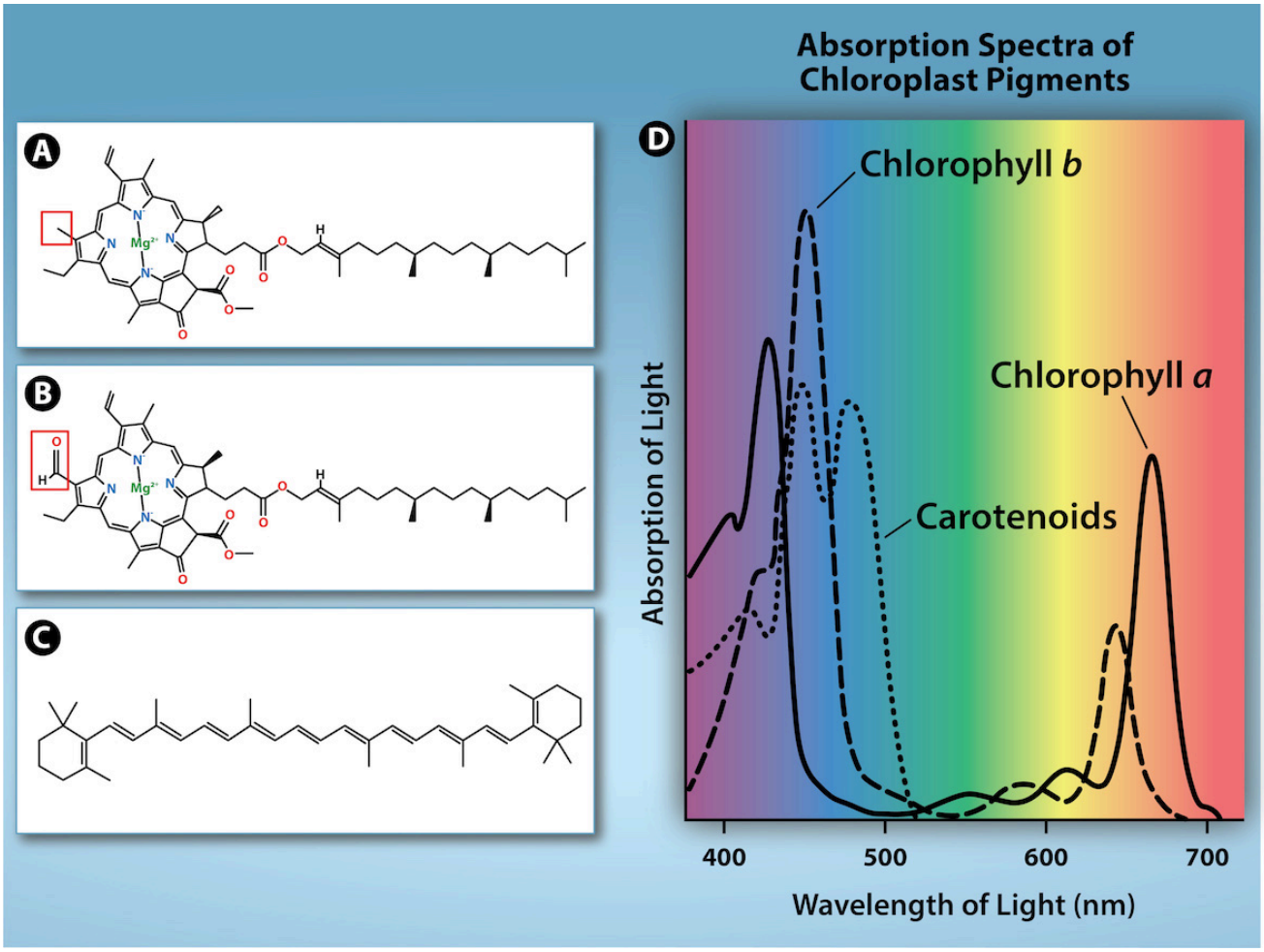

Figure 8.14 (a) Chlorophyll a, (b) chlorophyll b, and (c) β-carotene are hydrophobic organic pigments found in the thylakoid membrane. Chlorophyll a and b, which are identical except for the part indicated in the red box, are responsible for the green color of leaves. β-carotene is responsible for the orange color in carrots. Each pigment has (d) a unique absorbance spectrum. Credit: Rao, A., Ryan, K., Tag, A., Fletcher, S. and Hawkins, A. Department of Biology, Texas A&M University.

Many photosynthetic organisms have a mixture of pigments, and by using these pigments, the organism can absorb energy from a wider range of wavelengths. Not all photosynthetic organisms have full access to sunlight. Some organisms grow underwater where light intensity and quality decrease and change with depth. Other organisms grow in competition for light. Plants on the rainforest floor must be able to absorb any bit of light that comes through, because the taller trees absorb most of the sunlight and scatter the remaining solar radiation (Figure 8.15).

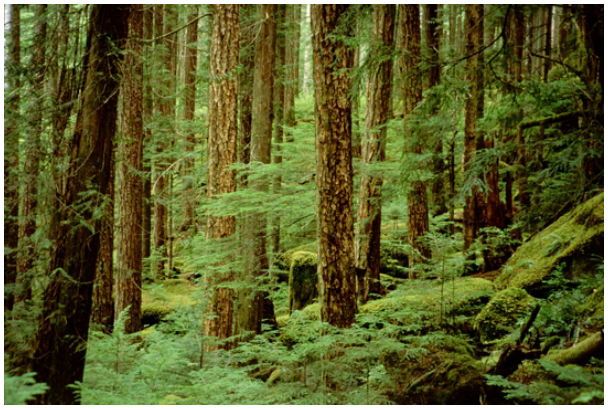

Figure 8.15 Plants that commonly grow in the shade have adapted to low levels of light by changing the relative concentrations of their chlorophyll pigments. (credit: Jason Hollinger)

When studying a photosynthetic organism, scientists can determine the types of pigments present by generating absorption spectra. An instrument called a **spectrophotometer** can differentiate which wavelengths of light a substance can absorb. Spectrophotometers measure transmitted light and compute from it the absorption. By extracting pigments from leaves and placing these samples into a spectrophotometer, scientists can identify which wavelengths of light an organism can absorb. Additional methods for the identification of plant pigments include various types of chromatography that separate the pigments by their relative affinities to solid and mobile phases.

How Light-Dependent Reactions Work

The overall function of light-dependent reactions is to convert solar energy into chemical energy in the form of NADPH and ATP. This chemical energy supports the light-independent reactions and fuels the assembly of sugar molecules. The light-dependent reactions are depicted in Figure 8.16. Protein complexes and pigment molecules work together to produce NADPH and ATP. The numbering of the photosystems is derived from the order in which they were discovered, not in the order of the transfer of electrons.

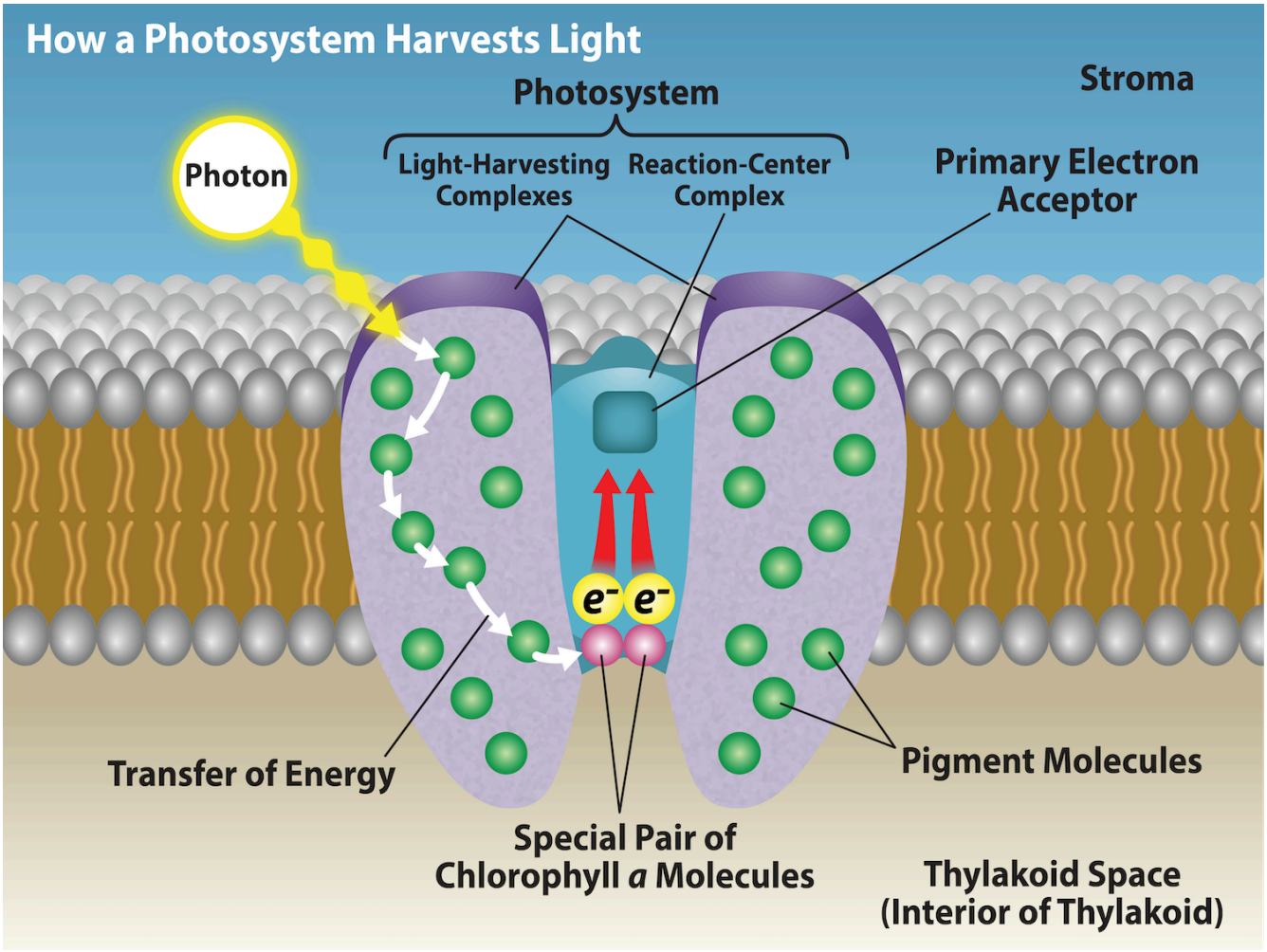

Figure 8.16 A photosystem consists of 1) a light-harvesting complex and 2) a reaction center. Pigments in the light-harvesting complex pass light energy to two special chlorophyll a molecules in the reaction center. The light excites an electron from the chlorophyll a pair, which passes to the primary electron acceptor. The excited electron must then be replaced. Credit: Rao, A., Ryan, K., Fletcher, S. and Hawkins, A. Department of Biology, Texas A&M University.

The actual step that converts light energy into chemical energy takes place in a multiprotein complex called a **photosystem**, two types of which are found embedded in the thylakoid membrane: **photosystem II** (PSII) and **photosystem I** (PSI) (Figure 8.17). The two complexes differ on the basis of what they oxidize (that is, the source of the low-energy electron supply) and what they reduce (the place to which they deliver their energized electrons).

Both photosystems have the same basic structure: a number of **antenna proteins** to which the chlorophyll molecules are bound surround the **reaction center** where the photochemistry takes place. Each photosystem is serviced by the **light-harvesting complex**, which passes energy from sunlight to the reaction center; it consists of multiple antenna proteins that contain a mixture of 300 to 400 chlorophyll *a* and *b* molecules as well as other pigments like carotenoids. The absorption of a single **photon** or distinct quantity or "packet" of light by any of the chlorophylls pushes that molecule into an excited state. In short, the light energy has now

been captured by biological molecules but is not stored in any useful form yet. The energy is transferred from chlorophyll to chlorophyll until eventually (after about a millionth of a second), it is delivered to the reaction center. Up to this point, only energy has been transferred between molecules, not electrons.

Visual Connection

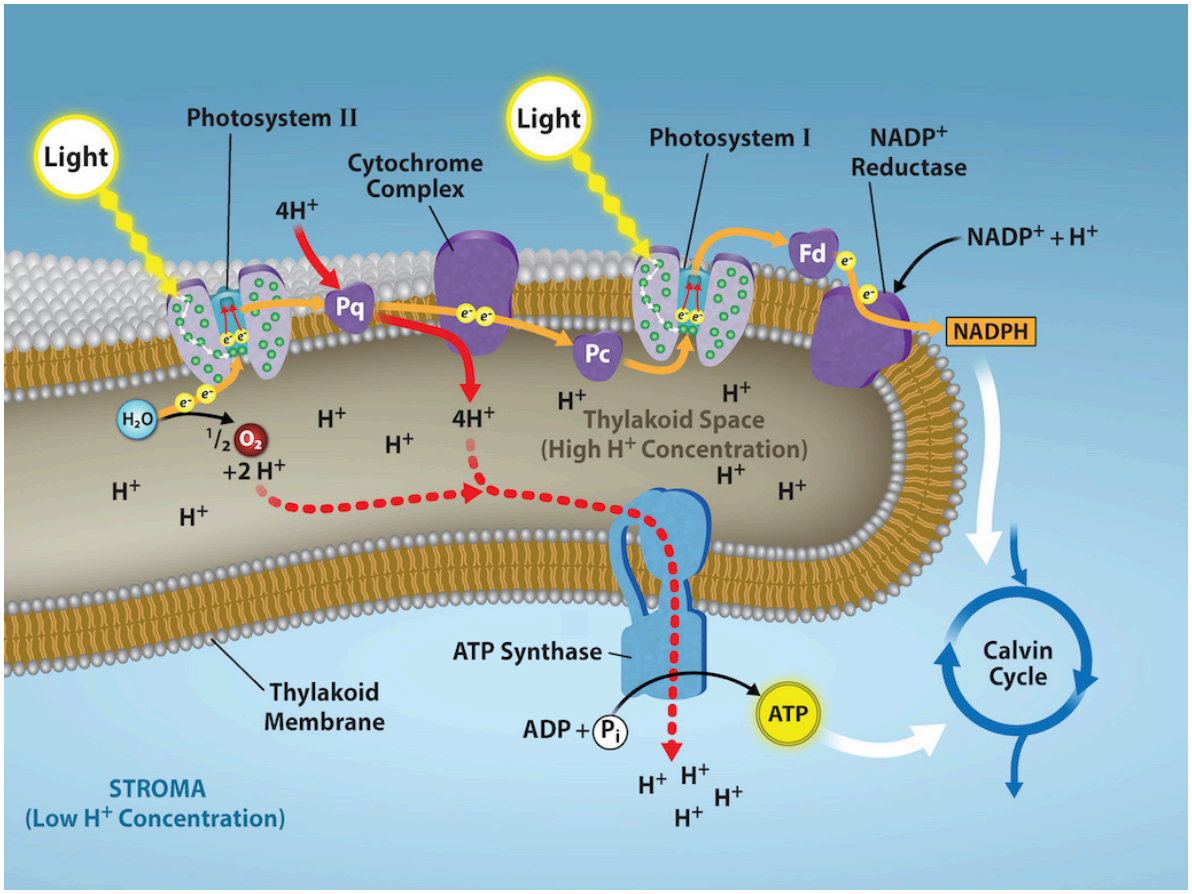

Figure 8.17 In the photosystem II (PSII) reaction center, energy from sunlight is used to extract electrons from water. The electrons travel through the chloroplast electron transport chain to photosystem I (PSI), which reduces NADP+ to NADPH. The electron transport chain moves protons across the thylakoid membrane into the lumen. At the same time, splitting of water adds protons to the lumen, and reduction of NADPH removes protons from the stroma. The net result is a low pH in the thylakoid lumen, and a high pH in the stroma. ATP synthase uses this electrochemical gradient to make ATP. Credit: Rao, A., Ryan, K., Fletcher, S. Department of Biology, Texas A&M University.

What is the initial source of electrons for the chloroplast electron transport chain?

1. water
2. oxygen
3. carbon dioxide
4. NADPH

The reaction center contains a pair of chlorophyll *a* molecules with a special property. Those two chlorophylls can undergo oxidation upon excitation; they can actually give up an electron in a process called a **photoact**. It is at this step in the reaction center during photosynthesis that light energy is converted into an excited electron. All of the subsequent steps involve getting that electron onto the energy carrier NADPH for delivery to the Calvin cycle where the electron is deposited onto carbon for long-term storage in the form of a carbohydrate. PSII and PSI are two major components of the photosynthetic **electron transport chain**, which also includes the *cytochrome complex*. The cytochrome complex, an enzyme composed of two protein complexes, transfers the electrons from the carrier molecule plastoquinone (Pq) to the protein plastocyanin (Pc), thus enabling both the transfer of protons across the thylakoid membrane and the transfer of electrons from PSII to PSI.

The reaction center of PSII (called **P680**) delivers its high-energy electrons, one at the time, to the **primary electron acceptor**, and through the electron transport chain (Pq to cytochrome complex to plastocyanine) to PSI. P680's missing electron is replaced by extracting a low-energy electron from water; thus, water is "split" during this stage of photosynthesis, and PSII is re-reduced after every photoact. Splitting one H_2O molecule releases two electrons, two hydrogen atoms, and one atom of oxygen. However, splitting two molecules is required to form one molecule of diatomic O_2 gas. About 10 percent of the oxygen is used by mitochondria in the leaf to support oxidative phosphorylation. The remainder escapes to the atmosphere where it is used by aerobic organisms to support respiration.

As electrons move through the proteins that reside between PSII and PSI, they lose energy. This energy is used to move hydrogen atoms from the stromal side of the membrane to the thylakoid lumen. Those hydrogen atoms, plus the ones produced by splitting water, accumulate in the thylakoid lumen and will be used synthesize ATP in a later step. Because the electrons have lost energy prior to their arrival at PSI, they must be re-energized by PSI, hence, another photon is absorbed by the PSI antenna. That energy is relayed to the PSI reaction center (called **P700**). P700 is oxidized and sends a high-energy electron to $NADP^+$ to form NADPH. Thus, PSII captures the energy to create proton gradients to make ATP, and PSI captures the energy to reduce $NADP^+$ into NADPH. The two photosystems work in concert, in part, to guarantee that the production of NADPH will roughly equal the production of ATP. Other mechanisms exist to fine-tune that ratio to exactly match the chloroplast's constantly changing energy needs.

Generating an Energy Carrier: ATP

As in the intermembrane space of the mitochondria during cellular respiration, the buildup of hydrogen ions inside the thylakoid lumen creates a *concentration gradient*. The passive diffusion of hydrogen ions from high concentration (in the thylakoid lumen) to low concentration (in the stroma) is harnessed to create ATP, just as in the electron transport chain of cellular respiration. The ions build up energy because of diffusion and because they all have the same electrical charge, repelling each other.

To release this energy, hydrogen ions will rush through any opening, similar to water jetting through a hole in a dam. In the thylakoid, that opening is a passage through a specialized protein channel called the ATP synthase. The energy released by the hydrogen ion stream allows ATP synthase to attach a third phosphate group to ADP, which forms a molecule of ATP (Figure 8.17). The flow of hydrogen ions through ATP synthase is called chemiosmosis because the ions move from an area of high to an area of low concentration through a semi-permeable structure of the thylakoid.

Link to Learning

Visit this site and click through the animation to view the process of photosynthesis within a leaf.

78.

USING LIGHT ENERGY TO MAKE ORGANIC MOLECULES

Learning Objectives

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

- Describe the Calvin cycle
- Define carbon fixation
- Explain how photosynthesis works in the energy cycle of all living organisms

After the energy from the sun is converted into chemical energy and temporarily stored in ATP and NADPH molecules, the cell has the fuel needed to build carbohydrate molecules for long-term energy storage. The products of the light-dependent reactions, ATP and NADPH, have lifespans in the range of millionths of seconds, whereas the products of the light-independent reactions (carbohydrates and other forms of reduced carbon) can survive almost indefinitely. The carbohydrate molecules made will have a backbone of carbon atoms. But where does the carbon come from? It comes from carbon dioxide—the gas that is a waste product of respiration in microbes, fungi, plants, and animals.

The Calvin Cycle

In plants, carbon dioxide (CO_2) enters the leaves through stomata, where it diffuses over short distances through intercellular spaces until it reaches the mesophyll cells. Once in the mesophyll cells, CO_2 diffuses into the stroma of the chloroplast—the site of light-independent reactions of photosynthesis. These reactions actually have several names associated with them. Another term, the **Calvin cycle**, is named for the man who discovered it, and because these reactions function as a cycle. Others call it the Calvin-Benson cycle to include

the name of another scientist involved in its discovery. The most outdated name is "dark reaction," because light is not directly required (Figure 8.18). However, the term dark reaction can be misleading because it implies incorrectly that the reaction only occurs at night or is independent of light, which is why most scientists and instructors no longer use it.

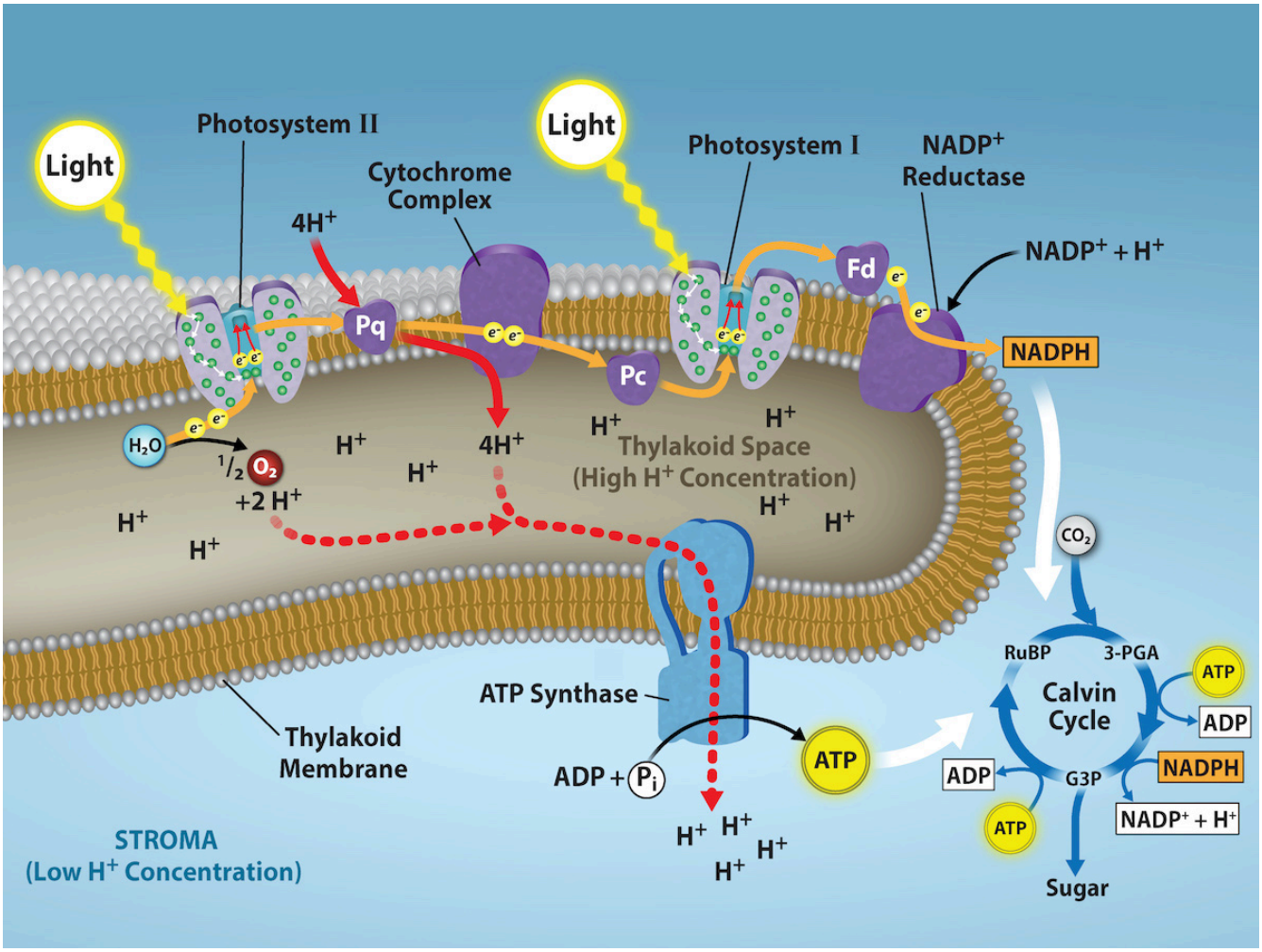

Figure 8.18 Light reactions harness energy from the sun to produce chemical bonds, ATP, and NADPH. These energy-carrying molecules are made in the stroma where carbon fixation takes place. Credit: Rao, A., Ryan, K., Tag, A., Fletcher, S. and Hawkins, A. Department of Biology, Texas A&M University.

The light-independent reactions of the Calvin cycle can be organized into three basic stages: *fixation, reduction, and regeneration.*

Stage 1: Fixation

In the stroma, in addition to CO_2, two other components are present to initiate the light-independent reactions: an enzyme called ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and three molecules

of ribulose bisphosphate (RuBP), as shown in Figure 8.19. RuBP has five atoms of carbon, flanked by two phosphates.

Visual Connection

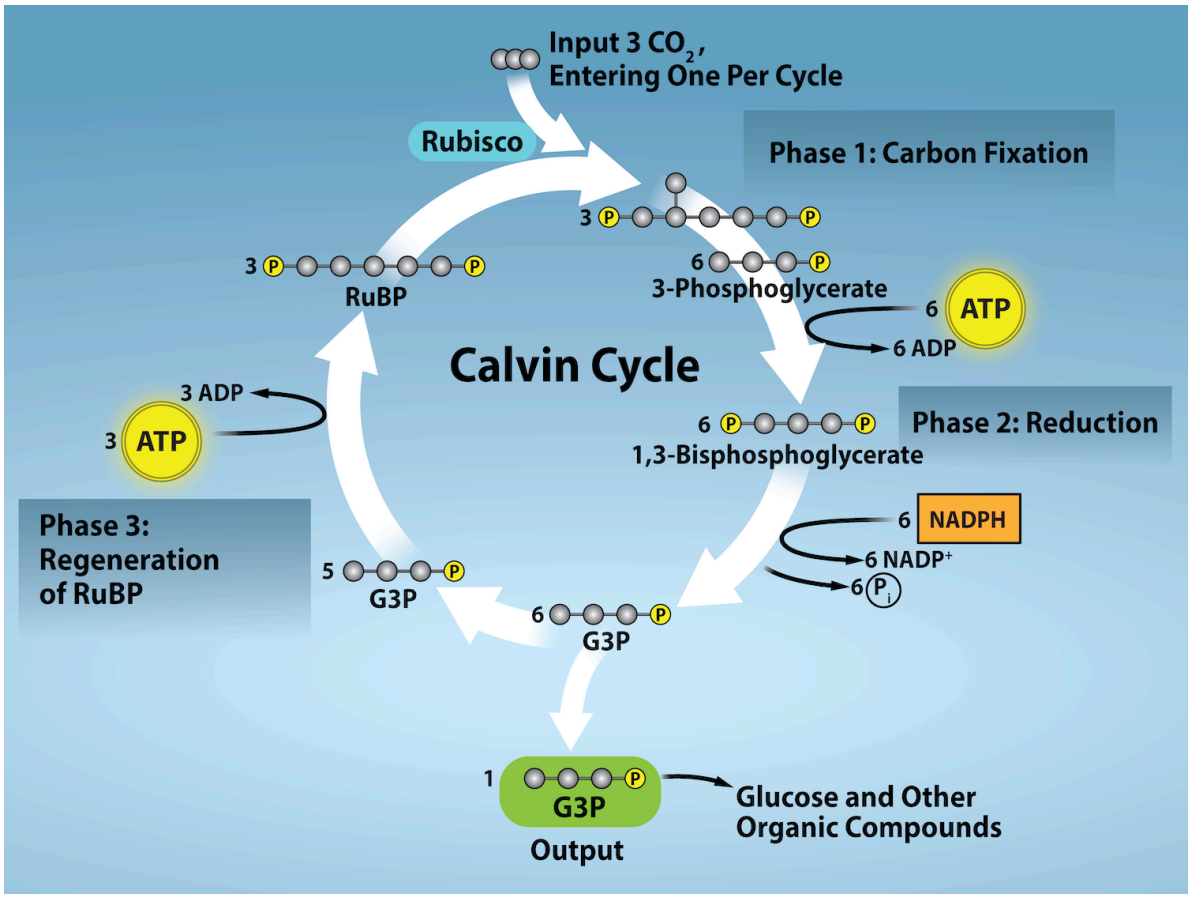

Figure 8.19 The Calvin cycle has three stages. In stage 1, the enzyme RuBisCO incorporates carbon dioxide into an organic molecule, 3-PGA. In stage 2, the organic molecule is reduced using electrons supplied by NADPH. In stage 3, RuBP, the molecule that starts the cycle, is regenerated so that the cycle can continue. Only one carbon dioxide molecule is incorporated at a time, so the cycle must be completed three times to produce a single three-carbon G3P molecule, and six times to produce a six-carbon glucose molecule. Credit: Rao, A., Ryan, K., Tag, A., Fletcher, S. and Hawkins, A. Department of Biology, Texas A&M University.

Which of the following statements is true?

1. In photosynthesis, oxygen, carbon dioxide, ATP, and NADPH are reactants. G3P and water are products.
2. In photosynthesis, chlorophyll, water, and carbon dioxide are reactants. G3P and oxygen are products.
3. In photosynthesis, water, carbon dioxide, ATP, and NADPH are reactants. RuBP and oxygen are products.
4. In photosynthesis, water and carbon dioxide are reactants. G3P and oxygen are products.

RuBisCO catalyzes a reaction between CO_2 and RuBP. For each CO_2 molecule that reacts with one RuBP, two molecules of another compound 3-phospho glyceric acid (3-PGA) form. PGA has three carbons and one phosphate. Each turn of the cycle involves only one RuBP and one carbon dioxide and forms two molecules of 3-PGA. The number of carbon atoms remains the same, as the atoms move to form new bonds during the reactions (3 C atoms from $3CO_2$ + 15 C atoms from 3RuBP = 18 C atoms in 6 molecules of 3-PGA). This process is called **carbon fixation**, because CO_2 is "fixed" from an inorganic form into organic molecules.

Stage 2: Reduction

ATP and NADPH are used to convert the six molecules of 3-PGA into six molecules of a chemical called glyceraldehyde 3-phosphate (G3P). That is a reduction reaction because it involves the gain of electrons by 3-PGA. (Recall that a **reduction** is the gain of an electron by an atom or molecule.) Six molecules of both ATP and NADPH are used. For ATP, energy is released with the loss of the terminal phosphate atom, converting it into ADP; for NADPH, both energy and a hydrogen atom are lost, converting it into $NADP^+$. Both of these molecules return to the nearby light-dependent reactions to be reused and re-energized.

Stage 3: Regeneration

Interestingly, at this point, only one of the G3P molecules leaves the Calvin cycle and is sent to the cytoplasm to contribute to the formation of other compounds needed by the plant. Because the G3P exported from the chloroplast has three carbon atoms, it takes three "turns" of the Calvin cycle to fix enough net carbon to export one G3P. But each turn makes two G3Ps, thus three turns make six G3Ps. One is exported while the remaining five G3P molecules remain in the cycle and are used to regenerate RuBP, which enables the system to prepare for more CO_2 to be fixed. Three more molecules of ATP are used in these regeneration reactions.

Link to Learning

This link leads to an animation of photosynthesis and the Calvin cycle.

Evolution Connection

Photosynthesis

During the evolution of photosynthesis, a major shift occurred from the bacterial type of photosynthesis that involves only one photosystem and is typically anoxygenic (does not generate oxygen) into modern oxygenic (does generate oxygen) photosynthesis, employing two photosystems. This modern oxygenic photosynthesis is used by many organisms—from giant tropical leaves in the rainforest to tiny cyanobacterial cells—and the process and components of this photosynthesis remain largely the same. Photosystems absorb light and use electron transport chains to convert energy into the chemical energy of ATP and NADH. The subsequent light-independent reactions then assemble carbohydrate molecules with this energy.

In the harsh dry heat of the desert, plants must conserve and use every drop of water to survive. Because stomata must open to allow for the uptake of CO_2, water escapes from the leaf during active photosynthesis. Desert plants have evolved processes to conserve water and deal with harsh conditions. Mechanisms to capture and store CO_2 allows plants to adapt to living with less water. Some plants such as cacti (Figure 8.20) can prepare materials for photosynthesis during the night by a temporary carbon fixation/storage process, because opening the stomata at this time conserves water due to cooler temperatures. During the day, cacti use the captured CO_2 for photosynthesis and keep their stomata closed.

Figure 8.20 The harsh conditions of the desert have led plants like these cacti to evolve variations of the light-independent reactions of photosynthesis. These variations increase the efficiency of water usage, helping to conserve water and energy. (credit: Piotr Wojtkowski)

The Energy Flow

Whether the organism is a bacterium, plant, or animal, all living things access energy by breaking down carbohydrate and other carbon-rich organic molecules. But if plants make carbohydrate molecules, why would they need to break them down, especially when it has been shown that the gas organisms release as a "waste product" (CO_2) acts as a substrate for the formation of more food in photosynthesis? Remember, living things need energy to perform life functions. In addition, an organism can either make its own food or eat another organism—either way, the food still needs to be broken down. Finally, in the process of breaking down food, called cellular respiration, heterotrophs release needed energy and produce "waste" in the form of CO_2 gas.

However, in nature, there is no such thing as "waste." Every single atom of matter and energy is conserved, recycled over and over infinitely. Substances change form or move from one type of molecule to another, but their constituent atoms never disappear (Figure 8.22).

In reality, CO_2 is no more a form of waste than oxygen is wasteful to photosynthesis. Both are byproducts of reactions that move on to other reactions. Photosynthesis absorbs light energy to build carbohydrates in chloroplasts, and aerobic cellular respiration releases energy by using oxygen to metabolize carbohydrates in the cytoplasm and mitochondria. Both processes use electron transport chains to capture the energy necessary

to drive other reactions. These two powerhouse processes, photosynthesis and cellular respiration, function in biological, cyclical harmony to allow organisms to access life-sustaining energy that originates millions of miles away in a burning star humans call the sun.

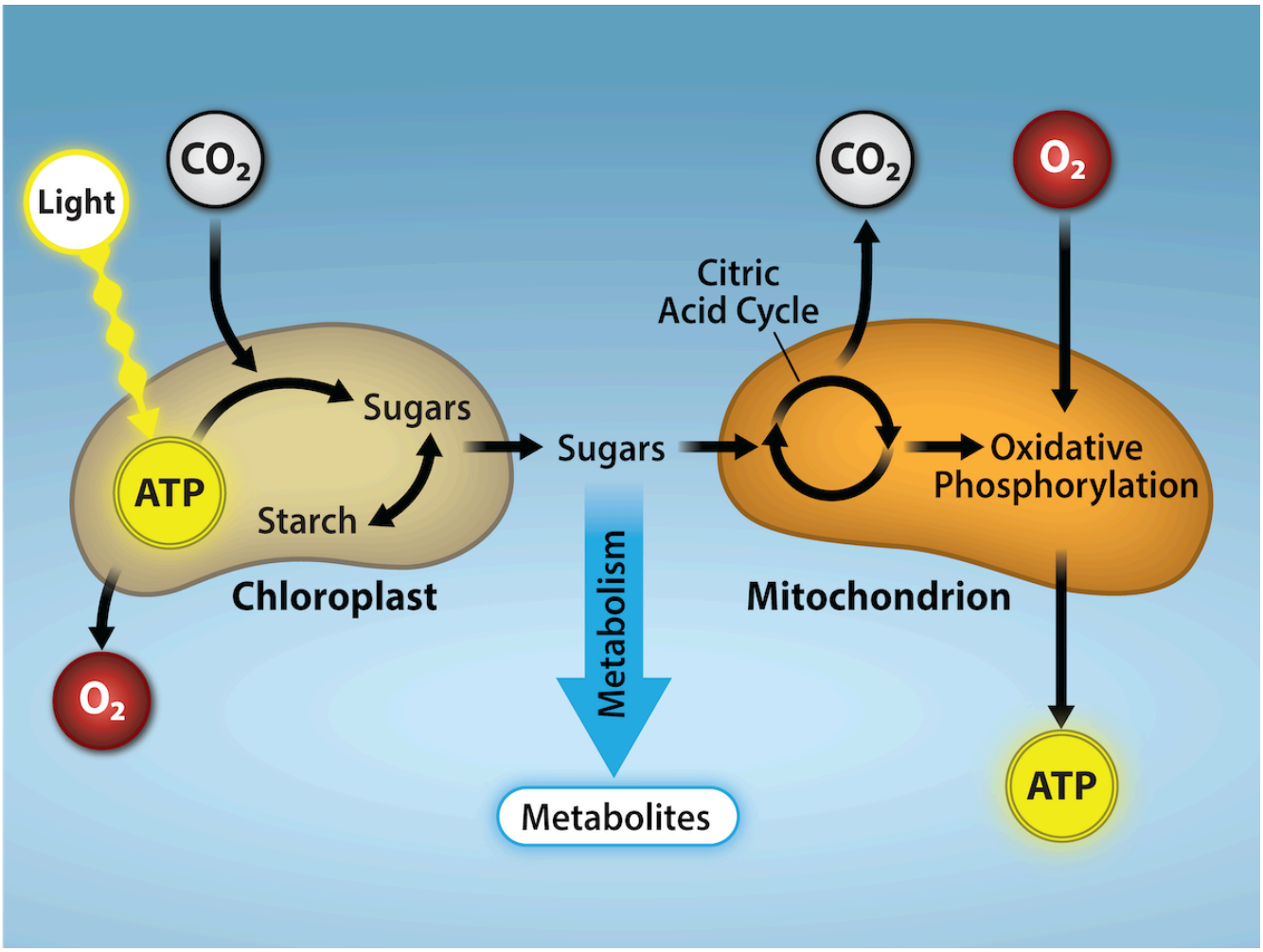

Figure 8.21 Connection between Photosynthesis and Respiration Photosynthesis in chloroplasts is the process by which light energy is converted to chemical energy and stored in sugars. Initially, the light energy is converted into chemical energy during ATP synthesis in a process that gives off oxygen. The energy in ATP is then used to reduce CO2 to simple sugars. In contrast, cellular respiration is the process in which the chemical energy stored in sugars is converted into ATP, a source of chemical energy that can be used by the rest of the cell. In the process of converting the energy stored in the sugars to ATP, CO2 is released and oxygen is consumed. Credit: Rao, A., Ryan, and Tag, A. Department of Biology, Texas A&M University.

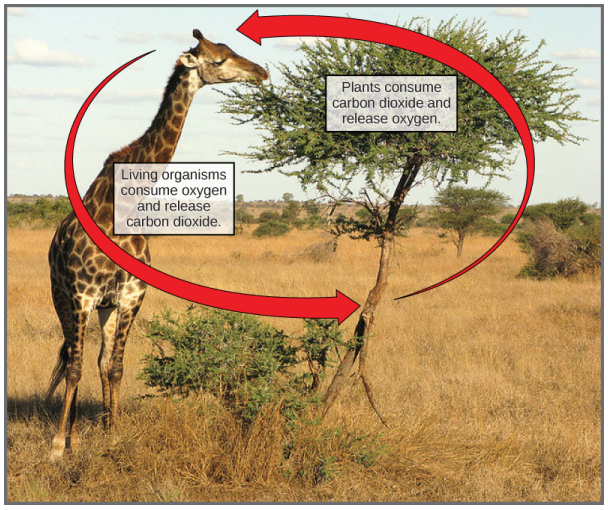

Figure 8.22 Photosynthesis consumes carbon dioxide and produces oxygen. Aerobic respiration consumes oxygen and produces carbon dioxide. These two processes play an important role in the carbon cycle. (credit: modification of work by Stuart Bassil)

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79.

KEY TERMS

absorption spectrum
: range of wavelengths of electromagnetic radiation absorbed by a given substance

antenna protein
: pigment molecule that directly absorbs light and transfers the energy absorbed to other pigment molecules

Calvin cycle
: light-independent reactions of photosynthesis that convert carbon dioxide from the atmosphere into carbohydrates using the energy and reducing power of ATP and NADPH

carbon fixation
: process of converting inorganic CO_2 gas into organic compounds

carotenoid
: photosynthetic pigment (yellow-orange-red) that functions to dispose of excess energy

chemoautotroph
: organism that can build organic molecules using energy derived from inorganic chemicals instead of sunlight

chlorophyll *a*
: form of chlorophyll that absorbs violet-blue and red light and consequently has a bluish-green color; the only pigment molecule that performs the photochemistry by getting excited and losing an electron to the electron transport chain

chlorophyll *b*
: accessory pigment that absorbs blue and red-orange light and consequently has a yellowish-green tint

chloroplast
: organelle in which photosynthesis takes place

cytochrome complex
: group of reversibly oxidizable and reducible proteins that forms part of the electron transport chain between photosystem II and photosystem I

electromagnetic spectrum
: range of all possible frequencies of radiation

electron transport chain
: group of proteins between PSII and PSI that pass energized electrons and use the energy released by the

electrons to move hydrogen ions against their concentration gradient into the thylakoid lumen

granum
: stack of thylakoids located inside a chloroplast

heterotroph
: organism that consumes organic substances or other organisms for food

light harvesting complex
: complex that passes energy from sunlight to the reaction center in each photosystem; it consists of multiple antenna proteins that contain a mixture of 300 to 400 chlorophyll *a* and *b* molecules as well as other pigments like carotenoids

light-dependent reaction
: first stage of photosynthesis where certain wavelengths of the visible light are absorbed to form two energy-carrying molecules (ATP and NADPH)

light-independent reaction
: second stage of photosynthesis, through which carbon dioxide is used to build carbohydrate molecules using energy from ATP and NADPH

mesophyll
: middle layer of chlorophyll-rich cells in a leaf

P680
: reaction center of photosystem II

P700
: reaction center of photosystem I

photoact
: ejection of an electron from a reaction center using the energy of an absorbed photon

photoautotroph
: organism capable of producing its own organic compounds from sunlight

photon
: distinct quantity or "packet" of light energy

photosystem
: group of proteins, chlorophyll, and other pigments that are used in the light-dependent reactions of photosynthesis to absorb light energy and convert it into chemical energy

photosystem I
: integral pigment and protein complex in thylakoid membranes that uses light energy to transport electrons from plastocyanin to $NADP^+$ (which becomes reduced to NADPH in the process)

photosystem II
: integral protein and pigment complex in thylakoid membranes that transports electrons from water to the electron transport chain; oxygen is a product of PSII

pigment
: molecule that is capable of absorbing certain wavelengths of light and reflecting others (which accounts for its color)

primary electron acceptor
: pigment or other organic molecule in the reaction center that accepts an energized electron from the reaction center

reaction center
: complex of chlorophyll molecules and other organic molecules that is assembled around a special pair of chlorophyll molecules and a primary electron acceptor; capable of undergoing oxidation and reduction

reduction
: gain of electron(s) by an atom or molecule

spectrophotometer
: instrument that can measure transmitted light and compute the absorption

stoma
: opening that regulates gas exchange and water evaporation between leaves and the environment, typically situated on the underside of leaves

stroma
: fluid-filled space surrounding the grana inside a chloroplast where the light-independent reactions of photosynthesis take place

thylakoid
: disc-shaped, membrane-bound structure inside a chloroplast where the light-dependent reactions of photosynthesis take place; stacks of thylakoids are called grana

thylakoid lumen
: aqueous space bound by a thylakoid membrane where protons accumulate during light-driven electron transport

wavelength
: distance between consecutive points of equal position (two crests or two troughs) of a wave in a graphic representation; inversely proportional to the energy of the radiation

80.

CHAPTER SUMMARY

8.1 Overview of Photosynthesis

The process of photosynthesis transformed life on Earth. By harnessing energy from the sun, the evolution of photosynthesis allowed living things access to enormous amounts of energy. Because of photosynthesis, living things gained access to sufficient energy that allowed them to build new structures and achieve the biodiversity evident today.

Only certain organisms (photoautotrophs), can perform photosynthesis; they require the presence of chlorophyll, a specialized pigment that absorbs certain wavelengths of the visible spectrum and can capture energy from sunlight. Photosynthesis uses carbon dioxide and water to assemble carbohydrate molecules and release oxygen as a byproduct into the atmosphere. Eukaryotic autotrophs, such as plants and algae, have organelles called chloroplasts in which photosynthesis takes place and starch accumulates. In prokaryotes, such as cyanobacteria, the process is less localized and occurs within folded membranes, extensions of the plasma membrane, and in the cytoplasm.

8.2 The Light-Dependent Reactions of Photosynthesis

The pigments of the first part of photosynthesis, the light-dependent reactions, absorb energy from sunlight. A photon strikes the antenna pigments of photosystem II to initiate photosynthesis. The energy travels to the reaction center that contains chlorophyll *a* and then to the electron transport chain, which pumps hydrogen ions into the thylakoid interior. This action builds up a high concentration of hydrogen ions. The hydrogen ions flow through ATP synthase during chemiosmosis to form molecules of ATP, which are used for the formation of sugar molecules in the second stage of photosynthesis. Photosystem I absorbs a second photon, which results in the formation of an NADPH molecule, another energy and reducing carrier for the light-independent reactions.

8.3 Using Light Energy to Make Organic Molecules

Using the energy carriers formed in the first steps of photosynthesis, the light-independent reactions, or the Calvin cycle, take in CO_2 from the atmosphere. An enzyme, RuBisCO, catalyzes a reaction with CO_2 and

another organic compound, RuBP. After three cycles, a three-carbon molecule of G3P leaves the cycle to become part of a carbohydrate molecule. The remaining G3P molecules stay in the cycle to be regenerated into RuBP, which is then ready to react with more CO_2. Photosynthesis forms an energy cycle with the process of cellular respiration. Because plants contain both chloroplasts and mitochondria, they rely upon both photosynthesis and respiration for their ability to function in both the light and dark, and to be able to interconvert essential metabolites.

81.

VISUAL CONNECTION QUESTIONS

1. Figure 8.6 On a hot, dry day, the guard cells of plants close their stomata to conserve water. What impact will this have on photosynthesis?

2. Figure 8.17 What is the initial source of electrons for the chloroplast electron transport chain?

a. Water
b. Oxygen
c. Carbon dioxide
d. NADPH

3. Figure 8.19 Which of the following statements is true?

a. In photosynthesis, oxygen, carbon dioxide, ATP, and NADPH are reactants. G3P and water are products.
b. In photosynthesis, chlorophyll, water, and carbon dioxide are reactants. G3P and oxygen are products.
c. In photosynthesis, water, carbon dioxide, ATP, and NADPH are reactants. RuBP and oxygen are products.
d. In photosynthesis, water and carbon dioxide are reactants. G3P and oxygen are products.

82.

REVIEW QUESTIONS

4. Which of the following components is not used by both plants and cyanobacteria to carry out photosynthesis?

a. chloroplasts
b. chlorophyll
c. carbon dioxide
d. water

5. What two main products result from photosynthesis?

a. oxygen and carbon dioxide
b. chlorophyll and oxygen
c. sugars/carbohydrates and oxygen
d. sugars/carbohydrates and carbon dioxide

6. In which compartment of the plant cell do the light-independent reactions of photosynthesis take place?

a. thylakoid
b. stroma
c. outer membrane
d. mesophyll

7. Which statement about thylakoids in eukaryotes is not correct?

a. Thylakoids are assembled into stacks.
b. Thylakoids exist as a maze of folded membranes.
c. The space surrounding thylakoids is called stroma.
d. Thylakoids contain chlorophyll.

8. Predict the end result if a chloroplast's light-independent enzymes developed a mutation that prevented them from activating in response to light.

a. G3P accumulation
b. ATP and NADPH accumulation
c. Water accumulation
d. Carbon dioxide depletion

9. How are the NADPH and G3P molecules made during photosynthesis similar?

a. They are both end products of photosynthesis.
b. They are both substrates for photosynthesis.
c. They are both produced from carbon dioxide.
d. They both store energy in chemical bonds.

10. Which of the following structures is not a component of a photosystem?

a. ATP synthase
b. antenna molecule
c. reaction center
d. primary electron acceptor

11. How many photons does it take to fully reduce one molecule of NADP+ to NADPH?

a. 1
b. 2
c. 4
d. 8

12. Which complex is not involved in the establishment of conditions for ATP synthesis?

a. photosystem I
b. ATP synthase
c. photosystem II
d. cytochrome complex

13. From which component of the light-dependent reactions does NADPH form most directly?

a. photosystem II
b. photosystem I
c. cytochrome complex

d. ATP synthase

14. Three of the same species of plant are each grown under a different colored light for the same amount of time. Plant A is grown under blue light, Plant B is grown under green light, and Plant C is grown under orange light. Assuming the plants use only chlorophyll a and chlorophyll b for photosynthesis, what would be the predicted order of the plants from most growth to least growth?

a. A, C, B
b. A, B, C
c. C, A, B
d. B, A, C

15. Plants containing only chlorophyll b are exposed to radiation with the following wavelengths: 10nm (x-rays), 450nm (blue light), 670nm (red light), and 800nm (infrared light). Which plants harness the most energy for photosynthesis?

a. X-ray irradiated plants
b. Blue light irradiated plants
c. Red light irradiated plants
d. Infrared irradiated plants

16. Which molecule must enter the Calvin cycle continually for the light-independent reactions to take place?

a. RuBisCO
b. RuBP
c. 3-PGA
d. CO_2

17. Which order of molecular conversions is correct for the Calvin cycle?

a. RuBP + G3P → 3-PGA → sugarRuBP + G3P → 3-PGA → sugar
b. RuBisCO → CO_2 → RuBP → G3PRuBisCO → CO2 → RuBP → G3P
c. RuBP + CO_2 → [RuBisCO] 3-PGA → G3PRuBP + CO2 → [RuBisCO] 3-PGA → G3P
d. CO_2 → 3-PGA → RuBP → G3PCO2 → 3-PGA → RuBP → G3P

18. Where in eukaryotic cells does the Calvin cycle take place?

a. thylakoid membrane

b. thylakoid lumen
c. chloroplast stroma
d. granum

19. Which statement correctly describes carbon fixation?

a. the conversion of CO_2 into an organic compound
b. the use of RuBisCO to form 3-PGA
c. the production of carbohydrate molecules from G3P
d. the formation of RuBP from G3P molecules
e. the use of ATP and NADPH to reduce CO_2

20. If four molecules of carbon dioxide enter the Calvin cycle (four "turns" of the cycle), how many G3P molecules are produced and how many are exported?

a. 4 G3P made, 1 G3P exported
b. 4 G3P made, 2 G3P exported
c. 8 G3P made, 1 G3P exported
d. 8 G3P made, 4 G3P exported

83.

CRITICAL THINKING QUESTIONS

21. What is the overall outcome of the light reactions in photosynthesis?

22. Why are carnivores, such as lions, dependent on photosynthesis to survive?

23. Why are energy carriers thought of as either "full" or "empty"?

24. Describe how the grey wolf population would be impacted by a volcanic eruption that spewed a dense ash cloud that blocked sunlight in a section of Yellowstone National Park.

25. How does the closing of the stomata limit photosynthesis?

26. Describe the pathway of electron transfer from photosystem II to photosystem I in light-dependent reactions.

27. What are the roles of ATP and NADPH in photosynthesis?

28. How and why would the end products of photosynthesis be changed if a plant had a mutation that eliminated its photosystem II complex?

29. Why is the third stage of the Calvin cycle called the regeneration stage?

30. Which part of the light-independent reactions would be affected if a cell could not produce the enzyme RuBisCO?

31. Why does it take three turns of the Calvin cycle to produce G3P, the initial product of photosynthesis?

32. Imagine a sealed terrarium containing a plant and a beetle. How does each organism provide resources for the other? Could each organism survive if it was the only living thing in the terrarium? Why or why not?

33. Compare the flow of energy with the flow of nutrients in a closed, sunny ecosystem consisting of a giraffe and a tree.

PART IX

CELL COMMUNICATION

84.

INTRODUCTION

Figure 9.1 Have you ever become separated from a friend while in a crowd? If so, you know the challenge of searching for someone when surrounded by thousands of other people. If you and your friend have cell phones, your chances of finding each other are good. Cell phone networks use various methods of encoding to ensure that the signals reach their intended recipients without interference. Similarly, cells must communicate using specific signals and receptors to ensure that messages are clear. (credit: modification of work by Vincent and Bella Productions)

Imagine what life would be like if you and the people around you could not communicate. You would not be able to express your wishes to others, nor could you ask questions about your location. Social organization is dependent on communication between the individuals that comprise that society; without communication, society would fall apart.

As with people, it is vital for individual cells to be able to interact with their environment. This is true for both a one-celled organism growing in a puddle and a large animal living on a savanna. In order to properly respond to external stimuli, cells have developed complex mechanisms of communication that can receive a message, transfer the information across the plasma membrane, and then produce changes within the cell in response to the message.

In multicellular organisms, cells send and receive chemical messages constantly to coordinate the actions of distant organs, tissues, and cells. The ability to send messages quickly and efficiently enables cells to coordinate and fine-tune their functions.

While the necessity for cellular communication in larger organisms seems obvious, even single-celled organisms communicate with each other. Yeast cells signal each other to aid in finding other yeast cells for

reproduction. Some forms of bacteria coordinate their actions in order to form large complexes called biofilms or to organize the production of toxins to remove competing organisms. The ability of cells to communicate through chemical signals originated in single cells and was essential for the evolution of multicellular organisms. The efficient and relatively error-free function of communication systems is vital for all life as we know it.

85.

SIGNALING MOLECULES AND CELLULAR RECEPTORS

Learning Objectives

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

- Describe four types of signaling mechanisms found in multicellular organisms
- Compare internal receptors with cell-surface receptors
- Recognize the relationship between a ligand's structure and its mechanism of action

There are two kinds of communication in the world of living cells. Communication between cells is called **intercellular signaling**, and communication within a cell is called **intracellular signaling**. An easy way to remember the distinction is by understanding the Latin origin of the prefixes: *inter-* means "between" (for example, intersecting lines are those that cross each other) and *intra-* means "inside" (as in intravenous).

Chemical signals are released by **signaling cells** in the form of small, usually volatile or soluble molecules called ligands. A **ligand** is a molecule that binds another specific molecule, in some cases, delivering a signal in the process. Ligands can thus be thought of as signaling molecules. Ligands interact with proteins in **target cells**, which are cells that are affected by chemical signals; these proteins are also called **receptors**. Ligands and receptors exist in several varieties; however, a specific ligand will have a specific receptor that typically binds only that ligand.

Forms of Signaling

There are four categories of chemical signaling found in multicellular organisms: paracrine signaling, endocrine signaling, autocrine signaling, and direct signaling across gap junctions (Figure 9.2). The main difference

between the different categories of signaling is the distance that the signal travels through the organism to reach the target cell. We should note here that not all cells are affected by the same signals.

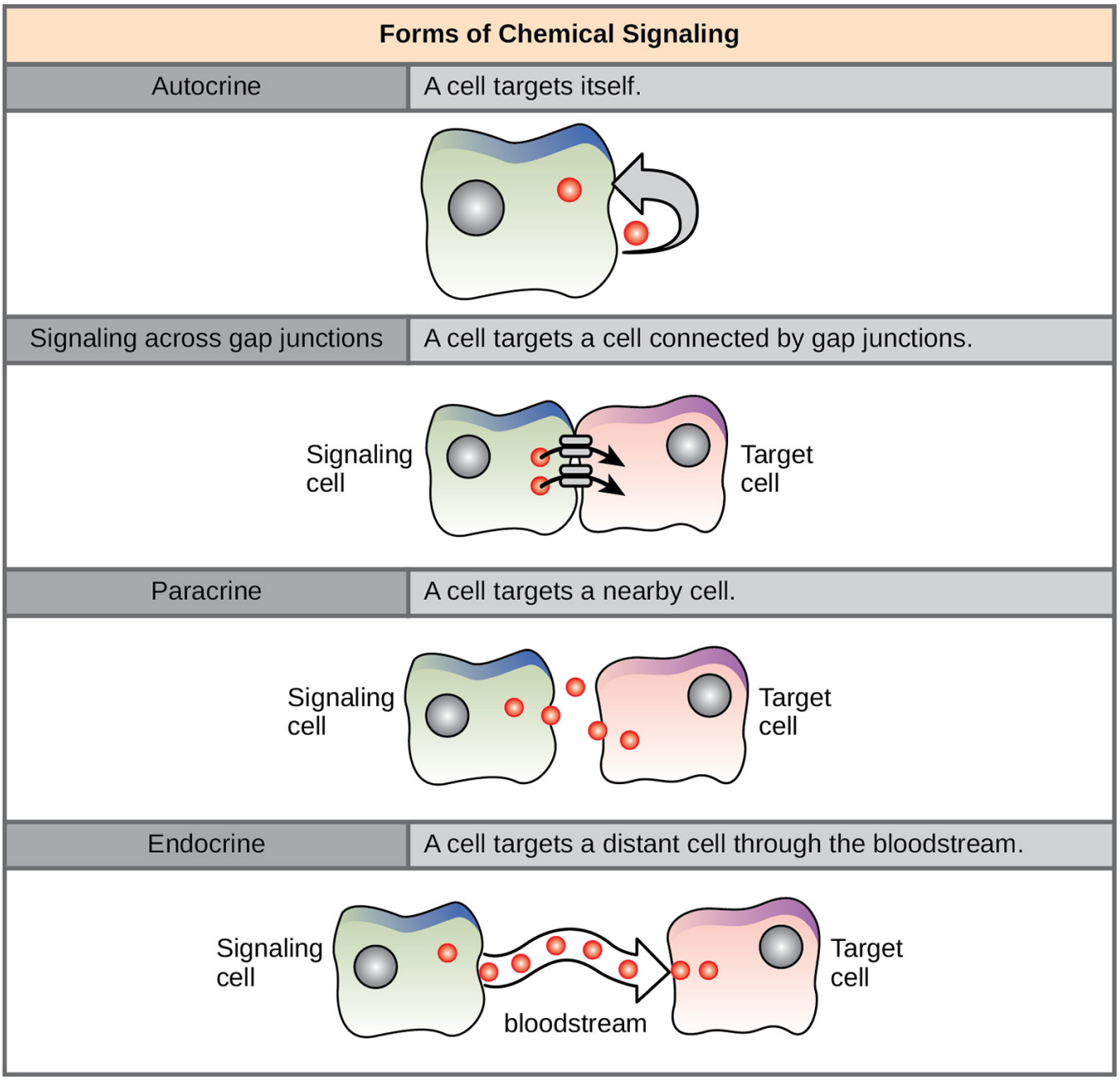


Figure 9.2 In chemical signaling, a cell may target itself (autocrine signaling), a cell connected by gap junctions, a nearby cell (paracrine signaling), or a distant cell (endocrine signaling). Paracrine signaling acts on nearby cells, endocrine signaling uses the circulatory system to transport ligands, and autocrine signaling acts on the signaling cell. Signaling via gap junctions involves signaling molecules moving directly between adjacent cells.

Paracrine Signaling

Signals that act locally between cells that are close together are called **paracrine signals**. Paracrine signals move by diffusion through the extracellular matrix. These types of signals usually elicit quick responses that last only a short period of time. In order to keep the response localized, paracrine ligand molecules are normally quickly degraded by enzymes or removed by neighboring cells. Removing the signals will reestablish the concentration gradient for the signal, allowing them to quickly diffuse through the intracellular space if released again.

One example of paracrine signaling is the transfer of signals across synapses between nerve cells. A nerve cell consists of a cell body, several short, branched extensions called dendrites that receive stimuli, and a long extension called an axon, which transmits signals to other nerve cells or muscle cells. The junction between nerve cells where signal transmission occurs is called a synapse. A **synaptic signal** is a chemical signal that travels between nerve cells. Signals within the nerve cells are propagated by fast-moving electrical impulses. When these impulses reach the end of the axon, the signal continues on to a dendrite of the next cell by the release of chemical ligands called **neurotransmitters** from the presynaptic cell (the cell emitting the signal). The neurotransmitters are transported across the very small distances (20–40 nanometers) between nerve cells, which are called **chemical synapses** (Figure 9.3). The small distance between nerve cells allows the signal to travel quickly; this enables an immediate response, such as, "Take your hand off the stove!"

When the neurotransmitter binds the receptor on the surface of the postsynaptic cell, the electrochemical potential of the target cell changes, and the next electrical impulse is launched. The neurotransmitters that are released into the chemical synapse are degraded quickly or get reabsorbed by the presynaptic cell so that the recipient nerve cell can recover quickly and be prepared to respond rapidly to the next synaptic signal.

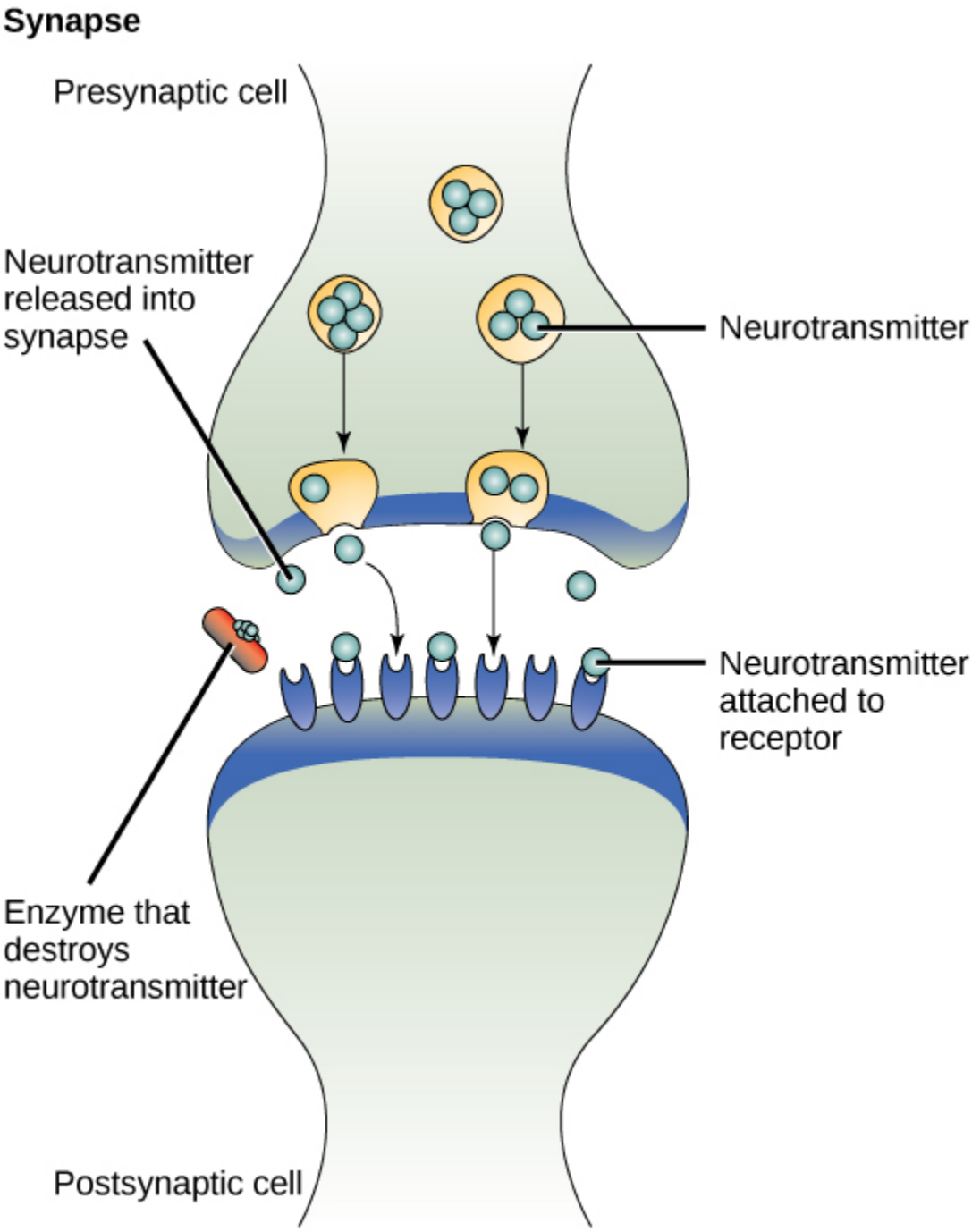


Figure 9.3 The distance between the presynaptic cell and the postsynaptic cell—called the synaptic gap—is very small and allows for rapid diffusion of the neurotransmitter. Enzymes in the synapatic gap degrade some types of neurotransmitters to terminate the signal.

Link to Learning

Review the following videos: Cell Signaling Types and Signal Transduction Animation

Endocrine Signaling

Signals from distant cells are called **endocrine signals**, and they originate from **endocrine cells**. (In the body, many endocrine cells are located in endocrine glands, such as the thyroid gland, the hypothalamus, and the pituitary gland.) These types of signals usually produce a slower response but have a longer-lasting effect. The ligands released in endocrine signaling are called hormones, signaling molecules that are produced in one part of the body but affect other body regions some distance away.

Hormones travel the large distances between endocrine cells and their target cells via the bloodstream, which is a relatively slow way to move throughout the body. Because of their form of transport, hormones become diluted and are present in low concentrations when they act on their target cells. This is different from paracrine signaling, in which local concentrations of ligands can be very high.

Autocrine Signaling

Autocrine signals are produced by signaling cells that can also bind to the ligand that is released. This means the signaling cell and the target cell can be the same or a similar cell (the prefix *auto-* means self, a reminder that the signaling cell sends a signal to itself). This type of signaling often occurs during the early development of an organism to ensure that cells develop into the correct tissues and take on the proper function. Autocrine signaling also regulates pain sensation and inflammatory responses. Further, if a cell is infected with a virus, the cell can signal itself to undergo programmed cell death, killing the virus in the process. In some cases, neighboring cells of the same type are also influenced by the released ligand. In embryological development, this process of stimulating a group of neighboring cells may help to direct the differentiation of identical cells into the same cell type, thus ensuring the proper developmental outcome.

Direct Signaling Across Gap Junctions

Gap junctions in animals and *plasmodesmata* in plants are connections between the plasma membranes of neighboring cells. These fluid-filled channels allow small signaling molecules, called **intracellular mediators**, to diffuse between the two cells. Small molecules or ions, such as calcium ions (Ca^{2+}), are able to move between cells, but large molecules like proteins and DNA cannot fit through the channels. The specificity of

the channels ensures that the cells remain independent but can quickly and easily transmit signals. The transfer of signaling molecules communicates the current state of the cell that is directly next to the target cell; this allows a group of cells to coordinate their response to a signal that only one of them may have received. In plants, *plasmodesmata* are ubiquitous, making the entire plant into a giant communication network.

Types of Receptors

Receptors are protein molecules in the target cell or on its surface that bind ligand. There are two types of receptors, internal receptors and cell-surface receptors.

Internal receptors

Internal receptors, also known as intracellular or cytoplasmic receptors, are found in the cytoplasm of the cell and respond to hydrophobic ligand molecules that are able to travel across the plasma membrane. Once inside the cell, many of these molecules bind to proteins that act as regulators of mRNA synthesis (transcription) to mediate gene expression. Gene expression is the cellular process of transforming the information in a cell's DNA into a sequence of amino acids, which ultimately forms a protein. When the ligand binds to the internal receptor, a conformational change is triggered that exposes a DNA-binding site on the protein. The ligand-receptor complex moves into the nucleus, then binds to specific regulatory regions of the chromosomal DNA and promotes the initiation of transcription (Figure 9.4). Transcription is the process of copying the information in a cell's DNA into a special form of RNA called messenger RNA (mRNA); the cell uses information in the mRNA (which moves out into the cytoplasm and associates with ribosomes) to link specific amino acids in the correct order, producing a protein. Internal receptors can directly influence gene expression without having to pass the signal on to other receptors or messengers.

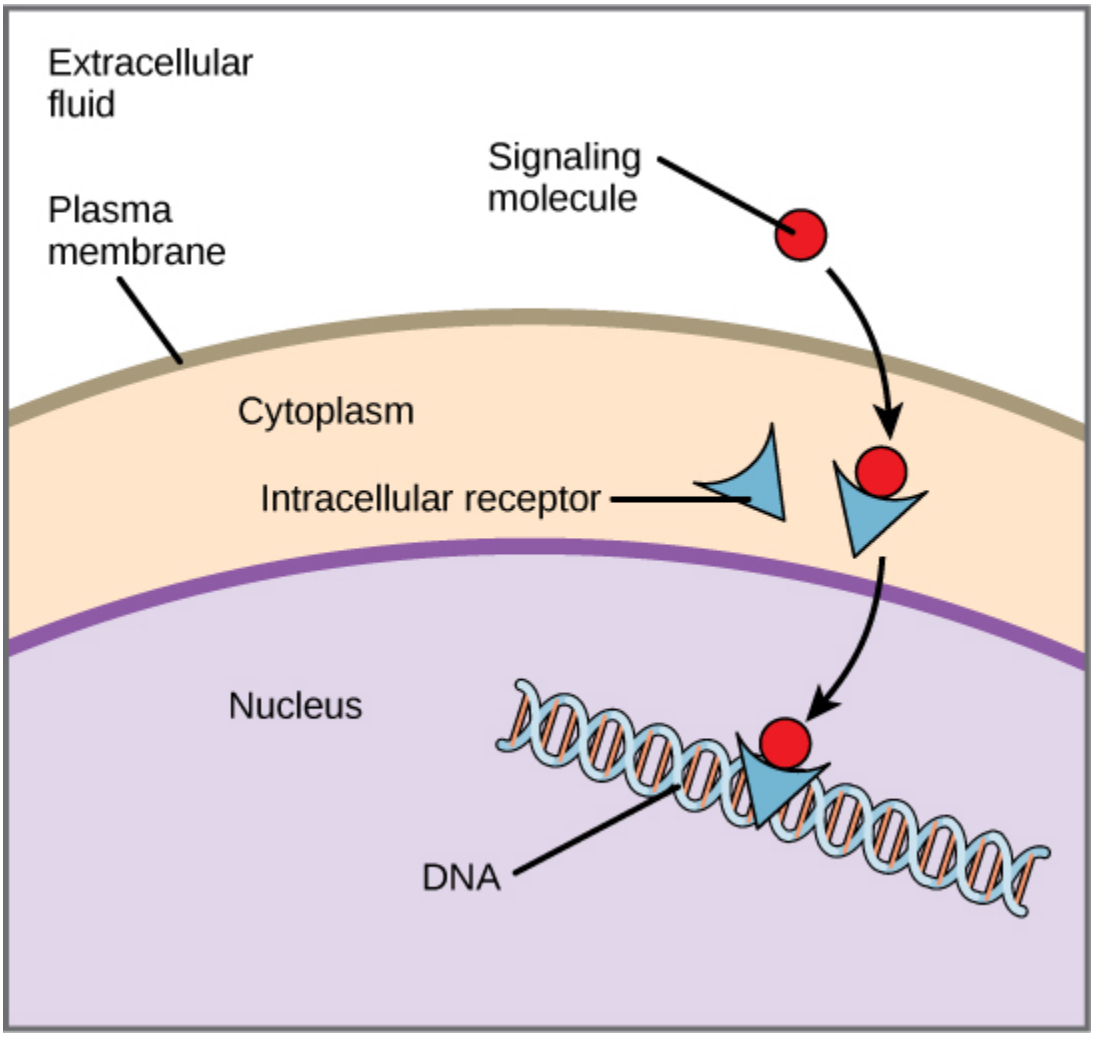


Figure 9.4 Hydrophobic signaling molecules typically diffuse across the plasma membrane and interact with intracellular receptors in the cytoplasm. Many intracellular receptors are transcription factors that interact with DNA in the nucleus and regulate gene expression.

Cell-Surface Receptors

Cell-surface receptors, also known as transmembrane receptors, are cell-surface, membrane-anchored (integral) proteins that bind to external ligand molecules. This type of receptor spans the plasma membrane and performs signal transduction, through which an extracellular signal is converted into an intracellular 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.

Because cell-surface receptor proteins are fundamental to normal cell functioning, it should come as no surprise that a malfunction in any one of these proteins could have severe consequences. Errors in the protein structures of certain receptor molecules have been shown to play a role in hypertension (high blood pressure), asthma, heart disease, and cancer.

Each cell-surface receptor has three main components: an external ligand-binding domain called the **extracellular domain**, a hydrophobic membrane-spanning region called a transmembrane domain, and an intracellular domain inside the cell. The size and extent of each of these domains vary widely, depending on the type of receptor.

Evolution Connection

How Viruses Recognize a Host

Unlike living cells, many viruses do not have a plasma membrane or any of the structures necessary to sustain metabolic life. Some viruses are simply composed of an inert protein shell enclosing 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) often 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.[1] Once a virus jumps the former "species barrier" 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.

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 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. In order to interact with the double layer of phospholipid fatty acid tails that form the center of the plasma membrane, many of the amino acids in the membrane-spanning region are hydrophobic in nature. Conversely, the amino acids that line the inside of the channel are hydrophilic to allow for the passage of water or ions. When a ligand binds to the extracellular region of the channel, there is a conformational change in the protein's structure that allows ions such as sodium, calcium, magnesium, and hydrogen to pass through (Figure 9.5).

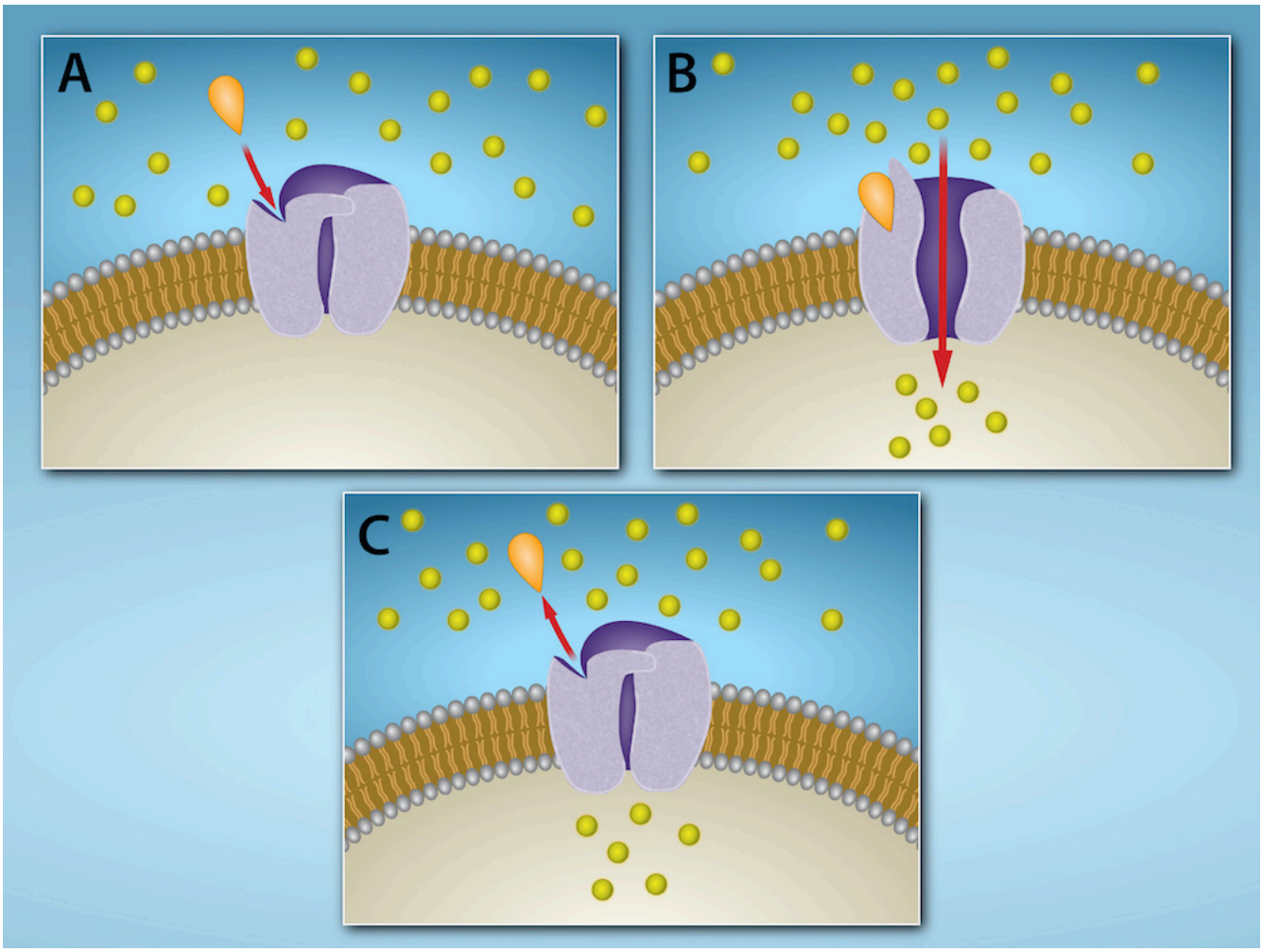


Figure 9.5 Gated ion channels located in the plasma membrane allow for the controlled flow of ions into and out of the cell. The channel protein remains closed until a signaling molecule (orange teardrop) binds to the channel protein. Then the channel protein changes conformation and allows ions (yellow circles) to flow into (or out of) the cell. When the signaling molecule is released, the channel protein resumes its closed conformation, preventing ion flow. Credit: Rao, A. and Fletcher, S. Department of Biology, Texas A&M University.

G-protein-linked 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 9.6). All

G-protein-linked receptors have seven transmembrane domains, but each receptor has its own specific extracellular domain and G-protein-binding site.

Cell signaling using G-protein-linked receptors occurs as a cyclic series of events. Before the ligand binds, the inactive G-protein can bind to a newly revealed site on the receptor specific for its binding. Once the G-protein binds to the receptor, the resulting change in shape activates the G-protein, which releases guanosine diphosphate (GDP) and picks up guanosine 3-phosphate (GTP). The subunits of the G-protein then split into the α subunit and the $\beta\gamma$ subunit. One or both of these G-protein fragments may be able to activate other proteins as a result. After a while, the GTP on the active α subunit of the G-protein is hydrolyzed to GDP and the $\beta\gamma$ subunit is deactivated. The subunits reassociate to form the inactive G-protein, and the cycle begins anew.

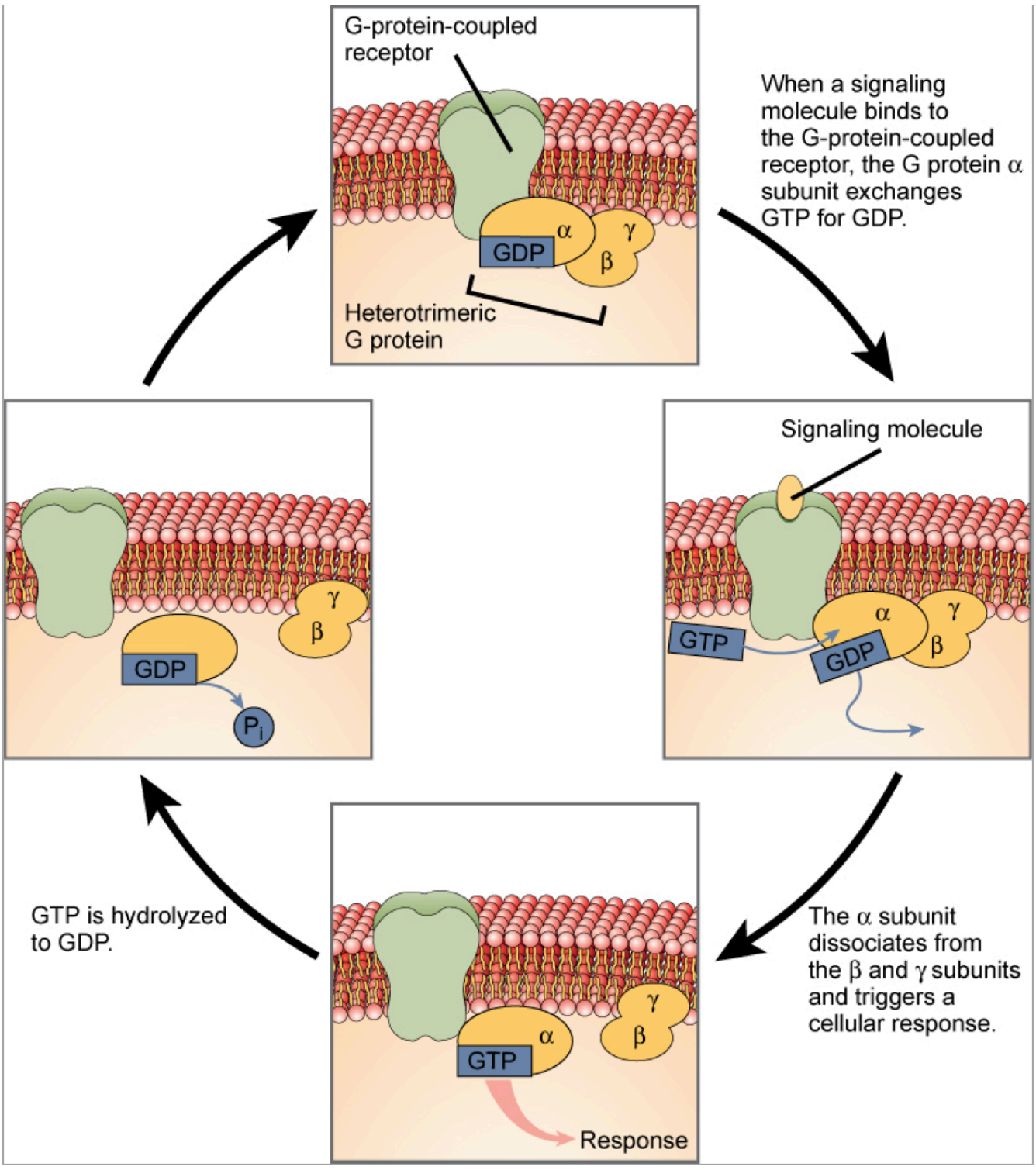
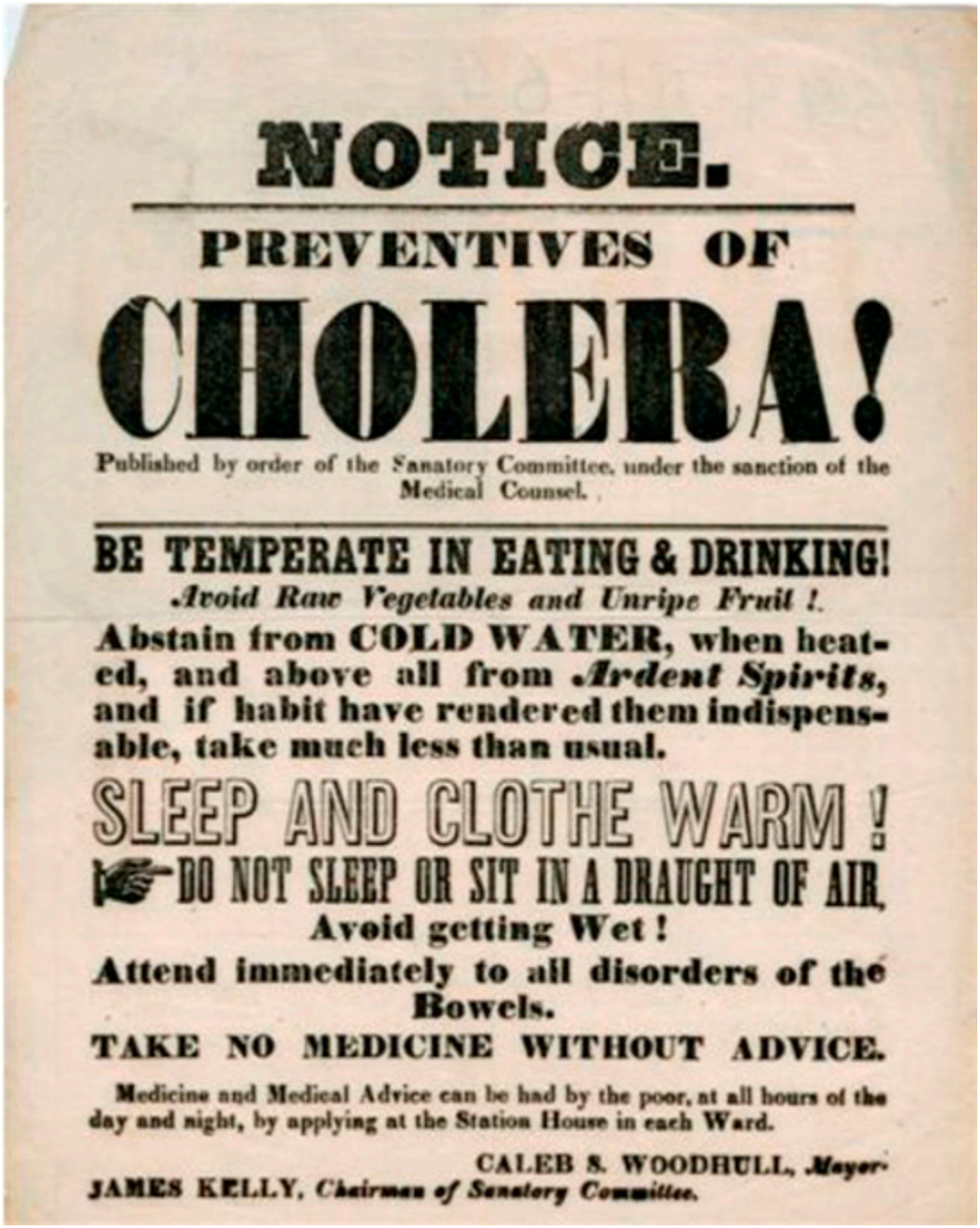


Figure 9.6 Heterotrimeric G-proteins have three subunits: α, β, and γ. When a signaling molecule binds to a G-protein-coupled receptor in the plasma membrane, a GDP molecule associated with the α subunit is exchanged for GTP. The β and γ subunits dissociate from the α subunit, and a cellular response is triggered either by the α subunit or the dissociated βγ pair. Hydrolysis of GTP to GDP terminates the signal.

G-protein-linked receptors have been extensively studied, and much has been learned about their roles in

maintaining health. Bacteria that are pathogenic to humans can release poisons that interrupt specific G-protein-linked receptor function, leading to illnesses such as pertussis, botulism, and cholera. In cholera (Figure 9.7), for example, the water-borne bacterium *Vibrio cholerae* produces a toxin, choleragen, that binds to cells lining the small intestine. The toxin then enters these intestinal cells, where it modifies a G-protein that controls the opening of a chloride channel and causes it to remain continuously active, resulting in large losses of fluids from the body and potentially fatal dehydration as a result.



NOTICE.

PREVENTIVES OF

CHOLERA!

Published by order of the Sanatory Committee, under the sanction of the Medical Counsel.

BE TEMPERATE IN EATING & DRINKING!

Avoid Raw Vegetables and Unripe Fruit !.

Abstain from COLD WATER, when heated, and above all from *Ardent Spirits*, and if habit have rendered them indispensable, take much less than usual.

SLEEP AND CLOTHE WARM !

DO NOT SLEEP OR SIT IN A DRAUGHT OF AIR.

Avoid getting Wet !

Attend immediately to all disorders of the Bowels.

TAKE NO MEDICINE WITHOUT ADVICE.

Medicine and Medical Advice can be had by the poor, at all hours of the day and night, by applying at the Station House in each Ward.

CALEB S. WOODHULL, *Mayor.*

JAMES KELLY, *Chairman of Sanatory Committee.*

Figure 9.7 Transmitted primarily through contaminated drinking water, cholera is a major cause of death in the developing world and in areas where natural disasters interrupt the availability of clean water. The cholera bacterium, Vibrio cholerae, creates a toxin that modifies G-protein-mediated cell signaling pathways in the intestines. Modern sanitation eliminates the threat of cholera outbreaks, such as the one that swept through New York City in 1866. This poster from that era shows how, at that time, the way that the disease was transmitted was not understood. (credit: New York City Sanitary Commission)

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. The enzyme-linked receptors normally have large extracellular and intracellular domains, but the membrane-spanning region consists of a single alpha-helical region of the peptide strand. 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. One example of this type of enzyme-linked receptor is the tyrosine kinase receptor (Figure 9.8). A kinase is an enzyme that transfers phosphate groups from ATP to another protein. The tyrosine kinase receptor transfers phosphate groups to tyrosine molecules (tyrosine residues). First, signaling molecules bind to the extracellular domain of two nearby tyrosine kinase receptors. The two neighboring receptors then bond together, or dimerize. Phosphates are then added to tyrosine residues on the intracellular domain of the receptors (phosphorylation). The phosphorylated residues can then transmit the signal to the next messenger within the cytoplasm.

Visual Connection

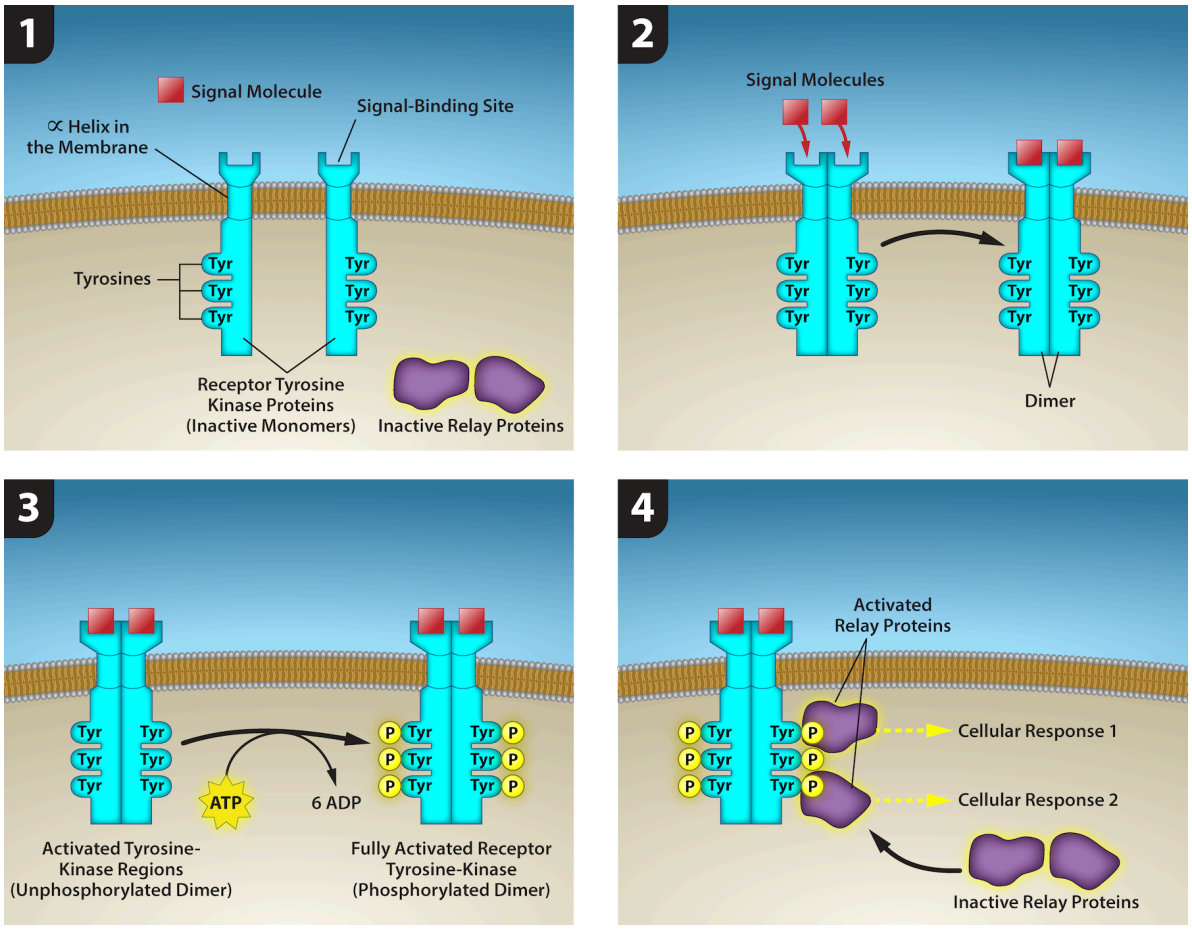


Figure 9.8 A receptor tyrosine kinase is an enzyme-linked receptor with a single helical transmembrane region, and extracellular and intracellular domains. 2) Binding of a signaling molecule to the extracellular domain causes the receptor to dimerize. 3) Tyrosine residues on the intracellular domain are then autophosphorylated, 4) triggering a downstream cellular response. The signal is terminated by a phosphatase that removes the phosphates from the phosphotyrosine residues. Credit: Rao, A., Ryan, K., Tag, A., Fletcher, S. and Hawkins, A. Department of Biology, Texas A&M University.

HER2 is a receptor tyrosine kinase. In 30 percent of human breast cancers, HER2 is permanently activated, resulting in unregulated cell division. Lapatinib, a drug used to treat breast cancer, inhibits HER2 receptor tyrosine kinase autophosphorylation (the process by which the receptor adds phosphates onto itself), thus reducing tumor growth by 50 percent. Besides autophosphorylation, which of the following steps would be inhibited by Lapatinib?

a. Signaling molecule binding, dimerization, and the downstream cellular response
b. Dimerization, and the downstream cellular response

c. The downstream cellular response
d. Phosphatase activity, dimerization, and the downstream cellular response

Signaling Molecules

Produced by signaling cells and the subsequent binding to receptors in target cells, ligands act as chemical signals that travel to the target cells to coordinate responses. The types of molecules that serve as ligands are incredibly varied and range from small proteins to small ions like calcium (Ca^{2+}).

Small Hydrophobic Ligands

Small hydrophobic ligands can directly diffuse through the plasma membrane and interact with internal receptors. Important members of this class of ligands are the steroid hormones. Steroids are lipids that have a hydrocarbon skeleton with four fused rings; different steroids have different functional groups attached to the carbon skeleton. Steroid hormones include the female sex hormone, estradiol, which is a type of estrogen; the male sex hormone, testosterone; and cholesterol, which is an important structural component of biological membranes and a precursor of steroid hormones (Figure 9.9). Other hydrophobic hormones include thyroid hormones and vitamin D. In order to be soluble in blood, hydrophobic ligands must bind to carrier proteins while they are being transported through the bloodstream.

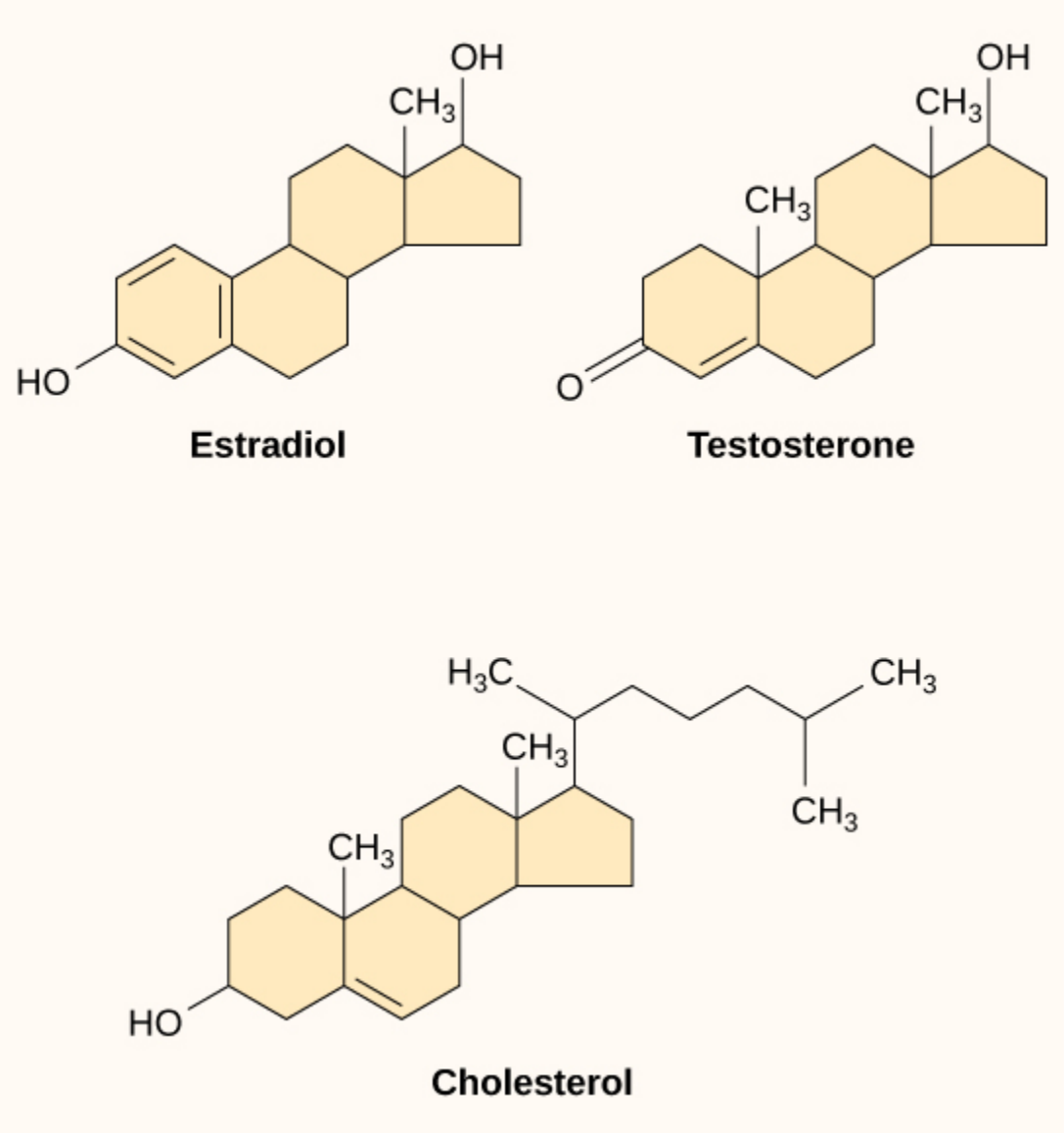


Figure 9.9 Steroid hormones have similar chemical structures to their precursor, cholesterol. Because these molecules are small and hydrophobic, they can diffuse directly across the plasma membrane into the cell, where they interact with internal receptors.

Water-Soluble Ligands

Water-soluble ligands are polar and, therefore, cannot pass through the plasma membrane unaided; sometimes, they are too large to pass through the membrane at all. Instead, most water-soluble ligands bind to the extracellular domain of cell-surface receptors. This group of ligands is quite diverse and includes small molecules, peptides, and proteins.

Other Ligands

Nitric oxide (NO) is a gas that also acts as a ligand. It is able to diffuse directly across the plasma membrane, and one of its roles is to interact with receptors in smooth muscle and induce relaxation of the tissue. NO has a very short half-life and, therefore, only functions over short distances. Nitroglycerin, a treatment for heart disease, acts by triggering the release of NO, which causes blood vessels to dilate (expand), thus restoring blood flow to the heart. NO has become better known recently because the pathway that it affects is targeted by prescription medications for erectile dysfunction, such as Viagra (erection involves dilated blood vessels).

Link to Learning

Review the video: Common cell signaling pathway

Footnotes

- 1A. B. Sigalov, The School of Nature. IV. Learning from Viruses, *Self/Nonself* 1, no. 4 (2010): 282-298. Y. Cao, X. Koh, L. Dong, X. Du, A. Wu, X. Ding, H. Deng, Y. Shu, J. Chen, T. Jiang, Rapid Estimation of Binding Activity of Influenza Virus Hemagglutinin to Human and Avian Receptors, *PLoS One* 6, no. 4 (2011): e18664.

86.

PROPAGATION OF THE SIGNAL

Learning Objectives

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

- Explain how the binding of a ligand initiates signal transduction throughout a cell
- Recognize the role of phosphorylation in the transmission of intracellular signals
- Evaluate the role of second messengers in signal transmission

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, which cannot interact with most components of the cell such as DNA. Only internal receptors are able to interact directly with DNA in the nucleus to initiate protein synthesis.

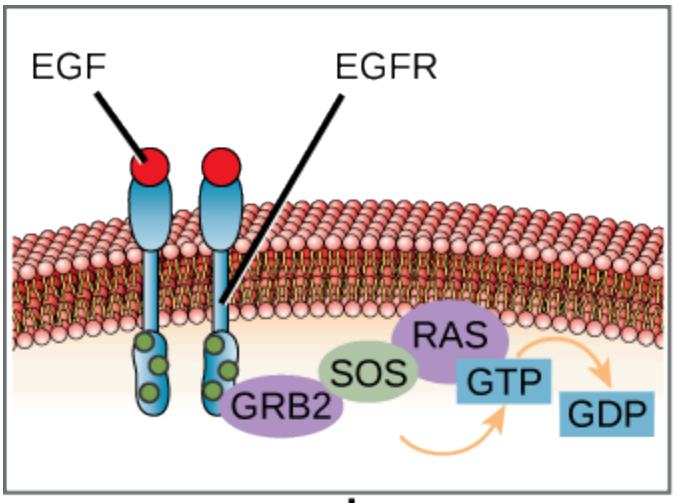
When a ligand binds to its receptor, conformational changes occur that affect the receptor's intracellular domain. Conformational changes of the extracellular domain upon ligand binding can propagate through the membrane region of the receptor and lead to activation of the intracellular domain or its associated proteins. In some cases, binding of the ligand causes **dimerization** of the receptor, which means that two receptors bind to each other to form a stable complex called a dimer. A **dimer** is a chemical compound formed when two molecules (often identical) join together. The binding of the receptors in this manner enables their intracellular domains to come into close contact and activate each other.

Binding Initiates a Signaling Pathway

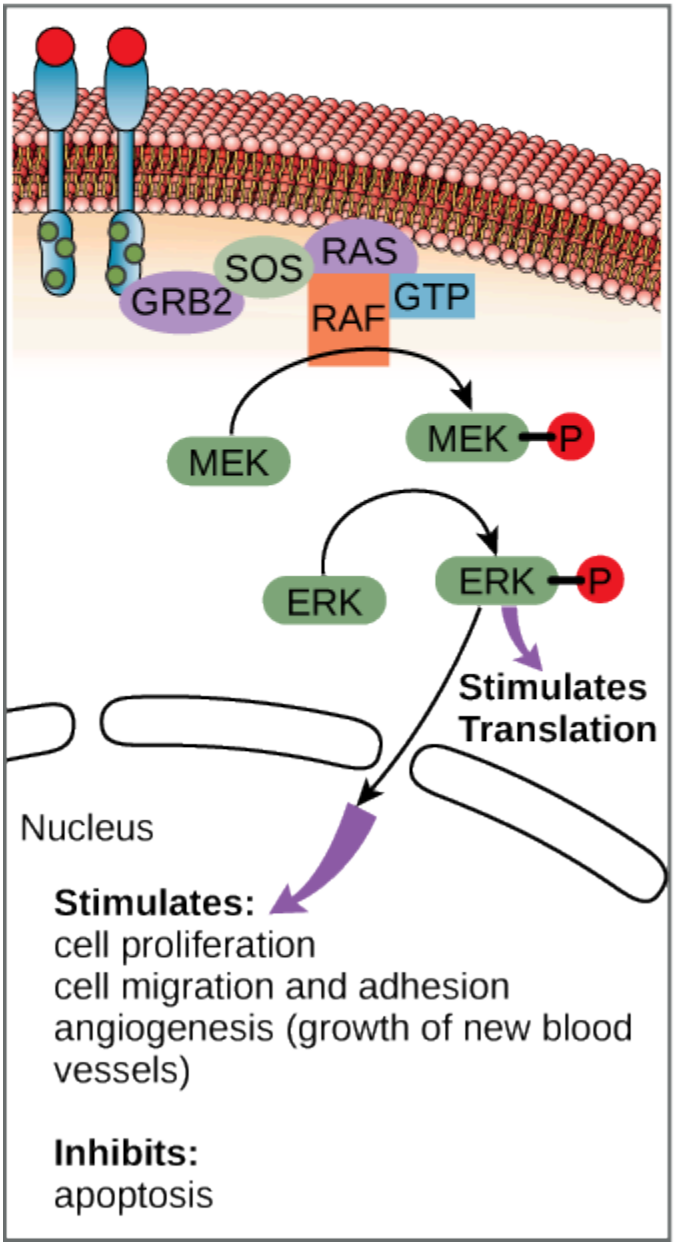
After the ligand binds to the cell-surface receptor, the activation of the receptor's intracellular components sets off a chain of events that is called a **signaling pathway**, sometimes called a signaling cascade. In a signaling

pathway, second messengers—enzymes—and activated proteins interact with specific proteins, which are in turn activated in a chain reaction that eventually leads to a change in the cell's environment (Figure 9.10), such as an increase in metabolism or specific gene expression. The events in the cascade occur in a series, much like a current flows in a river. Interactions that occur before a certain point are defined as upstream events, and events after that point are called downstream events.

Visual Connection



Upon binding of epidermal growth factor (EGF) to the EGF receptor (EGFR), two proteins associated with the receptor called GRB2 and SOS activate RAS, a small G-protein.



A protein kinase called RAF is activated by RAS-GTP. RAF phosphorylates MEK, which in turn phosphorylates ERK, a MAP kinase. The phosphorylated ERK enters the nucleus, where it triggers a cellular response.

Figure 9.10 The epidermal growth factor (EGF) receptor (EGFR) is a receptor tyrosine kinase involved in the regulation of cell growth, wound healing, and tissue repair. When EGF binds to the EGFR, a cascade of downstream events causes the cell to grow and divide. If EGFR is activated at inappropriate times, uncontrolled cell growth (cancer) may occur.

In certain cancers, the GTPase activity of the RAS G-protein is inhibited. This means that the RAS protein can no longer hydrolyze GTP into GDP. What effect would this have on downstream cellular events?

You can see that 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.

Link to Learning

Observe an animation of cell signaling at this site.

Explore videos on cell signaling at this site and this site.

Methods of Intracellular Signaling

The induction of a signaling pathway depends on the modification of a cellular component by an enzyme. There are numerous 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 phosphate can be added to a nucleotide such as GMP to form GDP or GTP. Phosphates are also often added to serine, threonine, and tyrosine residues of proteins, where they replace the hydroxyl group of the amino acid (Figure 9.11). The transfer of the phosphate is catalyzed by an enzyme called a **kinase**. Various kinases are named for the substrate they phosphorylate. Phosphorylation of serine and threonine residues often activates enzymes. Phosphorylation of tyrosine residues can either affect the activity of an enzyme or 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.

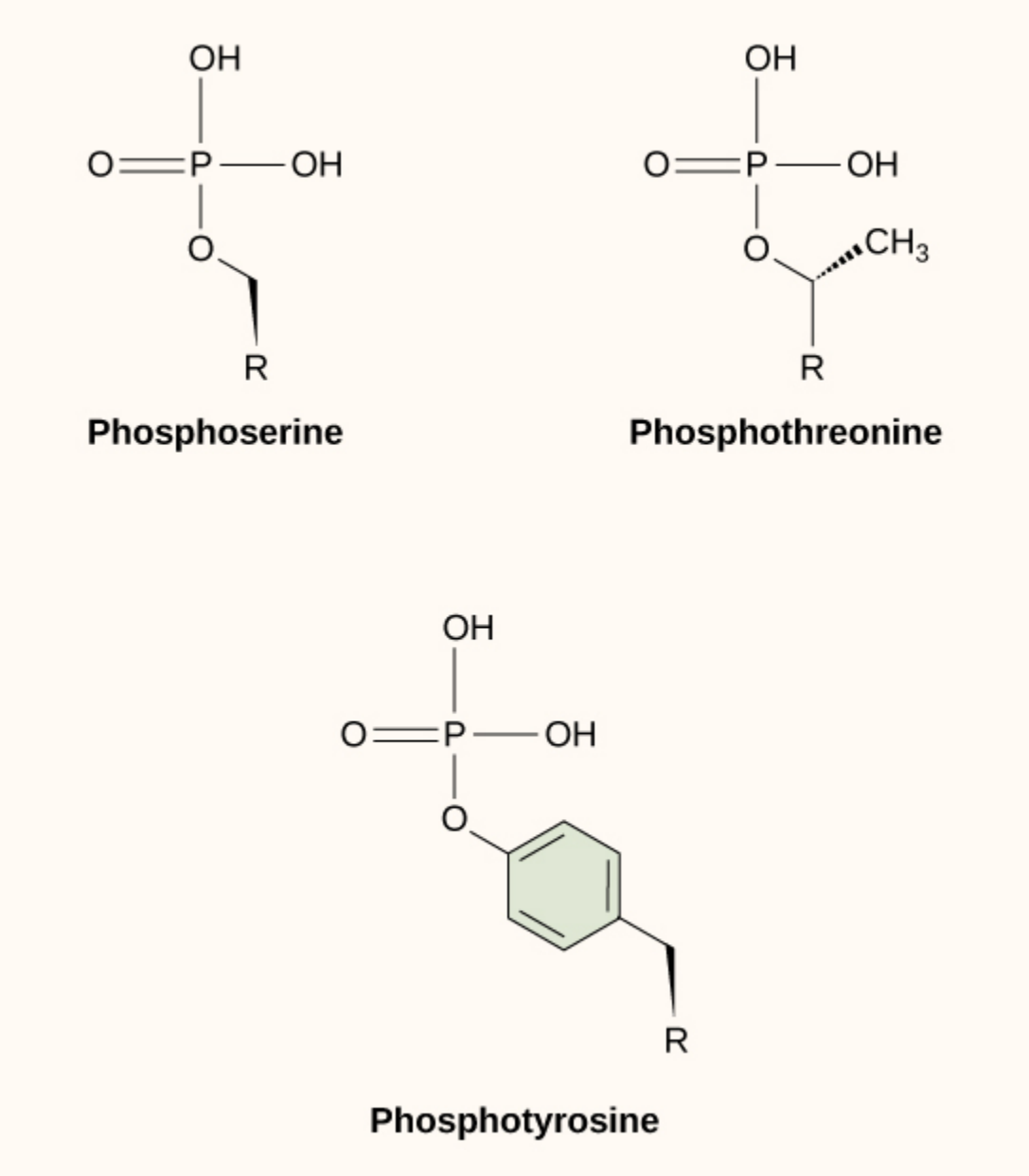


Figure 9.11 In protein phosphorylation, a phosphate group (PO4 -3) is added to residues of the amino acids serine, threonine, and tyrosine.

Second Messengers

Second messengers are small molecules that propagate a signal after it has been initiated by the binding of the signaling molecule to the receptor. These molecules help to spread a signal through the cytoplasm by altering the behavior of certain cellular proteins.

Calcium ion is a widely used second messenger. The free concentration of calcium ions (Ca^{2+}) within a cell is very low because ion pumps in the plasma membrane continuously remove it by using adenosine-5′-triphosphate (ATP). For signaling purposes, Ca^{2+} is stored in cytoplasmic vesicles, such as the

endoplasmic reticulum, or accessed from outside the cell. When signaling occurs, ligand-gated calcium ion channels allow the higher levels of Ca^{2+} that are present outside the cell (or in intracellular storage compartments) to flow into the cytoplasm, which raises the concentration of cytoplasmic Ca^{2+}. The response to the increase in Ca^{2+} varies and depends on the cell type involved. For example, in the β-cells of the pancreas, Ca^{2+} signaling leads to the release of insulin, and in muscle cells, an increase in Ca^{2+} leads to muscle contractions.

Another second messenger utilized in many different cell types is **cyclic AMP (cAMP)**. Cyclic AMP is synthesized by the enzyme adenylyl cyclase from ATP (Figure 9.12). The main role of cAMP in cells is to bind to and activate an enzyme called **cAMP-dependent kinase (A-kinase)**. A-kinase regulates many vital metabolic pathways: It phosphorylates serine and threonine residues of its target proteins, activating them in the process. A-kinase is found in many different types of cells, and the target proteins in each kind of cell are different. Differences give rise to the variation of the responses to cAMP in different cells.

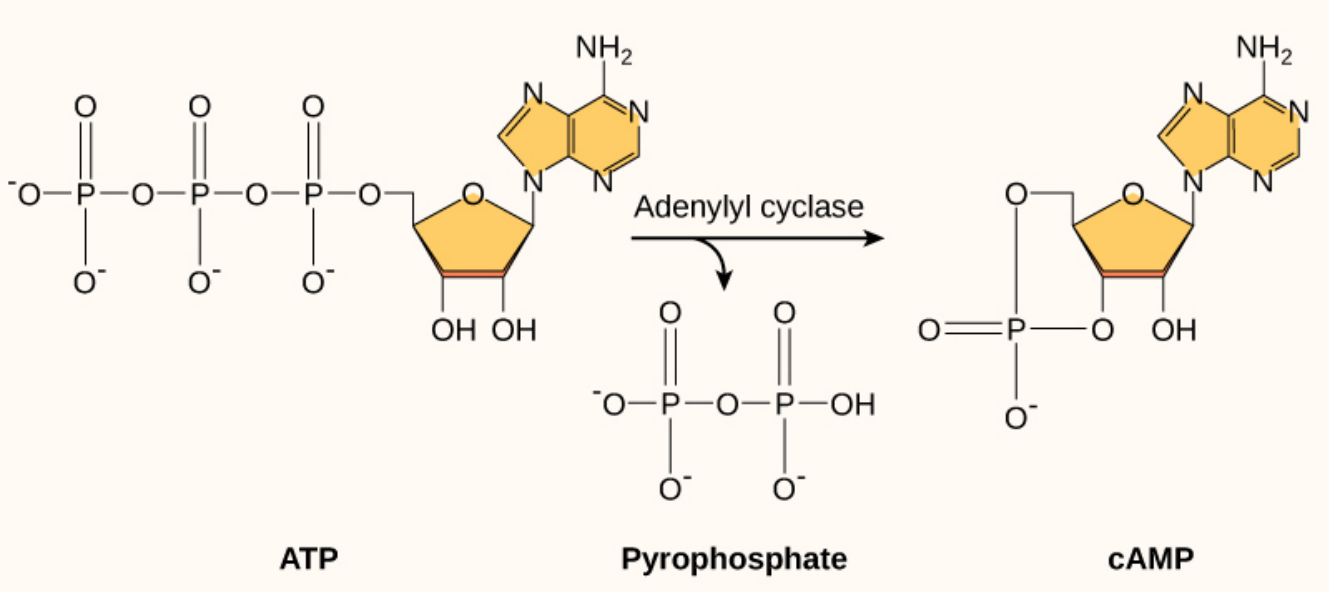


Figure 9.12 This diagram shows the mechanism for the formation of cyclic AMP (cAMP). cAMP serves as a second messenger to activate or inactivate proteins within the cell. Termination of the signal occurs when an enzyme called phosphodiesterase converts cAMP into AMP.

Present in small concentrations in the plasma membrane, **inositol phospholipids** are lipids that can also be converted into second messengers. Because these molecules are membrane components, they are located near membrane-bound receptors and can easily interact with them. Phosphatidylinositol (PI) is the main phospholipid that plays a role in cellular signaling. Enzymes known as kinases phosphorylate PI to form PI-phosphate (PIP) and PI-bisphosphate (PIP_2).

The enzyme phospholipase C cleaves PIP_2 to form **diacylglycerol (DAG)** and **inositol triphosphate (IP_3)** (Figure 9.13). These products of the cleavage of PIP_2 serve as second messengers. Diacylglycerol (DAG)

remains in the plasma membrane and activates protein kinase C (PKC), which then phosphorylates serine and threonine residues in its target proteins. IP_3 diffuses into the cytoplasm and binds to ligand-gated calcium channels in the endoplasmic reticulum to release Ca^{2+} that continues the signal cascade.

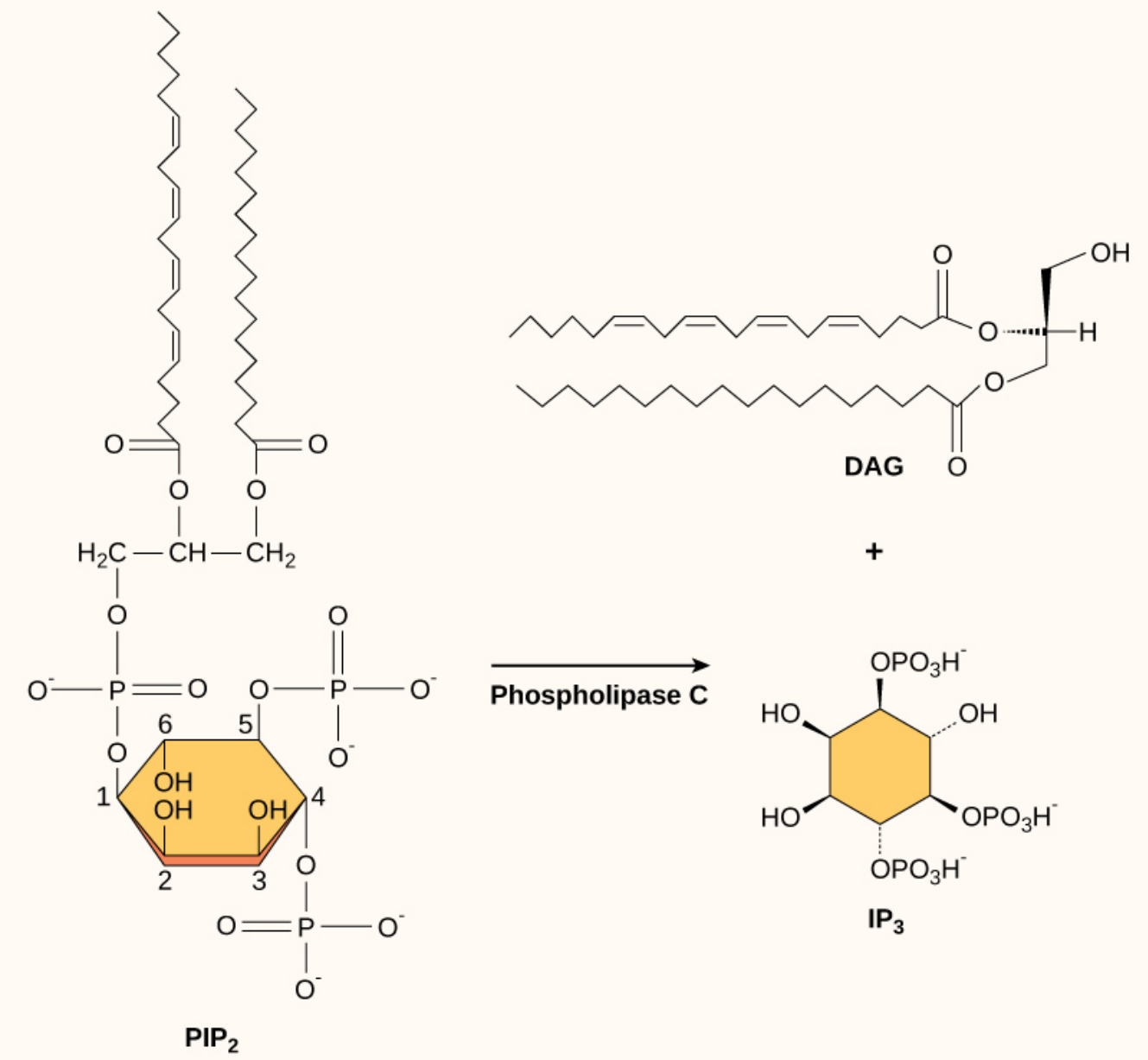


Figure 9.13 The enzyme phospholipase C breaks down PIP2 into IP3 and DAG, both of which serve as second messengers.

Link to Learning

Explore videos on intracellular signaling at this site and receptors and intracellular signaling at this site.

87.

RESPONSE TO THE SIGNAL

Learning Objectives

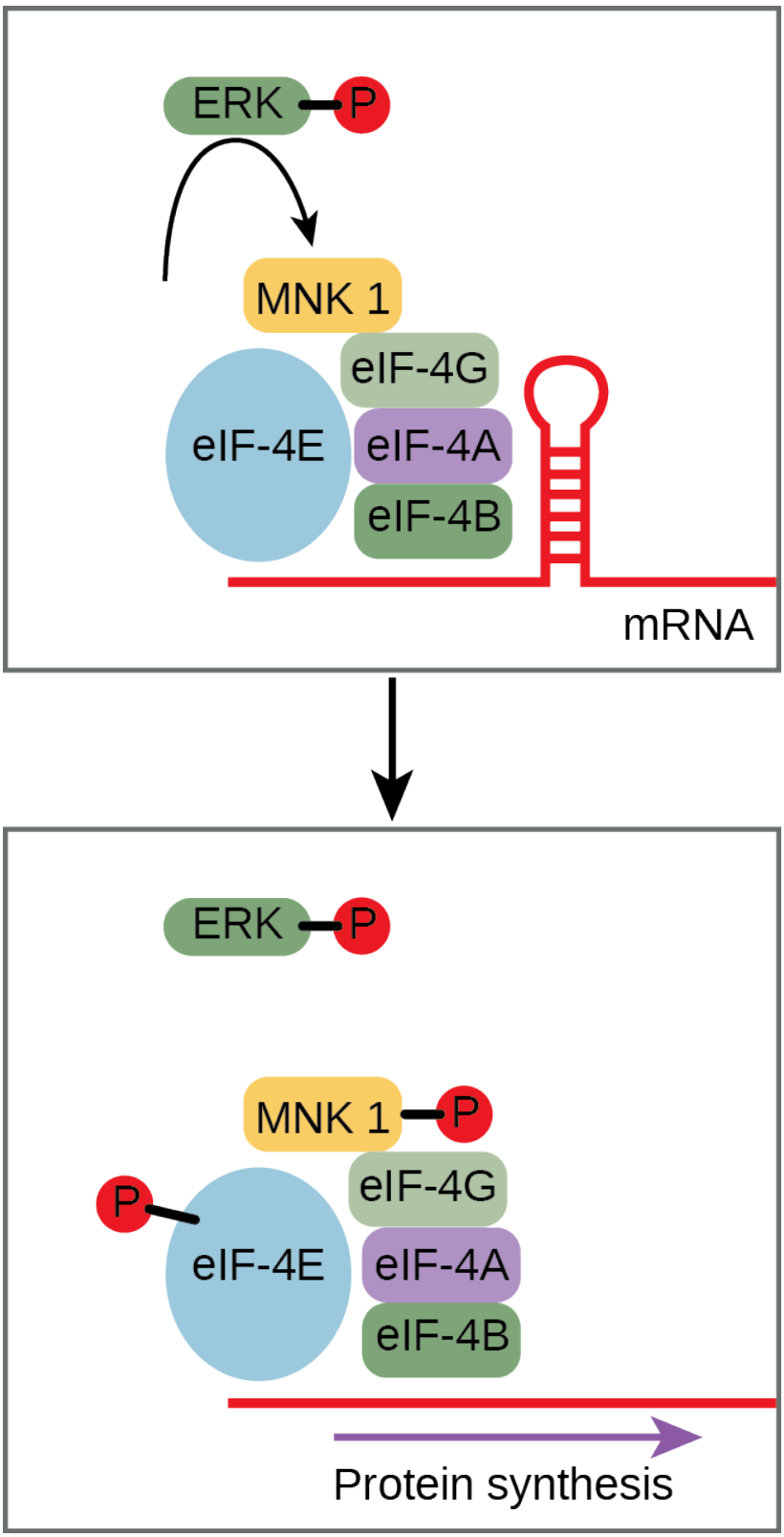
By the end of this section, you will be able to do the following:

- Describe how signaling pathways direct protein expression, cellular metabolism, and cell growth
- Identify the function of PKC in signal transduction pathways
- Recognize the role of apoptosis in the development and maintenance of a healthy organism

Inside the cell, ligands bind to their internal receptors, allowing them to directly affect the cell's DNA and protein-producing machinery. Using signal transduction pathways, receptors in the plasma membrane produce a variety of effects on the cell. 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 transcription of RNA. Others regulate the translation of proteins from mRNA. An example of a protein that regulates translation in the nucleus is the MAP kinase ERK. The MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the nuclear DNA. ERK is activated in a phosphorylation cascade when epidermal growth factor (EGF) binds the EGF receptor (see Figure 9.10). Upon phosphorylation, ERK enters the nucleus and activates a protein kinase that, in turn, regulates protein translation (Figure 9.14).



The MAP kinase ERK phosphorylates MNK1. MNK1 in turn phosphorylates eIF-4E, which is associated with mRNA. The mRNA unfolds and protein sythesis begins.

Figure 9.14 ERK is a MAP kinase that activates translation when it is phosphorylated. 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. (See Figure 9.10 for the phosphorylation pathway that activates

ERK.)

Another mechanism of gene regulation involves PKC, which is a protein that acts as an inhibitor. An **inhibitor** is a molecule that binds to a protein and prevents it from functioning or reduces its function. In this case, the inhibitor is a protein called Iκ-B, which binds to the regulatory protein NF-κB. (The symbol κ represents the Greek letter kappa.) When Iκ-B is bound to NF-κB, the complex cannot enter the nucleus of the cell, but when Iκ-B is phosphorylated by PKC, it can no longer bind NF-κB, and NF-κB (a transcription factor) can enter the nucleus and initiate RNA transcription. In this case, the effect of phosphorylation is to inactivate an inhibitor and thereby activate the process of transcription.

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 located on top of 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 its constituent glucose monomers. (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 9.15). 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.

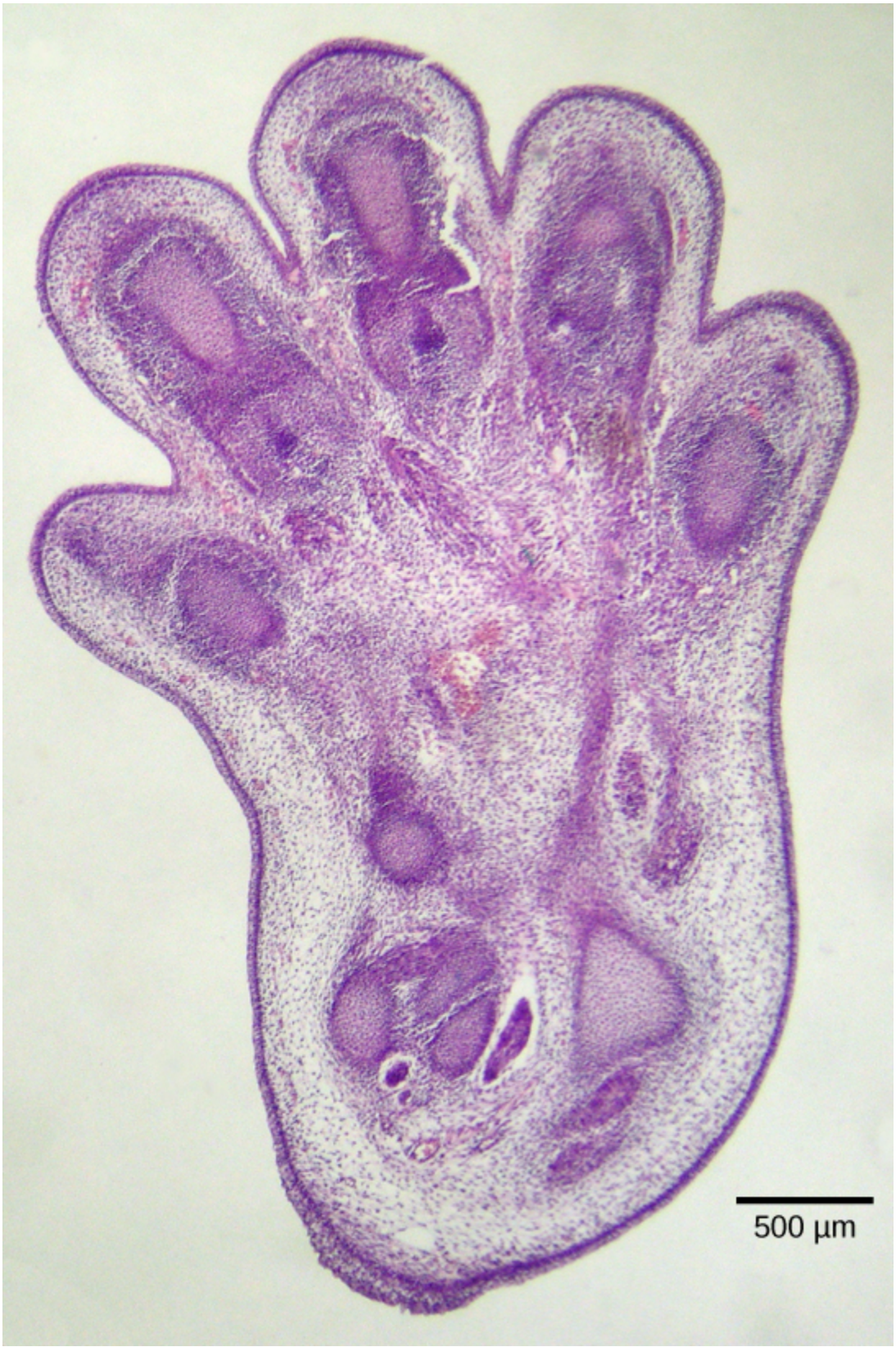


Figure 9.15 The histological section of a foot of a 15-day-old mouse embryo, visualized using light microscopy, reveals areas of tissue between the toes, which apoptosis will eliminate before the mouse reaches its full gestational age at 27 days. (credit:

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.

88.

SIGNALING IN SINGLE-CELLED ORGANISMS

Learning Objectives

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

- Describe how single-celled yeasts use cell signaling to communicate with one another
- Relate the role of quorum sensing to the ability of some bacteria to form biofilms

Within-cell signaling allows bacteria to respond to environmental cues, such as nutrient levels. Some single-celled organisms also release molecules to signal to each other.

Signaling in Yeast

Yeasts are eukaryotes (fungi), and the components and processes found in yeast signals are similar to those of cell-surface receptor signals in multicellular organisms. Budding yeasts (Figure 9.16) are able to participate in a process that is similar to sexual reproduction that entails two haploid cells (cells with one-half the normal number of chromosomes) combining to form a diploid cell (a cell with two sets of each chromosome, which is what normal body cells contain). In order to find another haploid yeast cell that is prepared to mate, budding yeasts secrete a signaling molecule called **mating factor**. When mating factor binds to cell-surface receptors in other yeast cells that are nearby, they stop their normal growth cycles and initiate a cell signaling cascade that includes protein kinases and GTP-binding proteins that are similar to G-proteins.

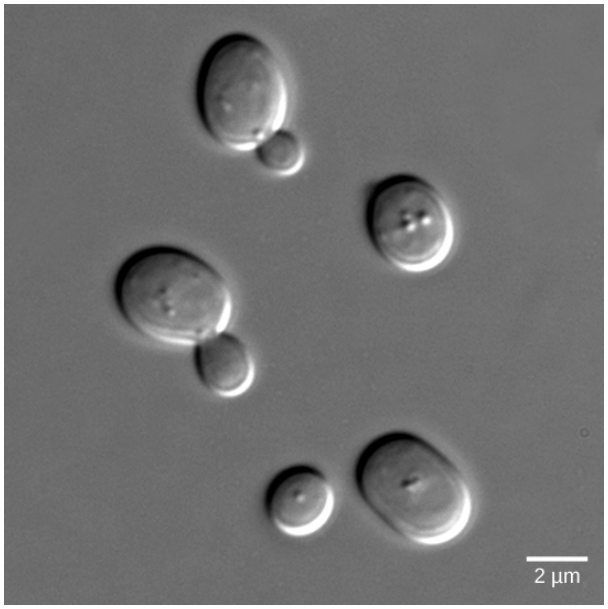


Figure 9.16 Budding Saccharomyces cerevisiae yeast cells can communicate by releasing a signaling molecule called mating factor. In this micrograph, they are visualized using differential interference contrast microscopy, a light microscopy technique that enhances the contrast of the sample.

Signaling in Bacteria

Signaling in bacteria enables bacteria to monitor extracellular conditions, ensure that there are sufficient amounts of nutrients, and ensure that hazardous situations are avoided. There are circumstances, however, when bacteria communicate with each other.

The first evidence of bacterial communication was observed in a bacterium that has a symbiotic relationship with Hawaiian bobtail squid. When the population density of the bacteria reaches a certain level, specific gene expression is initiated, and the bacteria produce bioluminescent proteins that emit light. Because the number of cells present in the environment (cell density) is the determining factor for signaling, bacterial signaling was named **quorum sensing**. In politics and business, a quorum is the minimum number of members required to be present to vote on an issue.

Quorum sensing uses autoinducers as signaling molecules. **Autoinducers** are signaling molecules secreted by bacteria to communicate with other bacteria of the same kind. The secreted autoinducers can be small, hydrophobic molecules, such as acyl-homoserine lactone (AHL), or larger peptide-based molecules; each type of molecule has a different mode of action. When AHL enters target bacteria, it binds to transcription factors, which then switch gene expression on or off. When the number of bacteria increases, so does the concentration of the autoinducer, triggering increased expression of certain genes, including autoinducers, which results in a self-amplifying cycle, also known as a positive feedback loop (Figure 9.17). The peptide

autoinducers stimulate more complicated signaling pathways that include bacterial kinases. The changes in bacteria following exposure to autoinducers can be quite extensive. The pathogenic bacterium *Pseudomonas aeruginosa* has 616 different genes that respond to autoinducers.

Some species of bacteria that use quorum sensing form biofilms, complex colonies of bacteria (often containing several species) that exchange chemical signals to coordinate the release of toxins that will attack the host. Bacterial biofilms (Figure 9.18) can sometimes be found on medical equipment; when biofilms invade implants such as hip or knee replacements or heart pacemakers, they can cause life-threatening infections.

Visual Connection

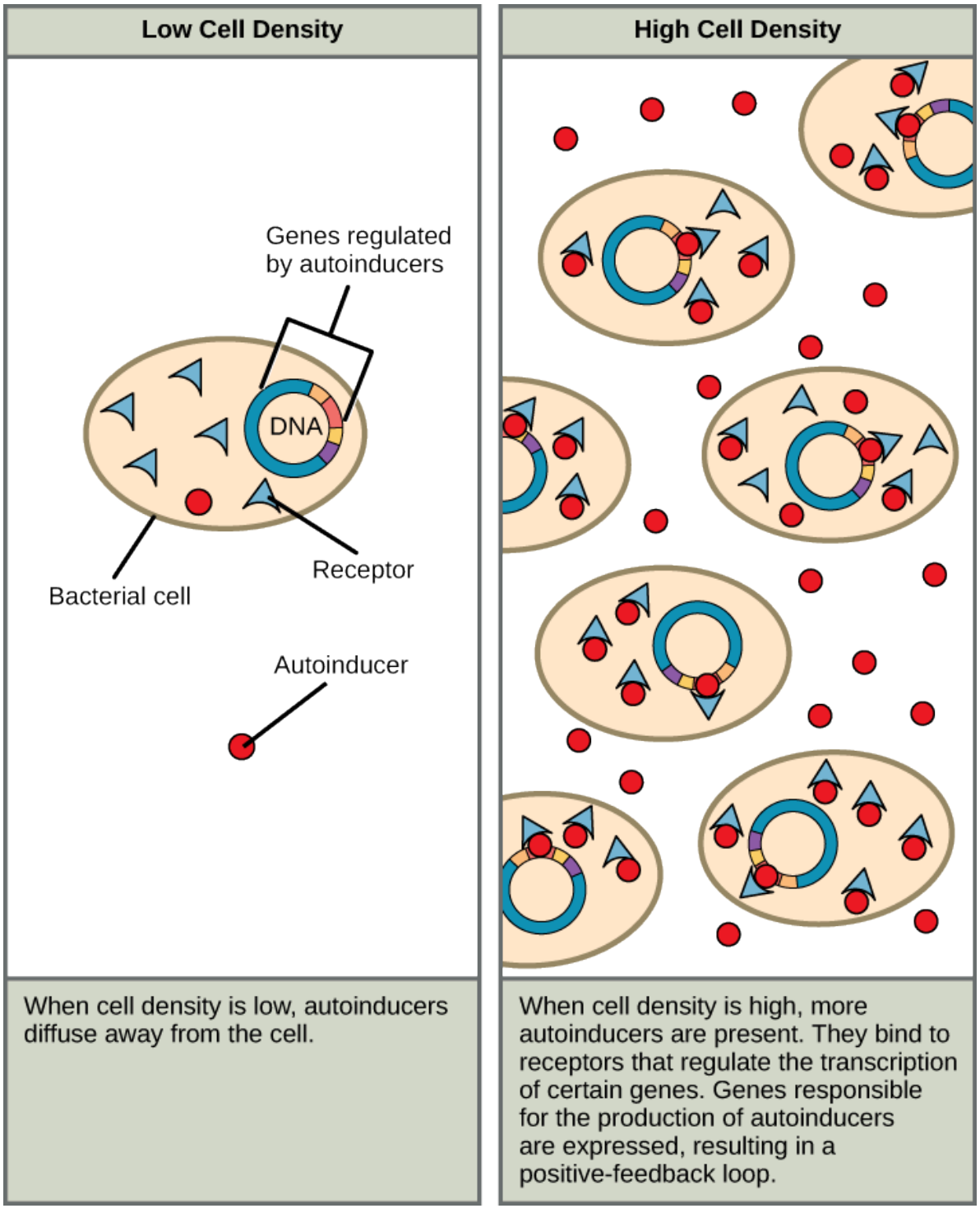


Figure 9.17 Autoinducers are small molecules or proteins produced by bacteria that regulate gene expression.

Which of the following statements about quorum sensing is false?

a. Autoinducer must bind to receptor to turn on transcription of genes responsible for the production of more autoinducer.
b. The receptor stays in the bacterial cell, but the autoinducer diffuses out.
c. Autoinducer can only act on a different cell: it cannot act on the cell in which it is made.
d. Autoinducer turns on genes that enable the bacteria to form a biofilm.

Visual Connection

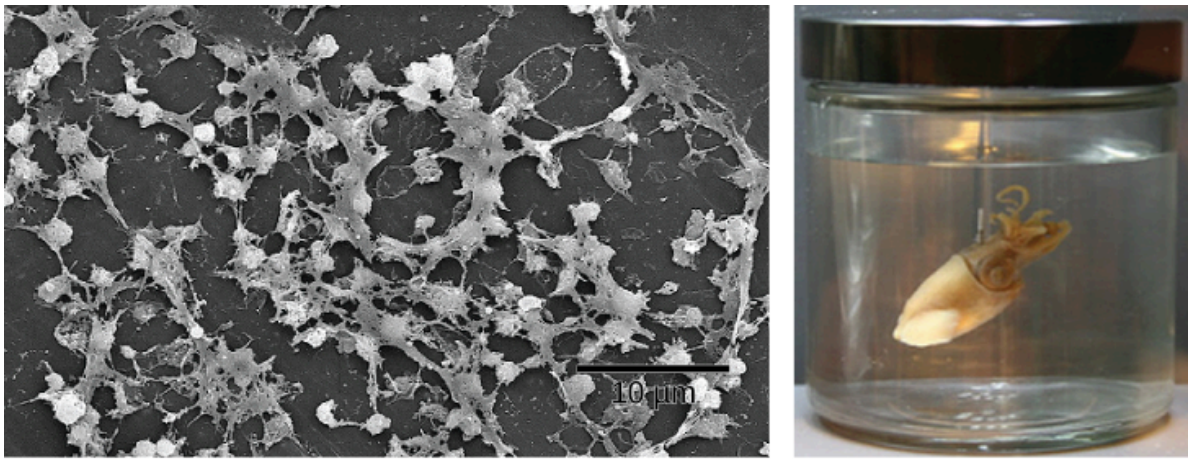


Figure 9.18 Cell-cell communication enables these (a) Staphylococcus aureus bacteria to work together to form a biofilm inside a hospital patient's catheter, seen here via scanning electron microscopy. S. aureus is the main cause of hospital-acquired infections. (b) Hawaiian bobtail squid have a symbiotic relationship with the bioluminescent bacteria Vibrio fischeri. The luminescence makes it difficult to see the squid from below because it effectively eliminates its shadow. In return for camouflage, the squid provides food for the bacteria. Free-living V. fischeri do not produce luciferase, the enzyme responsible for luminescence, but V. fischeri living in a symbiotic relationship with the squid do. Quorum sensing determines whether the bacteria should produce the luciferase enzyme. (credit a: modifications of work by CDC/Janice Carr; credit b: modifications of work by Cliff1066/Flickr)

What advantage might biofilm production confer on the *S. aureus* inside the catheter?

Research on the details of quorum sensing has led to advances in growing bacteria for industrial purposes.

Recent discoveries suggest that it may be possible to exploit bacterial signaling pathways to control bacterial growth; this process could replace or supplement antibiotics that are no longer effective in certain situations.

Link to Learning

Watch geneticist Bonnie Bassler discuss her discovery of quorum sensing in biofilm bacteria in squid.

Evolution Connection

Cellular Communication in Yeasts

The first cellular form of life on our planet likely consisted of single-celled prokaryotic organisms that had limited interaction with each other. While some external signaling occurs between different species of single-celled organisms, the majority of signaling within bacteria and yeasts concerns only other members of the same species. The evolution of cellular communication is an absolute necessity for the development of multicellular organisms, and this innovation is thought to have required approximately 2 billion years to appear in early life forms.

Yeasts are single-celled eukaryotes and, therefore, have a nucleus and organelles characteristic of more complex life forms. Comparisons of the genomes of yeasts, nematode worms, fruit flies, and humans illustrate the evolution of increasingly complex signaling systems that allow for the efficient inner workings that keep humans and other complex life forms functioning correctly.

Kinases are a major component of cellular communication, and studies of these enzymes illustrate the evolutionary connectivity of different species. Yeasts have 130 types of kinases. More complex organisms such as nematode worms and fruit flies have 454 and 239 kinases, respectively. Of the 130 kinase types in yeast, 97 belong to the 55 subfamilies of kinases that are found in other eukaryotic organisms. The only obvious deficiency seen in yeasts is the complete absence of tyrosine kinases. It is hypothesized that phosphorylation of tyrosine residues is needed to control the more sophisticated functions of development, differentiation, and cellular communication used in multicellular organisms.

Because yeasts contain many of the same classes of signaling proteins as humans, these

organisms are ideal for studying signaling cascades. Yeasts multiply quickly and are much simpler organisms than humans or other multicellular animals. Therefore, the signaling cascades are also simpler and easier to study, although they contain similar counterparts to human signaling.[2]

Link to Learning

Watch this collection of interview clips with biofilm researchers in "What Are Bacterial Biofilms?"

Click to view content

Footnotes

- 2G. Manning, G.D. Plowman, T. Hunter, S. Sudarsanam, "Evolution of Protein Kinase Signaling from Yeast to Man," *Trends in Biochemical Sciences* 27, no. 10 (2002): 514–520.

89.

KEY TERMS

apoptosis
: programmed cell death

autocrine signal
: signal that is sent and received by the same or similar nearby cells

autoinducer
: signaling molecule secreted by bacteria to communicate with other bacteria of its kind

cell-surface receptor
: cell-surface protein that transmits a signal from the exterior of the cell to the interior, even though the ligand does not enter the cell

chemical synapse
: small space between axon terminals and dendrites of nerve cells where neurotransmitters function

cyclic AMP (cAMP)
: second messenger that is derived from ATP

cyclic AMP-dependent kinase
: (also, protein kinase A, or PKA) kinase that is activated by binding to cAMP

diacylglycerol (DAG)
: cleavage product of PIP_2 that is used for signaling within the plasma membrane

dimer
: chemical compound formed when two molecules join together

dimerization
: (of receptor proteins) interaction of two receptor proteins to form a functional complex called a dimer

endocrine cell
: cell that releases ligands involved in endocrine signaling (hormones)

endocrine signal
: long-distance signal that is delivered by ligands (hormones) traveling through an organism's circulatory system from the signaling cell to the target cell

enzyme-linked receptor
: cell-surface receptor with intracellular domains that are associated with membrane-bound enzymes

extracellular domain
: region of a cell-surface receptor that is located on the cell surface

G-protein-linked receptor
: cell-surface receptor that activates membrane-bound G-proteins to transmit a signal from the receptor to nearby membrane components

growth factor
: ligand that binds to cell-surface receptors and stimulates cell growth

inhibitor
: molecule that binds to a protein (usually an enzyme) and keeps it from functioning

inositol phospholipid
: lipid present at small concentrations in the plasma membrane that is converted into a second messenger; it has inositol (a carbohydrate) as its hydrophilic head group

inositol triphosphate (IP_3)
: cleavage product of PIP_2 that is used for signaling within the cell

intercellular signaling
: communication between a cell

internal receptor
: (also, intracellular receptor) receptor protein that is located in the cytosol of a cell and binds to ligands that pass through the plasma membrane

intracellular mediator
: (also, second messenger) small molecule that transmits signals within a cell

intracellular signaling
: communication within cells

ion channel-linked receptor
: cell-surface receptor that forms a plasma membrane channel, which opens when a ligand binds to the extracellular domain (ligand-gated channels)

kinase
: enzyme that catalyzes the transfer of a phosphate group from ATP to another molecule

ligand
: molecule produced by a signaling cell that binds with a specific receptor, delivering a signal in the process

mating factor
: signaling molecule secreted by yeast cells to communicate to nearby yeast cells that they are available to mate

neurotransmitter
: chemical ligand that carries a signal from one nerve cell to the next

paracrine signal
: signal between nearby cells that is delivered by ligands traveling in the liquid medium in the space between the cells

phosphatase
enzyme that removes the phosphate group from a molecule that has been previously phosphorylated

phosphodiesterase
enzyme that degrades cAMP, producing AMP, to terminate signaling

quorum sensing
method of cellular communication used by bacteria that informs them of the abundance of similar (or different) bacteria in the environment

receptor
protein in or on a target cell that bind to ligands

second messenger
small, non-protein molecule that propagates a signal within the cell after activation of a receptor causes its release

signal integration
interaction of signals from two or more different cell-surface receptors that merge to activate the same response in the cell

signal transduction
propagation of the signal through the cytoplasm (and sometimes also the nucleus) of the cell

signaling cell
cell that releases signal molecules that allow communication with another cell

signaling pathway
(also signaling cascade) chain of events that occurs in the cytoplasm of the cell to propagate the signal from the plasma membrane to produce a response

synaptic signal
chemical signal (neurotransmitter) that travels between nerve cells

target cell
cell that has a receptor for a signal or ligand from a signaling cell

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CHAPTER SUMMARY

9.1 Signaling Molecules and Cellular Receptors

Cells communicate by both inter- and intracellular signaling. Signaling cells secrete ligands that bind to target cells and initiate a chain of events within the target cell. The four categories of signaling in multicellular organisms are paracrine signaling, endocrine signaling, autocrine signaling, and direct signaling across gap junctions. Paracrine signaling takes place over short distances. Endocrine signals are carried long distances through the bloodstream by hormones, and autocrine signals are received by the same cell that sent the signal or other nearby cells of the same kind. Gap junctions allow small molecules, including signaling molecules, to flow between neighboring cells.

Internal receptors are found in the cell cytoplasm. Here, they bind ligand molecules that cross the plasma membrane; these receptor-ligand complexes move to the nucleus and interact directly with cellular DNA. Cell-surface receptors transmit a signal from outside the cell to the cytoplasm. Ion channel-linked receptors, when bound to their ligands, form a pore through the plasma membrane through which certain ions can pass. G-protein-linked receptors interact with a G-protein on the cytoplasmic side of the plasma membrane, promoting the exchange of bound GDP for GTP and interacting with other enzymes or ion channels to transmit a signal. Enzyme-linked receptors transmit a signal from outside the cell to an intracellular domain of a membrane-bound enzyme. Ligand binding causes activation of the enzyme. Small hydrophobic ligands (like steroids) are able to penetrate the plasma membrane and bind to internal receptors. Water-soluble hydrophilic ligands are unable to pass through the membrane; instead, they bind to cell-surface receptors, which transmit the signal to the inside of the cell.

9.2 Propagation of the Signal

Ligand binding to the receptor allows for signal transduction through the cell. The chain of events that conveys the signal through the cell is called a signaling pathway or cascade. Signaling pathways are often very complex because of the interplay between different proteins. A major component of cell signaling cascades is the phosphorylation of molecules by enzymes known as kinases. Phosphorylation adds a phosphate group to serine, threonine, and tyrosine residues in a protein, changing their shapes, and activating or inactivating the protein. Small molecules like nucleotides can also be phosphorylated. Second messengers are small,

non-protein molecules that are used to transmit a signal within a cell. Some examples of second messengers are calcium ions (Ca^{2+}), cyclic AMP (cAMP), diacylglycerol (DAG), and inositol triphosphate (IP_3).

9.3 Response to the Signal

The initiation of a signaling pathway is a response to external stimuli. This response can take many different forms, including protein synthesis, a change in the cell's metabolism, cell growth, or even cell death. Many pathways influence the cell by initiating gene expression, and the methods utilized are quite numerous. Some pathways activate enzymes that interact with DNA transcription factors. Others modify proteins and induce them to change their location in the cell. Depending on the status of the organism, cells can respond by storing energy as glycogen or fat, or making it available in the form of glucose. A signal transduction pathway allows muscle cells to respond to immediate requirements for energy in the form of glucose. Cell growth is almost always stimulated by external signals called growth factors. Uncontrolled cell growth leads to cancer, and mutations in the genes encoding protein components of signaling pathways are often found in tumor cells. Programmed cell death, or apoptosis, is important for removing damaged or unnecessary cells. The use of cellular signaling to organize the dismantling of a cell ensures that harmful molecules from the cytoplasm are not released into the spaces between cells, as they are in uncontrolled death, necrosis. Apoptosis also ensures the efficient recycling of the components of the dead cell. Termination of the cellular signaling cascade is very important so that the response to a signal is appropriate in both timing and intensity. Degradation of signaling molecules and dephosphorylation of phosphorylated intermediates of the pathway by phosphatases are two ways to terminate signals within the cell.

9.4 Signaling in Single-Celled Organisms

Yeasts and multicellular organisms have similar signaling mechanisms. Yeasts use cell-surface receptors and signaling cascades to communicate information on mating with other yeast cells. The signaling molecule secreted by yeasts is called mating factor.

Bacterial signaling is called quorum sensing. Bacteria secrete signaling molecules called autoinducers that are either small, hydrophobic molecules or peptide-based signals. The hydrophobic autoinducers, such as AHL, bind transcription factors and directly affect gene expression. The peptide-based molecules bind kinases and initiate signaling cascades in the cells.

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VISUAL CONNECTION QUESTIONS

1. Figure 9.8 HER2 is a receptor tyrosine kinase. In 30 percent of human breast cancers, HER2 is permanently activated, resulting in unregulated cell division. Lapatinib, a drug used to treat breast cancer, inhibits HER2 receptor tyrosine kinase autophosphorylation (the process by which the receptor adds phosphates onto itself), thus reducing tumor growth by 50 percent. Besides autophosphorylation, which of the following steps would be inhibited by Lapatinib?

a. Signaling molecule binding, dimerization, and the downstream cellular response.
b. Dimerization, and the downstream cellular response.
c. The downstream cellular response.
d. Phosphatase activity, dimerization, and the downstream cellular response.

2. Figure 9.10 In certain cancers, the GTPase activity of the RAS G-protein is inhibited. This means that the RAS protein can no longer hydrolyze GTP into GDP. What effect would this have on downstream cellular events?

3. Figure 9.17 Which of the following statements about quorum sensing is false?

a. Autoinducer must bind to receptor to turn on transcription of genes responsible for the production of more autoinducer.
b. The receptor stays in the bacterial cell, but the autoinducer diffuses out.
c. Autoinducer can only act on a different cell: it cannot act on the cell in which it is made.
d. Autoinducer turns on genes that enable the bacteria to form a biofilm.

4. Figure 9.18 What advantage might biofilm production confer on the S. aureus inside the catheter?

92.

REVIEW QUESTIONS

5. What property prevents the ligands of cell-surface receptors from entering the cell?

a. The molecules bind to the extracellular domain.
b. The molecules are hydrophilic and cannot penetrate the hydrophobic interior of the plasma membrane.
c. The molecules are attached to transport proteins that deliver them through the bloodstream to target cells.
d. The ligands are able to penetrate the membrane and directly influence gene expression upon receptor binding.

6. The secretion of hormones by the pituitary gland is an example of _____.

a. autocrine signaling
b. paracrine signaling
c. endocrine signaling
d. direct signaling across gap junctions

7. Why are ion channels necessary to transport ions into or out of a cell?

a. Ions are too large to diffuse through the membrane.
b. Ions are charged particles and cannot diffuse through the hydrophobic interior of the membrane.
c. Ions do not need ion channels to move through the membrane.
d. Ions bind to carrier proteins in the bloodstream, which must be removed before transport into the cell.

8. Endocrine signals are transmitted more slowly than paracrine signals because _____.

a. the ligands are transported through the bloodstream and travel greater distances
b. the target and signaling cells are close together
c. the ligands are degraded rapidly
d. the ligands don't bind to carrier proteins during transport

9. A scientist notices that when she adds a small, water-soluble molecule to a dish of cells, the cells turn off transcription of a gene. She hypothesizes that the ligand she added binds to a(n) _____ receptor.

a. Intracellular
b. Hormone
c. Enzyme-linked
d. Gated ion channel-linked

10. Where do DAG and IP_3 originate?

a. They are formed by phosphorylation of cAMP.
b. They are ligands expressed by signaling cells.
c. They are hormones that diffuse through the plasma membrane to stimulate protein production.
d. They are the cleavage products of the inositol phospholipid, PIP_2.

11. What property enables the residues of the amino acids serine, threonine, and tyrosine to be phosphorylated?

a. They are polar.
b. They are non-polar.
c. They contain a hydroxyl group.
d. They occur more frequently in the amino acid sequence of signaling proteins.

12. Histamine binds to the H1 G-protein-linked receptor to initiate the itchiness and airway constriction associated with an allergic response. If a mutation in the associated G-protein's alpha subunit prevented the hydrolysis of GTP how would the allergic response change?

a. More severe allergic response compared to normal G-protein signaling.
b. Less severe allergic response compared to normal G-protein signaling.
c. No allergic response.
d. No change compared to normal G-protein signaling.

13. A scientist observes a mutation in the transmembrane region of EGFR that eliminates its ability to be stabilized by binding interactions during dimerization after ligand binding. Which hypothesis regarding the effect of this mutation on EGF signaling is most likely to be correct?

a. EGF signaling cascades would be active for longer in the cell.
b. EGF signaling cascades would be active for a shorter period of time in the cell.

c. EGF signaling cascades would not occur.
d. EGF signaling would be unaffected.

14. What is the function of a phosphatase?

a. A phosphatase removes phosphorylated amino acids from proteins.
b. A phosphatase removes the phosphate group from phosphorylated amino acid residues in a protein.
c. A phosphatase phosphorylates serine, threonine, and tyrosine residues.
d. A phosphatase degrades second messengers in the cell.

15. How does NF-κB induce gene expression?

a. A small, hydrophobic ligand binds to NF-κB, activating it.
b. Phosphorylation of the inhibitor Iκ-B dissociates the complex between it and NF-κB, and allows NF-κB to enter the nucleus and stimulate transcription.
c. NF-κB is phosphorylated and is then free to enter the nucleus and bind DNA.
d. NF-κB is a kinase that phosphorylates a transcription factor that binds DNA and promotes protein production.

16. Apoptosis can occur in a cell when the cell is _____.

a. damaged
b. no longer needed
c. infected by a virus
d. all of the above

17. What is the effect of an inhibitor binding an enzyme?

a. The enzyme is degraded.
b. The enzyme is activated.
c. The enzyme is inactivated.
d. The complex is transported out of the cell.

18. How does PKC's signaling role change in response to growth factor signaling versus an immune response?

a. PKC interacts directly with signaling molecules in both cascades, but only exhibits kinase activity during growth factor signaling.
b. PKC interacts directly with signaling molecules in growth factor cascades, but interacts with signaling

inhibitors during immune signaling.

c. PKC amplifies growth factor cascades, but turns off immune cascades.
d. PKC is activated during growth factor cascades, but is inactivated during immune response cascades.

19. A scientist notices that a cancer cell line fails to die when they add an inducer of apoptosis to his culture of cells. Which hypothesis could explain why the cells fail to die?

a. The cells have a mutation that prevents the initiation of apoptosis signaling.
b. The cells have lost expression of the receptor for the apoptosis-inducing ligand.
c. The cells overexpress a growth factor pathway that inhibits apoptosis.
d. All of the above.

20. Which type of molecule acts as a signaling molecule in yeasts?

a. steroid
b. autoinducer
c. mating factor
d. second messenger

21. Quorum sensing is triggered to begin when _____.

a. treatment with antibiotics occurs
b. bacteria release growth hormones
c. bacterial protein expression is switched on
d. a sufficient number of bacteria are present

22. A doctor is researching new ways to treat biofilms on artificial joints. Which approach would best help prevent bacterial colonization of the medical implants?

a. Increase antibiotic dosing
b. Create implants with rougher surfaces
c. Vaccinate patients against all pathogenic bacteria
d. Inhibit quorum sensing

93.

CRITICAL THINKING QUESTIONS

23. What is the difference between intracellular signaling and intercellular signaling?

24. How are the effects of paracrine signaling limited to an area near the signaling cells?

25. What are the differences between internal receptors and cell-surface receptors?

26. Cells grown in the laboratory are mixed with a dye molecule that is unable to pass through the plasma membrane. If a ligand is added to the cells, observations show that the dye enters the cells. What type of receptor did the ligand bind to on the cell surface?

27. Insulin is a hormone that regulates blood sugar by binding to its receptor, insulin receptor tyrosine kinase. How does insulin's behavior differ from steroid hormone signaling, and what can you infer about its structure?

28. The same second messengers are used in many different cells, but the response to second messengers is different in each cell. How is this possible?

29. What would happen if the intracellular domain of a cell-surface receptor was switched with the domain from another receptor?

30. If a cell developed a mutation in its MAP2K1 gene (encodes the MEK protein) that prevented MEK from being recognized by phosphatases, how would the EGFR signaling cascade and the cell's behavior change?

31. What is a possible result of a mutation in a kinase that controls a pathway that stimulates cell growth?

32. How does the extracellular matrix control the growth of cells?

33. A scientist notices that a cancer cell line shows high levels of phosphorylated ERK in the absence of EGF. What are two possible explanations for the increase in phosphorylated ERK? Be specific in which proteins are involved.

34. What characteristics make yeasts a good model for learning about signaling in humans?

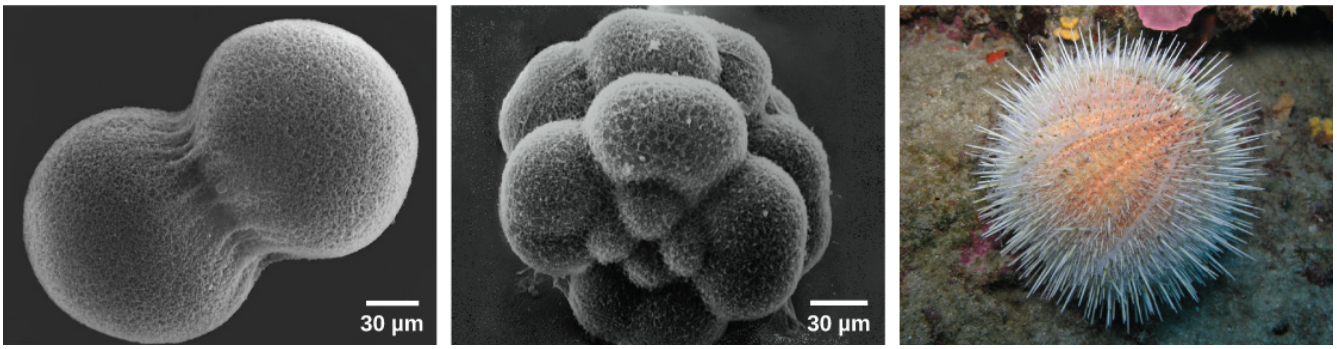
35. Why is signaling in multicellular organisms more complicated than signaling in single-celled organisms?

36. Pseudomonas infections are very common in hospital settings. Why would it be important for doctors to determine the bacterial load before treating an infected patient?

PART X
CELL REPRODUCTION

94.

INTRODUCTION



(a) **(b)** **(c)**

Figure 10.1 A sea urchin begins life as a single diploid cell (zygote) that (a) divides through cell division to form two genetically identical daughter cells, visible here through scanning electron microscopy (SEM). After four rounds of cell division, (b) there are 16 cells, as seen in this SEM image. After many rounds of cell division, the individual develops into a complex, multicellular organism, as seen in this (c) mature sea urchin. (credit a: modification of work by Evelyn Spiegel, Louisa Howard; credit b: modification of work by Evelyn Spiegel, Louisa Howard; credit c: modification of work by Marco Busdraghi; scale-bar data from Matt Russell)

Cell division is critical to life. When a prokaryotic cell divides by mitosis, it has basically reproduced a new organism. Single-celled eukaryotic organisms may also use cell division as their method of reproduction. A multicellular eukaryotic organism, on the other hand, has a more complex reproduction. Sexually reproducing organisms reproduce by cell division called meiosis, which produces sperms and eggs. A human, like every sexually reproducing organism, begins life as a fertilized egg (embryo), or **zygote**. In our species, billions of cell divisions subsequently must occur in a controlled manner in order to produce a complex, multicellular human comprising trillions of cells. Thus, the original single-celled zygote is literally the ancestor of all cells in the body. However, once a human is fully grown, cell reproduction is still necessary to repair and regenerate tissues, and sometimes to increase our size! In fact, all multicellular organisms use cell division for growth and the maintenance and repair of cells and tissues. Cell division is closely regulated, and the occasional failure of this regulation can have life-threatening consequences.

95.

CELL DIVISION

Learning Objectives

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

- Describe the structure of prokaryotic and eukaryotic genomes
- Describe the mechanisms of chromosome compaction

The continuity of life is the ability of organisms to reproduce their own kind. Thus, cell division from one cell to another has its foundation in the cell cycle. The cell cycle is an orderly sequence of events that describes the stages of a cell's life from the division of a single parent cell to the production of two new genetically identical daughter cells.

Genomic DNA

Before discussing the steps a cell must undertake to replicate and divide its DNA, a deeper understanding of the structure and function of a cell's genetic information is necessary. DNA is the mainstay of the genetic material present in a cell or organism. A cell's DNA comprises the complete set of genes packaged as a double-stranded DNA molecule, commonly called its **genome**. Prokaryotes (domains Archaea and Bacteria) are single-celled organisms that lack a nucleus. They have a single piece of circular DNA in the nucleoid area of the cell. In prokaryotes, which lack a nucleus, the genome is composed of a single, double-stranded DNA molecule in the form of a loop or circle found in a region or area called the nucleoid (Figure 10.2). This single, double-stranded DNA molecule must not be confused with separate smaller loops of DNA called plasmids that can be found in some of these cells but are not essential for normal growth. Bacteria can exchange these plasmids with other bacteria, sometimes receiving beneficial new genes that the recipient can add to their chromosomal DNA.

Antibiotic resistance is one such trait that often spreads through a bacterial colony through plasmid exchange from resistant donors to recipient cells. Naturally occurring plasmids are usually very small, containing a few useful additional genes during certain conditions. However, their study has enabled bacterial transformation involving the design of artificial or synthetic plasmids, which are widely used in recombinant DNA technology as vectors in molecular cloning.

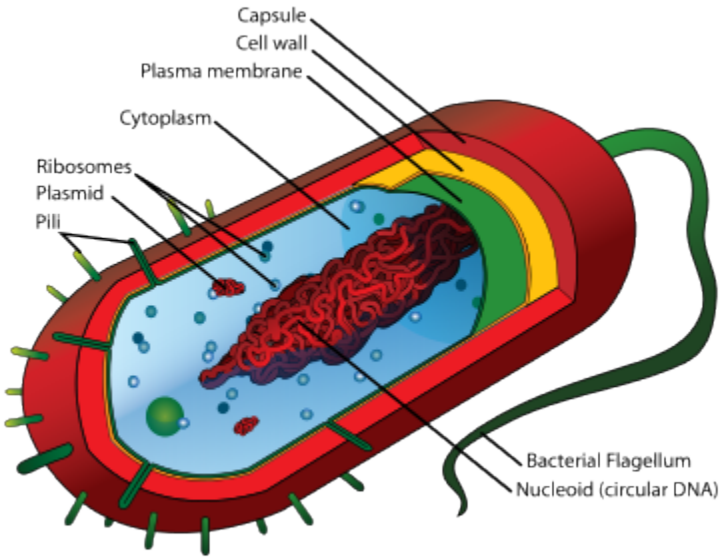


Figure 10.2 Prokaryotes, including both Bacteria and Archaea, have a single, circular chromosome located in a central region called the nucleoid. (https://en.wikipedia.org/wiki/Wikipedia:Featured_pictures/Other_lifeforms/Bacteria)

In eukaryotes, a nucleus is present and encloses the genome, which consists of several double-stranded linear DNA molecules (Figure 10.3). Each species of eukaryotes has a characteristic number of chromosomes in the nuclei of its cells. Human body (**somatic**) cells have 46 chromosomes, while human **gametes** (sperm or eggs) have 23 chromosomes each. The number of chromosomes is not the same among all species. For example, it is 48 in chimpanzees and 18 in cabbage plants. A typical body cell contains two matched or homologous sets of chromosomes (one set from each biological parent)—a configuration known as **diploid**. (Note: 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 chromosomes are called gametes, or sex cells; these are egg and sperm, and are designated *1n*, or **haploid**.

Upon fertilization, each gamete contributes one set of chromosomes, creating a diploid cell containing matched pairs of chromosomes called **homologous** ("same knowledge") **chromosomes**.

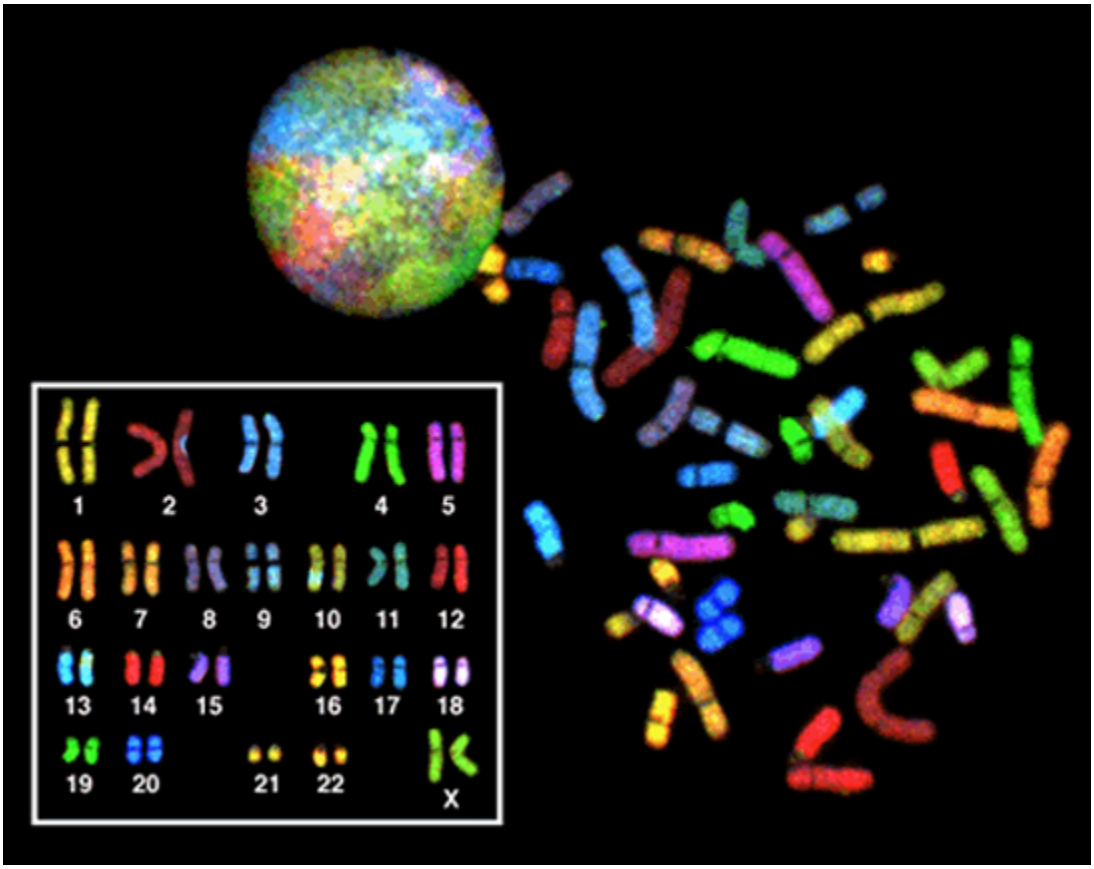


Figure 10.3 There are 23 pairs of homologous chromosomes in a female human somatic cell. The condensed chromosomes are viewed within the nucleus (top), removed from a cell during mitosis (also called karyokinesis or nuclear division) and spread out on a slide (right), and artificially arranged according to length (left); an arrangement like this is called a karyotype. In this image, the chromosomes were exposed to fluorescent stains for differentiation of the different chromosomes. A method of staining called "chromosome painting" employs fluorescent dyes that highlight chromosomes in different colors. (credit: National Human Genome Project/NIH)

Apparently minor variations of traits, such as blood type, eye color, and handedness, contribute to the natural variation found within a species, but even though they seem minor, these traits may be connected with the expression of other traits as of yet unknown characteristics. However, if the entire DNA sequence from any pair of human homologous chromosomes is compared, the difference is much less than one percent. The sex chromosomes, X and Y, are the single exception to the rule of homologous chromosome uniformity: Other than a small amount of homology that is necessary to accurately produce gametes, the genes found on the X and Y chromosomes are different.

Link to Learning

An explanation of the sex chromosomes, x and Y being the single exception to the rule of homologous chromosome uniformity, is available via this video.

Eukaryotic Chromosomal Structure and Compaction

Chromosomes are the basic structures into which the DNA molecules are packaged. In simple terms, the overall DNA length in a eukaryotic cell is enormous. Take a human cell, for example. If the DNA from all 46 chromosomes in its nucleus were laid out end-to-end, it would measure approximately two meters, a length about 250,000 times greater than the cell's diameter; however, its diameter would be only 2 nm! Considering that the size of a typical human cell is about 10 μm (100,000 cells lined up to equal one meter), DNA must be *tightly packaged* to fit in the cell's nucleus. At the same time, it must also be readily available and accessible for the genes to be expressed. For this reason, the long strands of DNA are condensed into compact chromosomes during certain stages of the cell cycle. There are a number of ways that chromosomes are compacted.

In the first level of compaction, short stretches of the DNA double helix wrap around a core of eight **histone proteins** at regular intervals along the entire length of the chromosome (Figure 10.4). The DNA-histone complex is called chromatin. The beadlike, histone DNA complex is called a **nucleosome**, and DNA connecting the nucleosomes is called linker DNA. A DNA molecule in this form is about seven times shorter than the double helix without the histones, and the beads are about 10 nm in diameter, in contrast with the 2-nm diameter of a DNA double helix.

The entire complex structure of DNA and proteins that functions as the building blocks of chromosomes is referred to as chromatin. This second level of compaction occurs as the nucleosomes and the linker DNA between them coil into a 30-nm chromatin fiber. This coiling further *condenses* the chromosome so that it is now about 50 times shorter than the extended form.

In the third level of compaction, a variety of *fibrous proteins* are used to "pack the chromatin." These fibrous proteins also ensure that each chromosome in a non-dividing cell occupies a particular area of the nucleus that does not overlap with that of any other chromosome (see the second top image in Figure 10.4).

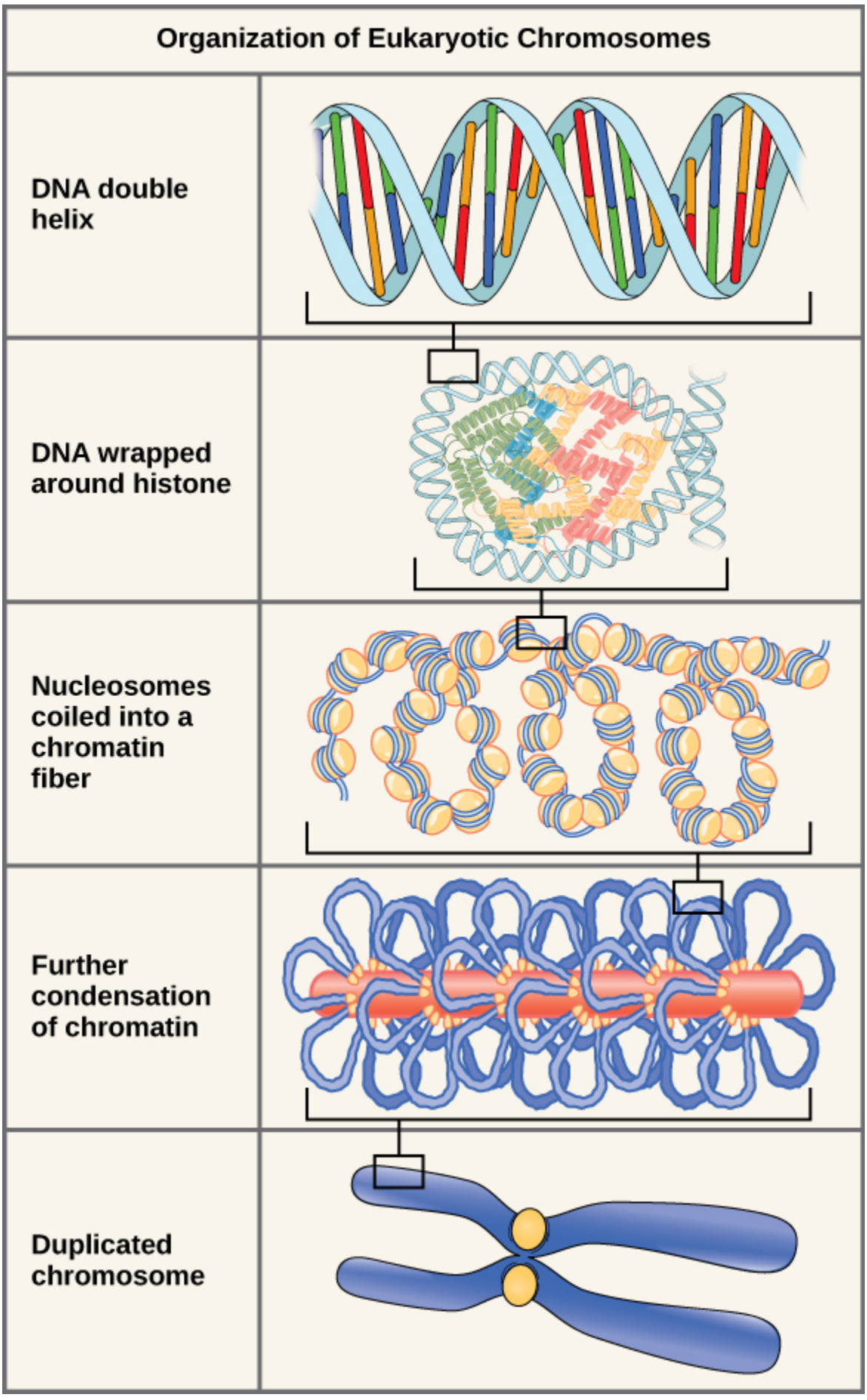

Organization of Eukaryotic Chromosomes
DNA double helix
DNA wrapped around histone
Nucleosomes coiled into a chromatin fiber
Further condensation of chromatin
Duplicated chromosome

Figure 10.4 Double-stranded DNA wraps around histone proteins to form nucleosomes that create the appearance of "beads on a string." The nucleosomes are coiled into a 30-nm chromatin fiber. When a cell undergoes mitosis, the chromosomes condense even further.

DNA replicates in the S phase of interphase, which technically is not a part of mitosis, but must always precede it. After replication, the chromosomes are composed of two linked sister **chromatids**. When fully compact, the pairs of identically packed chromosomes are bound to each other by cohesin proteins. The connection between the sister chromatids is closest in a region called the **centromere**. The conjoined sister chromatids, with a diameter of about 1 μm, are visible under a light microscope. The centromeric region is highly condensed and is visible as a constricted area.

Link to Learning

This animation illustrates the different levels of chromosome packing.

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96.

THE CELL CYCLE

Learning Objectives

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

- Describe the three stages of interphase
- Discuss the behavior of chromosomes during karyokinesis/mitosis
- Explain how the cytoplasmic content is divided during cytokinesis
- Define the quiescent G_0 phase

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 nuclear and cytoplasmic division that ultimately produces two identical (clone) cells. In both prokaryotic and eukaryotic cell divisions, there is reproduction of identical genetic material and their distribution into two daughter cells. The cell cycle has two major phases: interphase, which is about 90% of the cycle and alternates with the mitotic phase, which is usually the shortest part of the cell cycle (Figure 10.5). During **interphase**, the cell grows and DNA is replicated. During the **mitotic phase**, the replicated DNA and cytoplasmic contents are separated, and the cell cytoplasm is typically partitioned by a third process of the cell cycle called **cytokinesis**. We should note, however, that interphase and mitosis (karyokinesis) may take place without cytokinesis, in which case cells with multiple nuclei (multinucleate cells) are produced.

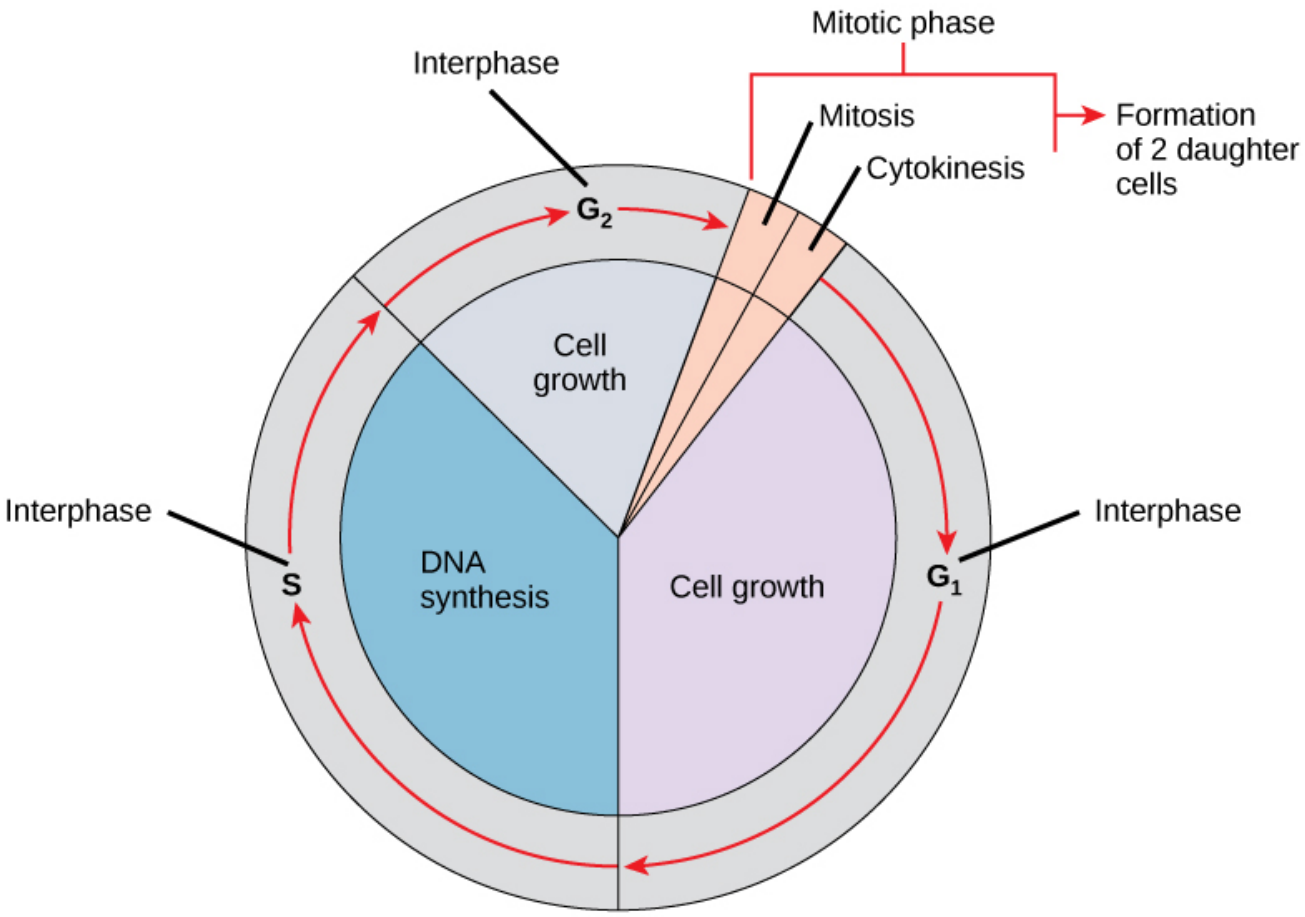

Figure 10.5a The cell cycle in multicellular organisms consists of interphase and the mitotic phase. During interphase, the cell grows and the nuclear DNA is duplicated. Interphase is followed by the mitotic phase. During the mitotic phase, the duplicated chromosomes are segregated and distributed into daughter nuclei. Following mitosis, the cytoplasm is usually divided as well by cytokinesis, resulting in two genetically identical daughter cells.

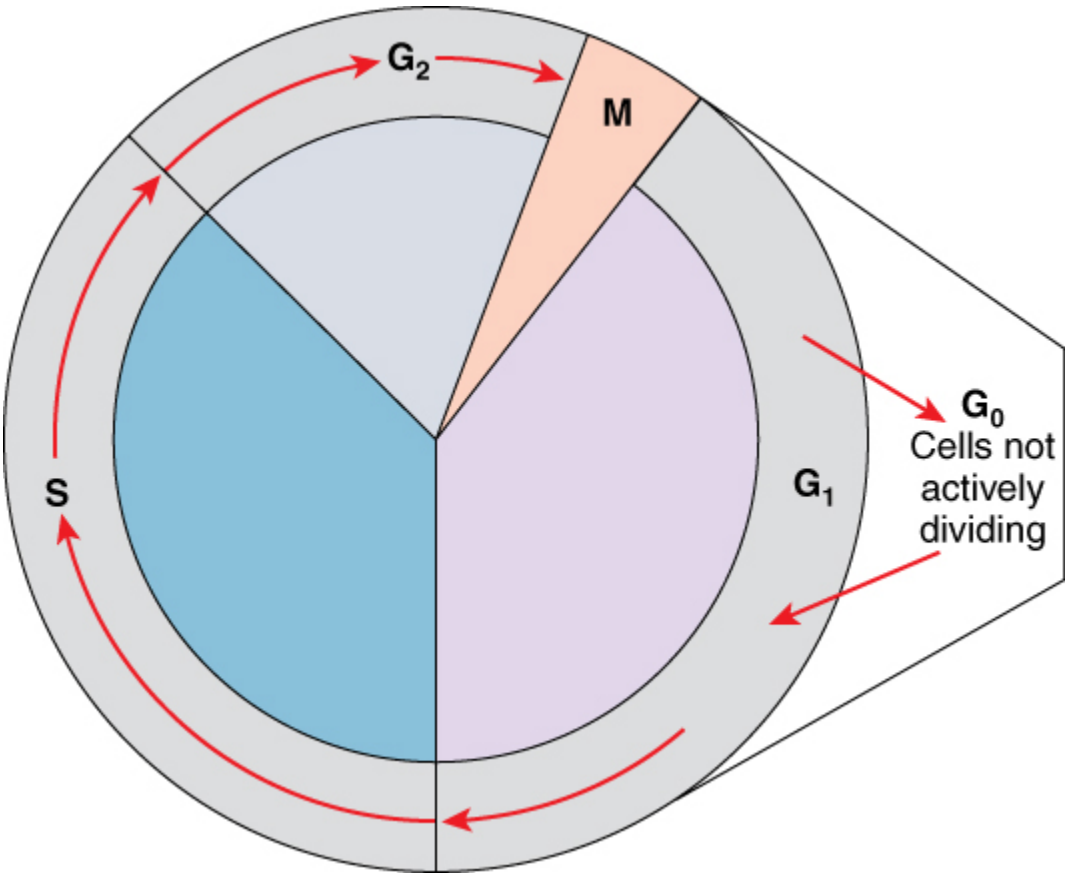

Figure 10.5b The cells are not always actively dividing. There are periods in which cells remain quiescent or dormant. G0 phase is a resting phase of the cell cycle. Typical examples include the more common episode of resting cells or cells that have temporarily stopped dividing and are resting and cells that have permanently stopped or ceased dividing like nerve cells. (http://oerpub.github.io/epubjs-demo-book/content/m46034.xhtml)

Mitotic Spindle

The mitotic spindle begins to form during prophase in the cytoplasm. The structure is mainly fibers of microtubules which forms the spindle from partial disassembly of the cytoskeleton. The spindle microtubules polymerize and depolymerize by incorporating more or losing subunits of the protein tubulin, activities that cause the spindle microtubules to elongate and shorten respectively. Assembly of spindle microtubules is typified in animal cells in the subcellular region of the centrosome where it starts. This “microtubule-organizing center” contains a pair of centrioles located at the center of the centrosome, but the pair of centrioles have been experimentally shown not to be significant in cell division and not even found in plant cells. Mitotic spindles form in plant cells.

Interphase

During interphase, the cell undergoes normal growth processes while also preparing for cell division. In animal cells, the centrosome duplicates into two centrosomes, which remain close to the nucleus. In order for a cell to move from interphase into the mitotic phase, many internal and external conditions must be met. The three stages of interphase are called *G1, S, and G2.*

G_1 Phase (First Gap)

The first stage of interphase is called the **G_1 phase** (first gap) because, from a microscopic point of view, 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 sufficient energy reserves to complete the task of replicating each chromosome in the nucleus.

S Phase (Synthesis of DNA)

Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the **S phase**, DNA replication can proceed through the mechanisms that result in the formation of identical pairs of DNA molecules—sister chromatids—that are firmly attached to the centromeric region. The centrosome is also duplicated during the S phase. The two centrosomes of homologous chromosomes will give rise to the **mitotic spindle**, the apparatus that orchestrates the movement of chromosomes during mitosis. For example, roughly at the center of each animal cell, the centrosomes are associated with a pair of rod-like objects, the **centrioles**, which are positioned at right angles to each other. Centrioles help organize cell division. We should note, however, that centrioles are not present in the centrosomes of other eukaryotic organisms, such as plants and most fungi.

G_2 Phase (Second Gap)

In the **G_2 phase**, the cell replenishes its energy stores and synthesizes proteins necessary for chromosome manipulation and movement. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic phase. 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

The mitotic phase alternates with longer interphase and is usually the shortest phase of the cell cycle. The mitotic phase is a multistep process during which the duplicated chromosomes are aligned, separated, and move into two new, identical daughter cells. The first portion of the mitotic phase is called **karyokinesis**,

or nuclear division. As we have just seen, the second portion of the mitotic phase (and often viewed as a process separate from and following mitosis) is called cytokinesis—the physical separation of the cytoplasmic components into the two daughter cells.

Link to Learning

Revisit the stages of mitosis at this site.

Karyokinesis (Mitosis)

Karyokinesis, also known as mitosis, is divided into a series of phases—prophase, prometaphase, metaphase, anaphase, and telophase—that result in the division of the cell nucleus (Figure 10.6).

Visual Connection

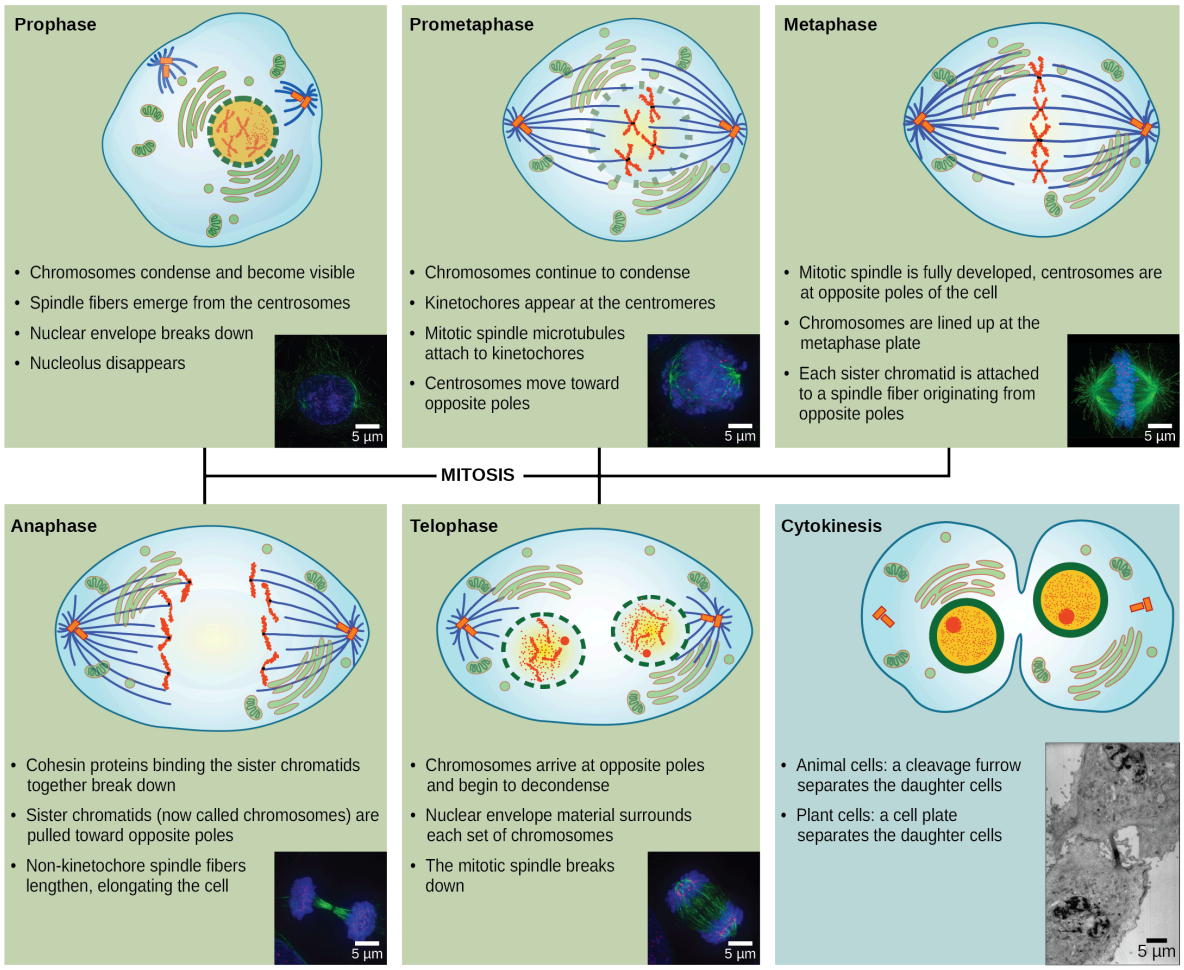

Figure 10.6 Karyokinesis (or mitosis) is divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase. The pictures at the bottom were taken by fluorescence microscopy (hence, the black background) of cells artificially stained by fluorescent dyes: blue fluorescence indicates DNA (chromosomes) and green fluorescence indicates microtubules (spindle apparatus). (credit "mitosis drawings": modification of work by Mariana Ruiz Villareal; credit "micrographs": modification of work by Roy van Heesbeen; credit "cytokinesis micrograph": Wadsworth Center/New York State Department of Health; 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 reforms and the cell divides. Cohesin proteins break

down and the sister chromatids separate.

b. The kinetochore becomes attached to the mitotic spindle. Cohesin proteins break down and the sister chromatids separate. Sister chromatids line up at the metaphase plate. The nucleus reforms and the cell divides.
c. The kinetochore becomes attached to the cohesin proteins. Sister chromatids line up at the metaphase plate. The kinetochore breaks down and the sister chromatids separate. The nucleus reforms and the cell divides.
d. The kinetochore becomes attached to the mitotic spindle. Sister chromatids line up at the metaphase plate. Cohesin proteins break down and the sister chromatids separate. The nucleus reforms and the cell divides.

Prophase (the "first phase"): the nuclear envelope starts to dissociate into small vesicles, and the membranous organelles (such as the Golgi complex [Golgi apparatus] and the endoplasmic reticulum), fragment and disperse toward the periphery of the cell. The nucleolus disappears (disperses) as well, and the centrosomes earlier formed begin to move to opposite poles of the cell as spindle microtubules grow out of these two subcellular structures and during prometaphase. Microtubules that will form the *mitotic spindle* extend between the centrosomes, *pushing them farther apart* as the microtubule fibers lengthen. The sister chromatids begin to coil more tightly with the aid of **condensin proteins** and now become visible under a light microscope.

Prometaphase (the "first change phase"): Many processes that began in prophase continue to advance. The remnants of the nuclear envelope fragment further, and the mitotic spindle continues to develop as more microtubules assemble and stretch across the length of the former nuclear area. In late prometaphase, the two centrosomes are distributed with one at each pole of the spindle, located at opposite ends of the cell. A radial array of short microtubules called an aster forms extensions from each centrosome. At this stage, the spindle primarily is made up of the centrosomes, the spindle microtubules and the asters. Chromosomes become even more condensed and discrete. Each sister chromatid develops a protein structure called a **kinetochore that assemble on specific parts of DNA** in its centromeric region (Figure 10.7). The proteins of the kinetochore attract and bind to the mitotic spindle microtubules. As the spindle microtubules extend from the centrosomes, some of these microtubules come into contact with and firmly bind to the kinetochores. Once a mitotic fiber attaches to a chromosome, the chromosome will be oriented until the kinetochores of sister chromatids face the *opposite poles*. Eventually, all the sister chromatids will be attached via their kinetochores to microtubules from opposing poles. Spindle microtubules that do not engage the chromosomes are called **polar microtubules**. These microtubules overlap each other midway between the two poles and contribute to

cell elongation. Astral microtubules are located near the poles, aid in spindle orientation, and are required for the regulation of mitosis.

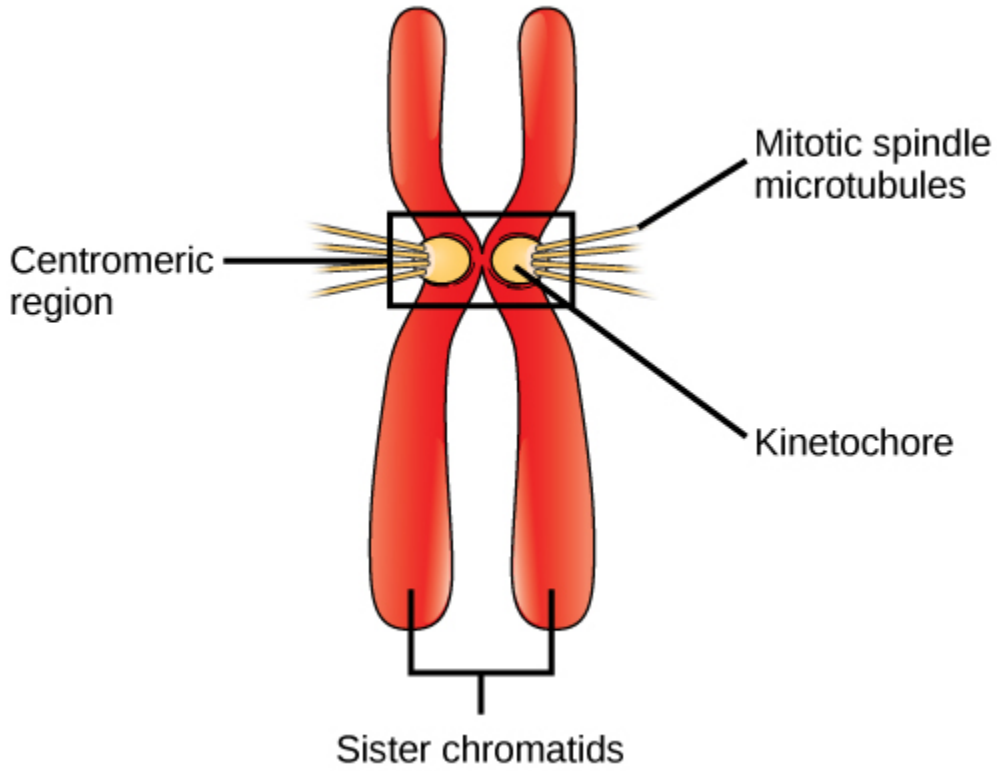

Figure 10.7 During prometaphase, mitotic spindle microtubules from opposite poles attach to each sister chromatid at the kinetochore. In anaphase, the connection between the sister chromatids breaks down, and the microtubules pull the chromosomes toward opposite poles.

Metaphase (the "change phase"): All the chromosomes are aligned in a plane which is imaginary called the **metaphase plate**, or the equatorial plane, roughly midway between the two poles of the cell. The sister chromatids are still tightly attached to each other by cohesin proteins. At this time, the chromosomes are maximally condensed. During this time, the microtubules of the asters have grown and established contact with the plasma membrane. At this stage the spindle is complete.

Anaphase ("upward phase"): The cohesin proteins degrade, and the sister chromatids separate at the centromere. Each chromatid, now called a single chromosome, is pulled rapidly toward the centrosome to which its microtubule is attached. The cell becomes visibly elongated (oval shaped) as the polar microtubules slide against each other at the metaphase plate where they overlap. In animal cells undergoing division, the nonkinetochore microtubules elongate the cells during anaphase. Duplicate chromosome groups arrive at opposite ends of the elongated parent cell

Telophase (the "distance phase"): the chromosomes reach the opposite poles and begin to *decondense* (unravel), relaxing once again into a stretched-out chromatin configuration. The mitotic spindles are depolymerized into tubulin monomers that will be used to assemble cytoskeletal components for each daughter cell. Nuclear envelopes form around the chromosomes, and nucleosomes appear within the nuclear area. The spindle undergoes disassembly by depolymerization of microtubules.

Link to Learning

Learn about Chromosome and Kinetochore via this video.

Cytokinesis

Cytokinesis, or "cell motion," is sometimes viewed as the second main stage of the mitotic phase, during which cell division is completed via the physical separation of the cytoplasmic components into two daughter cells. This physical separation is proceeding by late telophase. However, as we have seen earlier, cytokinesis can also be viewed as a separate phase, which may or may not take place following mitosis. If cytokinesis does take place, cell division is not complete until the cell components have been apportioned and completely separated into the 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 animal cells, cytokinesis typically starts during late anaphase by a process called cleavage. 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 is called the **cleavage furrow**. The furrow deepens as the actin ring contracts, and eventually the membrane of the parent cell is pinched or cleaved in two producing two separate cells each with its own nucleus, cytosol, organelles and other substructures in cells (Figure 10.8).

In plant cells, which have cell walls, cytokinesis is significantly different. There is no cleavage furrow, and 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 into vesicles and dispersing throughout the dividing cell. During telophase, these Golgi vesicles are transported on microtubules to form a *phragmoplast* (a vesicular structure) at the metaphase plate. There, the vesicles fuse and coalesce 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 walls at the periphery of the cell. Enzymes use the glucose that has accumulated between

the membrane layers to build a new cell wall. The Golgi membranes become parts of the plasma membrane on either side of the new cell wall (Figure 10.8).

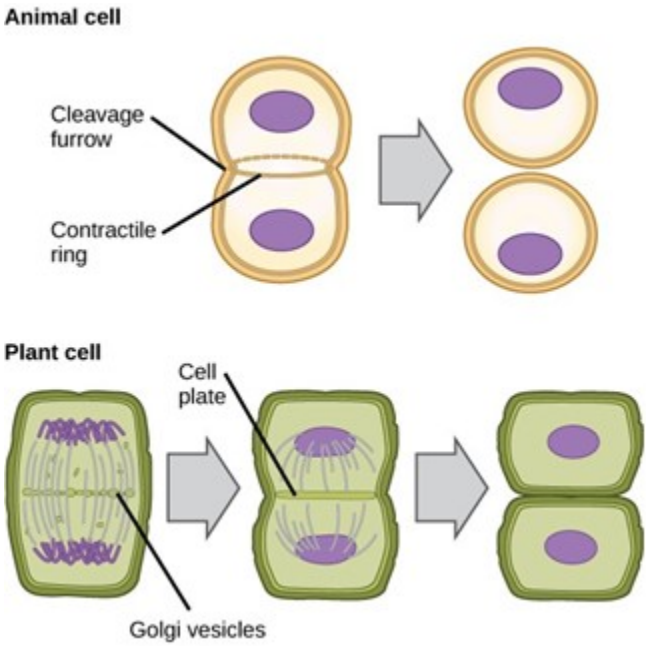

Figure 10.8 During cytokinesis in animal cells, a ring of actin filaments forms at the metaphase plate. The ring contracts, forming a cleavage furrow, which divides the cell in two. In plant cells, Golgi vesicles coalesce at the former metaphase plate, forming a phragmoplast. A cell plate formed by the fusion of the vesicles of the phragmoplast grows from the center toward the cell walls, and the membranes of the vesicles fuse to form a plasma membrane that divides the cell in two.

G_0 Phase

Not all cells adhere to the classic cell-cycle pattern in which a newly formed daughter cell immediately enters the preparatory phases of interphase, closely followed by the mitotic phase, and cytokinesis. Cells in G_0 phase are not actively preparing to divide. The cell is in a quiescent (inactive) stage that occurs when cells exit the cell cycle. Some cells enter G_0 temporarily due to environmental conditions such as availability of nutrients, or stimulation by growth factors. The cell will remain in this phase until conditions improve or 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.

Scientific Method Connection

Determine the Time Spent in Cell-Cycle Stages

Problem: How long does a cell spend in interphase compared to each stage of mitosis?

Background: A prepared microscope slide of whitefish blastula cross-sections will show cells arrested in various stages of the cell cycle. (Note: It is not visually possible to separate the stages of interphase from each other, but the mitotic stages are readily identifiable.) If 100 cells are examined, the number of cells in each identifiable cell-cycle stage will give an estimate of the time it takes for the cell to complete that stage.

Problem Statement: Given the events included in all of interphase and those that take place in each stage of mitosis, estimate the length of each stage based on a 24-hour cell cycle. Before proceeding, state your hypothesis.

Test your hypothesis: Test your hypothesis by doing the following:

1. Place a fixed and stained microscope slide of whitefish blastula cross-sections under the scanning objective of a light microscope.
2. Locate and focus on one of the sections using the low-power objective of your microscope. Notice that the section is a circle composed of dozens of closely packed individual cells.
3. Switch to the medium-power objective and refocus. With this objective, individual cells are clearly visible, but the chromosomes will still be very small.
4. Switch to the high-power objective and slowly move the slide left to right, and up and down to view all the cells in the section (Figure 10.9). As you scan, you will notice that most of the cells are not undergoing mitosis but are in the interphase period of the cell cycle.

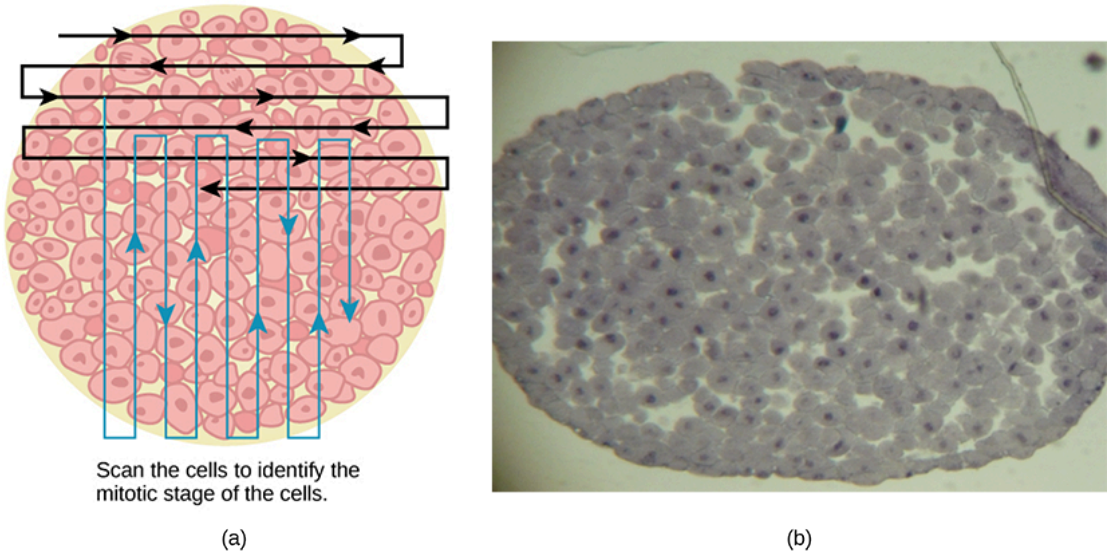

Figure 10.9 Slowly scan whitefish blastula cells with the high-power objective as illustrated in image (a) to identify their mitotic stage. (b) A microscopic image of the scanned cells is shown. (credit "micrograph": modification of work by Linda Flora; scale-bar data from Matt Russell)

5. Practice identifying the various stages of the cell cycle, using the drawings of the stages as a guide (Figure 10.6).
6. Once you are confident about your identification, begin to record the stage of each cell you encounter as you scan left to right, and top to bottom across the blastula section.
7. Keep a tally of your observations and stop when you reach 100 cells identified.
8. The larger the sample size (total number of cells counted), the more accurate the results. If possible, gather and record group data prior to calculating percentages and making estimates.

Record your observations: Make a table similar to Table 10.1 within which to record your observations.

Results of Cell Stage Identification

Phase or Stage	Individual Totals	Group Totals	Percent
Interphase			
Prophase			
Metaphase			
Anaphase			
Telophase			
Cytokinesis			
Totals	100	100	100 percent

Table 10.1

Analyze your data/report your results: To find the length of time whitefish blastula cells spend in each stage, multiply the percent (recorded as a decimal) by 24 hours. Make a table similar to Table 10.2 to illustrate your data.

Estimate of Cell Stage Length

Phase or Stage	Percent	Time in Hours
Interphase		
Prophase		
Metaphase		
Anaphase		
Telophase		
Cytokinesis		

Table 10.2

Draw a conclusion: Did your results support your estimated times? Were any of the outcomes unexpected? If so, discuss those events in that stage that may have contributed to the calculated time.

97.

CONTROL OF THE CELL CYCLE

Learning Objectives

Type your learning objectives here.

- Understand how the cell cycle is controlled by mechanisms that are both internal and external to the cell
- Explain how the three internal "control checkpoints" occur at the end of G_1, at the G_2/M transition, and during metaphase
- Describe the molecules that control the cell cycle through positive and negative regulation

The length of the cell cycle is highly variable, even within the cells of a single 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, and 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, as this process is not fixed and rigid. When rapidly dividing mammalian cells are grown in a culture (outside the body under optimal growing conditions), the length of the cell cycle is about 24 hours. In rapidly dividing human cells with a 24-hour cell cycle, the G1 phase lasts approximately nine hours, the S phase lasts 10 hours, the G2 phase lasts about four and one-half hours, and the M phase lasts approximately one-half hour. By comparison, in fertilized eggs (and early embryos) of fruit flies, the cell cycle is completed in about eight minutes. This is because the nucleus of the fertilized egg divides many times by mitosis but does not go through cytokinesis until a multinucleate "zygote" has been produced, with many nuclei located along the periphery of the cell membrane, thereby shortening the time of the cell division cycle. The timing of events in the cell cycle of both "invertebrates" and "vertebrates" is controlled by mechanisms that are both internal and external to the cell.

Regulation of the Cell Cycle by External Events

Both the initiation and inhibition of cell division are triggered by events external to the cell when it is about to begin the replication process. An event may be as simple as the death of nearby cells or as sweeping as the release of growth-promoting hormones, such as **human growth hormone (HGH or hGH)**. A lack of HGH can *inhibit* cell division, resulting in dwarfism, whereas too much HGH can result in gigantism. Crowding of cells can also inhibit cell division. In contrast, a factor that can initiate cell division is the size of the cell: As a cell grows, it becomes physiologically inefficient due to its decreasing surface-to-volume ratio. The solution to this problem is to divide.

Whatever the source of the message, the cell receives the signal, and a series of events within the cell allows it to proceed into interphase. Moving forward from this initiation point, every parameter required during each cell cycle phase must be met or the cycle cannot progress.

Regulation at Internal Checkpoints

It is essential that the daughter cells produced 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 an abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main **cell-cycle checkpoints**: A checkpoint is one of several points in the eukaryotic cell cycle at which the progression of a cell to the next stage in the cycle can be halted until conditions are favorable. These checkpoints occur near the end of G1, at the G2/M transition, and during metaphase (Figure 10.10).

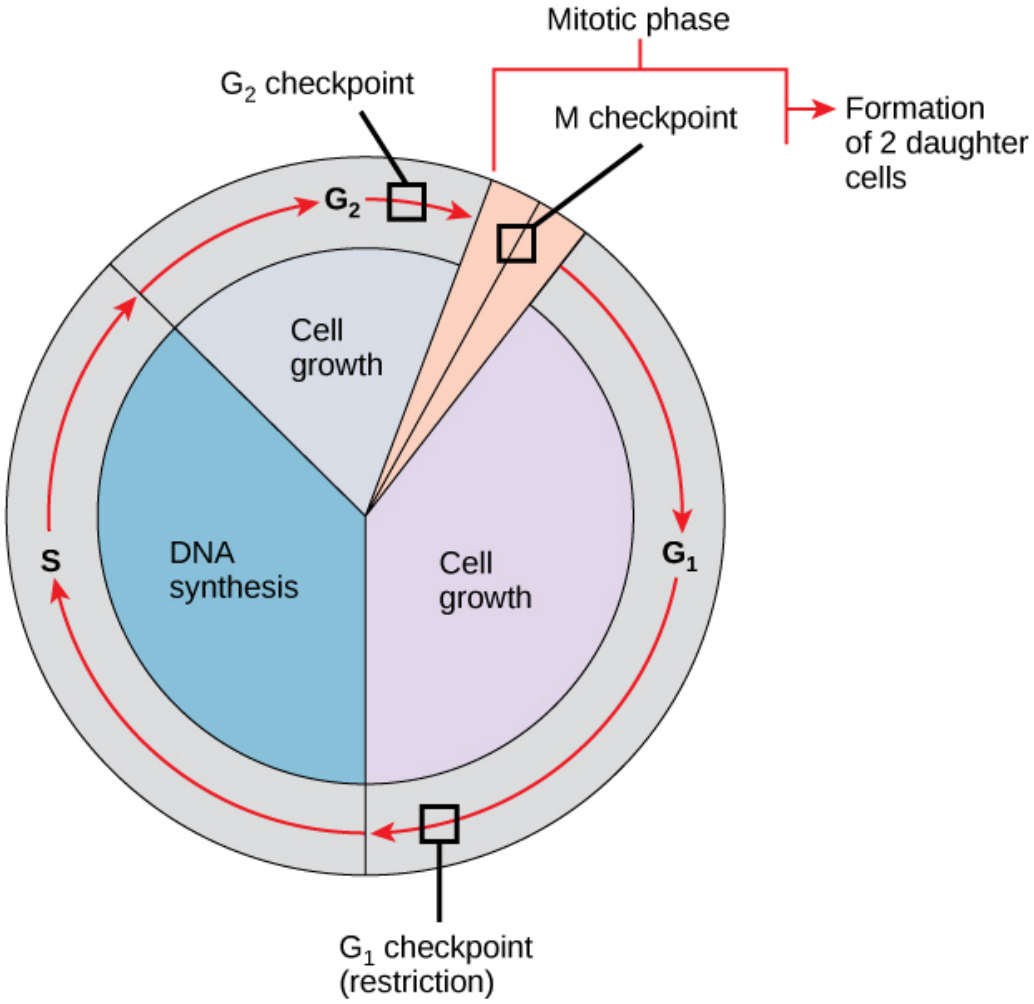

Figure 10.10 The cell cycle is controlled at three checkpoints. The integrity of the DNA is assessed at the G1 checkpoint. Proper chromosome duplication is assessed at the G2 checkpoint. Attachment of each kinetochore to a spindle fiber is assessed at the M checkpoint.

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 (in yeast), is a point at which the cell irreversibly commits to the cell division process. External influences, such as growth factors, play a large role in carrying the cell past the G_1 checkpoint. In addition to adequate reserves and cell size, there is a check for genomic DNA damage at the G_1 checkpoint. A cell that does not meet all the requirements will not be allowed to progress into the S phase. The cell can halt the cycle and attempt to remedy the problematic condition, or the cell can advance into G_0 and await further signals when conditions improve.

The G_2 Checkpoint

The G_2 checkpoint bars entry into the mitotic phase if certain conditions are not met. As at 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. If the checkpoint mechanisms detect problems with the DNA, the cell cycle is halted, and the cell attempts to either complete DNA replication or repair the damaged DNA.

The M Checkpoint

The M checkpoint occurs near the end of the metaphase stage of karyokinesis. The M checkpoint is also known as the spindle checkpoint because it determines whether 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 at least two spindle fibers arising from opposite poles of the cell.

Link to Learning

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

Regulator Molecules of the Cell Cycle

In addition to the internally controlled checkpoints, there are two groups of intracellular molecules that regulate the cell cycle. These regulatory molecules either promote progress of the cell to the next phase (positive regulation) or halt the cycle (negative regulation). Regulator molecules may act individually, or they can influence the activity or production of other regulatory proteins. Therefore, the failure of a single regulator may have almost no effect on the cell cycle, especially if more than one mechanism controls the same event. However, the effect of a deficient or non-functioning regulator can be wide-ranging and possibly fatal to the cell if multiple processes are affected.

Positive Regulation of the Cell Cycle

Two groups of proteins, called **cyclins** and **cyclin-dependent kinases** (Cdks), are termed positive regulators. They are responsible for the progress of the cell through the various checkpoints. The levels of the four

cyclin proteins fluctuate throughout the cell cycle in a predictable pattern (Figure 10.11). Increases in the concentration of cyclin proteins are triggered by both external and internal signals. After the cell moves to the next stage of the cell cycle, the cyclins that were active in the previous stage are degraded by cytoplasmic enzymes, as shown in Figure 10.11 below.

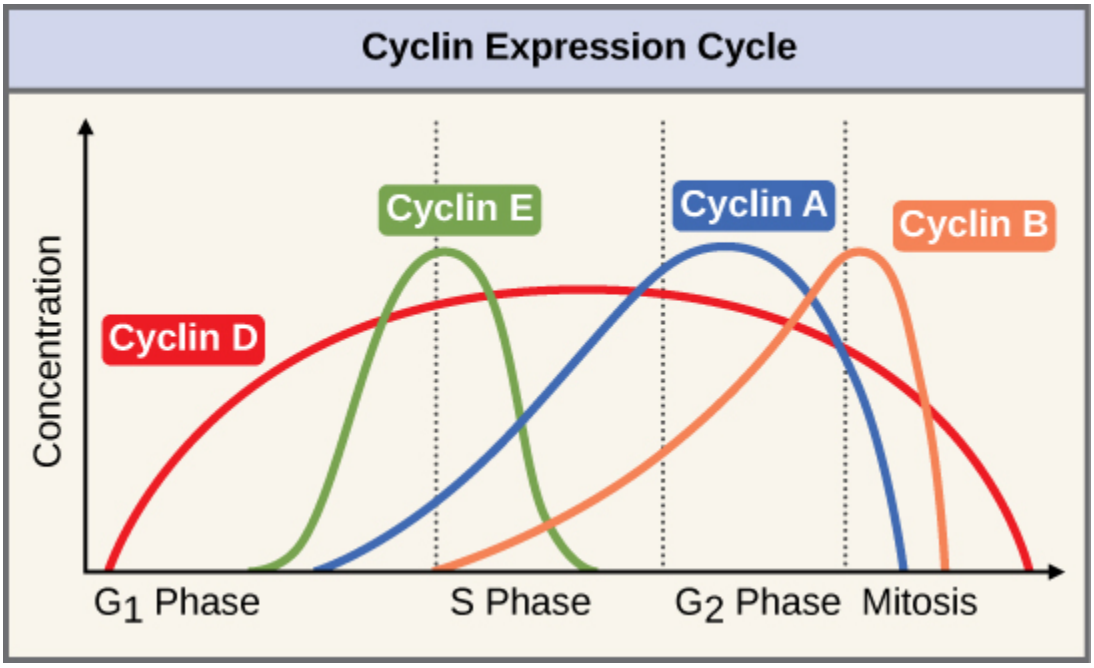

Figure 10.11 The concentrations of cyclin proteins change throughout the cell cycle. There is a direct correlation between cyclin accumulation and the three major cell-cycle checkpoints. Also note the sharp decline of cyclin levels following each checkpoint (the transition between phases of the cell cycle), as cyclin is degraded by cytoplasmic enzymes. (credit: modification of work by "WikiMiMa"/Wikimedia Commons)

Cyclins regulate the cell cycle only when they are tightly bound to Cdks. To be fully active, the Cdk/cyclin complex must also be phosphorylated in specific locations to activate the complex. Like all kinases, Cdks are enzymes (*kinases*) that in turn phosphorylate other proteins. Phosphorylation activates the protein by changing its shape. The proteins phosphorylated by Cdks are involved in advancing the cell to the next phase. (Figure 10.12). The levels of Cdk proteins are relatively stable throughout the cell cycle; however, the concentrations of cyclin fluctuate and determine when Cdk/cyclin complexes form. The different cyclins and Cdks bind at specific points in the cell cycle and thus regulate different checkpoints.

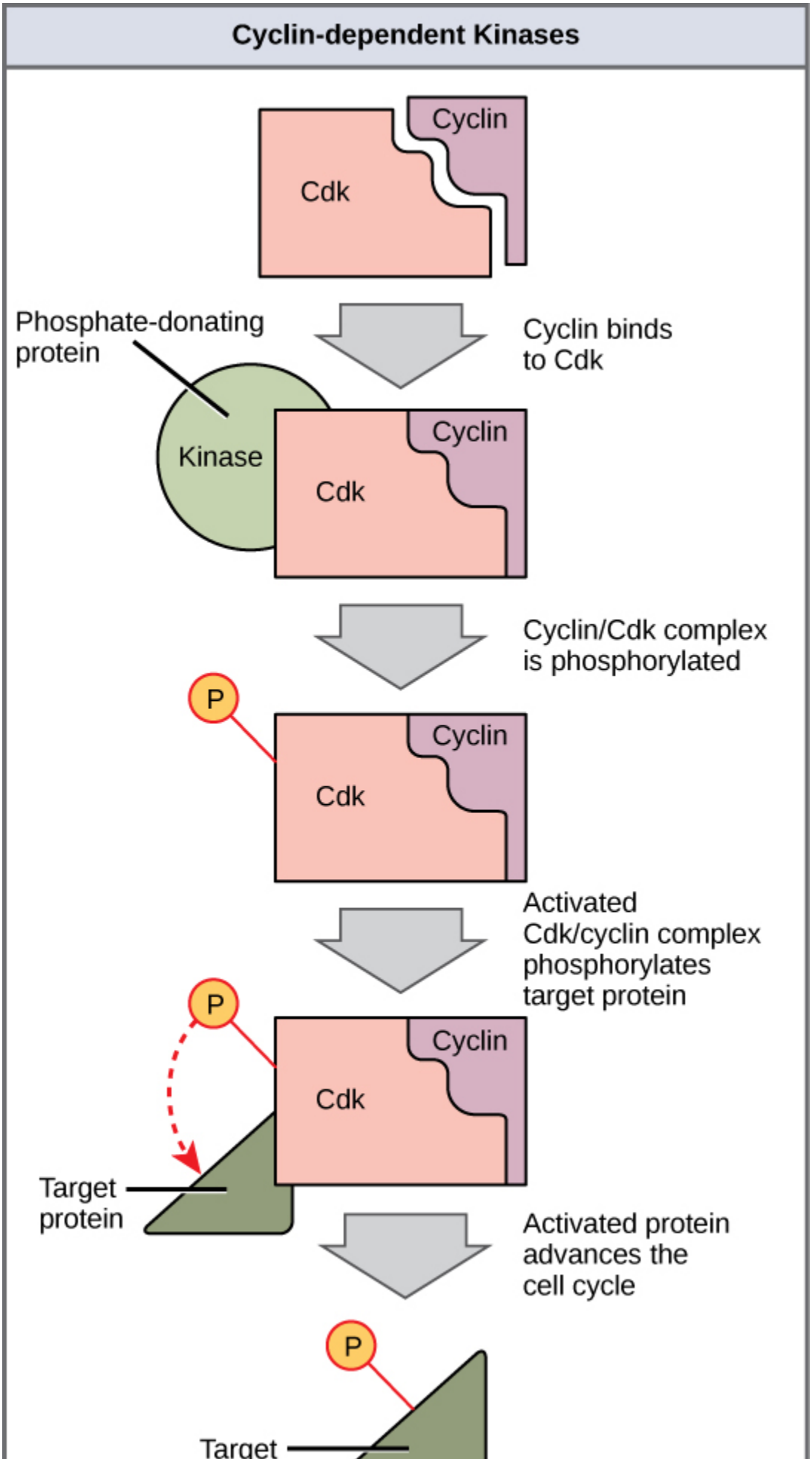
Cyclin-dependent Kinases
Cyclin
Cdk
Phosphate-donating protein
Cyclin binds to Cdk
Kinase
Cyclin
Cdk
Cyclin/Cdk complex is phosphorylated
P
Cyclin
Cdk
Activated Cdk/cyclin complex phosphorylates target protein
P
Cyclin
Cdk
Target protein
Activated protein advances the cell cycle
P
Target

Figure 10.12 Cyclin-dependent kinases (Cdks) are protein kinases that, when fully activated, can phosphorylate and thus activate other proteins that advance the cell cycle past a checkpoint. To become fully activated, a Cdk must bind to a cyclin protein and then be phosphorylated by another kinase.

Because the cyclic fluctuations of cyclin levels are largely based on the *timing of the cell cycle* and not on specific events, regulation of the cell cycle usually occurs by either the Cdk molecules alone or the Cdk/cyclin complexes. Without a specific concentration of fully activated cyclin/Cdk complexes, the cell cycle cannot proceed through the checkpoints.

Although the cyclins are the main regulatory molecules that determine the forward momentum of the cell cycle, there are several other mechanisms that fine-tune the progress of the cycle with negative, rather than positive, effects. These mechanisms essentially block the progression of the cell cycle until problematic conditions are resolved. Molecules that prevent the full activation of Cdks are called Cdk inhibitors. Many of these inhibitor molecules directly or indirectly monitor a particular cell-cycle event. The block placed on Cdks by inhibitor molecules will not be removed until the specific event that the inhibitor monitors is completed.

Negative Regulation of the Cell Cycle

The second group of cell-cycle regulatory molecules are *negative regulators*, which stop the cell cycle. Remember that in positive regulation, active molecules cause the cycle to progress.

The best understood negative regulatory molecules are **retinoblastoma protein (Rb)**, **p53**, and **p21**. Retinoblastoma proteins are a group of *tumor-suppressor proteins* common in many cells. We should note here that the 53 and 21 designations refer to the functional molecular masses of the proteins (p) in kilodaltons (a dalton is equal to an *atomic mass unit*, which is equal to one proton or one neutron or 1 g/mol). Much of what is known about cell-cycle regulation comes from research conducted with cells that have *lost regulatory control*. All three of these regulatory proteins were discovered to be damaged or non-functional in cells that had begun to replicate uncontrollably (i.e., became cancerous). In each case, the main cause of the unchecked progress through the cell cycle was a faulty copy of the regulatory protein.

Rb, p53, and p21 act primarily at the G_1 checkpoint. p53 is a multi-functional protein that has a major impact on the commitment of a cell to division because it acts when there is damaged DNA in cells that are undergoing the preparatory processes during G_1. If damaged DNA is detected, p53 halts the cell cycle and then recruits specific enzymes to repair the DNA. If the DNA cannot be repaired, p53 can trigger apoptosis, or cell suicide, to prevent the duplication of damaged chromosomes. As p53 levels rise, the production of p21 is triggered. p21 enforces the halt in the cycle dictated by p53 by binding to and inhibiting the activity of the Cdk/cyclin complexes. As a cell is exposed to more stress, higher levels of p53 and p21 accumulate, making it less likely that the cell will move into the S phase.

Rb, which largely monitors cell size, exerts its regulatory influence on other positive regulator proteins. In the *active*, dephosphorylated state, Rb binds to proteins called *transcription factors*, most commonly, E2F

(Figure 10.13). Transcription factors "turn on" specific genes, allowing the production of proteins encoded by that gene. When Rb is bound to E2F, production of proteins necessary for the G_1/S transition is blocked. As the cell increases in size, Rb is slowly phosphorylated until it becomes *inactivated*. Rb releases E2F, which can now turn on the gene that produces the transition protein, and this particular block is removed. For the cell to move past each of the checkpoints, all positive regulators must be "turned on," and all negative regulators must be "turned off."

Visual Connection

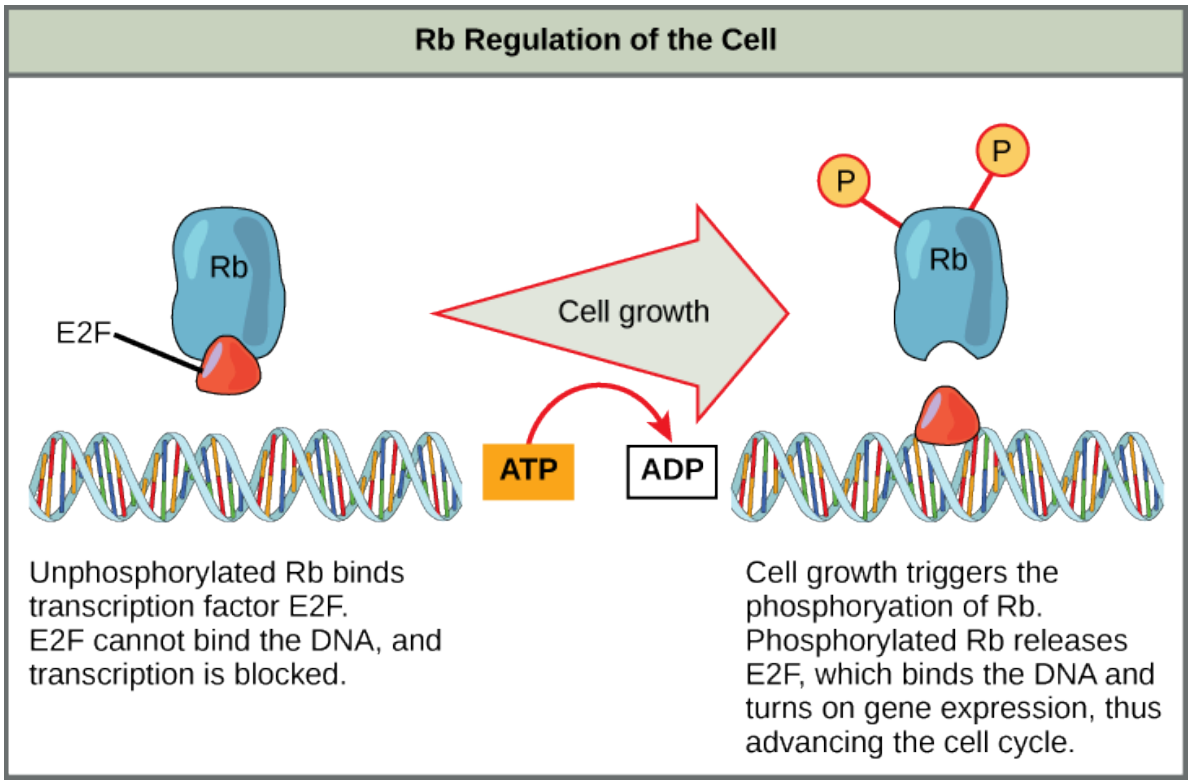

Figure 10.13 Rb halts the cell cycle and releases its hold in response to cell growth.

Rb and other proteins that negatively regulate the cell cycle are sometimes called tumor suppressors. Why do you think the name tumor suppressor might be appropriate for these proteins?

98.

CANCER AND THE CELL CYCLE

Learning Objectives

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

- Describe how cancer is caused by uncontrolled cell growth
- Understand how proto-oncogenes are normal cell genes that, when mutated, become oncogenes
- Describe how tumor suppressors function
- Explain how mutant tumor suppressors cause cancer

Cancer comprises many different diseases caused by a common mechanism: uncontrolled cell growth. Despite the redundancy and overlapping levels of cell-cycle control, errors do 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 changes to the DNA nucleotide sequence occur within a coding portion of a gene and are not corrected, a gene mutation results. All cancers start when a gene mutation gives rise to a faulty protein that plays a key role in cell reproduction.

The change in the cell that results from the malformed protein may be minor: perhaps a slight delay in the binding of Cdk to cyclin or an Rb protein that detaches from its target DNA while still phosphorylated. Even minor mistakes, however, may allow subsequent mistakes to occur more readily. Over and over, small uncorrected errors are passed from the parent cell to the daughter cells and amplified as each generation 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 ("-oma") can result.

Proto-oncogenes

The genes that code for the positive cell-cycle regulators are called **proto-oncogenes**. Proto-oncogenes are normal genes that, when mutated in certain ways, become **oncogenes**—genes that cause a cell to become cancerous. 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 carried forward. If a cell cannot reproduce, the mutation is not propagated and the damage is minimal. Occasionally, however, a gene mutation causes a change that increases the activity of a positive regulator. For example, a mutation that allows Cdk to be activated without being partnered with cyclin 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 undergo further cell divisions, the mutation would not be propagated and no harm would come to the organism. However, if the atypical daughter cells are able to undergo further cell divisions, subsequent generations of cells may accumulate even more mutations, some possibly in additional genes that regulate the cell cycle.

The Cdk gene in the above 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. An oncogene is any gene that, when altered, leads to an increase in the rate of cell-cycle progression.

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https://louis.pressbooks.pub/generalbiology1leclab/?p=406#h5p-46

Tumor Suppressor Genes

Like proto-oncogenes, many of the negative cell-cycle regulatory proteins were discovered in cells that had become cancerous. **Tumor suppressor genes** are segments of DNA that code for negative regulator proteins, the type of regulators that, when activated, can prevent the cell from undergoing uncontrolled division. The collective function of the best-understood tumor suppressor gene proteins, Rb, p53, and p21, is to put up a roadblock to cell-cycle progression 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. Tumor suppressors are similar to brakes in a vehicle: Malfunctioning brakes can contribute to a car crash!

Mutated p53 genes have been identified in more than 50 percent 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. A cell with a faulty p53 may fail to detect errors present in the genomic DNA (Figure 10.14). Even if a partially functional p53 does identify the mutations, it may no longer be able to signal the necessary DNA repair enzymes. Either way, damaged DNA will remain uncorrected. At this point, a functional p53 will deem the cell unsalvageable and trigger programmed cell death (apoptosis). The damaged version of p53 found in cancer cells, however, cannot trigger apoptosis.

Visual Connection

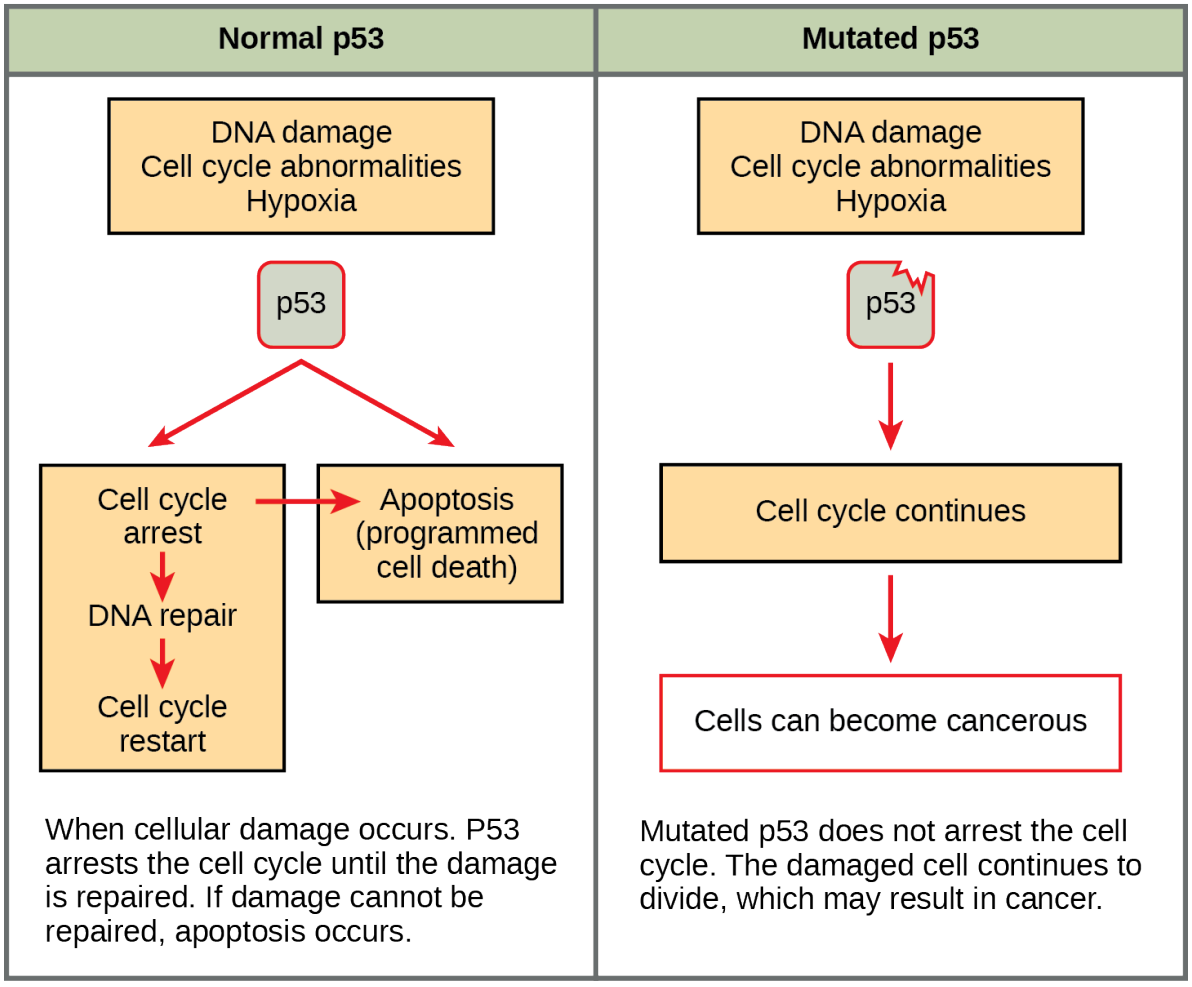

Figure 10.14 The role of normal p53 is to monitor DNA and the supply of oxygen (hypoxia is a condition of reduced oxygen supply). If damage is detected, p53 triggers repair mechanisms. If repairs are unsuccessful, p53 signals apoptosis. A cell with an abnormal p53 protein cannot repair damaged DNA and thus cannot signal apoptosis. Cells with abnormal p53 can become cancerous. (credit: modification of work by Thierry Soussi)

Human papillomavirus can cause cervical cancer. The virus encodes E6, a protein that binds p53. Based on this fact and what you know about p53, what effect do you think E6 binding has on p53 activity?

a. E6 activates p53
b. E6 inactivates p53
c. E6 mutates p53

d. E6 binding marks p53 for degradation

The loss of p53 function has other repercussions for the cell cycle. Mutated p53 might lose its ability to trigger p21 production. Without adequate levels of p21, there is no effective block on Cdk activation. Essentially, without a fully functional p53, the G_1 checkpoint is severely compromised and the cell proceeds directly from G_1 to S regardless of internal and external conditions. At the completion of this shortened cell cycle, two daughter cells are produced that have inherited the mutated p53 gene. Given the non-optimal conditions under which the parent cell reproduced, it is likely that the daughter cells will have acquired other mutations in addition to the faulty tumor-suppressor gene. Cells such as these daughter cells quickly accumulate both oncogenes and non-functional tumor-suppressor genes. Again, the result is tumor growth.

Link to Learning

Watch an animation of how cancer results from errors in the cell cycle.

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99.

PROKARYOTIC CELL DIVISION

Learning Objectives

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

- Describe the process of binary fission in prokaryotes
- Explain how FtsZ and tubulin proteins are examples of homology

Prokaryotes (bacteria and archaea) can undergo reproduction in which the cell increases to about double its original size and then undergoes division to form two daughter cells. For unicellular organisms, cell division is the only method to produce new individuals. In both prokaryotic and eukaryotic cells, the outcome of cell reproduction is a pair of daughter cells that are genetically identical to the parent cell. In unicellular organisms, daughter cells are individuals.

To achieve the outcome of cloned offspring, certain steps are essential. The genomic DNA must be replicated and then allocated into the daughter cells; the cytoplasmic contents must also be divided to give both new cells the cellular machinery to sustain life. As we've seen with bacterial cells, the genome consists of a single, circular DNA chromosome; therefore, the process of cell division is simplified. Karyokinesis is unnecessary because there is no true nucleus and thus no need to direct one copy of the multiple chromosomes into each daughter cell. This type of cell division is called **binary (prokaryotic) fission**.

Binary Fission

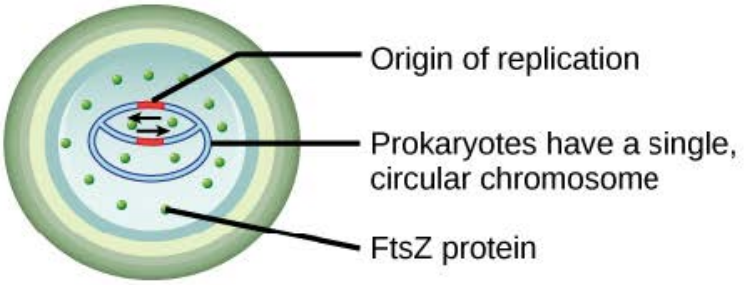
Due to the relative simplicity of the prokaryotes, the cell division process is a less complicated and much more rapid process than cell division in complex eukaryotes. Binary fission, meaning "division in half," is the process by which prokaryotes reproduce from a parental cell into two daughter cells, each inheriting a

complete genome and each a new individual. Binary fission also refers to the process of asexual reproduction of single-celled eukaryotes like amoeba, which involves mitosis, unlike prokaryotes like bacteria, which does not involve mitosis. As a review of the general information on cell division we discussed at the beginning of this chapter, recall that the single, circular DNA chromosome of bacteria occupies a specific location, the nucleoid region, within the cell (Figure 10.2). Although the DNA of the nucleoid is associated with proteins that aid in packaging the molecule into a compact size, there are no histone proteins and thus no nucleosomes in prokaryotes. The packing proteins of bacteria are, however, related to the cohesin and condensin proteins involved in the chromosome compaction of eukaryotes.

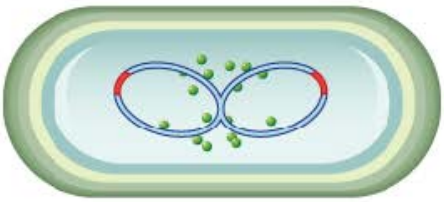
The bacterial chromosome is attached to the plasma membrane at about the midpoint of the cell. The starting point of replication, the **origin**, is close to the binding site of the chromosome to the plasma membrane (Figure 10.15a) and cells with plasmids proceed accordingly (Figure 10.15b). Replication of the DNA is bidirectional, moving away from the origin on both strands of the loop simultaneously. As the new double strands are formed, each origin point moves away from the cell wall attachment toward the opposite ends of the cell. As the cell elongates, the growing membrane aids in the transport of the chromosomes. After the chromosomes have cleared the midpoint of the elongated cell, cytoplasmic separation begins. The formation of a ring composed of repeating units of a protein called **FtsZ** (short for "filamenting temperature-sensitive mutant Z") directs the partition between the nucleoids. Formation of the FtsZ ring triggers the accumulation of other proteins that work together to recruit new membrane and cell wall materials to the site. A **septum** is formed between the daughter nucleoids, extending gradually from the periphery toward the center of the cell. When the new cell walls are in place, the daughter cells separate.

Binary Fission in Prokaryotes

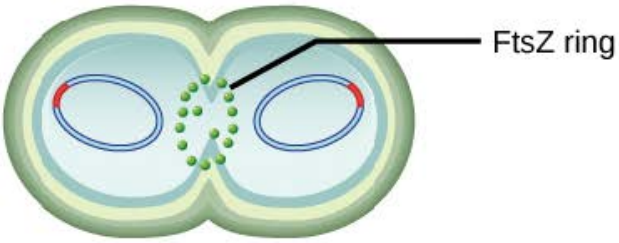
Replication of the circular prokaryotic chromosome begins at the origin of replication and continues in both directions at once. 1



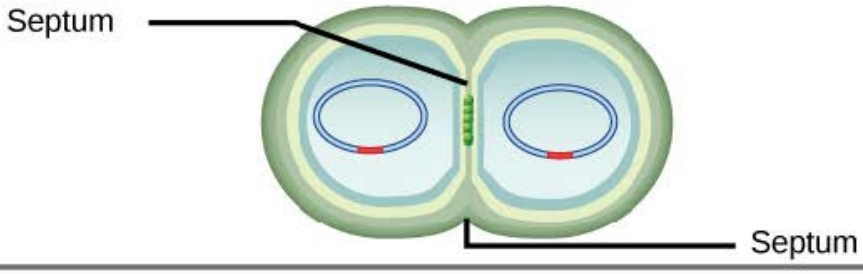
The cell begins to elongate. FtsZ proteins migrate toward the midpoint of the cell. 2



The duplicated chromosomes separate and continue to move away from each other toward opposite ends of the cell. FtsZ proteins form a ring around the periphery of the midpoint between the chromosomes. 3



The FtsZ ring directs the formation of a septum that divides the cell. Plasma membrane and cell wall materials accumulate. 4



After the septum is complete, the cell pinches in two, forming two daughter cells. FtsZ is dispersed throughout the cytoplasm of the new cells. 5

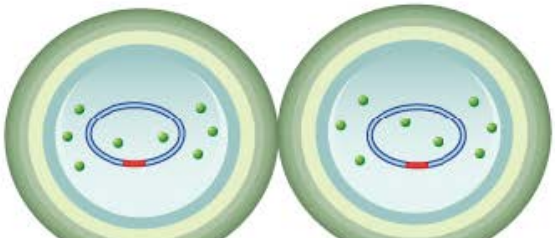


Figure 10.15a These images show the steps of the replication of the chromosome, beginning at the origin of replication in the consequent binary fission in prokaryotes. (credit: modification of work by "Mcstrother"/Wikimedia Commons)

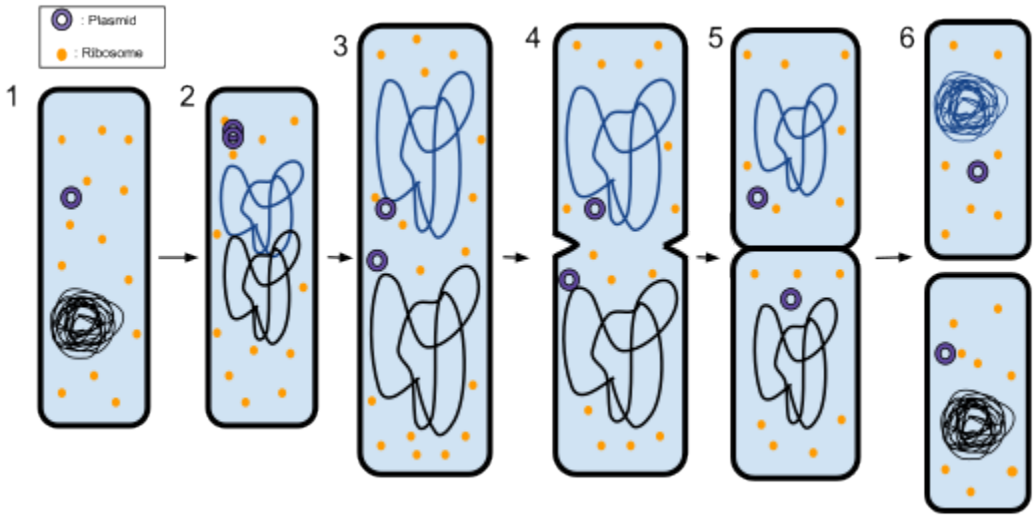


Figure 10.15b The steps highlight cell contents during binary fission 1: Tightly coiled DNA in bacterium before binary fission; 2: Replicated uncoiled DNA; 3: The DNA is pulled to the separate poles of the large cell to prepare for splitting; 4: New cell wall begins to emerge in the separation; 5: The new cell wall fully develops, producing two separate daughter cells; 6: Each cell is a new individual and has tightly coiled DNA, ribosomes, and plasmids. (https://simple.wikipedia.org/wiki/Binary_fission)

Evolution Connection

Mitotic Spindle Apparatus

Did mitosis evolve from prokaryotic cell reproduction? There is a relatedness; however, we cannot observe evidence of extinct species' cell divisions directly except to hypothesize. The precise timing and formation of the mitotic spindle is critical to the success of eukaryotic cell division. Prokaryotic cells, on the other hand, do not undergo karyokinesis and therefore have no need for a mitotic spindle. However, the FtsZ protein that plays such a vital role in prokaryotic cytokinesis is structurally and functionally very similar to tubulin, the building block of the microtubules, which make up the mitotic spindle fibers that are necessary for eukaryotic

nuclear division. FtsZ proteins can form filaments, rings, and other three-dimensional structures that resemble the way tubulin forms microtubules, centrioles, and various cytoskeletal components. In addition, both FtsZ and tubulin employ the same energy source, GTP (guanosine triphosphate), to rapidly assemble and disassemble complex structures.

FtsZ and tubulin are considered to be homologous structures derived from common evolutionary origins. In this example, FtsZ is the ancestor protein to tubulin (an evolutionarily derived protein). While both proteins are found in extant organisms, tubulin function has evolved and diversified tremendously since evolving from its FtsZ prokaryotic origin. A survey of mitotic assembly components found in present-day unicellular eukaryotes reveals crucial intermediary steps to the complex membrane-enclosed genomes of multicellular eukaryotes (Table 10.3).

Cell Division Apparatus among Various Organisms

	Structure of genetic material	Division of nuclear material	Separation of daughter cells
Prokaryotes	There is no nucleus. The single, circular chromosome exists in a region of cytoplasm called the nucleoid.	Occurs through binary fission. As the chromosome is replicated, the two copies move to opposite ends of the cell by an unknown mechanism.	FtsZ proteins assemble into a ring that pinches the cell in two.
Some protists	Linear chromosomes exist in the nucleus.	Chromosomes attach to the nuclear envelope, which remains intact. The mitotic spindle passes through the envelope and elongates the cell. No centrioles exist.	Microfilaments form a cleavage furrow that pinches the cell in two.
Other protists	Linear chromosomes wrapped around histones exist in the nucleus.	A mitotic spindle forms from the centrioles and passes through the nuclear membrane, which remains intact. Chromosomes attach to the mitotic spindle, which separates the chromosomes and elongates the cell.	Microfilaments form a cleavage furrow that pinches the cell in two.
Animal cells	Linear chromosomes exist in the nucleus.	A mitotic spindle forms from the centrosomes. The nuclear envelope dissolves. Chromosomes attach to the mitotic spindle, which separates the chromosomes and elongates the cell.	Microfilaments form a cleavage furrow that pinches the cell in two.

Table 10.3

100.

KEY TERMS

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

binary fission
: prokaryotic cell division process

cell cycle
: ordered series of events involving cell growth and cell division that produces two new daughter cells

cell plate
: structure formed during plant cell cytokinesis by Golgi vesicles, forming a temporary structure (phragmoplast) and fusing at the metaphase plate; ultimately leads to the formation of cell walls that separate the two daughter cells

cell-cycle checkpoint
: mechanism that monitors the preparedness of a eukaryotic cell to advance through the various cell-cycle stages

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

centromere
: region at which sister chromatids are bound together; a constricted area in condensed chromosomes

chromatid
: single DNA molecule of two strands of duplicated DNA and associated proteins held together at the centromere

cleavage furrow
: constriction formed by an actin ring during cytokinesis in animal cells that leads to cytoplasmic division

condensin
: proteins that help sister chromatids coil during prophase

cyclin
: one of a group of proteins that act in conjunction with cyclin-dependent kinases to help regulate the cell cycle by phosphorylating key proteins; the concentrations of cyclins fluctuate throughout the cell cycle

cyclin-dependent kinase (Cdk)
: one of a group of protein kinases that helps to regulate the cell cycle when bound to cyclin; it functions to phosphorylate other proteins that are either activated or inactivated by phosphorylation

cytokinesis
division of the cytoplasm following mitosis that forms two daughter cells.

diploid
cell, nucleus, or organism containing two sets of chromosomes ($2n$)

FtsZ
tubulin-like protein component of the prokaryotic cytoskeleton that is important in prokaryotic cytokinesis (name origin: **F**ilamenting **t**emperature-**s**ensitive mutant **Z**)

G_0 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) first phase of interphase centered on cell growth during mitosis

G_2 phase
(also, second gap) third phase of interphase during which the cell undergoes final preparations for mitosis

gamete
haploid reproductive cell or sex cell (sperm, pollen grain, or egg)

gene
physical and functional unit of heredity, a sequence of DNA that codes for a protein.

genome
total genetic information of a cell or organism

haploid
cell, nucleus, or organism containing one set of chromosomes (n)

histone
one of several similar, highly conserved, low molecular weight, basic proteins found in the chromatin of all eukaryotic cells; associates with DNA to form nucleosomes

homologous chromosomes
chromosomes of the same morphology with genes in the same location; diploid organisms have pairs of homologous chromosomes (homologs), with each homolog derived from a different parent

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

karyokinesis
mitotic nuclear division

kinetochore
protein structure associated with the centromere of each sister chromatid that attracts and binds spindle microtubules during prometaphase

locus
: position of a gene on a chromosome

metaphase
: stage of mitosis during which chromosomes are aligned at the metaphase plate

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

mitosis
: (also, karyokinesis) period of the cell cycle during which the duplicated chromosomes are separated into identical nuclei; includes prophase, prometaphase, metaphase, anaphase, and telophase

mitotic phase
: period of the cell cycle during which duplicated chromosomes are distributed into two nuclei and cytoplasmic contents are divided; includes karyokinesis (mitosis) and cytokinesis

mitotic spindle
: apparatus composed of microtubules that orchestrates the movement of chromosomes during mitosis

nucleosome
: subunit of chromatin composed of a short length of DNA wrapped around a core of histone proteins

oncogene
: mutated version of a normal gene involved in the positive regulation of the cell cycle

origin
: (also, ORI) region of the prokaryotic chromosome where replication begins (origin of replication)

p21
: cell-cycle regulatory protein that inhibits the cell cycle; its levels are controlled by p53

p53
: cell-cycle regulatory protein that regulates cell growth and monitors DNA damage; it halts the progression of the cell cycle in cases of DNA damage and may induce apoptosis

prometaphase
: stage of mitosis during which the nuclear membrane breaks down and mitotic spindle fibers attach to kinetochores

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

proto-oncogene
: normal gene that when mutated becomes an oncogene

quiescent
: refers to a cell that is performing normal cell functions and has not initiated preparations for cell division

retinoblastoma protein (Rb)
: regulatory molecule that exhibits negative effects on the cell cycle by interacting with a transcription factor (E2F)

S phase
: second, or synthesis, stage of interphase during which DNA replication occurs

septum
: structure formed in a bacterial cell as a precursor to the separation of the cell into two daughter cells

telophase
: stage of mitosis during which chromosomes arrive at opposite poles, decondense, and are surrounded by a new nuclear envelope

tumor suppressor gene
: segment of DNA that codes for regulator proteins that prevent the cell from undergoing uncontrolled division

101.

CHAPTER SUMMARY

10.1 Cell Division

Prokaryotes have a single circular chromosome composed of double-stranded DNA, whereas eukaryotes have multiple, linear chromosomes composed of chromatin wrapped around histones, all of which are surrounded by a nuclear membrane. The 46 chromosomes of human somatic cells are composed of 22 pairs of autosomes (matched pairs) and a pair of sex chromosomes, which may or may not be matched. This is the $2n$ or diploid state. Human gametes have 23 chromosomes, or one complete set of chromosomes; a set of chromosomes is complete with either one of the sex chromosomes, X or Y. This is the n or haploid state. Genes are segments of DNA that code for a specific functional molecule (a protein or RNA). An organism's traits are determined by the genes inherited from each parent. Duplicated chromosomes are composed of two sister chromatids. Chromosomes are compacted using a variety of mechanisms during certain stages of the cell cycle. Several classes of protein are involved in the organization and packing of the chromosomal DNA into a highly condensed structure. The condensing complex compacts chromosomes, and the resulting condensed structure is necessary for chromosomal segregation during mitosis.

10.2 The Cell Cycle

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, during which chromosomes are replicated. Interphase is divided into G_1, S, and G_2 phases. The mitotic phase begins with karyokinesis (mitosis), which consists of five stages: prophase, prometaphase, metaphase, anaphase, and telophase. The final stage of the cell division process, and sometimes viewed as the final stage of the mitotic phase, is 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).

10.3 Control of the Cell Cycle

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. Positive regulator molecules allow the cell cycle to advance to the next stage of cell division. Negative regulator molecules monitor cellular conditions and can halt the cycle until specific requirements are met.

10.4 Cancer and the Cell Cycle

Cancer is the result of unchecked cell division caused by a breakdown of the mechanisms that regulate the cell cycle. The loss of control begins with a change in the DNA sequence of a gene that codes for one of the regulatory molecules. Faulty instructions lead to a protein that does not function as it should. Any disruption of the monitoring system can allow other mistakes to be passed on to the daughter cells. Each successive cell division will give rise to daughter cells with even more accumulated damage. Eventually, all checkpoints become nonfunctional, and rapidly reproducing cells crowd out normal cells, resulting in a tumor or leukemia (blood cancer).

10.5 Prokaryotic Cell Division

In both prokaryotic and eukaryotic cell division, the genomic DNA is replicated and then each copy is allocated into a daughter cell. In addition, the cytoplasmic contents are divided evenly and distributed to the new cells. However, there are many differences between prokaryotic and eukaryotic cell division. Bacteria have a single, circular DNA chromosome but no nucleus. Therefore, mitosis (karyokinesis) is not necessary in bacterial cell division. Bacterial cytokinesis is directed by a ring composed of a protein called FtsZ. Ingrowth of membrane and cell wall material from the periphery of the cells results in the formation of a septum that eventually constructs the separate cell walls of the daughter cells.

102.

VISUAL CONNECTION QUESTIONS

1. Figure 10.13 Rb and other proteins that negatively regulate the cell cycle are sometimes called tumor suppressors. Why do you think the name tumor suppressor might be appropriate for these proteins?

2. Figure 10.14 Human papillomavirus can cause cervical cancer. The virus encodes E6, a protein that binds p53. Based on this fact and what you know about p53, what effect do you think E6 binding has on p53 activity?

a. E6 activates p53
b. E6 inactivates p53
c. E6 mutates p53
d. E6 binding marks p53 for degradation

103.

REVIEW QUESTIONS

3. A diploid cell has_____ the number of chromosomes as a haploid cell.

a. one-fourth
b. half
c. twice
d. four times

4. An organism's traits are determined by the specific combination of inherited _____.

a. cells.
b. genes.
c. proteins.
d. chromatids.

5. The first level of DNA organization in a eukaryotic cell is maintained by which molecule?

a. cohesin
b. condensin
c. chromatin
d. histone

6. Identical copies of chromatin held together by cohesin at the centromere are called _____.

a. histones.
b. nucleosomes.
c. chromatin.
d. sister chromatids.

7. Chromosomes are duplicated during what stage of the cell cycle?

a. G1 phase

b. S phase
c. prophase
d. prometaphase

8. Which of the following events does not occur during some stages of interphase?

a. DNA duplication
b. organelle duplication
c. increase in cell size
d. separation of sister chromatids

9. The mitotic spindles arise from which cell structure?

a. centromere
b. centrosome
c. kinetochore
d. cleavage furrow

10. Attachment of the mitotic spindle fibers to the kinetochores is a characteristic of which stage of mitosis?

a. prophase
b. prometaphase
c. metaphase
d. anaphase

11. Unpacking of chromosomes and the formation of a new nuclear envelope is a characteristic of which stage of mitosis?

a. prometaphase
b. metaphase
c. anaphase
d. telophase

12. Separation of the sister chromatids is a characteristic of which stage of mitosis?

a. prometaphase
b. metaphase
c. anaphase

d. telophase

13. The chromosomes become visible under a light microscope during which stage of mitosis?

a. prophase
b. prometaphase
c. metaphase
d. anaphase

14. The fusing of Golgi vesicles at the metaphase plate of dividing plant cells forms what structure?

a. cell plate
b. actin ring
c. cleavage furrow
d. mitotic spindle

15. Figure 10.6 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 reforms and the cell divides. Cohesin proteins break down and the sister chromatids separate.
b. The kinetochore becomes attached to the mitotic spindle. Cohesin proteins break down and the sister chromatids separate. Sister chromatids line up at the metaphase plate. The nucleus reforms and the cell divides.
c. The kinetochore becomes attached to the cohesin proteins. Sister chromatids line up at the metaphase plate. The kinetochore breaks down and the sister chromatids separate. The nucleus reforms and the cell divides.
d. The kinetochore becomes attached to the mitotic spindle. Sister chromatids line up at the metaphase plate. Cohesin proteins break down and the sister chromatids separate. The nucleus reforms and the cell divides.

16. At which of the cell-cycle checkpoints do external forces have the greatest influence?

a. G_1 checkpoint
b. G_2 checkpoint
c. M checkpoint
d. G_0 checkpoint

17. What is the main prerequisite for clearance at the G_2 checkpoint?

a. cell has reached a sufficient size
b. an adequate stockpile of nucleotides
c. accurate and complete DNA replication
d. proper attachment of mitotic spindle fibers to kinetochores

18. If the M checkpoint is not cleared, what stage of mitosis will be blocked?

a. prophase
b. prometaphase
c. metaphase
d. anaphase

19. Which protein is a positive regulator that phosphorylates other proteins when activated?

a. p53
b. retinoblastoma protein (Rb)
c. cyclin
d. cyclin-dependent kinase (Cdk)

20. Many of the negative regulator proteins of the cell cycle were discovered in what type of cells?

a. gametes
b. cells in G_0
c. cancer cells
d. stem cells

21. Which negative regulatory molecule can trigger cell suicide (apoptosis) if vital cell cycle events do not occur?

a. p53
b. p21
c. retinoblastoma protein (Rb)
d. cyclin-dependent kinase (Cdk)

22. _____ are changes to the order of nucleotides in a segment of DNA that codes for a protein.

a. Proto-oncogenes
b. Tumor suppressor genes
c. Gene mutations
d. Negative regulators

23. A gene that codes for a positive cell-cycle regulator is called a(n) _____.

a. kinase inhibitor.
b. tumor suppressor gene.
c. proto-oncogene.
d. oncogene.

24. A mutated gene that codes for an altered version of Cdk that is active in the absence of cyclin is a(n) _____.

a. kinase inhibitor.
b. tumor suppressor gene.
c. proto-oncogene.
d. oncogene.

25. Which molecule is a Cdk inhibitor that is controlled by p53?

a. cyclin
b. anti-kinase
c. Rb
d. p21

26. Which eukaryotic cell-cycle event is missing in binary fission?

a. cell growth
b. DNA duplication
c. karyokinesis
d. cytokinesis

27. FtsZ proteins direct the formation of a _____ that will eventually form the new cell walls of the daughter cells.

a. contractile ring
b. cell plate

c. cytoskeleton
d. septum

104.

CRITICAL THINKING QUESTIONS

28. Compare and contrast a human somatic cell to a human gamete.

29. What is the relationship between a genome, chromosomes, and genes?

30. Eukaryotic chromosomes are thousands of times longer than a typical cell. Explain how chromosomes can fit inside a eukaryotic nucleus.

31. Briefly describe the events that occur in each phase of interphase.

32. Chemotherapy drugs such as vincristine (derived from Madagascar periwinkle plants) and colchicine (derived from autumn crocus plants) disrupt mitosis by binding to tubulin (the subunit of microtubules) and interfering with microtubule assembly and disassembly. Exactly what mitotic structure is targeted by these drugs and what effect would that have on cell division?

33. Describe the similarities and differences between the cytokinesis mechanisms found in animal cells versus those in plant cells.

34. List some reasons why a cell that has just completed cytokinesis might enter the G_0 phase instead of the G_1 phase.

35. What cell-cycle events will be affected in a cell that produces mutated (non-functional) cohesin protein?

36. Describe the general conditions that must be met at each of the three main cell-cycle checkpoints.

37. Compare and contrast the roles of the positive cell-cycle regulators negative regulators.

38. What steps are necessary for Cdk to become fully active?

39. Rb is a negative regulator that blocks the cell cycle at the G_1 checkpoint until the cell achieves a requisite size. What molecular mechanism does Rb employ to halt the cell cycle?

40. Outline the steps that lead to a cell becoming cancerous.

41. Explain the difference between a proto-oncogene and a tumor-suppressor gene.

42. List the regulatory mechanisms that might be lost in a cell producing faulty p53.

43. p53 can trigger apoptosis if certain cell-cycle events fail. How does this regulatory outcome benefit a multicellular organism?

44. Name the common components of eukaryotic cell division and binary fission.

45. Describe how the duplicated bacterial chromosomes are distributed into new daughter cells without the direction of the mitotic spindle.

PART XI

MEIOSIS AND SEXUAL REPRODUCTION

105.

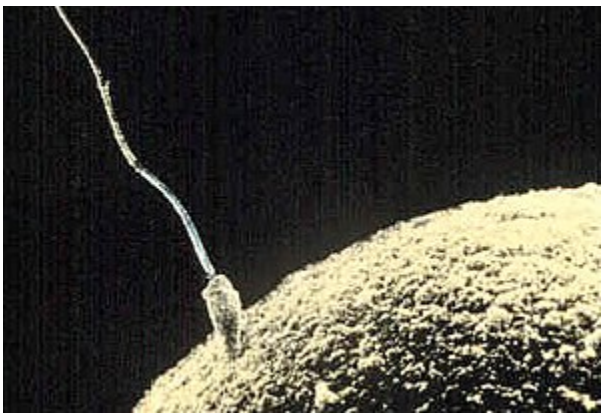
INTRODUCTION

Figure 11.1 Each of us, like the organisms shown above, begins life as a fertilized egg (zygote). After trillions of cell divisions, each of us develops into a complex, multicellular organism. (credit a: modification of work by Frank Wouters; credit b: modification of work by Ken Cole, USGS; credit c: modification of work by Martin Pettitt).

The ability to reproduce is a basic characteristic of all organisms: Hippopotamuses give birth to hippopotamus calves; Joshua trees produce seeds from which Joshua tree seedlings emerge; and adult flamingos lay eggs that hatch into flamingo chicks. However, unlike the organisms shown above, offspring may or may not resemble their parents. For example, in the case of most insects such as butterflies (with a complete metamorphosis), the larval forms rarely resemble the adult forms.

Although many unicellular organisms and a few multicellular organisms can produce genetically identical clones of themselves through ***asexual reproduction***, many single-celled organisms and most multicellular organisms reproduce regularly using another method—***sexual reproduction***. This highly evolved method involves the production by parents of two haploid cells and the fusion of two haploid cells to form a single, genetically recombined diploid cell—a genetically unique organism. In almost all sexually reproducing species, these two haploid cells differ in size, with the smaller cell called "male" and the larger one called "female." These haploid cells are produced by a type of cell division called meiosis. Sexual reproduction, involving both meiosis and fertilization, introduces variation into offspring that may account for the evolutionary success of sexual reproduction. The vast majority of eukaryotic organisms, both multicellular and unicellular, can or must employ some form of meiosis and fertilization to reproduce.

In most plants and animals, the zygote formed by fertilization, through thousands of rounds of mitotic cell division, will develop into an adult organism.



Sperm fusing (fertilizing) with an egg (ovum); the size of the sperm as it makes contact is emphasized by the egg. (https://en.wikipedia.org/wiki/Fertilisation)

106.

THE PROCESS OF MEIOSIS

Learning Objectives

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

- Describe the behavior of chromosomes during meiosis, and the differences between the first and second meiotic divisions
- Describe the cellular events that take place during meiosis
- Explain the differences between meiosis and mitosis
- Explain the mechanisms within the meiotic process that produce genetic variation among the haploid gametes

Sexual reproduction requires the union of two specialized cells, called **gametes**, each of which contains one set of chromosomes. When gametes unite, they form a **zygote**, or a fertilized egg that contains two sets of chromosomes. (Note: Cells that contain one set of chromosomes are called **haploid**; cells containing two sets of chromosomes are called **diploid**). If the reproductive cycle is to continue for any sexually reproducing species, then the diploid cell must somehow maintain their two sets of chromosomes and do so by reducing its number of chromosome sets to produce haploid gametes (reduction division); otherwise, the number of chromosome sets will double with every future round of fertilization.

Therefore, sexual reproduction requires a nuclear division that reduces the number of chromosome sets by half.

Link to Learning

Watch this video on Meiosis: The Reduction Division

In humans, 46 chromosomes can be found in each somatic cell (n= 23, haploid and 2n= 46, diploid). These chromosomes condense sufficiently to be seen under a compound light microscope. This makes them distinguishable from each other by size, the centromere position and the pattern of chromatin-binding stained colored bands is demonstrated as the karyotype; mitosis reveals there are two chromosomes each of 23 types, each of these pairs have the same length, centromere position and chromatin-staining pattern and are called homologous chromosomes (or homologs). These chromosome pairs carry information controlling the same or similar hereditary characters giving rise to traits. Generally, in gender determination, in human somatic cells, the two chromosomes referred to as X and Y do not follow this homologous rule; human females are chromosomally homologous (XX), while males have two sex chromosomes, the X and Y, which are non-homologous.

In summary, animals and plants and many unicellular organisms are diploid and therefore have two sets of chromosomes. In each **somatic cell** of the organism (all cells of a multicellular organism except the gametes or reproductive cells), the nucleus contains two copies of each chromosome, called **homologous chromosomes**. Homologous chromosomes are matched pairs containing the same genetic information in identical locations along their lengths. Diploid organisms inherit one copy of each homologous chromosome from each parent.

Mitosis vs. Meiosis

Meiosis is the *nuclear division* that forms haploid cells from diploid cells, and it employs many of the same cellular mechanisms as mitosis. However, as you have learned, **mitosis** produces daughter cells whose nuclei are genetically identical to the original parent nucleus. In mitosis, both the parent and the daughter nuclei are at the same "ploidy level"—diploid in the case of most multicellular animals. Plants use mitosis to grow as sporophytes and to grow and produce eggs and sperm as gametophytes, so they use mitosis for both haploid and diploid cells (as well as for all other ploidies). In meiosis, the starting nucleus is always diploid and the daughter nuclei that result are haploid. To achieve this reduction in chromosome number, meiosis consists of one round of chromosome replication followed by two rounds of nuclear division.

Genes are the units of inheritance passed on from parents to offspring. Genetic information endows offspring with their traits through its transmission, which has its molecular basis in the replication of DNA. The units of inheritance are a genetic program written in the language of specific sequences of DNA. In plants

and animals, reproductive cells or gametes (male-sperms and female-eggs) are the carriers of this information and, through fusion or fertilization, pass on these units of inheritance from both parents to the offspring. Small amounts of DNA are carried in the mitochondria and chloroplasts, which are independent of the DNA of a eukaryotic cell that is contained or packaged into chromosomes within the nucleus. A gene's location along the length of a chromosome is specified as the locus (plural loci; Latin meaning "place"). A single chromosome comprises of a single long DNA molecule, closely coiled in association with various proteins, and includes hundreds to a few thousand genes, each made up of a precise sequence of nucleotides along the DNA molecule.

Asexually reproducing organisms have genetically identical copies as offspring and are clones. Thus, amoeba or yeast cells are sole parents who transmit all their genetic information or pass copies of their entire chromosomes through mitosis cell division to their offspring without the involvement of fusion of gametes.

Sexually reproducing organisms are two parents who produce gametes by distinct cell division called meiosis. These gametes fuse during fertilization giving rise to offspring that have unique combinations of genes inherited from both parents. Therefore, these offspring are not clones because they genetically vary from each other and collectively present family resemblance but not exact replicas (picture below). Because the events that occur during each of the division stages are analogous to the events of mitosis, the same stage names are assigned. However, because there are two rounds of division, the major process and the stages are designated with a "I" or a "II." Thus, **meiosis I** is the first round of meiotic division and consists of prophase I, prometaphase I, and so on. Likewise, **Meiosis II** (during which the second round of meiotic division takes place) includes prophase II, prometaphase II, and so on. The two essential parts of reproduction are fertilization and meiosis, and they alternate in sexual life cycles. In the human life cycle, for fertilization, the haploid sperm (n= 23) fuses with the haploid egg (n= 23) to form the diploid zygote (2n= 46), which divides by mitosis into the embryo, which will develop into the fetus as it attaches in the uterus and eventually be born and become sexually mature, and the meiosis process repeats again in the gonads.

A family of four typifying varying inheritance of combinations of genes with siblings showing some semblance to their parents. (https://humanbiology.pressbooks.tru.ca/chapter/5-11-sexual-reproduction-meiosis-and-gametogenesis/)

Meiosis I

Meiosis is preceded by an interphase consisting of G_1, S, and G_2 phases, which are nearly identical to the phases preceding mitosis. The G_1 phase (the "first gap phase") is focused on cell growth. During the S phase—the second phase of interphase—the cell copies or *replicates* the DNA of the chromosomes. Finally, in the G_2 phase (the "second gap phase") the cell undergoes the final preparations for meiosis.

During DNA duplication in the S phase, each chromosome is replicated to produce two identical copies—*sister chromatids*—that are held together at the centromere by **cohesin** proteins, which hold the chromatids together until anaphase II.

Prophase I

Early in prophase I, before the chromosomes can be seen clearly with a microscope, the homologous chromosomes are attached at their tips to the nuclear envelope by proteins. As the nuclear envelope begins to break down, the proteins associated with homologous chromosomes bring the pair closer together. Recall that in mitosis, homologous chromosomes do not pair together. The **synaptonemal complex**, a lattice of proteins between the homologous chromosomes, first forms at specific locations and then spreads outward to cover the entire length of the chromosomes. The tight pairing of the homologous chromosomes is called *synapsis*. In **synapsis**, the genes on the chromatids of the homologous chromosomes are aligned precisely with each other. The synaptonemal complex supports the exchange of chromosomal segments between homologous nonsister chromatids—a process called **crossing over** (crossover). The crossing over can be observed visually after the exchange as *chiasmata* (singular = chiasma) (Figure 11.2).

In humans, even though the X and Y sex chromosomes are not completely homologous (that is, most of their genes differ), there is a small region of homology that allows the X and Y chromosomes to pair up during prophase I. A partial synaptonemal complex develops only between the regions of homology.

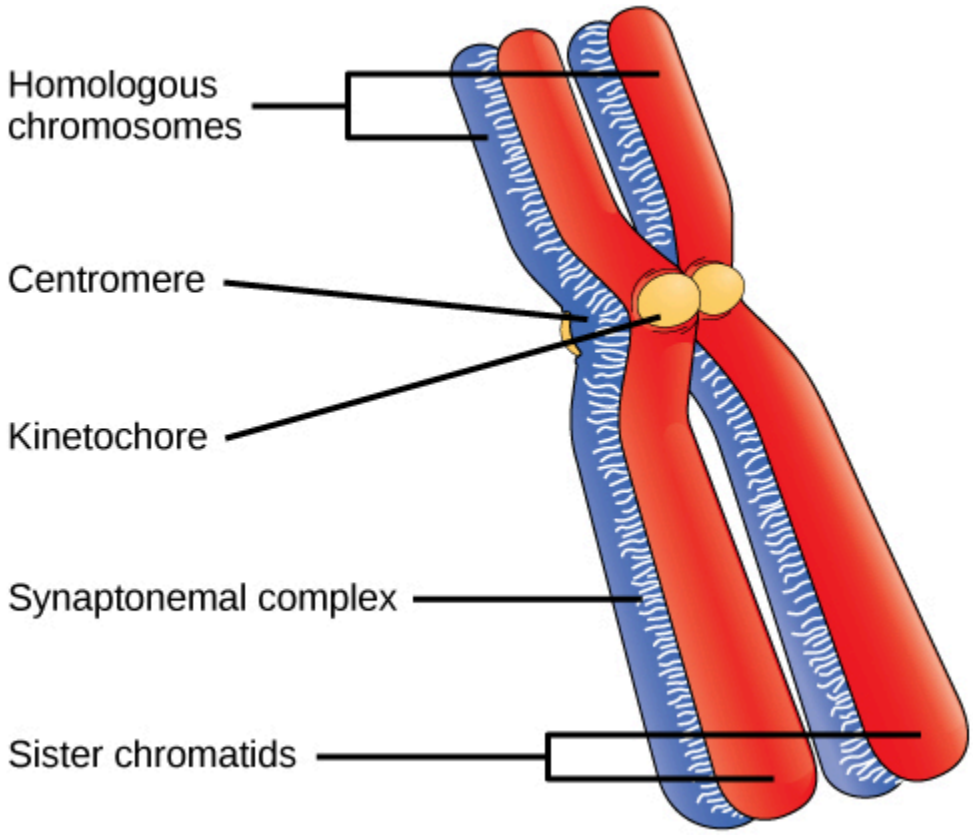


Figure 11.2 Early in prophase I, homologous chromosomes come together to form a synapse. The chromosomes are bound tightly together and in perfect alignment by a protein lattice called a synaptonemal complex and by cohesin proteins at the centromere.

Located at intervals along the synaptonemal complex are large protein assemblies called **recombination nodules**. These assemblies mark the points of later chiasmata and mediate the multistep process of crossing over—or genetic recombination—between the nonsister chromatids. Near the recombination nodule, the double-stranded DNA of each chromatid is cleaved, the cut ends are modified, and a new connection is made between the nonsister chromatids. As prophase I progresses, the synaptonemal complex begins to break down and the chromosomes begin to condense. When the synaptonemal complex is gone, the homologous chromosomes remain attached to each other at the centromere and at chiasmata. The chiasmata remain until anaphase I. The number of chiasmata varies according to the species and the length of the chromosome. There must be at least one chiasma per chromosome for proper separation of homologous chromosomes during meiosis I, but there may be as many as 25. Following crossing over, the synaptonemal complex breaks down, and the cohesin connection between homologous pairs is removed. At the end of prophase I, the pairs are held together only at the chiasmata (Figure 11.3). These pairs are called **tetrads** because a total of four sister chromatids of each pair of homologous chromosomes are now visible.

The crossing over events are the first source of genetic variation in the nuclei produced by meiosis. A single crossing over event between homologous nonsister chromatids leads to a reciprocal exchange of equivalent

DNA between an egg-derived chromosome and a sperm-derived chromosome. When a recombinant sister chromatid is moved into a gamete cell, it will carry a combination of maternal and paternal genes that did not exist before the crossing over. The crossing over events can occur almost anywhere along the length of the synapsed chromosomes. Different cells undergoing meiosis will therefore produce different recombinant chromatids, with varying combinations of maternal and parental genes. Multiple crossing overs in an arm of the chromosome have the same effect, exchanging segments of DNA to produce genetically recombined chromosomes.

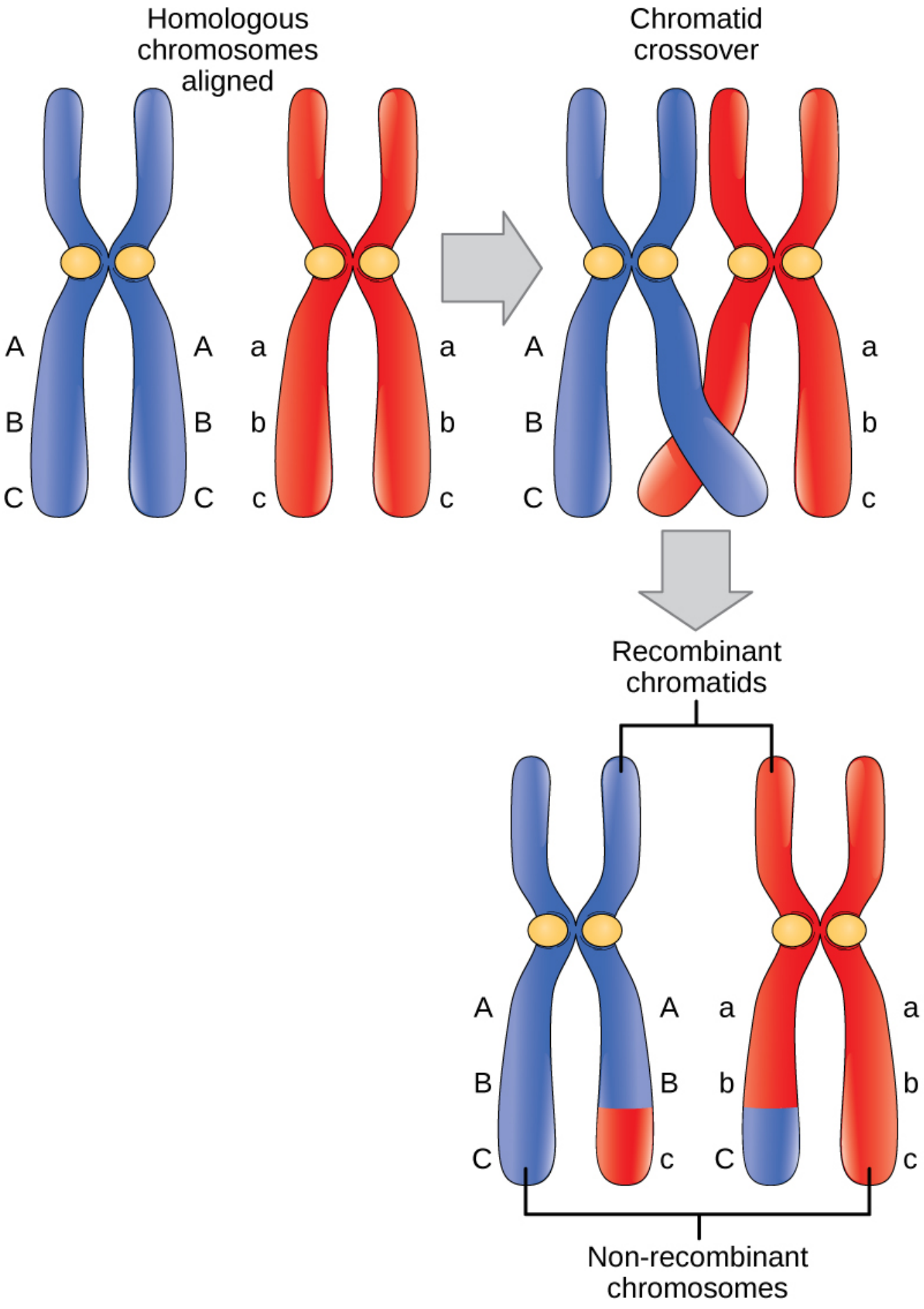

Homologous chromosomes aligned
Chromatid crossover
A
B
C
A
B
C
a
b
c
a
b
c
A
B
C
a
b
c
Recombinant chromatids
A
B
C
A
B
C
a
b
C
a
b
c
Non-recombinant chromosomes

Figure 11.3 Crossing over (crossover) occurs between nonsister chromatids of homologous chromosomes. The result is an exchange of genetic material between homologous chromosomes.

Prometaphase I

The key event in prometaphase I is the attachment of the spindle fiber microtubules to the kinetochore proteins at the centromeres. Kinetochore proteins are multiprotein complexes that bind the centromeres of a chromosome to the microtubules of the mitotic spindle. Microtubules grow from microtubule-organizing centers (MTOCs). In animal cells, MTOCs are centrosomes located at opposite poles of the cell. The microtubules from each pole move toward the middle of the cell and attach to one of the kinetochores of the two fused homologous chromosomes. Each member of the homologous pair attaches to a microtubule extending from opposite poles of the cell so that in the next phase, the microtubules can pull the homologous pair apart. A spindle fiber that has attached to a kinetochore is called a *kinetochore microtubule*. At the end of prometaphase I, each tetrad is attached to microtubules from both poles, with one homologous chromosome facing each pole. The homologous chromosomes are still held together at the chiasmata. In addition, the nuclear membrane has broken down entirely.

Metaphase I

During metaphase I, the homologous chromosomes are arranged at the **metaphase plate**—roughly in the midline of the cell, with the kinetochores facing opposite poles. Each homologous pair is oriented randomly at the equator. For example, if the two homologous members of chromosome 1 are labeled *a* and *b*, then the chromosomes could line up a-b or b-a. This is important in determining the genes carried by a gamete, as each will only receive one of the two homologous chromosomes. (Recall that homologous chromosomes are not identical. They contain different versions of the same genes, and after recombination during crossing over, each gamete will have a unique genetic makeup that has never existed before).

The randomness in the alignment of recombined chromosomes at the metaphase plate, coupled with the crossing over events between nonsister chromatids, is responsible for much of the genetic variation in the offspring. To clarify this further, remember that the homologous chromosomes of a sexually reproducing organism are originally inherited as two separate sets, one from each parent. Using humans as an example, one set of 23 chromosomes is present in the egg cell, often called maternal chromosomes because the genetic contributor is often the mother. The other set of 23 chromosomes is contained in the sperm, and the genetic contributor is called a father, providing the paternal chromosomes. Every cell of the multicellular offspring has copies of the original two sets of homologous chromosomes. When the offspring human creates their own gametes through meiosis, the two sets of chromosomes will be rearranged. In prophase I of meiosis, the homologous chromosomes form the tetrads. In metaphase I, these pairs line up at the midway point between the two poles of the cell to form the metaphase plate. Because there is an equal chance that a microtubule fiber will encounter a maternally or paternally inherited chromosome, the arrangement of the tetrads at the

metaphase plate is random. Thus, any maternally inherited chromosome may face either pole. Likewise, any paternally inherited chromosome may also face either pole. *The orientation of each tetrad is independent of the orientation of the other 22 tetrads.*

This event—the *random* (or *independent*) assortment of homologous chromosomes at the metaphase plate—is the second mechanism that introduces variation into the gametes or spores. In each cell that undergoes meiosis, the arrangement of the tetrads is different. The number of variations is dependent on the number of chromosomes making up a set. There are two possibilities for orientation at the metaphase plate; the possible number of alignments therefore equals 2^n in a diploid cell, where n is the number of chromosomes per haploid set. Humans have 23 chromosome pairs, which results in over eight million (2^{23}) possible genetically distinct gametes just from the random alignment of chromosomes at the metaphase plate. This number does not include the variability that was previously produced by crossing over between the nonsister chromatids. Given these two mechanisms, it is highly unlikely that any two haploid cells resulting from meiosis will have the same genetic composition (Figure 11.4).

To summarize, meiosis I creates genetically diverse gametes in two ways. First, during prophase I, crossing over events between the nonsister chromatids of each homologous pair of chromosomes generate recombinant chromatids with new combinations of maternal and paternal genes. Second, the random assortment of tetrads on the metaphase plate produces unique combinations of maternal and paternal chromosomes that will make their way into the gametes.

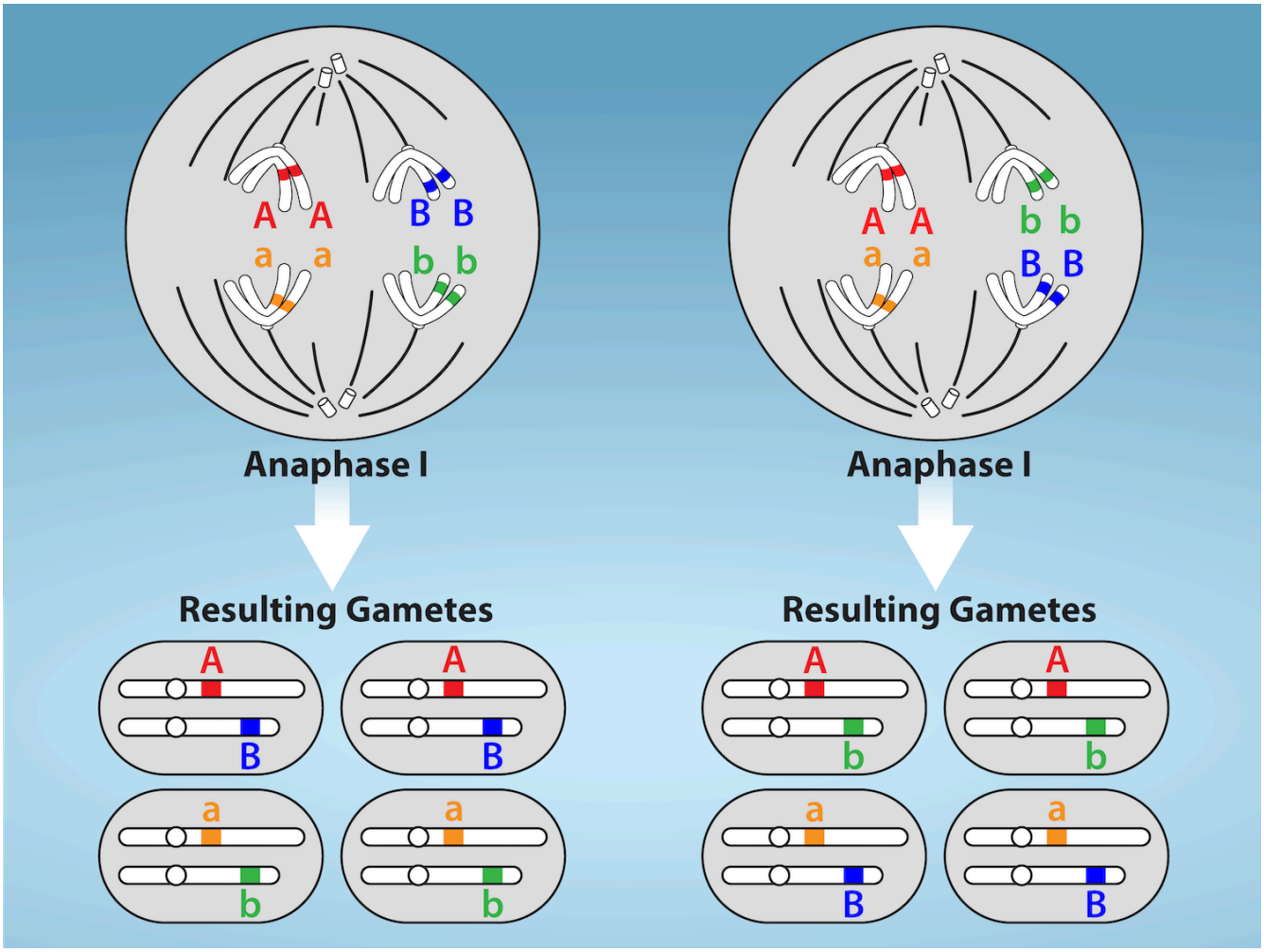


Figure 11.4 Random, independent assortment during metaphase I can be demonstrated by considering a cell with a set of two chromosomes (n = 2). In this case, there are two possible arrangements at the equatorial plane in metaphase I. The total possible number of different gametes is 2n, where n equals the number of chromosomes in a set. In this example, there are four possible genetic combinations for the gametes. With n = 23 in human cells, there are over eight million possible combinations of paternal and maternal chromosomes.

Anaphase I

In anaphase I, the microtubules pull the linked chromosomes apart. The sister chromatids remain tightly bound together at the centromere. The chiasmata are broken in anaphase I as the microtubules attached to the fused kinetochores pull the homologous chromosomes apart **(Figure 11.4)**

Telophase I and Cytokinesis

In telophase, the separated chromosomes arrive at opposite poles. The remainder of the typical telophase events may or may not occur, depending on the species. In some organisms, the chromosomes "decondense" and nuclear envelopes form around the separated sets of chromatids produced during telophase I. In other

organisms, **cytokinesis**—the physical separation of the cytoplasmic components into two daughter cells—occurs without reformation of the nuclei. In nearly all species of animals and some fungi, cytokinesis separates the cell contents via a *cleavage furrow* (constriction of the actin ring that leads to cytoplasmic division). In plants, a *cell plate* is formed during cell cytokinesis by Golgi vesicles fusing at the metaphase plate. This cell plate will ultimately lead to the formation of cell walls that separate the two daughter cells.

Two haploid cells are the result of the first meiotic division of a diploid cell. The cells are haploid because at each pole, there is just one of each pair of the homologous chromosomes. Therefore, only one full set of the chromosomes is present. This is why the cells are considered haploid—there is only one chromosome set, *even though each chromosome still consists of two sister chromatids.* Recall that sister chromatids are merely duplicates of one of the two homologous chromosomes (except for changes that occurred during crossing over). In meiosis II, these two sister chromatids will separate, creating four haploid daughter cells.

Link to Learning

Review the process of meiosis, observing how chromosomes align and migrate, at Meiosis: An Interactive Animation.

Meiosis II

In some species, cells enter a brief interphase, or **interkinesis**, before entering meiosis II. Interkinesis lacks an S phase, so chromosomes are not duplicated. The two cells produced in meiosis I go through the events of meiosis II in synchrony. During meiosis II, the sister chromatids within the two daughter cells separate, forming four new haploid gametes. The mechanics of meiosis II are similar to mitosis, except that each dividing cell has only one set of homologous chromosomes, each with two chromatids. Therefore, each cell has half the number of sister chromatids to separate out as a diploid cell undergoing mitosis. In terms of chromosomal content, cells at the start of meiosis II are similar to haploid cells in G_2, preparing to undergo mitosis.

Prophase II

If the chromosomes decondensed in telophase I, they condense again. If nuclear envelopes were formed, they fragment into vesicles. The MTOCs that were duplicated during interkinesis move away from each other toward opposite poles, and new spindles are formed.

Prometaphase II

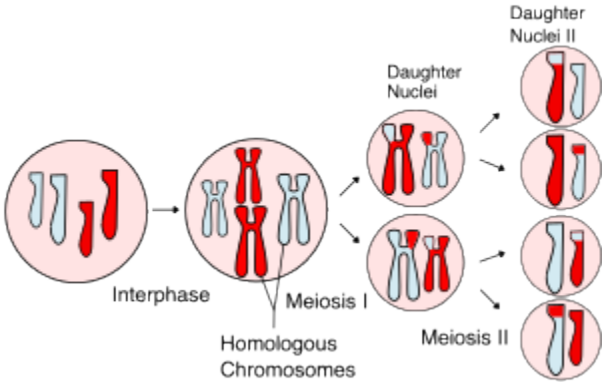
The nuclear envelopes are completely broken down, and the spindle is fully formed. Each sister chromatid forms an individual kinetochore that attaches to microtubules from opposite poles.

Metaphase II

The sister chromatids are maximally condensed and aligned at the equator of the cell.

Anaphase II

The sister chromatids are pulled apart by the kinetochore microtubules and move toward opposite poles. Nonkinetochore microtubules elongate the cell.



The process of chromosome alignment differs between meiosis I and meiosis II and entirely separate from chromosome alignment in mitosis. In prometaphase I, microtubules attach to the fused kinetochores of homologous chromosomes, and the homologous chromosomes are arranged at the midline of the cell (the metaphase plate) in metaphase I. In anaphase I, the homologous chromosomes separate. In prometaphase II, microtubules attach to the kinetochores of sister chromatids, and the sister chromatids are arranged at the midpoint of the cells in metaphase II. In anaphase II, the sister chromatids separate. (https://en.wikipedia.org/wiki/Meiosis)

YouTube: https://www.youtube.com/watch?v=q78mPCTvnvg

Telophase II and Cytokinesis

The chromosomes arrive at opposite poles and begin to decondense. Nuclear envelopes form around the chromosomes. If the parent cell was diploid, as is most commonly the case, then cytokinesis now separates the two cells into four unique haploid cells. The cells produced are genetically unique because of the random assortment of paternal and maternal homologs and because of the recombination of maternal and paternal segments of chromosomes (with their sets of genes) that occurs during crossover. Thus, the four daughter cells after meiosis I and II are genetically distinct from one another and the parent cell, which means they are not clones. The entire process of meiosis is outlined in Figure 11.6.

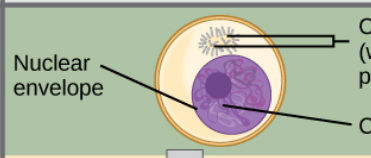
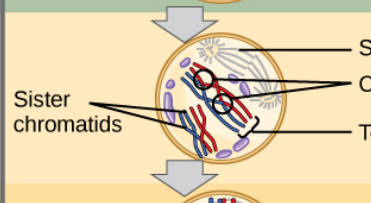
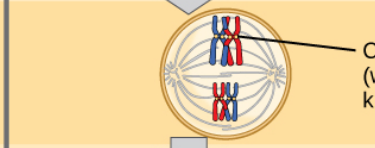
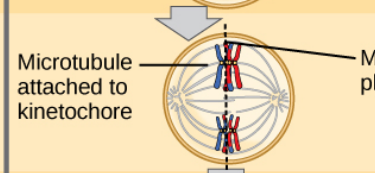
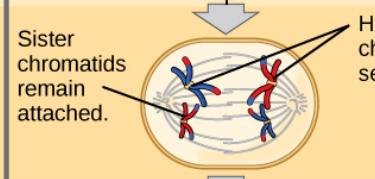
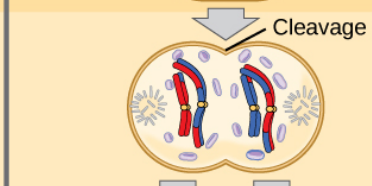
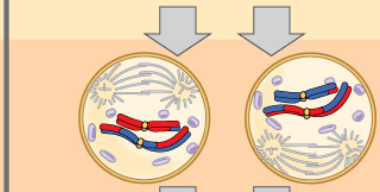
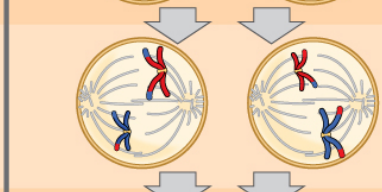
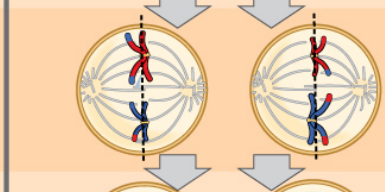
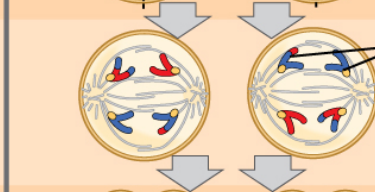
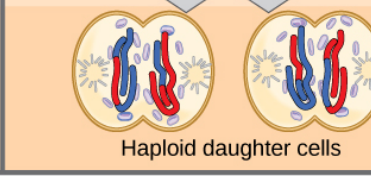
	Stage	Event	Outcome
INTERPHASE	S phase		Chromosomes are duplicated during interphase. The resulting sister chromatids are held together at the centromere. The centrosomes are also duplicated.
MEIOSIS I	Prophase I		Chromosomes condense, and the nuclear envelope fragments. Homologous chromosomes bind firmly together along their length, forming a tetrad. Chiasmata form between non-sister chromatids. Crossing over occurs at the chiasmata. Spindle fibers emerge from the centrosomes.
	Prometaphase I		Homologous chromosomes are attached to spindle microtubules at the fused kinetochore shared by the sister chromatids. Chromosomes continue to condense, and the nuclear envelope completely disappears.
	Metaphase I		Homologous chromosomes randomly assemble at the metaphase plate, where they have been maneuvered into place by the microtubules.
	Anaphase I		Spindle microtubules pull the homologous chromosomes apart. The sister chromatids are still attached at the centromere.
	Telophase I and Cytokinesis		Sister chromatids arrive at the poles of the cell and begin to decondense. A nuclear envelope forms around each nucleus and the cytoplasm is divided by a cleavage furrow. The result is two haploid cells. Each cell contains one duplicated copy of each homologous chromosome pair.
MEIOSIS II	Prophase II		Sister chromatids condense. A new spindle begins to form. The nuclear envelope starts to fragment.
	Prometaphase II		The nuclear envelope disappears, and the spindle fibers engage the individual kinetochores on the sister chromatids.
	Metaphase II		Sister chromatids line up at the metaphase plate.
	Anaphase II		Sister chromatids are pulled apart by the shortening of the kinetochore microtubules. Non-kinetochore microtubules lengthen the cell.
	Telophase II and Cytokinesis		Chromosomes arrive at the poles of the cell and decondense. Nuclear envelopes surround the four nuclei. Cleavage furrows divide the two cells into four haploid cells.

Figure 11.6 An animal cell with a diploid number of four (2n = 4) proceeds through the stages of meiosis to form four haploid daughter cells.

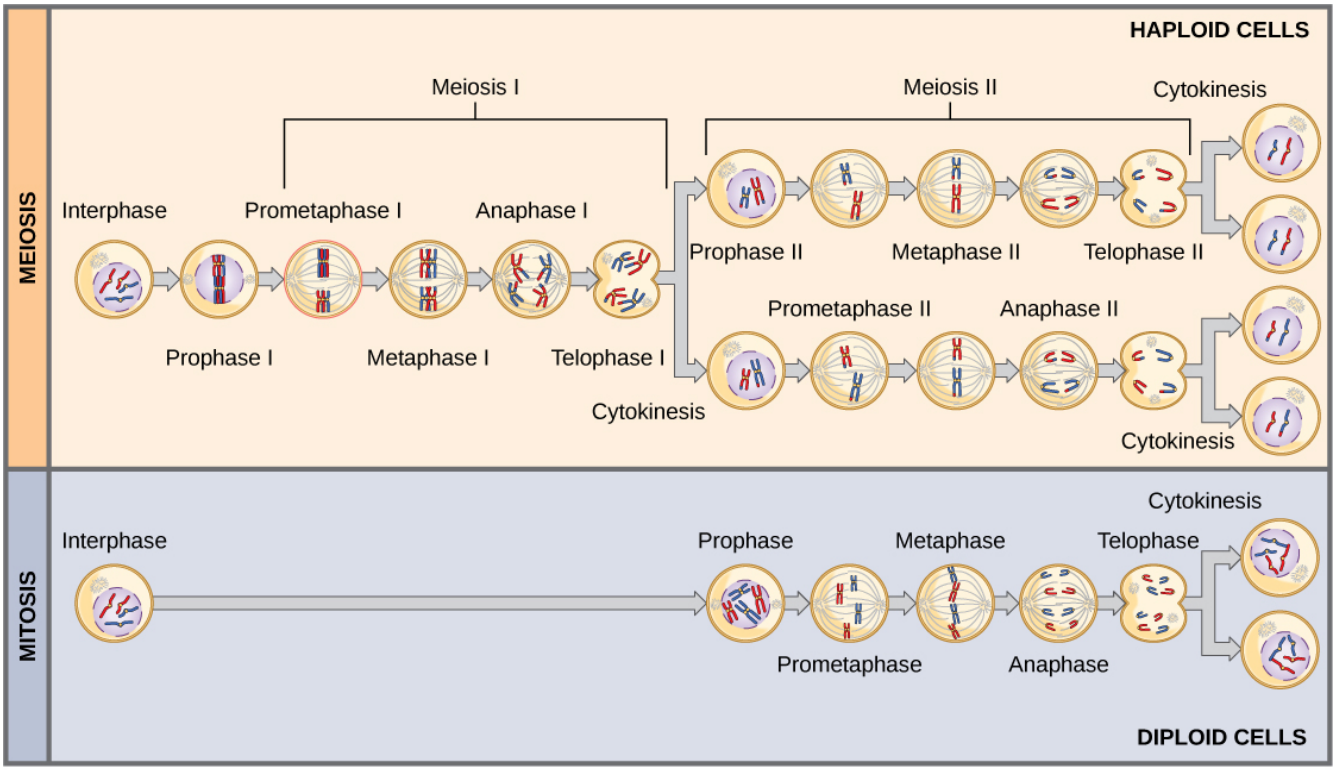
Comparing Meiosis and Mitosis

Mitosis and meiosis are both forms of division of the nucleus in eukaryotic cells. They share some similarities, but also exhibit a number of distinct processes that lead to very different outcomes (Figure 11.7). Mitosis is a single nuclear division that results in two nuclei that are usually partitioned into two new cells. The nuclei resulting from a mitotic division are genetically identical to the original nucleus. They have the same number of sets of chromosomes: one set in the case of haploid cells and two sets in the case of diploid cells. In contrast, meiosis consists of two nuclear divisions resulting in four nuclei that are usually partitioned into four new, genetically distinct cells. The four nuclei produced during meiosis are not genetically identical, and they contain one chromosome set only. This is half the number of chromosome sets of the original cell, which is diploid.

The main differences between mitosis and meiosis occur in meiosis I, which is a very different nuclear division than mitosis. In meiosis I, the homologous chromosome pairs physically meet and are bound together with the synaptonemal complex. Following this, the chromosomes develop chiasmata and undergo crossover between nonsister chromatids. In the end, the chromosomes line up along the metaphase plate as tetrads—with kinetochore fibers from opposite spindle poles attached to each kinetochore of a homolog to form a tetrad. *All of these events occur only in meiosis I.*

When the chiasmata resolve and the tetrad is broken up with the homologous chromosomes moving to one pole or another, the ploidy level—the number of sets of chromosomes in each future nucleus—has been reduced from two to one. For this reason, meiosis I is referred to as a **reductional division**. There is no such reduction in ploidy level during mitosis.

Meiosis II is analogous to a mitotic division. In this case, the duplicated chromosomes (only one set of them) line up on the metaphase plate with divided kinetochores attached to kinetochore fibers from opposite poles. During anaphase II, as in mitotic anaphase, the kinetochores divide and one sister chromatid—now referred to as a chromosome—is pulled to one pole while the other sister chromatid is pulled to the other pole. If it were not for the fact that there had been crossover, the two products of each individual meiosis II division would be identical (as in mitosis). Instead, they are different because there has always been at least one crossover per chromosome. Meiosis II is not a reduction division because although there are fewer copies of the genome in the resulting cells, there is still one set of chromosomes, as there was at the end of meiosis I.



						OUTCOME
PROCESS	DNA synthesis	Synapsis of homologous chromosomes	Crossover	Homologous chromosomes line up at metaphase plate	Sister chromatids line up at metaphase plate	Number and genetic composition of daughter cells
MEIOSIS	Occurs in S phase of interphase	During prophase I	During prophase I	During metaphase I	During metaphase II	Four haploid cells at the end of meiosis II
MITOSIS	Occurs in S phase of interphase	Does not occur in mitosis	Does not occur in mitosis	Does not occur in mitosis	During metaphase	Two diploid cells at the end of mitosis

Figure 11.7 Meiosis and mitosis are both preceded by one cycle of DNA replication; however, meiosis includes two nuclear divisions. The four daughter cells resulting from meiosis are haploid and genetically distinct. The daughter cells resulting from mitosis are diploid and identical to the parent cell.

Evolution Connection

The Mystery of the Evolution of Meiosis

Some characteristics of organisms are so widespread and fundamental that it is sometimes difficult to remember that they evolved like other simple traits. Meiosis is such an extraordinarily complex series of cellular events that biologists have had trouble testing hypotheses concerning how it may have evolved. Although meiosis is inextricably entwined with sexual reproduction and its advantages and disadvantages, it is important to separate the questions of the evolution of meiosis and the evolution of sex because early meiosis may have been advantageous for different reasons than it is now. Thinking outside the box and imagining what the early benefits of meiosis might have been is one approach to uncovering how it may have evolved.

Meiosis and mitosis share obvious cellular processes, and it makes sense that meiosis evolved from mitosis. The difficulty lies in the clear differences between meiosis I and mitosis. Adam Wilkins and Robin Holliday[1] summarized the unique events that needed to occur for the evolution of meiosis from mitosis. These steps are homologous chromosome pairing and synapsis, crossover exchanges, sister chromatids remaining attached during anaphase, and suppression of DNA replication in interphase. They argue that the first step is the hardest and most important and that understanding how it evolved would make the evolutionary process clearer. They suggest genetic experiments that might shed light on the evolution of synapsis.

There are other approaches to understanding the evolution of meiosis in progress. Different forms of meiosis exist in single-celled protists. Some appear to be simpler or more "primitive" forms of meiosis. Comparing the meiotic divisions of different protists may shed light on the evolution of meiosis. Marilee Ramesh and colleagues[2] compared the genes involved in meiosis in protists to understand when and where meiosis might have evolved. Although research is still ongoing, recent scholarship into meiosis in protists suggests that some aspects of meiosis may have evolved later than others. This kind of genetic comparison can tell us what aspects of meiosis are the oldest and what cellular processes they may have borrowed from in earlier cells.

Link to Learning

Click through the steps of this interactive animation to compare the meiotic process of cell division to that of mitosis: How Cells Divide.

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https://louis.pressbooks.pub/generalbiology1leclab/?p=431#h5p-48

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https://louis.pressbooks.pub/generalbiology1leclab/?p=431#h5p-49

Footnotes

- 1 Adam S. Wilkins and Robin Holliday, "The Evolution of Meiosis from Mitosis," *Genetics* 181 (2009): 3–12.
- 2 Marilee A. Ramesh, Shehre-Banoo Malik and John M. Logsdon, Jr, "A Phylogenetic Inventory of Meiotic Genes: Evidence for Sex in *Giardia* and an Early Eukaryotic Origin of Meiosis," *Current Biology* 15 (2005):185–91.

107.

SEXUAL REPRODUCTION

Learning Objectives

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

- Explain that meiosis and sexual reproduction are highly evolved traits
- Identify variation among offspring as a potential evolutionary advantage of sexual reproduction
- Describe the three different life-cycle types among sexually reproducing multicellular organisms.

Sexual reproduction was likely an early evolutionary innovation after the appearance of eukaryotic cells. Sexual reproduction appears to have been very successful because most eukaryotes are able to reproduce sexually, and in many animals, it is the only mode of reproduction. And yet, scientists also recognize some real disadvantages to sexual reproduction. On the surface, creating offspring that are genetic clones of the parent appears to be a better system. If the parent organism is successfully occupying a habitat, offspring with the same traits should be similarly successful. There is also the obvious benefit to an organism that can produce offspring whenever circumstances are favorable by asexual budding, fragmentation, or producing eggs asexually. These methods of reproduction do not require a partner with which to reproduce. Indeed, some organisms that lead a solitary lifestyle have retained the ability to reproduce asexually. In addition, in asexual populations, every individual is capable of reproduction. In sexual populations, the males are not producing the offspring themselves, so hypothetically an asexual population could grow twice as fast.

However, multicellular organisms that exclusively depend on asexual reproduction are exceedingly rare. Why are meiosis and sexual reproductive strategies so common? These are important (and as yet unanswered) questions in biology, even though they have been the focus of much research beginning in the latter half of the 20th century. There are several possible explanations, one of which is that the variation that sexual

reproduction creates among offspring is very important to the survival and reproduction of the population. Thus, on average, a sexually reproducing population will leave more descendants than an otherwise similar asexually reproducing population. The only source of variation in asexual organisms is mutation. Mutations that take place during the formation of germ cell lines are also a source of variation in sexually reproducing organisms. However, in contrast to mutation during asexual reproduction, the mutations during sexual reproduction can be continually reshuffled from one generation to the next when different parents combine their unique genomes and the genes are mixed into different combinations by crossovers during prophase I and random assortment at metaphase I.

Evolution Connection

The Red Queen Hypothesis

Genetic variation is the outcome of sexual reproduction, but why are ongoing variations necessary, even under seemingly stable environmental conditions? Enter the Red Queen hypothesis, first proposed by Leigh Van Valen in 1973.[3] The concept was named in reference to the Red Queen's race in Lewis Carroll's book *Through the Looking-Glass*.

All species **coevolve** (evolve together) with other organisms. For example, predators evolve with their prey, and parasites evolve with their hosts. Each tiny advantage gained by favorable variation gives a species a reproductive edge over close competitors, predators, parasites, or even prey. However, survival of any given genotype or phenotype in a population is dependent on the reproductive fitness of other genotypes or phenotypes within a given species. The only method that will allow a coevolving species to maintain its own share of the resources is to also *continually improve* its **fitness** (the capacity of the members to produce more reproductively viable offspring relative to others within a species). As one species gains an advantage, this increases selection on the other species; they must also develop an advantage or they will be outcompeted. No single species progresses too far ahead because genetic variation among the progeny of sexual reproduction provides all species with a mechanism to improve rapidly. Species that cannot keep up become extinct. The Red Queen's catchphrase was, "It takes all the running you can do to stay in the same place." This is an apt description of coevolution between competing species.

Life Cycles of Sexually Reproducing Organisms

Fertilization and meiosis alternate in sexual **life cycles**. What happens between these two events depends on

the organism's "reproductive strategy." The process of meiosis reduces the chromosome number by half. Fertilization, the joining of two haploid gametes, restores the diploid condition. Some organisms have a multicellular diploid stage that is most obvious and only produce haploid reproductive cells. Animals, including humans, have this type of life cycle. Other organisms, such as fungi, have a multicellular haploid stage that is most obvious. Plants and some algae have alternation of generations, in which they have multicellular diploid and haploid life stages that are apparent to different degrees depending on the group.

Nearly all animals employ a diploid-dominant life-cycle strategy in which the only haploid cells produced by the organism are the gametes. Early in the development of the embryo, specialized diploid cells, called **germ cells**, are produced within the gonads (such as the testes and ovaries). Germ cells are capable of mitosis to perpetuate the germ cell line and meiosis to produce haploid gametes. Once the haploid gametes are formed, they lose the ability to divide again. There is no multicellular haploid life stage. Fertilization occurs with the fusion of two gametes, usually from different individuals, restoring the diploid state (Figure 11.8).

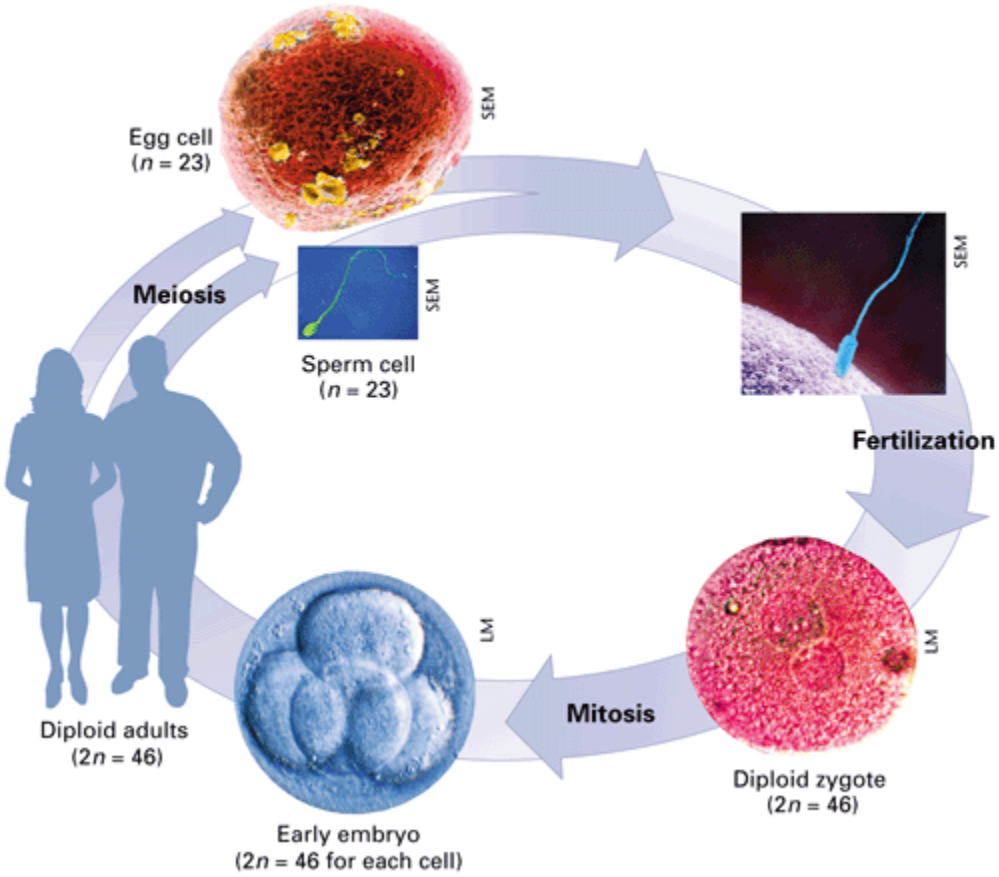

Figure 11.8 In animals, sexually reproducing adults form haploid gametes, called egg and sperm, from diploid germ cells. Fusion of the two gametes gives rise to a fertilized egg cell, or zygote. The zygote will undergo multiple rounds of mitosis to produce a multicellular offspring. The germ cells are generated early in the development of the zygote.

Most fungi and algae employ a life-cycle type in which the "body" of the organism—the ecologically important part of the life cycle—is haploid. The haploid cells that make up the tissues of the dominant multicellular stage are formed by mitosis. During sexual reproduction, specialized haploid cells from two individuals—designated the (+) and (−) mating types—join to form a diploid zygote. The zygote immediately undergoes meiosis to form four haploid cells called *spores*. Although these spores are haploid like the "parents," they contain a new genetic combination from two parents. The spores can remain dormant for various time periods. Eventually, when conditions are favorable, the spores form multicellular haploid structures through many rounds of mitosis (**Figure 11.9**).

Visual Connection

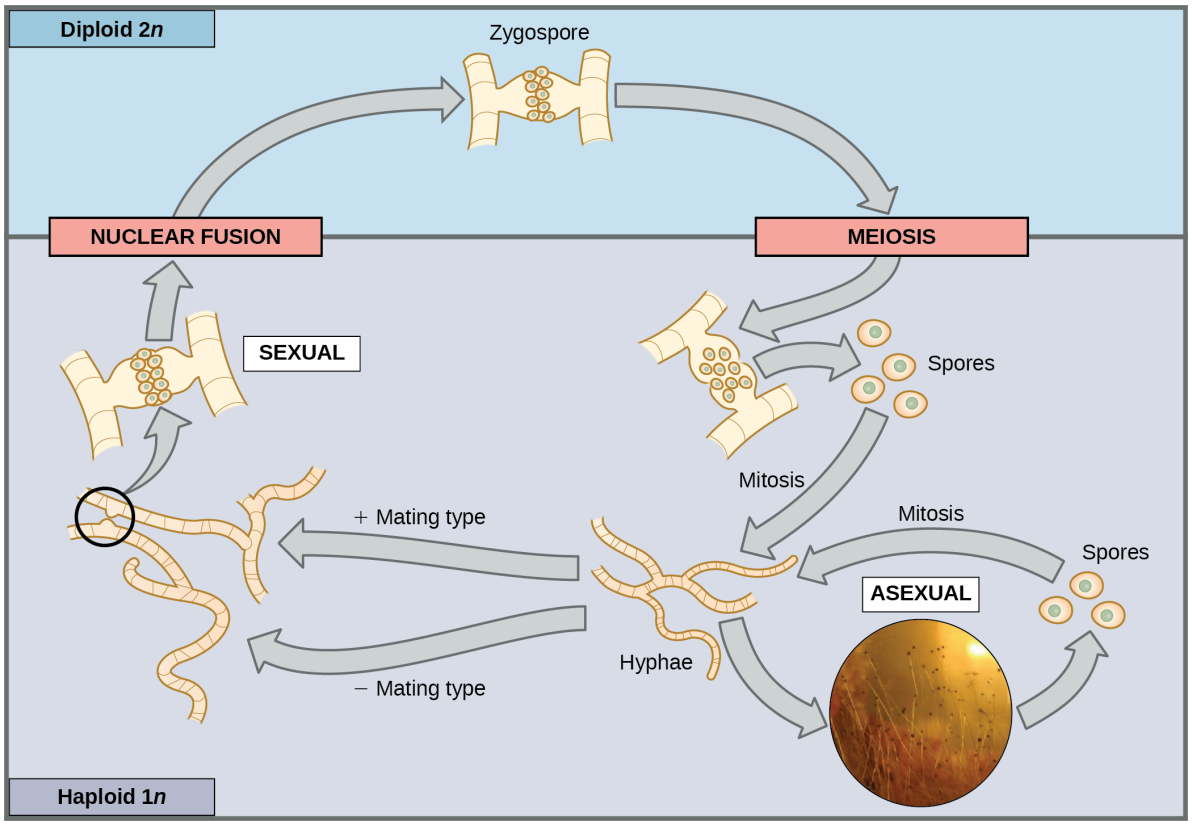

Figure 11.9 Fungi, such as black bread mold (Rhizopus nigricans), have a haploid multicellular stage that produces specialized haploid cells by mitosis that fuse to form a diploid zygote. The zygote undergoes meiosis to produce haploid spores. Each spore gives rise to a multicellular haploid organism by mitosis. Above, different mating hyphae types (denoted as + and –) join to form a zygospore through nuclear fusion. (credit "zygomycota" micrograph: modification of work by "Fanaberka"/Wikimedia Commons)

If a mutation occurs so that a fungus is no longer able to produce a minus mating type, will it still be able to reproduce?

The third life-cycle type, employed by some algae and all plants, is a blend of the haploid-dominant and diploid-dominant extremes. Species with alternation of generations have both haploid and diploid multicellular organisms as part of their life cycle. The haploid multicellular plants are called **gametophytes**, because they produce gametes from specialized cells. Meiosis is not directly involved in the production of gametes in this case, because the organism that produces the gametes is already haploid. Fertilization between the gametes forms a diploid zygote. The zygote will undergo many rounds of mitosis and give rise to a diploid

multicellular plant called a **sporophyte**. Specialized cells of the sporophyte will undergo meiosis and produce haploid spores. The spores will subsequently develop into the gametophytes (Figure 11.10).

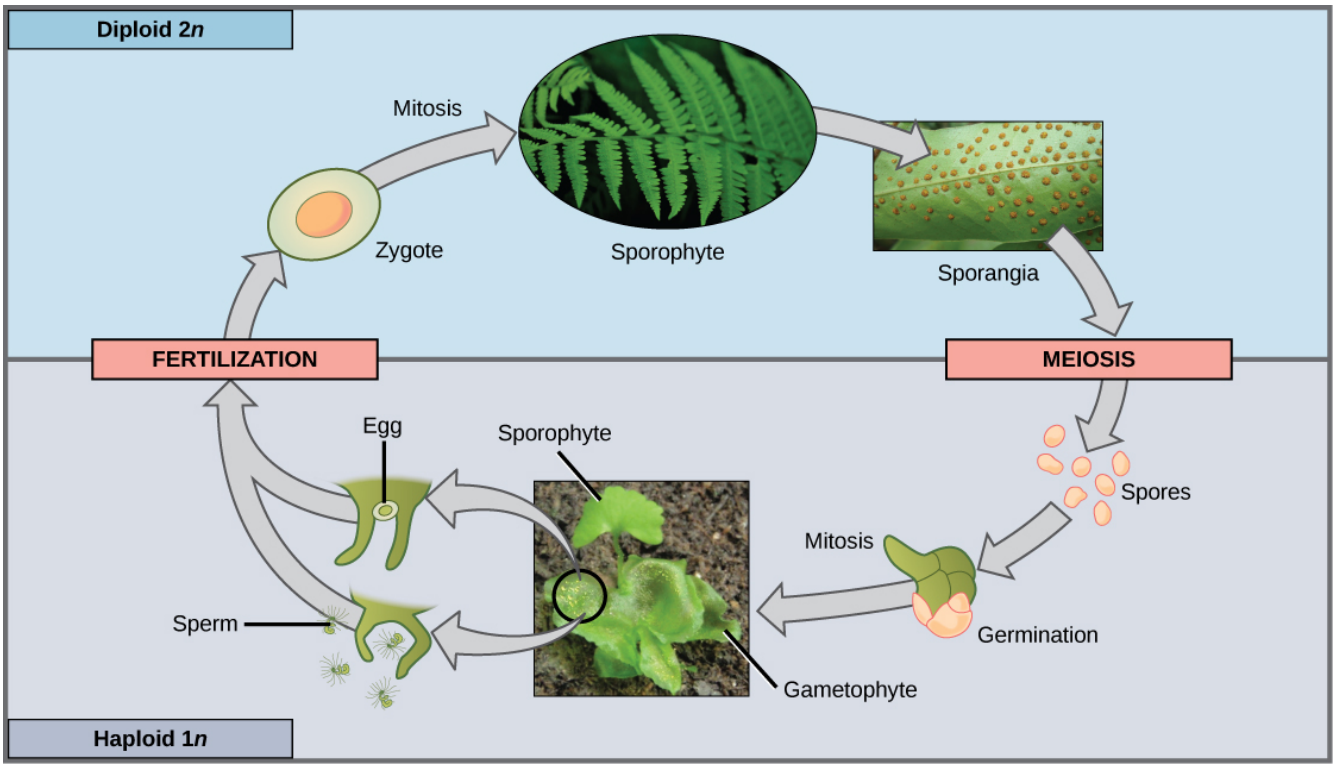

Figure 11.10 Plants have a life cycle that alternates between a multicellular haploid organism and a multicellular diploid organism. In some plants, such as ferns, both the haploid and diploid plant stages are free-living. The diploid plant is called a sporophyte because it produces haploid spores by meiosis. The spores develop into multicellular, haploid plants that are called gametophytes because they produce gametes. The gametes of two individuals will fuse to form a diploid zygote that becomes the sporophyte. (credit "fern": modification of work by Cory Zanker; credit "sporangia": modification of work by "Obsidian Soul"/Wikimedia Commons; credit "gametophyte and sporophyte": modification of work by "Vlmastra"/Wikimedia Commons)

Although all plants utilize some version of the alternation of generations, the relative size of the sporophyte and the gametophyte and the relationship between them vary greatly. In plants such as moss, the gametophyte organism is the free-living plant, and the sporophyte is physically dependent on the gametophyte. In other plants, such as ferns, both the gametophyte and sporophyte plants are free-living; however, the sporophyte is much larger. In seed plants, such as magnolia trees and daisies, the gametophyte is composed of only a few cells and, in the case of the female gametophyte, is completely retained within the sporophyte.

Sexual reproduction takes many forms in multicellular organisms. The fact that nearly every multicellular organism on Earth employs sexual reproduction is strong evidence for the benefits of producing offspring with unique gene combinations, though there are other possible benefits as well.

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Footnotes

- 3 Leigh Van Valen, "A New Evolutionary Law," *Evolutionary Theory* 1 (1973): 1–30

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KEY TERMS

alternation of generations
: life-cycle type in which the diploid and haploid stages alternate

chiasmata
: (singular, *chiasma*) the structure that forms at the crossover points after genetic material is exchanged

cohesin
: proteins that form a complex that seals sister chromatids together at their centromeres until anaphase II of meiosis

crossover
: exchange of genetic material between nonsister chromatids resulting in chromosomes that incorporate genes from both parents of the organism

fertilization
: union of two haploid cells from two individual organisms

gametophyte
: a multicellular haploid life-cycle stage that produces gametes

germ cells
: specialized cell line that produces gametes, such as eggs or sperm

interkinesis
: (also, *interphase II*) brief period of rest between meiosis I and meiosis II

life cycle
: the sequence of events in the development of an organism and the production of cells that produce offspring

meiosis
: a nuclear division process that results in four haploid cells

meiosis I
: first round of meiotic cell division; referred to as *reduction division* because the ploidy level is reduced from diploid to haploid

meiosis II
: second round of meiotic cell division following meiosis I; sister chromatids are separated into individual chromosomes, and the result is four unique haploid cells

recombination nodules
: protein assemblies formed on the synaptonemal complex that mark the points of crossover events and mediate the multistep process of genetic recombination between nonsister chromatids of a homologous pair

reduction division
: nuclear division that produces daughter nuclei each having one-half as many chromosome sets as the parental nucleus; meiosis I is a reduction division

somatic cell
: all the cells of a multicellular organism except the gametes or reproductive cells

spore
: haploid cell that can produce a haploid multicellular organism or can fuse with another spore to form a diploid cell

sporophyte
: a multicellular diploid life-cycle stage that produces haploid spores by meiosis

synapsis
: formation of a close association between homologous chromosomes during prophase I

synaptonemal complex
: protein lattice that forms between homologous chromosomes during prophase I, supporting crossover

tetrad
: two duplicated homologous chromosomes (four chromatids) bound together by chiasmata during prophase I

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CHAPTER SUMMARY

11.1 The Process of Meiosis

Sexual reproduction requires that organisms produce cells that can fuse during fertilization to produce offspring. In most organisms, fertilization occurs between two haploid cells, the larger being called "female" or "egg" and the smaller being called "male" or "sperm." In most animals, meiosis is used to produce haploid egg and sperm from diploid parent cells so that the fusion of an egg and sperm produces a diploid zygote. As with mitosis, DNA replication occurs prior to meiosis during the S-phase of the cell cycle so that each chromosome becomes a pair of sister chromatids. In meiosis, there are two rounds of nuclear division resulting in four nuclei and usually four daughter cells, each with half the number of chromosomes as the parent cell. The first division separates homologous chromosomes, and the second—like mitosis—separates chromatids into individual chromosomes. Meiosis generates variation in the daughter nuclei during crossover in prophase I as well as during the random alignment of tetrads at metaphase I. The cells that are produced by meiosis are genetically unique.

Meiosis and mitosis share similar processes, but have distinct outcomes. Mitotic divisions are single nuclear divisions that produce genetically identical daughter nuclei (i.e., each daughter nucleus has the same number of chromosome sets as the original cell). In contrast, meiotic divisions include two nuclear divisions that ultimately produce four genetically different daughter nuclei that have only one chromosome set (instead of the two sets of chromosomes in the parent cell). The main differences between the two nuclear division processes take place during the first division of meiosis: homologous chromosomes pair, crossover, and exchange homologous nonsister chromatid segments. The homologous chromosomes separate into different nuclei during meiosis I, causing a *reduction of ploidy level in the first division*. The second division of meiosis is similar to a mitotic division, except that the daughter cells do not contain identical genomes because of crossover and chromosome recombination in prophase I.

11.2 Sexual Reproduction

Nearly all eukaryotes undergo sexual reproduction. The variation introduced into the reproductive cells by meiosis provides an important advantage that has made sexual reproduction evolutionarily successful. Meiosis and fertilization alternate in sexual life cycles. The process of meiosis produces unique reproductive cells called gametes, which have half the number of chromosomes as the parent cell. When two haploid gametes fuse,

this restores the diploid condition in the new zygote. Thus, most sexually reproducing organisms alternate between haploid and diploid stages. However, the ways in which reproductive cells are produced and the timing between meiosis and fertilization vary greatly.

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VISUAL CONNECTION QUESTIONS

1. Figure 11.9 If a mutation occurs so that a fungus is no longer able to produce a minus (-) mating type, will it still be able to reproduce?”

111.

REVIEW QUESTIONS

2. Meiosis usually produces _____ daughter cells.

a. two haploid
b. two diploid
c. four haploid
d. four diploid

3. What structure is most important in forming the tetrads?

a. centromere
b. synaptonemal complex
c. chiasma
d. kinetochore

4. At which stage of meiosis are sister chromatids separated from each other?

a. prophase I
b. prophase II
c. anaphase I
d. anaphase II

5. At metaphase I, homologous chromosomes are connected only at what structures?

a. chiasmata
b. recombination nodules
c. microtubules
d. kinetochores

6. Which of the following is not true in regard to crossover?

a. Spindle microtubules guide the transfer of DNA across the synaptonemal complex.

b. Nonsister chromatids exchange genetic material.
c. Chiasmata are formed.
d. Recombination nodules mark the crossover point.

7. What phase of mitotic interphase is missing from meiotic interkinesis?

a. G_0 phase
b. G_1 phase
c. S phase
d. G_2 phase

8. The part of meiosis that is similar to mitosis is _____.

a. meiosis I
b. anaphase I
c. meiosis II
d. interkinesis

9. If a muscle cell of a typical organism has 32 chromosomes, how many chromosomes will be in a gamete of that same organism?

a. 8
b. 16
c. 32
d. 64

10. Which statement best describes the genetic content of the two daughter cells in prophase II of meiosis?

a. haploid with one copy of each gene
b. haploid with two copies of each gene
c. diploid with two copies of each gene
d. diploid with four copies of each gene

11. The pea plants used in Mendel's genetic inheritance studies were diploid, with 14 chromosomes in somatic cells. Assuming no crossing over events occur, how many unique gametes could one pea plant produce?

a. 28
b. 128

c. 196
d. 16,384

12. How do telophase I and telophase II differ during meiosis in animal cells?

a. Cells remain diploid at the end of telophase I, but are haploid at the end of telophase II.
b. Daughter cells form a cell plate to divide during telophase I, but divide by cytokinesis during telophase II.
c. Cells enter interphase after telophase I, but not after telophase II.
d. Chromosomes can remain condensed at the end of telophase I, but decondense after telophase II.

13. What is a likely evolutionary advantage of sexual reproduction over asexual reproduction?

a. Sexual reproduction involves fewer steps.
b. There is a lower chance of using up the resources in a given environment.
c. Sexual reproduction results in variation in the offspring.
d. Sexual reproduction is more cost-effective.

14. Which type of life cycle has both a haploid and diploid multicellular stage?

a. asexual life cycles
b. most animal life cycles
c. most fungal life cycles
d. alternation of generations

15. What is the ploidy of the most conspicuous form of most fungi?

a. diploid
b. haploid
c. alternation of generations
d. asexual

16. A diploid, multicellular life-cycle stage that gives rise to haploid cells by meiosis is called a _____.

a. sporophyte
b. gametophyte
c. spore
d. gamete

17. Hydras and jellyfish both live in a freshwater lake that is slowly being acidified by the runoff from a chemical plant built upstream. Which population is predicted to be better able to cope with the changing environment?

a. jellyfish
b. hydra
c. The populations will be equally able to cope.
d. Both populations will die.

18. Many farmers are worried about the decreasing genetic diversity of plants associated with generations of artificial selection and inbreeding. Why is limiting random sexual reproduction of food crops concerning?

a. Mutations during asexual reproduction decrease plant fitness.
b. Consumers do not trust identical-appearing produce.
c. Larger portions of the plant populations are susceptible to the same diseases.
d. Spores are not viable in an agricultural setting.

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CRITICAL THINKING QUESTIONS

19. Describe the process that results in the formation of a tetrad.

20. Explain how the random alignment of homologous chromosomes during metaphase I contributes to the variation in gametes produced by meiosis.

21. What is the function of the fused kinetochore found on sister chromatids in prometaphase I?

22. In a comparison of the stages of meiosis to the stages of mitosis, which stages are unique to meiosis and which stages have the same events in both meiosis and mitosis?

23. Why would an individual with a mutation that prevented the formation of recombination nodules be considered less fit than other members of its species?

24. Does crossing over occur during prophase II? From an evolutionary perspective, why is this advantageous?

25. List and briefly describe the three processes that lead to variation in offspring with the same parents.

26. Animals and plants both have diploid and haploid cells. How does the animal life cycle differ from the alternation of generations exhibited by plants?

27. Explain why sexual reproduction is beneficial to a population but can be detrimental to an individual offspring.

28. How does the role of meiosis in gamete production differ between organisms with a diploid-dominant life cycle and organisms with an alternation of generations life cycle?

29. How do organisms with haploid-dominant life cycles ensure continued genetic diversification in offspring without using a meiotic process to make gametes?

PART XII

MENDEL'S EXPERIMENTS AND HEREDITY

113.

INTRODUCTION

Figure 12.1 Experimenting with thousands of garden peas, Mendel uncovered the fundamentals of genetics. (credit: modification of work by Jerry Kirkhart)

Genetics is the study of heredity. Johann Gregor Mendel set the framework for genetics long before chromosomes or genes had been identified, at a time when meiosis was not well understood. Mendel selected a simple biological system and conducted methodical, quantitative analyses using large sample sizes. Because of Mendel's work, the fundamental principles of heredity were revealed. We now know that genes, carried on chromosomes, are the basic functional units of heredity with the capability to be replicated, expressed, or mutated. Today, the postulates put forth by Mendel form the basis of classical, or Mendelian, genetics. Not all genes are transmitted from parents to offspring according to **Mendelian genetics,** but Mendel's experiments serve as an excellent starting point for thinking about inheritance.

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114.

MENDEL'S EXPERIMENTS AND THE LAWS OF PROBABILITY

Learning Objectives

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

- Describe the scientific reasons for the success of Mendel's experimental work
- Describe the expected outcomes of monohybrid crosses involving dominant and recessive alleles
- Apply the sum and product rules to calculate probabilities

Figure 12.2 Johann Gregor Mendel is considered the father of genetics.

Johann Gregor Mendel (1822–1884) (Figure 12.2) 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**. 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 from parents to offspring independently of other traits and in dominant and recessive patterns. In 1866, he published his work *Experiments in Plant Hybridization*[1] in the proceedings of the Natural History Society of Brünn.

Mendel's work went virtually unnoticed by the scientific community, which believed, incorrectly, that the process of inheritance involved a blending of parental traits that produced an intermediate physical appearance in offspring. The **blending theory of inheritance** asserted that the original parental traits were lost or

absorbed by the blending in the offspring, but we now know that this is not the case. The long-held belief was the "blending" hypothesis in which genetic material contributed by the two parents mixes to literally form a blend, giving rise to uniformity in a population after several mating generations, in contrast to Mendel's "particulate" hypothesis of inheritance, the real gene idea of parents passing discrete heritable genes that retain their separate original identities in offspring and are randomly inherited and can appear as traits or remain discrete in generations where the traits are skipped.

Another complicating observation in **genetics** is that traits can fall in the category of discontinuous traits or continuous traits. Discontinuous traits are of the "one or the other" type. For example, the peas of a pea plant are either green or yellow. Another example is the color of the flower in the pea plant. It is either violet or it is white. Now, it can be more complicated if a trait is a continuous trait. Continuous traits are of the "range of" phenotypes. An example could be the range of height in adult humans. Some are 4 feet (ft), some are 4 feet 1 inch (in), 4 ft 2 in, 4 ft 3 in, 5ft 9 in, 6ft 4 in, and so on. Studying the underlying genetics by looking at phenotypes of continuous traits is more complicated. Mendel chose to look at the less complicated discontinuous traits and tried to understand the underlying genetics. Mendel worked with traits that were inherited as discontinuous (specifically, violet versus white flowers). Mendel's choice of these kinds of traits allowed him to see experimentally that the traits were not blended in the offspring, nor were they absorbed; rather, they kept their distinctness and could be passed on. 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 Model System

Mendel's seminal work was accomplished using the garden pea, *Pisum sativum*, to study inheritance. This species naturally self-fertilizes, such that pollen encounters ova within individual flowers. The flower petals remain sealed tightly until after pollination, preventing pollination from 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.

Mendelian Crosses

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. In plants, pollen carries the male gametes (sperm) to the stigma, a sticky organ that traps pollen and allows the sperm to move down the pistil to the female gametes (ova) below. To prevent the pea plant that was receiving pollen from self-fertilizing and confounding his results, Mendel painstakingly removed all of the anthers from the plant's flowers before they had a chance to mature.

Plants used in first-generation crosses were called **P_0**, or parental generation one (Figure 12.3). After each cross, Mendel collected the seeds belonging to the P_0 plants and grew them the following season. These offspring were called the **F_1**, or the first filial (*filial* = offspring, 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 and F_4 generations, and so on, but it was the ratio of characteristics in the P_0–F_1–F_2 generations that were the most intriguing and became the basis for Mendel's postulates.

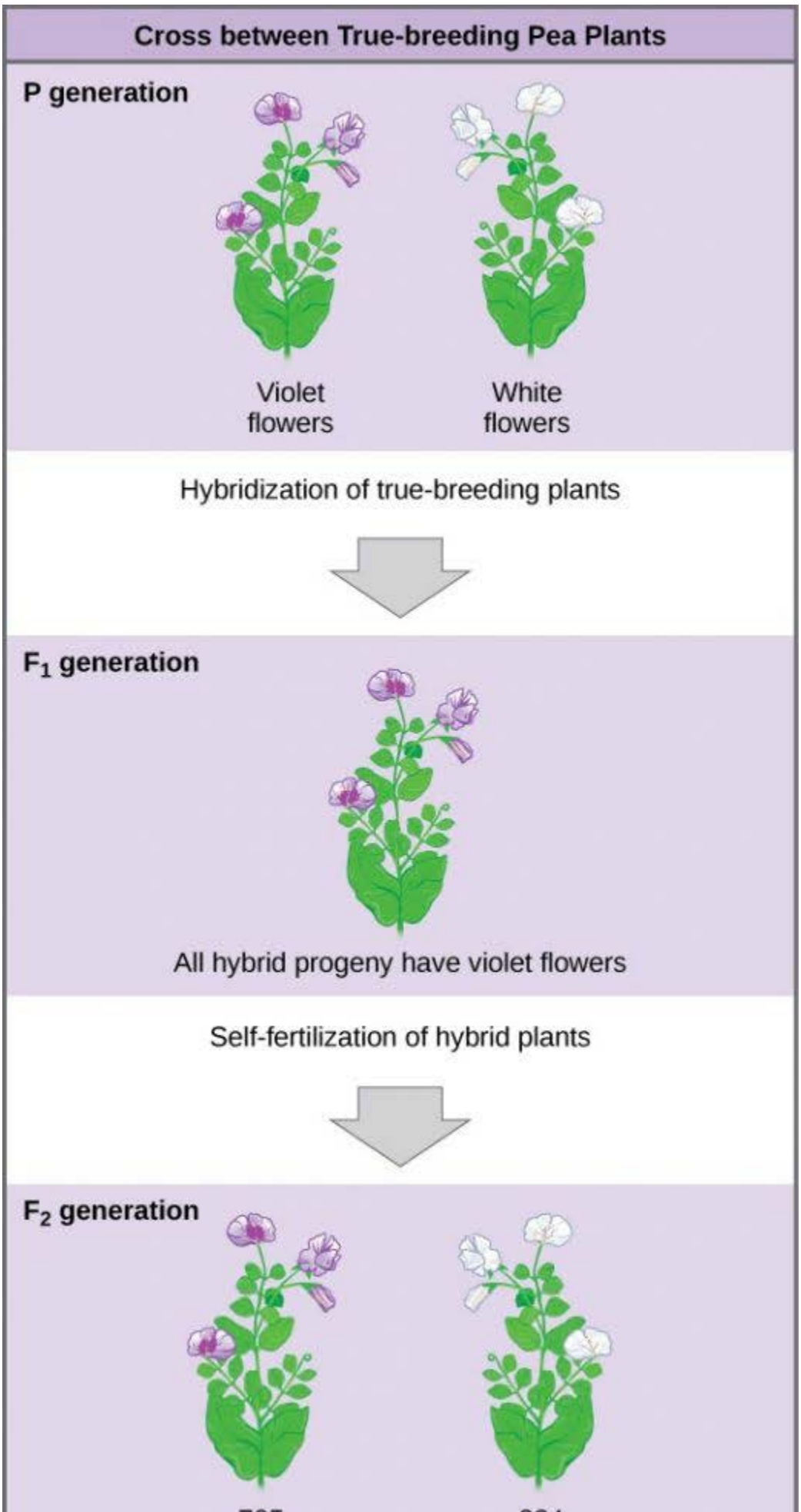

Cross between True-breeding Pea Plants
P generation
Violet flowers
White flowers
Hybridization of true-breeding plants
F1 generation
All hybrid progeny have violet flowers
Self-fertilization of hybrid plants
F2 generation

Figure 12.3 In one of his experiments on inheritance patterns, Mendel crossed plants that were true-breeding for violet flower color with plants true-breeding for white flower color (the P generation). The resulting hybrids in the F1 generation all had violet flowers. In the F2 generation, approximately three quarters of the plants had violet flowers, and one quarter had white flowers.

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, reporting results from 19,959 F_2 plants alone. His findings were consistent. What results did Mendel find in his crosses for flower color? First, Mendel confirmed that he had plants that bred true for white or violet flower color. Regardless of how many generations 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.

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*. Mendel's results demonstrated that the white flower trait in the F_1 generation had completely disappeared. Thereby, he disproved the conventional wisdom at that time of the blending theory which would have predicted the hybrid flowers to be pale violet or for hybrid plants to have equal numbers of white and violet flowers.

Importantly, Mendel did not stop his experimentation there. He discovered that the white flower trait re-appeared. He allowed the F_1 plants to self-fertilize and found that, of F_2-generation plants, 705 had violet flowers and 224 had white flowers. This was a ratio of 3.15 violet flowers per one white flower, or approximately 3:1. The reappearance of white flowers is important, as it hinted at something called recessive traits (see further explanation later in this paragraph). When Mendel transferred pollen from a plant with violet flowers to the stigma of a plant with white flowers and vice versa, he obtained about the same ratio regardless 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 Mendel examined, the F_1 and F_2 generations behaved in the same way as they had for flower color. One of the two traits would disappear or **recede** completely from the F_1 generation only to reappear in the F_2 generation at a ratio of approximately 3:1 (Table 12.1).

The Results of Mendel's Garden Pea Hybridizations

Characteristic	Contrasting P_0 Traits	F_1 Offspring Traits	F_2 Offspring Traits	F_2 Trait Ratios
Flower color	Violet vs. white	100 percent violet	• 705 violet • 224 white	3.15:1
Flower position	Axial vs. terminal	100 percent axial	• 651 axial • 207 terminal	3.14:1
Plant height	Tall vs. dwarf	100 percent tall	• 787 tall • 277 dwarf	2.84:1
Seed texture	Round vs. wrinkled	100 percent round	• 5,474 round • 1,850 wrinkled	2.96:1
Seed color	Yellow vs. green	100 percent yellow	• 6,022 yellow • 2,001 green	3.01:1
Pea pod texture	Inflated vs. constricted	100 percent inflated	• 882 inflated • 299 constricted	2.95:1
Pea pod color	Green vs. yellow	100 percent green	• 428 green • 152 yellow	2.82:1

Table 12.1

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, respectively, dominant and recessive traits. **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-flower trait. For this same characteristic (flower color), white-colored flowers are a recessive trait. The fact that the recessive trait reappeared in the F2 generation meant that the traits remained separate (not blended) in the plants of the F_1 generation. Mendel also proposed that plants possessed two copies of the trait for the flower-color characteristic, and that each parent transmitted

one of its two copies to its 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.

So why did Mendel repeatedly obtain 3:1 ratios in his crosses? To understand how Mendel deduced the basic mechanisms of inheritance that lead to such ratios, we must first review the laws of probability.

Probability Basics

Probabilities are mathematical measures of likelihood. The empirical probability of an event is calculated by dividing the number of times the event occurs by the total number of opportunities for the event to occur. It is also possible to calculate theoretical probabilities by dividing the number of times that an event is *expected* to occur by the number of times that it could occur. Empirical probabilities come from observations, like those of Mendel. Theoretical probabilities, on the other hand, come from knowing how the events are produced and assuming that the probabilities of individual outcomes are equal. A probability of one for some event indicates that it is guaranteed to occur, whereas a probability of zero indicates that it is guaranteed not to occur. An example of a genetic event is a round seed produced by a pea plant.

In one experiment, Mendel demonstrated that the probability of the event "round seed" occurring was one in the F_1 offspring of true-breeding parents, one of which has round seeds and one of which has wrinkled seeds. When the F_1 plants were subsequently self-crossed, the probability of any given F_2 offspring having round seeds was now three out of four. In other words, in a large population of F_2 offspring chosen at random, 75 percent were expected to have round seeds, whereas 25 percent were expected to have wrinkled seeds. Using large numbers of crosses, Mendel was able to calculate probabilities and use these to predict the outcomes of other crosses.

The Product Rule and Sum Rule

The **product rule** of probability can be applied to this phenomenon of the independent transmission of characteristics. The product rule states that the probability of two independent events occurring together can be calculated by multiplying the individual probabilities of each event occurring alone. To demonstrate the product rule, imagine that you are rolling a six-sided die (D) and flipping a penny (P) at the same time. The die may roll any number from 1–6 ($D_{\#}$), whereas the penny may turn up heads (P_H) or tails (P_T). The outcome of rolling the die has no effect on the outcome of flipping the penny and vice versa. There are 12 possible outcomes of this action (Table 12.2), and each event is expected to occur with equal probability.

Twelve Equally Likely Outcomes of Rolling a Die and Flipping a Penny

Rolling Die	Flipping Penny
D_1	P_H
D_1	P_T
D_2	P_H
D_2	P_T
D_3	P_H
D_3	P_T
D_4	P_H
D_4	P_T
D_5	P_H
D_5	P_T
D_6	P_H
D_6	P_T

Table 12.2

Of the 12 possible outcomes, the die has a 2/12 (or 1/6) probability of rolling a two, and the penny has a 6/12 (or 1/2) probability of coming up heads. By the product rule, the probability that you will obtain the combined outcome 2 and heads is: $(D_2) \times (P_H) = (1/6) \times (1/2)$ or 1/12 (Table 12.3). Notice the word "and" in the description of the probability. The "and" is a signal to apply the product rule. For example, consider how the product rule is applied to the dihybrid cross: the probability of having both dominant traits (using for example the traits for seed, yellow and round) in the F_2 progeny is the product of the probabilities of having the dominant trait for each characteristic, as shown here:

$$3/4 \times 3/4 = 9/16$$

On the other hand, the **sum rule** of probability is applied when considering two mutually exclusive outcomes that can come about by more than one pathway. The sum rule states that the **probability of the occurrence of one event or the other event, of two mutually exclusive events, is the sum of their individual probabilities.** Notice the word "or" in the description of the probability. The "or" indicates that you should apply the sum rule. In this case, let's imagine you are flipping a penny (P) and a quarter (Q). What is the probability of one coin coming up heads and one coin coming up tails? This outcome can be achieved by two cases: the penny may be heads (P_H) and the quarter may be tails (Q_T), or the quarter may be heads (Q_H) and the penny may be tails (P_T). Either case fulfills the outcome. By the sum rule, we calculate the probability of obtaining one head and one tail as $[(P_H) \times (Q_T)] + [(Q_H) \times (P_T)] = [(1/2) \times (1/2)] + [(1/2) \times (1/2)] = 1/2$. You should also notice that we used the product rule to calculate the probability of P_H and Q_T, and

also the probability of P_T and Q_H, before we summed them. Again, the sum rule can be applied to show the probability of having at least one dominant trait in the F_2 generation of a dihybrid cross:

$$(1/4\times3/4)+(3/4\times1/4)=3/16+3/16=6/16=3/8$$

The major differences of the "product rule" and the "sum rule" are summarized in (Table 12.3).

The Product Rule and Sum Rule

Product Rule	**Sum Rule**
For independent events A and B, the probability (P) of them both occurring (A *and* B) is ($P_A \times P_B$)	For mutually exclusive events A and B, the probability (P) that at least one occurs (A *or* B) is ($P_A + P_B$)

Table 12.3

To use probability laws in practice, we must work with large sample sizes because small sample sizes are prone to deviations caused by chance. The large quantities of pea plants that Mendel examined allowed him to calculate the probabilities of the traits appearing in his F_2 generation. As you will learn, this discovery meant that when parental traits were known, the offspring's traits could be predicted accurately even before fertilization.

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=451#h5p-57

Footnotes

- 1 Johann Gregor Mendel, *Versuche über Pflanzenhybriden Verhandlungen des naturforschenden Vereines in Brünn, Bd. IV für das Jahr*, 1865 Abhandlungen, 3–47. [for English translation see http://www.mendelweb.org/Mendel.plain.html]

115.

CHARACTERISTICS AND TRAITS

Learning Objectives

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

- Explain the relationship between genotypes and phenotypes in dominant and recessive gene systems
- Develop a Punnett square to calculate the expected proportions of genotypes and phenotypes in a monohybrid cross
- Explain the purpose and methods of a test cross
- Identify non-Mendelian inheritance patterns such as incomplete dominance, codominance, recessive lethals, multiple alleles, and sex linkage

Physical characteristics are expressed through **genes** carried on **chromosomes**. The genetic makeup of peas consists of two similar, or homologous, copies of each chromosome, one from each parent. Each pair of **homologous chromosomes** has the same linear order of **genes**. In other words, peas are diploid organisms in that they have two copies of each chromosome. The same is true for many other plants and for virtually all animals. Diploid organisms produce haploid gametes, which contain one copy of each homologous chromosome that unite at fertilization to create a diploid zygote.

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. **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 physically visible and non-expressed alleles**, is called its **genotype**. Mendel's hybridization experiments demonstrate the difference between phenotype and genotype. When true-breeding plants in which one parent had yellow pods and one had green pods were cross-fertilized, all of the F_1 hybrid offspring had yellow pods. That is, the hybrid offspring were phenotypically identical to the true-breeding parent with yellow pods. However, we know that the allele donated by the parent with green pods was not simply lost because it reappeared in some of the F_2 offspring. Therefore, the F_1 plants must have been genotypically different from the parent with yellow pods.

The P_1 plants that Mendel used in his experiments were each homozygous for the trait he was studying. Diploid organisms that are **homozygous** at a given gene, or locus, have **two identical alleles for that gene on their homologous chromosomes**. Mendel's parental pea plants always bred true because both of the gametes produced carried the same trait. When P_1 plants with contrasting traits were cross-fertilized, all of the offspring were heterozygous for the contrasting trait, meaning that their genotype reflected that they had different alleles for the gene being examined.

Dominant and Recessive Alleles

Our discussion of homozygous and heterozygous organisms brings us to why the F1 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 chromosome pairs. 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*). The *recessive allele will only be observed as phenotype in homozygous recessive individuals*. Examples of dominant and recessive alleles in humans are shown in Table 12.4.

Human Inheritance in Dominant and Recessive Patterns

Dominant Traits	Recessive Traits
Achondroplasia	Albinism
Brachydactyly	Cystic fibrosis
Huntington's disease	Duchenne muscular dystrophy
Marfan syndrome	Galactosemia
Neurofibromatosis	Phenylketonuria
Widow's peak	Sickle-cell anemia
Wooly hair	Tay-Sachs disease

Table 12.4

Conventions for Referring to Genes and Alleles

Several conventions exist for referring to genes and alleles. For the purposes of this chapter, we will **abbreviate genes** most of the time using the **first letter** of the gene's corresponding **dominant trait**. For example, violet is the dominant trait for a pea plant's flower color, so the flower-color gene would be abbreviated as *V* (note that it is customary to *italicize* gene designations). Furthermore, we will use **uppercase** (*V*) and **lowercase** (*v*) letters to represent dominant and recessive alleles, respectively. Therefore, we would refer to the genotype of a homozygous dominant pea plant with violet flowers as *VV*, a homozygous recessive pea plant with white flowers as *vv*, and a heterozygous pea plant with violet flowers as *Vv*.

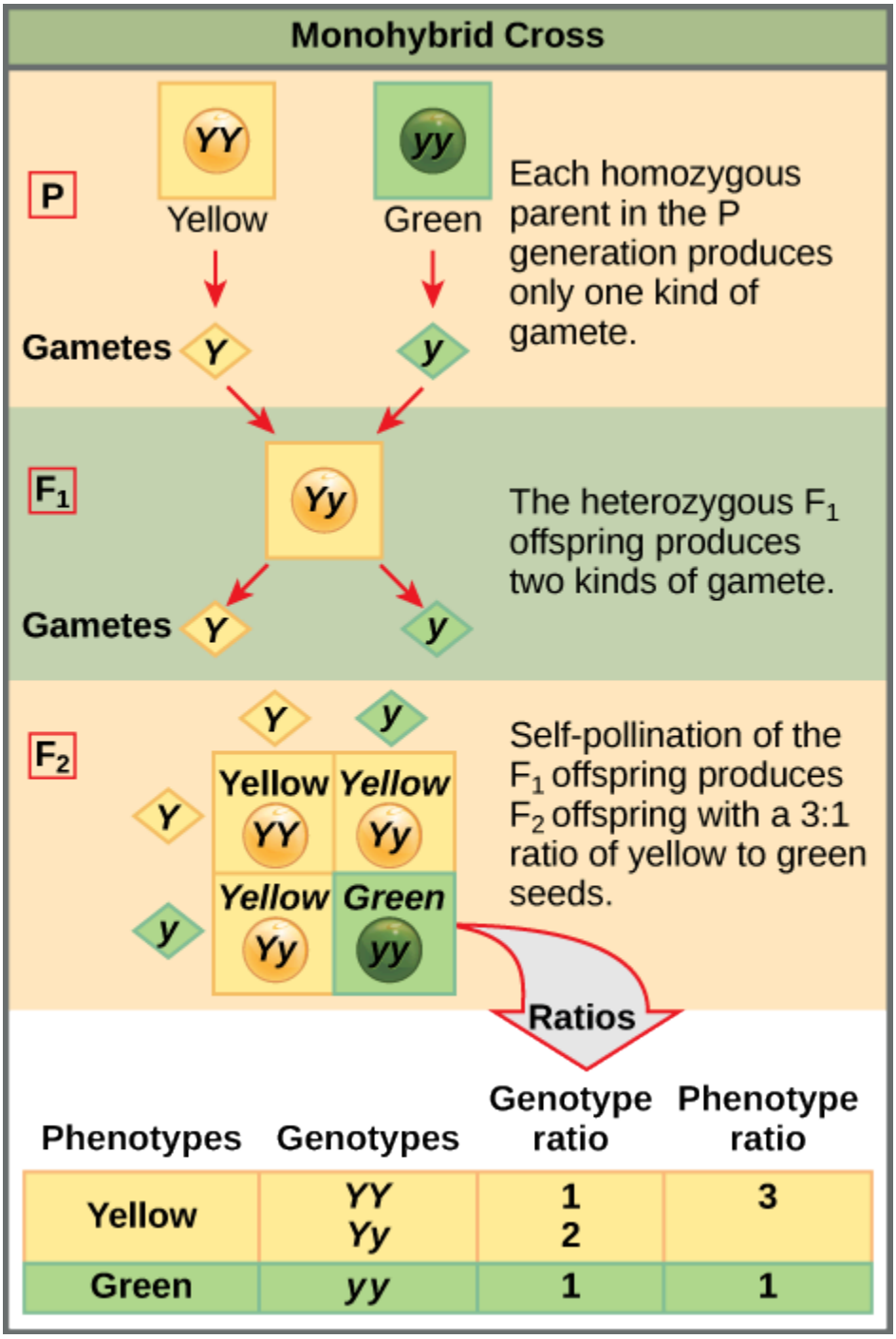
Genes can also be named after a **mutant phenotype**. If the mutant is **dominant**, then the letter is **uppercase**. If the mutant is a **recessive mutant**, then the letter is **lowercase**. An example is the recessive mutant for white (w) eye color in the fruit fly. Fruit flies with white eyes would be *ww*. The dominant allele *W* causes red eyes for flies with *WW* and *Ww*, but flies with *ww* have white eyes (see Figure 12.11 and Figure 12.12). Mutations to X chromosomes are written as superscripts to X chromosome. The white eye mutant is located on the X chromosome. Therefore, some examples of genotypes could be *XWXW, XWXw, and XwXw* (see Figure 12.11 and Figure 12.12).

The Punnett Square Approach for a Monohybrid Cross

When fertilization occurs between two true-breeding parents that **differ in only one characteristic**, the process is called a **monohybrid** cross, and the resulting offspring are monohybrids. Mendel performed seven monohybrid crosses involving contrasting traits for each characteristic. On the basis of his results in F1 and F2 generations, 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.

To demonstrate a monohybrid cross, consider the case of true-breeding pea plants with yellow versus green pea 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, respectively. A **Punnett square**, devised by the British geneticist Reginald Punnett, can be drawn that applies the rules of probability to **predict the possible outcomes** of a genetic cross or mating and their **expected frequencies**. To prepare a Punnett square, all possible combinations of the parental alleles are listed along the top (for one parent) and side (for the other parent) of a grid, representing their meiotic segregation into haploid gametes. Then the combinations of egg and sperm are made in the boxes in the table to show which alleles are combining. Each box then represents the diploid genotype of a zygote, or fertilized egg, that could result from this mating. Because each possibility is equally likely, genotypic ratios can be determined from a Punnett square. If the pattern of inheritance (dominant or 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. All offspring are *Yy* and have yellow seeds (Figure 12.4).



In the P generation, pea plants that are true-breeding for the dominant yellow phenotype are crossed with plants with the recessive green phenotype. This cross produces F1 heterozygotes with a yellow phenotype. Punnett square analysis can be used to predict the genotypes of the F2 generation.

A self-cross of one of the *Yy* heterozygous offspring can be represented in a 2 × 2 Punnett square because each parent can donate one of two different alleles. Therefore, the offspring can potentially have one of four

allele combinations: *YY*, *Yy*, *yY*, or *yy* (Figure 12.4). Notice that there are two ways to obtain the *Yy* genotype: a *Y* from the egg and a *y* from the sperm, or a *y* from the egg and a *Y* from the sperm. Both of these possibilities must be counted. Recall that Mendel's pea-plant characteristics behaved in the same way in reciprocal crosses. Therefore, the two possible heterozygous combinations produce offspring that are genotypically and phenotypically identical despite their dominant and recessive alleles deriving from different parents. They are grouped together. Because fertilization is a random event, we expect each combination to be equally likely and for the offspring to exhibit a ratio of *YY*:*Yy*:*yy* genotypes of 1:2:1 (Figure 12.4). Furthermore, because the *YY* and *Yy* offspring have yellow seeds and are phenotypically identical, applying the sum rule of probability, we expect the offspring to exhibit a phenotypic ratio of 3 yellow:1 green. Indeed, working with large sample sizes, Mendel observed approximately this ratio in every F_2 generation resulting from crosses for individual traits.

Mendel validated these results by performing an F_3 cross in which he self-crossed the dominant- and recessive-expressing F_2 plants. When he self-crossed the plants expressing green seeds, all of the offspring had green seeds, confirming that all green seeds had homozygous genotypes of *yy*. When he self-crossed the F_2 plants expressing yellow seeds, he found that one-third of the plants bred true, and two-thirds of the plants segregated at a 3:1 ratio of yellow:green seeds. In this case, the true-breeding plants had homozygous (*YY*) genotypes, whereas the segregating plants corresponded to the heterozygous (*Yy*) genotype. When these plants self-fertilized, the outcome was just like the F_1 self-fertilizing cross.

The Test Cross Distinguishes the Dominant Phenotype

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 F1 offspring will be heterozygotes expressing the dominant trait (Figure 12.5). Alternatively, if the dominant expressing organism is a heterozygote, the F1 offspring will exhibit a 1:1 ratio of heterozygotes and recessive homozygotes (Figure 12.5). The test cross further validates Mendel's postulate that pairs of unit factors segregate equally.

Visual Connection

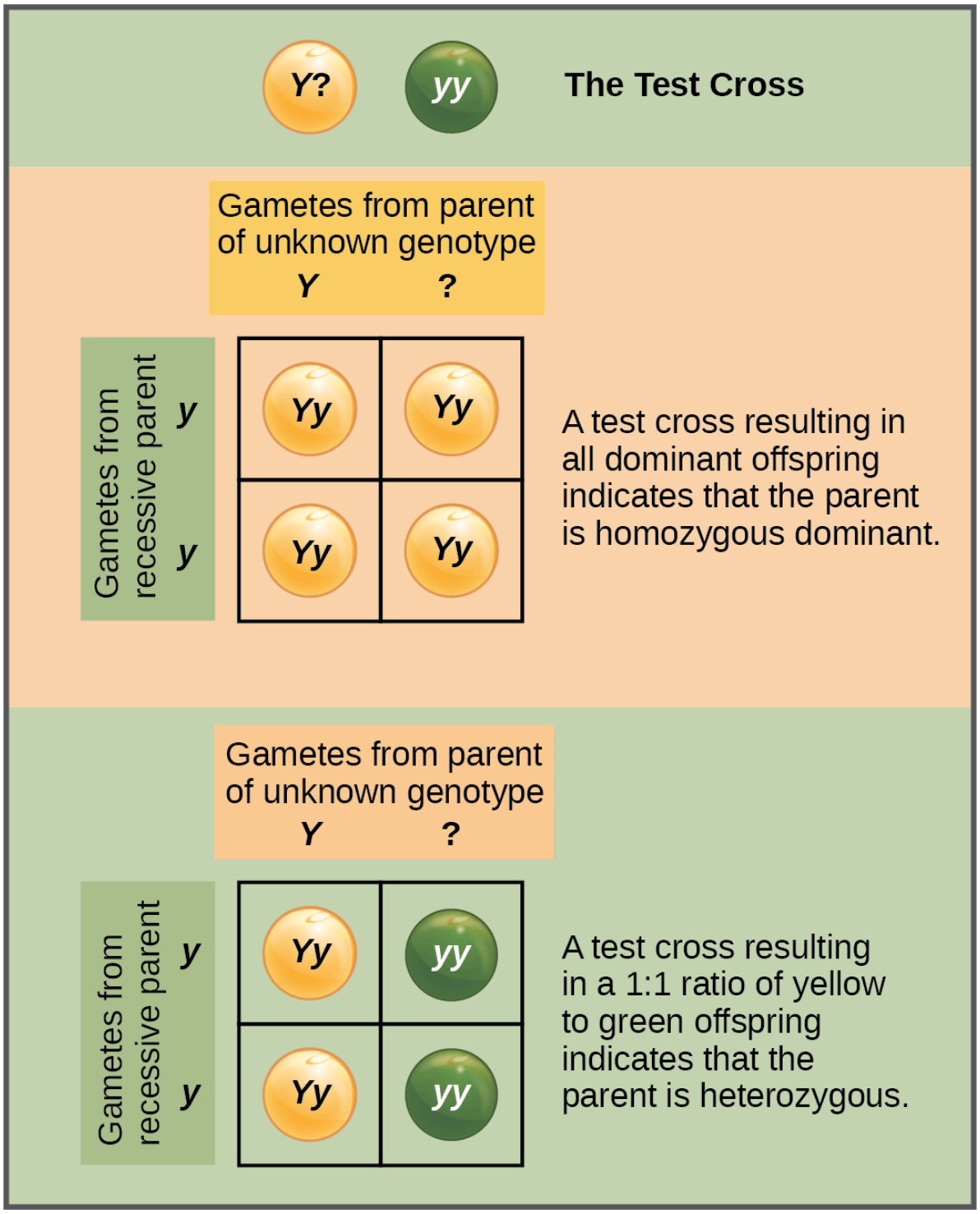


Figure 12.5 A test cross can be performed to determine whether an organism expressing a dominant trait is a homozygote or a heterozygote.

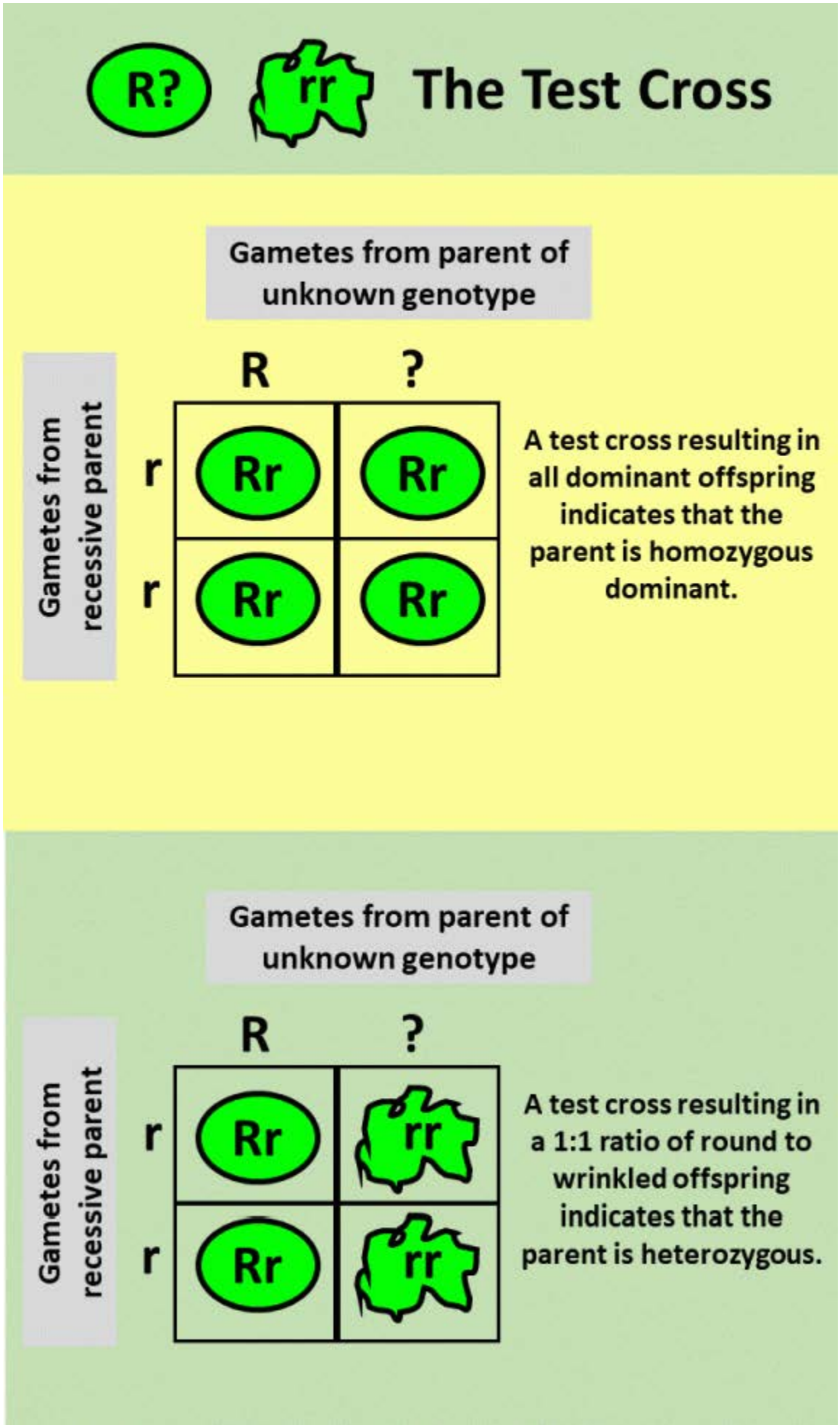

R?
rr
The Test Cross
Gametes from parent of unknown genotype
Gametes from recessive parent
R
?
r
r
Rr
Rr
Rr
Rr
A test cross resulting in all dominant offspring indicates that the parent is homozygous dominant.
Gametes from parent of unknown genotype
Gametes from recessive parent
R
?
r
r
Rr
rr
Rr
rr
A test cross resulting in a 1:1 ratio of round to wrinkled offspring indicates that the parent is heterozygous.

Figure 12.5 B A test cross can be performed to determine whether an organism expressing a dominant trait is a homozygote or a heterozygote. Image credit: I. Tietzel CC BY SA

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 round pea parent plant is homozygous dominant or heterozygous? If the round pea parent plant is heterozygous, what is the probability that a random sample of 3 progeny peas will all be round?

Many human diseases are genetically inherited. A healthy person in a family in which some members suffer from a recessive genetic disorder may want to know if they have the disease-causing gene and what risk exists of passing the disorder on to their offspring. Of course, doing a test cross in humans is unethical and impractical. Instead, geneticists use **pedigree analysis** to study the inheritance pattern of human genetic diseases (Figure 12.6).

Visual Connection

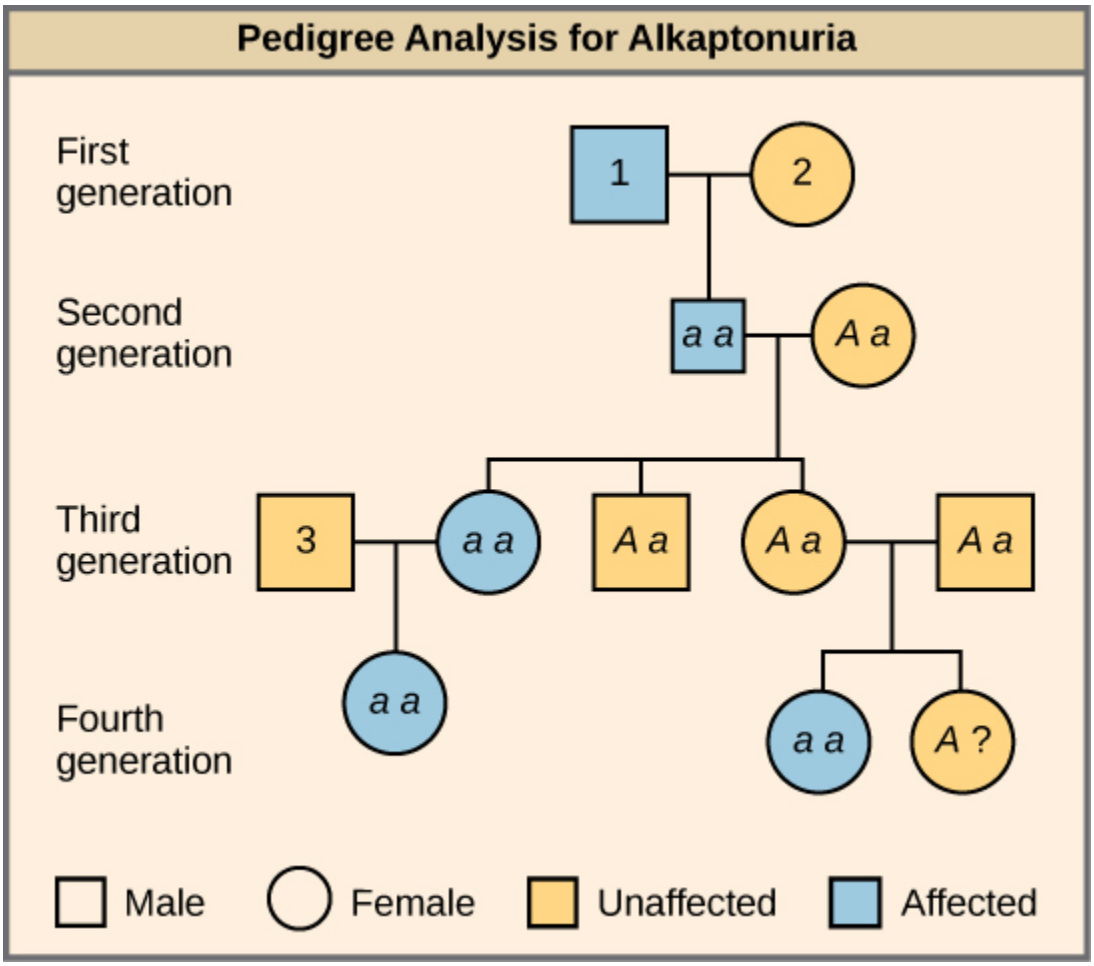


Figure 12.6 Alkaptonuria is a recessive genetic disorder in which two amino acids, phenylalanine and tyrosine, are not properly metabolized. Affected individuals may have darkened skin and brown urine, and may suffer joint damage and other complications. In this pedigree, individuals with the disorder are indicated in blue and have the genotype aa. Unaffected individuals are indicated in yellow and have the genotype AA or Aa. Note that it is often possible to determine a person's genotype from the genotype of their offspring. For example, if neither parent has the disorder but their child does, they must be heterozygous. Two individuals on the pedigree have an unaffected phenotype but unknown genotype. Because they do not have the disorder, they must have at least one normal allele, so their genotype gets the "A?" designation. Circles symbolize biological females. Square symbols indicate biological males.

What are the genotypes of the individuals labeled 1, 2, and 3?

Alternatives to Dominance and Recessiveness

Mendel's experiments with pea plants suggested that: (1) two "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 and makes 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**." Further genetic studies in other plants and animals 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 Mendelian genetics. If Mendel had chosen an experimental system that exhibited these genetic complexities, it's possible that he would not have understood what his results meant.

Incomplete Dominance

Mendel's results, 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 12.7), 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**, denoting the expression of two contrasting alleles such that the individual displays an intermediate phenotype. 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.

Figure 12.7 A: These pink flowers of a heterozygote snapdragon result from incomplete dominance. (credit: “storebukkebruse”/Flickr)

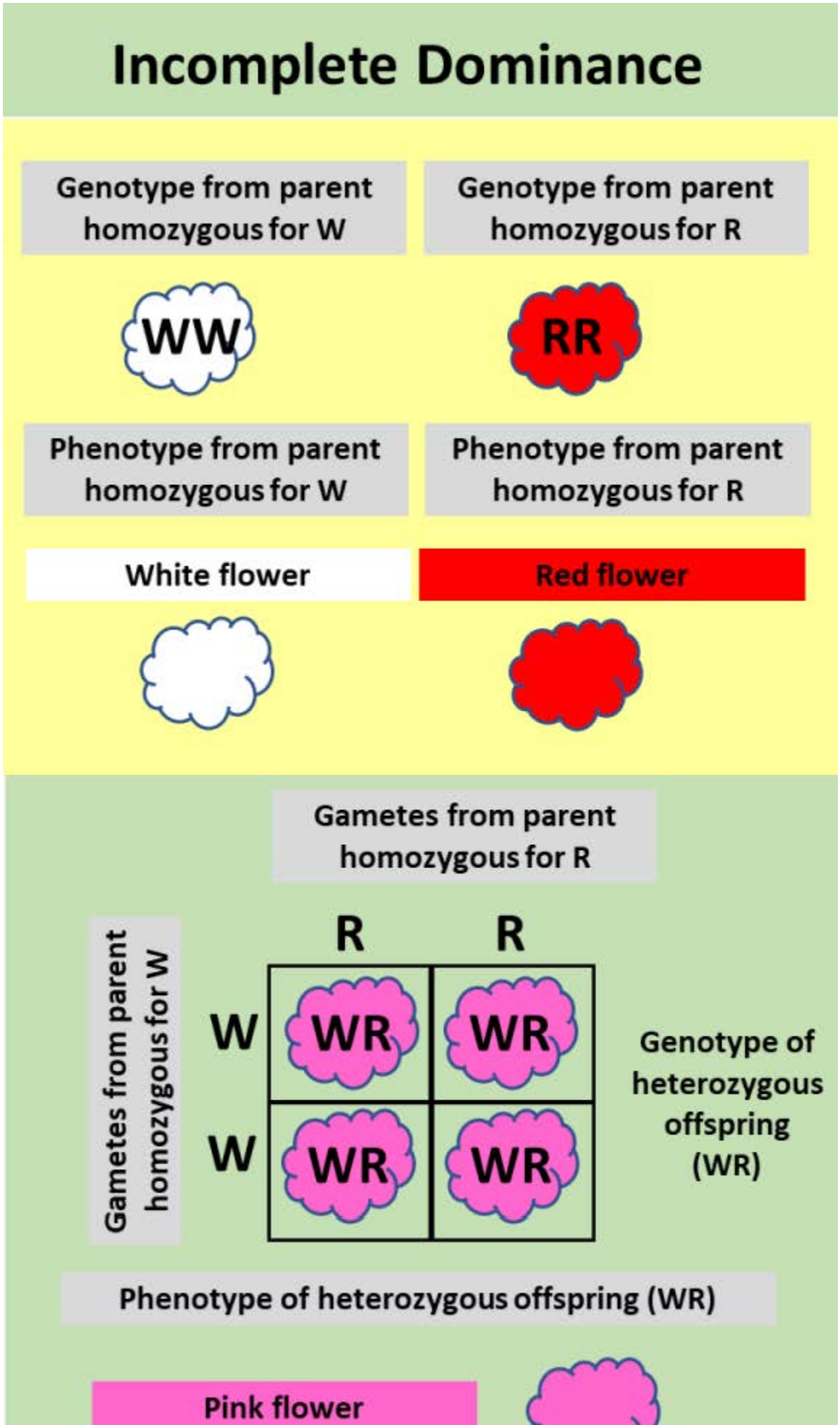

Incomplete Dominance
Genotype from parent homozygous for W
Genotype from parent homozygous for R
WW
RR
Phenotype from parent homozygous for W
Phenotype from parent homozygous for R
White flower
Red flower
Gametes from parent homozygous for R
R
R
Gametes from parent homozygous for W
W
W
WR
WR
WR
WR
Genotype of heterozygous offspring (WR)
Phenotype of heterozygous offspring (WR)
Pink flower

Figure 12.7 B: Genotypes and phenotypes observed for incomplete dominance. The example of the snapdragon is used. Image credit: I. Tietzel CC BY SA

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 is the MN blood groups of humans. The M and N alleles are expressed in the form of an M or N antigen present on the surface of red blood cells. Homozygotes ($L^{M}L^{M}$ and $L^{N}L^{N}$) express either the M or the N allele, and heterozygotes ($L^{M}L^{N}$) express both alleles equally. 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.

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 among wild animals as the **wild type** (often abbreviated "+"); this is considered the standard or norm. All other phenotypes or genotypes are considered **variants** of this standard, meaning that they deviate from the wild type. The variant may be recessive or dominant to the wild-type allele.

An example of multiple alleles is coat color in rabbits (Figure 12.8). Here, four alleles exist for the *c* gene. The wild-type version, $C^{+}C^{+}$, is expressed as brown fur. The chinchilla phenotype, $c^{ch}c^{ch}$, is expressed as black-tipped white fur. The Himalayan phenotype, $c^{h}c^{h}$, has black fur on the extremities and white fur elsewhere. Finally, the albino, or "colorless" phenotype, *cc*, is expressed as white fur. In cases of multiple alleles, dominance hierarchies can exist. In this case, the wild-type allele is dominant over all the others, chinchilla is incompletely dominant over Himalayan and albino, and Himalayan is dominant over albino. This hierarchy, or allelic series, was revealed by observing the phenotypes of each possible heterozygote offspring.

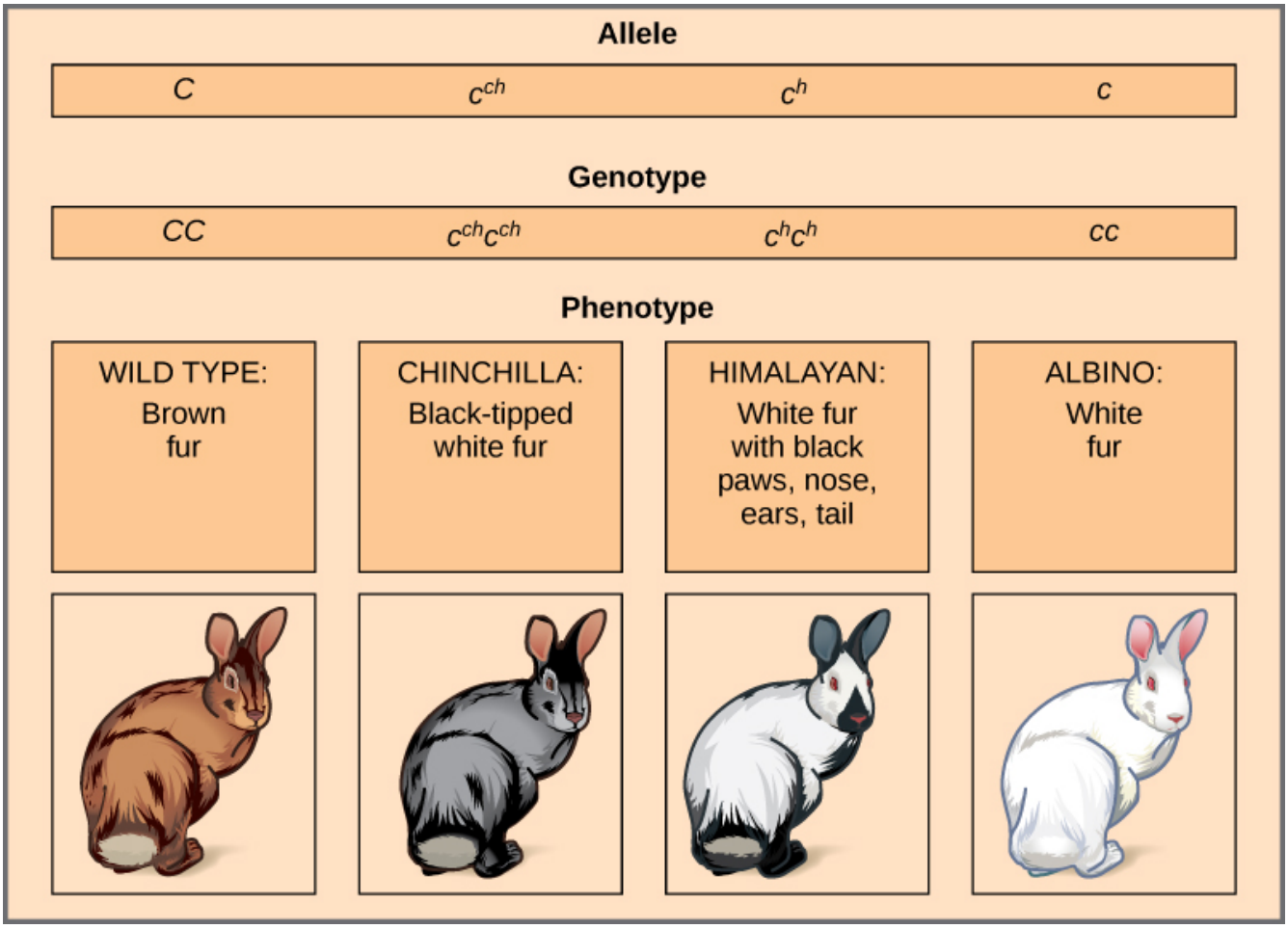


Figure 12.8 Four different alleles exist for the rabbit coat color (C) gene.

The complete dominance of a wild-type phenotype over all other mutants often occurs as an effect of "dosage" of a specific gene product, such that the wild-type allele supplies the correct amount of gene product whereas the mutant alleles cannot. For the allelic series in rabbits, the wild-type allele may supply a given dosage of fur pigment, whereas the mutants supply a lesser dosage or none at all. Interestingly, the Himalayan phenotype is the result of an allele that produces a temperature-sensitive gene product that only produces pigment in the cooler extremities of the rabbit's body.

Alternatively, one mutant allele can be dominant over all other phenotypes, including the wild type. This may occur when the mutant allele somehow interferes with the genetic message so that even a heterozygote with one wild-type allele copy expresses the mutant phenotype. One way in which the mutant allele can interfere is by enhancing the function of the wild-type gene product or changing its distribution in the body. One example of this is the *Antennapedia* mutation in *Drosophila* (Figure 12.9). In this case, the mutant allele expands the distribution of the gene product, and as a result, the *Antennapedia* heterozygote develops legs on its head where its antennae should be.

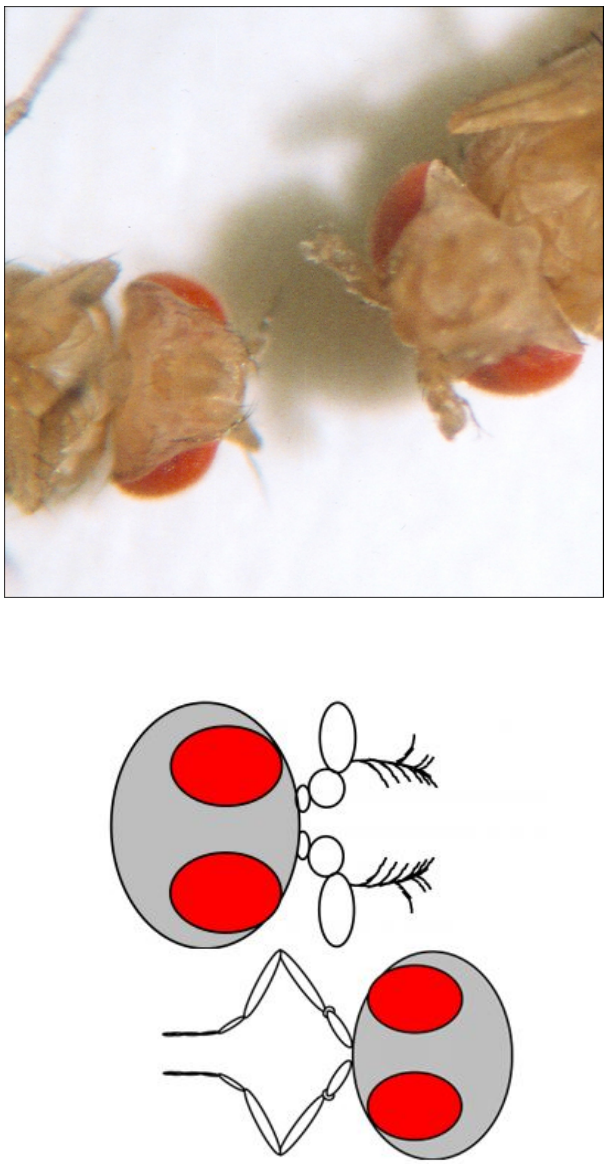
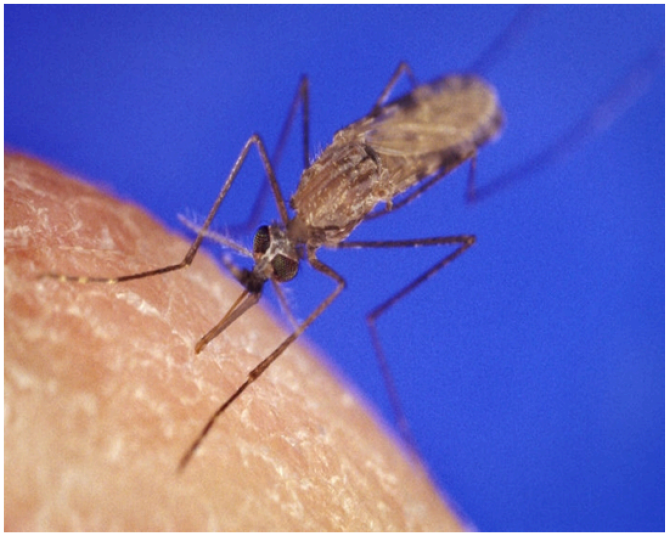


Figure 12.9 Photograph (top image) and schematic (bottom image) of the Antennapedia mutant in Drosophila. The wild-type *Drosophila is shown* on the left and the *Antennapedia* mutant on the right. The *Antennapedia* mutant has legs on its head in place of antennae. Image credit of the cartoon-like schematic. I Tietzel. CC BY SA

Evolution Connection

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* (Figure 12.10a), 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 (Figure 12.10b). 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.



(a)

(b)

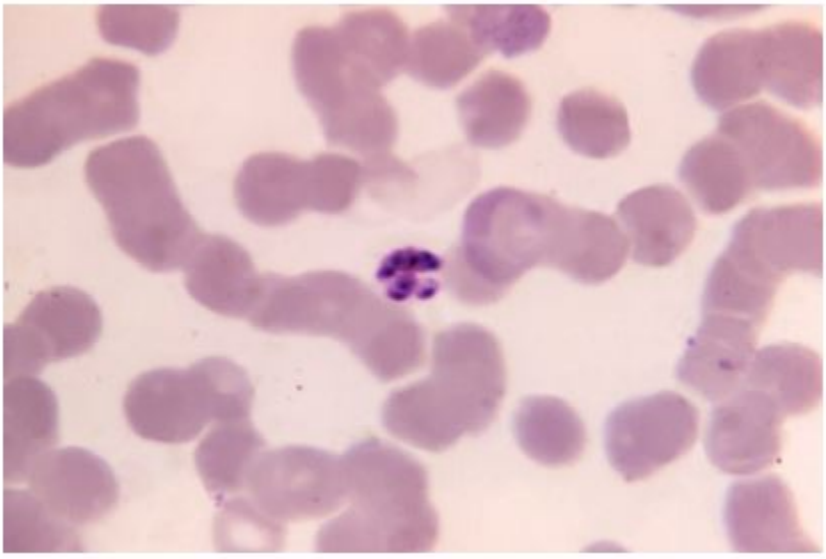


Figure 12.10 The (a) *Anopheles gambiae*, or African malaria mosquito, acts as a vector in the transmission to humans of the malaria-causing parasite (b) *Plasmodium falciparum*, here visualized using false-color transmission electron microscopy. (credit a: James D. Gathany; credit b: Ute Frevert; false color by Margaret Shear; scale-bar data from Matt Russell) (c) A 1125X photomicrograph magnification of a Giemsa stained, thin film blood smear, revealed a mature, Plasmodium malariae schizont. Malaria is seen in darker violet color. Original image sourced from US Government department: Public Health Image Library, Centers for Disease Control and Prevention.

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 infectious 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 occurs 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 where 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.[2]

X-Linked Traits

In humans, as well as in many other animals and some plants, the sex of the individual is determined by sex chromosomes. The sex chromosomes are 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 many fewer genes. In fact, when Nettie Stevens discovered that the X and Y chromosomes were the determinants of sex, she differentiated them only by size. (Note that in this case and in the description below, the terms X and Y chromosome were not used at the time.) When a gene being examined is present on the X chromosome, but not on the Y chromosome, it is said to be **X-linked**.

Eye color in *Drosophila* was one of the first X-linked traits to be identified. Thomas Hunt Morgan mapped this trait to what became known as 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 it is dominant to white eye color (X^w) (Figure 12.11). Because of the location of the eye-color gene, reciprocal crosses do not produce the same offspring ratios. Males are said to be hemizygous, because they have only one allele for any X-linked characteristic. Hemizygosity makes the descriptions of dominance and recessiveness irrelevant for XY males. *Drosophila* males lack a second allele copy 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.

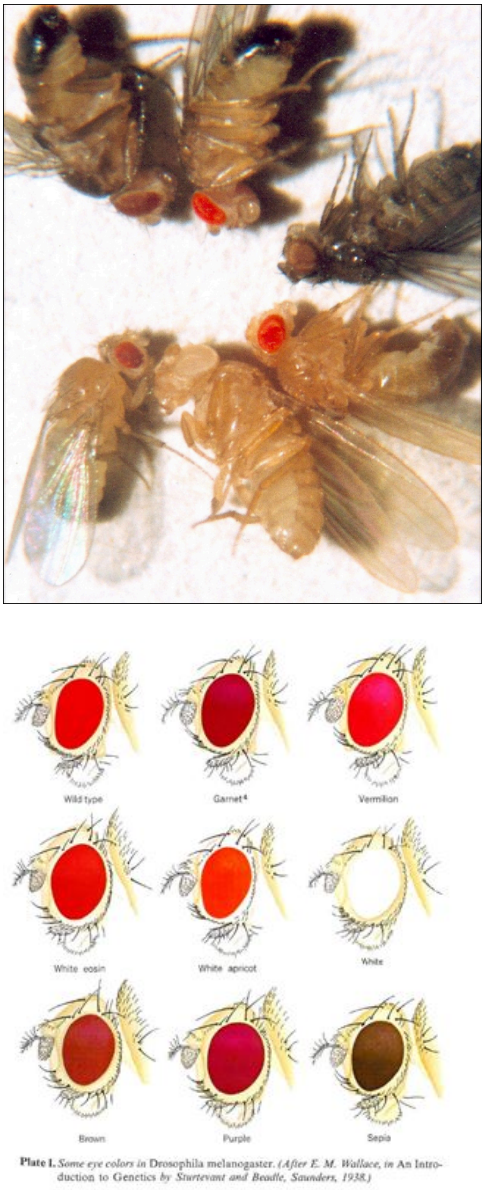


Plate I. *Some eye colors in* Drosophila melanogaster. *(After E. M. Wallace, in* An Introduction to Genetics *by Sturtevant and Beadle, Saunders, 1938.)*

Figure 12.11 In *Drosophila*, several genes determine eye color. The genes for white and vermilion eye colors are located on the X chromosome. Others are located on the autosomes. Top panel: Photograph of Drosophila phenotypes for eye color. Clockwise from top left are brown, cinnabar, sepia, vermilion, white, and red. Red eye color is wild-type and is dominant to white eye color. Bottom panel: Schematic diagrams of phenotypes for eye color of Drosophila.

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_1 generation. With regard to *Drosophila* eye color, when the P_1 male expresses the white-eye phenotype and the female is homozygous red-eyed, all members of the F_1 generation exhibit red eyes (Figure 12.12). 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_1 female and their Y chromosome from the P_1 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).

Visual Connection

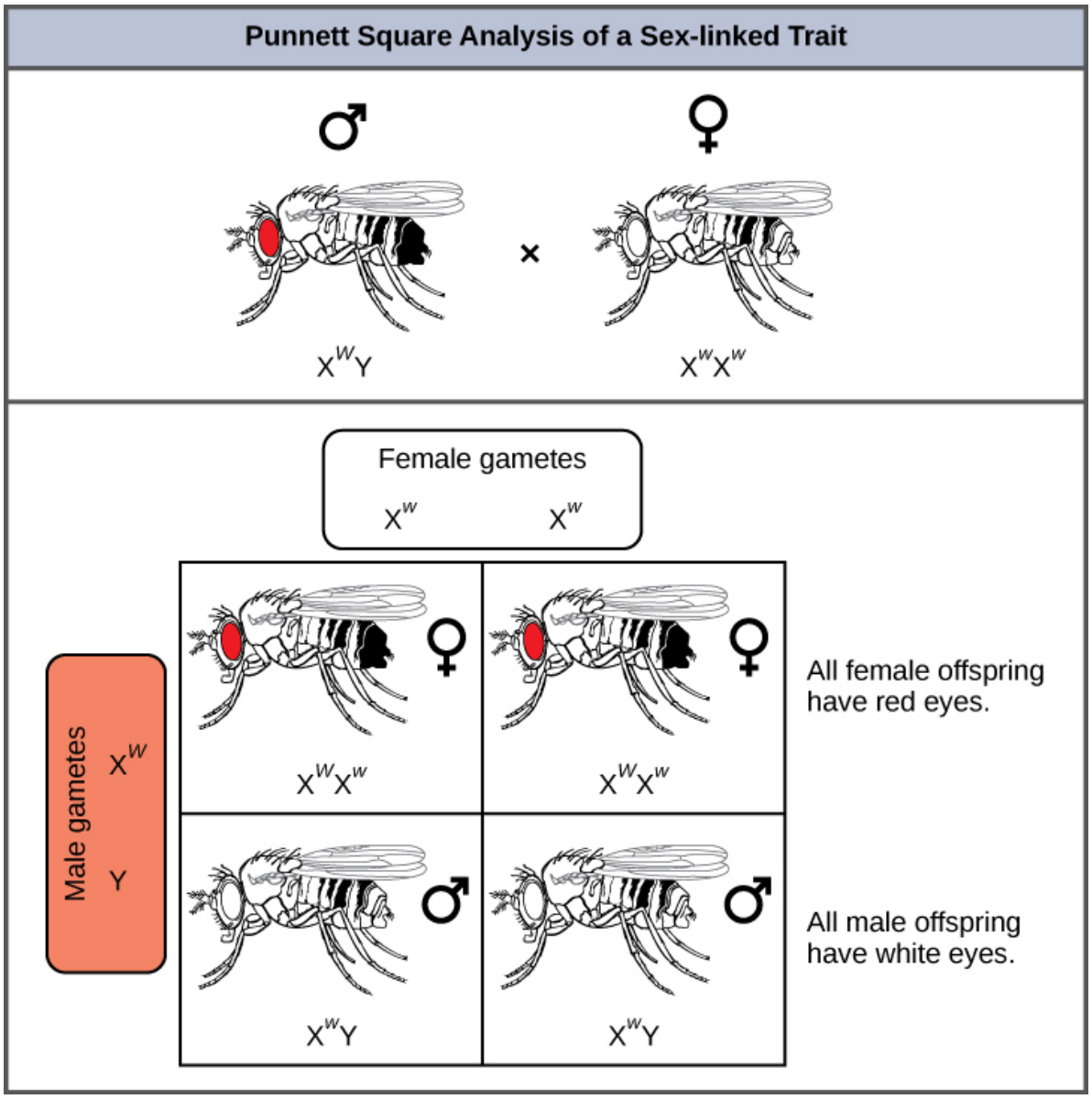


Figure 12.12 Punnett square analysis is used to determine the ratio of offspring from a cross between a red-eyed male fruit fly and a white-eyed female fruit fly.

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 offspring. Her male offspring are, therefore, destined to express the trait, as they will inherit their father's Y chromosome. In humans, the alleles

for certain conditions (some forms of 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, recessive 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 which they are hemizygous.

Human Sex-linked Disorders

Sex-linkage studies in Morgan's laboratory provided the fundamentals for understanding X-linked recessive disorders in humans, which include red-green color blindness, and Types A and B hemophilia. Because human males need to inherit only one recessive mutant X allele to be affected, X-linked disorders are disproportionately observed in males. Females must inherit recessive X-linked alleles from both of their parents in order to express the trait. When they inherit one recessive X-linked mutant allele and one dominant X-linked wild-type allele, they are carriers of the trait and are typically unaffected. Carrier females can manifest mild forms of the trait due to the inactivation of the dominant allele located on one of the X chromosomes. However, female carriers can contribute the trait to their male children, resulting in the male exhibiting the trait, or they can contribute the recessive allele to their female children, resulting in the children being carriers of the trait (Figure 12.13). Although some Y-linked recessive disorders exist, typically they are associated with infertility in males and are therefore not transmitted to subsequent generations.

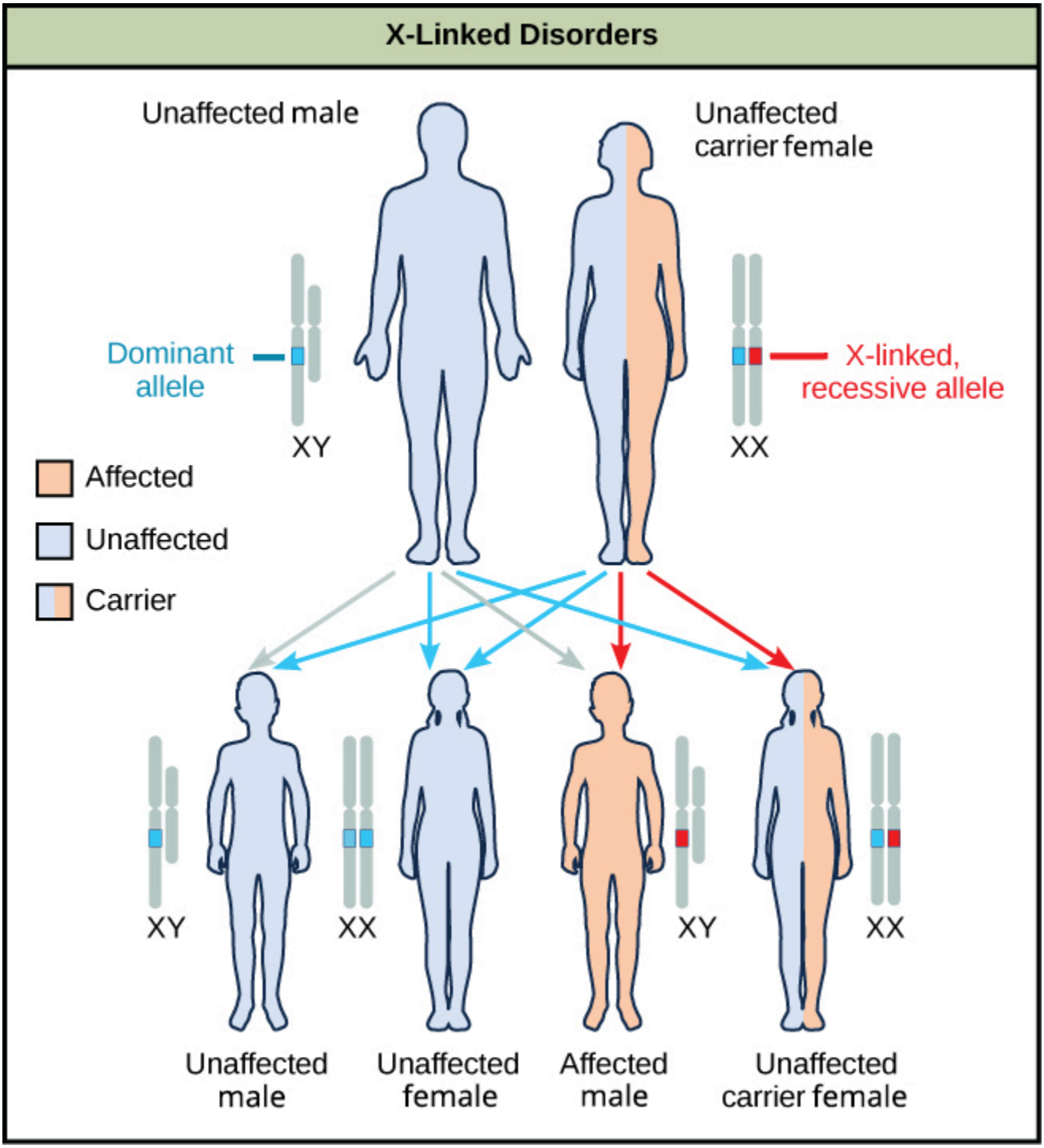


Figure 12.13 The male offspring of a person who is a carrier of a recessive X-linked disorder will have a 50 percent chance of being affected. A female will not be affected, but she will have a 50 percent chance of being a carrier like the female parent.

Link to Learning

Watch this video to learn more about sex-linked traits.

Click to view content

Lethality

A large proportion of genes in an individual's genome are essential for survival. Occasionally, a nonfunctional allele for an essential gene can arise by mutation and be transmitted in a population as long as individuals with this allele also have a wild-type, functional copy. The wild-type allele functions at a capacity sufficient to sustain life and is therefore considered to be dominant over the nonfunctional allele. However, consider two heterozygous parents that have a genotype of wild-type/nonfunctional mutant for a hypothetical essential gene. In one quarter of their offspring, we would expect to observe individuals that are homozygous recessive for the nonfunctional allele. Because the gene is essential, these individuals might fail to develop past fertilization, die *in utero*, or die later in life, depending on what life stage requires this gene. An inheritance pattern in which an allele is only lethal in the homozygous form and in which the heterozygote may be normal or have some altered nonlethal phenotype is referred to as **recessive lethal**.

For crosses between heterozygous individuals with a recessive lethal allele that causes death before birth when homozygous, only wild-type homozygotes and heterozygotes would be observed. The genotypic ratio would therefore be 2:1. In other instances, the recessive lethal allele might also exhibit a dominant (but not lethal) phenotype in the heterozygote. For instance, the recessive lethal *Curly* allele in *Drosophila* affects wing shape in the heterozygote form but is lethal in the homozygote.

A single copy of the wild-type allele is not always sufficient for normal functioning or even survival. The **dominant lethal** inheritance pattern is one in which an allele is lethal both in the homozygote and the heterozygote; this allele can only be transmitted if the lethality phenotype occurs after reproductive age. Individuals with mutations that result in dominant lethal alleles fail to survive even in the heterozygote form. Dominant lethal alleles are very rare because, as you might expect, the allele only lasts one generation and is not transmitted. However, just as the recessive lethal allele might not immediately manifest the phenotype of death, dominant lethal alleles also might not be expressed until adulthood. Once the individual reaches reproductive age, the allele may be unknowingly passed on, resulting in a delayed death in both generations. An example of this in humans is Huntington's disease, in which the nervous system gradually wastes away (Figure 12.14). People who are heterozygous for the dominant Huntington allele (*Hh*) will inevitably develop the fatal disease. However, the onset of Huntington's disease may not occur until age 40, at which point the afflicted persons may have already passed the allele to 50 percent of their offspring.

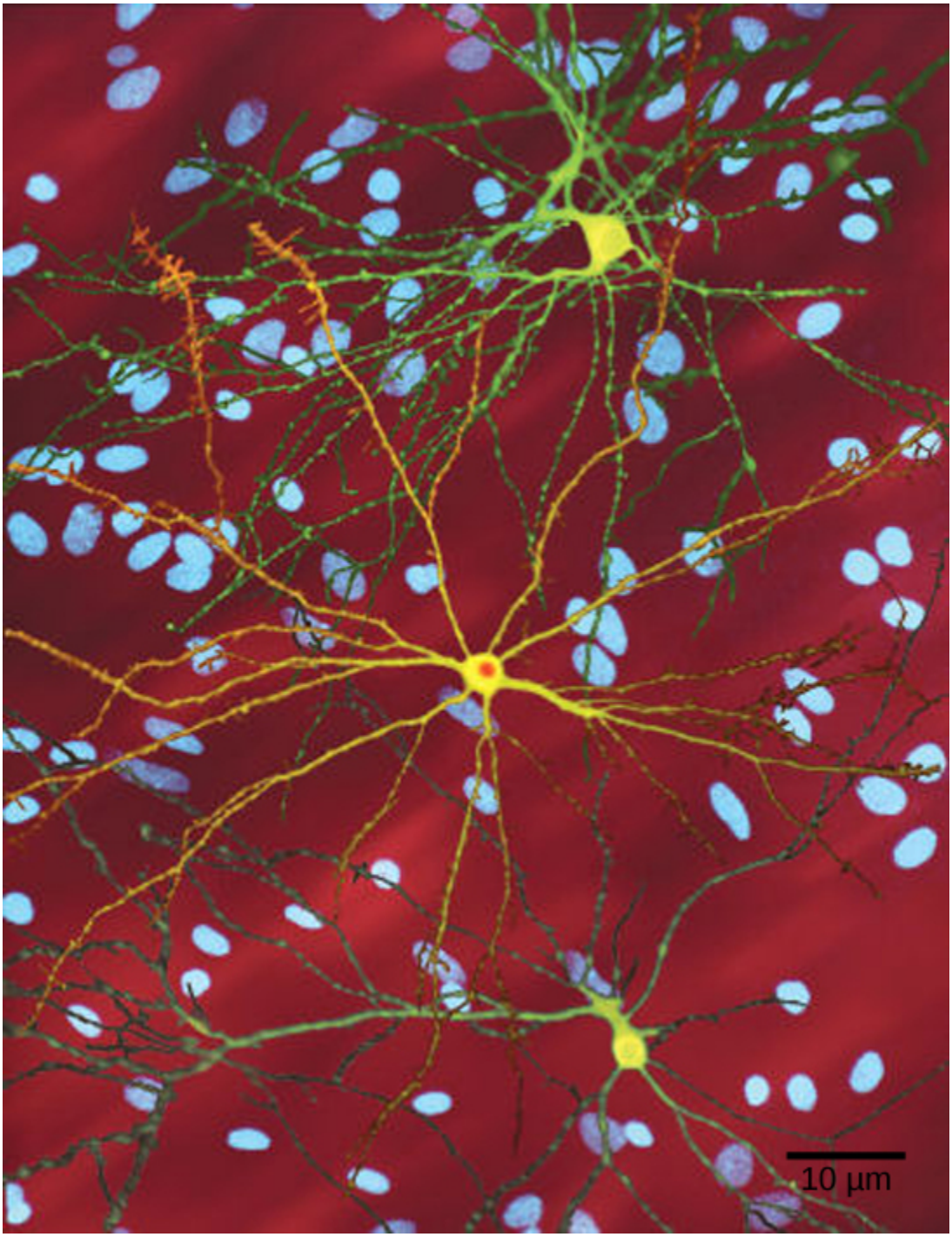


Figure 12.14 The neuron in the center of this micrograph (yellow) has nuclear inclusions characteristic of Huntington's disease (orange area in the center of the neuron). Huntington's disease occurs when an abnormal dominant allele for the Huntington gene is present. (credit: Dr. Steven Finkbeiner, Gladstone Institute of Neurological Disease, The Taube-Koret Center for Huntington's Disease Research, and the University of California San Francisco/Wikimedia)

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=454#h5p-58

Footnotes

- 2 Sumiti Vinayak, et al., "Origin and Evolution of Sulfadoxine Resistant *Plasmodium falciparum*," *Public Library of Science Pathogens* 6, no. 3 (2010): e1000830, doi:10.1371/journal.ppat.1000830.

116.

LAWS OF INHERITANCE

Learning Objectives

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

- Explain Mendel's law of segregation and independent assortment in terms of genetics and the events of meiosis
- Use the forked-line method and the probability rules to calculate the probability of genotypes and phenotypes from multiple gene crosses
- Explain the effect of linkage and recombination on gamete genotypes
- Explain the phenotypic outcomes of epistatic effects between genes

Mendel generalized the results of his pea-plant experiments into four postulates, some of which are sometimes called "laws," that describe the basis of dominant and recessive inheritance in diploid organisms. As you have learned, more complex extensions of Mendelism exist that do not exhibit the same F_2 phenotypic ratios (3:1). Nevertheless, these laws summarize the basics of classical genetics.

Pairs of Unit Factors, or Genes

Mendel proposed first that paired unit factors of heredity were transmitted faithfully from generation to generation by the dissociation and reassociation of paired factors during gametogenesis and fertilization, respectively. After he crossed peas with contrasting traits and found that the recessive trait resurfaced in the F_2 generation, Mendel deduced that hereditary factors must be inherited as discrete units. This finding contradicted the belief at that time that parental traits were blended in the offspring.

Alleles Can Be Dominant or Recessive

Mendel's **law of dominance** states that in a heterozygote, one trait will conceal the presence of another trait for the same characteristic. 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 by the same manner in which the dominant allele is transmitted. The recessive trait will only be expressed by offspring that have two copies of this allele (Figure 12.15), and these offspring will breed true when self-crossed.

Since Mendel's experiments with pea plants, researchers have found that the law of dominance does not always hold true. Instead, several different patterns of inheritance have been found to exist.

Figure 12.15 A: Alligators with albinism and without albinism. ("Albino Alligator" by mrjoro is marked with CC BY-NC 2.0.)

Figure 12.15 B: Deer without albinism and with albinism. ("illinois true albino deer" by Illinois Wildlife lover is licensed under CC BY 2.0.)

Equal Segregation of Alleles

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 could 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. The role of the meiotic segregation of chromosomes in sexual reproduction was not understood by the scientific community during Mendel's lifetime.

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. The 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 green, wrinkled seeds (*yyrr*) and another that has yellow,

round seeds (*YYRR*). Because each parent is homozygous, the law of segregation indicates that the gametes for the green/wrinkled plant all are *yr*, and the gametes for the yellow/round plant are all *YR*. Therefore, the F_1 generation of offspring all are *YyRr* (Figure 12.16).

Visual Connection

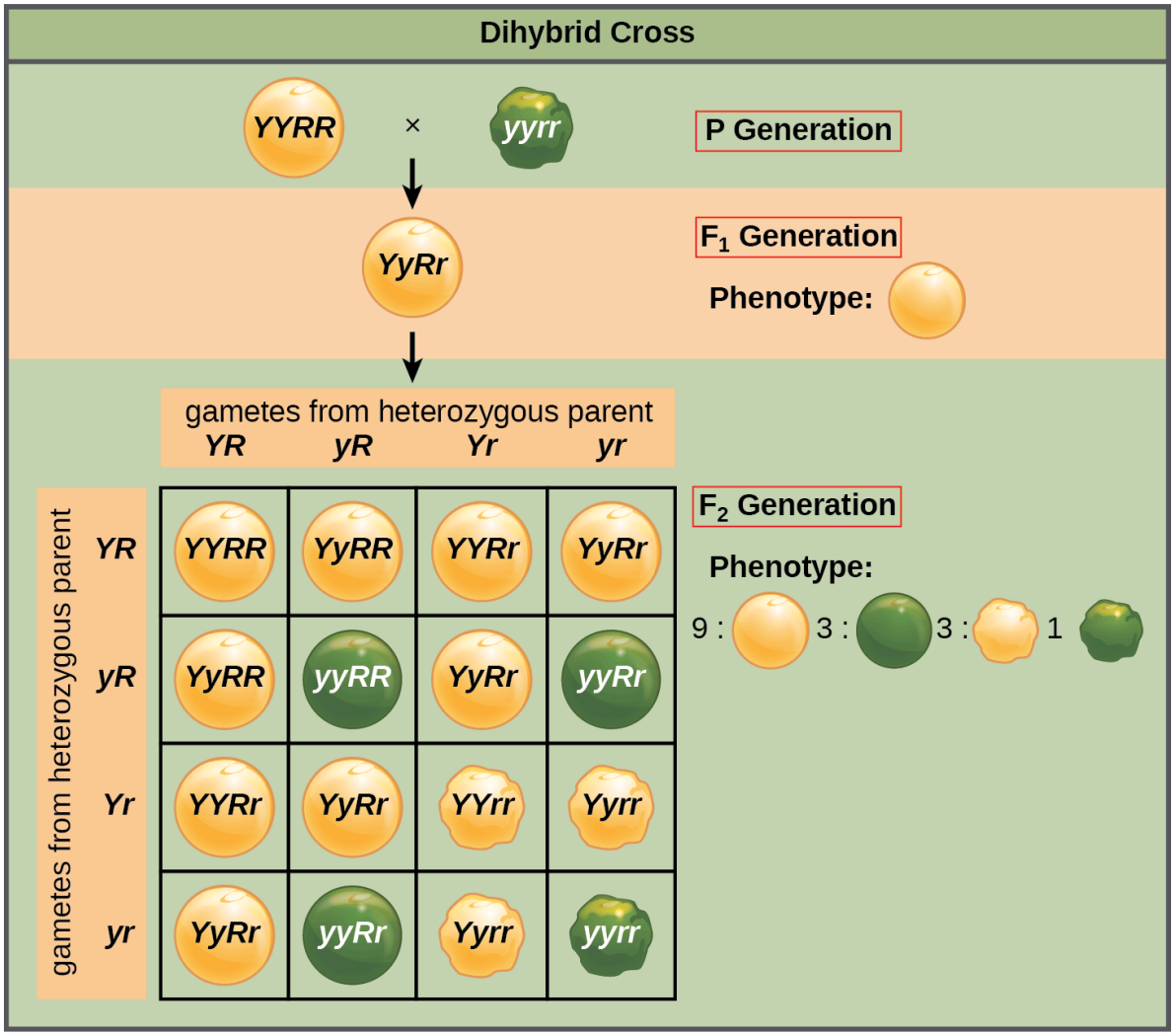


Figure 12.16 This dihybrid cross of pea plants involves the genes for seed color and texture.

In pea plants, round seed shape (R) is dominant to wrinkled seed shape (r) and yellow peas (Y) are dominant to green peas (y). What are the possible genotypes and phenotypes for a cross between RrYY and rrYy pea plants? How many squares do you

need to do a Punnett square analysis of this cross?

For the F2 generation, the law of segregation requires that each gamete receive either an *R* allele or an *r* allele along with either a *Y* allele or a *y* allele. The law of independent assortment states that a gamete into which an *r* allele sorted would be equally likely to contain either a *Y* allele or a *y* allele. Thus, there are four equally likely gametes that can be formed when the *YyRr* heterozygote is self-crossed, as follows: *YR*, *Yr*, *yR*, and *yr*. Arranging these gametes along the top and left of a 4 × 4 Punnett square (Figure 12.16) gives us 16 equally likely genotypic combinations. From these genotypes, we infer a phenotypic ratio of 9 round/yellow:3 round/green:3 wrinkled/yellow:1 wrinkled/green (Figure 12.16). These are the offspring ratios we would expect, assuming we performed the crosses with a large enough sample size.

Because of independent assortment and dominance, the 9:3:3:1 dihybrid phenotypic ratio can be collapsed into two 3:1 ratios, characteristic of any monohybrid cross that follows a dominant and recessive pattern. Ignoring seed color and considering only seed texture in the above dihybrid cross, we would expect that three quarters of the F_2 generation offspring would be round, and one quarter would be wrinkled. Similarly, isolating only seed color, we would assume that three quarters of the F_2 offspring would be yellow and one quarter would be green. The sorting of alleles for texture and color are independent events, so we can apply the product rule. Therefore, the proportion of round and yellow F_2 offspring is expected to be (3/4) × (3/4) = 9/16, and the proportion of wrinkled and green offspring is expected to be (1/4) × (1/4) = 1/16. These proportions are identical to those obtained using a Punnett square. Round, green and wrinkled, yellow offspring can also be calculated using the product rule, as each of these genotypes includes one dominant and one recessive phenotype. Therefore, the proportion of each is calculated as (3/4) × (1/4) = 3/16.

The law of independent assortment also indicates that a cross between yellow, wrinkled (*YYrr*) and green, round (*yyRR*) parents would yield the same F_1 and F_2 offspring as in the *YYRR* x *yyrr* cross.

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.

Forked-Line Method

When more than two genes are being considered, the Punnett-square method becomes unwieldy. For instance, examining a cross involving four genes would require a 16 × 16 grid containing 256 boxes. It would be extremely cumbersome to manually enter each genotype. For more complex crosses, the forked-line and probability methods are preferred.

To prepare a forked-line diagram for a cross between F_1 heterozygotes resulting from a cross

between *AABBCC* and *aabbcc* parents, we first create rows equal to the number of genes being considered, and then segregate the alleles in each row on forked lines according to the probabilities for individual monohybrid crosses (Figure 12.17). We then multiply the values along each forked path to obtain the F_2 offspring probabilities. Note that this process is a diagrammatic version of the product rule. The values along each forked pathway can be multiplied because each gene assorts independently. For a trihybrid cross, the F_2 phenotypic ratio is 27:9:9:9:3:3:3:1.

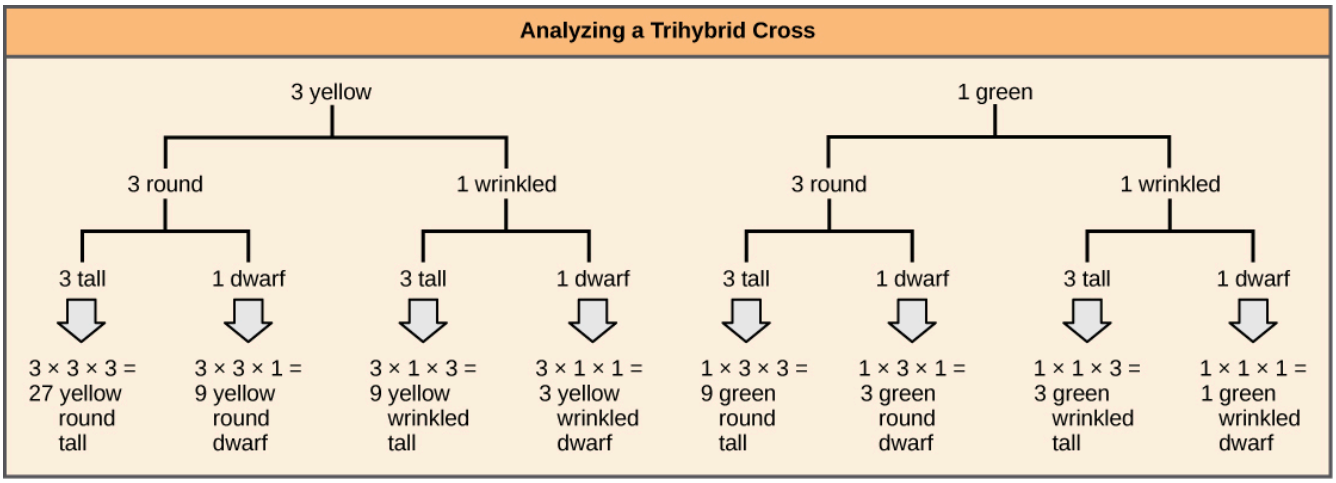


Figure 12.17 The forked-line method can be used to analyze a trihybrid cross. Here, the probability for color in the F2 generation occupies the top row (3 yellow:1 green). The probability for shape occupies the second row (3 round: 1 wrinkled), and the probability for height occupies the third row (3 tall:1 dwarf). The probability for each possible combination of traits is calculated by multiplying the probability for each individual trait. Thus, the probability of F2 offspring having yellow, round, and tall traits is 3 × 3 × 3, or 27.0.

Probability Method

While the forked-line method is a diagrammatic approach to keeping track of probabilities in a cross, the probability method gives the proportions of offspring expected to exhibit each phenotype (or genotype) without the added visual assistance. Both methods make use of the product rule and consider the alleles for each gene separately. Earlier, we examined the phenotypic proportions for a trihybrid cross using the forked-line method; now we will use the probability method to examine the genotypic proportions for a cross with even more genes.

For a trihybrid cross, writing out the forked-line method is tedious, albeit not as tedious as using the Punnett-square method. To fully demonstrate the power of the probability method, however, we can consider specific genetic calculations. For instance, for a tetrahybrid cross between individuals that are heterozygotes for all four genes, and in which all four genes are sorting independently and in a dominant and recessive pattern, what proportion of the offspring will be expected to be homozygous recessive for all four alleles? Rather than writing out every possible genotype, we can use the probability method. We know that for each gene, the

fraction of homozygous recessive offspring will be 1/4. Therefore, multiplying this fraction for each of the four genes, (1/4) × (1/4) × (1/4) × (1/4), we determine that 1/256 of the offspring will be quadruply homozygous recessive.

For the same tetrahybrid cross, what is the expected proportion of offspring that have the dominant phenotype at all four loci? We can answer this question using phenotypic proportions, but let's do it the hard way—using genotypic proportions. The question asks for the proportion of offspring that are 1) homozygous dominant at *A* or heterozygous at *A,* and 2) homozygous at *B* or heterozygous at *B*, and so on. Noting the "or" and "and" in each circumstance makes clear where to apply the sum and product rules. The probability of a homozygous dominant at *A* is 1/4 and the probability of a heterozygote at *A* is 1/2. The probability of the homozygote or the heterozygote is 1/4 + 1/2 = 3/4 using the sum rule. The same probability can be obtained in the same way for each of the other genes, so that the probability of a dominant phenotype at *A* and *B* and *C* and *D* is, using the product rule, equal to 3/4 × 3/4 × 3/4 × 3/4, or 81/256. If you are ever unsure about how to combine probabilities, returning to the forked-line method should make it clear.

Rules for Multihybrid Fertilization

Predicting the genotypes and phenotypes of offspring from given crosses is the best way to test your knowledge of Mendelian genetics. Given a multihybrid cross that obeys independent assortment and follows a dominant and recessive pattern, several generalized rules exist; you can use these rules to check your results as you work through genetics calculations (Table 12.5). To apply these rules, first you must determine *n*, the number of heterozygous gene pairs (the number of genes segregating two alleles each). For example, a cross between *AaBb* and *AaBb* heterozygotes has an *n* of 2. In contrast, a cross between *AABb* and *AABb* has an *n* of 1 because *A* is not heterozygous.

General Rules for Multihybrid Crosses

General Rule	Number of Heterozygous Gene Pairs
Number of different F_1 gametes	2^n
Number of different F_2 genotypes	3^n
Given dominant and recessive inheritance, the number of different F_2 phenotypes	2^n

Table 12.5

Linked Genes Violate the Law of Independent Assortment

Although all of Mendel's pea 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's consider the biological basis of gene linkage and recombination.

Homologous chromosomes possess the same genes in the same linear order. The alleles may differ on homologous chromosome pairs, but the genes to which they correspond do not. In preparation for the first division of meiosis, homologous chromosomes replicate and synapse. Like genes on the homologs align with each other. At this stage, segments of homologous chromosomes exchange linear segments of genetic material (Figure 12.18). 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.

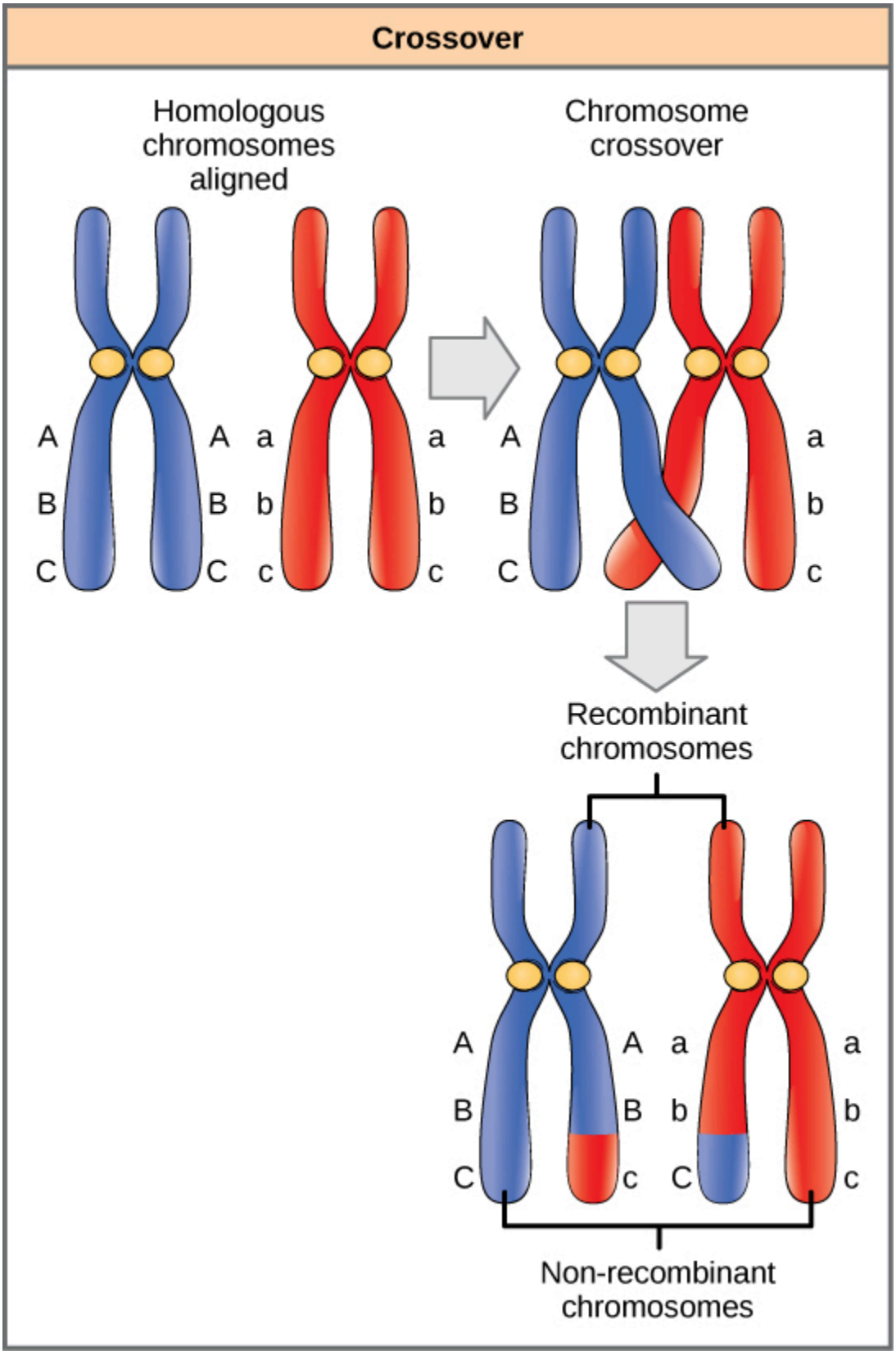


Figure 12.18 The process of crossover, or recombination, occurs when two homologous chromosomes align during meiosis and exchange a segment of genetic material. Here, the alleles for gene C were exchanged. The result is two recombinant and two

non-recombinant chromosomes.

When two genes are located in close proximity 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 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 the 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 elaborate 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 pairs of 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.

Scientific Method Connection

Testing the Hypothesis of Independent Assortment

To better appreciate the amount of labor and ingenuity that went into Mendel's experiments, proceed through one of Mendel's dihybrid crosses.

Question: What will be the offspring of a dihybrid cross?

Background: Consider that pea plants mature in one growing season, and you have access to a large garden in which you can cultivate thousands of pea plants. There are several true-breeding plants with the following pairs of traits: tall plants with inflated pods, and dwarf plants with constricted pods. Before the plants have matured, you remove the pollen-producing

organs from the tall/inflated plants in your crosses to prevent self-fertilization. Upon plant maturation, the plants are manually crossed by transferring pollen from the dwarf/constricted plants to the stigmata of the tall/inflated plants.

Hypothesis: Both trait pairs will sort independently according to Mendelian laws. When the true-breeding parents are crossed, all of the F_1 offspring are tall and have inflated pods, which indicates that the tall and inflated traits are dominant over the dwarf and constricted traits, respectively. A self-cross of the F_1 heterozygotes results in 2,000 F_2 progeny.

Test the hypothesis: Because each trait pair sorts independently, the ratios of tall:dwarf and inflated:constricted are each expected to be 3:1. The tall/dwarf trait pair is called *T/t*, and the inflated/constricted trait pair is designated *I/i*. Each member of the F_1 generation therefore has a genotype of *TtIi*. Construct a grid analogous to Figure 12.16, in which you cross two *TtIi* individuals. Each individual can donate four combinations of two traits: *TI*, *Ti*, *tI*, or *ti*, meaning that there are 16 possibilities of offspring genotypes. Because the *T* and *I* alleles are dominant, any individual having one or two of those alleles will express the tall or inflated phenotypes, respectively, regardless if they also have a *t* or *i* allele. Only individuals that are *tt* or *ii* will express the dwarf and constricted alleles, respectively. As shown in Figure 12.19, you predict that you will observe the following offspring proportions: tall/inflated:tall/constricted:dwarf/inflated:dwarf/constricted in a 9:3:3:1 ratio. Notice from the grid that when considering the tall/dwarf and inflated/constricted trait pairs in isolation, they are each inherited in 3:1 ratios.

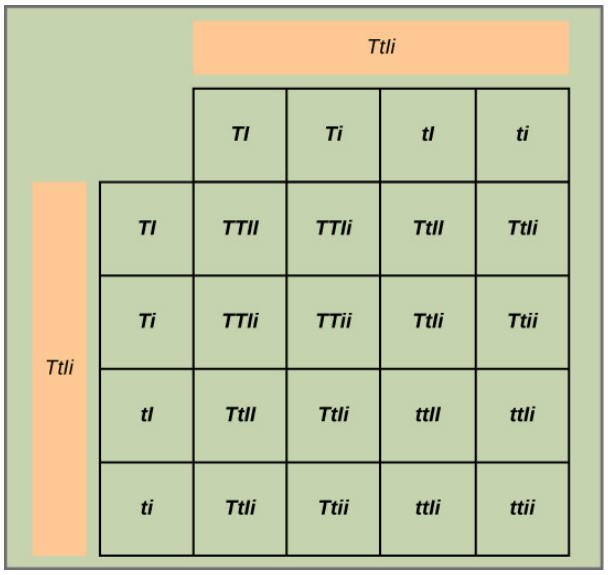


Figure 12.19 This figure shows all possible combinations of offspring resulting from a dihybrid cross of pea plants that are heterozygous for the tall/dwarf and inflated/constricted alleles.

Test the hypothesis: You cross the dwarf and tall plants and then self-cross the offspring. For best results, this is repeated with hundreds or even thousands of pea plants. What special precautions should be taken in the crosses and in growing the plants?

Analyze your data: You observe the following plant phenotypes in the F_2 generation: 2706 tall/inflated, 930 tall/constricted, 888 dwarf/inflated, and 300 dwarf/constricted. Reduce these findings to a ratio and determine if they are consistent with Mendelian laws.

Form a conclusion: Were the results close to the expected 9:3:3:1 phenotypic ratio? Do the results support the prediction? What might be observed if far fewer plants were used, given that alleles segregate randomly into gametes? Try to imagine growing that many pea plants, and consider the potential for experimental error. For instance, what would happen if it was extremely windy one day?

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.

Link to Learning

Eye color in humans is determined by multiple genes. 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 need to be expressed simultaneously to affect a phenotype. Genes may also oppose each other, with one gene modifying 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 that mean "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 the 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*) is necessary for pigment production. A mouse with a recessive *c* allele at this locus is unable to produce pigment and is albino regardless of the allele present at locus *A* (Figure 12.20). 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 solid color:4 albino (Figure 12.20). In this case, the *C* gene is epistatic to the *A* gene.

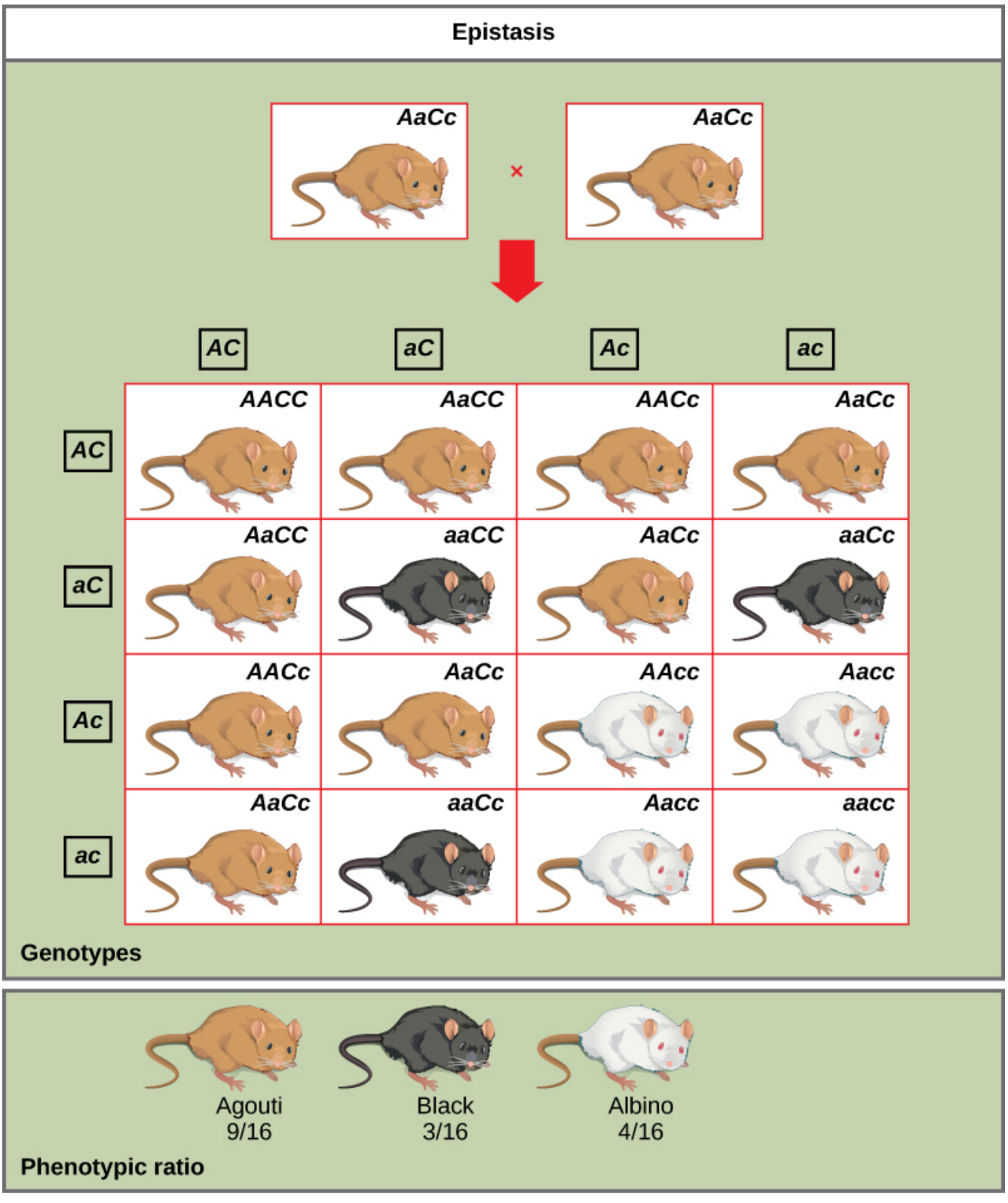


Figure 12.20 In mice, the mottled agouti coat color (A) is dominant to a solid coloration, such as black or gray. A gene at a separate locus (C) is responsible for pigment production. The recessive c allele does not produce pigment, and a mouse with the homozygous recessive cc genotype is albino regardless of the allele present at the A locus. Thus, the C gene is epistatic to the A gene.

Epistasis can also occur when a dominant allele masks expression at a separate gene. Fruit color in summer squash is expressed in this way. Homozygous recessive expression of the *W* gene (*ww*) coupled with homozygous dominant or heterozygous expression of the *Y* gene (*YY* or *Yy*) generates yellow fruit, and the *wwyy* genotype produces green fruit. However, if a dominant copy of the *W* gene is present in the homozygous or heterozygous form, the summer squash will produce white fruit regardless of the *Y* alleles. A cross between white heterozygotes for both genes (*WwYy* × *WwYy*) would produce offspring with a phenotypic ratio of 12 white:3 yellow:1 green.

Finally, epistasis can be reciprocal such that either gene, when present in the dominant (or recessive) form, expresses the same phenotype. In the shepherd's purse plant (*Capsella bursa-pastoris*), the characteristic of seed shape is controlled by two genes in a dominant epistatic relationship. When the genes *A* and *B* are both homozygous recessive (*aabb*), the seeds are ovoid. If the dominant allele for either of these genes is present, the result is triangular seeds. That is, every possible genotype other than *aabb* results in triangular seeds, and a cross between heterozygotes for both genes (*AaBb* x *AaBb*) would yield offspring with a phenotypic ratio of 15 triangular:1 ovoid.

As you work through genetics problems, keep in mind that any single characteristic that results in a phenotypic ratio that totals 16 is typical of a two-gene interaction. Recall the phenotypic inheritance pattern for Mendel's dihybrid cross, which considered two noninteracting genes—9:3:3:1. Similarly, we would expect interacting gene pairs to also exhibit ratios expressed as 16 parts. Note that we are assuming the interacting genes are not linked; they are still assorting independently into gametes.

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https://louis.pressbooks.pub/generalbiology1leclab/?p=458#h5p-59

117.

KEY TERMS

allele
: gene variations that arise by mutation and exist at the same relative locations on homologous chromosomes

autosomes
: any of the non-sex chromosomes

blending theory of inheritance
: hypothetical inheritance pattern in which parental traits are blended together in the offspring to produce an intermediate physical appearance

carriers
: heterozygous individual or organism that carries a recessive allele that is not contributing to the phenotype. Relevant in medicine for recessive alleles that cause genetic disease(s) in homozygous offspring

chromosomal basic of heredity
: also known as Chromosomal Theory of Inheritance. A theory proposing that chromosomes are the genes' vehicles and that their behavior during meiosis is the physical basis of the inheritance patterns that Mendel observed

codominance
: in a heterozygote, complete and simultaneous expression of both alleles for the same characteristic

continuous variation
: inheritance pattern in which a character shows a range of trait values with small gradations rather than large gaps between them

dihybrid
: result of a cross between two true-breeding parents that express different traits for two characteristics

discontinuous variation
: inheritance pattern in which traits are distinct and are transmitted independently of one another

dominant
: trait which confers the same physical appearance whether an individual has two copies of the trait or one copy of the dominant trait and one copy of the recessive trait

dominant lethal
: inheritance pattern in which an allele is lethal both in the homozygote and the heterozygote; this allele

can only be transmitted if the lethality phenotype occurs after reproductive age

epistasis
: antagonistic interaction between genes such that one gene masks or interferes with the expression of another

F_1
: first filial generation in a cross; the offspring of the parental generation

F_2
: second filial generation produced when F_1 individuals are self-crossed or fertilized with each other

genetics
: the study of heredity

genotype
: underlying genetic makeup, consisting of both physically visible and non-expressed alleles, of an organism

hemizygous
: presence of only one allele for a characteristic, as in X-linkage; hemizygosity makes descriptions of dominance and recessiveness irrelevant

heterozygous
: having two different alleles for a given gene on the homologous chromosome

homozygous
: having two identical alleles for a given gene on the homologous chromosome

hybridization
: process of mating two individuals that differ with the goal of achieving a certain characteristic in their offspring

incomplete dominance
: in a heterozygote, expression of two contrasting alleles such that the individual displays an intermediate phenotype

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

linkage
: phenomenon in which alleles that are located in close proximity to each other on the same chromosome are more likely to be inherited together

Mendelian genetics
: patterns of inheritance discovered by Gregor Mendel that include law of segregation, law of independent assortment, genotypes and phenotypes

model system
: species or biological system used to study a specific biological phenomenon to be applied to other different species

monohybrid
: result of a cross between two true-breeding parents that express different traits for only one characteristic

P_0
: parental generation in a cross

phenotype
: observable traits expressed by an organism

product rule
: probability of two independent events occurring simultaneously can be calculated by multiplying the individual probabilities of each event occurring alone

Punnett square
: 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

recessive
: trait that appears "latent" or non-expressed when the individual also carries a dominant trait for that same characteristic; when present as two identical copies, the recessive trait is expressed

recessive lethal
: inheritance pattern in which an allele is only lethal in the homozygous form; the heterozygote may be normal or have some altered, nonlethal phenotype

reciprocal cross
: 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

sex-linked
: any gene on a sex chromosome

sum rule
: probability of the occurrence of at least one of two mutually exclusive events is the sum of their individual probabilities

test cross
: 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

trait
: variation in the physical appearance of a heritable characteristic

true breeding
: parental organisms that always produce offspring that look like the parent (because they are homozygous for that trait)

wild type
: term for most common phenotype or genotype among wild animals of a gene

X-linked
: gene present on the X, but not the Y chromosome

118.

CHAPTER SUMMARY

12.1 Mendel's Experiments and the Laws of Probability

Working with garden pea plants, Mendel found that crosses between parents that differed by one trait produced F_1 offspring that all expressed the traits of one parent. Observable traits are referred to as dominant, and non-expressed traits are described as recessive. When the offspring 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_0 parent. Reciprocal crosses generated identical F_1 and F_2 offspring ratios. By examining sample sizes, Mendel showed that his crosses behaved reproducibly according to the laws of probability, and that the traits were inherited as independent events.

Two rules in probability can be used to find the expected proportions of offspring of different traits from different crosses. To find the probability of two or more independent events occurring together, apply the product rule and multiply the probabilities of the individual events. The use of the word "and" suggests the appropriate application of the product rule. To find the probability of two or more events occurring in combination, apply the sum rule and add their individual probabilities together. The use of the word "or" suggests the appropriate application of the sum rule.

12.2 Characteristics and Traits

When true-breeding or homozygous individuals that differ for a certain trait are crossed, all of the offspring will be heterozygotes 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.

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 of 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. Finally, some alleles can be lethal. Recessive lethal alleles are only lethal in homozygotes, but dominant lethal alleles are fatal in heterozygotes as well.

12.3 Laws of Inheritance

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, alleles are generally not more likely to segregate into a gamete with a particular allele of another gene. A dihybrid cross demonstrates independent assortment when the genes in question are on different chromosomes or distant from each other on the same chromosome. For crosses involving more than two genes, use the forked line or probability methods to predict offspring genotypes and phenotypes rather than a Punnett square.

Although chromosomes sort independently into gametes during meiosis, Mendel's law of independent assortment refers to genes, not chromosomes, and a single chromosome may carry more than 1,000 genes. 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.

119.

VISUAL CONNECTION QUESTIONS

1. Figure 12.5 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 round pea parent plant is homozygous dominant or heterozygous? If the round pea parent plant is heterozygous, what is the probability that a random sample of 3 progeny peas will all be round?

2. Figure 12.6 What are the genotypes of the individuals labeled 1, 2, and 3?

3. Figure 12.12 What ratio of offspring would result from a cross between a white-eyed male and a female that is heterozygous for red eye color?

4. Figure 12.16 In pea plants, round seed shape (R) is dominant to wrinkled seed shape (r) and yellow peas (Y) are dominant to green peas (y). What are the possible genotypes and phenotypes for a cross between RrYY and rrYy pea plants? How many squares do you need to do a Punnett square analysis of this cross?

120.

REVIEW QUESTIONS

5. Mendel performed hybridizations by transferring pollen from the _____ of the male plant to the female ova.

a. anther
b. pistil
c. stigma
d. seed

6. Which is one of the seven characteristics that Mendel observed in pea plants?

a. flower size
b. seed texture
c. leaf shape
d. stem color

7. Imagine you are performing a cross involving seed color in garden pea plants. What F1 offspring would you expect if you cross true-breeding parents with green seeds and yellow seeds? Yellow seed color is dominant over green.

a. 100 percent yellow-green seeds
b. 100 percent yellow seeds
c. 50 percent yellow seeds, 50 percent green seeds
d. 25 percent green seeds, 75 percent yellow seeds

8. Consider a cross to investigate the pea pod texture trait, involving constricted or inflated pods. Mendel found that the traits behave according to a dominant/recessive pattern in which inflated pods were dominant. If you performed this cross and obtained 650 inflated-pod plants in the F_2 generation, approximately how many constricted-pod plants would you expect to have?

a. 600
b. 165

c. 217
d. 468

9. A scientist pollinates a true-breeding pea plant with violet, terminal flowers with pollen from a true-breeding pea plant with white, axial flowers. Which of the following observations would most accurately describe the F_2 generation?

a. 75% violet flowers, 75% terminal flowers
b. 75% white flowers in a terminal position
c. 75% violet flowers, 75% axial flowers
d. 75% violet flowers in an axial position

10. The observable traits expressed by an organism are described as its _____.

a. phenotype
b. genotype
c. alleles
d. zygote

11. A recessive trait will be observed in individuals that are _____ for that trait.

a. heterozygous
b. homozygous or heterozygous
c. homozygous
d. diploid

12. 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

13. 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, IB 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 will

have 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

14. In a mating between two individuals that are heterozygous for a recessive lethal allele that is expressed in utero, what genotypic ratio (homozygous dominant:heterozygous:homozygous recessive) would you expect to observe in the offspring?

a. 1:2:1
b. 3:1:1
c. 1:2:0
d. 0:2:1

15. If the allele encoding polydactyly (six fingers) is dominant, why do most people have five fingers?

a. Genetic elements suppress the polydactyl gene.
b. Polydactyly is embryonic lethal.
c. The sixth finger is removed at birth.
d. The polydactyl allele is very rare in the human population.

16. A farmer raises black and white chickens. To his surprise, when the first generation of eggs hatch, all the chickens are black with white speckles throughout their feathers. What should the farmer expect when the eggs laid after interbreeding the speckled chickens hatch?

a. All the offspring will be speckled.
b. 75% of the offspring will be speckled, and 25% will be black.
c. 50% of the offspring will be speckled, 25% will be black, and 25% will be white.
d. 50% of the offspring will be black, and 50% of the offspring will be white.

17. Assuming no gene linkage, in a dihybrid cross of AABB x aabb with AaBb F_1 heterozygotes, what is the ratio of the F_1 gametes (AB, aB, Ab, ab) that will give rise to the F_2 offspring?

a. 1:1:1:1
b. 1:3:3:1

c. 1:2:2:1
d. 4:3:2:1

18. The forked line and probability methods make use of what probability rule?

a. test cross
b. product rule
c. monohybrid rule
d. sum rule

19. How many different offspring genotypes are expected in a trihybrid cross between parents heterozygous for all three traits when the traits behave in a dominant and recessive pattern? How many phenotypes?

1. 64 genotypes; 16 phenotypes
2. 16 genotypes; 64 phenotypes
3. 8 genotypes; 27 phenotypes
4. 27 genotypes; 8 phenotypes

20. Labrador retrievers' fur color is controlled by two alleles, E and B. Any dog with the ee__ genotype develops into a yellow lab, while B_E_ dogs become black labs and bbE_ dogs become chocolate labs. This is an example of _____.

a. epistasis
b. codominance
c. incomplete dominance
d. linkage

21. Which of the following situations does not follow the Law of Independent Assortment?

a. A blond person and a brown-haired person produce three offspring over time, all of who have blond hair.
b. A white cow crossed with a brown bull produces roan cattle.
c. Mating a hog with a sow produces six female piglets.
d. Men are more likely to experience hemophilia than women.

121.

CRITICAL THINKING QUESTIONS

22. Choose one of the following two questions (option a or option b):

- Option a) Describe one of the reasons why the garden pea was an excellent choice of model system for studying inheritance.
- Option b) What organism would you choose as a model system for studying inheritance? Name one reason for choosing this organism.

23. How would you perform a reciprocal cross for the characteristic of stem height in the garden pea?

24. Mendel performs a cross using a true-breeding pea plant with round, yellow seeds and a true-breeding pea plant with green, wrinkled seeds. What is the probability that offspring will have green, round seeds? Calculate the probability for the F_1 and F_2 generations.

25. Choose one of the following two questions (option a or option b):

- Option a) Calculate the probability of selecting a heart or a face card from a standard deck of cards. Is this outcome more or less likely than selecting a heart suit face card?
- Option b) A computer software can generate either random numbers or either random letters. Calculate the probability of the software selecting the number combination 77 and calculate the probability selecting the letter combination ZB. Which combination is less likely to occur?

26. The gene for flower position in pea plants exists as axial or terminal alleles. Given that axial is dominant to terminal, list all of the possible F_1 and F_2 genotypes and phenotypes from a cross involving parents that are homozygous for each trait. Express genotypes with conventional genetic abbreviations.

27. 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?

28. Can a human male be a carrier of red-green color blindness?

29. Why is it more efficient to perform a test cross with a homozygous recessive donor than a homozygous dominant donor? How could the same information still be found with a homozygous dominant donor?

30. Use the probability method to calculate the genotypes and genotypic proportions of a cross between AABBCc and Aabbcc parents.

31. Explain epistasis in terms of its Greek-language roots (“standing upon”).

32. In Section 12.3, "Laws of Inheritance," an example of epistasis was given for the summer squash. Cross white WwYy heterozygotes to prove the phenotypic ratio of 12 white:3 yellow:1 green that was given in the text.

33. People with trisomy 21 develop Down's syndrome. What law of Mendelian inheritance is violated in this disease? What is the most likely way this occurs?

34. A heterozygous pea plant produces violet flowers and yellow, round seeds. Describe the expected genotypes of the gametes produced by Mendelian inheritance. If all three genes are found on the same arm of one chromosome, should a scientist predict that inheritance patterns will follow Mendelian genetics?

PART XIII

MODERN UNDERSTANDINGS OF INHERITANCE

122.

INTRODUCTION

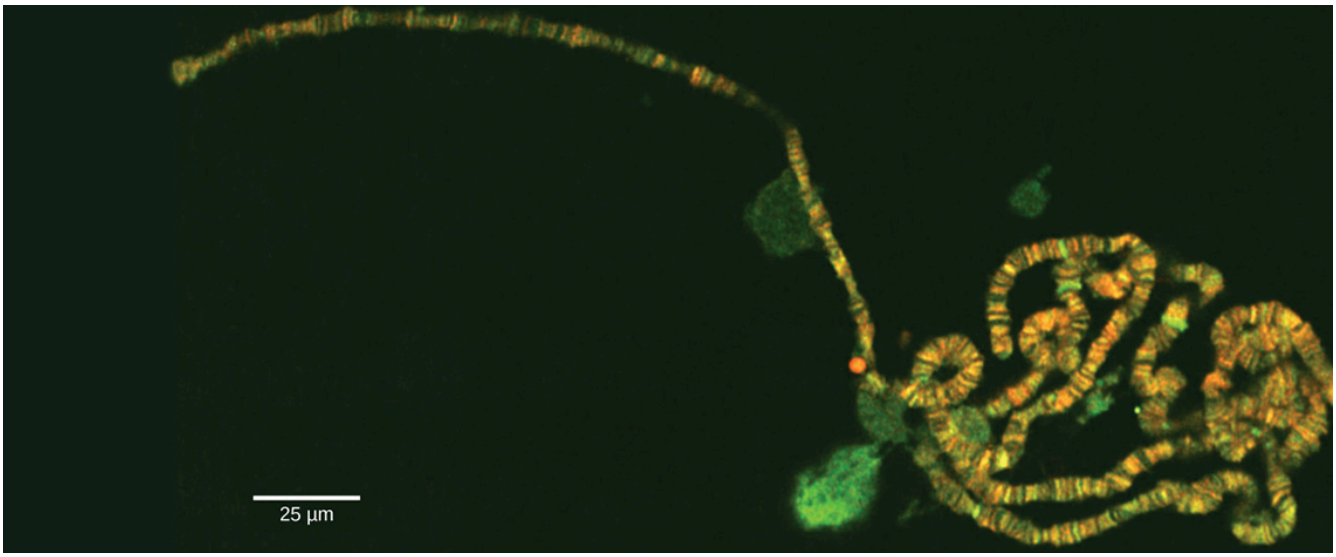


Figure 13.1 Chromosomes are threadlike nuclear structures consisting of DNA and proteins that serve as the repositories for genetic information. The chromosomes depicted here were isolated from a fruit fly's salivary gland, stained with dye, and visualized under a microscope. Akin to miniature bar codes, chromosomes absorb different dyes to produce characteristic banding patterns, which allows for their routine identification. (credit: modification of work by "LPLT"/Wikimedia Commons; scale-bar data from Matt Russell)

The gene is the physical unit of inheritance, and genes are arranged in a linear order on chromosomes. Chromosome behavior and interaction during meiosis explain, at a cellular level, inheritance patterns that we observe in populations. Genetic disorders involving alterations in chromosome number or structure may have dramatic effects and can prevent a fertilized egg from developing.

123.

CHROMOSOMAL THEORY AND GENETIC LINKAGE

Learning Objectives

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

- Discuss Sutton's Chromosomal Theory of Inheritance
- Describe genetic linkage
- Explain the process of homologous recombination, or crossing over

Long before scientists visualized chromosomes under a microscope, the father of modern genetics, Gregor Mendel, began studying heredity in 1843. With improved microscopic techniques during the late 1800s, cell biologists could stain and visualize subcellular structures with dyes and observe their actions during cell division and meiosis. With each mitotic division, chromosomes replicated, condensed from an amorphous (no constant shape) nuclear mass into distinct X-shaped bodies (pairs of identical sister chromatids), and migrated to separate cellular poles.

Chromosomal Theory of Inheritance

The speculation that chromosomes might be the key to understanding heredity led several scientists to examine Mendel's publications and reevaluate his model in terms of chromosome behavior during mitosis and meiosis. In 1902, Theodor Boveri observed that proper sea urchin embryonic development does not occur unless chromosomes are present. That same year, Walter Sutton observed chromosome separation into daughter cells during meiosis (Figure 13.2). Together, these observations led to the **Chromosomal Theory of Inheritance**, which identified chromosomes as the genetic material responsible for Mendelian inheritance.

Figure 13.2 (a) Walter Sutton and (b) Theodor Boveri developed the Chromosomal Theory of Inheritance, which states that chromosomes carry the unit of heredity (genes).

The Chromosomal Theory of Inheritance was consistent with Mendel's laws, which the following observations supported:

- During meiosis, homologous chromosome pairs migrate as discrete structures that are independent of other chromosome pairs.
- Chromosome sorting from each homologous pair into pre-gametes appears to be random.
- Each parent synthesizes gametes that contain only half their chromosomal complement.
- Even though male and female gametes (sperm and egg) differ in size and morphology, they have the same number of chromosomes, suggesting equal genetic contributions from each parent.
- The gametic chromosomes combine during fertilization to produce offspring with the same chromosome number as their parents.

Despite the lack of direct evidence that chromosomes carry traits, the compelling correlation between chromosome behavior during meiosis and Mendel's abstract laws led scientists to propose the Chromosomal Theory of Inheritance. Critics pointed out that individuals had far more independently segregating traits than they had chromosomes. About ten years after the theory was proposed, Eleanor Carothers was the first to discover physical evidence supporting it; she observed independent chromosome assortment in grasshoppers. Then, after several years of carrying out crosses with the fruit fly, *Drosophila melanogaster*, Thomas Hunt Morgan provided additional experimental evidence to support the Chromosomal Theory of Inheritance.

Genetic Linkage and Distances

Mendel's work suggested that traits are inherited independently of each other. Morgan identified a 1:1 correspondence between a segregating trait and the X chromosome, suggesting that random chromosome segregation was the physical basis of Mendel's model. This also demonstrated that linked genes disrupt Mendel's predicted outcomes. That each chromosome can carry many linked genes explains how individuals can have many more traits than they have chromosomes. However, researchers in Morgan's laboratory suggested that alleles positioned on the same chromosome were not always inherited together. During meiosis, linked genes somehow became unlinked.

Homologous Recombination

In 1909, Frans Janssen observed chiasmata—the point at which chromatids are in contact with each other and may exchange segments—prior to the first meiosis division. He suggested that alleles become unlinked and chromosomes physically exchange segments. As chromosomes condensed and paired with their homologs, they appeared to interact at distinct points. Janssen suggested that these points corresponded to regions in which chromosome segments exchanged. We now know that the pairing and interaction between homologous chromosomes, or synapsis, does more than simply organize the homologs for migration to separate daughter cells. When synapsed, homologous chromosomes undergo reciprocal physical exchanges at their arms in **homologous recombination**, or more simply, "crossing over."

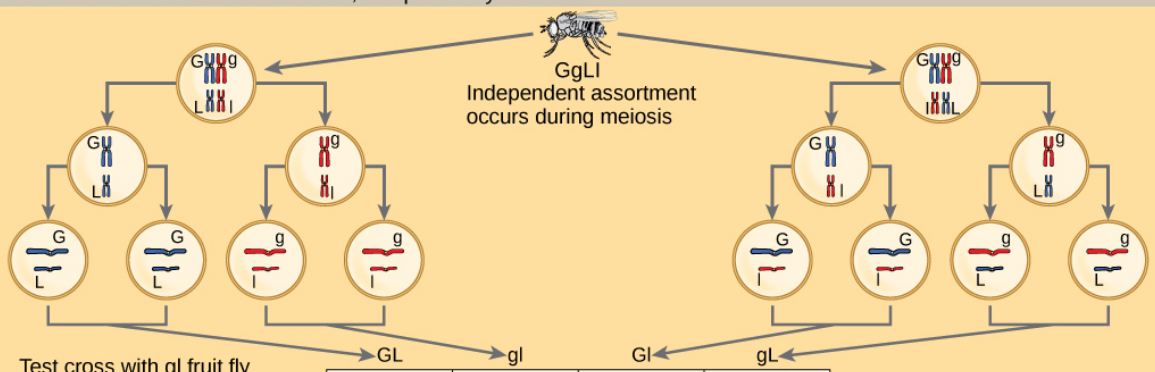
To better understand the type of experimental results that researchers were obtaining at this time, consider a heterozygous individual that inherited dominant maternal alleles for two genes on the same chromosome (such as *AB*) and two recessive paternal alleles for those same genes (such as *ab*). If the genes are linked, one would expect this individual to produce gametes that are either *AB* or *ab* with a 1:1 ratio. If the genes are unlinked, the individual should produce *AB*, *Ab*, *aB*, and *ab* gametes with equal frequencies, according to the Mendelian concept of independent assortment. Because they correspond to new allele combinations, the genotypes Ab and aB are **nonparental types** that result from homologous recombination during meiosis. **Parental types** are progeny that exhibit the same allelic combination as their parents. Morgan and his colleagues, however, found that when they test crossed such heterozygous individuals to a homozygous recessive parent (*AaBb* × *aabb*), both parental and nonparental cases occurred. For example, 950 offspring might be recovered that were either *AaBb* or *aabb*, but 50 offspring would also result that were either *Aabb* or *aaBb*. These results suggested that linkage occurred most often, but a significant minority of offspring were the products of recombination.

Visual Connection

Inheritance Pattern of Linked and Unlinked Genes

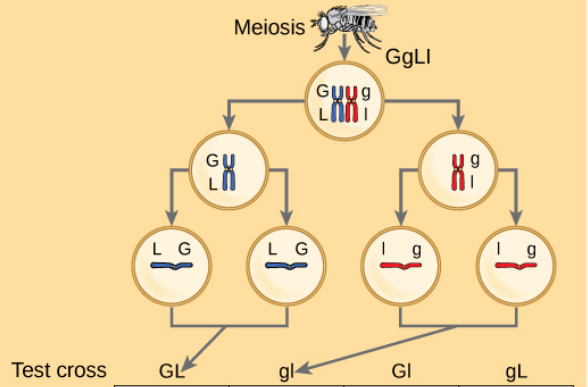
Three hypothetical inheritance patterns for a test cross between a heterozygote and a homozygous recessive individual, based on gene placement, are shown in A through C. The actual experimental results published by Thomas Hunt Morgan in 1912 are shown in D.

A. Genes on different chromosomes, independently assorted



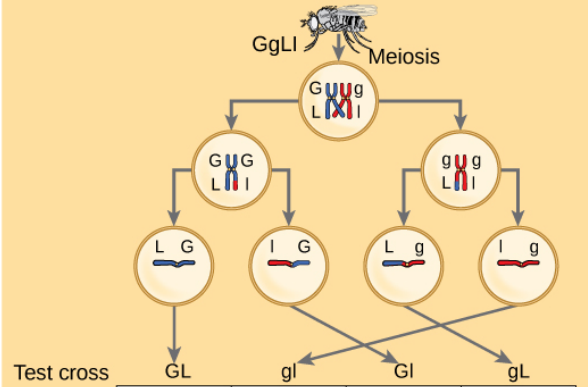
50% of the offspring will be recombinant.

B. Genes on the same chromosome, no crossover occurs



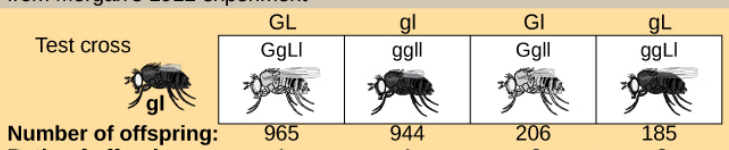
None of the offspring will be recombinant.

C. Genes on the same chromosome, crossover occurs 100% of the time



50% of the offspring will be recombinant.

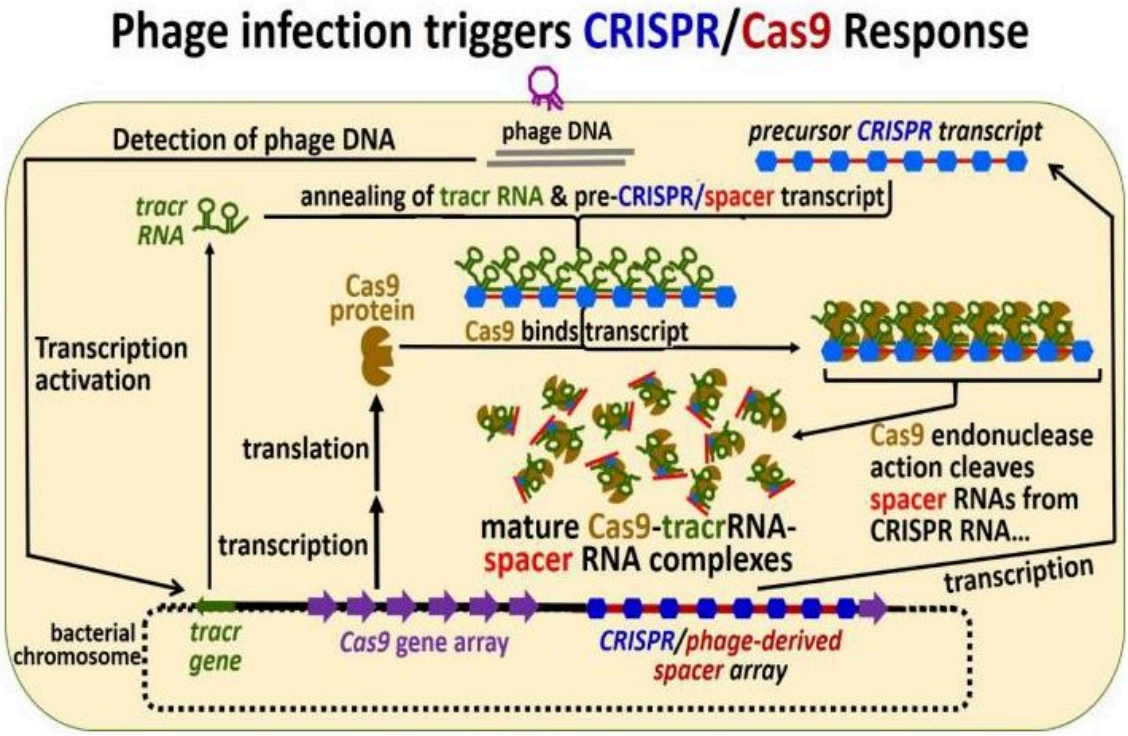
D. Results from Morgan's 1912 experiment



17% of the offspring are recombinant, indicating that the genes are on the same chromosome and crossover occurs some of the time.

Figure 13.3 This figure shows unlinked and linked gene inheritance patterns. In (a), two genes are located on different chromosomes so independent assortment occurs during meiosis. The offspring have an equal chance of being the parental type (inheriting the same combination of traits as the parents) or a nonparental type (inheriting a different combination of traits than the parents). In (b), two genes are very close together on the same chromosome so that no crossing over occurs between them. Therefore, the genes are always inherited together and all the offspring are the parental type. In (c), two genes are far apart on the chromosome such that crossing over occurs during every meiotic event. The recombination frequency will be the same as if the genes were on separate chromosomes. (d) The actual recombination frequency of fruit fly wing length and body color that Thomas Morgan observed in 1912 was 17 percent. A crossover frequency between 0 percent and 50 percent indicates that the genes are on the same chromosome and crossover sometimes occurs.

In a test cross for two characteristics such as the one here, can the recombinant offspring's predicted frequency be 60 percent? Why or why not?



Phage infection triggers formation of CRISPR/Cas9 array

Genetic Maps

Janssen did not have the technology to demonstrate crossing over, so it remained an abstract idea that scientists

did not widely believe. Scientists thought chiasmata were a variation on synapsis and could not understand how chromosomes could break and rejoin. Yet, the data were clear that linkage did not always occur. Ultimately, it took a young undergraduate student and an "all-nighter" to mathematically elucidate the linkage and recombination problem.

In 1913, Alfred Sturtevant, a student in Morgan's laboratory, gathered results from researchers in the laboratory, and took them home one night to mull them over. By the next morning, he had created the first "chromosome map," a linear representation of gene order and relative distance on a chromosome (Figure 13.4).

As Figure 13.4 shows, by using recombination frequency to predict genetic distance, we can infer the relative gene order on chromosome 2. The values represent map distances in centimorgans (cM), which correspond to recombination frequencies (in percent). Therefore, the genes for body color and wing size were 65.5 – 48.5 = 17 cM apart, indicating that the maternal and paternal alleles for these genes recombine in 17 percent of offspring, on average.

To construct a chromosome map, Sturtevant assumed that genes were ordered serially on threadlike chromosomes. He also assumed that the incidence of recombination between two homologous chromosomes could occur with equal likelihood anywhere along the chromosome's length. Operating under these assumptions, Sturtevant postulated that alleles that were far apart on a chromosome were more likely to dissociate during meiosis simply because there was a larger region over which recombination could occur. Conversely, alleles that were close to each other on the chromosome were likely to be inherited together. The average number of crossovers between two alleles—that is, their **recombination frequency**—correlated with their genetic distance from each other, relative to the locations of other genes on that chromosome. Considering the example cross between *AaBb* and *aabb* above, we could calculate the recombination's frequency as 50/1000 = 0.05. That is, the likelihood of a crossover between genes *A/a* and *B/b* was 0.05, or 5 percent. Such a result would indicate that the genes were definitively linked, but that they were far enough apart for crossovers to occasionally occur. Sturtevant divided his genetic map into map units, or **centimorgans (cM)**, in which a 0,01 recombination frequency corresponds to 1 cM.

VISUAL CONNECTION

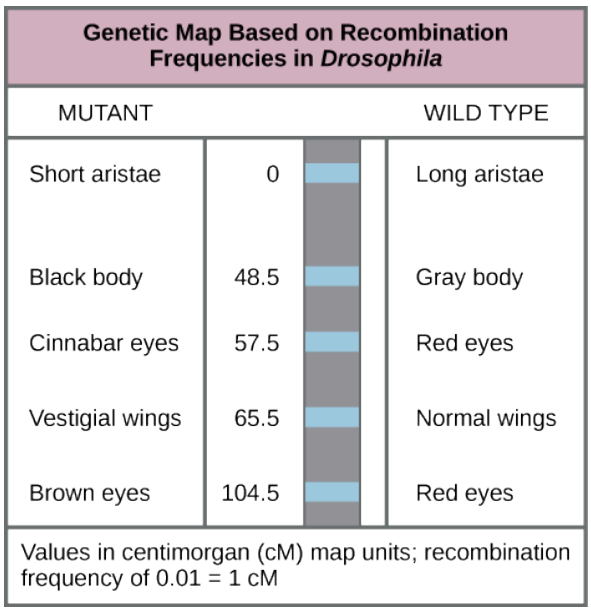


Figure 13.4 This genetic map orders Drosophila genes on the basis of recombination frequency.

Which of the following statements is true?

1. Recombination of the body color and red/cinnabar eye alleles will occur more frequently than recombination of the alleles for wing length and aristae length.
2. Recombination of the body color and aristae length alleles will occur more frequently than recombination of red/brown eye alleles and the aristae length alleles.
3. Recombination of the gray/black body color and long/short aristae alleles will not occur.
4. Recombination of the red/brown eye and long/short aristae alleles will occur more frequently than recombination of the alleles for wing length and body color.

By representing alleles in a linear map, Sturtevant suggested that genes can range from linking perfectly (recombination frequency = 0) to unlinking perfectly (recombination frequency = 0.5) when genes are on different chromosomes or genes separate very far apart on the same chromosome. Perfectly unlinked genes correspond to the frequencies Mendel predicted to assort independently in a dihybrid cross. A 0.5 recombination frequency indicates that 50 percent of offspring are recombinants and the other 50 percent are parental types. That is, every type of allele combination is represented with equal frequency. This representation allowed Sturtevant to additively calculate distances between several genes on the same chromosome. However, as the genetic distances approached 0.50, his predictions became less accurate because it was not clear whether the genes were very far apart on the same or on different chromosomes.

In 1931, Barbara McClintock and Harriet Creighton demonstrated the crossover of homologous chromosomes in corn plants. Weeks later, Curt Stern demonstrated microscopically homologous

recombination in *Drosophila*. Stern observed several X-linked phenotypes that were associated with a structurally unusual and dissimilar X chromosome pair in which one X was missing a small terminal segment, and the other X was fused to a piece of the Y chromosome. By crossing flies, observing their offspring, and then visualizing the offspring's chromosomes, Stern demonstrated that every time the offspring allele combination deviated from either of the parental combinations, there was a corresponding exchange of an X chromosome segment. Using mutant flies with structurally distinct X chromosomes was the key to observing the products of recombination because DNA sequencing and other molecular tools were not yet available. We now know that homologous chromosomes regularly exchange segments in meiosis by reciprocally breaking and rejoining their DNA at precise locations. Aurora Ruiz-Herrera, for example, studies the occurrence of genetic breakpoints at locations in the chromosomes known as fragile sites. By identifying chromosomal fragile sites that are shared between humans and other primates, Ruiz-Herrera has provided a deeper understanding of mammalian and specifically human evolution.

Homologous recombination is a common genetic process, yet Mendel never observed it. Had he investigated both linked and unlinked genes, it would have been much more difficult for him to create a unified model of his data on the basis of probabilistic calculations. Researchers who have since mapped the seven traits that Mendel investigated onto a pea plant genome's seven chromosomes have confirmed that all the genes he examined are either on separate chromosomes or are sufficiently far apart as to be statistically unlinked. Some have suggested that Mendel was enormously lucky to select only unlinked genes, whereas others question whether Mendel discarded any data suggesting linkage. In any case, Mendel consistently observed independent assortment because he examined genes that were effectively unlinked.

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CHROMOSOMAL BASIS OF INHERITED DISORDERS

Learning Objectives

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

- Describe how a karyogram is created
- Explain how nondisjunction leads to disorders in chromosome number
- Compare disorders that aneuploidy causes
- Describe how errors in chromosome structure occur through inversions and translocations

Inherited disorders can arise when chromosomes behave abnormally during meiosis. We can divide chromosome disorders into two categories: abnormalities in chromosome number and chromosomal structural rearrangements. Because even small chromosome segments can span many genes, chromosomal disorders are characteristically dramatic and often fatal.

Chromosome Identification

Chromosome isolation and microscopic observation form the basis of cytogenetics and are the primary methods by which clinicians detect chromosomal abnormalities in humans. A karyotype is the number and appearance of chromosomes, and includes their length, banding pattern, and centromere position. To obtain a view of an individual's **karyotype**, cytologists photograph the chromosomes and then cut and paste each chromosome into a chart, or **karyogram**. Another name is an ideogram (Figure 13.5).

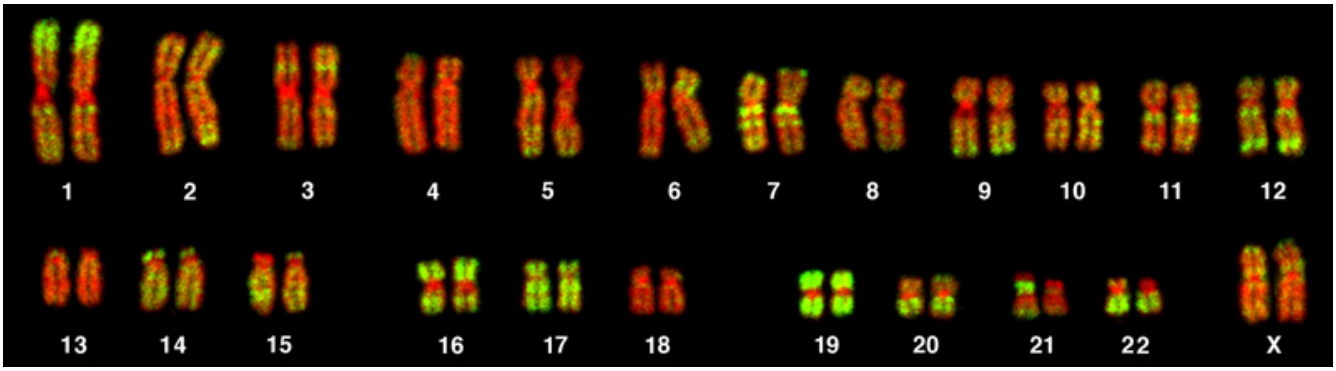

Figure 13.5 This karyotype is of a female human. Notice that homologous chromosomes are the same size, and have the same centromere positions and banding patterns. A human male would have an XY chromosome pair instead of the XX pair. (credit: Andreas Blozer et al.)

In a given species, we can identify chromosomes by their number, size, centromere position, and banding pattern. In a human karyotype, **autosomes** or "body chromosomes" (all of the non–sex chromosomes) are generally organized in approximate order of size from largest (chromosome 1) to smallest (chromosome 22). The X and Y chromosomes are not autosomes. However, chromosome 21 is actually shorter than chromosome 22. Researchers discovered this after naming Down syndrome as trisomy 21, reflecting how this disorder results from possessing one extra chromosome 21 (three total). Not wanting to change the name of this important disorder, scientists retained the numbering of chromosome 21 despite describing it having the shortest set of chromosomes. We may designate the chromosome "arms" projecting from either end of the centromere as short or long, depending on their relative lengths. We abbreviate the short arm *p* (for "petite"), whereas we abbreviate the long arm *q* (because it follows "p" alphabetically). Numbers further subdivide and denote each arm. Using this naming system, we can describe chromosome locations consistently in the scientific literature.

Visual Connection

Geneticists Use Karyotypes to Identify Chromosomal Aberrations

Although we refer to Mendel as the "father of modern genetics," he performed his experiments with none of the tools that the geneticists of today routinely employ. One such powerful cytological technique is karyotyping, a method in which geneticists can identify traits characterized by chromosomal abnormalities from a single cell. To observe an individual's karyotype, a geneticist first collects a person's cells (like white blood cells) from a blood sample or other tissue. In the laboratory, the geneticist stimulates the isolated cells to begin actively dividing. The geneticist then applies the chemical colchicine to cells to arrest condensed

chromosomes in metaphase. The geneticist then induces swelling in the cells using a hypotonic solution so the chromosomes spread apart. Finally, the geneticist preserves the sample in a fixative and applies it to a slide.

The geneticist then stains chromosomes with one of several dyes to better visualize each chromosome pair's distinct and reproducible banding patterns. Following staining, the geneticist views the chromosomes using bright-field microscopy. A common stain choice is the Giemsa stain. Giemsa staining results in approximately 400–800 bands (of tightly coiled DNA and condensed proteins) arranged along all 23 chromosome pairs. An experienced geneticist can identify each band. In addition to the banding patterns, geneticists further identify chromosomes on the basis of size and centromere location. To obtain the classic depiction of the karyotype in which homologous chromosome pairs align in numerical order from longest to shortest, the geneticist obtains a digital image, identifies each chromosome, and manually arranges the chromosomes into this pattern (Figure 13.5).

At its most basic, the karyogram may reveal genetic abnormalities in which an individual has too many or too few chromosomes per cell. Examples of this are Down Syndrome, which one identifies by a third copy of chromosome 21, and Turner Syndrome, which is characterized by the presence of only one X chromosome in females instead of the normal two. Geneticists can also identify large DNA deletions or insertions. For instance, geneticists can identify Jacobsen Syndrome—which involves distinctive facial features as well as heart and bleeding defects—by a deletion on chromosome 11. Finally, the karyotype can pinpoint **translocations**, which occur when a segment of genetic material breaks from one chromosome and reattaches to another chromosome or to a different part of the same chromosome. Translocations are implicated in certain cancers, including chronic myelogenous leukemia.

During Mendel's lifetime, inheritance was an abstract concept that one could only infer by performing crosses and observing the traits that offspring expressed. By observing a karyotype, today's geneticists can actually visualize an individual's chromosomal composition to confirm or predict genetic abnormalities in offspring, even before birth.

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https://louis.pressbooks.pub/generalbiology1leclab/?p=479#h5p-42

Chromosome Number Disorders

Of all of the chromosomal disorders, chromosome number abnormalities are the most obviously identifiable from a karyotype. Chromosome number disorders include duplicating or losing entire chromosomes, as well as changes in the number of complete sets of chromosomes. They are caused by **nondisjunction**, which occurs when homologous chromosome pairs or sister chromatids fail to separate during meiosis. Misaligned or incomplete synapsis, or a spindle apparatus dysfunction that facilitates chromosome migration, can cause nondisjunction. The risk of nondisjunction occurring increases with the parents' age.

Nondisjunction can occur during either meiosis I or II, with differing results (Figure 13.6). If homologous chromosomes fail to separate during meiosis I, the result is two gametes that lack that particular chromosome and two gametes with two chromosome copies. If sister chromatids fail to separate during meiosis II, the result is one gamete that lacks that chromosome, two normal gametes with one chromosome copy, and one gamete with two chromosome copies.

Evolution Connection

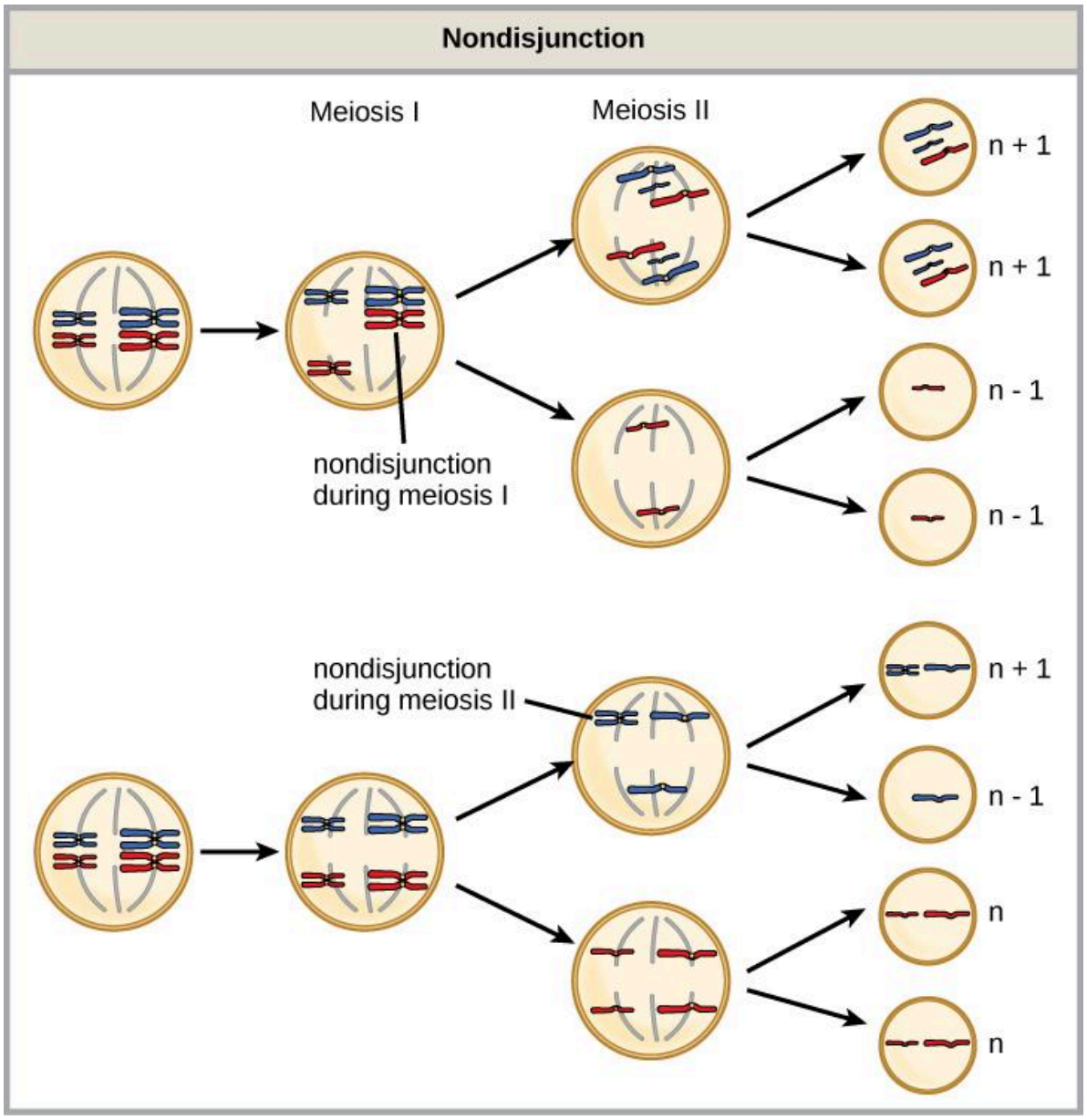

Figure 13.6 Nondisjunction occurs when homologous chromosomes or sister chromatids fail to separate during meiosis, resulting in an abnormal chromosome number. Nondisjunction may occur during meiosis I or meiosis II. Credit: Rao, A. and Tag, A. Department of Biology, Texas A&M University.

Which of the following statements about nondisjunction is true?

a. Nondisjunction only results in gametes with n+1 or n–1 chromosomes.
b. Nondisjunction occurring during meiosis II results in 50 percent normal gametes.
c. Nondisjunction during meiosis I results in 50 percent normal gametes.

d. Nondisjunction always results in four different kinds of gametes.

Aneuploidy

Scientists call an individual with the appropriate number of chromosomes for their species **euploid**. In humans, euploidy corresponds to 22 pairs of autosomes and one pair of sex chromosomes. An individual with an error in chromosome number is described as **aneuploid**, a term that includes **monosomy** (losing one chromosome) or **trisomy** (gaining an extraneous chromosome). Monosomic human zygotes missing any one copy of an autosome invariably fail to develop to birth because they lack essential genes. This underscores the importance of "gene dosage" in humans. Most autosomal trisomies also fail to develop to birth; however, duplications of some smaller chromosomes (13, 15, 18, 21, or 22) can result in offspring that survive for several weeks to many years. Trisomic individuals suffer from a different type of genetic imbalance: an excess in gene dose. Individuals with an extra chromosome may synthesize an abundance of the gene products, which that chromosome encodes. This extra dose (150 percent) of specific genes can lead to a number of functional challenges and often precludes development. The most common trisomy among viable births is that of chromosome 21, which corresponds to Down Syndrome. Short stature and stunted digits, facial distinctions that include a broad skull and large tongue, and significant developmental delays characterize individuals with this inherited disorder. We can correlate the incidence of Down syndrome with maternal age. Older people are more likely to become pregnant with fetuses carrying the trisomy 21 genotype (Figure 13.7).

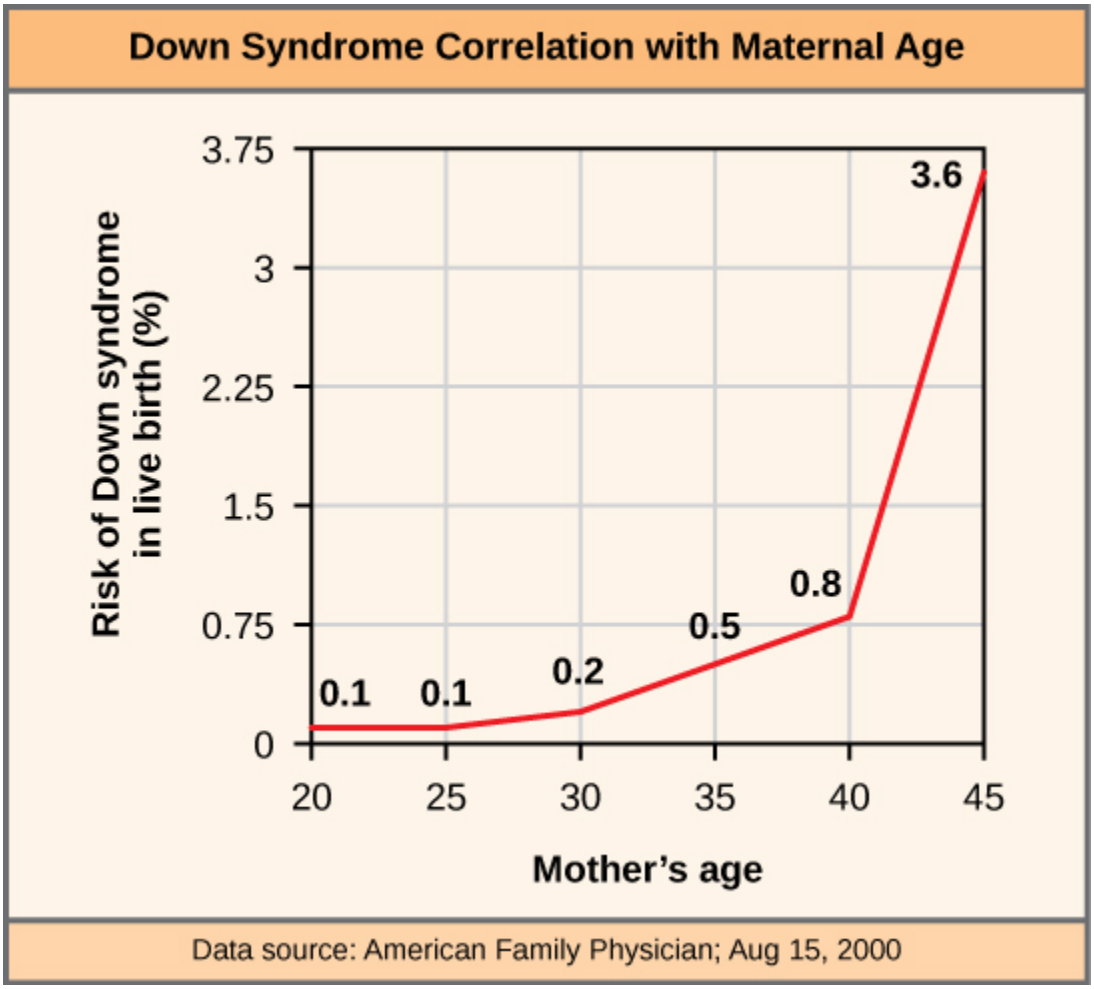

Figure 13.7 The incidence of having a fetus with trisomy 21 increases dramatically with maternal age.

Link to Learning

Visualize adding a chromosome that leads to Down syndrome in this video simulation (http://openstax.org/l/down_syndrome)

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online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=479#h5p-43

Polyploidy

We call an individual with more than the correct number of chromosome sets (two for diploid species) **polyploid**. For instance, fertilizing an abnormal diploid egg with a normal haploid sperm would yield a triploid zygote. Polyploid animals are extremely rare, with only a few examples among the flatworms, crustaceans, amphibians, fish, and lizards. Polyploid animals are sterile because meiosis cannot proceed normally and instead produces mostly aneuploid daughter cells that cannot yield viable zygotes. Rarely, polyploid animals can reproduce asexually by haplodiploidy, in which an unfertilized egg divides mitotically to produce offspring. In contrast, polyploidy is very common in the plant kingdom, and polyploid plants tend to be larger and more robust than euploids of their species (Figure 13.8).

Figure 13.8 As with many polyploid plants, this triploid orange daylily (Hemerocallis fulva) is particularly large and robust, and grows flowers with triple the number of petals of its diploid counterparts. (credit: Steve Karg)

X-Chromosome Inactivation

Humans display dramatic deleterious effects with autosomal trisomies and monosomies. Therefore, it may seem counterintuitive that human females and males can function normally, despite carrying different numbers of the X chromosome. Rather than a gain or loss of autosomes, variations in the number of sex

chromosomes occur with relatively mild effects. In part, this happens because of the molecular process **X inactivation**. Early in development, when female mammalian embryos consist of just a few thousand cells (relative to trillions in the newborn), one X chromosome in each cell inactivates by tightly condensing into a quiescent (dormant) structure, or a Barr body. The chance that an X chromosome (maternally or paternally derived) inactivates in each cell is random, but once this occurs, all cells derived from that one will have the same inactive X chromosome or Barr body. By this process, females compensate for their double genetic dose of X chromosome. In so-called tortoiseshell cats, we observe embryonic X inactivation as color variegation (Figure 13.9). Females that are heterozygous for an X-linked coat color gene will express one of two different coat colors over different regions of their body, corresponding to whichever X chromosome inactivates in that region's embryonic cell progenitor.

Figure 13.9 In cats, the gene for coat color is located on the X chromosome. In female cats' embryonic development, one of the two X chromosomes randomly inactivates in each cell, resulting in a tortoiseshell pattern if the cat has two different alleles for coat color. Male cats, having only one X chromosome, never exhibit a tortoiseshell coat color. (credit: Michael Bodega)

An individual carrying an abnormal number of X chromosomes will inactivate all but one X chromosome in each of her cells. However, even inactivated X chromosomes continue to express a few genes, and X chromosomes must reactivate for the proper maturation of female ovaries. As a result, X-chromosomal abnormalities typically occur with mild intellectual and physical disorders or disabilities, as well as sterility. If the X chromosome is absent altogether, the individual will not develop in utero.

Sex Chromosome Nondisjunction in Humans

Scientists have identified and characterized several errors in sex chromosome number. Individuals with three X chromosomes, triplo-X, are phenotypically female but express developmental delays and reduced fertility. The XXY genotype, corresponding to one type of Klinefelter syndrome, corresponds to phenotypically male individuals with small testes, enlarged breasts, and reduced body hair. More complex types of Klinefelter syndrome exist in which the individual has as many as five X chromosomes. In all types, every X chromosome except one undergoes inactivation to compensate for the excess genetic dosage. We see this as several Barr bodies in each cell nucleus. Turner syndrome, characterized as an X0 genotype (i.e., only a single sex chromosome), corresponds to a phenotypically female individual with short stature, webbed skin in the neck region, hearing and cardiac impairments, and sterility.

Duplications and Deletions

In addition to losing or gaining an entire chromosome, a chromosomal segment may duplicate or lose itself. Duplications and deletions often produce offspring that survive but exhibit abnormalities. Duplicated chromosomal segments may fuse to existing chromosomes or may be free in the nucleus. Cri-du-chat (from the French for “cry of the cat”) is a syndrome that occurs with nervous system abnormalities and identifiable physical features that result from a deletion of most 5p (the small arm of chromosome 5) (Figure 13.10). Infants with this genotype emit a characteristic high-pitched cry on which the disorder’s name is based.

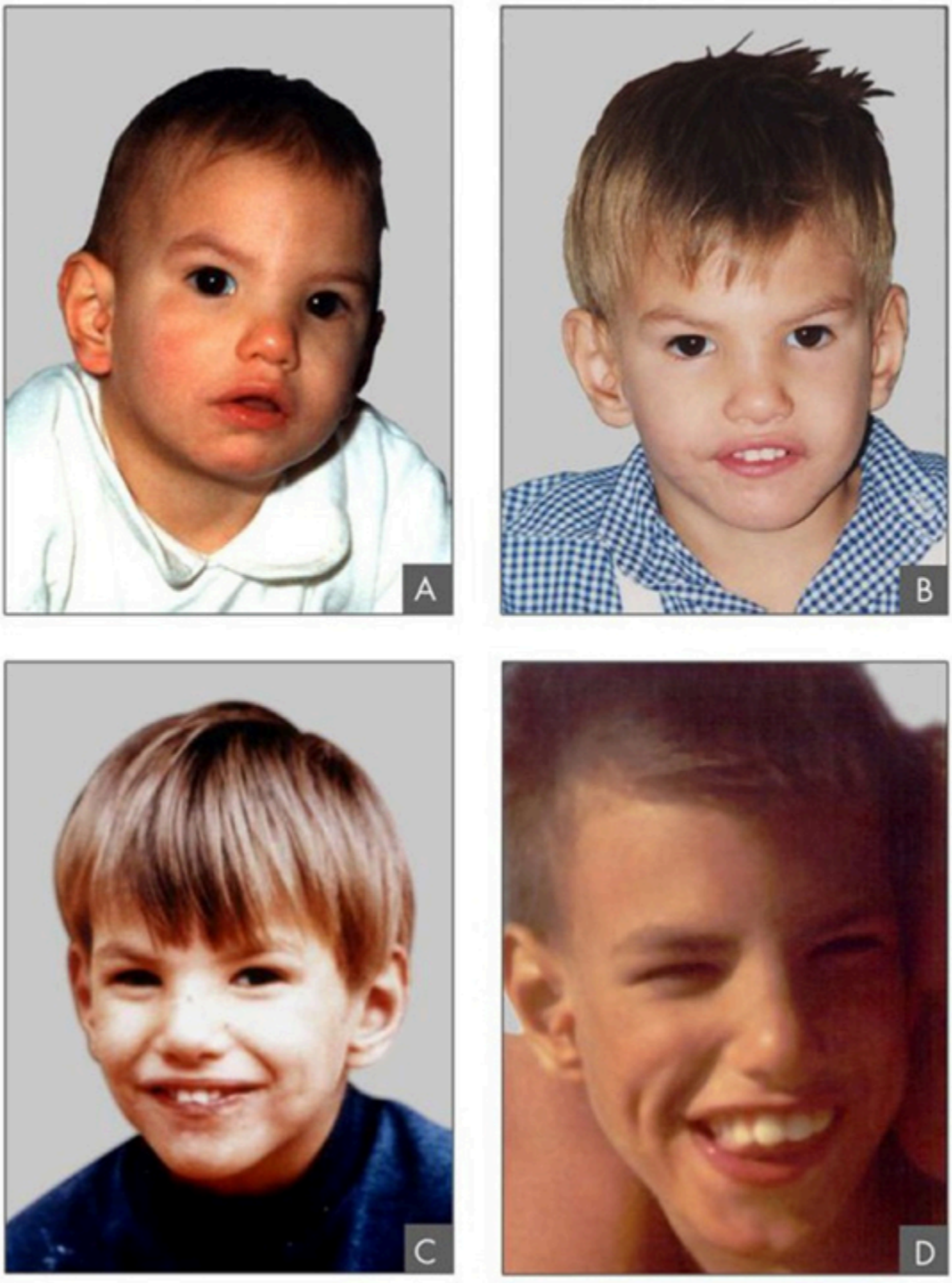


Figure 13.10 This figure shows an individual with cri-du-chat syndrome at two, four, nine, and 12 years of age. (credit: Paola Cerruti Mainardi)

Chromosomal Structural Rearrangements

Cytologists have characterized numerous structural rearrangements in chromosomes, but chromosome

inversions and translocations are the most common. We can identify both during meiosis by the adaptive pairing of rearranged chromosomes with their former homologs to maintain appropriate gene alignment. If the genes on two homologs are not oriented correctly, a recombination event could result in losing genes from one chromosome and gaining genes on the other. This would produce aneuploid gametes.

Chromosome Inversions

A **chromosome inversion** is the detachment, 180° rotation, and reinsertion of part of a chromosome. Inversions may occur in nature as a result of mechanical shear, or from transposable elements' action (special DNA sequences capable of facilitating rearranging chromosome segments with the help of enzymes that cut and paste DNA sequences). Unless they disrupt a gene sequence, inversions only change gene orientation and are likely to have more mild effects than aneuploid errors. However, altered gene orientation can result in functional changes because regulators of gene expression could move out of position with respect to their targets, causing aberrant levels of gene products.

An inversion can be **pericentric** and include the centromere, or **paracentric** and occur outside the centromere (Figure 13.11). A pericentric inversion that is asymmetric about the centromere can change the chromosome arms' relative lengths, making these inversions easily identifiable.

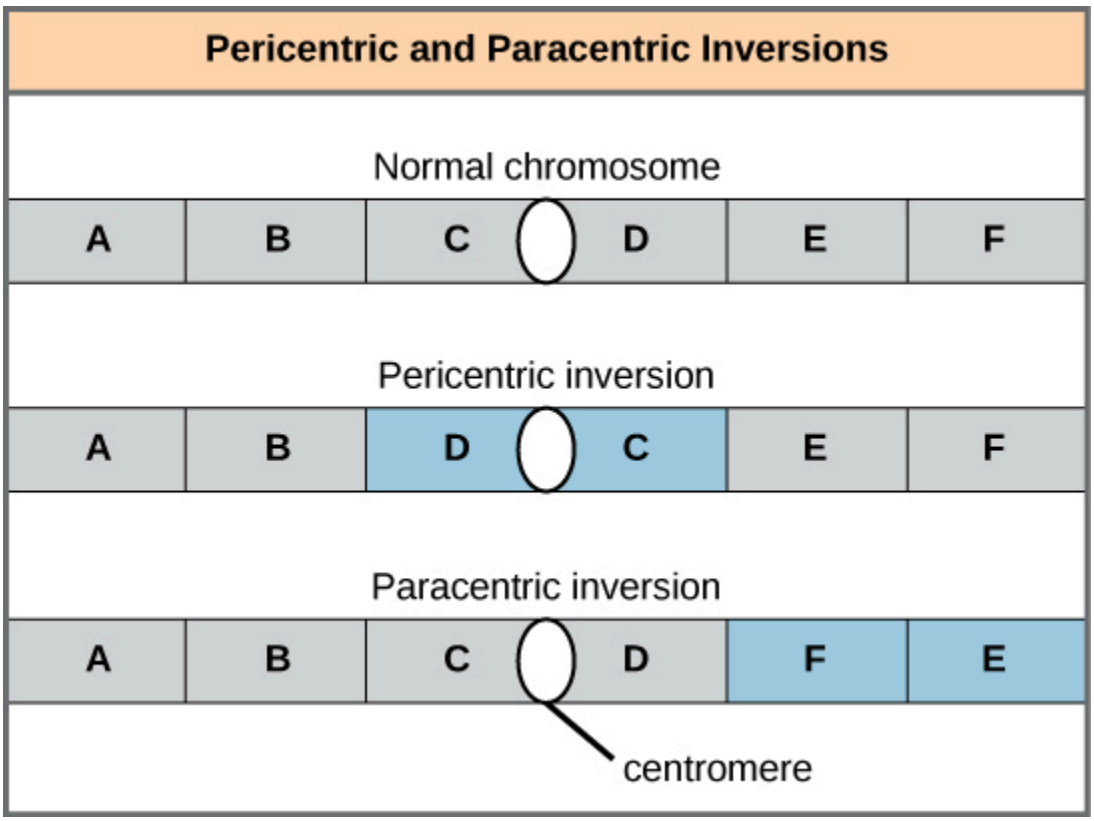


Figure 13.11 Pericentric inversions include the centromere, and paracentric inversions do not. A pericentric inversion can change the chromosome arms' relative lengths. A paracentric inversion cannot.

When one homologous chromosome undergoes an inversion but the other does not, the individual is an inversion heterozygote. To maintain point-for-point synapsis during meiosis, one homolog must form a loop, and the other homolog must mold around it. Although this topology can ensure that the genes correctly align, it also forces the homologs to stretch and can occur with imprecise synapsis regions (Figure 13.12).

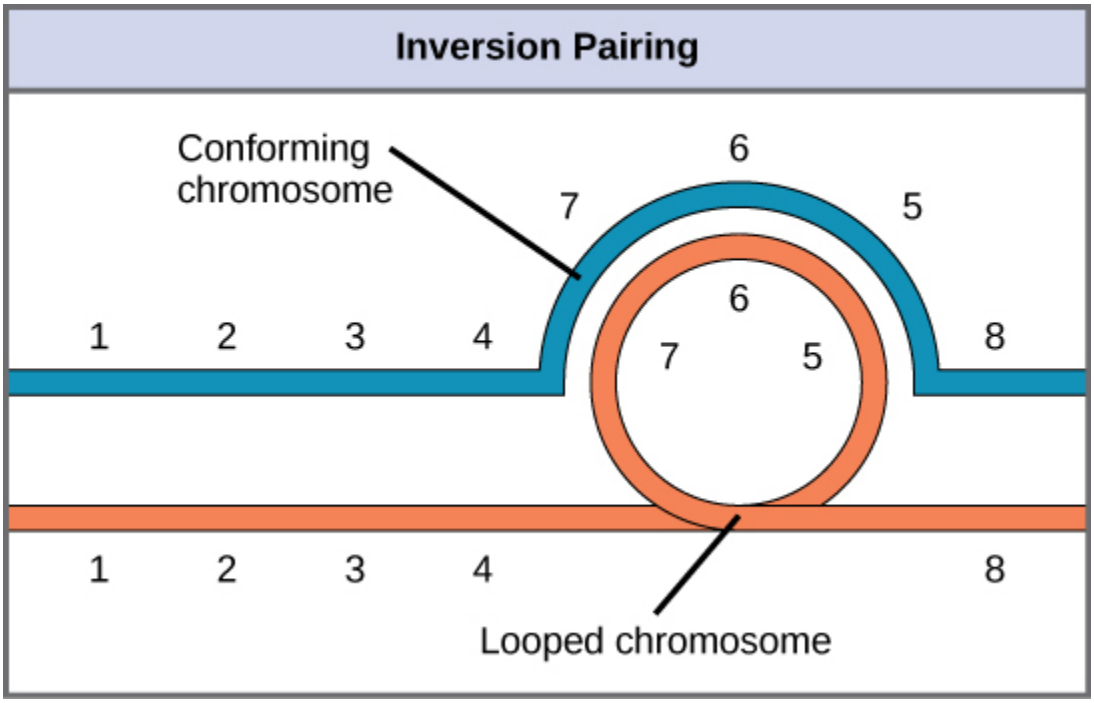


Figure 13.12 When one chromosome undergoes an inversion but the other does not, one chromosome must form an inverted loop to retain point-for-point interaction during synapsis. This inversion pairing is essential to maintaining gene alignment during meiosis and to allow for recombination.

Visual Connection

The Chromosome 18 Inversion

Not all chromosomes' structural rearrangements produce nonviable, impaired, or infertile individuals. In rare instances, such a change can result in new species evolving. In fact, a pericentric inversion in chromosome 18 appears to have contributed to human evolution. This inversion is not present in our closest genetic relatives, the chimpanzees. Humans and chimpanzees differ cytogenetically by pericentric inversions on several chromosomes and by the fusion of two separate chromosomes in chimpanzees that correspond to chromosome two in humans.

Scientists believe the pericentric chromosome 18 inversion occurred in early humans following their divergence from a common ancestor with chimpanzees approximately five million years ago. Researchers characterizing this inversion have suggested that approximately 19,000

nucleotide bases were duplicated on 18p, and the duplicated region inverted and reinserted on chromosome 18 of an ancestral human.

A comparison of human and chimpanzee genes in the region of this inversion indicates that two genes—*ROCK1* and *USP14*—that are adjacent on chimpanzee chromosome 17 (which corresponds to human chromosome 18) are more distantly positioned on human chromosome 18. This suggests that one of the inversion breakpoints occurred between these two genes. Interestingly, humans and chimpanzees express *USP14* at distinct levels in specific cell types, including cortical cells and fibroblasts. Perhaps the chromosome 18 inversion in an ancestral human repositioned specific genes and reset their expression levels in a useful way. Because both *ROCK1* and *USP14* encode cellular enzymes, a change in their expression could alter cellular function. We do not know how this inversion contributed to hominid evolution, but it appears to be a significant factor in the divergence of humans from other primates.[1]

Translocations

A **translocation** occurs when a chromosome segment dissociates and reattaches to a different, nonhomologous chromosome. Translocations can be benign or have devastating effects depending on how the positions of genes are altered with respect to regulatory sequences. Notably, specific translocations have occurred with several cancers and with schizophrenia. Reciprocal translocations result from exchanging chromosome segments between two nonhomologous chromosomes such that there is no genetic information gain or loss (Figure 13.13).

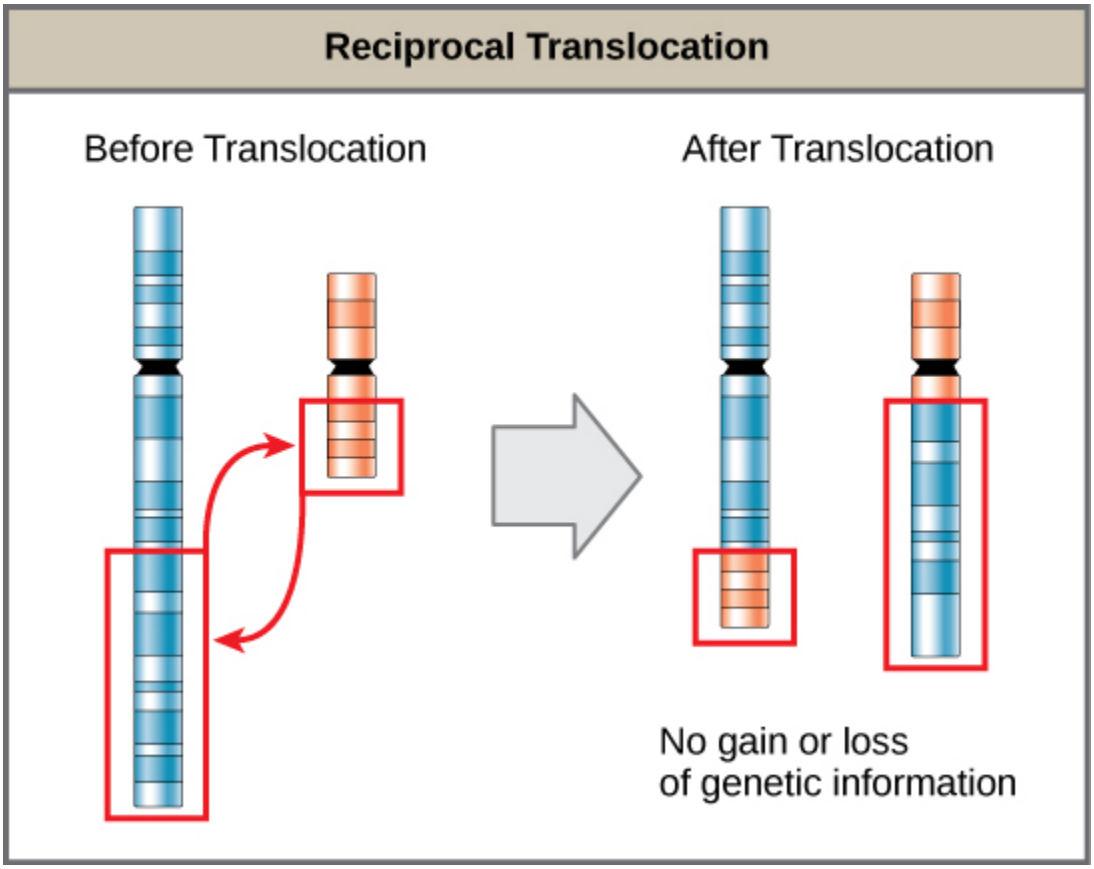


Figure 13.13 A reciprocal translocation occurs when a DNA segment transfers from one chromosome to another, nonhomologous chromosome. (credit: modification of work by National Human Genome Research/USA)

Footnotes

- 1 Violaine Goidts et al., "Segmental duplication associated with the human-specific inversion of chromosome 18: a further example of the impact of segmental duplications on karyotype and genome evolution in primates," *Human Genetics*. 115 (2004):116-122

125.

KEY TERMS

aneuploid
: individual with an error in chromosome number; includes chromosome segment deletions and duplications

autosome
: any of the non-sex chromosomes

centimorgan (cM)
: (also, map unit) relative distance that corresponds to a 0,01 recombination frequency

Chromosomal Theory of Inheritance
: theory proposing that chromosomes are the genes' vehicles and that their behavior during meiosis is the physical basis of the inheritance patterns that Mendel observed

chromosome inversion
: detachment, 180° rotation, and chromosome arm reinsertion

euploid
: individual with the appropriate number of chromosomes for their species

homologous recombination
: process by which homologous chromosomes undergo reciprocal physical exchanges at their arms, also crossing over

karyogram
: a karyotype's photographic image

karyotype
: an individual's chromosome number and appearance; includes the size, banding patterns, and centromere position

monosomy
: otherwise diploid genotype in which one chromosome is missing

nondisjunction
: failure of synapsed homologs to completely separate and migrate to separate poles during the meiosis' first cell division

nonparental (recombinant) type
: progeny resulting from homologous recombination that exhibits a different allele combination compared with its parents

paracentric
: inversion that occurs outside the centromere

parental types
: progeny that exhibits the same allelic combination as its parents

pericentric
: inversion that involves the centromere

polyploid
: individual with an incorrect number of chromosome sets

recombination frequency
: average number of crossovers between two alleles; observed as the number of nonparental types in a progeny's population

translocation
: process by which one chromosome segment dissociates and reattaches to a different, nonhomologous chromosome

trisomy
: otherwise diploid genotype in which one entire chromosome duplicates

X inactivation
: condensing X chromosomes into Barr bodies during embryonic development in females to compensate for the double genetic dose

126.

CHAPTER SUMMARY

13.1 Chromosomal Theory and Genetic Linkage

Sutton and Boveri's Chromosomal Theory of Inheritance states that chromosomes are the vehicles of genetic heredity. Neither Mendelian genetics nor gene linkage is perfectly accurate. Instead, chromosome behavior involves segregation, independent assortment, and occasionally, linkage. Sturtevant devised a method to assess recombination frequency and infer linked genes' relative positions and distances on a chromosome on the basis of the average number of crossovers in the intervening region between the genes. Sturtevant correctly presumed that genes are arranged in serial order on chromosomes and that recombination between homologs can occur anywhere on a chromosome with equal likelihood. Whereas linkage causes alleles on the same chromosome to be inherited together, homologous recombination biases alleles toward an independent inheritance pattern.

13.2 Chromosomal Basis of Inherited Disorders

The number, size, shape, and banding pattern of chromosomes make them easily identifiable in a karyotype and allow for the assessment of many chromosomal abnormalities. Disorders in chromosome number, or aneuploidies, are typically lethal to the embryo, although a few trisomic genotypes are viable. Because of X inactivation, aberrations in sex chromosomes typically have milder phenotypic effects. Aneuploidies also include instances in which a chromosome's segments duplicate or delete themselves. Inversion or translocation also may rearrange chromosome structures. Both of these aberrations can result in problematic phenotypic effects. Because they force chromosomes to assume unnatural topologies during meiosis, inversions and translocations often occur with reduced fertility because of the likelihood of nondisjunction.

127.

VISUAL CONNECTION QUESTIONS

1. Figure 13.3 In a test cross for two characteristics such as the one shown here, can the predicted frequency of recombinant offspring be 60 percent? Why or why not?

2. Figure 13.4 Which of the following statements is true?

a. Recombination of the body color and red/cinnabar eye alleles will occur more frequently than recombination of the alleles for wing length and aristae length.
b. Recombination of the body color and aristae length alleles will occur more frequently than recombination of red/brown eye alleles and the aristae length alleles.
c. Recombination of the gray/black body color and long/short aristae alleles will not occur.
d. Recombination of the red/brown eye and long/short aristae alleles will occur more frequently than recombination of the alleles for wing length and body color.

3. Figure 13.6 Which of the following statements about nondisjunction is true?

a. Nondisjunction only results in gametes with n+1 or n−1 chromosomes.
b. Nondisjunction occurring during meiosis II results in 50 percent normal gametes.
c. Nondisjunction during meiosis I results in 50 percent normal gametes.
d. Nondisjunction always results in four different kinds of gametes.

128.

REVIEW QUESTIONS

4. X-linked recessive traits in humans (or in Drosophila) are observed _____.

a. in more males than females
b. in more females than males
c. in males and females equally
d. in different distributions depending on the trait

5. The first suggestion that chromosomes may physically exchange segments came from the microscopic identification of _____.

a. synapsis
b. sister chromatids
c. chiasmata
d. alleles

6. Which recombination frequency corresponds to independent assortment and the absence of linkage?

a. 0
b. 0.25
c. 0.50
d. 0.75

7. Which recombination frequency corresponds to perfect linkage and violates the law of independent assortment?

a. 0
b. 0.25
c. 0.50
d. 0.75

8. Which of the following codes describes position 12 on the long arm of chromosome 13?

a. 13p12
b. 13q12
c. 12p13
d. 12q13

9. In agriculture, polyploid crops (like coffee, strawberries, or bananas) tend to produce _____.

a. more uniformity
b. more variety
c. larger yields
d. smaller yields

10. The genotype XXY corresponds to

a. Klinefelter syndrome
b. Turner syndrome
c. Triplo-X
d. Jacob syndrome

11. Abnormalities in the number of X chromosomes tend to have milder phenotypic effects than the same abnormalities in autosomes because of _____.

a. deletions
b. nonhomologous recombination
c. synapsis
d. X inactivation

12. By definition, a pericentric inversion includes the _____.

a. centromere
b. chiasma
c. telomere
d. synapse

129.

CRITICAL THINKING QUESTIONS

13. Explain how the Chromosomal Theory of Inheritance helped to advance our understanding of genetics.
14. Using diagrams, illustrate how nondisjunction can result in an aneuploid zygote.

PART XIV

DNA STRUCTURE AND FUNCTION

130.

INTRODUCTION

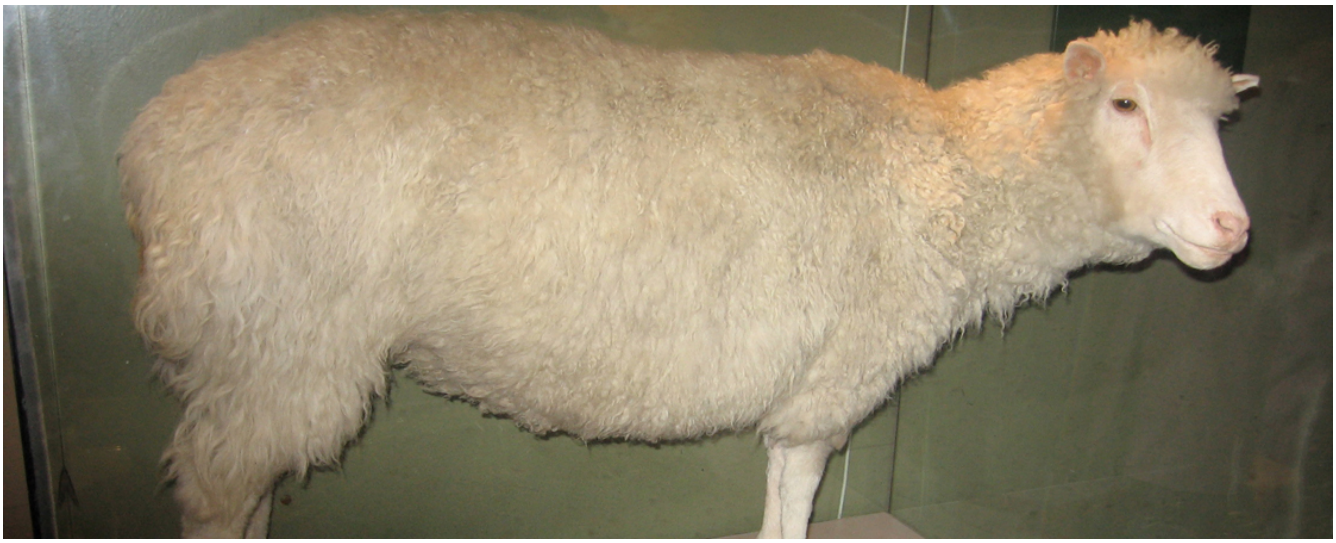


Figure 14.1 Dolly the sheep was the first large mammal to be cloned.

The three letters "DNA" have now become synonymous with crime solving and genetic testing. DNA can be retrieved from hair, blood, or saliva. Each person's DNA is unique, and it is possible to detect differences between individuals within a species on the basis of these unique features.

DNA analysis has many practical applications beyond forensics. In humans, DNA testing is applied to numerous uses: determining paternity, tracing genealogy, identifying pathogens, archeological research, tracing disease outbreaks, and studying human migration patterns. In the medical field, DNA is used in diagnostics, new vaccine development, and cancer therapy. It is now possible to determine predisposition to diseases by looking at genes.

Each human cell has 23 pairs of chromosomes: one set of chromosomes is inherited from the female parent and the other set is inherited from the male parent. There is also a mitochondrial genome, inherited exclusively from the female parent, which can be involved in inherited genetic disorders. On each chromosome, there are thousands of genes that are responsible for determining the genotype and phenotype of the individual. A gene is defined as a sequence of DNA that codes for a functional product. The human haploid genome contains 3 billion base pairs and has between 20,000 and 25,000 functional genes.

131.

HISTORICAL BASIS OF MODERN UNDERSTANDING

Learning Objectives

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

- Explain transformation of DNA
- Describe the key experiments that helped identify that DNA is the genetic material
- State and explain Chargaff's rules

Our current understanding of DNA began with the discovery of nucleic acids followed by the development of the double-helix model. In the 1860s, Friedrich Miescher (Figure 14.2), a physician by profession, isolated phosphate-rich chemicals from white blood cells (leukocytes). He named these chemicals (which would eventually be known as DNA) *nuclein* because they were isolated from the nuclei of the cells.

Figure 14.2 Friedrich Miescher (1844–1895) discovered nucleic acids.

Link to Learning

To see Miescher conduct his experiment that led to his discovery of DNA and associated proteins in the nucleus, click through this review.

A half century later, in 1928, British bacteriologist Frederick Griffith reported the first demonstration of bacterial **transformation**—a process in which external DNA is taken up by a cell, thereby changing its morphology and physiology. Griffith conducted his experiments with *Streptococcus pneumoniae,* a bacterium that causes pneumonia. Griffith worked with two strains of this bacterium called rough (R) and smooth (S). (The two cell types were called "rough" and "smooth" after the appearance of their colonies grown on a nutrient agar plate.)

The R strain is non-pathogenic (does not cause disease). The S strain is pathogenic (disease-causing), and has a capsule outside its cell wall. The capsule allows the cell to escape the immune responses of the host mouse.

When Griffith injected the living S strain into mice, they died from pneumonia. In contrast, when Griffith injected the live R strain into mice, they survived. In another experiment, when he injected mice with the heat-killed S strain, they also survived. This experiment showed that the capsule alone was not the cause of death. In a third set of experiments, a mixture of live R strain and heat-killed S strain were injected into mice, and—to his surprise—the mice died. Upon isolating the live bacteria from the dead mouse, only the S strain of bacteria was recovered. When this isolated S strain was injected into fresh mice, the mice died. Griffith concluded that something had passed from the heat-killed S strain into the live R strain and transformed it into the pathogenic S strain. He called this the *transforming principle* (Figure 14.3). These experiments are now known as Griffith's transformation experiments.

Mouse injected with heat-killed virulant S strain lives.

Mouse injected with both heat-killed S strain and live non-virulant R strain dies.

Figure 14.3 Two strains of S. pneumoniae were used in Griffith's transformation experiments. The R strain is non-pathogenic, whereas the S strain is pathogenic and causes death. When Griffith injected a mouse with the heat-killed S strain and a live R strain, the mouse died. The S strain was recovered from the dead mouse. Griffith concluded that something had passed from the heat-killed S strain to the R strain, transforming the R strain into the S strain in the process. (credit "living mouse": modification of work by NIH; credit "dead mouse": modification of work by Sarah Marriage)

Scientists Oswald Avery, Colin MacLeod, and Maclyn McCarty (1944) were interested in exploring this transforming principle further. They isolated the S strain from the dead mice and isolated the proteins and nucleic acids (RNA and DNA) as these were possible candidates for the molecule of heredity. They used enzymes that specifically degraded each component and then used each mixture separately to transform the R strain. They found that when DNA was degraded, the resulting mixture was no longer able to transform the bacteria, whereas all of the other combinations were able to transform the bacteria. This led them to conclude that DNA was the transforming principle.

Career Connection

Forensic Scientist

Forensic Scientists used DNA analysis evidence for the first time to solve an immigration case. The story started with a teenage boy returning to London from Ghana to be with his mother. Immigration authorities at the airport were suspicious of him, thinking that he was traveling on a forged passport. After much persuasion, he was allowed to go live with his mother, but the immigration authorities did not drop the case against him. All types of evidence, including

photographs, were provided to the authorities, but deportation proceedings were started nevertheless. Around the same time, Dr. Alec Jeffreys of Leicester University in the United Kingdom had invented a technique known as **DNA fingerprinting**. The immigration authorities approached Dr. Jeffreys for help. He took DNA samples from the mother and three of her children, as well as an unrelated mother, and compared the samples with the boy's DNA. Because the biological father was not in the picture, DNA from the three children was compared with the boy's DNA. He found a match in the boy's DNA for both the mother and his three siblings. He concluded that the boy was indeed the mother's son.

Forensic scientists analyze many items, including documents, handwriting, firearms, and biological samples. They analyze the DNA content of hair, semen, saliva, and blood and compare it with a database of DNA profiles of known criminals. Analysis includes DNA isolation, sequencing, and sequence analysis. Forensic scientists are expected to appear at court hearings to present their findings. They are usually employed in crime labs of city and state government agencies. Geneticists experimenting with DNA techniques also work for scientific and research organizations, pharmaceutical industries, and college and university labs. Students wishing to pursue a career as a forensic scientist should have at least a bachelor's degree in chemistry, biology, or physics, and preferably some experience working in a laboratory.

Although the experiments of Avery, McCarty and McLeod had demonstrated that DNA was the informational component transferred during transformation, DNA was still considered to be too simple a molecule to carry biological information. Proteins, with their 20 different amino acids, were regarded as more likely candidates. The decisive experiment, conducted by Martha Chase and Alfred Hershey in 1952, provided confirmatory evidence that DNA was indeed the genetic material and not proteins. Chase and Hershey were studying a **bacteriophage**—a virus that infects bacteria. Viruses typically have a simple structure: a protein coat, called the capsid, and a nucleic acid core that contains the genetic material (either DNA or RNA). The bacteriophage infects the host bacterial cell by attaching to its surface, and then it injects its nucleic acids inside the cell. The phage DNA makes multiple copies of itself using the host machinery, and eventually the host cell bursts, releasing a large number of bacteriophages. Hershey and Chase selected radioactive elements that would specifically distinguish the protein from the DNA in infected cells. They labeled one batch of phage with radioactive sulfur, ^{35}S, to label the protein coat. Another batch of phage were labeled with radioactive phosphorus, ^{32}P. Because phosphorous is found in DNA, but not protein, the DNA and not the protein would be tagged with radioactive phosphorus. Likewise, sulfur is absent from DNA, but present in several amino acids such as methionine and cysteine.

Each batch of phage was allowed to infect the cells separately. After infection, the phage bacterial suspension

was put in a blender, which caused the phage coat to detach from the host cell. Cells exposed long enough for infection to occur were then examined to see which of the two radioactive molecules had entered the cell. The phage and bacterial suspension was spun down in a centrifuge. The heavier bacterial cells settled down and formed a pellet, whereas the lighter phage particles stayed in the supernatant. In the tube that contained phage labeled with ^{35}S, the supernatant contained the radioactively labeled phage, whereas no radioactivity was detected in the pellet. In the tube that contained the phage labeled with ^{32}P, the radioactivity was detected in the pellet that contained the heavier bacterial cells, and no radioactivity was detected in the supernatant. Hershey and Chase concluded that it was the phage DNA that was injected into the cell and carried information to produce more phage particles, thus providing evidence that DNA was the genetic material and not proteins (Figure 14.4).

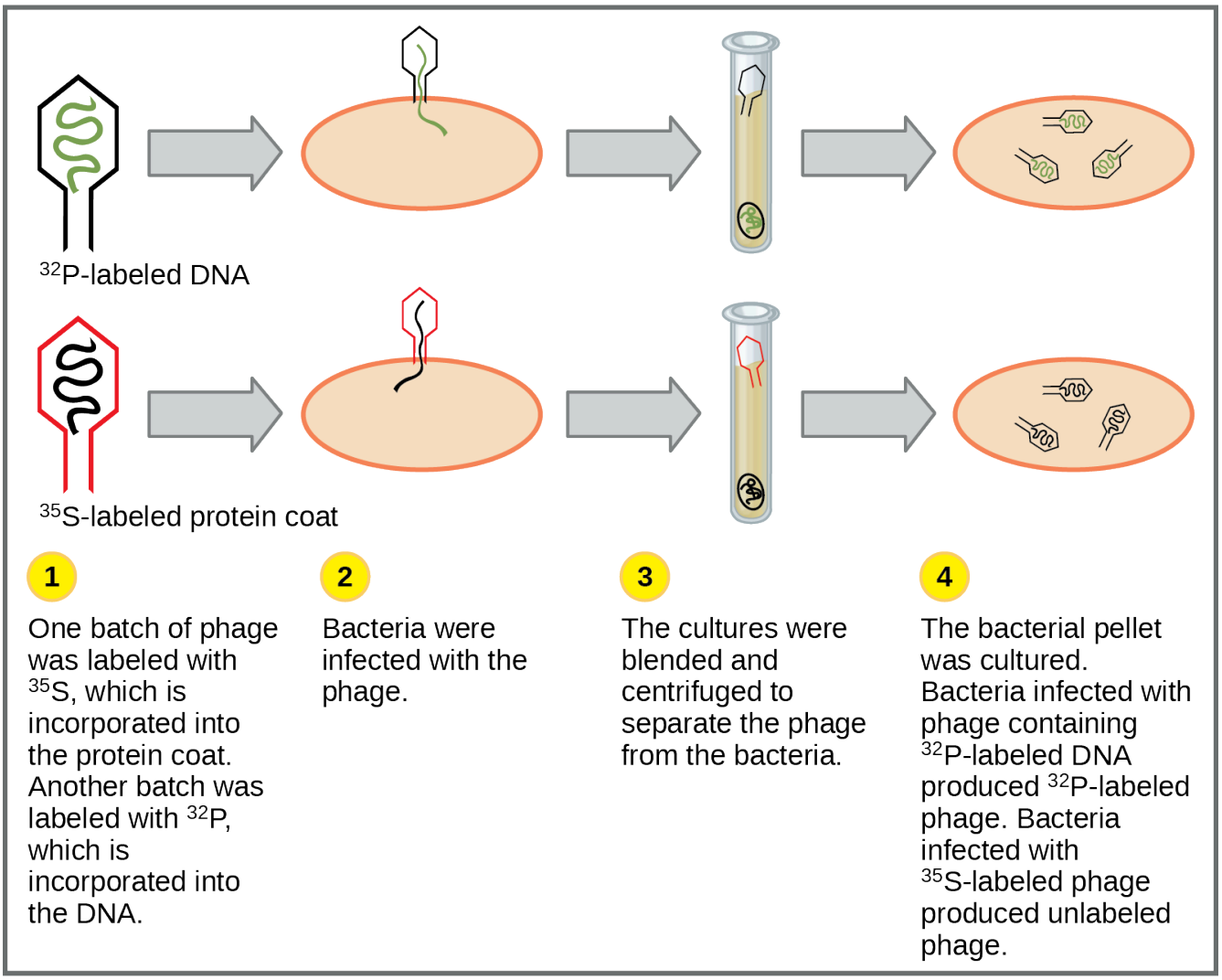


Figure 14.4 In Hershey and Chase's experiments, bacteria were infected with phage radiolabeled with either 35S, which labels protein, or 32P, which labels DNA. Only 32P entered the bacterial cells, indicating that DNA is the genetic material.

Around this same time, Austrian biochemist Erwin Chargaff examined the content of DNA in different species and found that the amounts of adenine, thymine, guanine, and cytosine were not found in equal quantities, and that relative concentrations of the four nucleotide bases varied from species to species, but not within tissues of the same individual or between individuals of the same species. He also discovered something unexpected: That the amount of adenine equaled the amount of thymine, and the amount of cytosine equaled the amount of guanine (that is, A = T and G = C). Different species had equal amounts of *purines* (A+G) and **pyrimidines** (T + C), but different ratios of A+T to G+C. These observations became known as **Chargaff's rules**. Chargaff's findings proved immensely useful when Watson and Crick were

getting ready to propose their DNA double helix model! You can see after reading the past few pages how science builds upon previous discoveries, sometimes in a slow and laborious process.

132.

DNA STRUCTURE AND SEQUENCING

Learning Objectives

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

- Describe the structure of DNA
- Explain the Sanger method of DNA sequencing
- Discuss the similarities and differences between eukaryotic and prokaryotic DNA

The building blocks of DNA are nucleotides. The important components of the nucleotide are a nitrogenous (nitrogen-bearing) base, a 5-carbon sugar (pentose), and a phosphate group (Figure 14.5). The nucleotide is named depending on the nitrogenous base. The nitrogenous base can be a purine such as adenine (A) and guanine (G), or a pyrimidine such as cytosine (C) and thymine (T).

Visual Connection

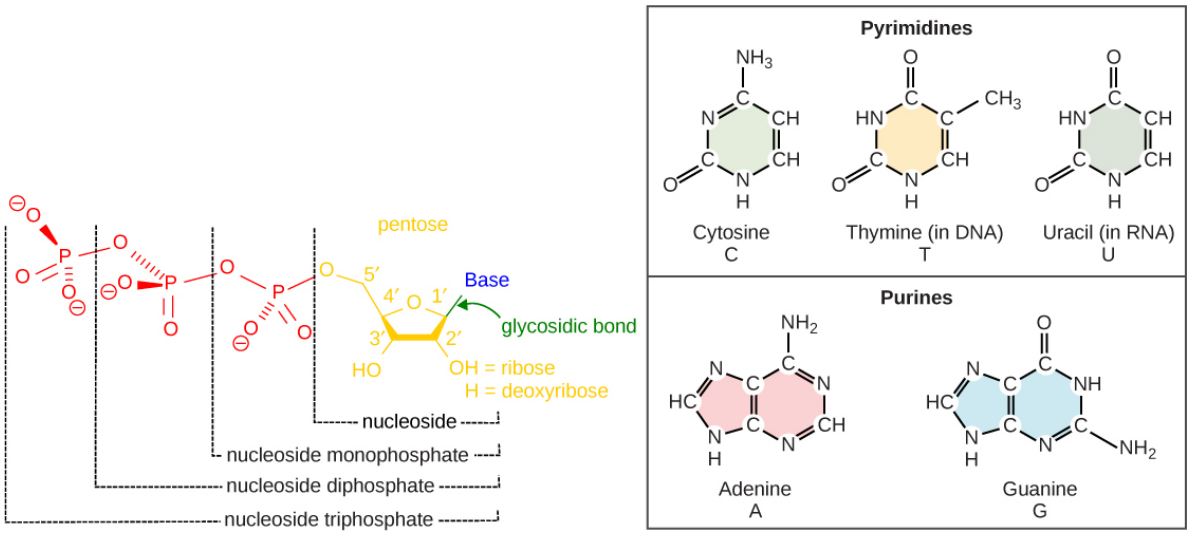


Figure 14.5 The purines have a double ring structure with a six-membered ring fused to a five-membered ring. Pyrimidines are smaller in size; they have a single six-membered ring structure.

The images above illustrate the five bases of DNA and RNA. Examine the images and explain why these are called "nitrogenous bases." How are the purines different from the pyrimidines? How is one purine or pyrimidine different from another, e.g., adenine from guanine? How is a nucleoside different from a nucleotide?

The purines have a double ring structure with a six-membered ring fused to a five-membered ring. Pyrimidines are smaller in size; they have a single six-membered ring structure.

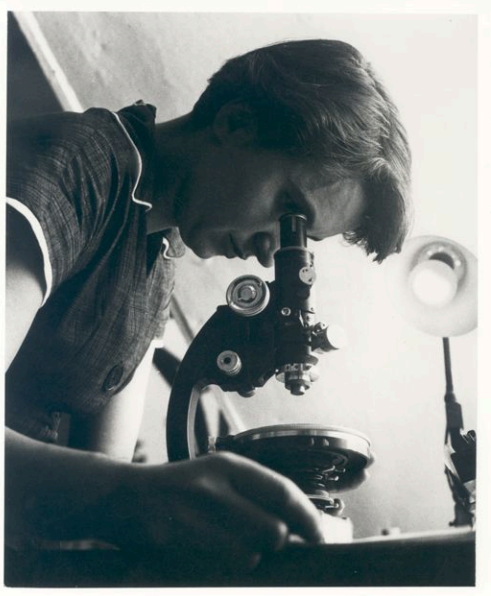
The sugar is deoxyribose in DNA and ribose in RNA. The carbon atoms of the five-carbon sugar are numbered 1′, 2′, 3′, 4′, and 5′ (1′ is read as "one prime"). The phosphate, which makes DNA and RNA acidic, is connected to the 5′ carbon of the sugar by the formation of an ester linkage between phosphoric acid and the 5′-OH group (an ester is an acid + an alcohol). In DNA nucleotides, the 3′ carbon of the sugar deoxyribose is attached to a hydroxyl (OH) group. In RNA nucleotides, the 2′ carbon of the sugar ribose also contains a hydroxyl group. The base is attached to the 1'carbon of the sugar.

The nucleotides combine with each other to produce phosphodiester bonds. The phosphate residue attached to the 5′ carbon of the sugar of one nucleotide forms a second ester linkage with the hydroxyl group of the 3′ carbon of the sugar of the next nucleotide, thereby forming a 5′-3′ phosphodiester bond. In a polynucleotide, one end of the chain has a free 5′ phosphate, and the other end has a free 3′-OH. These are called the 5′ and 3′ ends of the chain.

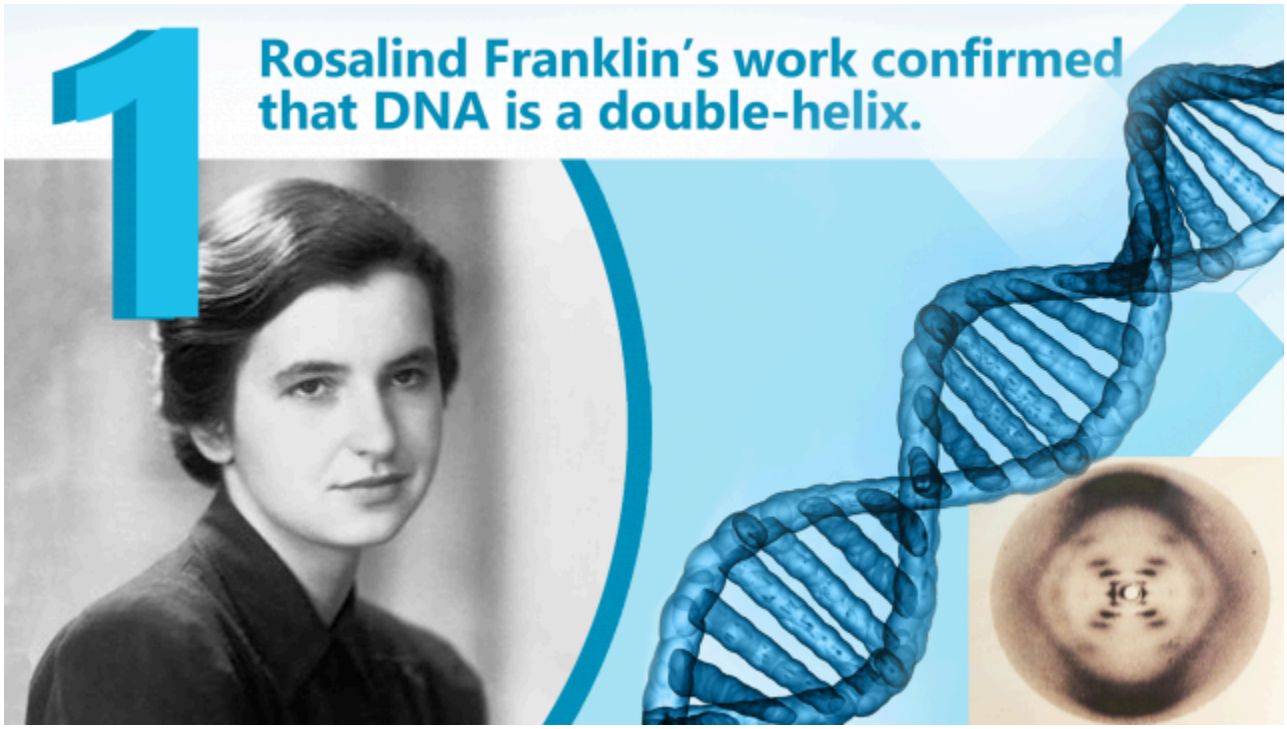
Rosalind Franklin joined the scientists at the Medical Research Unit, King's College when John Randall recruited her to work on the structure of DNA. DNA (deoxyribonucleic acid) was originally discovered in

1898 by Johann Miescher, and it was known that it was a key to genetics. But it was not until the middle of the 20th century when scientific methods had developed to where the actual structure of the molecule could be discovered, and Rosalind Franklin's work was key to that methodology.

Rosalind Franklin worked on the DNA molecule from 1951 until 1953. Using x-ray crystallography, she took photographs of the B version of the molecule. A co-worker with whom Franklin did not have a good working relationship, Maurice H.F. Wilkins, showed Franklin's photographs of DNA to James Watson—without Franklin's permission. Watson and his research partner Francis Crick were working independently on the structure of DNA, and Watson realized that these photographs were the scientific evidence they needed to prove that the DNA molecule was a double-stranded helix.



Rosalind Elsie Franklin is best known for her role in the discovery of the structure of DNA. License info: Rosalind Franklin (retouched).jpg from MagentaGreen and Wikimedia licensed CC-BY-SA 4.0



Rosalind Franklin used radiation science to advance the biological sciences. She used x-ray crystallography to obtain an image of the double-helix structure of DNA. Scientists James Watson and Francis Crick used the image in their work on DNA, for which they would win the Nobel Prize in biology. Dr. Franklin's contribution was not recognized by the Nobel Committee. Remix of "Photo of DNA Molecule by Rosalind Franklin" by Ryan Somma/CC BY

In the 1950s, Francis Crick and James Watson worked together to determine the structure of DNA at the University of Cambridge, England. Other scientists like Linus Pauling and Maurice Wilkins were also actively exploring this field. Pauling previously had discovered the secondary structure of proteins using X-ray crystallography. In Wilkins' lab, researcher Rosalind Franklin was using X-ray diffraction methods to understand the structure of DNA. Watson and Crick were able to piece together the puzzle of the DNA molecule on the basis of Franklin's data because Crick had also studied X-ray diffraction (Figure 14.6). In 1962, James Watson, Francis Crick, and Maurice Wilkins were awarded the Nobel Prize in Medicine. Unfortunately, by then Franklin had died, and Nobel prizes are not awarded posthumously.

(a) (b)

Figure 14.6 The work of pioneering scientists (a) James Watson, Francis Crick, and Maclyn McCarty led to our present day understanding of DNA. 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, Public Library of Science)

Watson and Crick proposed that DNA is made up of two strands that are twisted around each other to form a right-handed helix. Base pairing takes place between a purine and pyrimidine on opposite strands, so that A pairs with T, and G pairs with C (suggested by Chargaff's Rules). Thus, adenine and thymine are complementary base pairs, and cytosine and guanine are also complementary base pairs. The base pairs are stabilized by hydrogen bonds: *adenine and thymine form two hydrogen bonds and cytosine and guanine form three hydrogen bonds.* The two strands are anti-parallel in nature; that is, the 3′ end of one strand faces the 5′ end of the other strand. The sugar and phosphate of the nucleotides form the backbone of the structure, whereas the nitrogenous bases are stacked inside, like the rungs of a ladder. Each base pair is separated from the next base pair by a distance of 0.34 nm, and each turn of the helix measures 3.4 nm. Therefore, 10 base pairs are present per turn of the helix. The diameter of the DNA double-helix is 2 nm, and it is uniform throughout. Only the pairing between a purine and pyrimidine and the antiparallel orientation of the two DNA strands can explain the uniform diameter. The twisting of the two strands around each other results in the formation of uniformly spaced major and minor grooves (Figure 14.7).

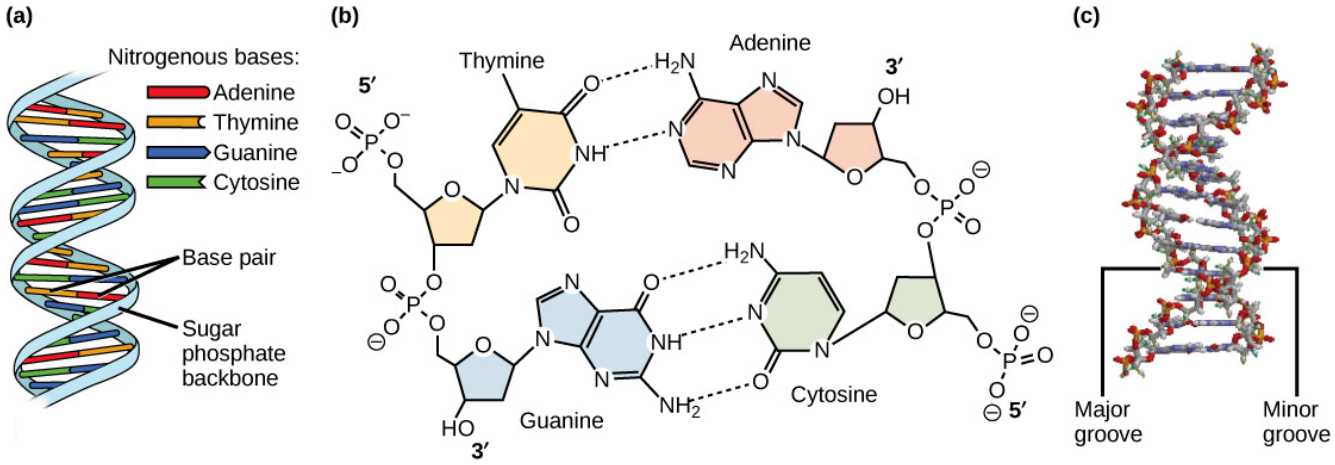


Figure 14.7 DNA has (a) a double helix structure and (b) phosphodiester bonds; the dotted lines between Thymine and Adenine and Guanine and Cytosine represent hydrogen bonds. The (c) major and minor grooves are binding sites for DNA binding proteins during processes such as transcription (the copying of RNA from DNA) and replication.

DNA Sequencing Techniques

DNA sequencing is the process of determining the nucleic acid sequence – the order of nucleotides in DNA. It includes any method or technology that is used to determine the order of the four bases: adenine, guanine, cytosine, and thymine. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. Until the 1990s, the sequencing of DNA (reading the sequence of DNA) was a relatively expensive and long process. Using radiolabeled nucleotides also compounded the problem through safety concerns. With currently available technology and automated machines, the process is cheaper, safer, and can be completed in a matter of hours. Fred Sanger developed the sequencing method used for the human genome sequencing project, which is widely used today (Figure 14.8).

Link to Learning

Visit this site to watch a video explaining the DNA sequence-reading technique that resulted from Sanger's work. These videos provide more information on DNA translation: DNA Translation: Initiation Phase & Ribosome FormationDNA Translation: mRNA to Protein, and tRNA's RoleDNA as Genetic material: Avery-MacLeod-McCarty experiment

The sequencing method is known as the dideoxy chain termination method. The method is based on the use of chain terminators, the dideoxynucleotides (ddNTPs). The ddNTPSs differ from the deoxynucleotides by

the lack of a free 3′ OH group on the five-carbon sugar. If a ddNTP is added to a growing DNA strand, the chain cannot be extended any further because the free 3′ OH group needed to add another nucleotide is not available. By using a predetermined ratio of deoxynucleotides to dideoxynucleotides, it is possible to generate DNA fragments of different sizes.

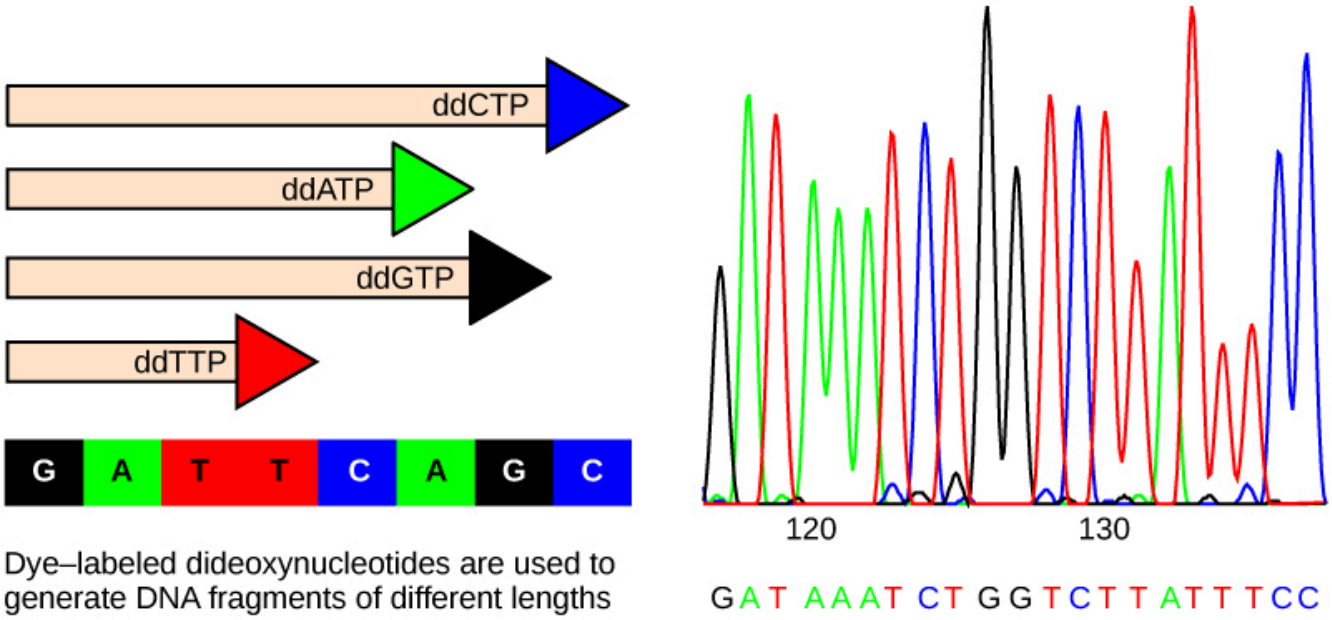


Figure 14.8 In Frederick Sanger's dideoxy chain termination method, dye-labeled dideoxynucleotides are used to generate DNA fragments that terminate at different points. The DNA is separated by capillary electrophoresis (not defined) on the basis of size, and from the order of fragments formed, the DNA sequence can be read. The DNA sequence readout is shown on an electropherogram (not defined) that is generated by a laser scanner.

The DNA sample to be sequenced is denatured (separated into two strands by heating it to high temperatures). The DNA is divided into four tubes in which a primer, DNA polymerase, and all four nucleoside triphosphates (A, T, G, and C) are added. In addition, limited quantities of one of the four dideoxynucleoside triphosphates (ddCTP, ddATP, ddGTP, and ddTTP) are added to each tube respectively. The tubes are labeled as A, T, G, and C according to the ddNTP added. For detection purposes, each of the four dideoxynucleotides carries a different fluorescent label. Chain elongation continues until a fluorescent dideoxy nucleotide is incorporated, after which no further elongation takes place. After the reaction is over, electrophoresis is performed. Even a difference in length of a single base can be detected. The sequence is read from a laser scanner that detects the fluorescent marker of each fragment. For his work on DNA sequencing, Sanger received a Nobel Prize in Chemistry in 1980.

Link to Learning

Sanger's genome sequencing has led to a race to sequence human genomes at rapid speed and low cost. Learn more by viewing the animation here.

Gel electrophoresis is a technique used to separate DNA fragments of different sizes. Usually the gel is made of a chemical called *agarose* (a polysaccharide polymer extracted from seaweed that is high in galactose residues). Agarose powder is added to a buffer and heated. After cooling, the gel solution is poured into a casting tray. Once the gel has solidified, the DNA is loaded on the gel and electric current is applied. The DNA has a net negative charge and moves from the negative electrode toward the positive electrode. The electric current is applied for sufficient time to let the DNA separate according to size; the smallest fragments will be farthest from the well (where the DNA was loaded), and the heavier molecular weight fragments will be closest to the well. Once the DNA is separated, the gel is stained with a DNA-specific dye for viewing it (Figure 14.9).

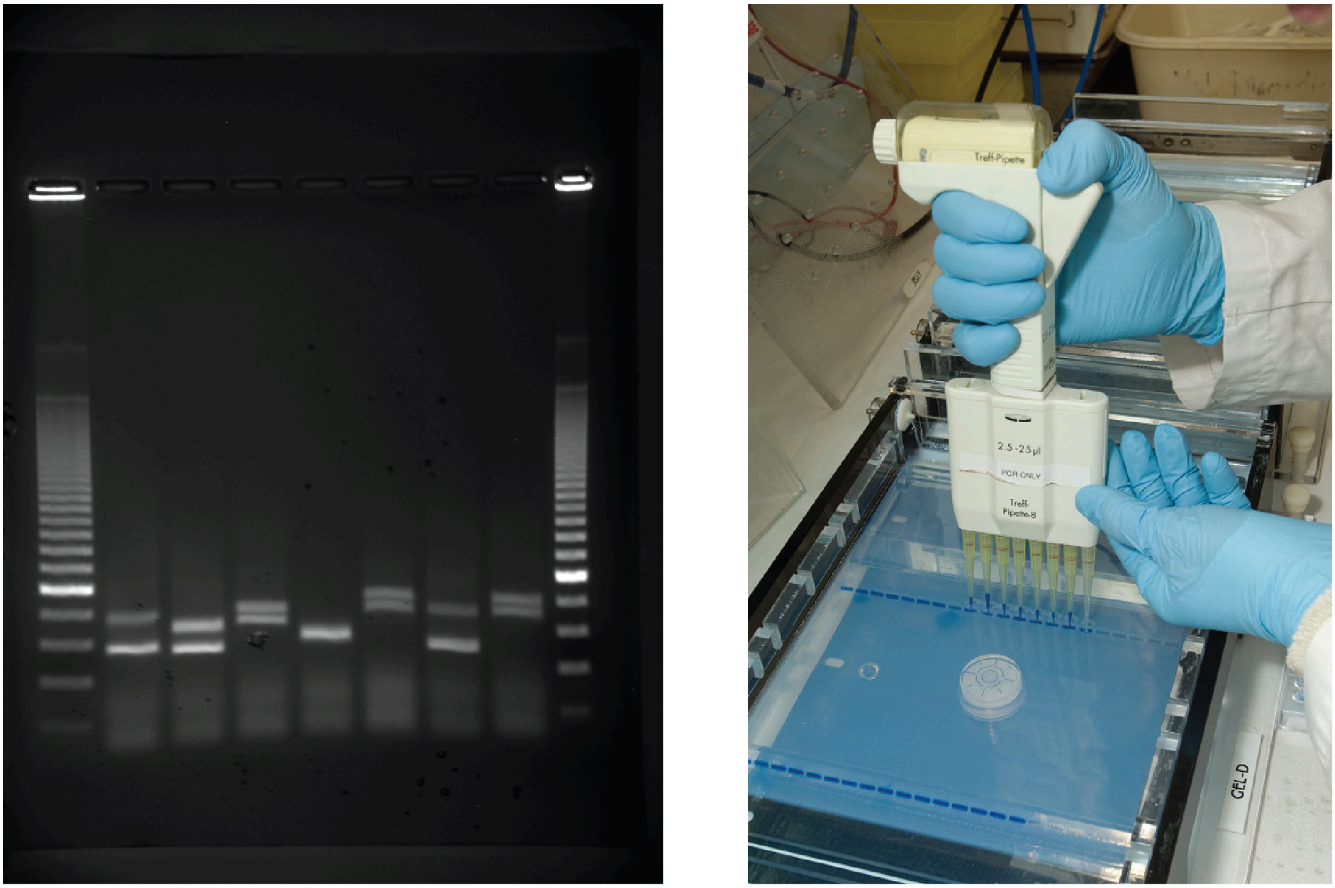


Figure 14.9 DNA can be separated on the basis of size using gel electrophoresis. (credit: James Jacob, Tompkins Cortland Community College)

Evolution Connection

Neanderthal Genome: How Are We Related? The first draft sequence of the Neanderthal genome was recently published by Richard E. Green et al. in 2010.[1] Neanderthals are the closest ancestors of present-day humans. They were known to have lived in Europe and Western Asia (and now, perhaps, in Northern Africa) before they disappeared from fossil records approximately 30,000 years ago. Green's team studied almost 40,000-year-old fossil remains that were selected from sites across the world. Extremely sophisticated means of sample preparation and DNA sequencing were employed because of the fragile nature of the bones and heavy microbial contamination. In their study, the scientists were able to sequence some four billion base pairs. The Neanderthal sequence was compared with that of present-day humans from across the world. After comparing the sequences, the researchers found that the

Neanderthal genome had 2 to 3 percent greater similarity to people living outside Africa than to people in Africa. While current theories have suggested that all present-day humans can be traced to a small ancestral population in Africa, the data from the Neanderthal genome suggest some interbreeding between Neanderthals and early modern humans.

Green and his colleagues also discovered DNA segments among people in Europe and Asia that are more similar to Neanderthal sequences than to other contemporary human sequences. Another interesting observation was that Neanderthals are as closely related to people from Papua New Guinea as to those from China or France. This is surprising because Neanderthal fossil remains have been located only in Europe and West Asia. Most likely, genetic exchange took place between Neanderthals and modern humans as modern humans emerged out of Africa, before the divergence of Europeans, East Asians, and Papua New Guineans. Several genes seem to have undergone changes from Neanderthals during the evolution of present-day humans. These genes are involved in cranial structure, metabolism, skin morphology, and cognitive development. One of the genes that is of particular interest is *RUNX2*, which is different in modern day humans and Neanderthals. This gene is responsible for the prominent frontal bone, bell-shaped rib cage, and dental differences seen in Neanderthals. It is speculated that an evolutionary change in *RUNX2* was important in the origin of modern-day humans, and this affected the cranium and the upper body.

Link to Learning

Watch Svante Pääbo's talk explaining the Neanderthal genome research at the 2011 annual TED (Technology, Entertainment, Design) conference. This review video demonstrates how DNA is packaged.

DNA Packaging in Cells

Prokaryotes are much simpler than eukaryotes in many of their features (Figure 14.10). Most prokaryotes contain a single, circular chromosome that is found in an area of the cytoplasm called the *nucleoid region*.

Visual Connection

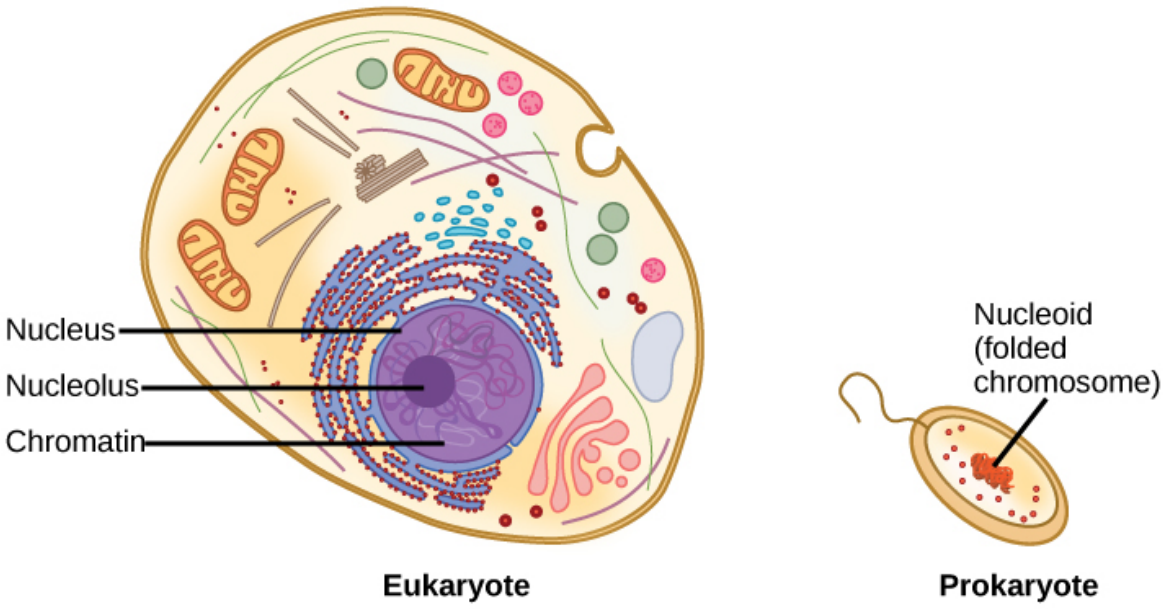


Figure 14.10 A eukaryote contains a well-defined nucleus, whereas in prokaryotes, the chromosome lies in the cytoplasm in an area called the nucleoid.

In eukaryotic cells, DNA and RNA synthesis occur in a separate compartment from protein synthesis. In prokaryotic cells, both processes occur together. What advantages might there be to separating the processes? What advantages might there be to having them occur together?

The size of the genome in one of the most well-studied prokaryotes, *E.coli*, is 4.6 million base pairs (approximately 1.1 mm, if cut and stretched out). So how does this fit inside a small bacterial cell? The DNA is twisted by what is known as supercoiling. Supercoiling suggests that DNA is either "under-wound" (less than one turn of the helix per 10 base pairs) or "over-wound" (more than 1 turn per 10 base pairs) from its normal relaxed state. Some proteins are known to be involved in the supercoiling; other proteins and enzymes such as DNA gyrase 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 14.11). At the most basic level, DNA is wrapped around proteins known as **histones** to form structures called nucleosomes. The histones are evolutionarily conserved proteins that are rich in basic amino acids and form an octamer composed of two molecules of each of four different histones. Their composition and properties

are important to understanding gene expression, and were partially uncovered based on research by Marie M. Daly and Alfred E. Mirsky in the early 1950s. The DNA (remember, it is negatively charged because of the phosphate groups) is wrapped tightly around the histone core. This nucleosome is linked to the next one with the help of a *linker DNA*. This is also known as the "beads on a string" structure. With the help of a fifth histone, a string of nucleosomes is further compacted into a 30-nm fiber, which is the diameter of the structure. Metaphase chromosomes are even further condensed by association with scaffolding proteins. At the metaphase stage, the chromosomes are at their most compact, approximately 700 nm in width. In interphase, eukaryotic chromosomes have two distinct regions that can be distinguished by staining. The tightly packaged region is known as heterochromatin, and the less dense region is known as euchromatin. Heterochromatin usually contains genes that are not expressed, and is found in the regions of the centromere and telomeres. The euchromatin usually contains genes that are transcribed, with DNA packaged around nucleosomes but not further compacted.

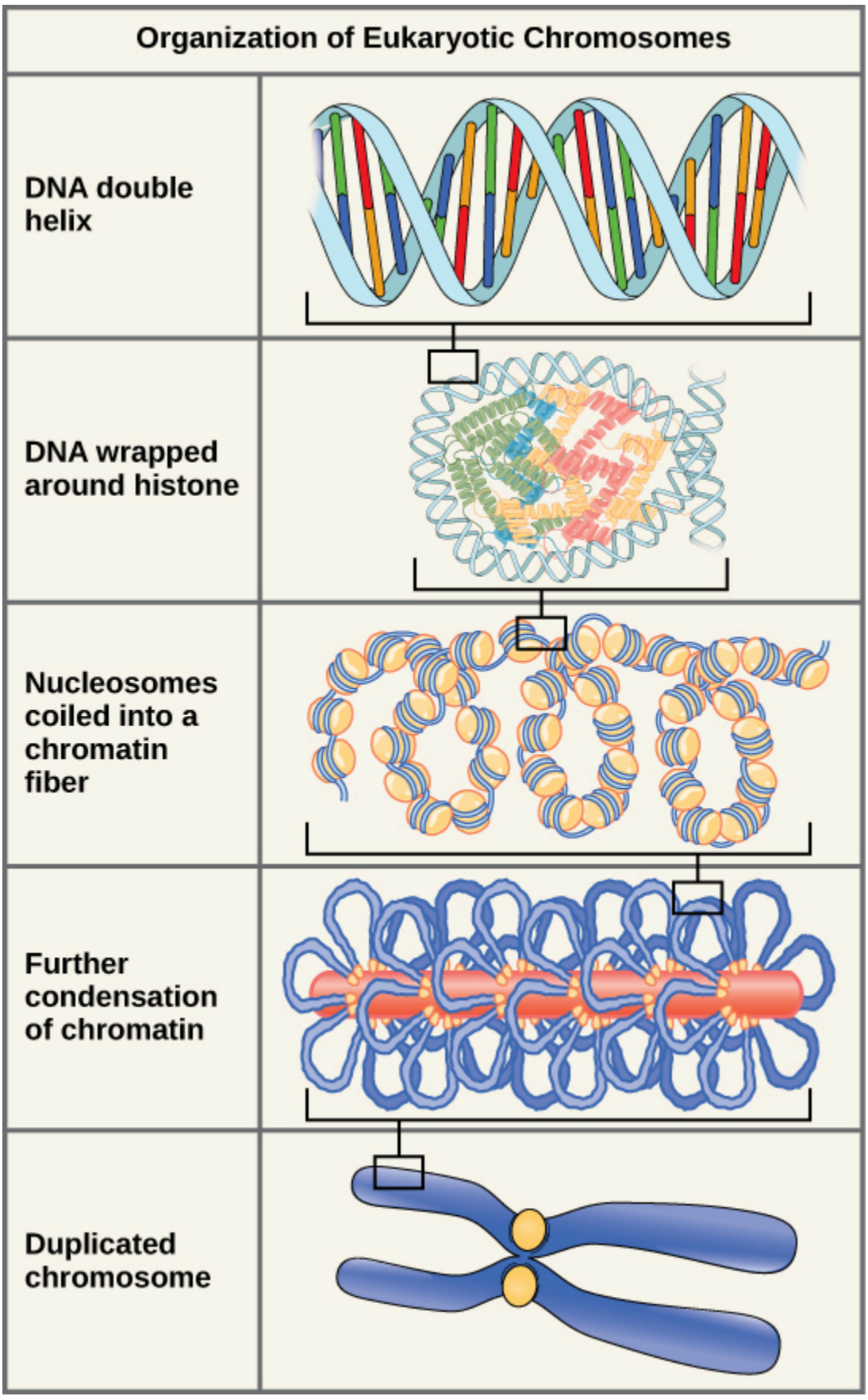

Organization of Eukaryotic Chromosomes
DNA double helix
DNA wrapped around histone
Nucleosomes coiled into a chromatin fiber
Further condensation of chromatin
Duplicated chromosome

Figure 14.11 These figures illustrate the compaction of the eukaryotic chromosome.

Footnotes

- 1 Richard E. Green et al., "A Draft Sequence of the Neandertal Genome," *Science* 328 (2010): 710-22.

133.

BASICS OF DNA REPLICATION

Learning Objectives

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

- Explain how the structure of DNA reveals the replication process
- Describe the Meselson and Stahl experiments

The elucidation of the structure of the double helix provided a hint as to how DNA divides and makes copies of itself. In their 1953 paper, Watson and Crick penned an incredible understatement: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." With specific base pairs, the sequence of one DNA strand can be predicted from its complement. The double-helix model 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. What was not clear was how the replication took place. There were three models suggested (Figure 14.12): *conservative, semi-conservative, and dispersive*.

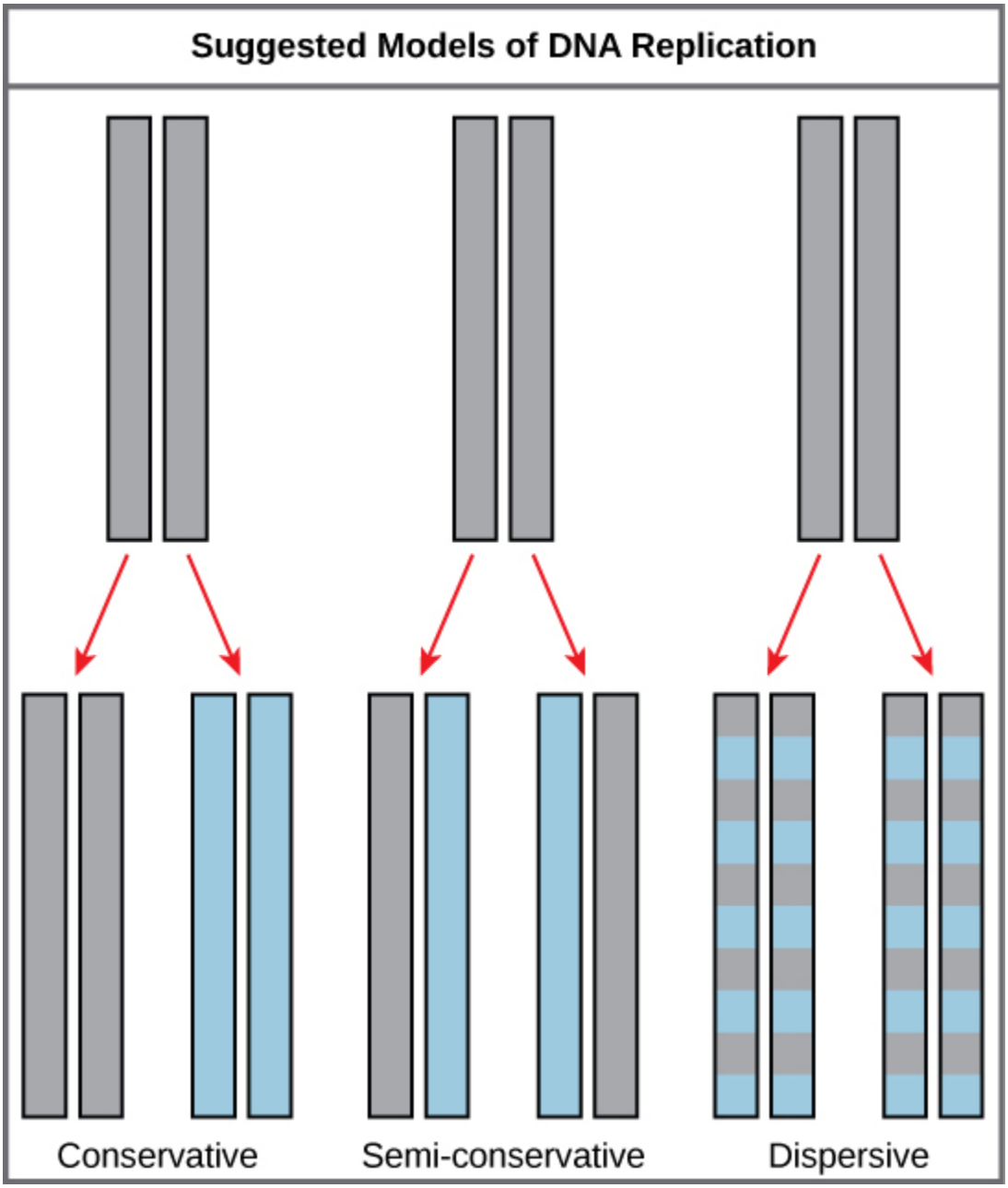


Figure 14.12 The three suggested models of DNA replication. Gray indicates the original DNA strands, and blue indicates newly synthesized DNA.

In conservative replication, the parental DNA remains together, and the newly formed daughter strands are together. The semi-conservative method suggests that each of the two parental DNA strands acts as a template for new DNA to be synthesized; after replication, each double-stranded DNA includes one parental or "old" strand and one "new" strand. In the dispersive model, both copies of DNA have double-stranded segments of parental DNA and newly synthesized DNA interspersed.

Meselson and Stahl were interested in understanding how DNA replicates. They grew *E. coli* for several

generations in a medium containing a "heavy" isotope of nitrogen (^{15}N), which gets incorporated into nitrogenous bases, and eventually into the DNA (Figure 14.13).

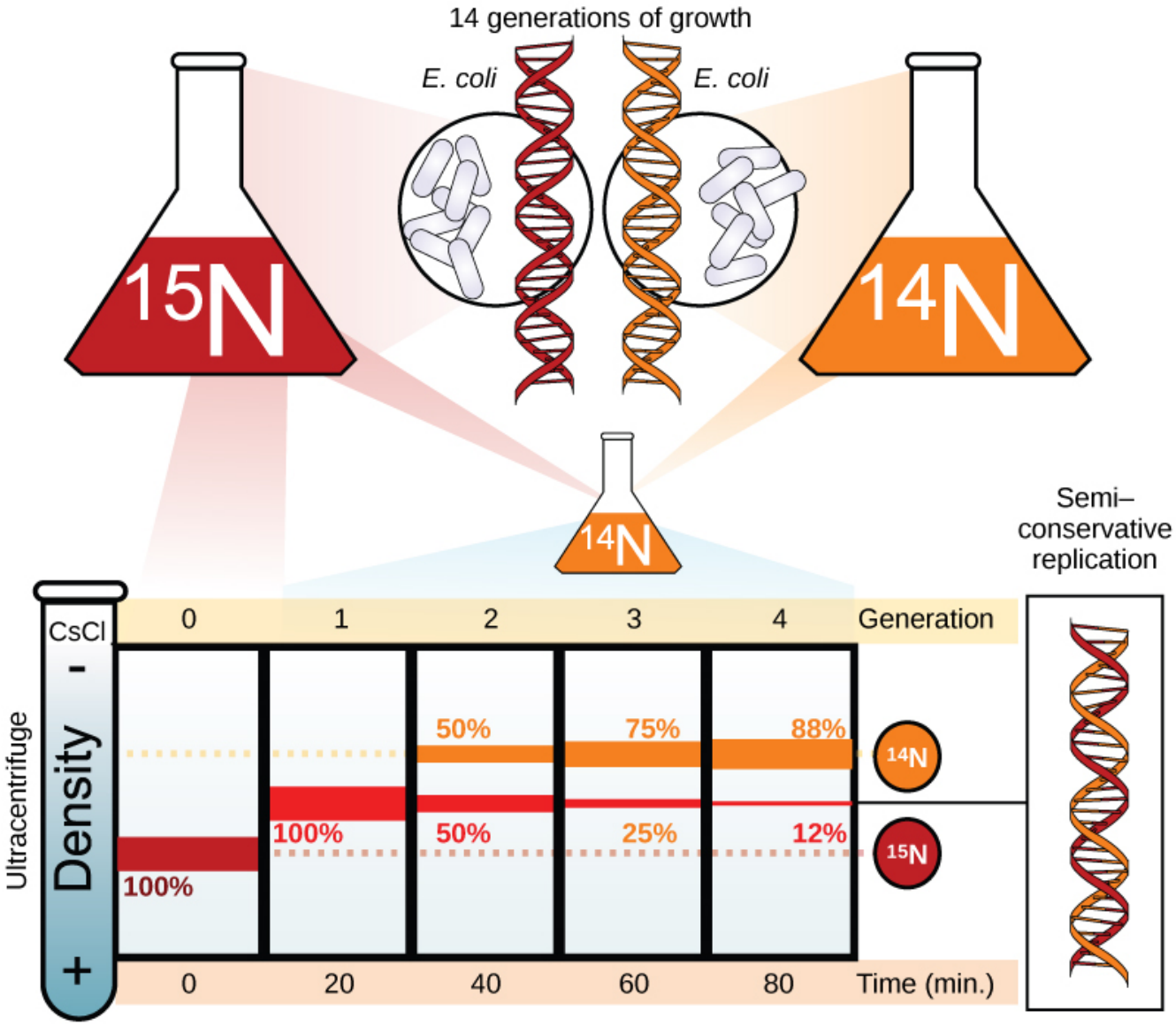


Figure 14.13 Meselson and Stahl experimented with E. coli grown first in heavy nitrogen (15N), then in 14N. DNA grown in 15N (red band) is heavier than DNA grown in 14N (orange band), and sediments to a lower level in cesium chloride solution in an ultracentrifuge. When DNA grown in 15N is switched to media containing 14N, after one round of cell division the DNA sediments halfway between the 15N and 14N levels, indicating that it now contains fifty percent 14N. In subsequent cell divisions, an increasing amount of DNA contains 14N only. These data support the semi-conservative replication model. (credit: modification of work by Mariana Ruiz Villareal)

The *E. coli* culture was then placed into a medium containing ^{14}N and allowed to grow for several generations. After each of the first few generations, the cells were harvested and the DNA was isolated, then centrifuged

at high speeds in an ultracentrifuge. During the centrifugation, the DNA was loaded into a *gradient* (typically a solution of salt such as cesium chloride or sucrose) and spun at high speeds of 50,000 to 60,000 rpm. Under these circumstances, the DNA will form a band according to its *buoyant density*: the density within the gradient at which it floats. DNA grown in ^{15}N will form a band at a higher density position (i.e., farther down the centrifuge tube) than that grown in ^{14}N. Meselson and Stahl noted that after one generation of growth in ^{14}N after they had been shifted from ^{15}N, the single band observed was intermediate in position in between DNA of cells grown exclusively in ^{15}N and ^{14}N. This suggested either a semi-conservative or dispersive mode of replication. The DNA harvested from cells grown for two generations in ^{14}N formed two bands: one DNA band was at the intermediate position between ^{15}N and ^{14}N, and the other corresponded to the band of ^{14}N DNA. These results could only be explained if DNA replicates in a semi-conservative manner. And for this reason, therefore, the other two models were ruled out.

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 strands will be complementary to the parental or "old" strands. When two daughter DNA copies are formed, they have the same sequence and are divided equally into the two daughter cells.

Link to Learning

View this video on DNA replication and this video of semi conservative replication.

134.

DNA REPLICATION IN PROKARYOTES

Learning Objectives

Type your learning objectives here.

- Explain the process of DNA replication in prokaryotes
- Discuss the role of different enzymes and proteins in supporting this process

DNA replication has been well studied in prokaryotes primarily because of the small size of the genome and because of the large variety of mutants that are available. *E. 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 site along the chromosome and proceeding around the circle in both directions. This means that approximately 1000 nucleotides are added per second. Thus, the process is quite rapid and occurs without many mistakes.

DNA replication employs a large number of structural proteins and enzymes, each of which plays a critical role during the process. One of the key players is the enzyme **DNA polymerase**, also known as DNA pol, which adds nucleotides one-by-one to the growing DNA chain that is complementary to the template strand. The addition of nucleotides requires energy; this energy is obtained from the nucleoside triphosphates ATP, GTP, TTP and CTP. Like ATP, the other **NTPs** (nucleoside triphosphates) are high-energy molecules that can serve both as the source of DNA nucleotides and the source of energy to drive the polymerization. When the bond between the phosphates is "broken," the energy released is used to form the phosphodiester bond between the incoming nucleotide and the growing chain. In prokaryotes, three main types of polymerases are known: DNA pol I, DNA pol II, and DNA pol III. It is now known that DNA pol III is the enzyme required for DNA synthesis; DNA pol I is an important accessory enzyme in DNA replication, and along with DNA pol II, is primarily required for repair.

How does the replication machinery know where to begin? It turns out that there are specific nucleotide

sequences called *origins of replication* where replication begins. In *E. coli,* which has a single origin of replication on its one chromosome (as do most prokaryotes), this origin of replication is approximately 245 base pairs long and is rich in AT sequences. The origin of replication is recognized by certain proteins that bind to this site. An enzyme called *helicase* unwinds the DNA by breaking the hydrogen bonds between the nitrogenous base pairs. ATP hydrolysis is required for this process. As the DNA opens up, Y-shaped structures called *replication forks* are formed. Two replication forks are formed at the origin of replication, and these get extended bi-directionally as replication proceeds. Single-strand binding proteins coat the single strands of DNA near the replication fork to prevent the single-stranded DNA from winding back into a double helix.

DNA polymerase has two important restrictions: it is able to add nucleotides only in the 5′ to 3′ direction (a new DNA strand can be only extended in this direction). It also requires a free 3′-OH group to which it can add nucleotides by forming a phosphodiester bond between the 3′-OH end and the 5′ phosphate of the next nucleotide. This essentially means that it cannot add nucleotides if a free 3′-OH group is not available. Then how does it add the first nucleotide? The problem is solved with the help of a primer that provides the free 3′-OH end. Another enzyme, RNA primase, synthesizes an RNA segment that is about five to ten nucleotides long and complementary to the template DNA. Because this sequence primes the DNA synthesis, it is appropriately called the primer. DNA polymerase can now extend this RNA primer, adding nucleotides one-by-one that are complementary to the template strand (Figure 14.14).

Link to Learning

Explore this video on DNA Polymerase in Prokaryotes and their mechanism of action (DNA Pol I, DNA Pol II and DNA Pol III).

This lecture Lac operon gene Regulation explains about the regulation of lac operon in prokaryotes including the catabolite repression of the lac operon regulated by the cyclic amp and cap protein. This lecture also explains the gene Regulation process of lac operon in the presence of glucose and lactose.

Visual Connection

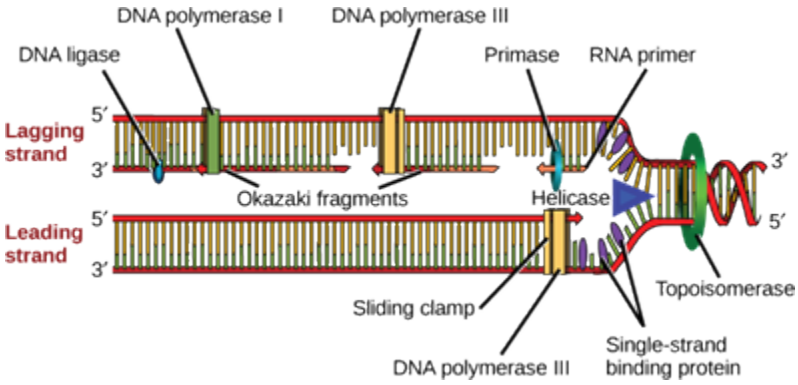


Figure 14.14 A replication fork is formed when helicase separates the DNA strands at the origin of replication. The DNA tends to become more highly coiled ahead of the replication fork. Topoisomerase breaks and reforms DNA's phosphate backbone ahead of the replication fork, thereby relieving the pressure that results from this "supercoiling." Single-strand binding proteins bind to the single-stranded DNA to prevent the helix from re-forming. Primase synthesizes an RNA primer. DNA polymerase III uses this primer to synthesize the daughter DNA strand. On the leading strand, DNA is synthesized continuously, whereas on the lagging strand, DNA is synthesized in short stretches called Okazaki fragments. DNA polymerase I replaces the RNA primer with DNA. DNA ligase seals the gaps between the Okazaki fragments, joining the fragments into a single DNA molecule. (credit: modification of work by Mariana Ruiz Villareal)

Question: You isolate a cell strain in which the joining 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?

The replication fork moves at the rate of 1000 nucleotides per second. Topoisomerase prevents the over-winding of the DNA double helix ahead of the replication fork as the DNA is opening up; it does so by causing temporary nicks in the DNA helix and then resealing it. Because DNA polymerase can only extend in the 5′ to 3′ direction, and because the DNA double helix is *antiparallel*, there is a slight problem at the replication fork. *The two template DNA strands have opposing orientations:* one strand is in the 5′ to 3′ direction, and the other is oriented in the 3′ to 5′ direction. Only one new DNA strand, the one that is complementary to the 3′ to 5′ parental DNA strand, can be synthesized continuously towards the replication fork. This continuously synthesized strand is known as the **leading strand**. The other strand, complementary to the 5′ to 3′ parental DNA, is extended away from the replication fork, in small fragments known as **Okazaki fragments**, each requiring a primer to start the synthesis. New primer segments are laid down in the direction

of the replication fork, but each pointing away from it. (Okazaki fragments are named after the Japanese scientist who first discovered them. The strand with the Okazaki fragments is known as the **lagging strand.**)

Link to Learning

Watch this video on DNA Replication – Leading Strand vs Lagging Strand & Okazaki Fragments

The leading strand can be extended from a single primer, whereas the lagging strand needs a new primer for each of the short Okazaki fragments. The overall direction of the lagging strand will be 3′ to 5′, and that of the leading strand 5′ to 3′. A protein called the sliding clamp holds the DNA polymerase in place as it continues to add nucleotides. The sliding clamp is a ring-shaped protein that binds to the DNA and holds the polymerase in place. As synthesis proceeds, the RNA primers are replaced by DNA. The primers are removed by the exonuclease activity of DNA pol I, which uses DNA behind the RNA as its own primer and fills in the gaps left by removal of the RNA nucleotides by the addition of DNA nucleotides. The nicks that remain between the newly synthesized DNA (that replaced the RNA primer) and the previously synthesized DNA are sealed by the enzyme DNA ligase, which catalyzes the formation of phosphodiester linkages between the 3′-OH end of one nucleotide and the 5′ phosphate end of the other fragment.

Once the chromosome has been completely replicated, the two DNA copies move into two different cells during cell division. The process of DNA replication can be summarized as follows:

1. DNA unwinds at the origin of replication.
2. Helicase opens up the DNA-forming replication forks; these are extended bidirectionally.
3. Single-strand binding proteins coat the DNA around the replication fork to prevent rewinding of the DNA.
4. Topoisomerase binds at the region ahead of the replication fork to prevent supercoiling.
5. Primase synthesizes RNA primers complementary to the DNA strand.
6. DNA polymerase III starts adding nucleotides to the 3′-OH end of the primer.
7. Elongation of both the lagging and the leading strand continues.
8. RNA primers are removed by exonuclease activity.
9. Gaps are filled by DNA pol I by adding dNTPs.
10. The gap between the two DNA fragments is sealed by DNA ligase, which helps in the formation of phosphodiester bonds.

Table 14.1 summarizes the enzymes involved in prokaryotic DNA replication and the functions of each.

Prokaryotic DNA Replication: Enzymes and Their Function

Enzyme/protein	Specific Function
DNA pol I	Removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5′-3′ direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Sliding Clamp	Helps to hold the DNA polymerase in place when nucleotides are being added
Topoisomerase	Helps relieve the strain on DNA when unwinding by causing breaks, and then resealing the DNA
Single-strand binding proteins (SSB)	Binds to single-stranded DNA to prevent DNA from rewinding back.

Table 14.1

Link to Learning

Review the full process of DNA replication here.

Watch the video DNA Polymerase in Prokaryotes and their mechanism of action (DNA Pol I, DNA Pol II and DNA Pol III).

135.

DNA REPLICATION IN EUKARYOTES

Learning Objectives

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

- Discuss the similarities and differences between DNA replication in eukaryotes and prokaryotes
- State the role of telomerase in DNA replication

Eukaryotic genomes are much more complex and larger in size than prokaryotic genomes. Eukaryotes also have a number of different linear chromosomes. The human genome has 3 billion base pairs per haploid set of chromosomes, and 6 billion base pairs are replicated during the S phase of the cell cycle. There are multiple origins of replication on each eukaryotic chromosome; humans can have up to 100,000 origins of replication across the genome. The rate of replication is approximately 100 nucleotides per second, much slower than prokaryotic replication. In yeast, which is a eukaryote, special sequences known as autonomously replicating sequences (ARS) are found on the chromosomes. These are equivalent to the origin of replication in *E. coli*.

The number of DNA polymerases in eukaryotes is much more than in prokaryotes: 14 are known, of which five are known to have major roles during replication and have been well studied. They are known as pol α, pol β, pol γ, pol δ, and pol ε.

The essential steps of replication are the same as in prokaryotes. Before replication can start, the DNA has to be made available as a template. Eukaryotic DNA is bound to basic proteins known as histones to form structures called nucleosomes. Histones must be removed and then replaced during the replication process, which helps to account for the lower replication rate in eukaryotes. The chromatin (the complex between DNA and proteins) may undergo some chemical modifications, so that the DNA may be able to slide off the proteins or be accessible to the enzymes of the DNA replication machinery. At the origin of replication, a

pre-replication complex is made with other initiator proteins. Helicase and other proteins are then recruited to start the replication process (Table 14.2).

Difference between Prokaryotic and Eukaryotic Replication

Property	**Prokaryotes**	**Eukaryotes**
Origin of replication	Single	Multiple
Rate of replication	1000 nucleotides/s	50 to 100 nucleotides/s
DNA polymerase types	5	14
Telomerase	Not present	Present
RNA primer removal	DNA pol I	RNase H
Strand elongation	DNA pol III	Pol α, pol δ, pol ε
Sliding clamp	Sliding clamp	PCNA

Table 14.2

A helicase using the energy from ATP hydrolysis opens up the DNA helix. Replication forks are formed at each replication origin as the DNA unwinds. The opening of the double helix causes over-winding, or supercoiling, in the DNA ahead of the replication fork. These are resolved with the action of topoisomerases. Primers are formed by the enzyme primase, and using the primer, DNA pol can start synthesis. Three major DNA polymerases are then involved: α, δ and ε. DNA pol α adds a short (20 to 30 nucleotides) DNA fragment to the RNA primer on both strands, and then hands off to a second polymerase. While the leading strand is continuously synthesized by the enzyme pol ε, the lagging strand is synthesized by pol δ. A sliding clamp protein known as PCNA (proliferating cell nuclear antigen) holds the DNA pol in place so that it does not slide off the DNA. As pol δ runs into the primer RNA on the lagging strand, it displaces it from the DNA template. The displaced primer RNA is then removed by RNase H (AKA flap endonuclease) and replaced with DNA nucleotides. The Okazaki fragments in the lagging strand are joined after the replacement of the RNA primers with DNA. The gaps that remain are sealed by DNA ligase, which forms the phosphodiester bond.

5 Steps of DNA Replication

DNA replication is the process through which a DNA molecule makes a copy of itself. We will

explore the enzymes involved in DNA replication, the concept of leading and lagging strands (Okazaki fragments), and walk you through the entire DNA replication step-by-step.

1. The first step in DNA replication is the separation of the double helix structure of the DNA molecule, which is carried out by DNA helicase. These separated strands serve as template strands in the DNA replication process.
2. Single stranded binding proteins (SSB) prevents the single template strands from re-annealing, which allows the template DNA strands to stay separated and available for the replication process. RNA primer segments are placed on this unwound DNA by the enzyme primase.
3. DNA polymerase (III) arrives at the site of the RNA primers to begin replication. The DNA polymerase moves along the template strand, and adds bases that are complimentary to our DNA template strand in an anti-parallel direction: DNA Polymerase reads the template strand in the 3' to 5' direction, but the new DNA strand is produced in the 5' to 3' direction.
4. Eventually, we differentiate into the leading and lagging strands of DNA. DNA polymerase binds to the leading strand, adding new complementary nucleotide bases to the strand of DNA in the 5’ to 3’ direction as helicase is unwinding the DNA. Chunks of DNA, called Okazaki fragments, are then added to the lagging strand also in the 5’ to 3’ direction. It is important to note that DNA replication always occurs in the 5' to 3' direction!
5. 5 In the lagging strands, RNAse H (aka DNA Pol I) removes primers as DNA Polymerase (III) approaches. From there, DNA ligase combines the Okazaki fragments.

Link to Learning

Review these videos for more information on:

- Enzymes and Proteins involved in DNA replication and their functions
- 5 Types of RNA: mRNA, tRNA, rRNA, HnRNA, and SnRNA
- 6 Steps of DNA Replication
- DNA Replication – Leading Strand vs Lagging Strand & Okazaki Fragments

Telomere replication

Unlike prokaryotic chromosomes, eukaryotic chromosomes are linear. As you've learned, the enzyme DNA pol can add nucleotides only in the 5′ to 3′ direction. In the leading strand, synthesis continues until the end of the chromosome is reached. On the lagging strand, DNA is synthesized in short stretches, each of which is initiated by a separate primer. When the replication fork reaches the end of the linear chromosome, there is no way to replace the primer on the 5' end of the lagging strand. The DNA at the ends of the chromosome thus remains unpaired, and over time these ends, called **telomeres,** may get progressively shorter as cells continue to divide.

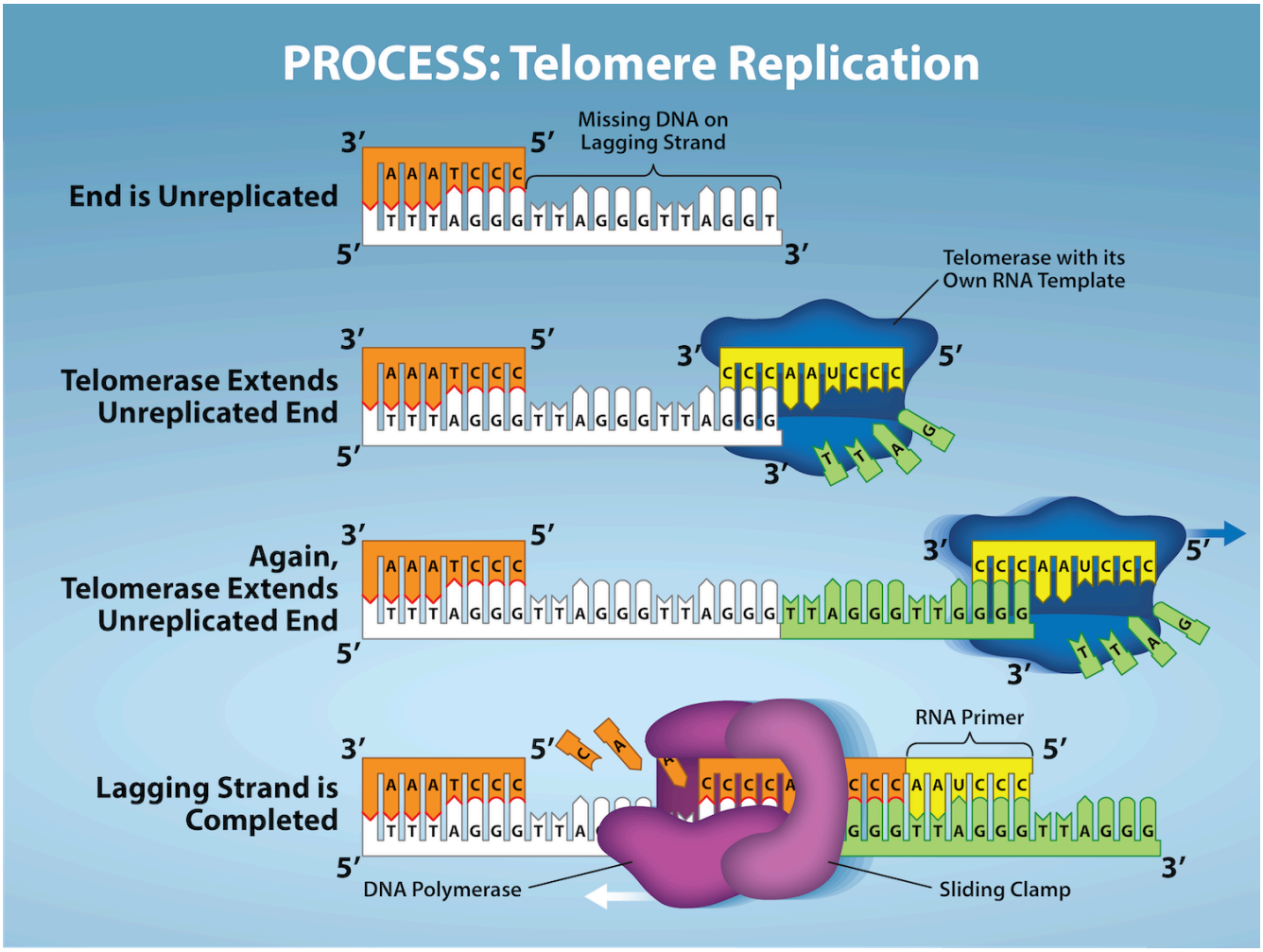


Figure 14.15 The ends of linear chromosomes are maintained by the action of the telomerase enzyme. Credit: Rao, A. and Fletcher, S. Department of Biology, Texas A&M University.

Telomeres comprise repetitive sequences that code for no particular gene. In humans, a six-base-pair sequence, TTAGGG, is repeated 100 to 1000 times in the telomere regions. In a way, these telomeres protect the genes from getting deleted as cells continue to divide. The telomeres are added to the ends of chromosomes by

a separate enzyme, telomerase (Figure 14.16), whose discovery helped in the understanding of how these repetitive chromosome ends are maintained. The **telomerase** enzyme contains a catalytic part and a built-in RNA template. It attaches to the end of the chromosome, and DNA nucleotides complementary to the RNA template are added on the 3′ end of the DNA strand. Once the 3′ end of the lagging strand template is sufficiently elongated, DNA polymerase can add the nucleotides complementary to the ends of the chromosomes. Thus, the ends of the chromosomes are replicated.

Telomerase is typically active in germ cells and adult stem cells. It is not active in adult somatic cells. For their discovery of telomerase and its action, Elizabeth Blackburn, Carol W. Greider, and Jack W. Szostak (Figure 14.16) received the Nobel Prize for Medicine and Physiology in 2009. Later research using HeLa cells (obtained from Henrietta Lacks) confirmed that telomerase is present in human cells. And in 2001, researchers including Diane L. Wright found that telomerase is necessary for cells in human embryos to rapidly proliferate.

Figure 14.16 Elizabeth Blackburn, 2009 Nobel Laureate, is one of the scientists who discovered how telomerase works. (credit: US Embassy Sweden)

Telomerase and Aging

Cells that undergo cell division continue to have their telomeres shortened because most somatic cells do not make telomerase. This essentially means that telomere shortening is associated with aging. With the advent of modern medicine, preventative health care, and healthier lifestyles, the human life span has increased, and there is an increasing demand for people to look younger and have a better quality of life as they grow older.

In 2010, scientists found that telomerase can reverse some age-related conditions in mice. This may have potential in regenerative medicine.[2] 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

the function of the testes, spleen, and intestines. Thus, telomere reactivation may have potential for treating age-related diseases in humans.

Cancer is characterized by uncontrolled cell division of abnormal cells. The cells accumulate mutations, proliferate uncontrollably, and can migrate to different parts of the body through a process called metastasis. Scientists have observed that cancerous cells have considerably shortened telomeres and that telomerase is active in these cells. Interestingly, only after the telomeres were shortened in the cancer cells did the telomerase become active. If the action of telomerase in these cells can be inhibited by drugs during cancer therapy, then the cancerous cells could potentially be stopped from further division.

Footnotes

- 2 Jaskelioff et al., "Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice," *Nature* 469 (2011): 102-7.

136.

DNA REPAIR

Learning Objectives

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

- Discuss the different types of mutations in DNA
- Explain DNA repair mechanisms

DNA replication is a highly accurate process, but mistakes can occasionally occur, such as a DNA polymerase inserting a wrong base. Uncorrected mistakes may sometimes lead to serious consequences, such as cancer. Repair mechanisms correct the mistakes. In rare cases, mistakes are not corrected, leading to mutations; in other cases, repair enzymes are themselves mutated or defective.

Most of the mistakes during DNA replication are promptly corrected by the proofreading ability of DNA polymerase itself. (Figure 14.17). In **proofreading**, the DNA pol reads the newly added base before adding the next one, so a correction can be made. The polymerase checks whether the newly added base has paired correctly with the base in the template strand. If it is the right base, the next nucleotide is added. If an incorrect base has been added, the enzyme makes a cut at the phosphodiester bond and releases the wrong nucleotide. This is performed by the 3′ exonuclease action of DNA pol. Once the incorrect nucleotide has been removed, it can be replaced by the correct one.

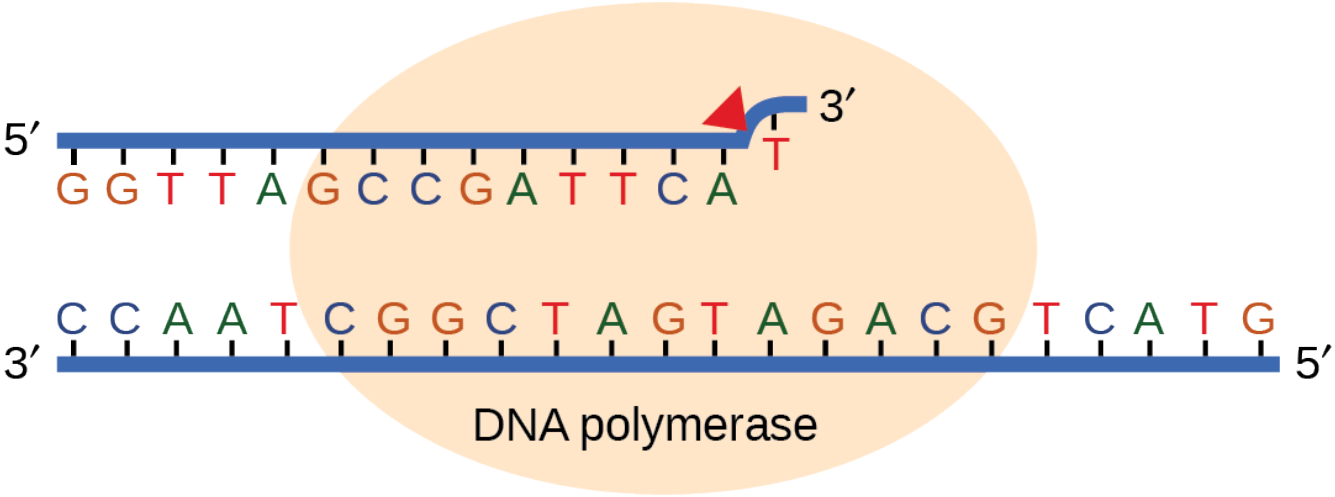


Figure 14.17 Proofreading by DNA polymerase corrects errors during replication.

Some errors are not corrected during replication, but are instead corrected after replication is completed; this type of repair is known as **mismatch repair** (Figure 14.18). Specific repair enzymes recognize the mispaired nucleotide and excise part of the strand that contains it; the excised region is then resynthesized. If the mismatch remains uncorrected, it may lead to more permanent damage when the mismatched DNA is replicated. How do mismatch repair enzymes recognize which of the two bases is the incorrect one? In *E. coli*, after replication, the nitrogenous base adenine acquires a methyl group; the parental DNA strand will have methyl groups, whereas the newly synthesized strand lacks them. Thus, DNA polymerase is able to remove the wrongly incorporated bases from the newly synthesized, non-methylated strand. In eukaryotes, the mechanism is not very well understood, but it is believed to involve recognition of unsealed nicks in the new strand, as well as a short-term continuing association of some of the replication proteins with the new daughter strand after replication has completed.

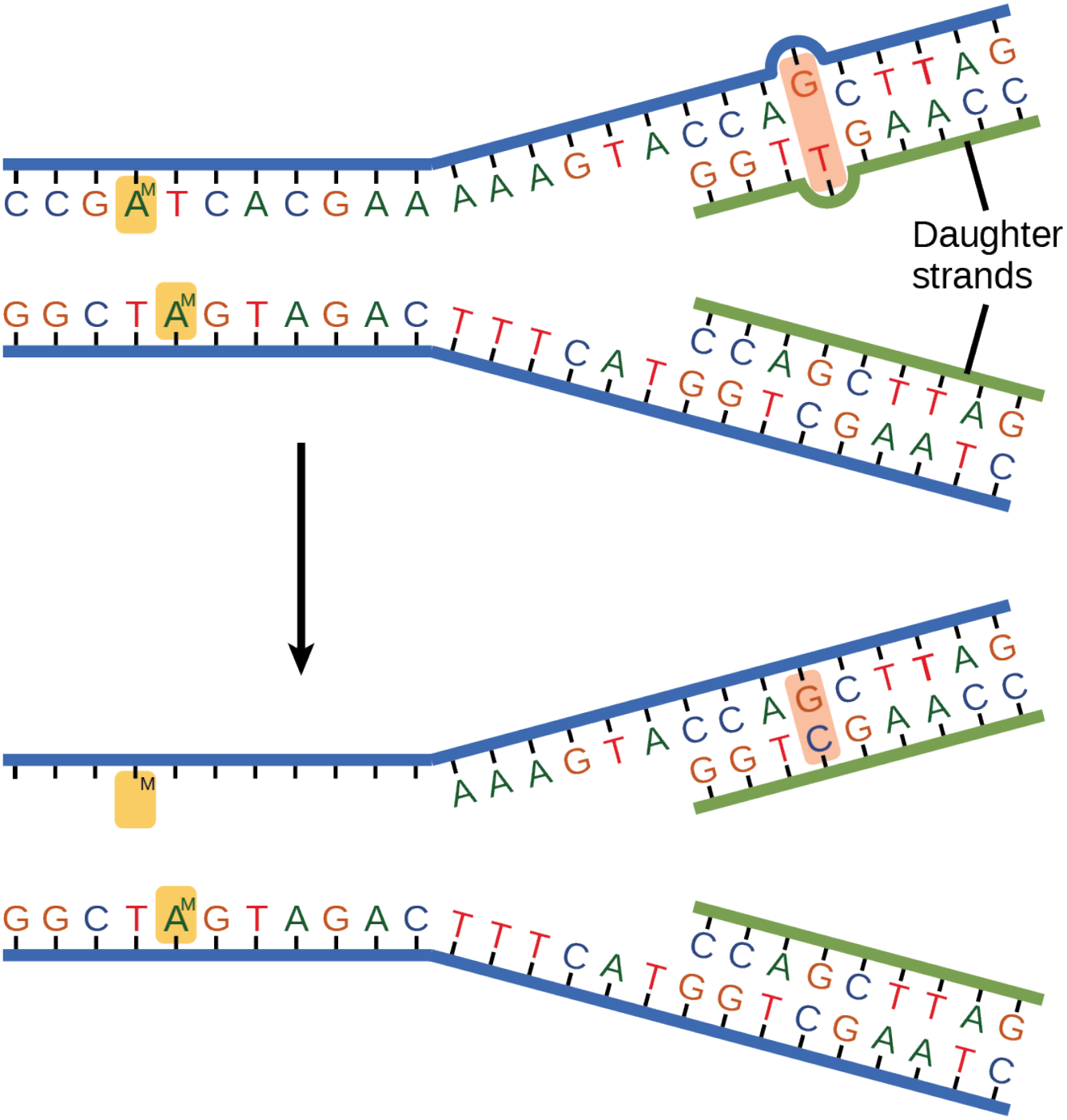


Figure 14.18 In mismatch repair, the incorrectly added base is detected after replication. The mismatch repair proteins detect this base and remove it from the newly synthesized strand by nuclease action. The gap is now filled with the correctly paired base.

Another type of repair mechanism, **nucleotide excision repair**, is similar to mismatch repair, except that it is used to remove damaged bases rather than mismatched ones. The repair enzymes replace abnormal bases by making a cut on both the 3′ and 5′ ends of the damaged base (Figure 14.19). The segment of DNA is removed and replaced with the correctly paired nucleotides by the action of DNA pol. Once the bases are filled in, the

remaining gap is sealed with a phosphodiester linkage catalyzed by DNA ligase. This repair mechanism is often employed when UV exposure causes the formation of pyrimidine dimers.

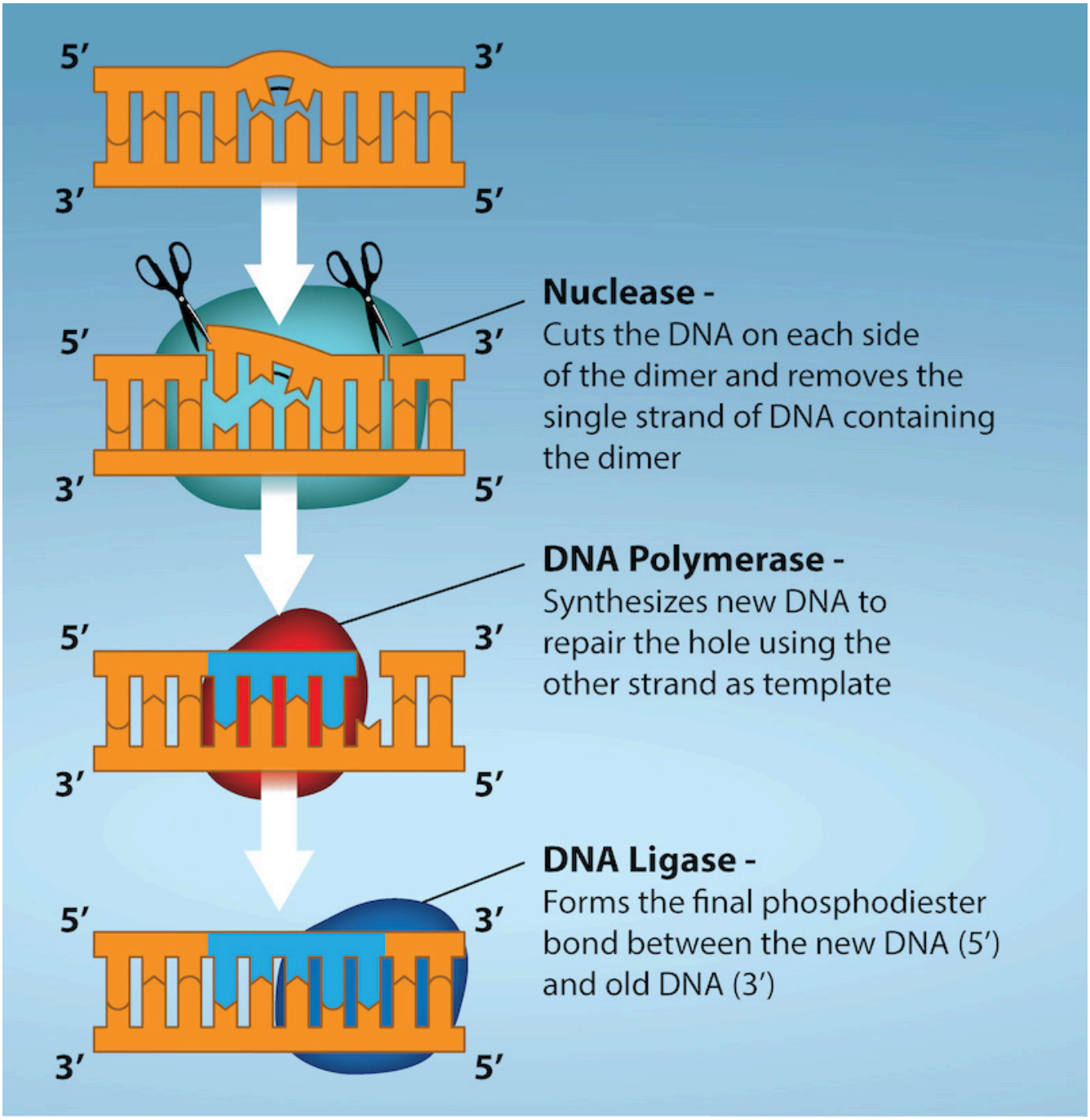


Figure 14.19 Nucleotide excision repairs thymine dimers. When exposed to UV light, thymines lying adjacent to each other can form thymine dimers. In normal cells, they are excised and replaced. Credit: Rao, A., Fletcher, S. and Tag, A. Department of Biology, Texas A&M University.

A well-studied example of mistakes not being corrected is seen in people suffering from xeroderma pigmentosa (Figure 14.20). Affected individuals have skin that is highly sensitive to UV rays from the sun. When individuals are exposed to UV light, pyrimidine dimers, especially those of thymine, are formed; people with xeroderma pigmentosa are not able to repair the damage. These are not repaired because of a defect in the nucleotide excision repair enzymes, whereas in normal individuals, the thymine dimers are excised and the defect is corrected. The thymine dimers distort the structure of the DNA double helix, and this may cause problems during DNA replication. People with xeroderma pigmentosa may have a higher risk of contracting skin cancer than those who don't have the condition.

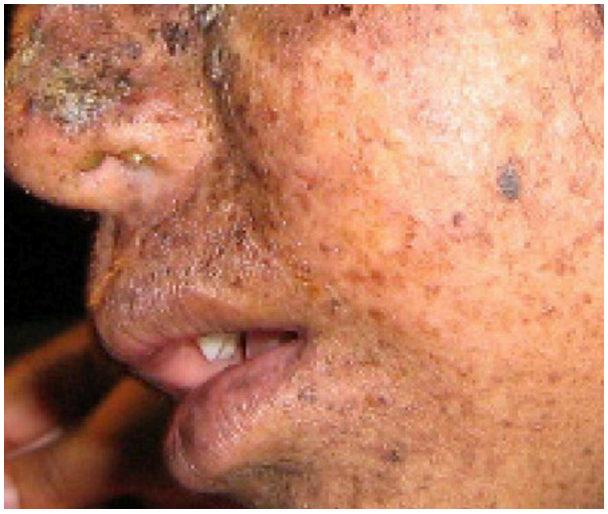


Figure 14.20 Xeroderma pigmentosa is a condition in which thymine dimerization from exposure to UV light is not repaired. Exposure to sunlight results in skin lesions. (credit: James Halpern et al.)

Errors during DNA replication are not the only reason why mutations arise in DNA. **Mutations**, variations in the nucleotide sequence of a genome, can also occur because of damage to DNA. Such mutations may be of two types: induced or spontaneous. **Induced mutations** are those that result from an exposure to chemicals, UV rays, x-rays, or some other environmental agent. For example, Charlotte Auerbach and J.M Robson discovered the mutation-inducing effects of mustard gas. **Spontaneous mutations** occur without any exposure to any environmental agent; they are a result of natural reactions taking place within the body.

Mutations may have a wide range of effects. Point mutations are those mutations that affect a single base pair. The most common nucleotide mutations are substitutions, in which one base is replaced by another. These substitutions can be of two types, either transitions or transversions. **Transition substitution** refers to a purine or pyrimidine being replaced by a base of the same kind; for example, a purine such as adenine may be replaced by the purine guanine. **Transversion substitution** refers to a purine being replaced by a pyrimidine, or vice versa; for example, cytosine, a pyrimidine, is replaced by adenine, a purine. Some point

mutations are not detectable in the final product; these are known as silent mutations. Silent mutations are usually due to a substitution in the third base of a codon, which often represents the same amino acid as the original codon. Other point mutations can result in the replacement of one amino acid by another, which may alter the function of the protein. Point mutations that generate a stop codon can terminate a protein early.

Some mutations can result in an increased number of copies of the same codon. These are called trinucleotide repeat expansions and result in repeated regions of the same amino acid. Mutations can also be the result of the addition of a base, known as an insertion, or the removal of a base, also known as deletion. If an insertion or deletion results in the alteration of the translational reading frame (a frameshift mutation), the resultant protein is usually nonfunctional. Sometimes a piece of DNA from one chromosome may get translocated to another chromosome or to another region of the same chromosome; this is also known as translocation. These mutation types are shown in Figure 14.21.

Visual Connection

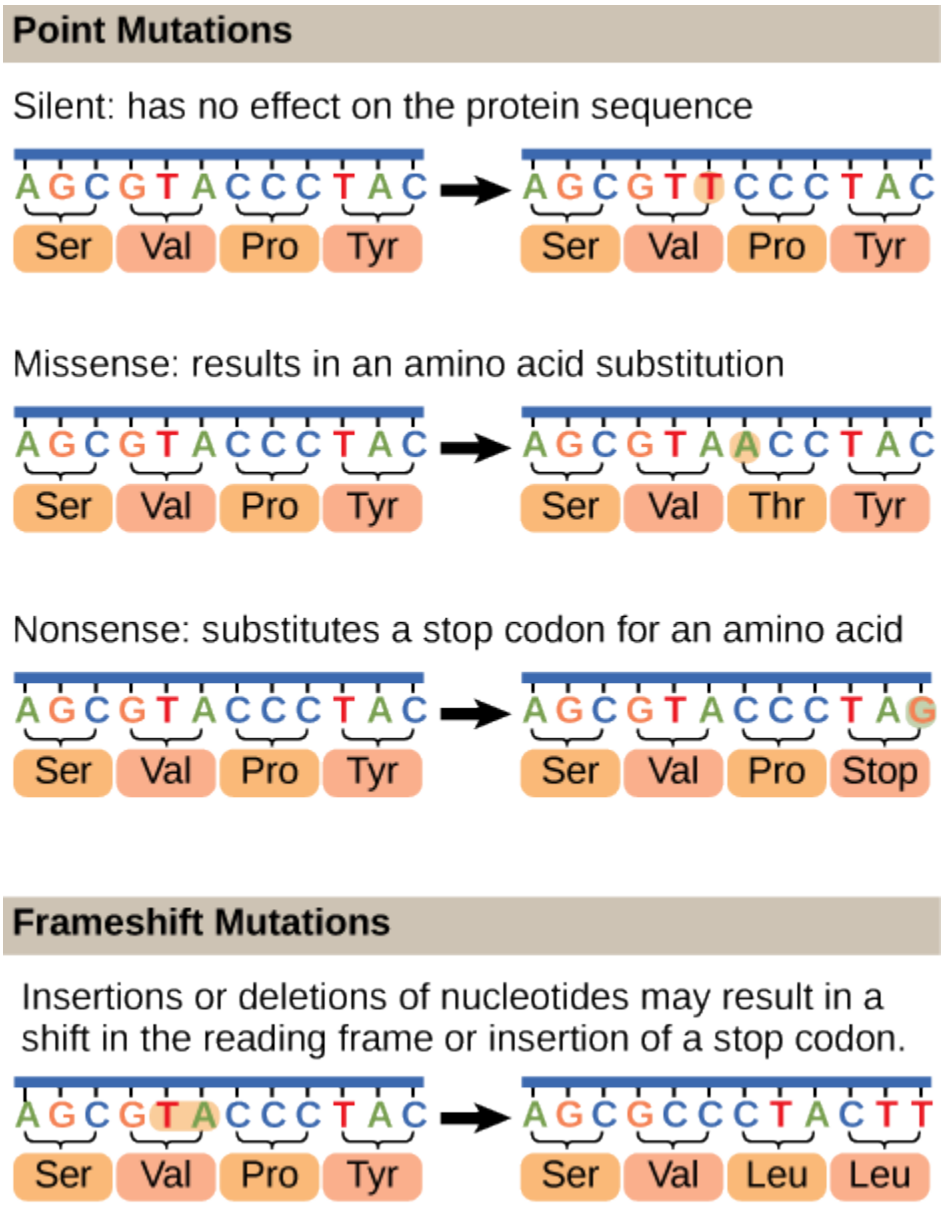


Figure 14.21 Mutations can lead to changes in the protein sequence encoded by the DNA.

A frameshift mutation that results in the insertion of three nucleotides is often less deleterious than a mutation that results in the insertion of one nucleotide. Why?

Link to Learning

Learn more by watching this video Mechanisms of DNA Damage and Repair

Mutations in repair genes have been known to cause cancer. Many mutated repair genes have been implicated in certain forms of pancreatic cancer, colon cancer, and colorectal cancer. Mutations can affect either somatic cells or germ cells. If many mutations accumulate in a somatic cell, they may lead to problems such as the uncontrolled cell division observed in cancer. If a mutation takes place in germ cells, the mutation will be passed on to the next generation, as in the case of hemophilia and xeroderma pigmentosa.

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137.

KEY TERMS

electrophoresis
: technique used to separate DNA fragments according to size

helicase
: during replication, this enzyme helps to open up the DNA helix by breaking the hydrogen bonds

induced mutation
: mutation that results from exposure to chemicals or environmental agents

lagging strand
: during replication, the strand that is replicated in short fragments and away from the replication fork

leading strand
: strand that is synthesized continuously in the 5′-3′ direction, which is synthesized in the direction of the replication fork

ligase
: enzyme that catalyzes the formation of a phosphodiester linkage between the 3′ OH and 5′ phosphate ends of the DNA

mismatch repair
: type of repair mechanism in which mismatched bases are removed after replication

mutation
: variation in the nucleotide sequence of a genome

nucleotide excision repair
: type of DNA repair mechanism in which the wrong base, along with a few nucleotides upstream or downstream, are removed

Okazaki fragment
: DNA fragment that is synthesized in short stretches on the lagging strand

point mutation
: mutation that affects a single base

primase
: enzyme that synthesizes the RNA primer; the primer is needed for DNA pol to start synthesis of a new DNA strand

primer
: short stretch of nucleotides that is required to initiate replication; in the case of replication, the primer

has RNA nucleotides

proofreading
: function of DNA pol in which it reads the newly added base before adding the next one

replication fork
: Y-shaped structure formed during initiation of replication

silent mutation
: mutation that is not expressed

single-strand binding protein
: during replication, protein that binds to the single-stranded DNA; this helps in keeping the two strands of DNA apart so that they may serve as templates

sliding clamp
: ring-shaped protein that holds the DNA pol on the DNA strand

spontaneous mutation
: mutation that takes place in the cells as a result of chemical reactions taking place naturally without exposure to any external agent

telomerase
: enzyme that contains a catalytic part and an inbuilt RNA template; it functions to maintain telomeres at chromosome ends

telomere
: DNA at the end of linear chromosomes

topoisomerase
: enzyme that prevents overwinding of DNA when DNA replication is taking place

transformation
: process in which external DNA is taken up by a cell

transition substitution
: when a purine is replaced with a purine or a pyrimidine is replaced with another pyrimidine

transversion substitution
: when a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine

138.

CHAPTER SUMMARY

14.1 Historical Basis of Modern Understanding

DNA was first isolated from white blood cells by Friedrich Miescher, who called it nuclein because it was isolated from nuclei. Frederick Griffith's experiments with strains of *Streptococcus pneumoniae* provided the first hint that DNA may be the transforming principle. Avery, MacLeod, and McCarty showed that DNA is required for the transformation of bacteria. Later experiments by Hershey and Chase using bacteriophage T2 proved that DNA is the genetic material. Chargaff found that the ratio of A = T and C = G, and that the percentage content of A, T, G, and C is different for different species.

14.2 DNA Structure and Sequencing

The currently accepted model of the double-helix structure of DNA was proposed by Watson and Crick. Some of the salient features are that the two strands that make up the double helix have complementary base sequences and anti-parallel orientations. Alternating deoxyribose sugars and phosphates form the backbone of the structure, and the nitrogenous bases are stacked like rungs inside. The diameter of the double helix, 2 nm, is uniform throughout. A purine always pairs with a pyrimidine; A pairs with T, and G pairs with C. One turn of the helix has 10 base pairs. Prokaryotes are much simpler than eukaryotes in many of their features. Most prokaryotes contain a single, circular chromosome. In general, eukaryotic chromosomes contain a linear DNA molecule packaged into nucleosomes, and have two distinct regions that can be distinguished by staining, reflecting different states of packaging and compaction.

14.3 Basics of DNA Replication

During cell division, each daughter cell receives a copy of each molecule of DNA by a process known as DNA replication. The single chromosome of a prokaryote or each chromosome of a eukaryote consists of a single continuous double helix. The model for DNA 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. In the conservative model of replication, the parental DNA is conserved, and the daughter DNA is newly synthesized. The semi-conservative model suggests that each of the two parental DNA strands acts as

a template for new DNA to be synthesized; after replication, each double-stranded DNA retains the parental or "old" strand and one "new" strand. The dispersive model suggested that the two copies of the DNA would have segments of parental DNA and newly synthesized DNA. The Meselson and Stahl experiment supported the semi-conservative model of replication, in which an entire replicated chromosome consists of one parental strand and one newly synthesized strand of DNA.

14.4 DNA Replication in Prokaryotes

Replication in prokaryotes starts from a sequence found on the chromosome called the origin of replication—the point at which the DNA opens up. Helicase opens up the DNA double helix, resulting in the formation of the replication fork. Single-strand binding proteins bind to the single-stranded DNA near the replication fork to keep the fork open. Primase synthesizes an RNA primer to initiate synthesis by DNA polymerase, which can add nucleotides only to the 3′ end of a previously synthesized primer strand. Both new DNA strands grow according to their respective 5′-3′ directions. 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, which creates phosphodiester bonds between the 3′-OH of one end and the 5′ phosphate of the other strand.

14.5 DNA Replication in Eukaryotes

Replication in eukaryotes starts at multiple origins of replication. The mechanism is quite similar to that in prokaryotes. A primer is required to initiate synthesis, which is then extended by DNA polymerase as it adds nucleotides one by one to the growing chain. The leading strand is synthesized continuously, whereas the lagging strand is synthesized in short stretches called Okazaki fragments. The RNA primers are replaced with DNA nucleotides; the DNA Okazaki fragments are linked into one continuous strand by DNA ligase. The ends of the chromosomes pose a problem as the primer RNA at the 5' ends of the DNA cannot be replaced with DNA, and the chromosome is progressively shortened. Telomerase, an enzyme with an inbuilt RNA template, extends the ends by copying the RNA template and extending one strand of the chromosome. DNA polymerase can then fill in the complementary DNA strand using the regular replication enzymes. In this way, the ends of the chromosomes are protected.

14.6 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 before proceeding with elongation. 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. In yet another type of repair, nucleotide excision repair, a damaged base is 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. The ends of the newly synthesized fragment are attached to the rest of the DNA using DNA ligase, which creates a phosphodiester bond.

Most mistakes are corrected, and if they are not, they may result in a mutation, defined as a permanent change in the DNA sequence. Mutations can be of many types, such as substitution, deletion, insertion, and trinucleotide repeat expansions. Mutations in repair genes may lead to serious consequences such as cancer. Mutations can be induced or may occur spontaneously.

139.

VISUAL CONNECTION QUESTIONS

1. Figure 14.10 In eukaryotic cells, DNA and RNA synthesis occur in a separate compartment from protein synthesis. In prokaryotic cells, both processes occur together. What advantages might there be to separating the processes? What advantages might there be to having them occur together?

2. Figure 14.14 You isolate a cell strain in which the joining 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?

3. Figure 14.21 A frameshift mutation that results in the insertion of three nucleotides is often less deleterious than a mutation that results in the insertion of one nucleotide. Why?

140.

REVIEW QUESTIONS

4. If DNA of a particular species was analyzed and it was found that it contains 27 percent A, what would be the percentage of C?

a. 27 percent
b. 30 percent
c. 23 percent
d. 54 percent

5. The experiments by Hershey and Chase helped confirm that DNA was the hereditary material on the basis of the finding that:

a. radioactive phage were found in the pellet
b. radioactive cells were found in the supernatant
c. radioactive sulfur was found inside the cell
d. radioactive phosphorus was found in the cell

6. Bacterial transformation is a major concern in many medical settings. Why might health care providers be concerned?

a. Pathogenic bacteria could introduce disease-causing genes in non-pathogenic bacteria.
b. Antibiotic resistance genes could be introduced to new bacteria to create “superbugs.”
c. Bacteriophages could spread DNA encoding toxins to new bacteria.
d. All of the above.

7. DNA double helix does not have which of the following?

a. antiparallel configuration
b. complementary base pairing
c. major and minor grooves
d. uracil

8. In eukaryotes, what is the DNA wrapped around?

a. single-stranded binding proteins
b. sliding clamp
c. polymerase
d. histones

9. Meselson and Stahl's experiments proved that DNA replicates by which mode?

a. conservative
b. semi-conservative
c. dispersive
d. none of the above

10. If the sequence of the 5′-3′ strand is AATGCTAC, then the complementary sequence has the following sequence:

a. 3′-AATGCTAC-5′
b. 3′-CATCGTAA-5′
c. 3′-TTACGATG-5′
d. 3′-GTAGCATT-5′

11. How did Meselson and Stahl support Watson and Crick's double-helix model?

a. They demonstrated that each strand serves as a template for synthesizing a new strand of DNA.
b. They showed that the DNA strands break and recombine without losing genetic material.
c. They proved that DNA maintains a double-helix structure while undergoing semi-conservative replication.
d. They demonstrated that conservative replication maintains the complementary base pairing of each DNA helix.

12. Which of the following components is not involved during the formation of the replication fork?

a. single-strand binding proteins
b. helicase
c. origin of replication
d. ligase

13. Which of the following does the enzyme primase synthesize?

a. DNA primer
b. RNA primer
c. Okazaki fragments
d. phosphodiester linkage

14. In which direction does DNA replication take place?

a. 5′-3′
b. 3′-5′
c. 5′
d. 3′

15. A scientist randomly mutates the DNA of a bacterium. She then sequences the bacterium's daughter cells, and finds that the daughters have many errors in their replicated DNA. The parent bacterium likely acquired a mutation in which enzyme?

a. DNA ligase
b. DNA pol II
c. Primase
d. DNA pol I

16. The ends of the linear chromosomes are maintained by

a. helicase
b. primase
c. DNA pol
d. telomerase

17. Which of the following is not a true statement comparing prokaryotic and eukaryotic DNA replication?

a. Both eukaryotic and prokaryotic DNA polymerases build off RNA primers made by primase.
b. Eukaryotic DNA replication requires multiple replication forks, while prokaryotic replication uses a single origin to rapidly replicate the entire genome.
c. DNA replication always occurs in the nucleus.
d. Eukaryotic DNA replication involves more polymerases than prokaryotic replication.

18. During proofreading, which of the following enzymes reads the DNA?

a. primase
b. topoisomerase
c. DNA pol
d. helicase

19. The initial mechanism for repairing nucleotide errors in DNA is _____.

a. mismatch repair
b. DNA polymerase proofreading
c. nucleotide excision repair
d. thymine dimers

20. A scientist creates fruit fly larvae with a mutation that eliminates the exonuclease function of DNA pol III. Which prediction about the mutational load in the adult fruit flies is most likely to be correct?

a. The adults with the DNA pol III mutation will have significantly more mutations than average.
b. The adults with the DNA pol III mutation will have slightly more mutations than average.
c. The adults with the DNA pol III mutation will have the same number of mutations as average.
d. The adults with the DNA pol III mutation will have fewer mutations than average.

141.

CRITICAL THINKING QUESTIONS

21. If a purine were substituted for a pyrimidine at a single position in one strand of a DNA double helix, what would happen?

22. The DNA double helix looks like a twisted ladder. What makes up each rung of the ladder? What holds the rungs together at the sides?

23. Is there mostly empty space between the atoms in a DNA double helix?

24. In a DNA double helix, why doesn't an A or T form two hydrogen bonds (out of the three possible) with G or C?

PART XV
GENES AND PROTEINS

142.

INTRODUCTION

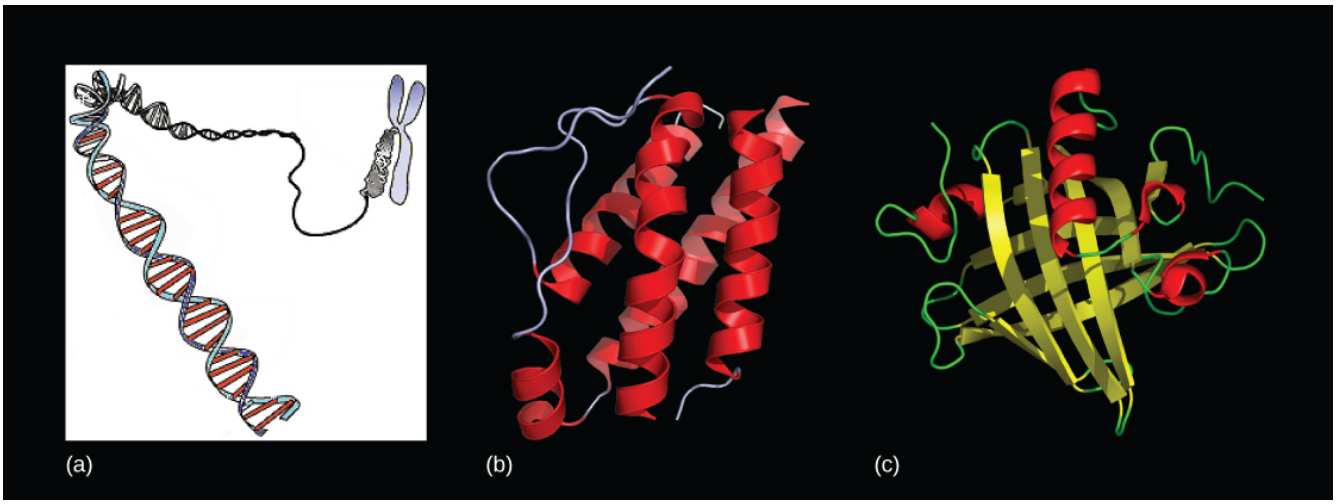

Figure 15.1 Genes, which are carried on (a) chromosomes, are linearly organized instructions for making the RNA and protein molecules that are necessary for all of the processes of life. The (b) interleukin-2 protein and (c) alpha-2u-globulin protein are just two examples of the array of different molecular 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)

Since the rediscovery of Mendel's work in 1900, the definition of the gene has progressed from an abstract unit of heredity to a tangible molecular entity capable of replication, expression, and mutation (Figure 15.1). Genes are composed of DNA and are linearly arranged on chromosomes. Genes specify the sequences of amino acids, which are the building blocks of proteins. In turn, proteins are responsible for orchestrating nearly every function of the cell. Both genes and the proteins they encode are absolutely essential to life as we know it.

143.

THE GENETIC CODE

Learning Objectives

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

- Explain the "central dogma" of DNA-protein synthesis
- Describe the genetic code and how the nucleotide sequence prescribes the amino acid and the protein sequence

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 on ribosomes converts nucleotide-based genetic information into a protein product. That is the central dogma of DNA-protein synthesis. Protein sequences consist of 20 commonly occurring amino acids; therefore, it can be said that the protein alphabet consists of 20 "letters" (Figure 15.2). Different amino acids have different chemistries (such as acidic versus basic, or polar and nonpolar) and different structural constraints. Variation in amino acid sequence is responsible for the enormous variation in protein structure and function.

Link to Learning

Review the video on the Genetic Code and the video on Eukaryotic 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 15.3), which states that genes specify the sequence of mRNAs, which in turn specify the sequence of amino acids making up all proteins. The decoding of one molecule to another is performed by specific proteins and RNAs. Because the information stored in DNA is so central to cellular function, it makes intuitive sense that the cell would make mRNA copies of this information for protein synthesis, while keeping the DNA itself intact and protected. The copying of DNA to RNA is relatively straightforward, with one nucleotide being added to the mRNA strand for every nucleotide read in the DNA strand. The translation to protein is a bit more complex because three mRNA nucleotides correspond to one amino acid in the polypeptide sequence. However, the translation to protein is still systematic and **colinear**, such that nucleotides 1 to 3 correspond to amino acid 1, nucleotides 4 to 6 correspond to amino acid 2, and so on.

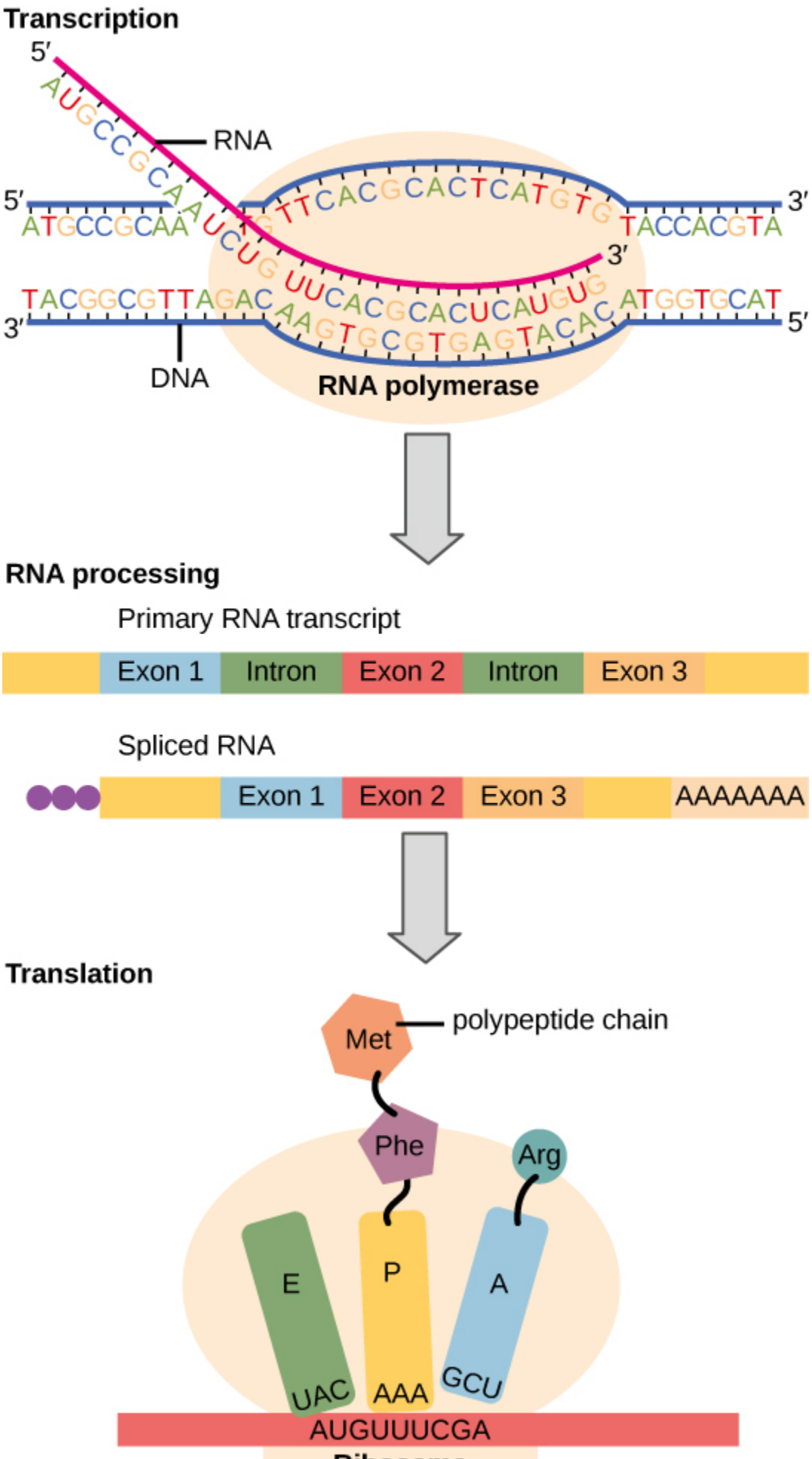
Transcription
5′
AUGCCGCAAUCUG
RNA
5′
ATGCCGCAA
TTCACGCACTCATGTG
TACCACGTA
3′
3′
TACGGCGTTAGAC
AAGTGCGTGAGTACAC
ATGGTGCAT
5′
3′
DNA
UUCACGCACUCAUGUG
RNA polymerase
RNA processing
Primary RNA transcript
Exon 1
Intron
Exon 2
Intron
Exon 3
Spliced RNA
Exon 1
Exon 2
Exon 3
AAAAAAA
Translation
Met
polypeptide chain
Phe
Arg
E
P
A
UAC
AAA
GCU
AUGUUUCGA

Figure 15.3 Instructions on DNA are transcribed onto messenger RNA. Ribosomes are able to read the genetic information inscribed on a strand of messenger RNA and use this information to string amino acids together into a protein.

The Genetic Code Is Degenerate and Universal

Each amino acid is defined by a three-nucleotide sequence called the triplet codon. Given the different numbers of "letters" in the mRNA and protein "alphabets," scientists theorized that single amino acids must be represented by combinations of nucleotides. Nucleotide doublets would not be sufficient to specify every amino acid because there are only 16 possible two-nucleotide combinations (4^2). In contrast, there are 64 possible nucleotide triplets (4^3), which is far more than the number of amino acids. Scientists theorized that amino acids were encoded by nucleotide triplets and that the genetic code was "**degenerate**." In other words, a given amino acid could be encoded by more than one nucleotide triplet. This was later confirmed experimentally: Francis Crick and Sydney Brenner used the chemical mutagen proflavin to insert one, two, or three nucleotides into the gene of a virus. When one or two nucleotides were inserted, the normal proteins were not produced. When three nucleotides were inserted, the protein was synthesized and functional. This demonstrated that the amino acids must be specified by groups of three nucleotides. These nucleotide triplets are called **codons**. The insertion of one or two nucleotides completely changed the triplet **reading frame**, thereby altering the message for every subsequent amino acid (Figure 15.5). Though insertion of three nucleotides caused an extra amino acid to be inserted during translation, the integrity of the rest of the protein was maintained.

Scientists painstakingly solved the genetic code by translating synthetic mRNAs in vitro and sequencing the proteins they specified (Figure 15.4).

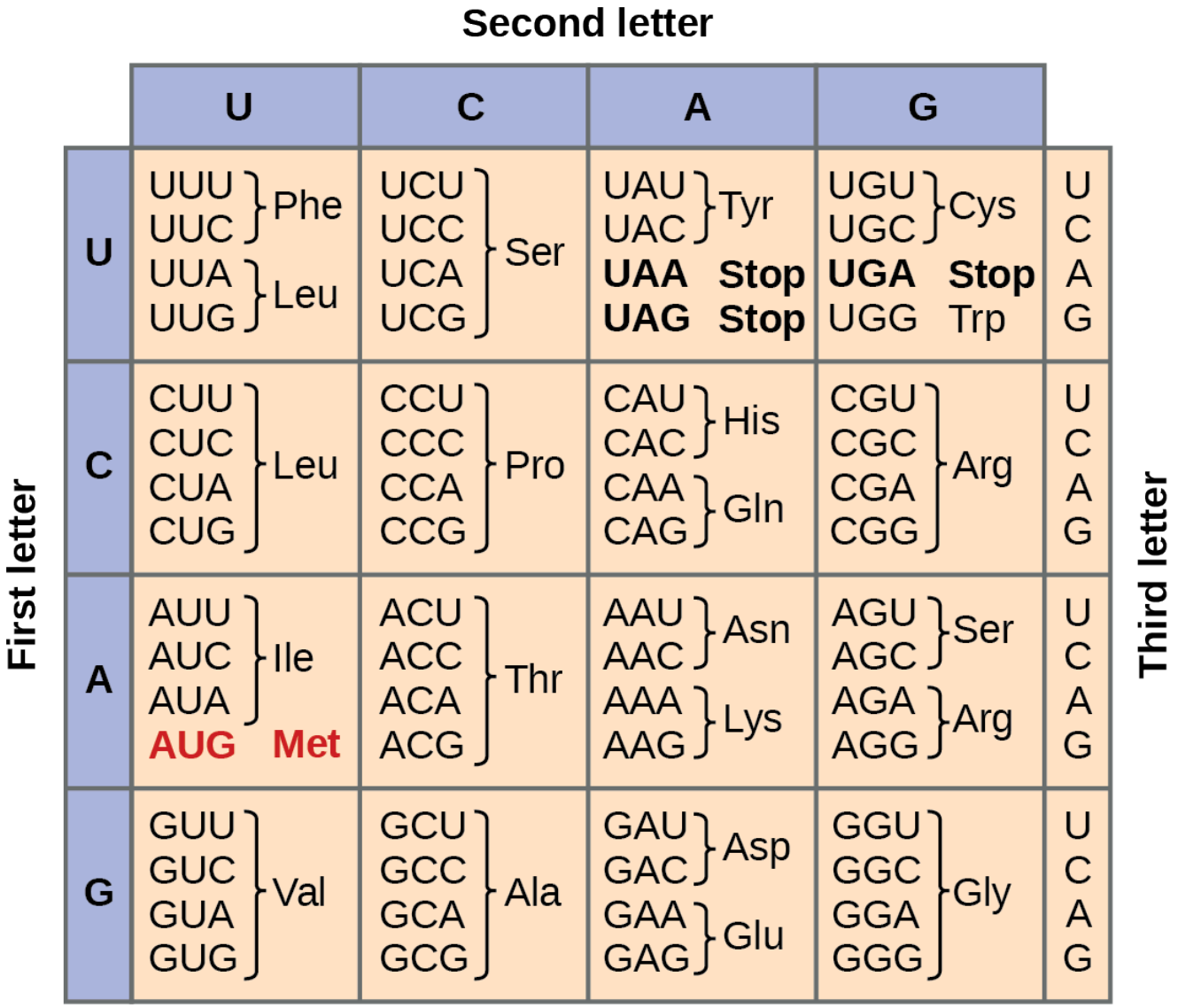

Figure 15.4 This figure shows the genetic code for translating each nucleotide triplet in mRNA into an amino acid or a termination signal in a protein. (credit: modification of work by NIH)

In addition to codons that instruct the addition of a specific amino acid to a polypeptide chain, three of the 64 codons terminate protein synthesis and release the polypeptide from the translation machinery. These triplets are called **nonsense codons**, or *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. Following the start codon, the mRNA is read in groups of three until a stop codon is encountered.

The arrangement of the coding table reveals the structure of the code. There are sixteen "blocks" of codons, each specified by the first and second nucleotides of the codons within the block, e.g., the "AC*" block that corresponds to the amino acid threonine (Thr). Some blocks are divided into a pyrimidine half, in which the codon ends with U or C, and a purine half, in which the codon ends with A or G. Some amino acids get a

whole block of four codons, like alanine (Ala), threonine (Thr) and proline (Pro). Some get the pyrimidine half of their block, like histidine (His) and asparagine (Asn). Others get the purine half of their block, like glutamate (Glu) and lysine (Lys). Note that some amino acids get a block and a half-block for a total of six codons.

The specification of a single amino acid by multiple similar codons is called "degeneracy." Degeneracy is believed to be a cellular mechanism to reduce the negative impact of random mutations. Codons that specify the same amino acid typically only differ by one nucleotide. In addition, amino acids with chemically similar side chains are encoded by similar codons. For example, aspartate (Asp) and glutamate (Glu), which occupy the GA* block, are both negatively charged. This nuance of the genetic code ensures that a single-nucleotide substitution mutation might specify the same amino acid but have no effect or specify a similar amino acid, preventing the protein from being rendered completely nonfunctional.

The genetic code is nearly universal. With a few minor exceptions, virtually all species use the same genetic code for protein synthesis. Conservation of codons means that a purified mRNA encoding the globin protein in horses could be transferred to a tulip cell, and the tulip would synthesize horse globin. That there is only one genetic code is powerful evidence that all of life on Earth shares a common origin, especially considering that there are about 10^{84} possible combinations of 20 amino acids and 64 triplet codons.

Link to Learning

Transcribe a gene and translate it to protein using complementary pairing and the genetic code at this site.

Frameshift Mutations

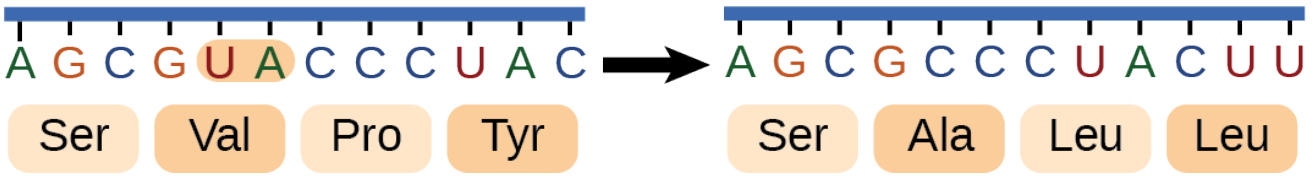

Figure 15.5 The deletion of two nucleotides shifts the reading frame of an mRNA and changes the entire protein message, creating a nonfunctional protein or terminating protein synthesis altogether.

Scientific Method Connection

Which Has More DNA: A Kiwi or a Strawberry?

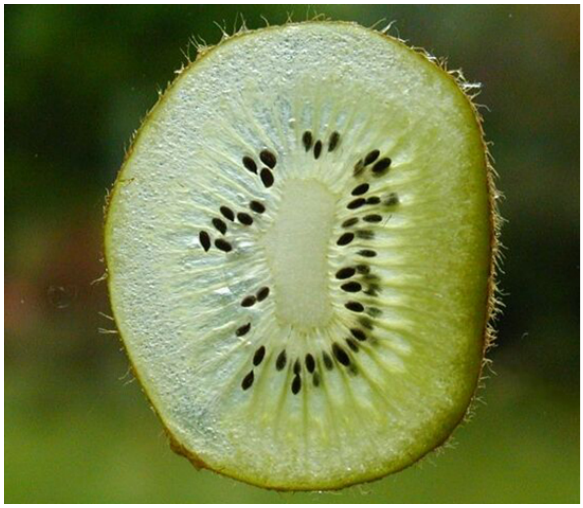

Figure 15.6 Do you think that a kiwi or a strawberry has more DNA per fruit? (credit "kiwi": "Kelbv"/Flickr; credit: "strawberry": Alisdair McDiarmid)

Question: Would a kiwi and strawberry that are approximately the same size (Figure 15.6) also have approximately the same amount of DNA?

Background: Genes are carried on chromosomes and are made of DNA. All mammals are diploid, meaning they have two copies of each chromosome. However, not all plants are diploid. The common strawberry is octoploid (8*n*) and the cultivated kiwi is hexaploid (6*n*). Research the total number of chromosomes in the cells of each of these fruits and think about how this might correspond to the amount of DNA in these fruits' cell nuclei. What other factors might contribute to the total amount of DNA in a single fruit? Read about the technique of DNA isolation to understand how each step in the isolation protocol helps liberate and precipitate DNA.

Hypothesis: Hypothesize whether you would be able to detect a difference in DNA quantity from similarly sized strawberries and kiwis. Which fruit do you think would yield more DNA?

Test your hypothesis: Isolate the DNA from a strawberry and a kiwi that are similarly sized. Perform the experiment in at least triplicate for each fruit

1. Prepare a bottle of DNA extraction buffer from 900 mL water, 50 mL dish detergent, and two teaspoons of table salt. Mix by inversion (cap it and turn it upside down a few times).
2. Grind a strawberry and a kiwi by hand in a plastic bag, or using a mortar and pestle, or with a metal bowl and the end of a blunt instrument. Grind for at least two minutes per fruit.
3. Add 10 mL of the DNA extraction buffer to each fruit, and mix well for at least one minute.
4. Remove cellular debris by filtering each fruit mixture through cheesecloth or porous cloth and into a funnel placed in a test tube or an appropriate container.
5. Pour ice-cold ethanol or isopropanol (rubbing alcohol) into the test tube. You should observe white, precipitated DNA.
6. Gather the DNA from each fruit by winding it around separate glass rods.

Record your observations: Because you are not quantitatively measuring DNA volume, you can record for each trial whether the two fruits produced the same or different amounts of DNA as observed by eye. If one or the other fruit produced noticeably more DNA, record this as well. Determine whether your observations are consistent with several pieces of each fruit.

Analyze your data: Did you notice an obvious difference in the amount of DNA produced by each fruit? Were your results reproducible?

Draw a conclusion: Given what you know about the number of chromosomes in each fruit, can you conclude that chromosome number necessarily correlates to DNA amount? Can you identify any drawbacks to this procedure? If you had access to a laboratory, how could you standardize your comparison and make it more quantitative?

144.

PROKARYOTIC TRANSCRIPTION

Learning Objectives

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

- List the different steps in prokaryotic transcription
- Discuss the role of promoters in prokaryotic transcription
- Describe how and when transcription is terminated

The prokaryotes, which include Bacteria and Archaea, are mostly single-celled organisms that, by definition, lack membrane-bound nuclei and other organelles. A bacterial chromosome is a closed circle that, unlike eukaryotic chromosomes, is not organized around histone proteins. The central region of the cell in which prokaryotic DNA resides is called the nucleoid region. In addition, prokaryotes often have abundant **plasmids**, which are shorter, circular DNA molecules that may only contain one or a few genes. Plasmids can be transferred independently of the bacterial chromosome during cell division and often carry traits such as those involved with antibiotic resistance.

Transcription in prokaryotes (and in eukaryotes) requires the DNA double helix to partially unwind in the region of mRNA synthesis. The region of unwinding is called a **transcription bubble**. Transcription always proceeds from the same DNA strand for each gene, 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**, or the coding strand. The only nucleotide difference is that in mRNA, all of the T nucleotides are replaced with U nucleotides (Figure 15.7). In an RNA double helix, A can bind U via two hydrogen bonds, just as in A–T pairing in a DNA double helix.

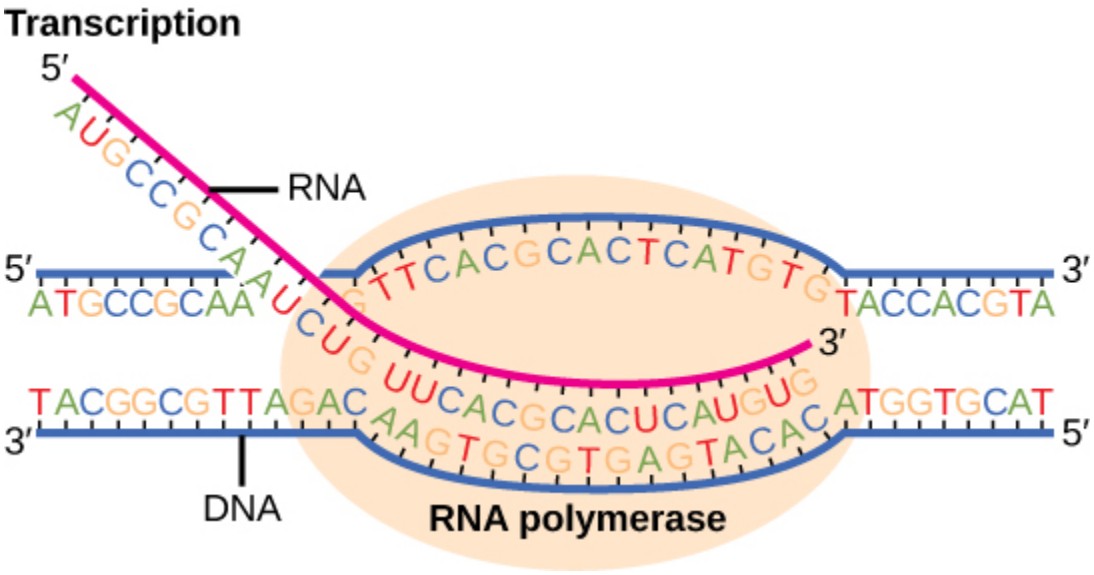

Figure 15.7 Messenger RNA is a copy of protein-coding information in the coding strand of DNA, with the substitution of U in the RNA for T in the coding sequence. However, new RNA nucleotides base pair with the nucleotides of the template strand. RNA is synthesized in its 5'-3' direction, using the enzyme RNA polymerase. As the template is read, the DNA unwinds ahead of the polymerase and then rewinds behind it.

The nucleotide pair in the DNA double helix that corresponds to the site from which the first 5′ mRNA nucleotide is transcribed is called the +1 site, or the initiation site. Nucleotides preceding the initiation site are denoted with a "-" and are designated *upstream nucleotides*. Conversely, nucleotides following the initiation site are denoted with "+" numbering and are called *downstream nucleotides*.

Initiation of Transcription in Prokaryotes

Prokaryotes do not have membrane-enclosed nuclei. Therefore, the processes of transcription, translation, and mRNA degradation can all occur simultaneously. The intracellular level of a bacterial protein can quickly be amplified by multiple transcription and translation events that occur concurrently on the same DNA template. Prokaryotic genomes are very compact, and prokaryotic transcripts often cover more than one gene or cistron (a coding sequence for a single protein). Polycistronic mRNAs are then translated to produce more than one kind of protein.

Our discussion here will exemplify transcription by describing this process in *Escherichia coli*, a well-studied eubacterial species. Although some differences exist between transcription in *E. coli* and transcription in archaea, an understanding of *E. coli* transcription can be applied to virtually all bacterial species.

Prokaryotic RNA Polymerase

Prokaryotes use the same RNA polymerase to transcribe all of their genes. In *E. coli*, the polymerase is

composed of five polypeptide subunits, two of which are identical. Four of these subunits, denoted α, α, β, and β', comprise the polymerase **core enzyme.** These subunits assemble every time a gene is transcribed, and they disassemble once transcription is complete. Each subunit has a unique role; the two α-subunits are necessary to assemble the polymerase on the DNA; the β-subunit binds to the ribonucleoside triphosphate that will become part of the nascent mRNA molecule; and the β' subunit binds the DNA template strand. The fifth subunit, σ, is involved only in transcription initiation. It confers transcriptional specificity such that the polymerase begins to synthesize mRNA from an appropriate initiation site. Without σ, the core enzyme would transcribe from random sites and would produce mRNA molecules that specified protein gibberish. The polymerase comprised of all five subunits is called the holoenzyme.

Prokaryotic Promoters

A **promoter** is a DNA sequence onto which the transcription machinery, including RNA polymerase, binds and initiates transcription. 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 the time, some of the time, or infrequently. Although promoters vary among prokaryotic genomes, a few elements are evolutionarily conserved in many species. At the -10 and -35 regions upstream of the initiation site, there are two *promoter consensus sequences*, or regions that are similar across all promoters and across various bacterial species (Figure 15.8). The -10 sequence, called the -10 region, has the consensus sequence TATAAT. The -35 sequence has the consensus sequence TTGACA. These consensus sequences are recognized and bound by σ. Once this interaction is made, the subunits of the core enzyme bind to the site. The A–T-rich -10 region facilitates unwinding of the DNA template, and several phosphodiester bonds are made. The transcription initiation phase ends with the production of abortive transcripts, which are polymers of approximately 10 nucleotides that are made and released.

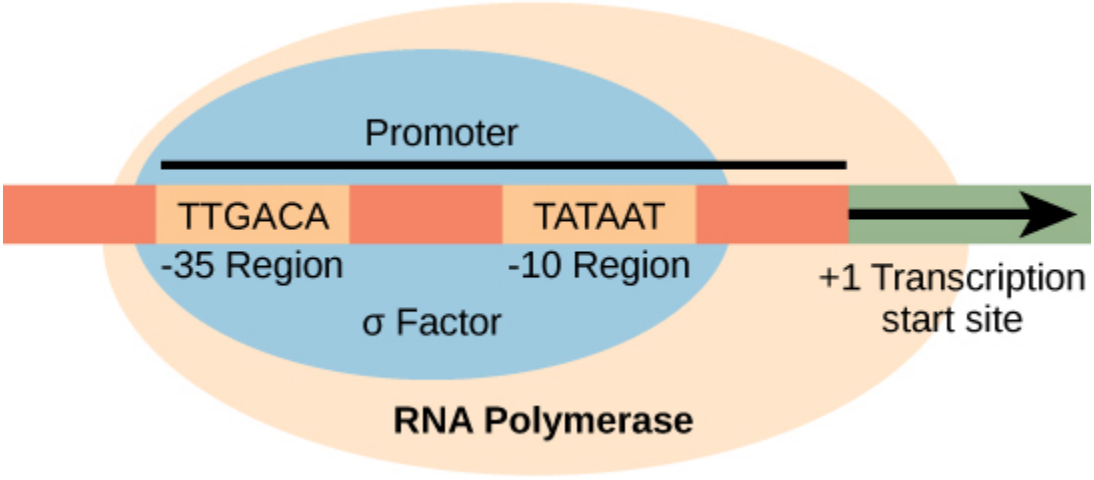

Figure 15.8 The σ subunit of prokaryotic RNA polymerase recognizes consensus sequences found in the promoter region upstream of the transcription start site. The σ subunit dissociates from the polymerase after transcription has been initiated.

Link to Learning

View this MolecularMovies animation to see the transcription process as it happens in the cell.

Elongation and Termination in Prokaryotes

The transcription elongation phase begins with the release of the σ subunit from the polymerase. The dissociation of σ allows the core enzyme to proceed along the DNA template, synthesizing mRNA in the 5′ to 3′ direction at a rate of approximately 40 nucleotides per second. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it. The base pairing between DNA and RNA is not stable enough to maintain the stability of the mRNA synthesis components. Instead, the RNA polymerase acts as a stable linker between the DNA template and the nascent RNA strands to ensure that elongation is not interrupted prematurely.

Prokaryotic Termination Signals

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. One is protein-based and the other is RNA-based. **Rho-dependent termination** is

controlled by the rho protein, which tracks along behind the polymerase on the growing mRNA chain. Near the end of the gene, the polymerase encounters a run of G nucleotides on the DNA template and it stalls. As a result, the rho protein collides with the polymerase. The interaction with rho releases the mRNA from the transcription bubble.

Rho-independent termination is controlled by specific sequences in the DNA template strand. As the polymerase nears the end of the gene being transcribed, it encounters a region rich in C–G nucleotides. The mRNA folds back on itself, and the complementary C–G nucleotides bind together. The result is a **stable hairpin** that causes the polymerase to stall as soon as it begins to transcribe a region rich in A–T nucleotides. The complementary U–A region of the mRNA transcript forms only a weak interaction with the template DNA. This, coupled with the stalled polymerase, induces enough instability for the core enzyme to break away and liberate the new mRNA transcript.

Upon termination, the process of transcription is complete. By the time termination occurs, the prokaryotic transcript would already have been used to begin synthesis of numerous copies of the encoded protein because these processes can occur concurrently. The unification of transcription, translation, and even mRNA degradation is possible because all of these processes occur in the same 5′ to 3′ direction, and because there is no membranous compartmentalization in the prokaryotic cell (Figure 15.9). In contrast, the presence of a nucleus in eukaryotic cells precludes simultaneous transcription and translation.

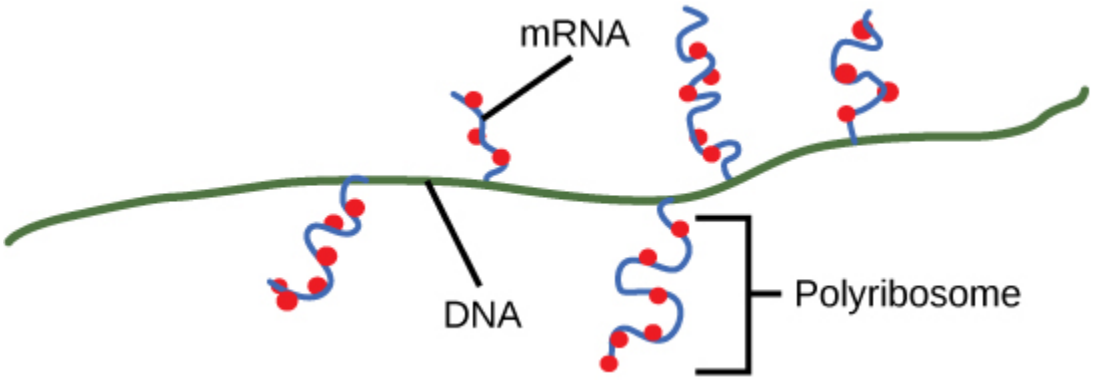

Figure 15.9 Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

Link to Learning

Visit this BioStudio animation to see the process of prokaryotic transcription.

145.

EUKARYOTIC TRANSCRIPTION

Learning Objectives

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

- List the steps in eukaryotic transcription
- Discuss the role of RNA polymerases in transcription
- Compare and contrast the three RNA polymerases
- Explain the significance of transcription factors

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few key differences. The most important difference between prokaryote and eukaryote transcription is due to the latter's membrane-bound nucleus and organelles. With the genes bound in a nucleus, the eukaryotic cell must be able to transport its mRNA to the cytoplasm and must protect its mRNA from degrading before it is translated. Eukaryotes also employ three different polymerases that each transcribe a different subset of genes. Eukaryotic mRNAs are usually *monogenic*, meaning that they specify a single protein.

Initiation of Transcription in Eukaryotes

Unlike the prokaryotic polymerase that can bind to a DNA template on its own, eukaryotes require several other proteins, called transcription factors, to first bind to the promoter region and then to help recruit the appropriate polymerase.

The Three Eukaryotic RNA Polymerases

The features of eukaryotic mRNA synthesis are markedly more complex than those of prokaryotes. Instead of

a single polymerase comprising five subunits, the eukaryotes have three polymerases that are each made up of 10 subunits or more. Each eukaryotic polymerase also requires a distinct set of transcription factors to bring it to the DNA template. RNA polymerase I is located in the nucleolus, a specialized nuclear substructure in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes (Table 15.1). The rRNA molecules are considered structural RNAs because they have a cellular role but are not translated into protein. The rRNAs are components of the ribosome and are essential to the process of translation. RNA polymerase I synthesizes all of the rRNAs from the tandemly duplicated set of 18S, 5.8S, and 28S ribosomal genes. (Note that the "S" designation applies to "Svedberg" units, a nonadditive value that characterizes the speed at which a particle sediments during centrifugation.)

Locations, Products, and Sensitivities of the Three Eukaryotic RNA Polymerases

RNA Polymerase	Cellular Compartment	Product of Transcription	α-Amanitin Sensitivity
I	Nucleolus	All rRNAs except 5S rRNA	Insensitive
II	Nucleus	All protein-coding nuclear pre-mRNAs	Extremely sensitive
III	Nucleus	5S rRNA, tRNAs, and small nuclear RNAs	Moderately sensitive

Table 15.1

RNA polymerase II is located in the nucleus and synthesizes all protein-coding nuclear pre-mRNAs. Eukaryotic pre-mRNAs undergo extensive processing after transcription but before translation. For clarity, this module's discussion of transcription and translation in eukaryotes will use the term "mRNAs" to describe only the mature, processed molecules that are ready to be translated. RNA polymerase II is responsible for transcribing the overwhelming majority of eukaryotic genes. *RNA polymerase III* is also located in the nucleus. This polymerase transcribes a variety of structural RNAs that includes the 5S pre-rRNA, transfer pre-RNAs (pre-tRNAs), and **small nuclear** pre-**RNAs**. The tRNAs have a critical role in translation; they serve as the "adaptor molecules" between the mRNA template and the growing polypeptide chain. Small nuclear RNAs have a variety of functions, including "splicing" pre-mRNAs and regulating transcription factors. A scientist characterizing a new gene can determine which polymerase transcribes it by testing whether the gene is expressed in the presence of α-amanitin, an oligopeptide toxin produced by the fly agaric toadstool mushroom and other species of *Amanita*. Interestingly, the α-amanitin affects the three polymerases very differently (Table 15.1). RNA polymerase I is completely insensitive to α-amanitin, meaning that the polymerase can transcribe DNA in vitro in the presence of this poison. RNA polymerase III is moderately sensitive to the toxin. In contrast, RNA polymerase II is extremely sensitive to α-amanitin. The toxin prevents the enzyme from progressing down the DNA, and thus inhibits transcription. Knowing the transcribing polymerase can provide clues as to the general function of the gene being studied. Because RNA polymerase II transcribes

the vast majority of genes, we will focus on this polymerase in our subsequent discussions about eukaryotic transcription factors and promoters.

RNA Polymerase II Promoters and Transcription Factors

Eukaryotic promoters are much larger and more intricate than prokaryotic promoters. However, both have a sequence similar to the -10 sequence of prokaryotes. In eukaryotes, this sequence is called the TATA box, and has the consensus sequence TATAAA on the coding strand. It is located at -25 to -35 bases relative to the initiation (+1) site (Figure 15.10). This sequence is not identical to the *E. coli* -10 box, but it conserves the A–T rich element. The thermostability of A–T bonds is low and this helps the DNA template to locally unwind in preparation for transcription.

Instead of the simple σ factor that helps bind the prokaryotic RNA polymerase to its promoter, eukaryotes assemble a complex of transcription factors required to recruit RNA polymerase II to a protein coding gene. Transcription factors that bind to the promoter are called *basal transcription factors*. These basal factors are all called TFII (for Transcription Factor/polymerase II) plus an additional letter (A-J). The core complex is TFIID, which includes a TATA-binding protein (TBP). The other transcription factors systematically fall into place on the DNA template, with each one further stabilizing the pre-initiation complex and contributing to the recruitment of RNA polymerase II.

Visual Connection

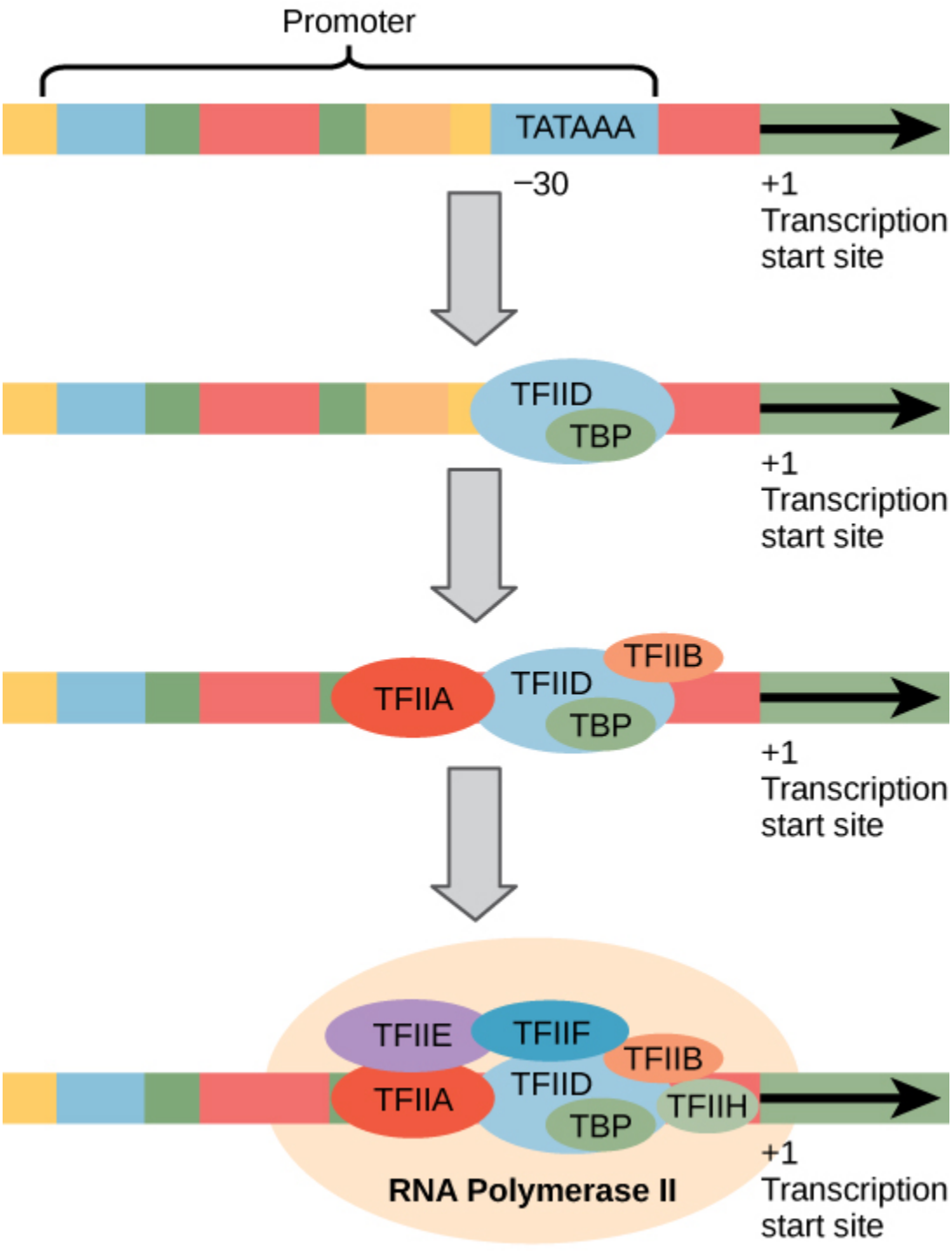

Figure 15.10 A generalized promoter of a gene transcribed by RNA polymerase II is shown. Transcription factors recognize the promoter. RNA polymerase II then binds and forms the transcription initiation complex.

A scientist splices a eukaryotic promoter in front of a bacterial gene and inserts the gene in a bacterial chromosome. Would you expect the bacteria to transcribe the gene?

A scientist splices a eukaryotic promoter in front of a bacterial gene and inserts the gene in a bacterial chromosome. Would you expect the bacteria to transcribe the gene?

Some eukaryotic promoters also have a conserved **CAAT box** (GGCCAATCT) at approximately -80. Further upstream of the TATA box, eukaryotic promoters may also contain one or more **GC-rich boxes** (GGCG) or **octamer boxes** (ATTTGCAT). These elements bind cellular factors that increase the efficiency of transcription initiation and are often identified in more "active" genes that are constantly being expressed by the cell.

Basal transcription factors are crucial in the formation of a preinitiation complex on the DNA template that subsequently recruits RNA polymerase II for transcription initiation. The complexity of eukaryotic transcription does not end with the polymerases and promoters. An army of other transcription factors, which bind to upstream enhancers and silencers, also help to regulate the frequency with which pre-mRNA is synthesized from a gene. Enhancers and silencers affect the efficiency of transcription but are not necessary for transcription to proceed.

Promoter Structures for RNA Polymerases I and III

The processes of bringing RNA polymerases I and III to the DNA template involve slightly less complex collections of transcription factors, but the general theme is the same.

The conserved promoter elements for genes transcribed by polymerases I and III differ from those transcribed by RNA polymerase II. RNA polymerase I transcribes genes that have two GC-rich promoter sequences in the -45 to +20 region. These sequences alone are sufficient for transcription initiation to occur, but promoters with additional sequences in the region from -180 to -105 upstream of the initiation site will further enhance initiation. Genes that are transcribed by RNA polymerase III have upstream promoters or promoters that occur within the genes themselves.

Eukaryotic transcription is a tightly regulated process that requires a variety of proteins to interact with each other and with the DNA strand. Although the process of transcription in eukaryotes involves a greater metabolic investment than in prokaryotes, it ensures that the cell transcribes precisely the pre-mRNAs that it needs for protein synthesis.

Evolution Connection

The Evolution of Promoters

The evolution of genes may be a familiar concept. Mutations can occur in genes during DNA replication, and the result may or may not be beneficial to the cell. By altering an enzyme, structural protein, or some other factor, the process of mutation can transform functions or physical features. However, eukaryotic promoters and other gene regulatory sequences may evolve as well. For instance, consider a gene that, over many generations, becomes more valuable to the cell. Maybe the gene encodes a structural protein that the cell needs to synthesize in abundance for a certain function. If this is the case, it would be beneficial to the cell for that gene's promoter to recruit transcription factors more efficiently and increase gene expression.

Scientists examining the evolution of promoter sequences have reported varying results. In part, this is because it is difficult to infer exactly where a eukaryotic promoter begins and ends. Some promoters occur within genes; others are located very far upstream, or even downstream, of the genes they are regulating. However, when researchers limited their examination to human core promoter sequences that were defined experimentally as sequences that bind the preinitiation complex, they found that promoters evolve even faster than protein-coding genes.

It is still unclear how promoter evolution might correspond to the evolution of humans or other complex organisms. However, the evolution of a promoter to effectively make more or less of a given gene product is an intriguing alternative to the evolution of the genes themselves.[1]

Eukaryotic Elongation and Termination

Following the formation of the preinitiation complex, the polymerase is released from the other transcription factors, and elongation is allowed to proceed as it does in prokaryotes with the polymerase synthesizing pre-mRNA in the 5′ to 3′ direction. As discussed previously, RNA polymerase II transcribes the major share of eukaryotic genes, so in this section we will focus on how this polymerase accomplishes elongation and termination.

Although the enzymatic process of elongation is essentially the same in eukaryotes and prokaryotes, the DNA template is considerably more complex. When eukaryotic cells are not dividing, their genes exist as a diffuse mass of DNA and proteins called chromatin. The DNA is tightly packaged around charged histone proteins

at repeated intervals. These *DNA–histone complexes*, collectively called nucleosomes, are regularly spaced and include 146 nucleotides of DNA wound around eight histones like thread around a spool.

For polynucleotide synthesis to occur, the transcription machinery needs to move histones out of the way every time it encounters a nucleosome. This is accomplished by a special protein complex called FACT, which stands for "*facilitates chromatin transcription*." This complex pulls histones away from the DNA template as the polymerase moves along it. Once the pre-mRNA is synthesized, the FACT complex replaces the histones to recreate the nucleosomes.

The termination of transcription is different for the different polymerases. Unlike in prokaryotes, elongation by RNA polymerase II in eukaryotes takes place 1,000 to 2,000 nucleotides beyond the end of the gene being transcribed. This pre-mRNA tail is subsequently removed by cleavage during mRNA processing. On the other hand, RNA polymerases I and III require termination signals. Genes transcribed by RNA polymerase I contain a specific 18-nucleotide sequence that is recognized by a termination protein. The process of termination in RNA polymerase III involves an mRNA hairpin similar to rho-independent termination of transcription in prokaryotes.

Link to Learning

Review these videos to learn more about concepts:

- Lac operon and the regulation of gene expressions in prokaryotes
- DNA Translation: Elongation and Ribosome Sites

Footnotes

- 1 H Liang et al., "Fast evolution of core promoters in primate genomes," *Molecular Biology and Evolution* 25 (2008): 1239–44.

146.

RNA PROCESSING IN EUKARYOTES

Learning Objectives

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

- Describe the different steps in RNA processing
- Understand the significance of exons, introns, and splicing for mRNAs
- Explain how tRNAs and rRNAs are processed

After transcription, eukaryotic pre-mRNAs must undergo several processing steps before they can be translated. Eukaryotic (and prokaryotic) tRNAs and rRNAs also undergo processing before they can function as components in the protein-synthesis machinery.

mRNA Processing

The eukaryotic pre-mRNA undergoes extensive processing before it is ready to be translated. Eukaryotic protein-coding sequences are not continuous, as they are in prokaryotes. The coding sequences (exons) are interrupted by noncoding introns, which must be removed to make a translatable mRNA. The additional steps involved in eukaryotic mRNA maturation also create a molecule with a much longer half-life than a prokaryotic mRNA. Eukaryotic mRNAs last for several hours, whereas the typical *E. coli* mRNA lasts no more than five seconds.

Pre-mRNAs are first coated in RNA-stabilizing proteins; these protect the pre-mRNA from degradation while it is processed and exported out of the nucleus. The three most important steps of pre-mRNA processing are the addition of stabilizing and signaling factors at the 5′ and 3′ ends of the molecule, and the removal of the introns (Figure 15.11). In rare cases, the mRNA transcript can be "edited" after it is transcribed.

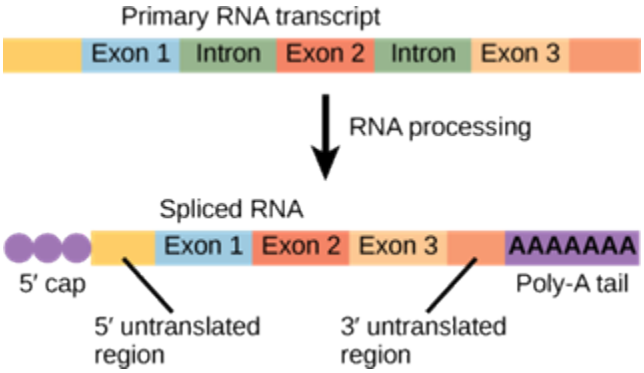

Figure 15.11 Eukaryotic mRNA contains introns that must be spliced out. A 5' cap and 3' poly-A tail are also added.

5′ Capping

While the pre-mRNA is still being synthesized, a **7-methylguanosine cap** is added to the 5′ end of the growing transcript by a phosphate linkage. This functional group protects the nascent mRNA from degradation. In addition, factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

3′ Poly-A Tail

Once elongation is complete, the pre-mRNA is cleaved by an endonuclease between an AAUAAA consensus sequence and a GU-rich sequence, leaving the AAUAAA sequence on the pre-mRNA. An enzyme called poly-A polymerase then adds a string of approximately 200 A residues, called the **poly-A tail**. This modification further protects the pre-mRNA from degradation and is also the binding site for a protein necessary for exporting the processed mRNA to the cytoplasm.

Pre-mRNA Splicing

Eukaryotic genes are composed of **exons**, which correspond to protein-coding sequences (*ex*-on signifies that they are *ex*pressed), and *int*ervening sequences called **introns** (*int*-ron denotes their *int*ervening role), which may be involved in gene regulation but are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins.

The discovery of introns came as a surprise to researchers in the 1970s who expected that pre-mRNAs would specify protein sequences without further processing, as they had observed in prokaryotes. The genes of higher eukaryotes very often contain one or more introns. These regions may correspond to regulatory sequences;

however, the biological significance of having many introns or having very long introns in a gene is unclear. It is possible that introns slow down gene expression because it takes longer to transcribe pre-mRNAs with lots of introns. Alternatively, introns may be nonfunctional sequence remnants left over from the fusion of ancient genes throughout the course of evolution. This is supported by the fact that separate exons often encode separate protein subunits or domains. For the most part, the sequences of introns can be mutated without ultimately affecting the protein product.

All of a pre-mRNA's introns must be completely and precisely removed before protein synthesis. If the process errs by even a single nucleotide, the reading frame of the rejoined exons would shift, and the resulting protein would be dysfunctional. The process of removing introns and reconnecting exons is called **splicing** (Figure 15.13). Introns are removed and degraded while the pre-mRNA is still in the nucleus. Splicing occurs by a sequence-specific mechanism that ensures introns will be removed and exons rejoined with the accuracy and precision of a single nucleotide. Although the intron itself is noncoding, the beginning and end of each intron is marked with specific nucleotides: GU at the 5′ end and AG at the 3′ end of the intron. The splicing of pre-mRNAs is conducted by complexes of proteins and RNA molecules called spliceosomes.

Visual Connection

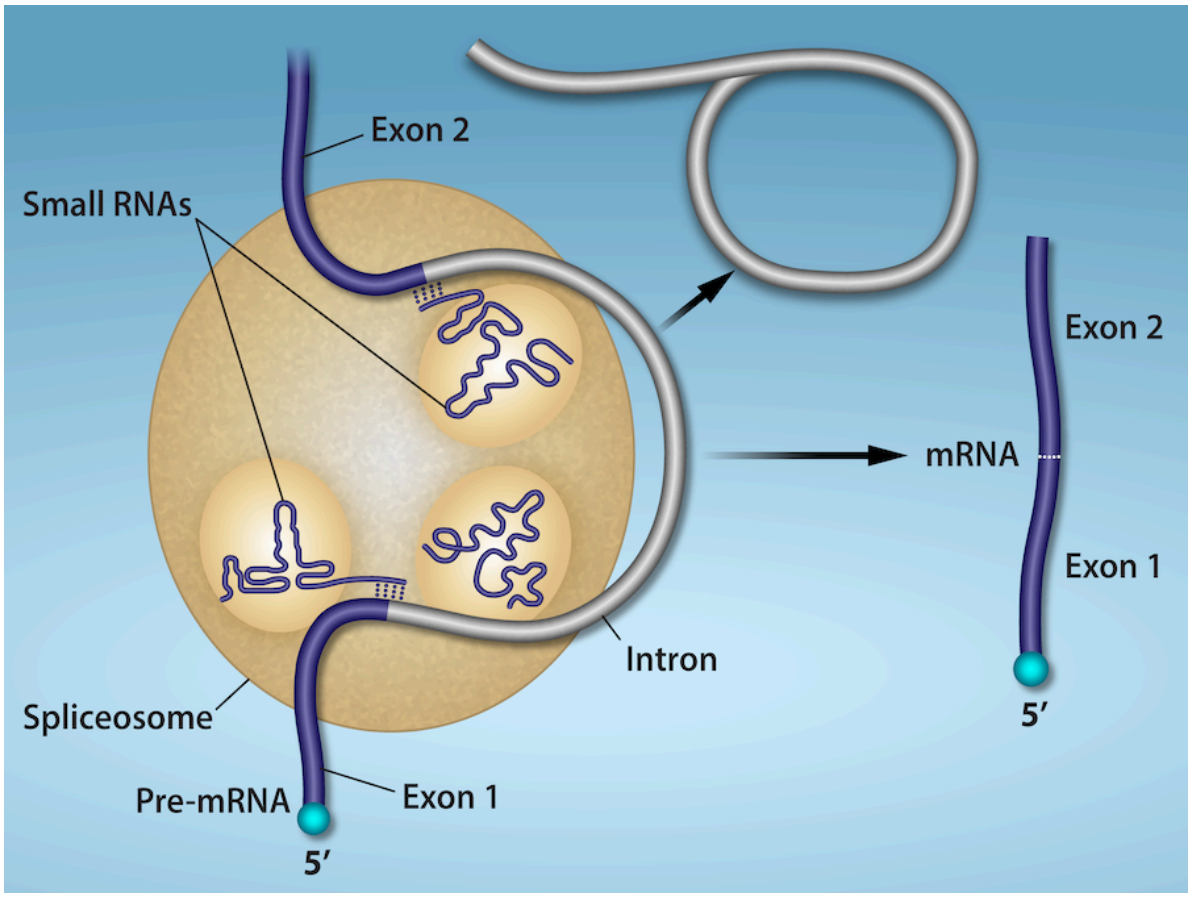

Figure 15.13 Pre-mRNA splicing involves the precise removal of introns from the primary RNA transcript. The splicing process is catalyzed by protein complexes called spliceosomes that are composed of proteins and RNA molecules called small nuclear RNAs (snRNAs). Spliceosomes recognize sequences at the 5' and 3' end of the intron. Rao, A. and Ryan, K. Department of Biology, Texas A&M University.

Errors in splicing are implicated in cancers and other human diseases. What kinds of mutations might lead to splicing errors? Think of different possible outcomes if splicing errors occur.

Note that more than 70 individual introns can be present, and each has to undergo the process of splicing—in addition to 5′ capping and the addition of a poly-A tail—just to generate a single, translatable mRNA molecule.

Link to Learning

See how introns are removed during RNA splicing at this website.

Watch this video to learn about eukaryotic RNA processing and modifications.

Processing of tRNAs and rRNAs

The tRNAs and rRNAs are structural molecules that have roles in protein synthesis; however, these RNAs are not themselves translated. Pre-rRNAs are transcribed, processed, and assembled into ribosomes in the nucleolus. Pre-tRNAs are transcribed and processed in the nucleus and then released into the cytoplasm, where they are linked to free amino acids for protein synthesis.

Most of the tRNAs and rRNAs in eukaryotes and prokaryotes are first transcribed as a long precursor molecule that spans multiple rRNAs or tRNAs. Enzymes then cleave the precursors into subunits corresponding to each structural RNA. Some of the bases of pre-rRNAs are *methylated*; that is, a $-CH_3$ methyl functional group is added for stability. Pre-tRNA molecules also undergo methylation. As with pre-mRNAs, subunit excision occurs in eukaryotic pre-RNAs destined to become tRNAs or rRNAs.

Mature rRNAs make up approximately 50 percent of each ribosome. Some of a ribosome's RNA molecules are purely structural, whereas others have catalytic or binding activities. Mature tRNAs take on a three-dimensional structure through local regions of base pairing stabilized by intramolecular hydrogen bonding. The tRNA folds to position the amino acid binding site at one end and the **anticodon** at the other end (Figure 15.14). The anticodon is a three-nucleotide sequence in a tRNA that interacts with an mRNA codon through complementary base pairing.

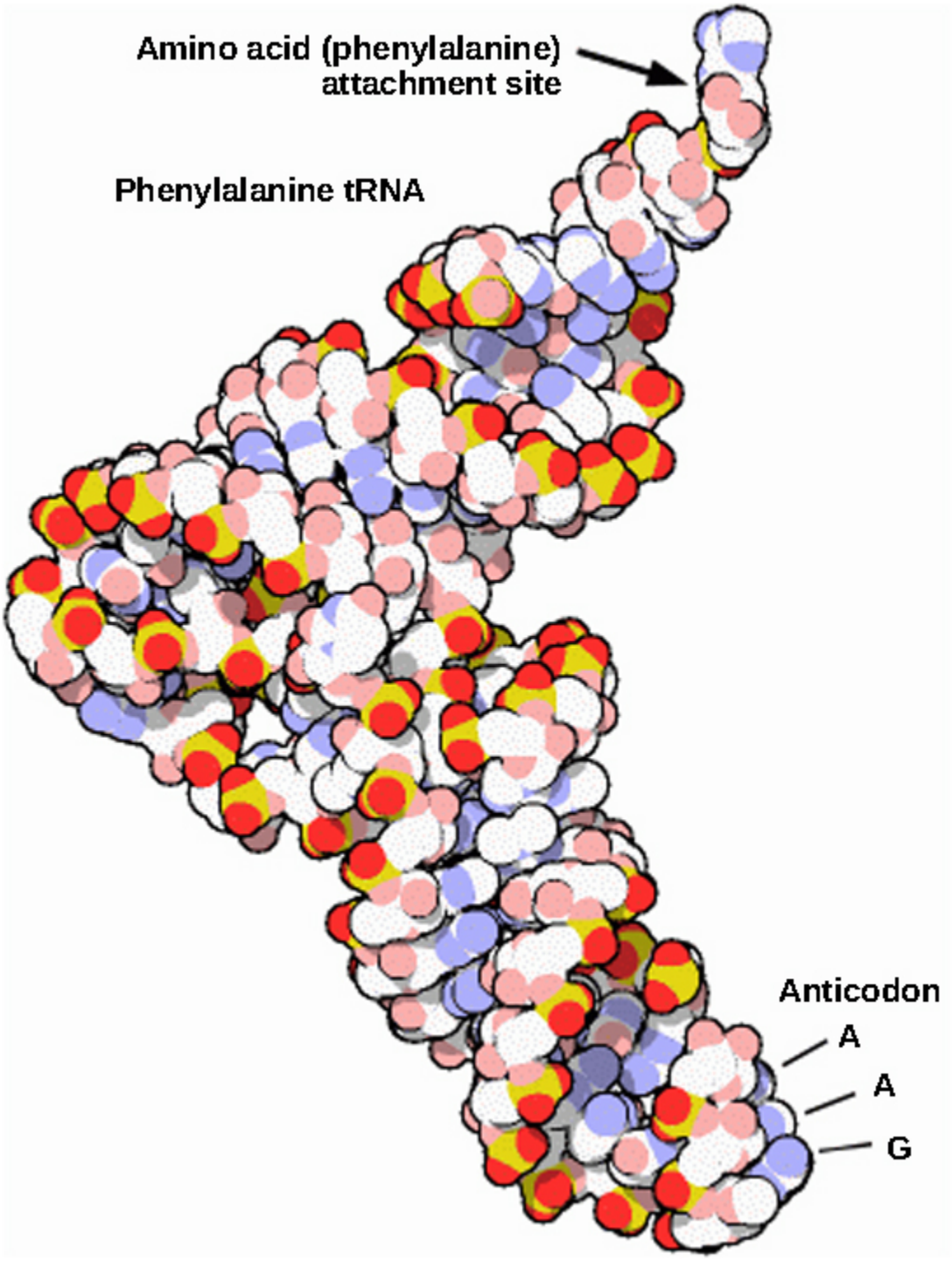

Figure 15.14 This is a space-filling model of a tRNA molecule that adds the amino acid phenylalanine to a growing polypeptide chain. The anticodon AAG binds the Codon UUC on the mRNA. The amino acid phenylalanine is attached to the other end of the tRNA.

147.

RIBOSOMES AND PROTEIN SYNTHESIS

Learning Objectives

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

- Describe the different steps in protein synthesis
- Discuss the role of ribosomes in protein synthesis

The synthesis of proteins consumes more of a cell's energy than any other metabolic process. In turn, proteins account for more mass than any other component of living organisms (with the exception of water), and proteins perform virtually every function of a cell. The process of translation, or protein synthesis, involves the decoding of an mRNA message into a polypeptide product. Amino acids are covalently strung together by interlinking peptide bonds in lengths ranging from approximately 50 to more than 1000 amino acid residues. Each individual amino acid has an amino group (NH_2) and a carboxyl (COOH) group. Polypeptides are formed when the amino group of one amino acid forms an amide (i.e., peptide) bond with the carboxyl group of another amino acid (Figure 15.15). This reaction is catalyzed by ribosomes and generates one water molecule.

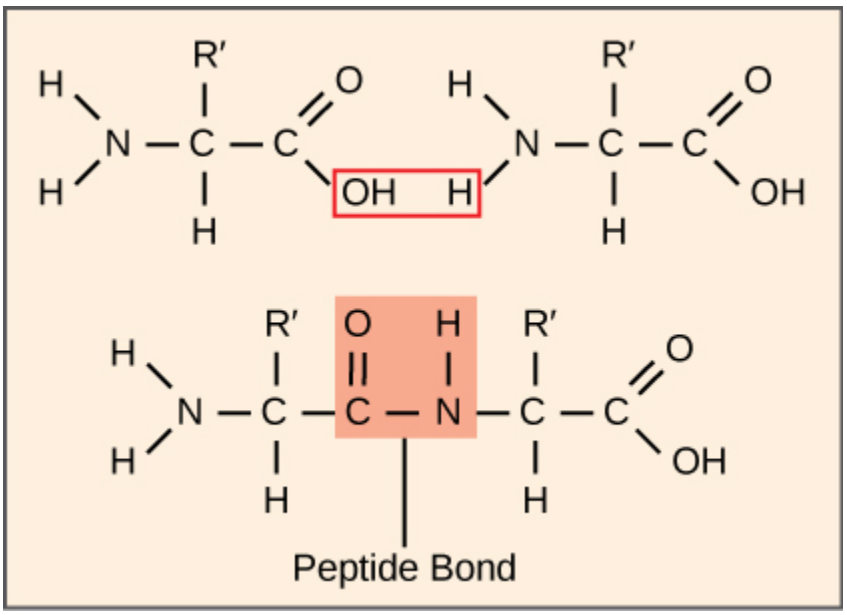

Figure 15.15 A peptide bond links the carboxyl end of one amino acid with the amino end of another, producing one water molecule during the process. For simplicity in this image, only the functional groups involved in the peptide bond are shown. The R and R' designations refer to the rest of each amino acid structure.

Link to Learning

Click through the steps of this PBS interactive to see protein synthesis in action.

Ribosomes

Even before an mRNA is translated, a cell must invest energy to build each of its ribosomes. In *E. coli*, there are between 10,000 and 70,000 ribosomes present in each 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 exist in the cytoplasm of prokaryotes and in the cytoplasm and rough endoplasmic reticulum of eukaryotes. Mitochondria and chloroplasts also have their own ribosomes in the matrix and stroma, which look more similar to prokaryotic ribosomes (and have similar drug sensitivities) than the ribosomes just outside their outer membranes in the cytoplasm. Ribosomes dissociate into large and small subunits when

they are not synthesizing proteins and reassociate during the initiation of translation. *In E. coli, the small subunit is described as 30S, and the large subunit is 50S, for a total of 70S (recall that Svedberg units are not additive).* Mammalian ribosomes have a small 40S subunit and a large 60S subunit, for a total of 80S. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs. Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction: reading the mRNA from 5′ to 3′ and synthesizing the polypeptide from the N terminus to the C terminus. The complete mRNA/poly-ribosome structure is called a **polysome**.

tRNAs

The tRNAs are structural RNA molecules that were transcribed from genes by RNA polymerase III. Depending on the species, 40 to 60 types of tRNAs exist in the cytoplasm. Transfer RNAs serve as adaptor molecules. Each tRNA carries a specific amino acid and recognizes one or more of the mRNA codons that define the order of amino acids in a protein. Aminoacyl-tRNAs bind to the ribosome 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.

Of the 64 possible mRNA codons—or triplet combinations of A, U, G, and C—three specify the termination of protein synthesis and 61 specify the addition of amino acids to the polypeptide chain. Of these 61, one codon (AUG) also encodes the initiation of translation. Each tRNA anticodon can base pair with one or more of the mRNA codons for its amino acid. For instance, if the sequence CUA occurred on an mRNA template in the proper reading frame, it would bind a leucine tRNA expressing the complementary sequence, GAU. The ability of some tRNAs to match more than one codon is what gives the genetic code its blocky structure.

As the adaptor molecules of translation, it is surprising that tRNAs can fit so much specificity into such a small package. Consider that tRNAs need to interact with three factors: 1) they must be recognized by the correct aminoacyl synthetase (see below); 2) they must be recognized by ribosomes; and 3) they must bind to the correct sequence in mRNA.

Aminoacyl tRNA Synthetases

The process of pre-tRNA synthesis by RNA polymerase III only creates the RNA portion of the adaptor molecule. The corresponding amino acid must be added later, once the tRNA is processed and exported to the cytoplasm. Through the process of tRNA "charging," each tRNA molecule is linked to its correct amino acid by one of a group of enzymes called **aminoacyl tRNA synthetases**. At least one type of aminoacyl tRNA synthetase exists for each of the 20 amino acids; the exact number of aminoacyl tRNA synthetases varies by species. These enzymes first bind and hydrolyze ATP to catalyze a high-energy bond between an amino acid and adenosine monophosphate (AMP); a pyrophosphate molecule is expelled in this reaction. The activated amino acid is then transferred to the tRNA, and AMP is released. The term "charging" is appropriate, since the

high-energy bond that attaches an amino acid to its tRNA is later used to drive the formation of the peptide bond. Each tRNA is named for its amino acid.

The Mechanism of Protein Synthesis

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'll explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between prokaryotic and eukaryotic translation.

Initiation of Translation

Protein synthesis begins with the formation of an **initiation complex**. In *E. coli*, this complex involves the small 30S ribosome, the mRNA template, three initiation factors (IFs; IF-1, IF-2, and IF-3), and a special initiator tRNA, called $tRNA^{Metf}$.

In *E. coli* mRNA, a sequence upstream of the first AUG codon, called the Shine-Dalgarno sequence (AGGAGG), interacts with the rRNA molecules that compose the ribosome. This interaction anchors the 30S ribosomal subunit at the correct location on the mRNA template. Guanosine triphosphate (GTP), which is a purine nucleotide triphosphate, acts as an energy source during translation—both at the start of elongation and during the ribosome's translocation. Binding of the mRNA to the 30S ribosome also requires IF-III.

The initiator tRNA then interacts with the **start codon** AUG (or rarely, GUG). This tRNA carries the amino acid methionine, which is formylated after its attachment to the tRNA. The formylation creates a "faux" peptide bond between the formyl carboxyl group and the amino group of the methionine. Binding of the fMet-$tRNA^{Metf}$ is mediated by the initiation factor IF-2. The fMet begins every polypeptide chain synthesized by *E. coli*, but it is usually removed after translation is complete. When an in-frame AUG is encountered during translation elongation, a non-formylated methionine is inserted by a regular Met-$tRNA^{Met}$. After the formation of the initiation complex, the 30S ribosomal subunit is joined by the 50S subunit to form the translation complex. In eukaryotes, a similar initiation complex forms, comprising mRNA, the 40S small ribosomal subunit, eukaryotic IFs, and nucleoside triphosphates (GTP and ATP). The methionine on the charged initiator tRNA, called Met-$tRNA_i$, is not formylated. However, Met-$tRNA_i$ is distinct from other Met-tRNAs in that it can bind IFs.

Instead of depositing at the Shine-Dalgarno sequence, the eukaryotic initiation complex recognizes the 7-methylguanosine cap at the 5′ end of the mRNA. A cap-binding protein (CBP) and several other IFs assist the movement of the ribosome to the 5′ cap. Once at the cap, the initiation complex tracks along the mRNA in the 5′ to 3′ direction, searching for the AUG start codon. Many eukaryotic mRNAs are translated from the first AUG, but this is not always the case. According to **Kozak's rules**, the nucleotides around the AUG indicate whether it is the correct start codon. Kozak's rules state that the following consensus sequence must appear

around the AUG of vertebrate genes: 5′-gccRccAUGG-3′. The R (for purine) indicates a site that can be either A or G, but cannot be C or U. Essentially, the closer the sequence is to this consensus, the higher the efficiency of translation.

Once the appropriate AUG is identified, the other proteins and CBP dissociate, and the 60S subunit binds to the complex of Met-tRNA$_i$, mRNA, and the 40S subunit. This step completes the initiation of translation in eukaryotes.

Translation, Elongation, and Termination

In prokaryotes and eukaryotes, the basics of elongation are the same, so we will review elongation from the perspective of *E. coli*. When the translation complex is formed, the tRNA binding region of the ribosome consists of three compartments. The A (aminoacyl) site binds incoming charged aminoacyl tRNAs. The P (peptidyl) site binds charged tRNAs carrying amino acids that have formed peptide bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The E (exit) site releases dissociated tRNAs so that they can be recharged with free amino acids. The initiating methionyl-tRNA, however, occupies the P site at the beginning of the elongation phase of translation in both prokaryotes and eukaryotes.

During translation elongation, the mRNA template provides tRNA binding specificity. As the ribosome moves along the mRNA, each mRNA codon comes into register, and specific binding with the corresponding charged tRNA anticodon is ensured. If mRNA were not present in the elongation complex, the ribosome would bind tRNAs nonspecifically and randomly.

Elongation proceeds with charged tRNAs sequentially entering and leaving the ribosome as each new amino acid is added to the polypeptide chain. Movement of a tRNA from A to P to E site is induced by conformational changes that advance the ribosome by three bases in the 3′ direction. The energy for each step along the ribosome is donated by elongation factors that hydrolyze GTP. GTP energy is required both for the binding of a new aminoacyl-tRNA to the A site and for its translocation to the P site after formation of the peptide bond. Peptide bonds form between the amino group of the amino acid attached to the A-site tRNA and the carboxyl group of the amino acid attached to the P-site tRNA. The formation of each peptide bond is catalyzed by **peptidyl transferase**, an RNA-based enzyme that is integrated into the 50S ribosomal subunit. The energy for each peptide bond formation is derived from the high-energy bond linking each amino acid to its tRNA. After peptide bond formation, the A-site tRNA that now holds the growing peptide chain moves to the P site, and the P-site tRNA that is now empty moves to the E site and is expelled from the ribosome (Figure 15.18). Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200-amino-acid protein can be translated in just 10 seconds.

Link to Learning

Watch this video Ribosomes and Protein Synthesis.

Visual Connection

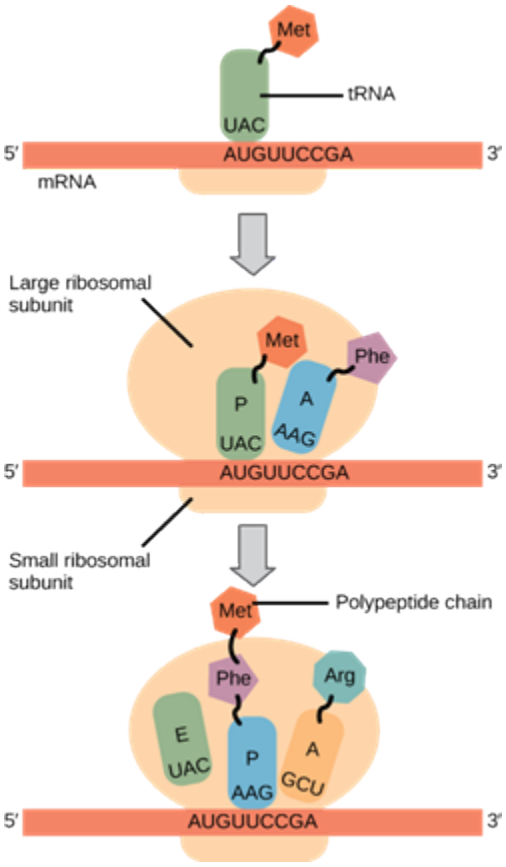

Figure 15.16 Translation begins when an initiator tRNA anticodon recognizes a start codon on mRNA bound to a small ribosomal subunit. The large ribosomal subunit joins the small subunit, and a second tRNA is recruited. As the mRNA moves relative to the ribosome, successive tRNAs move through the ribosome and the polypeptide chain is formed. Entry of a release factor into the A site terminates translation and the components dissociate.

Termination of translation occurs when a nonsense codon (UAA, UAG, or UGA) is encountered. Upon aligning with the A site, these nonsense codons are recognized by *protein release factors* that resemble tRNAs. The releasing factors in both prokaryotes and eukaryotes instruct peptidyl transferase to add a water molecule

to the carboxyl end of the P-site amino acid. This reaction forces the P-site amino acid to detach from its tRNA, and the newly made protein is released. The small and large ribosomal subunits dissociate from the mRNA and from each other; they are recruited almost immediately into another translation initiation complex. After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction.

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148.

KEY TERMS

7-methylguanosine cap
: modification added to the 5′ end of pre-mRNAs to protect mRNA from degradation and assist translation

aminoacyl tRNA synthetase
: enzyme that "charges" tRNA molecules by catalyzing a bond between the tRNA and a corresponding amino acid

anticodon
: three-nucleotide sequence in a tRNA molecule that corresponds to an mRNA codon

CAAT box
: (GGCCAATCT) essential eukaryotic promoter sequence involved in binding transcription factors

central dogma
: states that genes specify the sequence of mRNAs, which in turn specify the sequence of proteins

codon
: three consecutive nucleotides in mRNA that specify the insertion of an amino acid or the release of a polypeptide chain during translation

colinear
: in terms of RNA and protein, three "units" of RNA (nucleotides) specify one "unit" of protein (amino acid) in a consecutive fashion

consensus
: DNA sequence that is used by many species to perform the same or similar functions

core enzyme
: prokaryotic RNA polymerase consisting of α, α, β, and β' but missing σ; this complex performs elongation

degeneracy
: (of the genetic code) describes that a given amino acid can be encoded by more than one nucleotide triplet; the code is degenerate, but not ambiguous

downstream
: nucleotides following the initiation site in the direction of mRNA transcription; in general, sequences that are toward the 3′ end relative to a site on the mRNA

exon

sequence present in protein-coding mRNA after completion of pre-mRNA splicing

FACT

complex that "facilitates chromatin transcription" by disassembling nucleosomes ahead of a transcribing RNA polymerase II and reassembling them after the polymerase passes by

GC-rich box

(GGCG) nonessential eukaryotic promoter sequence that binds cellular factors to increase the efficiency of transcription; may be present several times in a promoter

hairpin

structure of RNA when it folds back on itself and forms intramolecular hydrogen bonds between complementary nucleotides

holoenzyme

prokaryotic RNA polymerase consisting of α, α, β, β', and σ; this complex is responsible for transcription initiation

initiation site

nucleotide from which mRNA synthesis proceeds in the 5′ to 3′ direction; denoted with a "+1"

initiator tRNA

in prokaryotes, called $tRNA\frac{Met}{f}$ and in eukaryotes, called $tRNA_i$; a tRNA that interacts with a start codon, binds directly to the ribosome P site, and links to a special methionine to begin a polypeptide chain

intron

non–protein-coding intervening sequences that are spliced from mRNA during processing

Kozak's rules

determines the correct initiation AUG in a eukaryotic mRNA; the following consensus sequence must appear around the AUG: 5'-GCC(**purine**)CCAUG**G**-3'; the bolded bases are most important

nonsense codon

one of the three mRNA codons that specifies termination of translation

nontemplate strand

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

Octamer box

(ATTTGCAT) nonessential eukaryotic promoter sequence that binds cellular factors to increase the efficiency of transcription; may be present several times in a promoter

peptidyl transferase

RNA-based enzyme that is integrated into the 50S ribosomal subunit and catalyzes the formation of peptide bonds

plasmid
: extrachromosomal, covalently closed, circular DNA molecule that may only contain one or a few genes; common in prokaryotes

poly-A tail
: modification added to the 3′ end of pre-mRNAs to protect mRNA from degradation and assist mRNA export from the nucleus

polysome
: mRNA molecule simultaneously being translated by many ribosomes all going in the same direction

preinitiation complex
: cluster of transcription factors and other proteins that recruit RNA polymerase II for transcription of a DNA template

promoter
: DNA sequence to which RNA polymerase and associated factors bind and initiate transcription

reading frame
: sequence of triplet codons in mRNA that specify a particular protein; a ribosome shift of one or two nucleotides in either direction completely abolishes synthesis of that protein

rho-dependent termination
: in prokaryotes, termination of transcription by an interaction between RNA polymerase and the rho protein at a run of G nucleotides on the DNA template

rho-independent
: termination sequence-dependent termination of prokaryotic mRNA synthesis; caused by hairpin formation in the mRNA that stalls the polymerase

RNA editing
: direct alteration of one or more nucleotides in an mRNA that has already been synthesized

Shine-Dalgarno sequence
: (AGGAGG); initiates prokaryotic translation by interacting with rRNA molecules comprising the 30S ribosome

signal sequence
: short tail of amino acids that directs a protein to a specific cellular compartment

small nuclear RNA
: molecules synthesized by RNA polymerase III that have a variety of functions, including splicing pre-mRNAs and regulating transcription factors

splicing
: process of removing introns and reconnecting exons in a pre-mRNA

start codon
: AUG (or rarely, GUG) on an mRNA from which translation begins; always specifies methionine

TATA box
: conserved promoter sequence in eukaryotes and prokaryotes that helps to establish the initiation site for transcription

template strand
: strand of DNA that specifies the complementary mRNA molecule

transcription bubble
: region of locally unwound DNA that allows for transcription of mRNA

upstream
: nucleotides preceding the initiation site; in general, sequences toward the 5′ end relative to a site on the mRNA

149.

CHAPTER SUMMARY

15.1 The Genetic Code

The genetic code refers to the DNA alphabet (A, T, C, G), the RNA alphabet (A, U, C, G), and the polypeptide alphabet (20 amino acids). 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 degenerate because 64 triplet codons in mRNA specify only 20 amino acids and three nonsense codons. Most amino acids have several similar codons. Almost every species on the planet uses the same genetic code.

15.2 Prokaryotic Transcription

In prokaryotes, mRNA synthesis is initiated at a promoter sequence on the DNA template comprising two consensus sequences that recruit RNA polymerase. The prokaryotic polymerase consists of a core enzyme of four protein subunits and a σ protein that assists only with initiation. Elongation synthesizes mRNA in the 5′ to 3′ direction at a rate of 40 nucleotides per second. Termination liberates the mRNA and occurs either by rho protein interaction or by the formation of an mRNA hairpin.

15.3 Eukaryotic Transcription

Transcription in eukaryotes involves one of three types of polymerases, depending on the gene being transcribed. RNA polymerase II transcribes all of the protein-coding genes, whereas RNA polymerase I transcribes the tandemly duplicated rRNA genes, and RNA polymerase III transcribes various small RNAs, like the 5S rRNA, tRNA, and small nuclear RNA genes. The initiation of transcription in eukaryotes involves the binding of several transcription factors to complex promoter sequences that are usually located upstream of the gene being copied. The mRNA is synthesized in the 5′ to 3′ direction, and the FACT complex moves and reassembles nucleosomes as the polymerase passes by. Whereas RNA polymerases I and III terminate transcription by protein- or RNA hairpin-dependent methods, RNA polymerase II transcribes for 1,000 or more nucleotides beyond the gene template and cleaves the excess during pre-mRNA processing.

15.4 RNA Processing in Eukaryotes

Eukaryotic pre-mRNAs are modified with a 5′ methylguanosine cap and a poly-A tail. These structures protect the mature mRNA from degradation and help export it from the nucleus. Pre-mRNAs also undergo splicing, in which introns are removed and exons are reconnected with single-nucleotide accuracy. Only finished mRNAs that have undergone 5′ capping, 3′ polyadenylation, and intron splicing are exported from the nucleus to the cytoplasm. Pre-rRNAs and pre-tRNAs may be processed by intramolecular cleavage, splicing, methylation, and chemical conversion of nucleotides. Rarely, RNA editing is also performed to insert missing bases after an mRNA has been synthesized.

15.5 Ribosomes and Protein Synthesis

The players in translation include the mRNA template, ribosomes, tRNAs, and various enzymatic factors. The small ribosomal subunit binds to the mRNA template either at the Shine-Dalgarno sequence (prokaryotes) or the 5′ cap (eukaryotes). Translation begins at the initiating AUG on the mRNA, specifying methionine. The formation of peptide bonds occurs between sequential amino acids matched to the mRNA template by their tRNAs according to the genetic code. Charged tRNAs enter the ribosomal A site, and their amino acid bonds with the amino acid at the P site. The entire mRNA is translated in three-nucleotide "steps" of the ribosome. When a nonsense codon is encountered, a release factor binds and dissociates the components and frees the new protein. Folding of the protein occurs during and after translation.

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VISUAL CONNECTION QUESTIONS

1. Figure 15.11 A scientist splices a eukaryotic promoter in front of a bacterial gene and inserts the gene in a bacterial chromosome. Would you expect the bacteria to transcribe the gene?

2. Figure 15.13 Errors in splicing are implicated in cancers and other human diseases. What kinds of mutations might lead to splicing errors? Think of different possible outcomes if splicing errors occur.

3. Figure 15.18 Many antibiotics inhibit bacterial protein synthesis. For example, tetracycline blocks the A site on the bacterial ribosome, and chloramphenicol blocks peptidyl transfer. What specific effect would you expect each of these antibiotics to have on protein synthesis?

Tetracycline would directly affect:

a. tRNA binding to the ribosome
b. ribosome assembly
c. growth of the protein chain

Chloramphenicol would directly affect

a. tRNA binding to the ribosome
b. ribosome assembly
c. growth of the protein chain

151.

REVIEW QUESTIONS

4. The AUC and AUA codons in mRNA both specify isoleucine. What feature of the genetic code explains this?

a. complementarity
b. nonsense codons
c. universality
d. degeneracy

5. How many nucleotides are in 12 mRNA codons?

a. 12
b. 24
c. 36
d. 48

6. Which event contradicts the central dogma of molecular biology?

a. Poly-A polymerase enzymes process mRNA in the nucleus.
b. Endonuclease enzymes splice out and repair damaged DNA.
c. Scientists use reverse transcriptase enzymes to make DNA from RNA.
d. Codons specifying amino acids are degenerate and universal.

7. Which subunit of the E. coli polymerase confers specificity to transcription?

a. α
b. β
c. β'
d. σ

8. The -10 and -35 regions of prokaryotic promoters are called consensus sequences because _____.

a. they are identical in all bacterial species
b. they are similar in all bacterial species
c. they exist in all organisms
d. they have the same function in all organisms

9. Three different bacteria species have the following consensus sequences upstream of a conserved gene.

	Species A	Species B	Species C
-10	TAATAAT	TTTAAT	TATATT
-35	TTGACA	TTGGCC	TTGAAA

Table 15.2

Order the bacteria from most to least efficient initiation of gene transcription.

a. A > B > C
b. B > C > A
c. C > B > A
d. A > C > B

10. Which feature of promoters can be found in both prokaryotes and eukaryotes?

a. GC box
b. TATA box
c. octamer box
d. -10 and -35 sequences

11. What transcripts will be most affected by low levels of α-amanitin?

a. 18S and 28S rRNAs
b. pre-mRNAs
c. 5S rRNAs and tRNAs
d. other small nuclear RNAs

12. How do enhancers and promoters differ?

a. Enhancers bind transcription factors to silence gene expression, while promoters activate transcription.

b. Enhancers increase the efficiency of gene expression, but are not essential for transcription. Promoter recognition is essential to transcription initiation.
c. Promoters bind transcription factors to increase the efficiency of transcription. Enhancers bind RNA polymerases to initiate transcription.
d. There is no difference. Both are transcription factor-binding sequences in DNA.

13. Which pre-mRNA processing step is important for initiating translation?

a. poly-A tail
b. RNA editing
c. splicing
d. 7-methylguanosine cap

14. What processing step enhances the stability of pre-tRNAs and pre-rRNAs?

a. methylation
b. nucleotide modification
c. cleavage
d. splicing

15. A scientist identifies a pre-mRNA with the following structure.

What is the predicted size of the corresponding mature mRNA in base pairs (bp), excluding the 5' cap and 3' poly-A tail?

a. 220bp
b. 295bp
c. 140bp
d. 435bp

16. The RNA components of ribosomes are synthesized in the _____.

a. cytoplasm
b. nucleus
c. nucleolus
d. endoplasmic reticulum

17. In any given species, there are at least how many types of aminoacyl tRNA synthetases?

a. 20
b. 40
c. 100
d. 200

18. A scientist introduces a mutation that makes the 60S ribosomal subunit nonfunctional in a human cell line. What would be the predicted effect on translation?

a. Translation stalls after the initiation AUG codon is identified.
b. The ribosome cannot catalyze the formation of peptide bonds between the tRNAs in the A and P sites.
c. The ribosome cannot interact with mRNAs.
d. tRNAs cannot exit the E site of the ribosome.

152.

CRITICAL THINKING QUESTIONS

19. If mRNA is complementary to the DNA template strand and the DNA template strand is complementary to the DNA non-template strand, then why are base sequences of mRNA and the DNA non-template strand not identical? Could they ever be?

20. A scientist observes that a cell has an RNA polymerase deficiency that prevents it from making proteins. Describe three additional observations that would together support the conclusion that a defect in RNA polymerase I activity, and not problems with the other polymerases, causes the defect.

21. Describe how transcription in prokaryotic cells can be altered by external stimulation such as excess lactose in the environment

22. Describe how controlling gene expression will alter the overall protein levels in the cell.

PART XVI
GENE EXPRESSION

153.

INTRODUCTION

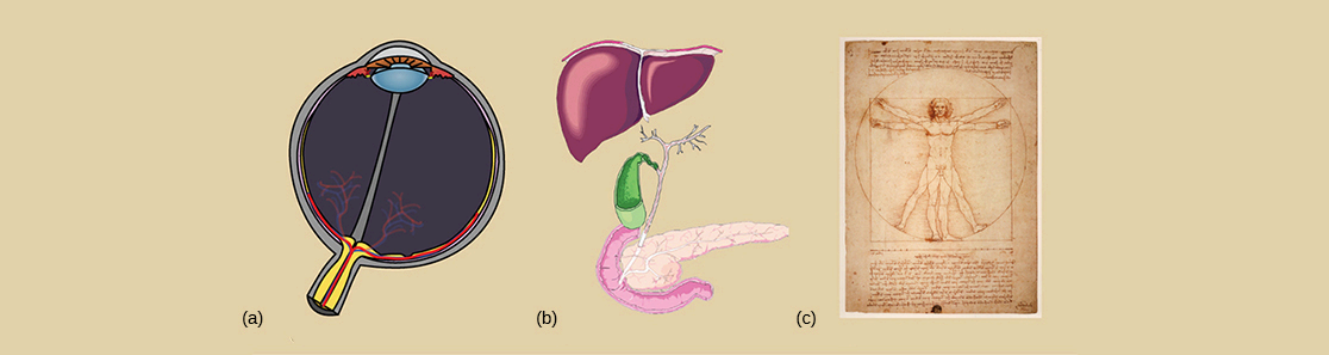

Figure 16.1 The genetic content of each somatic cell in an organism is the same, but not all genes are expressed in every cell. The control of which genes are expressed dictates whether a cell is, for example, (a) an eye cell or (b) a liver cell. It is the differential gene expression patterns that arise in different cells that give rise to (c) a complete organism.

Each somatic cell in the body generally contains the same DNA. A few exceptions include red blood cells, which contain no DNA in their mature state, and some immune system cells that rearrange their DNA while producing antibodies. In general, however, the genes that determine whether you have green eyes, brown hair, and how fast you metabolize food are the same in the cells in your eyes and your liver, even though these organs function quite differently. If each cell has the same DNA, how is it that cells or organs are different? Why do cells in the eye differ so dramatically from cells in the liver?

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.

154.

REGULATION OF GENE EXPRESSION

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

For a cell to function properly, necessary proteins must be synthesized at the proper time and place. All cells control or regulate the synthesis of proteins from information encoded in their DNA. The process of turning on a gene to produce RNA and protein is called **gene expression**. Whether in a simple unicellular organism or a complex multi-cellular organism, each cell controls when and how its genes are expressed. For this to occur, there must be internal chemical mechanisms that control when a gene is expressed to make RNA and protein, how much of the protein is made, and when it is time to stop making that protein because it is no longer needed.

The regulation of gene expression conserves energy and space. It would require a significant amount of energy for an organism to express every gene at all times, so it is more energy efficient to turn on the genes only when they are required. In addition, only expressing a subset of genes in each cell saves space because DNA must be unwound from its tightly coiled structure to transcribe and translate the DNA. Cells would have to be enormous if every protein were expressed in every cell all the time.

The control of gene expression is extremely complex. Malfunctions in this process are detrimental to the cell and can lead to the development of many diseases, including cancer.

Prokaryotic versus Eukaryotic Gene Expression

To understand how gene expression is regulated, we must first understand how a gene codes for a functional protein in a cell. The process occurs in both prokaryotic and eukaryotic cells, just in slightly different manners.

Prokaryotic organisms are single-celled organisms that lack a cell nucleus, and their DNA therefore floats freely in the cell cytoplasm. To synthesize a protein, the processes of transcription and translation occur almost simultaneously. When the resulting protein is no longer needed, transcription stops. As a result, the primary method to control what type of protein and how much of each protein is expressed in a prokaryotic cell is the regulation of DNA transcription. All of the subsequent steps occur automatically. When more protein is required, more transcription occurs. Therefore, in prokaryotic cells, the control of gene expression is mostly at the transcriptional level.

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 16.3). Regulation may occur when the DNA is uncoiled and loosened from nucleosomes to bind transcription factors (**epigenetic** level), when the RNA is transcribed (**transcriptional** level), when the RNA is processed and exported to the cytoplasm after it is transcribed **(post-transcriptional** level), when the RNA is translated into protein (**translational** level), or after the protein has been made (**post-translational** level).

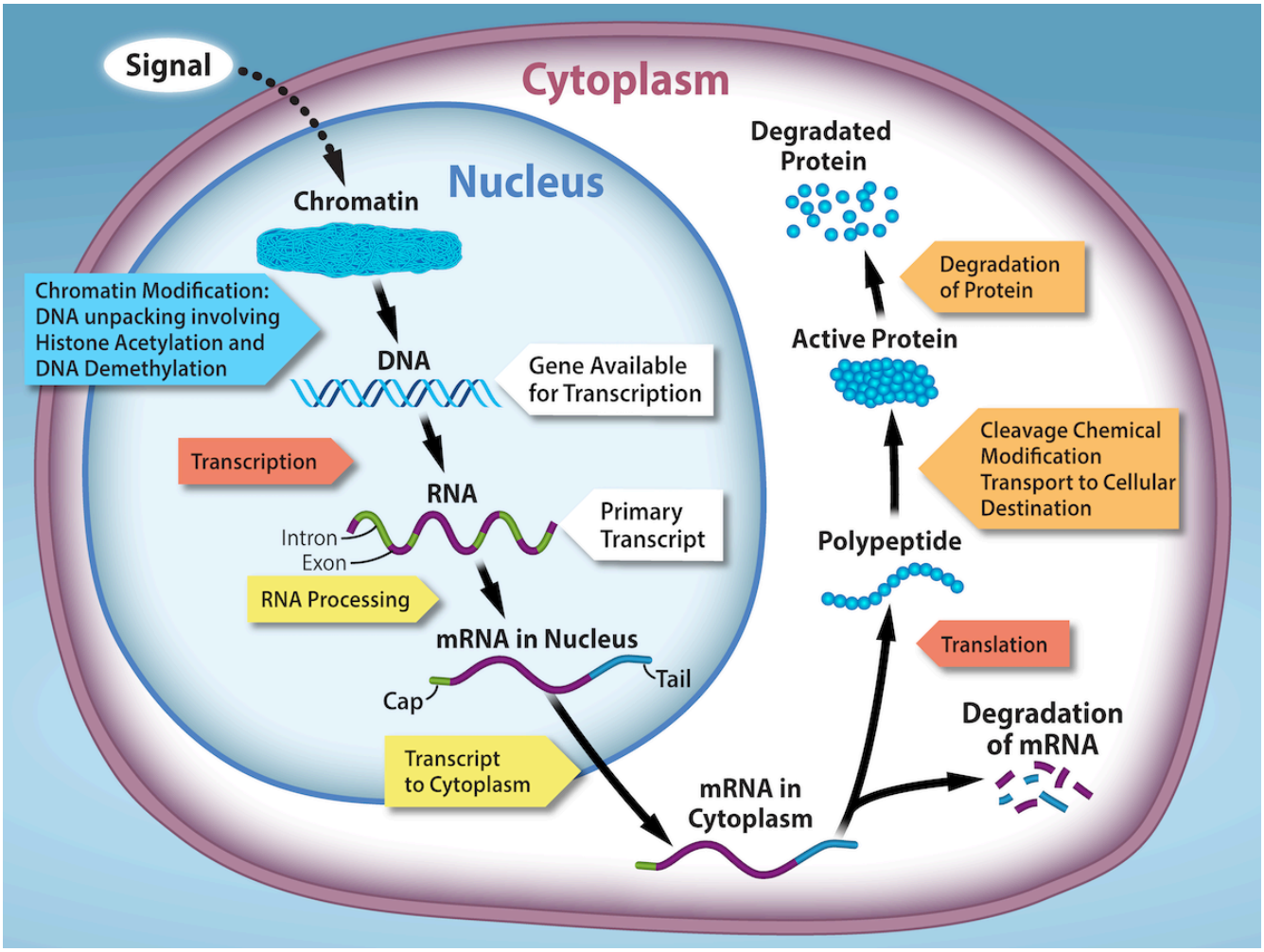

Figure 16.2 Locations of gene regulation. The regulation of gene expression occurs at multiple steps going from DNA to the functional gene product, usually a protein. It begins with chromatin structure making the DNA more or less accessible for transcription by RNA polymerase. In eukaryotes, the primary 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.

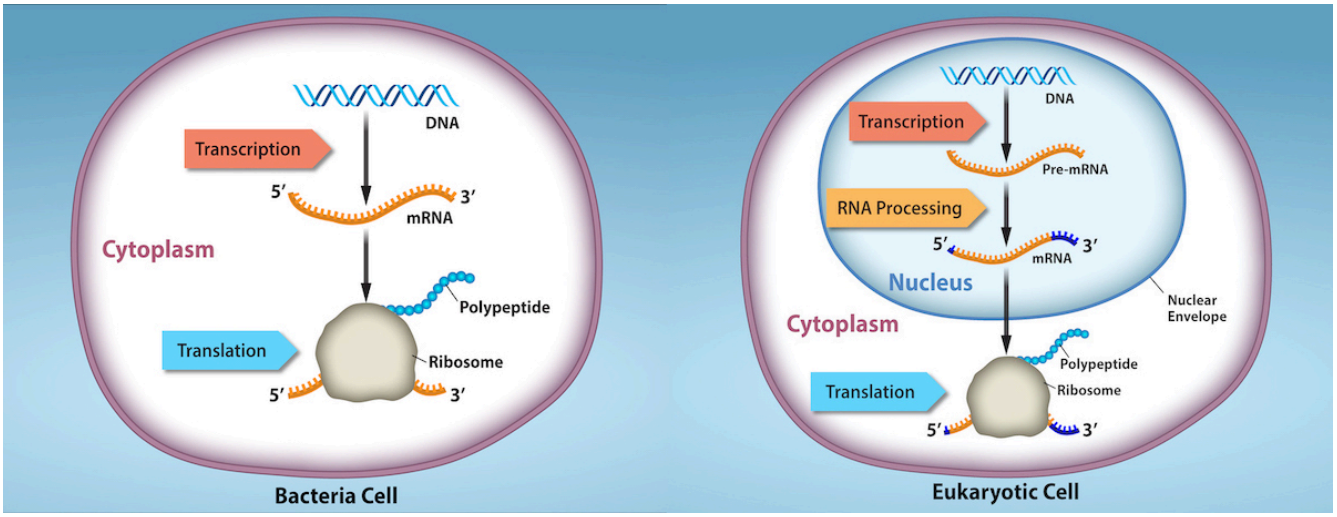

Figure 16.3 Regulation in prokaryotes and eukaryotes. A. Prokaryotic transcription and translation occur simultaneously in the cytoplasm, and regulation occurs primarily at the transcriptional level. B. Eukaryotic gene expression is regulated during transcription and RNA processing, which take place 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.

The differences in the regulation of gene expression between prokaryotes and eukaryotes are summarized in Table 16.1. The regulation of gene expression is discussed in detail in subsequent modules.

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, nuclear shuttling, post-transcriptional, translational, and post-translational)

Table 16.1

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.

155.

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 16.4). 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.

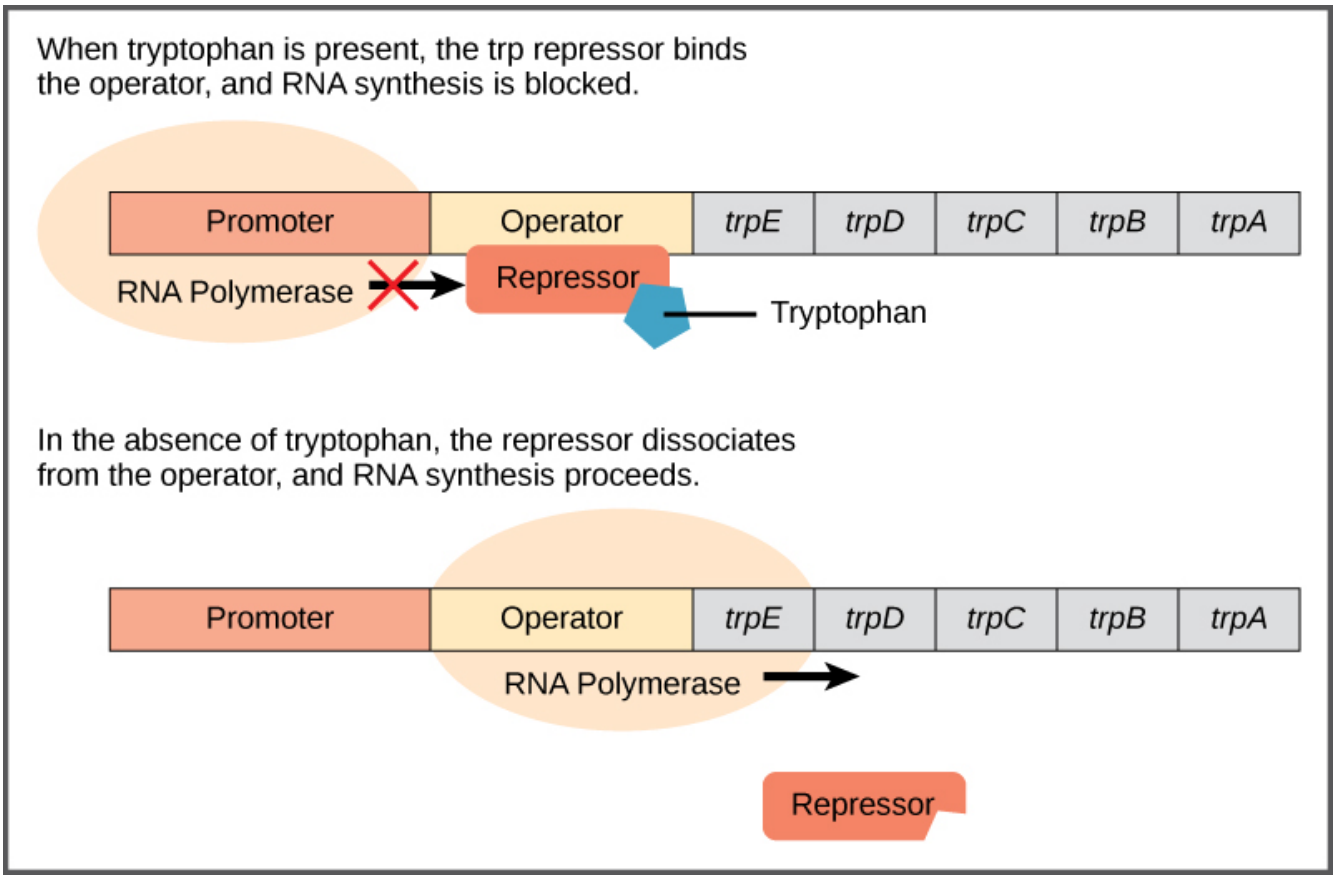

Figure 16.4 The tryptophan operon. The five genes that are needed to synthesize tryptophan in E. coli are located next to each other in the trp operon. When tryptophan is plentiful, two tryptophan molecules bind the repressor protein at the operator sequence. This physically blocks the RNA polymerase from transcribing the tryptophan genes. When tryptophan is absent, the repressor protein does not bind to the operator and the genes are transcribed.

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

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 16.5). 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.

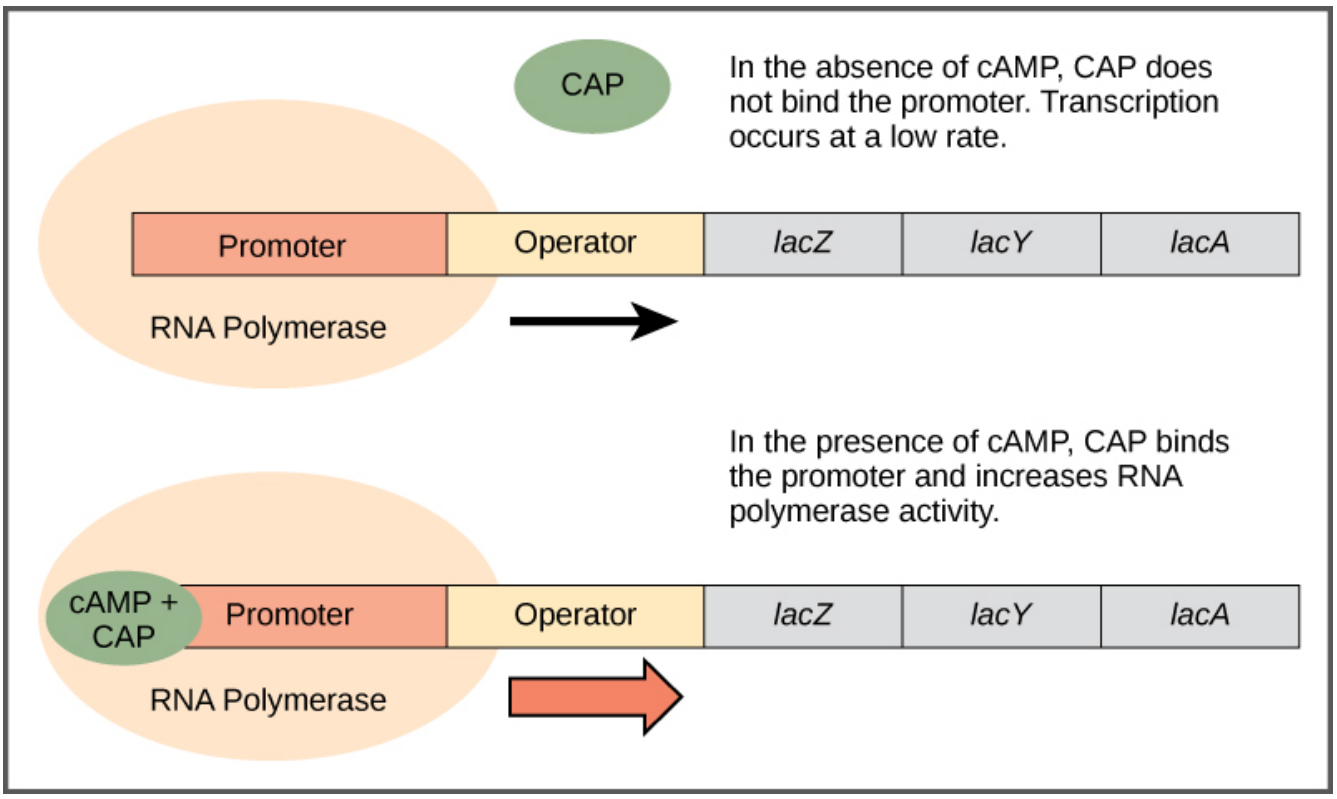

Figure 16.5 Transcriptional activation by the CAP protein. When glucose levels fall, E. coli may use other sugars for fuel but must transcribe new genes to do so. As glucose supplies become limited, cAMP levels increase. This cAMP binds to the CAP protein, a positive regulator that binds to a promoter region upstream of the genes required to use other sugar sources.

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 16.6). 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

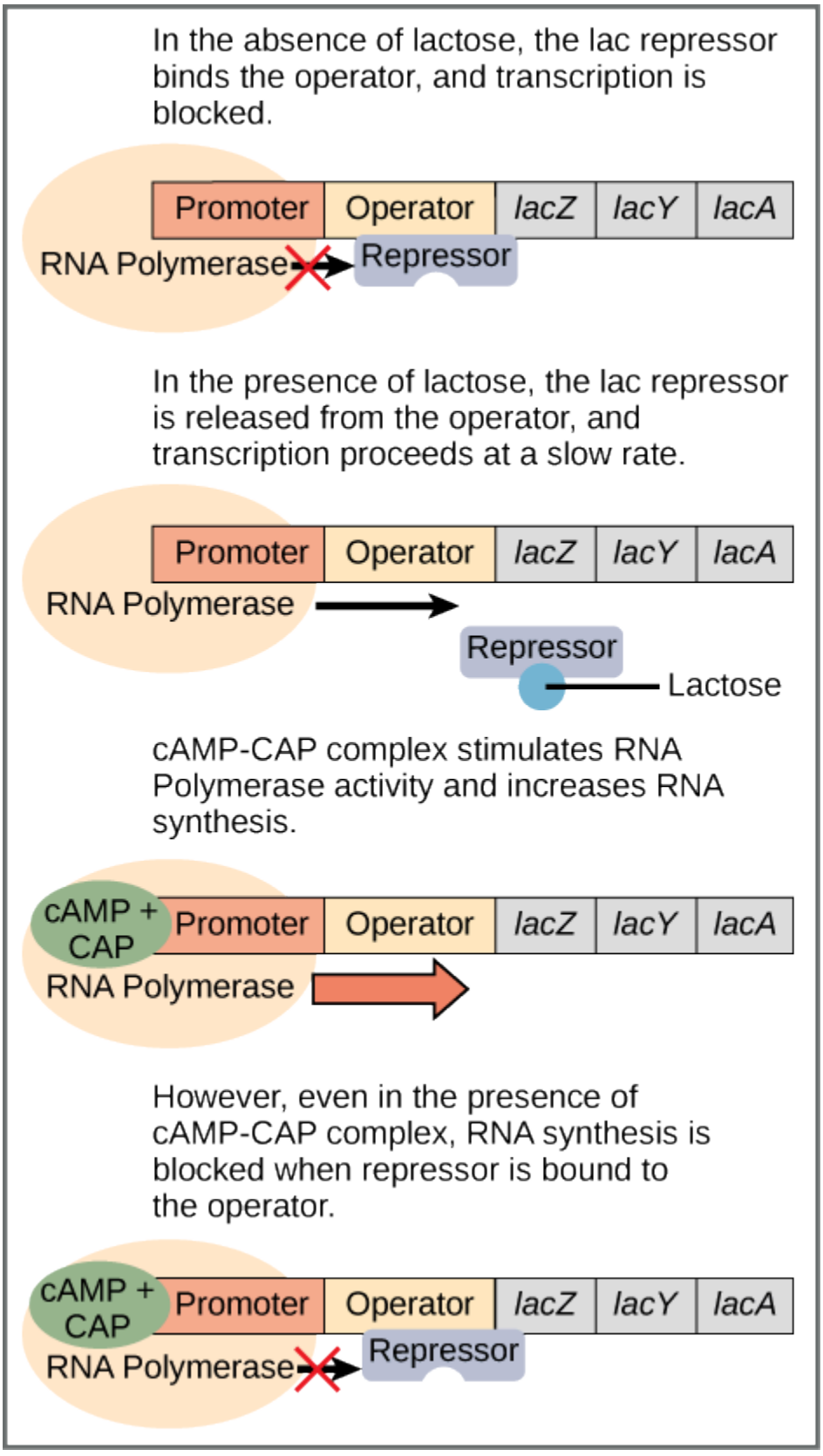
In the absence of lactose, the lac repressor binds the operator, and transcription is blocked.
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase
Repressor
In the presence of lactose, the lac repressor is released from the operator, and transcription proceeds at a slow rate.
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase
Repressor
Lactose
cAMP-CAP complex stimulates RNA Polymerase activity and increases RNA synthesis.
cAMP + CAP
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase
However, even in the presence of cAMP-CAP complex, RNA synthesis is blocked when repressor is bound to the operator.
cAMP + CAP
Promoter
Operator
lacZ
lacY
lacA
RNA Polymerase
Repressor

Figure 16.6 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.

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 16.2).

Signals that Induce or Repress Transcription of the *lac* Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	–	–	+	No
+	–	+	–	Some
–	+	–	+	No
–	+	+	–	Yes

Table 16.2

Link to Learning

Watch an animated tutorial about the workings of *lac* operon here.

Click to view content

156.

EUKARYOTIC EPIGENETIC GENE REGULATION

Learning Objectives

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

- Explain how chromatin remodeling controls transcriptional access
- Describe how access to DNA is controlled by histone modification
- Describe how DNA methylation is related to epigenetic gene changes

Eukaryotic gene expression is more complex than prokaryotic gene expression because the processes of transcription and translation are physically separated. Unlike prokaryotic cells, eukaryotic cells can regulate gene expression at many different levels. Epigenetic changes are inheritable changes in gene expression that do not result from changes in the DNA sequence. Eukaryotic gene expression begins with control of access to the DNA. Transcriptional access to the DNA can be controlled in two general ways: chromatin remodeling and DNA methylation. Chromatin remodeling changes the way that DNA is associated with chromosomal histones. DNA methylation is associated with developmental changes and gene silencing.

Epigenetic Control: Regulating Access to Genes within the Chromosome

The human genome encodes over 20,000 genes, with hundreds to thousands of genes on each of the 23 human chromosomes. The DNA in the nucleus is precisely wound, folded, and compacted into chromosomes so that it will fit into the nucleus. It is also organized so that specific segments can be accessed as needed by a specific cell type.

The first level of organization, or **packing**, is the winding of DNA strands around histone proteins. Histones package and order DNA into structural units called nucleosome complexes, which can control the access of proteins to the DNA regions (Figure 16.7a). Under the electron microscope, this winding of DNA around histone proteins to form nucleosomes looks like small beads on a string (Figure 16.7b).

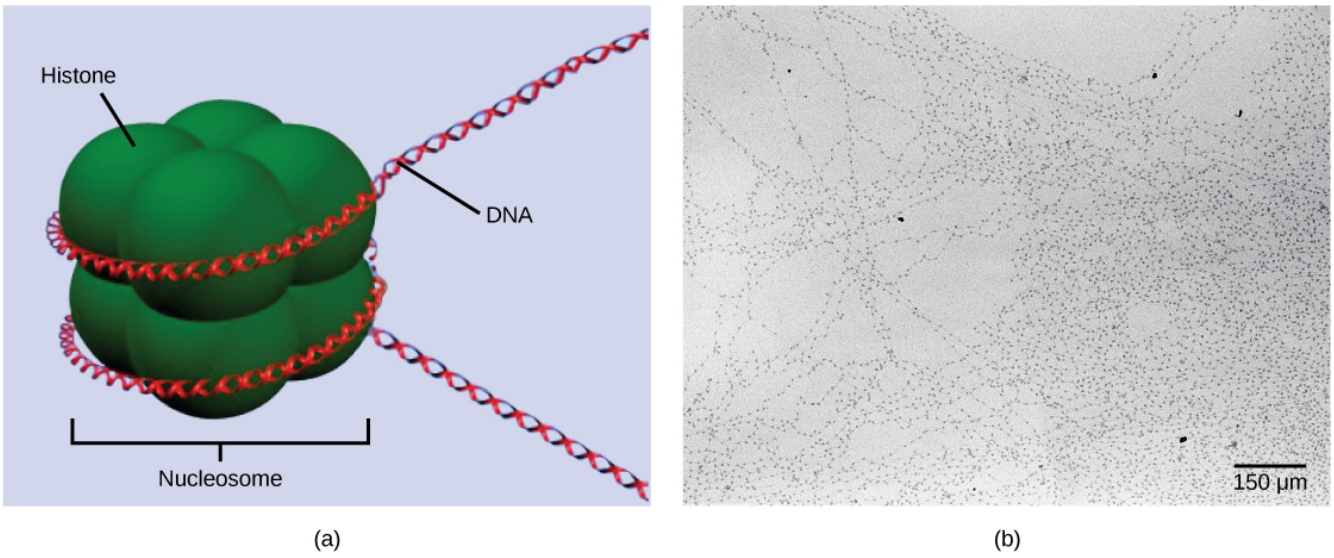

Figure 16.7 DNA is folded around histone proteins to create (a) nucleosome complexes. These nucleosomes control the access of proteins to the underlying DNA. When viewed through an electron microscope (b), the nucleosomes look like beads on a string. (credit "micrograph": modification of work by Chris Woodcock)

These beads (histone proteins) can move along the string (DNA) to expose different sections of the molecule. If DNA encoding a specific gene is to be transcribed into RNA, the nucleosomes surrounding that region of DNA can slide down the DNA to open that specific chromosomal region and allow for the transcriptional machinery (RNA polymerase) to initiate transcription (Figure 16.8).

Visual Connection

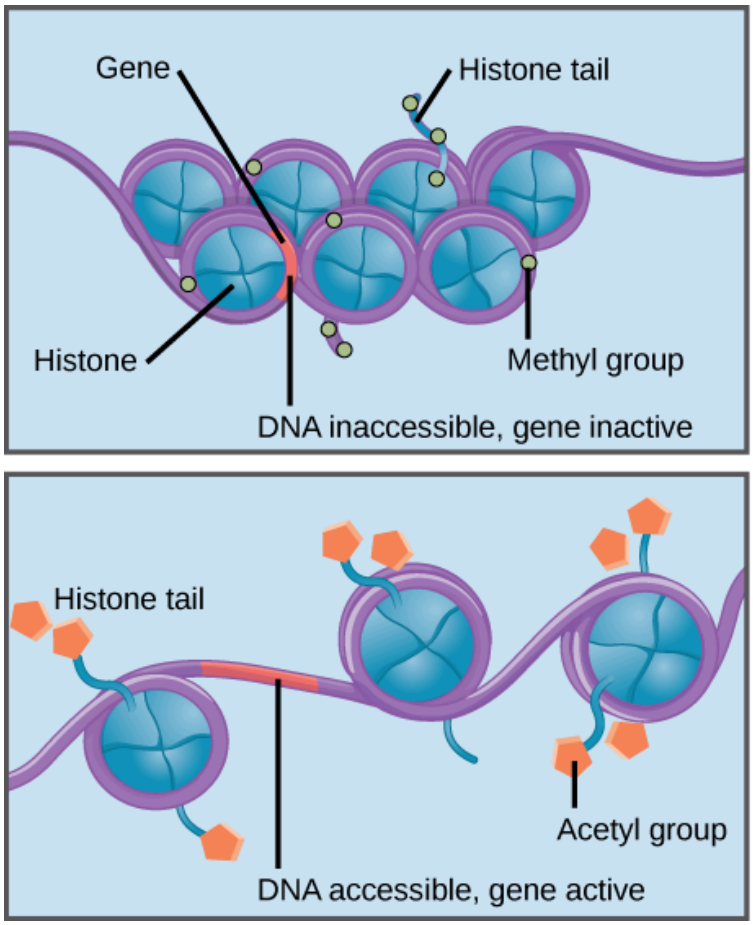

Figure 16.8 Nucleosomes can slide along DNA. When nucleosomes are spaced closely together (top), transcription factors cannot bind and gene expression is turned off. When the nucleosomes are spaced far apart (bottom), the DNA is exposed. Transcription factors can bind, allowing gene expression to occur. 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?

How closely the histone proteins associate with the DNA is regulated by signals found on both the histone proteins and on the DNA. These signals are functional groups added to histone proteins or to DNA and determine whether a chromosomal region should be open or closed (Figure 16.9 depicts modifications to histone proteins and DNA). These tags are not permanent, but may be added or removed as needed. Some chemical groups (phosphate, methyl, or acetyl groups) are attached to specific amino acids in histone "tails" at the N-terminus of the protein. These groups do not alter the DNA base sequence, but they do alter how

tightly wound the DNA is around the histone proteins. 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.

The DNA molecule itself can also be modified by methylation. DNA methylation occurs within very specific regions called CpG islands. These are stretches with a high frequency of cytosine and guanine dinucleotide DNA pairs (CG) found in the promoter regions of genes. The cytosine member of the CG pair can be methylated (a methyl group is added). Methylated genes are usually silenced, although methylation may have other regulatory effects. In some cases, genes that are silenced during the development of the gametes of one parent are transmitted in their silenced condition to the offspring. Such genes are said to be imprinted. Parental diet or other environmental conditions may also affect the methylation patterns of genes, which in turn modifies gene expression. Changes in chromatin organization interact with DNA methylation. DNA methyltransferases appear to be attracted to chromatin regions with specific histone modifications. Highly methylated (*hypermethylated*) DNA regions with deacetylated histones are tightly coiled and transcriptionally inactive.

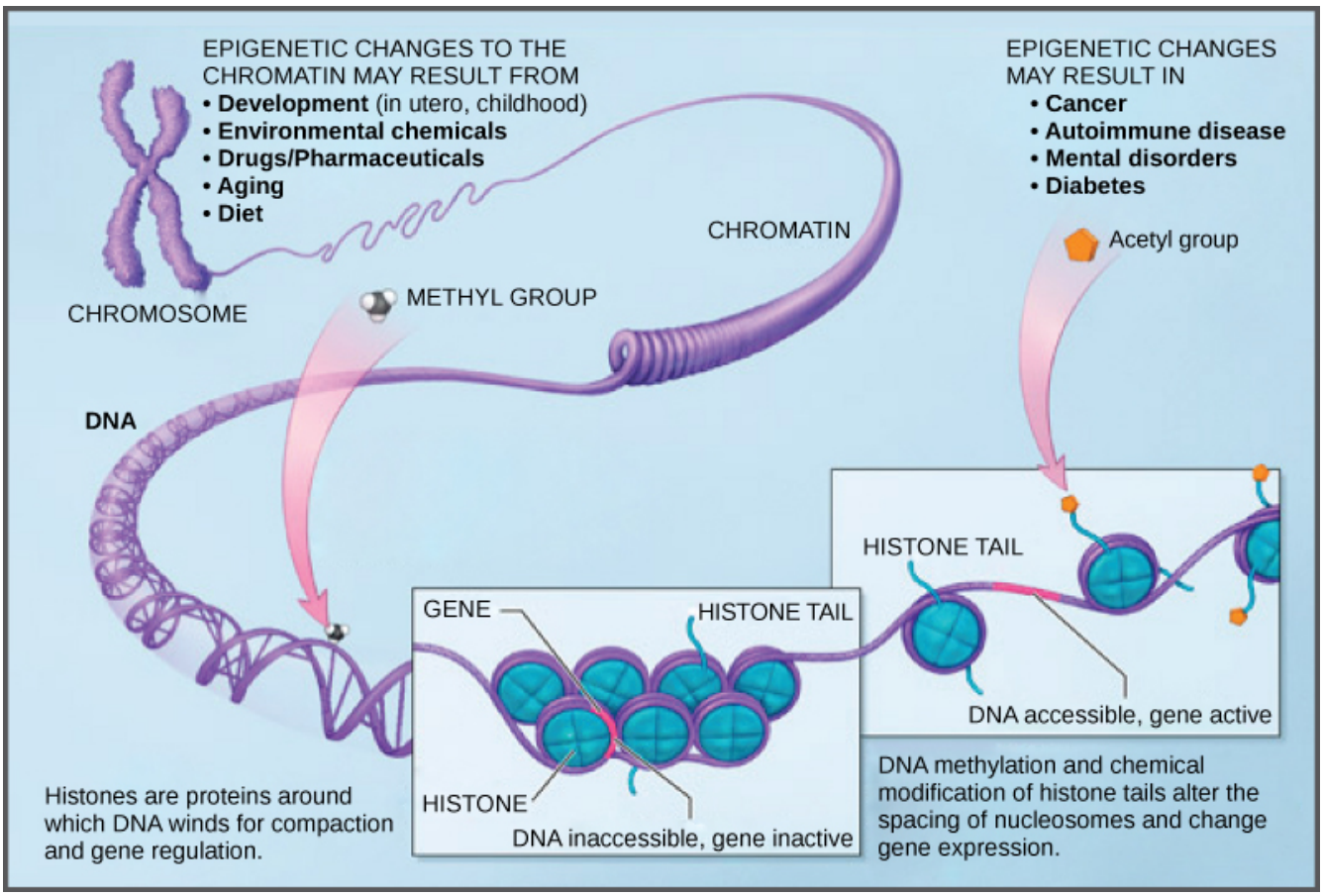

Figure 16.9 Histone proteins and DNA nucleotides can be modified chemically. Modifications affect nucleosome spacing and gene expression. (credit: modification of work by NIH)

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 16.9).

Link to Learning

View this video that describes how epigenetic regulation controls gene expression.

Click to view content

157.

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

Like prokaryotic cells, the transcription of genes in eukaryotes requires the action of an RNA polymerase to bind to a DNA sequence upstream of a gene in order to initiate transcription. However, unlike prokaryotic cells, the eukaryotic RNA polymerase requires other proteins, or transcription factors, to facilitate transcription initiation. RNA polymerase by itself cannot initiate transcription in eukaryotic cells. There are two types of transcription factors that regulate eukaryotic transcription: *General (or basal) transcription factors* bind to the core promoter region to assist with the binding of RNA polymerase. *Specific transcription factors* bind to various regions outside of the core promoter region and interact with the proteins at the core promoter to enhance or repress the activity of the polymerase.

Link to Learning

View the process of transcription—the making of RNA from a DNA template.

Click to view content

The Promoter and the Transcription Machinery

Genes are organized to make the control of gene expression easier. The promoter region is immediately upstream of the coding sequence. This region can be short (only a few nucleotides in length) or quite long (hundreds of nucleotides long). The longer the promoter, the more available space for proteins to bind. This also adds more control to the transcription process. The length of the promoter is gene-specific and can differ dramatically between genes. Consequently, the level of control of gene expression can also differ quite dramatically between genes. The purpose of the **promoter** is to bind transcription factors that control the initiation of transcription.

Within the core promoter region, 25 to 35 bases upstream of the transcriptional start site, resides the TATA box. The TATA box has the consensus sequence of 5'-TATAAA-3'. The TATA box is the binding site for a protein complex called TFIID, which contains a TATA-binding protein. Binding of TFIID recruits other transcription factors, including TFIIB, TFIIE, TFIIF, and TFIIH. Some of these transcription factors help to bind the RNA polymerase to the promoter, and others help to activate the transcription initiation complex.

In addition to the TATA box, other binding sites are found in some promoters. Some biologists prefer to restrict the range of the eukaryotic promoter to the core promoter, or polymerase binding site, and refer to these additional sites as promoter-proximal elements, because they are usually found within a few hundred base pairs upstream of the transcriptional start site. Examples of these elements are the CAAT box, with the consensus sequence 5'-CCAAT-3' and the GC box, with the consensus sequence 5'-GGGCGG-3'. Specific transcription factors can bind to these promoter-proximal elements to regulate gene transcription. A given gene may have its own combination of these specific transcription-factor binding sites. There are hundreds of transcription factors in a cell, each of which binds specifically to a particular DNA sequence motif. When transcription factors bind to the promoter just upstream of the encoded gene, it is referred to as a **cis-acting element** because it is on the same chromosome just next to the gene. Transcription factors respond to environmental stimuli that cause the proteins to find their binding sites and initiate transcription of the gene that is needed.

Enhancers and Transcription

In some eukaryotic genes, there are additional regions that help increase or enhance transcription. These regions, called **enhancers**, are not necessarily close to the genes they enhance. They can be located upstream of a gene, within the coding region of the gene, downstream of a gene, or may be thousands of nucleotides away.

Enhancer regions are binding sequences, or sites, for specific transcription factors. When a protein transcription factor binds to its enhancer sequence, the shape of the protein changes, allowing it to interact with proteins at the promoter site. However, since the enhancer region may be distant from the promoter, the DNA must bend to allow the proteins at the two sites to come into contact. DNA bending proteins help to bend the DNA and bring the enhancer and promoter regions together (Figure 16.10). This shape change allows for the interaction of the specific activator proteins bound to the enhancers with the general transcription factors bound to the promoter region and the RNA polymerase.

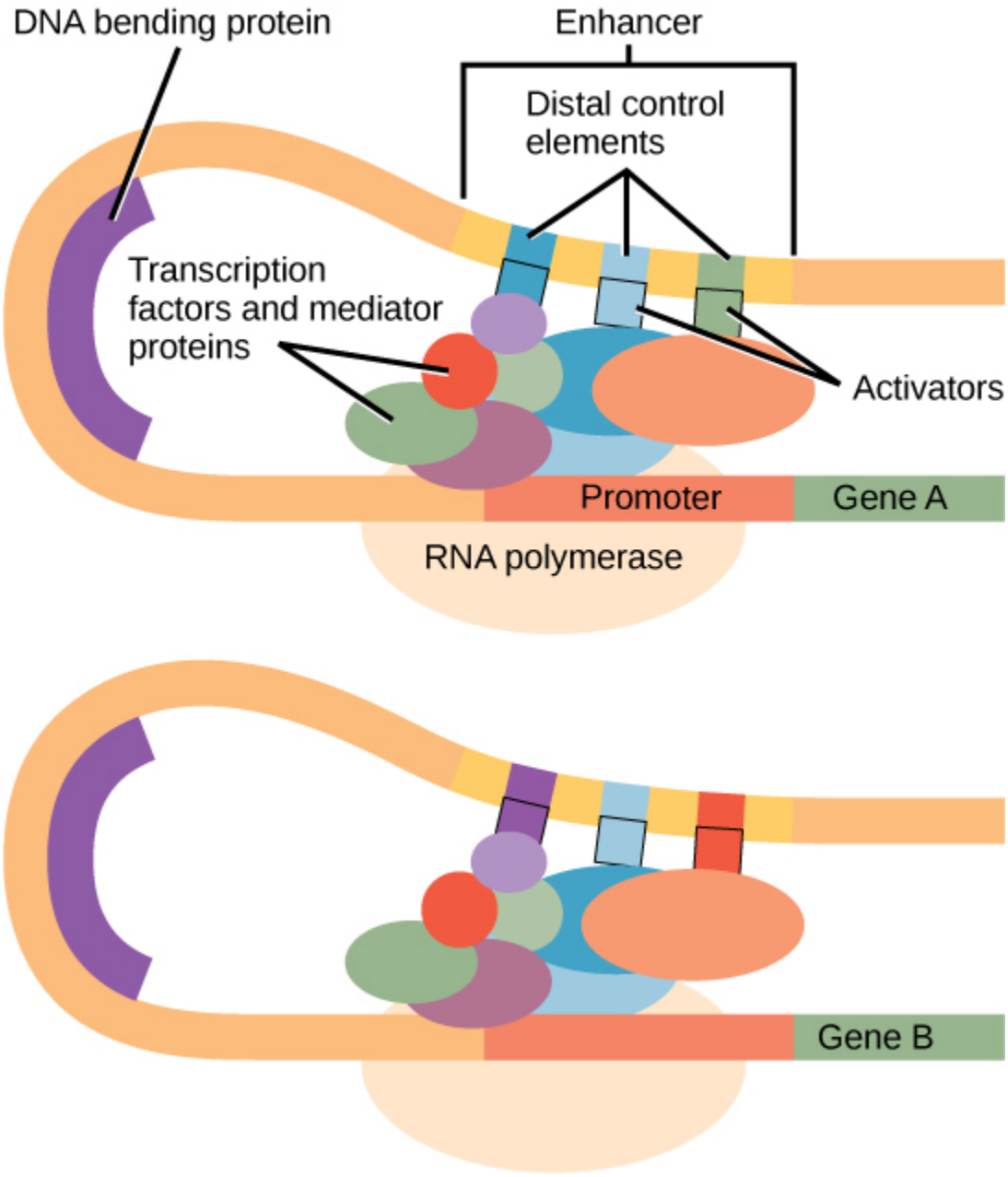

Figure 16.10 Interaction between proteins at the promoter and enhancer sites. An enhancer is a DNA sequence that promotes transcription. Each enhancer is made up of short DNA sequences called distal control elements. Activators bound to the distal control elements interact with mediator proteins and transcription factors. Two different genes may have the same promoter but different distal control elements, enabling differential gene expression.

Turning Genes Off: Transcriptional Repressors

Like prokaryotic cells, eukaryotic cells also have mechanisms to prevent transcription. Transcriptional

repressors can bind to promoter or enhancer regions and block transcription. Like the transcriptional activators, repressors respond to external stimuli to prevent the binding of activating transcription factors.

158.

EUKARYOTIC POST-TRANSCRIPTIONAL GENE REGULATION

Learning Objectives

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

- Understand RNA splicing and explain its role in regulating gene expression
- Describe the importance of RNA stability in gene regulation

RNA is transcribed, but must be processed into a mature form before translation can begin. This processing that takes place after an RNA molecule has been transcribed, but before it is translated into a protein, is called *post-transcriptional modification*. As with the epigenetic and transcriptional stages of processing, this post-transcriptional step can also be regulated to control gene expression in the cell. If the RNA is not processed, shuttled, or translated, then no protein will be synthesized.

RNA Splicing, the First Stage of Post-transcriptional Control

In eukaryotic cells, the RNA transcript often contains regions, called introns, that are removed prior to translation. The regions of RNA that code for protein are called **exons**. (Figure 16.11). After an RNA molecule has been transcribed, but prior to its departure from the nucleus to be translated, the RNA is processed and the introns are removed by splicing. Splicing is done by spliceosomes, ribonucleoprotein complexes that can recognize the two ends of the intron, cut the transcript at those two points, and bring the exons together for ligation.

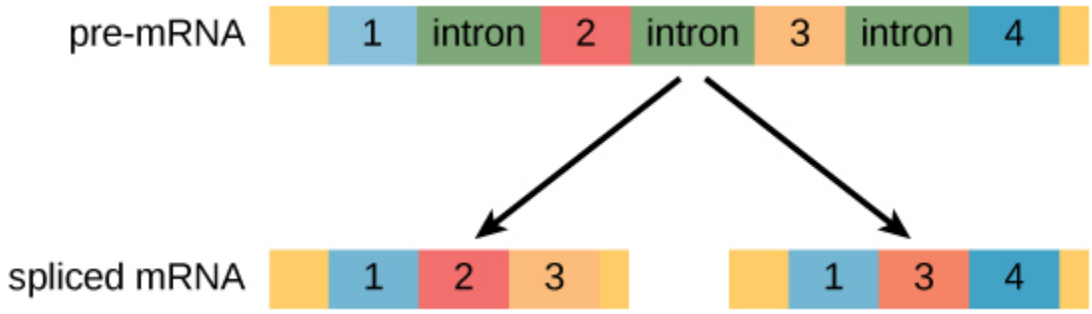

Figure 16.11 Pre-mRNA can be alternatively spliced to create different proteins.

Evolution Connection

Alternative RNA Splicing

In the 1970s, genes were first observed that exhibited alternative RNA splicing. Alternative RNA splicing is a mechanism that allows different protein products to be produced from one gene when different combinations of exons are combined to form the mRNA (Figure 16.12). This alternative splicing can be haphazard, but more often it is controlled and acts as a mechanism of *gene regulation*, with the frequency of different splicing alternatives controlled by the cell as a way to control the production of different protein products in different cells or at different stages of development. Alternative splicing is now understood to be a common mechanism of gene regulation in eukaryotes; according to one estimate, 70 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.

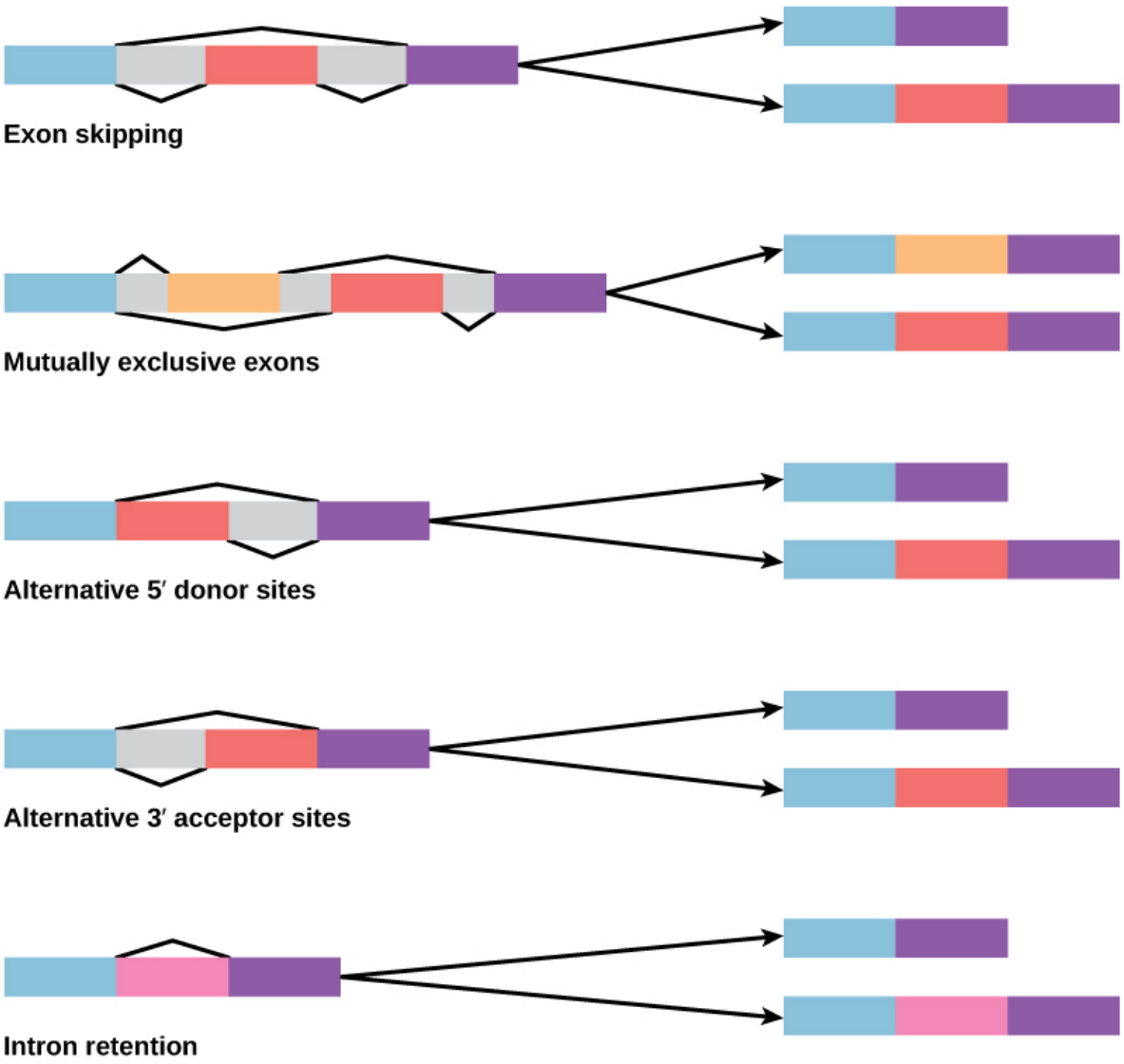

Figure 16.12 There are five basic modes of alternative splicing.

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. However, alternative splicing could possibly create a protein variant

without the loss of the original protein, opening up possibilities for adaptation of the new variant to new functions. Gene duplication has played an important role in the evolution of new functions in a similar way by providing genes that may evolve without eliminating the original, functional protein.

Question: In the corn snake *Pantherophis guttatus*, there are several different color variants, including amelanistic snakes whose skin patterns display only red and yellow pigments. The cause of amelanism in these snakes was recently identified as the insertion of a transposable element into an intron in the OCA2 (oculocutaneous albinism) gene. How might the insertion of extra genetic material into an intron lead to a nonfunctional protein?

Link to Learning

Visualize how mRNA splicing happens by watching the process in action in this video.

Click to view content

Before the mRNA leaves the nucleus, it is given two protective "caps" that prevent the ends of the strand from degrading during its journey. 5′ and 3′ exonucleases can degrade unprotected RNAs. The **5′ cap**, which is placed on the 5′ end of the mRNA, is usually composed of a methylated guanosine triphosphate molecule (GTP). The GTP is placed "backward" on the 5′ end of the mRNA, so that the 5′ carbons of the GTP and the terminal nucleotide are linked through three phosphates. The **poly-A tail**, which is attached to the 3′ end, is usually composed of a long chain of adenine nucleotides. These changes protect the two ends of the RNA from exonuclease attack.

Once the RNA is transported to the cytoplasm, the length of time that the RNA resides there can be controlled. Each RNA molecule has a defined lifespan and decays at a specific rate. This rate of decay can influence how much protein is in the cell. If the decay rate is increased, the RNA will not exist in the cytoplasm as long, shortening the time available for translation of the mRNA to occur. Conversely, if the rate of decay is decreased, the mRNA molecule will reside in the cytoplasm longer and more protein can be translated. This rate of decay is referred to as the RNA stability. If the RNA is stable, it will be detected for longer periods of time in the cytoplasm.

Binding of proteins to the RNA can also influence its stability. Proteins called **RNA-binding proteins**, or RBPs, can bind to the regions of the mRNA just upstream or downstream of the protein-coding region. These regions in the RNA that are not translated into protein are called the untranslated regions, or UTRs. They are not introns (those have been removed in the nucleus). Rather, these are regions that regulate mRNA localization, stability, and protein translation. The region just before the protein-coding region is called the **5′ UTR**, whereas the region after the coding region is called the **3′ UTR** (Figure 16.13). The binding of RBPs to these regions can increase or decrease the stability of an RNA molecule, depending on the specific RBP that binds.

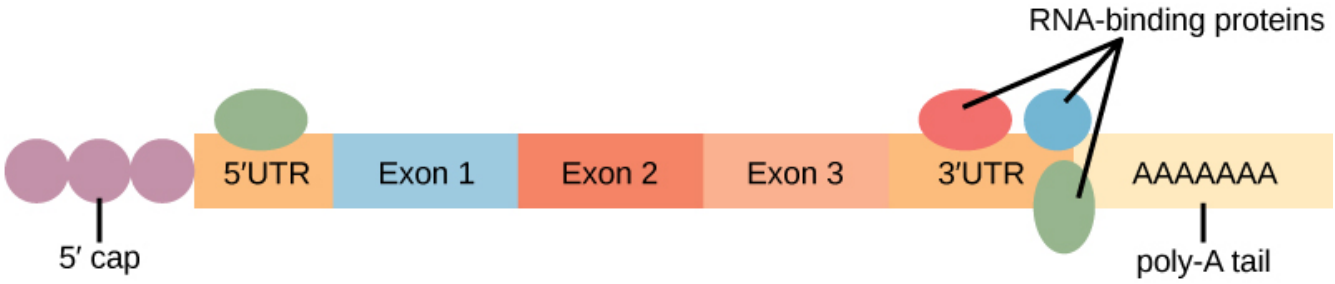

Figure 16.13 RNA-binding proteins. The protein-coding region of this processed mRNA is flanked by 5' and 3' untranslated regions (UTRs). The presence of RNA-binding proteins at the 5' or 3' UTR influences the stability of the RNA molecule.

RNA Stability and microRNAs

In addition to RBPs that bind to and control (increase or decrease) RNA stability, other elements called microRNAs can bind to the RNA molecule. These **microRNAs**, or miRNAs, are short RNA molecules that are only 21 to 24 nucleotides in length. The miRNAs are made in the nucleus as longer pre-miRNAs. These pre-miRNAs are chopped into mature miRNAs by a protein called ***Dicer***. Like transcription factors and RBPs, mature miRNAs recognize a specific sequence and bind to the RNA; however, miRNAs also associate with a ribonucleoprotein complex called the **RNA-induced silencing complex (RISC)**. The RNA component of the RISC base-pairs with complementary sequences on an mRNA and either impede translation of the message or lead to the degradation of the mRNA.

159.

EUKARYOTIC TRANSLATIONAL AND 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

After RNA has been transported to the cytoplasm, it is translated into protein. Control of this process is largely dependent on the RNA molecule. As previously discussed, the stability of the RNA will have a large impact on its translation into a protein. As the stability changes, the amount of time that it is available for translation also changes.

The Initiation Complex and Translation Rate

Like transcription, translation is controlled by proteins that bind and initiate the process. In translation, the complex that assembles to start the process is referred to as the translation **initiation complex**. In eukaryotes, translation is initiated by binding the initiating met-tRNAi to the 40S ribosome. This tRNA is brought to the 40S ribosome by a protein initiation factor, **eukaryotic initiation factor-2 (eIF-2)**. The eIF-2 protein binds to the high-energy molecule **guanosine triphosphate (GTP)**. The tRNA-eIF2-GTP complex then binds to the 40S ribosome. A second complex forms on the mRNA. Several different initiation factors recognize the 5′ cap of the mRNA and proteins bound to the poly-A tail of the same mRNA, forming the mRNA into a

loop. The cap-binding protein eIF4F brings the mRNA complex together with the 40S ribosome complex. The ribosome then scans along the mRNA until it finds a start codon AUG. When the anticodon of the initiator tRNA and the start codon are aligned, the GTP is hydrolyzed, the initiation factors are released, and the large **60S ribosomal subunit** binds to form the translation complex. The binding of eIF-2 to the RNA is controlled by phosphorylation. If eIF-2 is phosphorylated, it undergoes a conformational change and cannot bind to GTP. Therefore, the initiation complex cannot form properly and translation is impeded (Figure 16.14). When eIF-2 remains unphosphorylated, the initiation complex can form normally and translation can proceed.

Visual Connection

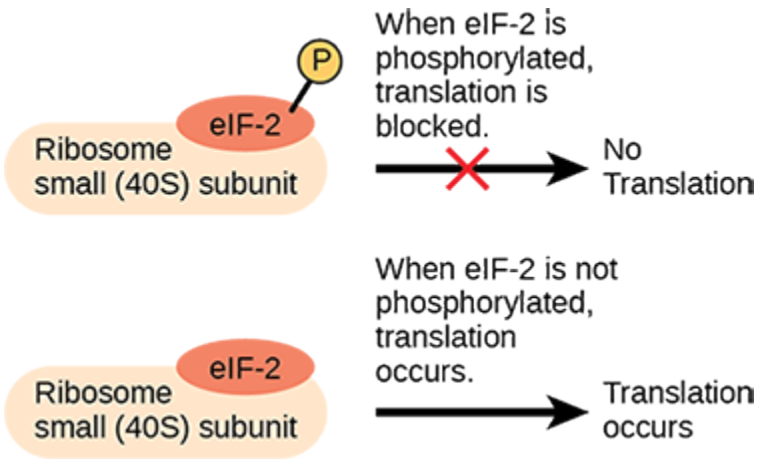

Figure 16.14 Gene expression can be controlled by factors that bind the translation initiation complex

An increase in phosphorylation levels of eIF-2 has been observed in patients with neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's. What impact do you think this might have on protein synthesis?

Proteins can be chemically modified with the addition of groups including methyl, phosphate, acetyl, and ubiquitin groups. The addition or removal of these groups from proteins regulates their activity or the length of time they exist in the cell. Sometimes these modifications can regulate where a protein is found in the cell—for example, in the nucleus, in the cytoplasm, or attached to the plasma membrane.

Chemical modifications occur in response to external stimuli such as stress, the lack of nutrients, heat, or ultraviolet light exposure. These changes can alter epigenetic accessibility, transcription, mRNA stability, or translation—all resulting in changes in expression of various genes. This is an efficient way for the cell to rapidly change the levels of specific proteins in response to the environment. Because proteins are involved in every stage of gene regulation, the phosphorylation of a protein (depending on the protein that is modified) can alter accessibility to the chromosome, can alter translation (by altering transcription factor binding or function), can change nuclear shuttling (by influencing modifications to the nuclear pore complex), can alter RNA stability (by binding or not binding to the RNA to regulate its stability), can modify translation (increase or decrease), or can change post-translational modifications (add or remove phosphates or other chemical modifications).

The addition of a ubiquitin group to a protein marks that protein for degradation. Ubiquitin acts like a flag indicating that the protein lifespan is complete. These proteins are moved to the **proteasome**, an organelle that functions to remove proteins, to be degraded (Figure 16.15). One way to control gene expression, therefore, is to alter the longevity of the protein.

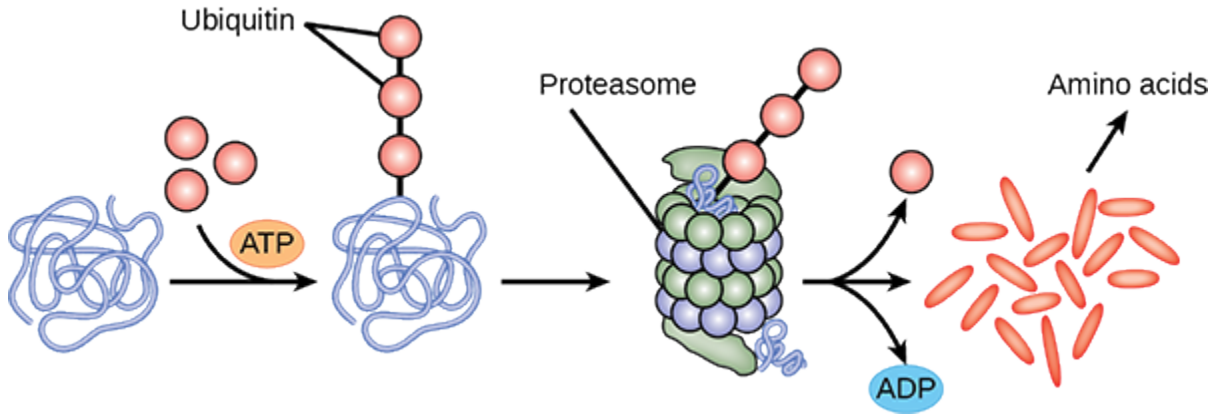

Figure 16.15 Proteins with ubiquitin tags are marked for degradation within the proteasome.

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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
- Explain how changes to gene expression at different levels can disrupt the cell cycle
- Discuss how understanding regulation of gene expression can lead to better drug design

Cancer is not a single disease but includes many different diseases. In cancer cells, mutations modify cell-cycle control, and cells don't stop growing as they normally would. Mutations can also alter the growth rate or the progression of the cell through the cell cycle. One example of a gene modification that alters the growth rate is increased phosphorylation of cyclin B, a protein that controls the progression of a cell through the cell cycle and serves as a cell-cycle checkpoint protein.

For cells to move through each phase of the cell cycle, the cell must pass through checkpoints. This ensures that the cell has properly completed the step and has not encountered any mutation that will alter its function. Many proteins, including cyclin B, control these checkpoints. The phosphorylation of cyclin B, a post-translational event, alters its function. As a result, cells can progress through the cell cycle unimpeded, even if mutations exist in the cell and its growth should be terminated. This post-translational change of cyclin B prevents it from controlling the cell cycle and contributes to the development of cancer.

Cancer: Disease of Altered Gene Expression

Cancer can be described as a disease of altered gene expression. There are many proteins that are turned on or off (gene activation or gene silencing) that dramatically alter the overall activity of the cell. A gene that is

not normally expressed in that cell can be switched on and expressed at high levels. This can be the result of gene mutation or changes in gene regulation (epigenetic, transcription, post-transcription, translation, or post-translation).

Changes in epigenetic regulation, transcription, RNA stability, protein translation, and post-translational control can be detected in cancer. While these changes don't occur simultaneously in one cancer, changes at each of these levels can be detected when observing cancer at different sites in different individuals. Therefore, changes in **histone acetylation** (epigenetic modification that leads to gene expression), activation of transcription factors by phosphorylation, increased RNA stability, increased translational control, and protein modification can all be detected at some point in various cancer cells. Scientists are working to understand the common changes that give rise to certain types of cancer or how a modification might be exploited to destroy a tumor cell.

Tumor Suppressor Genes, Oncogenes, and Cancer

In normal cells, some genes function to prevent excess, inappropriate cell growth. These are tumor-suppressor genes, which are active in normal cells to prevent uncontrolled cell growth. There are many tumor-suppressor genes in cells. The most studied tumor-suppressor gene is p53, which is mutated in over 50 percent of all cancer types. The p53 protein itself functions as a transcription factor. It can bind to sites in the promoters of genes to initiate transcription. Therefore, the mutation of p53 in cancer will dramatically alter the transcriptional activity of its target genes.

Link to Learning

Watch this animation to learn more about the use of p53 in fighting cancer.

Proto-oncogenes are positive cell-cycle regulators. When mutated, proto-oncogenes can become oncogenes and cause cancer. Overexpression of the oncogene can lead to uncontrolled cell growth. This is because oncogenes can alter transcriptional activity, stability, or protein translation of another gene that directly or indirectly controls cell growth. An example of an oncogene involved in cancer is a protein called myc. **Myc** is a transcription factor that is aberrantly activated in Burkett's Lymphoma, a cancer of the lymph system. Overexpression of myc transforms normal B cells into cancerous cells that continue to grow uncontrollably. High B-cell numbers can result in tumors that can interfere with normal bodily function. Patients with Burkett's lymphoma can develop tumors on their jaw or in their mouth that interfere with the ability to eat.

Cancer and Epigenetic Alterations

Silencing genes through epigenetic mechanisms is also very common in cancer cells. There are characteristic modifications to histone proteins and DNA that are associated with silenced genes. In cancer cells, the DNA in the promoter region of silenced genes is methylated on cytosine DNA residues in CpG islands. Histone proteins that surround that region lack the acetylation modification that is present when the genes are expressed in normal cells. This combination of DNA methylation and histone deacetylation (epigenetic modifications that lead to gene silencing) is commonly found in cancer. When these modifications occur, the gene present in that chromosomal region is silenced. Increasingly, scientists understand how epigenetic changes are altered in cancer. Because these changes are temporary and can be reversed—for example, by preventing the action of the histone deacetylase protein that removes acetyl groups, or by DNA methyl transferase enzymes that add methyl groups to cytosines in DNA—it is possible to design new drugs and new therapies to take advantage of the reversible nature of these processes. Indeed, many researchers are testing how a silenced gene can be switched back on in a cancer cell to help re-establish normal growth patterns.

Genes involved in the development of many other illnesses, ranging from allergies to inflammation to autism, are thought to be regulated by epigenetic mechanisms. As our knowledge of how genes are controlled deepens, new ways to treat diseases like cancer will emerge.

Cancer and Transcriptional Control

Alterations in cells that give rise to cancer can affect the transcriptional control of gene expression. Mutations that activate transcription factors, such as increased phosphorylation, can increase the binding of a transcription factor to its binding site in a promoter. This could lead to increased transcriptional activation of that gene that results in modified cell growth. Alternatively, a mutation in the DNA of a promoter or enhancer region can increase the binding ability of a transcription factor. This could also lead to the increased transcription and aberrant gene expression that is seen in cancer cells.

Researchers have been investigating how to control the transcriptional activation of gene expression in cancer. Identifying how a transcription factor binds, or a pathway that activates where a gene can be turned off, has led to new drugs and new ways to treat cancer. In breast cancer, for example, many proteins are overexpressed. This can lead to increased phosphorylation of key transcription factors that increase transcription. One such example is the overexpression of the epidermal growth-factor receptor (EGFR) in a subset of breast cancers. The EGFR pathway activates many protein kinases that, in turn, activate many transcription factors that control genes involved in cell growth. New drugs that prevent the activation of EGFR have been developed and are used to treat these cancers.

Cancer and Post-transcriptional Control

Changes in the post-transcriptional control of a gene can also result in cancer. Recently, several groups of researchers have shown that specific cancers have altered expression of miRNAs. Because miRNAs bind to the 3′ UTR of RNA molecules to degrade them, overexpression of these miRNAs could be detrimental to normal cellular activity. Too many miRNAs could dramatically decrease the RNA population, leading to a decrease in protein expression. Several studies have demonstrated a change in the miRNA population in specific cancer types. It appears that the subset of miRNAs expressed in breast cancer cells is quite different from the subset expressed in lung cancer cells or even from normal breast cells. This suggests that alterations in miRNA activity can contribute to the growth of breast cancer cells. These types of studies also suggest that if some miRNAs are specifically expressed only in cancer cells, they could be potential drug targets. It would, therefore, be conceivable that new drugs that turn off miRNA expression in cancer could be an effective method to treat cancer.

Cancer and Translational/Post-translational Control

There are many examples of how translational or post-translational modifications of proteins arise in cancer. Modifications are found in cancer cells from the increased translation of a protein to changes in protein phosphorylation to alternative splice variants of a protein. An example of how the expression of an alternative form of a protein can have dramatically different outcomes is seen in colon cancer cells. The c-Flip protein, a protein involved in mediating the cell-death pathway, comes in two forms: long (c-FLIPL) and short (c-FLIPS). Both forms appear to be involved in initiating controlled cell-death mechanisms in normal cells. However, in colon cancer cells, expression of the long form results in increased cell growth instead of cell death. Clearly, the expression of the wrong protein dramatically alters cell function and contributes to the development of cancer.

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 overexpression 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.

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KEY TERMS

3' UTR
: 3′ untranslated region; region just downstream of the protein-coding region in an RNA molecule that is not translated

5' cap
: a methylated guanosine triphosphate (GTP) molecule that is attached to the 5′ end of a messenger RNA to protect the end from degradation

5' UTR
: 5′ untranslated region; region just upstream of the protein-coding region in an RNA molecule that is not translated

activator
: protein that binds to prokaryotic operators to increase transcription

catabolite activator protein (CAP)
: protein that complexes with cAMP to bind to the promoter sequences of operons which control sugar processing when glucose is not available

cis-acting element
: transcription factor binding sites within the promoter that regulate the transcription of a gene adjacent to it

Dicer
: enzyme that chops the pre-miRNA into the mature form of the miRNA

DNA methylation
: epigenetic modification that leads to gene silencing; a process involving adding a methyl group to the DNA molecule

enhancer
: segment of DNA that is upstream, downstream, perhaps thousands of nucleotides away, or on another chromosome that influence the transcription of a specific gene

epigenetic
: heritable changes that do not involve changes in the DNA sequence

eukaryotic initiation factor-2 (eIF-2)
: protein that binds first to an mRNA to initiate translation

gene expression
: processes that control the turning on or turning off of a gene

guanine diphosphate (GDP)
: molecule that is left after the energy is used to start translation

guanine triphosphate (GTP)
: energy-providing molecule that binds to eIF-2 and is needed for translation

histone acetylation
: epigenetic modification that leads to gene expression; a process involving adding or removing an acetyl functional group

inducible operon
: operon that can be activated or repressed depending on cellular needs and the surrounding environment

initiation complex
: protein complex containing eIF-2 that starts translation

lac operon
: operon in prokaryotic cells that encodes genes required for processing and intake of lactose

large 60S ribosomal subunit
: second, larger ribosomal subunit that binds to the RNA to translate it into protein

microRNA (miRNA)
: small RNA molecules (approximately 21 nucleotides in length) that bind to RNA molecules to degrade them

myc
: oncogene that causes cancer in many cancer cells

negative regulator
: protein that prevents transcription

operator
: region of DNA outside of the promoter region that binds activators or repressors that control gene expression in prokaryotic cells

operon
: collection of genes involved in a pathway that are transcribed together as a single mRNA in prokaryotic cells

poly-A tail
: a series of adenine nucleotides that are attached to the 3′ end of an mRNA to protect the end from degradation

positive regulator
: protein that increases transcription

post-transcriptional
: control of gene expression after the RNA molecule has been created but before it is translated into

protein

post-translational
: control of gene expression after a protein has been created

promoter
: short DNA segment where RNA polymerase connects for the first time to start transcription of the group gene

proteasome
: organelle that degrades proteins

repressor
: protein that binds to the operator of prokaryotic genes to prevent transcription

RISC
: protein complex that binds along with the miRNA to the RNA to degrade it

RNA stability
: how long an RNA molecule will remain intact in the cytoplasm

RNA-binding protein (RBP)
: protein that binds to the 3′ or 5′ UTR to increase or decrease the RNA stability

small 40S ribosomal subunit
: ribosomal subunit that binds to the RNA to translate it into protein

trans-acting element
: transcription factor binding site found outside the promoter or on another chromosome that influences the transcription of a particular gene

transcription factor
: protein that binds to the DNA at the promoter or enhancer region and that influences transcription of a gene

transcription factor binding site
: sequence of DNA to which a transcription factor binds

transcriptional start site
: site at which transcription begins

trp operon
: series of genes necessary to synthesize tryptophan in prokaryotic cells

tryptophan
: amino acid that can be synthesized by prokaryotic cells when necessary

untranslated region
: segment of the RNA molecule that is not translated into protein. These regions lie before (upstream or 5′) and after (downstream or 3′) the protein-coding region

162.

CHAPTER SUMMARY

16.1 Regulation of Gene Expression

While all somatic 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.

16.2 Prokaryotic Gene Regulation

The regulation of gene expression in prokaryotic cells occurs at the transcriptional level. There are two majors kinds of proteins that control prokaryotic transcription: repressors and activators. Repressors bind to an operator region to block the action of RNA polymerase. Activators bind to the promoter to enhance the binding of RNA polymerase. Inducer molecules can increase transcription either by inactivating repressors or by activating activator proteins. In the *trp* operon, the *trp* repressor is itself activated by binding to tryptophan. Therefore, if tryptophan is not needed, the repressor is bound to the operator and transcription remains off. The *lac* operon is activated by the CAP (catabolite activator protein), which binds to the promoter to stabilize RNA polymerase binding. CAP is itself activated by cAMP, whose concentration rises as the concentration of glucose falls. However, the *lac* operon also requires the presence of lactose for transcription to occur. Lactose inactivates the *lac* repressor, and prevents the repressor protein from binding to the *lac* operator. With the repressor inactivated, transcription may proceed. Therefore glucose must be absent and lactose must be present for effective transcription of the *lac* operon.

16.3 Eukaryotic Epigenetic Gene Regulation

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.

16.4 Eukaryotic Transcription Gene Regulation

To start transcription, general transcription factors, such as TFIID, TFIIB, and others, must first bind to the TATA box and recruit RNA polymerase to that location. Additional transcription factors may also bind to other regulatory elements at the promoter to increase or prevent transcription. In addition to promoter sequences, enhancer regions help augment transcription. Enhancers can be upstream, downstream, within a gene itself, or on other chromosomes. Specific transcription factors bound to enhancer regions may either increase or prevent transcription.

16.5 Eukaryotic Post-transcriptional Gene Regulation

Post-transcriptional control can occur at any stage after transcription, including RNA splicing and RNA stability. Once RNA is transcribed, it must be processed to create a mature RNA that is ready to be translated. This involves the removal of introns that do not code for protein. Spliceosomes bind to the signals that mark the exon/intron border to remove the introns and ligate the exons together. Once this occurs, the RNA is mature and can be translated. Alternative splicing can produce more than one mRNA from a given transcript. Different splicing variants may be produced under different conditions.

RNA is created and spliced in the nucleus, but needs to be transported to the cytoplasm to be translated. RNA is transported to the cytoplasm through the nuclear pore complex. Once the RNA is in the cytoplasm, the length of time it resides there before being degraded, called RNA stability, can also be altered to control the overall amount of protein that is synthesized. The RNA stability can be increased, leading to longer residency time in the cytoplasm, or decreased, leading to shortened time and less protein synthesis. RNA stability is controlled by RNA-binding proteins (RPBs) and microRNAs (miRNAs). These RPBs and miRNAs bind to the 5′ UTR or the 3′ UTR of the RNA to increase or decrease RNA stability. MicroRNAs associated with RISC complexes may repress translation or lead to mRNA breakdown.

16.6 Eukaryotic Translational and Post-translational Gene Regulation

Changing the status of the RNA or the protein itself can affect the amount of protein, the function of the protein, or how long it is found in the cell. To translate the protein, a protein initiator complex must assemble on the RNA. Modifications (such as phosphorylation) of proteins in this complex can prevent proper translation from occurring. Once a protein has been synthesized, it can be modified (phosphorylated, acetylated, methylated, or ubiquitinated). These post-translational modifications can greatly impact the stability, degradation, or function of the protein.

16.7 Cancer and Gene Regulation

Cancer can be described as a disease of altered gene expression. Changes at every level of eukaryotic gene expression can be detected in some form of cancer at some point in time. In order to understand how changes to gene expression can cause cancer, it is critical to understand how each stage of gene regulation works in normal cells. By understanding the mechanisms of control in normal, non-diseased cells, it will be easier for scientists to understand what goes wrong in disease states including complex ones like cancer.

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VISUAL CONNECTION QUESTIONS

1. Figure 16.6 In E. coli, the trp operon is on by default, while the lac operon is off. Why do you think that this is the case?

2. Figure 16.8 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?

3. Figure 16.14 An increase in phosphorylation levels of eIF-2 has been observed in patients with neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's. What impact do you think this might have on protein synthesis?

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REVIEW QUESTIONS

4. 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

5. 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

6. How does the regulation of gene expression support continued evolution of more complex organisms?

a. Cells can become specialized within a multicellular organism.
b. Organisms can conserve energy and resources.
c. Cells grow larger to accommodate protein production.
d. Both A and B.

7. If glucose is absent, but so is lactose, the lac operon will be _____.

a. activated
b. repressed
c. activated, but only partially
d. mutated

8. Prokaryotic cells lack a nucleus. Therefore, the genes in prokaryotic cells are:

a. all expressed, all of the time

b. transcribed and translated almost simultaneously
c. transcriptionally controlled because translation begins before transcription ends
d. b and c are both true

9. The ara operon is an inducible operon that controls the breakdown of the sugar arabinose. When arabinose is present in a bacterium it binds to the protein AraC, and the complex binds to the initiator site to promote transcription. In this scenario, AraC is a(n) _____.

a. activator
b. inducer
c. repressor
d. operator

10. What are epigenetic modifications?

a. the addition of reversible changes to histone proteins and DNA
b. the removal of nucleosomes from the DNA
c. the addition of more nucleosomes to the DNA
d. mutation of the DNA sequence

11. Which of the following are true of epigenetic changes?

a. allow DNA to be transcribed
b. move histones to open or close a chromosomal region
c. are temporary
d. all of the above

12. The binding of _____ is required for transcription to start.

a. a protein
b. DNA polymerase
c. RNA polymerase
d. a transcription factor

13. What will result from the binding of a transcription factor to an enhancer region?

a. decreased transcription of an adjacent gene
b. increased transcription of a distant gene

c. alteration of the translation of an adjacent gene
d. initiation of the recruitment of RNA polymerase

14. A scientist compares the promoter regions of two genes. Gene A's core promoter plus proximal promoter elements encompasses 70bp. Gene B's core promoter plus proximal promoter elements encompasses 250bp. Which of the scientist's hypotheses is most likely to be correct?

a. More transcripts will be made from Gene B.
b. Transcription of Gene A involves fewer transcription factors.
c. Enhancers control Gene B's transcription.
d. Transcription of Gene A is more controlled than transcription of Gene B.

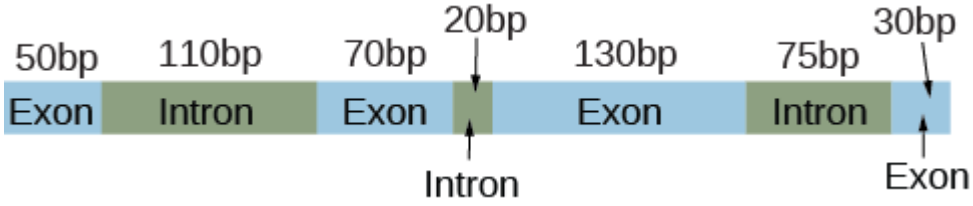
15. Which of the following are involved in post-transcriptional control?

a. control of RNA splicing
b. control of RNA shuttling
c. control of RNA stability
d. all of the above

16. Binding of an RNA binding protein will _____ the stability of the RNA molecule.

a. increase
b. decrease
c. neither increase nor decrease
d. either increase or decrease

17. An unprocessed pre-mRNA has the following structure.



Which of the following is not a possible size (in bp) of the mature mRNA?

a. 205bp
b. 180bp
c. 150bp
d. 100bp

18. Alternative splicing has been estimated to occur in more than 95% of multi-exon genes. Which of the following is not an evolutionary advantage of alternative splicing?

a. Alternative splicing increases diversity without increasing genome size.
b. Different gene isoforms can be expressed in different tissues.
c. Alternative splicing creates shorter mRNA transcripts.
d. Different gene isoforms can be expressed during different stages of development.

19. Post-translational modifications of proteins can affect which of the following?

a. protein function
b. transcriptional regulation
c. chromatin modification
d. all of the above

20. A scientist mutates eIF-2 to eliminate its GTP hydrolysis capability. How would this mutated form of eIF-2 alter translation?

a. Initiation factors would not be able to bind to mRNA.
b. The large ribosomal subunit would not be able to interact with mRNA transcripts.
c. tRNAi-Met would not scan mRNA transcripts for the start codon.
d. eIF-2 would not be able to interact with the small ribosomal subunit.

21. Cancer causing genes are called _____.

a. transformation genes
b. tumor suppressor genes
c. oncogenes
d. mutated genes

22. Targeted therapies are used in patients with a set gene expression pattern. A targeted therapy that prevents the activation of the estrogen receptor in breast cancer would be beneficial to which type of patient?

a. patients who express the EGFR receptor in normal cells
b. patients with a mutation that inactivates the estrogen receptor
c. patients with lots of the estrogen receptor expressed in their tumor
d. patients that have no estrogen receptor expressed in their tumor

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CRITICAL THINKING QUESTIONS

23. Name two differences between prokaryotic and eukaryotic cells and how these differences benefit multicellular organisms.

24. Describe how controlling gene expression will alter the overall protein levels in the cell.

25. Describe how transcription in prokaryotic cells can be altered by external stimulation such as excess lactose in the environment.

26. What is the difference between a repressible and an inducible operon?

27. In cancer cells, alteration to epigenetic modifications turns off genes that are normally expressed. Hypothetically, how could you reverse this process to turn these genes back on?

28. A scientific study demonstrated that rat mothering behavior impacts the stress response in their pups. Rats that were born and grew up with attentive mothers showed low activation of stress-response genes later in life, while rats with inattentive mothers had high activation of stress-response genes in the same situation. An additional study that swapped the pups at birth (i.e., rats born to inattentive mothers grew up with attentive mothers and vice versa) showed the same positive effect of attentive mothering. How do genetics and/or epigenetics explain the results of this study?

29. Some autoimmune diseases show a positive correlation with dramatically decreased expression of histone deacetylase 9 (HDAC9, an enzyme that removes acetyl groups from histones). Why would the decreased expression of HDAC9 cause immune cells to produce inflammatory genes at inappropriate times?

30. A mutation within the promoter region can alter transcription of a gene. Describe how this can happen.

31. What could happen if a cell had too much of an activating transcription factor present?

32. A scientist identifies a potential transcription regulation site 300bp downstream of a gene and hypothesizes that it is a repressor. What experiment (with results) could he perform to support this hypothesis?

33. Describe how RBPs can prevent miRNAs from degrading an RNA molecule.

34. How can external stimuli alter post-transcriptional control of gene expression?

35. Protein modification can alter gene expression in many ways. Describe how phosphorylation of proteins can alter gene expression.

36. Alternative forms of a protein can be beneficial or harmful to a cell. What do you think would happen if too much of an alternative protein bound to the 3′ UTR of an RNA and caused it to degrade?

37. Changes in epigenetic modifications alter the accessibility and transcription of DNA. Describe how environmental stimuli, such as ultraviolet light exposure, could modify gene expression.

38. A scientist discovers a virus encoding a Protein X that degrades a subunit of the eIF4F complex.

Knowing that this virus transcribes its own mRNAs in the cytoplasm of human cells, why would Protein X be an effective virulence factor?

39. New drugs are being developed that decrease DNA methylation and prevent the removal of acetyl groups from histone proteins. Explain how these drugs could affect gene expression to help kill tumor cells.

40. How can understanding the gene expression pattern in a cancer cell tell you something about that specific form of cancer?

PART XVII

ETHICS & SOCIETAL RESPONSIBILITY

Figure 17.1 A dense column of smoke rises more than 60,000 feet into the air over the Japanese port of Nagasaki, the result of an atomic bomb, the second ever used in warfare, dropped on the industrial center on August 8, 1945, from a U.S. B-29 Superfortress. Image credit: "atomic-bomb" by vaXzine is marked with CC BY-NC-ND 2.0

Chapter Outline

17.1. **Definition of Ethics & Societal Responsibility>**

17.2. **History of Ethics in STEM Science>**

17.3. **Atomic Fission Albert Einstein & Atomic Bomb>**

17.4. **Tuskegee Syphilis Experiment>**

17.5. **Helsinki Protocol for Human Research>**

17.6. **Henrietta Lacks & HeLa cells>**
17.7. **Gene Therapy and Death in 2000 >**
17.8. **Gene Therapy for Sickle Cell Disease 2018>**
17.9. **Ethics of Medical Disease Diagnostics>**
17.10. **Ethics of Genetic Testing>**
17.11. **Ethics of Gene Therapy>**
17.12. **Ethics of DNA testing in Forensic Science & Criminal Justice>**
17.13. **Importance of Ethics in Personal Experiences of Healthcare>**

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ETHICS AND SOCIETAL RESPONSIBILITY: INTRODUCTION

Introduction

This chapter explores the questions of the role of ethics in science, the role of responsibility of science for society, and the responsibility society has in science. It is not meant as an in-depth study of ethics in general. Please look at philosophy books for a thorough study of ethics.

Figure 17.1 A dense column of smoke rises more than 60,000 feet into the air over the Japanese port of Nagasaki, the result of an atomic bomb, the second ever used in warfare, dropped on the industrial center on August 8, 1945, from a U.S. B-29 Superfortress. Image credit: "atomic-bomb" by vaXzine is marked with CC BY-NC-ND 2.0

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HISTORY OF ETHICS IN STEM

During the history of science, technology, engineering, and mathematics (STEM), ethical questions or problems arose. Only very few examples are given, and chances are high that there are more important or instructive ones. The later sections list some additional examples in more depth.

Galileo Galilei (1564–1642) and Heliocentrism:

The Italian scientist Galileo proposed a model of planets, the sun, and other bodies with the sun at its center and planets of the solar system circling around it. This clashed with existing models proposing the planet Earth at the center and everything else moving around Earth. It clashed also with religious authorities and beliefs. It is conceivable that Galileo had ethical questions and problems because of his contemporary situation. He was jailed, condemned, and forced to deny his own research findings.[1]

- Had Galileo Galilei a conflict between his scientific views and his religious beliefs?
- Was it ethical for Galileo Galilei to disregard the warnings of the religious authorities at his time and to continue to pursue the scientific research and scientific dissemination of his research?
- Was it ethical for the religious authorities to prosecute Galileo Galilei and condemn him for his research and public dissemination of his findings?

1. Further reading is in this open access article Zanatta A, Zampieri F, Basso C, Thiene G. Galileo Galilei: Science vs. faith, Global Cardiology Science and Practice 2017:10 http://dx.doi.org/10.21542/gcsp.2017. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5871402/

Figure 17.2: Drawing of Galileo Galilei. Image credit: 'Galileo Galilei' by Biblioteca Rector Machado y Nuñez is marked with CC PDM 1.0.

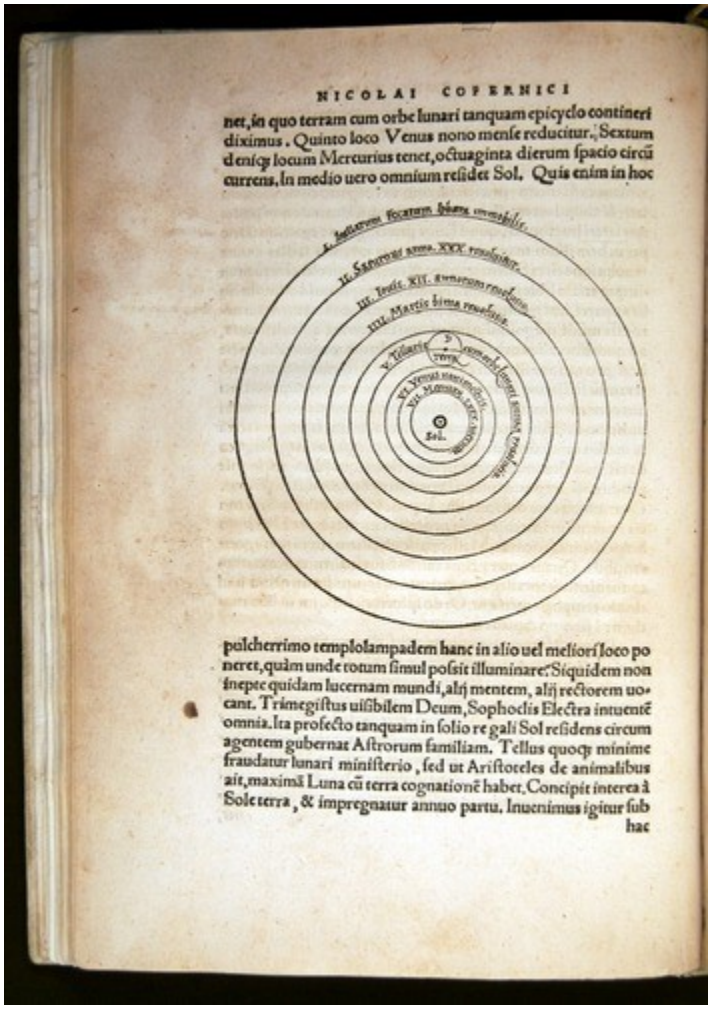

Fig 17.3: Heliocentric model proposed by Copernicus and defended by Galileo. (image courtesy History of Science Collections, University of Oklahoma Libraries, marked with CC0 1.0.)

Fig. 17.4: Charles Darwin Bicentenary Statue, Cambridge 17-01-2010. (Image credit: "Charles Darwin Bicentenary Statue, Cambridge 17-01-2010" by Karen Roe is licensed under CC BY 2.0.)

Charles Darwin (1809–1882) and the Origin of Species:

Charles Darwin proposed to his contemporaries that animal species evolved and were not created. His explanations for biological diversity and species raised concerns with prevailing religious views of the origins of animals and biological diversity arising from a creator. Charles Darwin wrote also a second book, entitled *The Descent of Man, and Selection in Relation to Sex*, wherein he proposes that morality and ethics in humans developed or evolved for biological reasons. It was interpreted as a biologicalization of ethics. However, that proposal of evolutionary ethics has received wide criticism even into contemporary times.[2]

Some of the following concerns were raised:

- Are there dangers if the concept of the survival of a "tribe" or "race" is used as a measuring stick to defend ethical standards in a society?
- Is a nation's war culture justified for ethical reasons if it allows the nation to survive better against other competing nations?
- Is it ethical to justify cannibalism if a society evolved where cannibalism is practiced?

Rosalind Franklin (1920–1958) and the DNA structure

In 1951, Rosalind Franklin was in charge of setting up a lab for a highly complicated, very advanced technology. She was building a lab for X-ray diffraction or crystallography. Combining physics, chemistry, and mathematics, the structure of a molecule can be calculated based on experimental data. X-rays hitting atoms of a molecule will be redirected or diffracted. A layer of X-ray sensitive film changes color when X-rays are hitting the material. The resulting pattern caused by diffracted X-rays is based on the molecular structure, but in an indirect way. Experience and knowledge of physics, chemistry, and mathematics are needed to make sense of the data and figure out the structure of the molecule. Within two years, Rosalind Franklin not only set up a working lab, but she also came up with a brand new analysis of the structure of DNA. She prepared a scientific

2. References: (1) Allhoff F. Evolutionary ethics from Darwin to Moore. Hist Philos Life Sci. 2003;25(1):51-79. doi: 10.1080/0391971031233127294 5. PMID: 15293515. https://pubmed.ncbi.nlm.nih.gov/15293515/ accessed 3/23/2022. (2) The difference of being human: Morality. Francisco J. Ayala. PNAS. May 5, 2010. 107 (supplement_2) 9015-9022 https://doi.org/10.1073/pnas.0914616107 accessed 3/23/2022).

manuscript in 1953 and published it. She describes a double helix with phosphates at the outside. She also delayed her publication until she had done the tedious mathematical calculations that supported her model structure. There were no modern-day computers that could cut down calculations from weeks or months to mere days. Rosalind Franklin studied the structure of DNA and provided the exact calculations needed.

Her findings were taken without her consent and knowledge and used by Watson and Crick, who received the credit and also the Nobel prize. Anne Sayre's book entitled *Rosalind Franklin and DNA* (published by Norton in New York in 1975) shines light on unethical or questionable behaviors. It was pointed out that Watson and Crick claim not to have known Rosalind Franklin's data when they devised their model, but the facts discovered by others indicate the opposite, as pointed out on this website. It is also pointed out by some that Watson and Crick allegedly did not do any hands-on work with DNA in a "wet lab." Their work was only theoretical, and they used the experimental findings of Rosalind Franklin without her consent. Rosalind Franklin's story raises a glaring ethics controversy in gender bias.[3]

- Is it ethical to take someone else's research findings without their consent?
- Is it ethical to take someone else's research findings to improve your own research without acknowledging the contribution of that work?
- Is it ethical to treat someone with less respect or give them less credit because they are a female?

3. There quite a few online resources available. Another one is https://onlineethics.org/cases/ethics-science-classroom/search-structure-dna accessed 3/23/2022.

Figure 17.5: Poster of Rosalind Franklin (Image credit: "File:Rosalind Franklin – Beyond Curie – March for Science Poster.png" by Amanda Phingbodhipakkiya is marked with CC BY-SA 4.0)

In Vitro Fertilization of Human Egg (1978 – "Test Tube Baby")

When it seemed possible to fertilize a human egg in a test tube, ethical concerns arose.[4] It was considered unethical experimentation on human beings. Additional arguments were that the parents' desire for a child should not prevail over a possibly unsafe method that could result in a child with severe health issues. In 1978, successful in vitro fertilization (IVF) occurred in England. Even though IVF is practiced today, ethical concerns are discussed.[5] IVF can help individuals in need. IVF can also raise ethical concerns. Ethical concerns are about the age limit of a mother for IVF, single women & same-sex couples, ownership of gametes & embryos,

4. Wymelenberg S; Institute of Medicine (US). Science and Babies: Private Decisions, Public Dilemmas. Washington (DC): National Academies Press (US); 1990. 7, New Technologies: The Ethical and Social Issues. Available from: https://www.ncbi.nlm.nih.gov/books/NBK235272/ accessed 3/23/2022

5. Use of in vitro fertilization—ethical issues. Kjell Asplund. Ups J Med Sci. 2020; 125(2): 192–199. doi: 10.1080/03009734.2019.1684405. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7721055/ accessed 3/23/2022 This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/

preimplantatory genetic testing, egg storage, egg sharing, surrogacy, commercialization, public funding, and religion. Some of the following questions were raised:

- Is it ethical for parents to have IVF at a high age when the parents will not live long enough to raise the child to adulthood?
- Is it ethical for a single woman to have a child through IVF?
- Is it ethical for same-sex couples to have a child through IVF?
- Is it ethical for woman to share some of her stored eggs with a female friend?
- Is it ethical for a person to offer eggs to others for profit reasons?

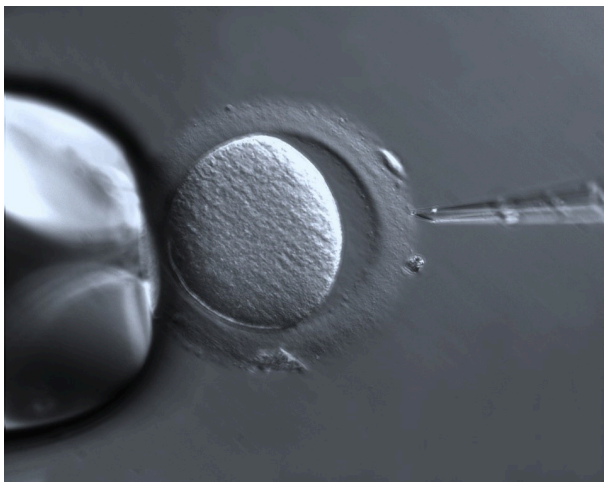

Figure 17.6: Oocyte with Zona pellucida. (Image credit: "Oocyte with Zona pellucida" by ZEISS Microscopy is marked with CC BY-SA 2.0)

Cloning of Dolly the Sheep (1996)

In 1996, scientists succeeded in cloning an adult sheep using its somatic cells instead of its gametes (egg or sperm cells). The offspring generated from the cloning was named Dolly. Cloning has different meanings in different contexts. It can mean to make identical genetic copies of a part of a gene, of a whole gene, of a whole cell, or of a whole organism.[6] For Dolly, scientists removed DNA from the nucleus of the tissue of an adult sheep. Then that DNA was inserted into the egg cell of another sheep. The egg cell has genetic information

6. Reference Authors: Mary Ann Clark, Matthew Douglas, Jung Choi. Publisher/website: OpenStax. Book title: Biology 2e. Publication date: Mar 28, 2018. Location: Houston, Texas. Book URL: https://openstax.org/books/biology-2e/pages/1-introduction Section URL: https://openstax.org/books/biology-2e/pages/17-1-biotechnology; accessed 3-24-2022

within mitochondria from the donating mother. Therefore, Dolly was not 100% a clone. Dolly was euthanized at around the age of 5 years due to health complications. Sheep are culled in agriculture around 5 years of age. The maximum lifespan for sheep is estimated to be close to 23 years.[7] Dolly raised ethical questions about animal cloning. Some of them are listed as follows:

- Is it ethical to do cloning if at that time the success rate was at best 12%, and 88% was the lowest failure rate (rates from A. Fiester. 2005)?
- Is it ethical to expose the female animal to treatments and surgery to retrieve a useful egg for animal cloning?
- Is it ethical to expose the surrogate mother animal to treatments to bring the cloned egg to birth?
- Is it ethical to conduct animal cloning for a commercial purpose?
- Is it ethical to conduct animal cloning to preserve a species?
- Is cloning animals opening the door for attempts to clone humans?

7. Hoffman JM, Valencak TG. A short life on the farm: aging and longevity in agricultural, large-bodied mammals. Geroscience. 2020;42(3):909-922. doi:10.1007/s11357-020-00190-4

Figure 17.7: The sheep Dolly as an example of cloned mammalian animals. (Image credit: The photo of "Dolly the Sheep" by Neil T is marked with CC BY-SA 2.0.)

Human Genome Project (1990–2003)

The Human Genome Project started officially in 1990 with the goal of sequencing all genes of the human genome, sequence all 3 billion base pairs that make up the entire human genome (non-coding and coding sequences), and study the ethical, legal, and societal issues (ELSI) of the project. The U.S. Department of Energy (DOE) and the National Institutes of Health (NIH) devoted 3% to 5% of their annual Human Genome Project (HGP) budgets toward studying the ethical, legal, and social issues (ELSI) surrounding availability of genetic information.[8] The Human Genome Project Information Archive lists the following areas and questions for ethical, legal, and social issues (ELSI):

8. Reference Human Genome Project Information Archive. 1990–2003. http://www.ornl.gov/hgmis

Fairness in the use of genetic information by insurers, employers, courts, schools, adoption agencies, and the military, among others. *Who should have access to personal genetic information, and how will it be used?*

Privacy and confidentiality of genetic information. *Who owns and controls genetic information?*

Psychological impact and stigmatization due to an individual's genetic differences. *How does personal genetic information affect an individual and society's perceptions of that individual? How does genomic information affect members of minority communities?*

Reproductive issues including adequate informed consent for complex and potentially controversial procedures, use of genetic information in reproductive decision making, and reproductive rights. *Do healthcare personnel properly counsel parents about the risks and limitations of genetic technology? How reliable and useful is fetal genetic testing? What are the larger societal issues raised by new reproductive technologies?*

Clinical issues including the education of doctors and other health service providers, patients, and the general public in genetic capabilities, scientific limitations, and social risks, and implementation of standards and quality-control measures in testing procedures. *How will genetic tests be evaluated and regulated for accuracy, reliability, and utility?*

Uncertainties associated with gene tests for susceptibilities and complex conditions (e.g., heart disease) linked to multiple genes and gene-environment interactions. *Should testing be performed when no treatment is available? Should parents have the right to have their minor children tested for adult-onset diseases? Are genetic tests reliable and interpretable by the medical community?*

Conceptual and philosophical implications regarding human responsibility, free will vs genetic determinism, and concepts of health and disease. *Do people's genes make them behave in a particular way? Can people always control their behavior? What is considered acceptable diversity? Where is the line between medical treatment and enhancement?*

Health and environmental issues concerning genetically modified foods (GM) and microbes. *Are GM foods and other products safe to humans and the environment? How will these technologies affect developing nations' dependence on the West?*

Commercialization of products including property rights (patents, copyrights, and trade secrets) and accessibility of data and materials. *Who owns genes and other pieces of DNA? Will patenting DNA sequences limit their accessibility and development into useful products?*

An example is Huntington's Disease. It raises questions about the psychological impact, clinical issues, and uncertainties. This disease is inherited, and often signs or symptoms show at the adult age of 30 to 40 years old. The damage to the nerve cells can impact movement, speech, thinking, and psyche. Unfortunately, the disease is progressive, and there is no cure. There is a psychological impact if someone finds out that they have a fatal debilitating disease. There are clinical issues. Doctors and the general public need to be educated about Huntington's disease, about the consequences of finding out about a genetic disease, and about the fact that the disease's severity has a range. The uncertainties include testing for an incurable disease such as

Huntington's disease. The disease's severity ranges also between the number of trinucleotide repeats, but the demarcations are clear cut. The following ethical questions could be raised:

- *Should testing be performed when no treatment is available?*
- *Should parents have the right to have their minor children tested for adult-onset diseases?*
- *Should an 18-year-old child tell their test results to their 35-year-old parent who does not want to get tested?*
- *Should an 18-year-old child tell their positive test result to a parent who doesn't want to get tested and doesn't want to know about their chances of having inherited that disease?*

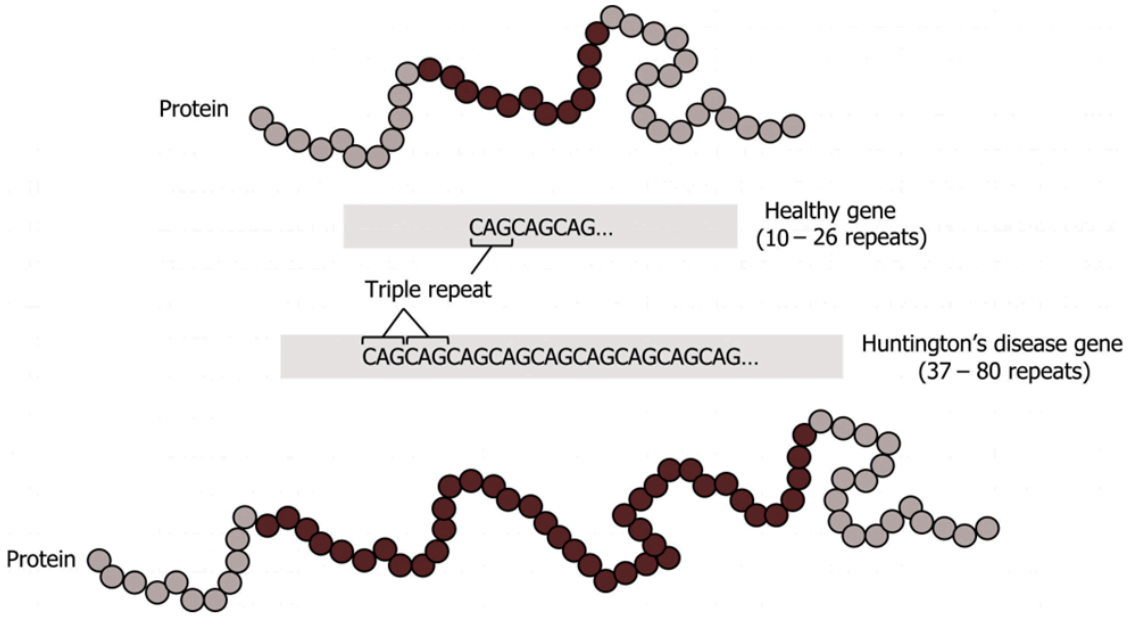

Figure 17.8: Huntington's Disease-Observed changes on the DNA level (gene) and protein level (gene product). (Image credit: Licensed with a Creative Commons Attribution NonCommercial-ShareAlike 4.0 License. Retrieved from LeClair, Renée (2021). Cell Biology, Genetics, and Biochemistry for Pre-Clinical Students. Roanoke: Virginia Tech Carilion School of Medicine. https://doi.org/10.21061/cellbio. Licensed with CC BY-NC-SA 4.0)

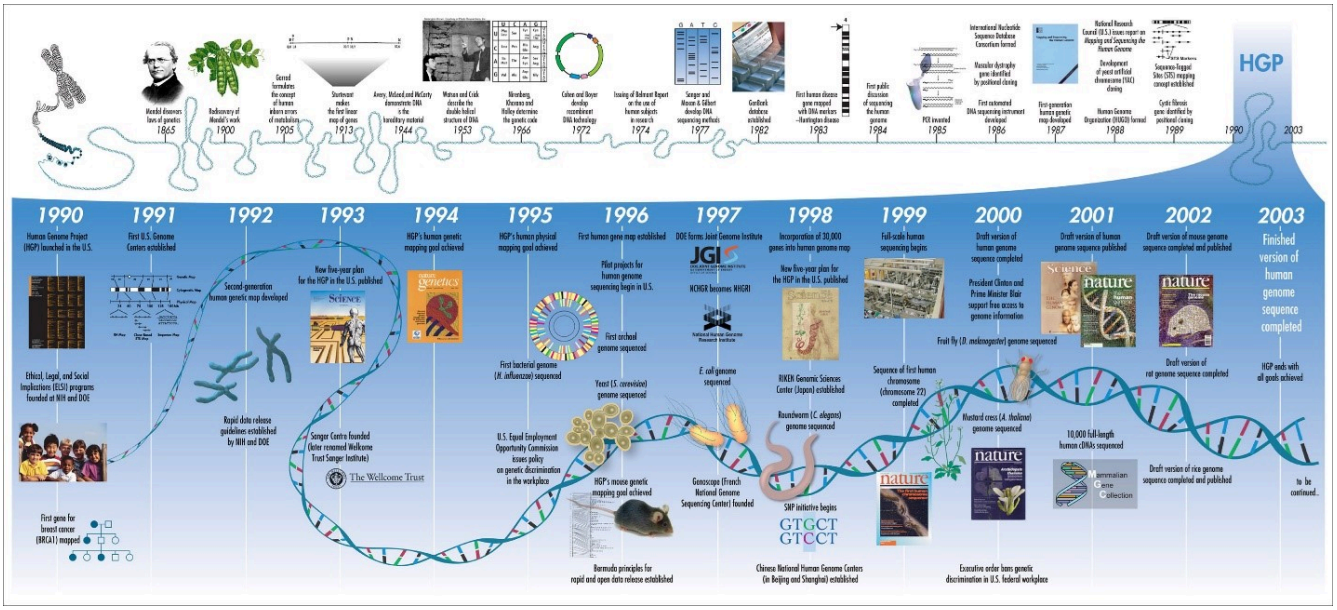

Figure 17.9: Timeline of genetic discoveries from genes by Gregor Mendel to the human genome project (HGP-in blue). (Image credit: Figure "human genome" by vaXzine is marked with CC BY-NC-ND 2.0. Also available at https://www.flickr.com/photos/56367751@N00/366134862)

Figure 17.9: Timeline of genetic discoveries from genes by Gregor Mendel to the human genome project (HGP-in blue).

Ownership of a Gene (1990–2013)

In the USA, the human genome project raised also questions about ownership of genes and patent rights for genes. Scientists, institutions including the National Institutes of Health (NIH), and commercial entities were endeavoring to patent genes or genetic information.[9] Patents were granted for different reasons such as diagnostic uses, compositions of matter, and functional uses. The genes BRCA1 & BRCA2 were patented for diagnostic uses related to breast cancer and ovarian cancer. Insulin protein made from obtaining the gene sequence of the insulin gene falls in the category of patents for compositions of matter. An example of a functional use patent is a product or drug that is regulating a gene involved in making someone healthier or preventing disease. In 2013, the Supreme Court of the USA ruled that human genes themselves can't be patented, but complementary DNA (cDNA) can. The patent claims by the company claiming broad patent infringements for the human BRCA1 & BRCA2 genes failed.

The following ethical questions can arise:

9. Reference Merz JF, Cho MK. What are gene patents and why are people worried about them? *Community Genet*. 2005;8(4):203-208

- If a research university is prevented from researching additional and deeper functions of a gene because research on the gene is prohibited by a gene patent, is that good, bad, neutral, and for whom?
- If patients can't afford to be tested for a genetic disease because a gene patent requires expensive license fees for that test, is that good, bad, neutral, and for whom?

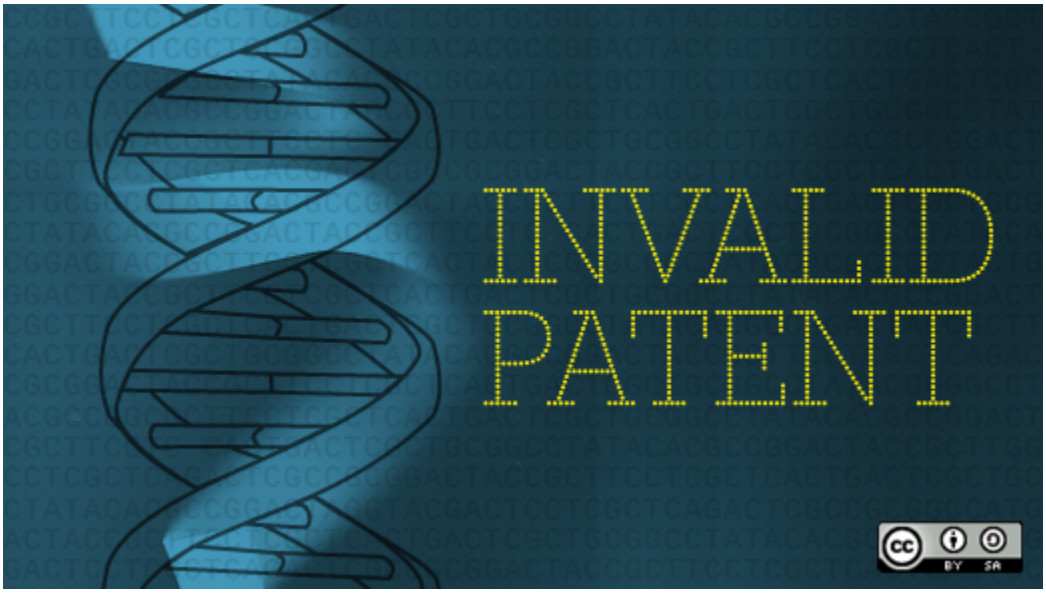

Figure 17.10 Invalid patent. (Image credit: "Gene patenting and free software: a breakthrough" by opensourceway is marked with CC BY-SA 2.0. Also available at https://www.flickr.com/photos/47691521@N07/4500295071)

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=1404#h5p-61

Further reading:

Merz, J. F., & Cho, M. K. (2005). What are gene patents and why are people worried about them?. *Community genetics*, *8*(4), 203–208. https://doi.org/10.1159/000087956

Can genes be patented?: MedlinePlus Genetics. (n.d.). https://medlineplus.gov/genetics/understanding/testing/genepatents/

Supreme Court of the USA. ASSOCIATION FOR MOLECULAR PATHOLOGY ET AL. v. MYRIAD

GENETICS, INC., ET AL. No. 12–398. 2013 free download at https://www.supremecourt.gov/opinions/12pdf/12-398_1b7d.pdf

Ownership of human genes. Correspondence: Richard M. Leibovitz. Robert Mullan Cook-Deegan Nature Vol. 382. page 17. 4 July 1996 https://www.nature.com/articles/382017a0.pdf?origin=ppub

Fiester, A. (2005). Ethical Issues in Animal Cloning. Retrieved from https://repository.upenn.edu/bioethics_papers/35 Reprinted with permission of Johns Hopkins University Press. The article originally appeared in Perspectives in Biology and Medicine, Volume 48, Issue 2, June 2005, pages 328-343. Publisher URL: http://muse.jhu.edu/journals/perspectives_in_biology_and_medicine/toc/pbm48.3.html

Harris J. "Goodbye Dolly?" The ethics of human cloning. Journal of Medical Ethics 1997;23:353-360. A version of this article is freely available as a pubmed central version using the link https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1377577/, accessed 3/24/2022

Barghachie, S. (2014, January 27). *The hype about Dolly | Ethical Issues in Health Care*. https://scholarblogs.emory.edu/philosophy316/2014/01/27/the-hype-about-dolly/

Cloned sheep raises ethical issues. (n.d.). The Canadian Encyclopedia. https://www.thecanadianencyclopedia.ca/en/article/cloned-sheep-raises-ethical-issues

Authors: Mary Ann Clark, Matthew Douglas, Jung Choi. Publisher/website: OpenStax. Book title: Biology 2e. Publication date: Mar 28, 2018. Location: Houston, Texas. Book URL: https://openstax.org/books/biology-2e/pages/1-introduction Section URL: https://openstax.org/books/biology-2e/pages/17-1-biotechnology; accessed 3-24-2022

Review of the Ethical, Legal and Social Implications Research Program and related Activities (1990-1995). (n.d.). https://www.genome.gov/10001747/elsi-program-review-19901995

Human Genome Project Information. (n.d.). http://www.ornl.gov/hgmis

Murray, T. H. (1991). Ethical issues in human genome research. *The FASEB Journal*, *5*(1), 55–60. https://doi.org/10.1096/fasebj.5.1.1825074

Huntington's disease – Diagnosis and treatment – Mayo Clinic. (2022, May 17). https://www.mayoclinic.org/diseases-conditions/huntingtons-disease/diagnosis-treatment/drc-20356122

168.

DEFINITION OF ETHICS AND SOCIETAL RESPONSIBILITY

Learning Objectives

This section explores the questions of the role of ethics in science, the role of responsibility of science for society, and the responsibility society has in science. It is not meant as an in-depth study of ethics in general. Please look at philosophy books for a thorough study of ethics.

What Is Meant by "Ethics"?

Ethics is the study of the standards of right and wrong that inform us as to how we ought to behave. These standards relate to unwritten rules that are necessary for humans to live among each other, such as "don't hurt others." We function better as a society when we treat each other well. Ethics can also refer to the standards themselves. They often pertain to rights, obligations, fairness, responsibilities, and specific virtues like honesty and loyalty. They are supported by consistent and well-founded reasons; as such, they have universal appeal. It's never good to have a society that supports hurting others as a general rule; honesty and loyalty are positive attributes. Can we think of instances when hurting others is condoned (such as in war) and where honesty or loyalty may be misplaced? Of course! That's one of the reasons why ethics are so complicated.

adapted from: Introduction to Ethics. Authors: Manuela A. Gomez, El Paso Community College. Introduction to Ethics by Lumen Learning is licensed under a Creative Commons CC BY. Attribution 4.0 International License, except where otherwise noted. accessed from https://www.oercommons.org/courses/introduction-to-ethics/ view on 3/16/2022

Types of Ethics

Ethics is a big field with different types and traditions. Only a few are listed in the following sections.

Personal Ethics

- Includes your personal values and moral qualities.
- Influenced by family, friends, culture, religion, education and many other factors.

- Examples: I believe using animals for research is morally wrong.
- Personal ethics can change and are chosen by an individual.

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Common Ethics

- Ethics that the majority of people agree on.
- Many philosophers argue there is no such ethics.
- Do we have the same ethics in the world? Do we have the same ethics in the U.S.? Does everyone in your family share the same ethics?
- Examples: Murdering people for the sake of murder is wrong.
- Notice how this would change in the context of self-defense.
- Common ethics have to be very general to avoid disagreement.

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Professional Ethics

Professional ethics can use formal codes. The purpose of these formal codes is to remove ambiguity regarding what is considered ethical and unethical behavior in that specific environment. For college students, there are formal codes about academic integrity. Formal codes about academic integrity include plagiarism. Louisiana

State University defines plagiarism as "the lack of appropriate citation, or the unacknowledged inclusion of someone else's words, structure, ideas, or data; failure to identify a source, or the submission of essentially the same work for two assignments without permission of the Instructor" (https://www.lsu.edu/saa/students/academicintegrity/index.php). Southern University and Agricultural & Mechanical College define plagiarism as "failing to identify sources, published or unpublished, copyrighted or uncopyrighted, from which information was taken" (https://www.subr.edu/page/851).

Some additional thoughts about professional ethics are as follows:

- Rules imposed on an employee in a company, or as a member of a profession. For instance, journalists, doctors, lawyers, etc.
- Imposed when you are a part of a professional setting or when you are being trained or educated for working for a specific profession.
- Examples: no gossiping, time management, punctuality, confidentiality, transparency.
- Not adhering to these may harm your professional reputation.

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Prescriptive Ethics

Adapted from Philosophical Ethics. George W. Matthews, Plymouth State University. Copyright Year: 2020. Attribution CC BY-SA (https://open.umn.edu/opentextbooks/textbooks/philosophical-ethics)

– What is really the right thing to do?

– What moral principles are really justified and should be followed?

This approach to ethics is the uniquely philosophical attempt to find the true basis of ethical thinking. This way of approaching ethics is not scientific, to the extent that science concerns itself with "value-neutral" descriptions and explanations of whatever phenomena it is addressing.

Applied Ethics

Philosophical Ethics. George W. Matthews, Plymouth State University. Copyright Year: 2020. Attribution CC BY-SA (https://open.umn.edu/opentextbooks/textbooks/philosophical-ethics)

– What is the right thing to do in real-world cases of ethical controversy?

– What assumptions and principles lie at the basis of ethical controversies?

How does all of this play out in real-life cases? Under this heading are also to be found discussions of ethical issues associated with some particular area of human life, profession, or subject matter—hence medical ethics, business ethics, legal ethics, environmental ethics, bioethics, and so on are sub-fields within applied ethics.

Ethical Approaches (for Personal Ethics)

From the earliest moments of recorded human consciousness, the ethical discipline has entailed four fundamental approaches, often called ethical decision-making frameworks: **Utilitarian Ethics** (outcome based), **Deontological Ethics** (duty based), **Virtue Ethics** (virtue based), and **Communitarian Ethics** (community based).

adapted from: Introduction to Ethics. Authors: Manuela A. Gomez, El Paso Community College. Introduction to Ethics by Lumen Learning is licensed under a Creative Commons CC BY. Attribution 4.0 International License, except where otherwise noted. accessed from https://www.oercommons.org/courses/introduction-to-ethics/ view on 3/16/2022

(2) The Primacy of the Public. Subtitle: Ethical Design for Technology Professionals. Author: Marcus Schultz-Bergin.

Research Ethics

Adapted from

The Practice of Science Versus the Uses of Science. (2021, February 20). https://socialsci.libretexts.org/@go/page/ 12555. CC BY-NC-SA 3.0

Research ethics has to do with both how research is conducted and how findings from that research are used and by whom. In this section, we'll consider research ethics from both angles.

Doing Science the Ethical Way

Adapted from

The Practice of Science Versus the Uses of Science. (2021, February 20). https://socialsci.libretexts.org/@go/page/ 12555. CC BY-NC-SA 3.0

As you should now be aware, researchers must consider their own personal ethical principles in addition to following those of their institution, their discipline, and their community.

The use of human beings as research subjects is exceptional because technically humans cannot be used in any way similar to animals in research. Considerations for human research subjects can be contemplated under systematic, scientific investigation in scenarios that can be either interventional (a "trial") or observational (no "test article"), and they are normally referred to as test subjects. Also, for the purpose of the Common

Rule, scientific research that only uses materials, including samples and specimen, from deceased persons is not typically considered human subjects research.

The ethical practice of research includes informing and protecting subjects if they are humans. But the practice of ethical research doesn't end once subjects have been identified and data have been collected. Scientists must also fully disclose their research procedures and findings. This means being honest about how research subjects were identified and recruited, how exactly data were collected and analyzed, and ultimately, what findings were reached.

If researchers fully disclose how they conducted their research, then those of us who use their work to build our own research projects, to create policies, or to make decisions about our lives can have some level of confidence in the work. By sharing how research was conducted, a researcher helps assure readers that he or she has conducted legitimate research and didn't simply come to whatever conclusions he or she *wanted* to find. A description or presentation of research findings that is not accompanied by information about research methodology is missing some relevant information. Sometimes methodological details are left out because there isn't time or space to share them. This is often the case with news reports of research findings. Other times, there may be a more insidious reason that that important information isn't there. This may be the case if sharing methodological details would call the legitimacy of a study into question. As researchers, it is our ethical responsibility to fully disclose our research procedures. As consumers of research, it is our ethical responsibility to pay attention to such details. We'll discuss this more in the section "Using Science the Ethical Way."

Full disclosure also includes the need to be honest about a study's strengths and weaknesses, both with oneself and with others. Being aware of the strengths and weaknesses of one's own work can help a researcher make reasonable recommendations about the next steps other researchers might consider taking in their inquiries. Awareness and disclosure of a study's strengths and weaknesses can also help highlight the theoretical or policy implications of one's work. In addition, openness about strengths and weaknesses helps those reading the research better evaluate the work and decide for themselves how or whether to rely on its findings. Finally, openness about a study's sponsors is crucial. How can we effectively evaluate research without knowing who paid the bills?

The standard of replicability along with openness about a study's strengths, weaknesses, and funders enable those who read the research to evaluate it fairly and completely. Knowledge of funding sources is often raised as an issue in medical research. Understandably, independent studies of new drugs may be more compelling to the Food and Drug Administration (FDA) than studies touting the virtues of a new drug that happen to have been funded by the company who created that drug. The FDA has rules in reporting research data and/or findings in R&D. One is not required to carry all manner of research on for example a new drug that has been developed by a company. In most cases, safety, efficacy and dosage are threshold findings that suffice. However, if one does any research investigation, findings reported must not be selective under any circumstances, all data must be made available to the FDA. Mandatory reporters (that is, manufacturers, device user facilities, and importers) are also required to make some reports for adverse events and product

problems about medical devices. Health care professionals, patients, caregivers and users may submit voluntary reports about adverse events that are serious adverse events associated with a medical device, use errors, poor product quality, therapeutic failures among others. Such reports and data obtained from additional sources, can provide crucial information beneficial to patient safety improvement.

Using Science the Ethical Way

Adapted from

The Practice of Science Versus the Uses of Science. (2021, February 20). https://socialsci.libretexts.org/@go/page/12555

Science has many uses. By "use," we mean the ways that science is understood and applied (as opposed to the way it is conducted). Some use science to create laws and social policies; others use it to understand themselves and those around them. Some people rely on science to improve their life conditions or those of other people, while still others use it to improve their businesses or other undertakings. In each case, the most ethical way for us to use science is to educate ourselves about the design and purpose of any studies we may wish to use or apply, to recognize our limitations in terms of scientific and methodological knowledge and how those limitations may impact our understanding of research, and to apply the findings of scientific investigation only in cases or to populations for which they are actually relevant.

When Are Research and Innovation Good for Society?

Adapted from

Responsible Innovation – 2nd edition: Ethics, Safety and Technology; 2nd edition. Authors: Joost Groot Kormelink;. TU Delft, Technology. Policy and Management

https://textbooks.open.tudelft.nl/textbooks/catalog/view/24/53/164-1 accessed 3/15/2022 DOI https://doi.org/10.5074/t.2019.006

ISBN 978-94-6366-202-4; PUBLICATION DATE October 7, 2019;

Innovation may bring a lot of good to society, but innovation is not a good in itself. History provides many examples of innovations and new technologies that had serious negative consequences, or simply failed to address significant problems and make meaningful contributions to society. Ethics is continuous. Well-known examples are carcinogenic asbestos or the ecological devastation caused by DDT. At times, science and society are intertwined in contentious ties. DDT manufacture was ethical at the time because it saved many agricultural products. DDT was shown to be persistent in the environment and to have harmful impacts on animal and human health as time went on. Once a product has been shown to be deleterious to society, there is a relentless obligation of science to pull those products from the environment. This is demonstrating ethics as well. Innovation itself can be controversial, as sometimes good intentions have had profoundly negative results with severe consequences and long-lasting impacts on society, populations, and the environment. The drug Thalidomide provides one of the best case studies that has continued to affect ethical conduct of research in numerous ways as the drug itself has found new uses. Thalidomide was original developed and tested on pregnant mice. Thalidomide was sent to the FDA for approval for clinical use but faced resistance due to insufficient data and the animal model used. Thalidomide found acceptance in the United Kingdom and generally countries in Europe and was mainly administered in the late 1950s and early 1960s for the treatment of nausea in pregnant women. By the 1960s, severe defects resulting from thalidomide treatment were being widely reported in several thousands of children. The United States escaped the scourge of the drug, but it was banned in most countries. Continuing research showed the drug to be useful in the treatment for leprosy and, later, multiple myeloma. Unfortunately, in rural areas of the world that lack extensive medical surveillance, initiatives continued to use thalidomide on pregnant women with leprosy, resulting in malformations. However, research on thalidomide has continued focusing mainly on mechanisms of action of the drug to delineate the molecular targets. Understanding how the drug works could lead to smart and safer drug design. Quite recently, it was successfully used in the treatment of some ulcers. This innovation originally brought tragedy but has led to improved toxicity testing. The United States, which initially was circumspect, has joined international regulatory agencies to develop systematic toxicity testing protocols. Thus, Thalidomide has served as a powerful tool in developmental biology, resulting in significant discoveries in the biochemical pathways of limb development. Notwithstanding the tragedies of thalidomide use, the Society of Toxicology regards its withdrawal from the market during the 1960s as a significant milestone. Ethical situations are difficult to contend, but revisiting these difficult lessons points to how far we have come and, going forward, the adjustments that have to be made and new things learnt.

It is therefore of the utmost importance—our duty, even—to define an adequate and shared conception of responsibility for our innovations and technologies. Just think about questions like the following:

- Can our innovations save lives?
- Will they produce more jobs?
- Can they save the planet, or do they only contribute more waste and pollution?
- Are they safe for users and secure from abusers?

- Do they respect the values and basic human rights we hold dear, like privacy, freedom, autonomy and equality? If not, how can we make them so? If not us, who? If not now, when?

Responsible Research and Innovation

In November 2014, the Council of the European Union issued the Rome Declaration **on Responsible Research and Innovation** in Europe. It declared that "Responsible Research and Innovation (RRI) is the ongoing process of aligning research and innovation to the values, needs, and expectations of society. Decisions in research and innovation must consider the principles on which the European Union is founded—i.e., the respect of human dignity, freedom, democracy, equality, the rule of law, and the respect of human rights, including the rights of persons belonging to minorities. RRI requires that all stakeholders in civil society are responsive to each other and take shared responsibility for the processes and outcomes of research and innovation. This means working together in science education, the definition of research agendas, the conduct of research, the access to research results, and the application of new knowledge in society—in full respect of gender equality, the gender dimension in research and ethics considerations. The benefits of Responsible Research and Innovation go beyond alignment with society: it ensures that research and innovation deliver on the promise of smart, inclusive and sustainable solutions to our societal challenges; it engages new perspectives, new innovators, and new talent from across our diverse society, allowing us to identify solutions which would otherwise go unnoticed; it builds trust between citizens and public and private institutions in supporting research and innovation; it reassures society about embracing innovative products and services; and it assesses the risks and the way these risks should be managed.

The document can be found online https://www.sis-rri-conference.eu/wp-content/uploads/2014/12/RomeDeclaration_Final.pdf

accessed 3/15/2022

International Organization for Standardization and Societal Responsibility

There are also industry standards for social responsibility. The ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). It developed a standard for societal responsibility called ISO 26000. This standard provides guidance on the underlying principles of social responsibility, recognizing social responsibility and engaging stakeholders, the core subjects and issues pertaining to social responsibility, and ways to integrate socially responsible behavior into the

organization. This International Standard emphasizes the importance of results and improvements in performance on social responsibility.

Adapted from ISO 26000:2010(en) Guidance on social responsibility, https://www.iso.org/iso-26000-social-responsibility.html, https://www.iso.org/standard/42546.html, https://www.iso.org/obp/ui/#iso:std:iso:26000:ed-1:v1:en. Accessed 3/16/2022

An interactive H5P element has been excluded from this version of the text. You can view it online here:

https://louis.pressbooks.pub/generalbiology1leclab/?p=1402#h5p-60

169.

ATOMIC FISSION, ALBERT EINSTEIN, AND THE ATOMIC BOMB

Albert Einstein was a well-known physicist concerned about research into the splitting of the atom (nuclear fission) and the threat of the atomic bomb by Nazi Germany.

Einstein Letter

In the 1930s, physicists from Europe fled the threat of Nazi Germany including Leo Szilard, Edward Teller, and Eugene Wigner. The physicists understood that Nazi Germany was researching atomic fission to build an atomic bomb. The physicists felt the ethical need to inform the American government about this threat and reached out to the German American physicist Albert Einstein. (References https://www.osti.gov/opennet/manhattan-project-history/Events/1939-1942/einstein_letter.htm accessed 3/23/2022; http://www.fdrlibrary.marist.edu/archives/pdfs/docsworldwar.pdf Accessed 3/23/2022)

In 1939, the physicists wrote a letter to warn the US President Franklin D. Roosevelt and Albert Einstein agreed to sign the letter to give it more importance in the eyes of the US government. The letter also encouraged the President to promote nuclear research in the US.

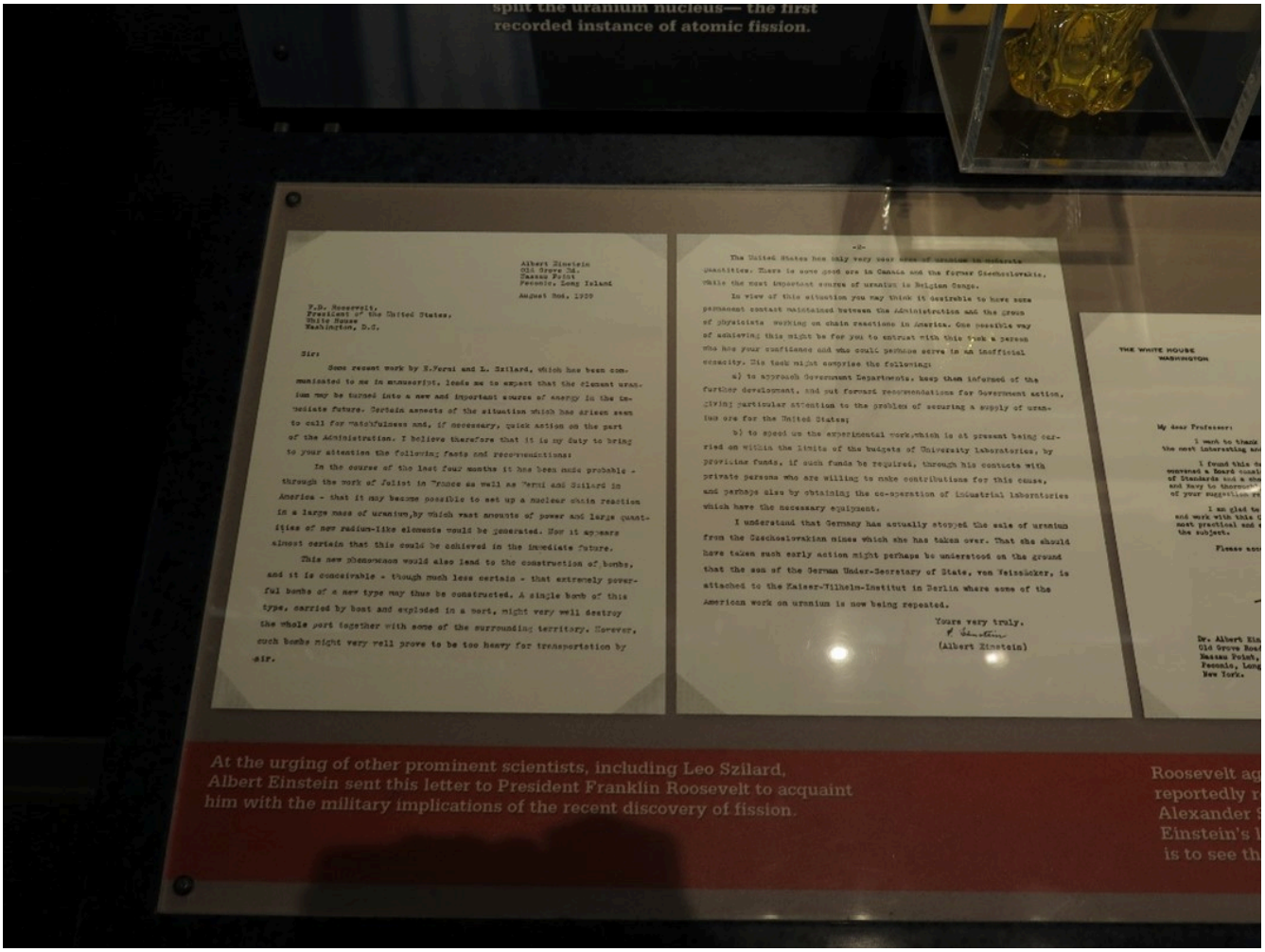

Figure 17.11 Albert Einstein's letter to President Franklin Roosevelt. (Image credit: "Albert Einstein's letter to President Franklin Roosevelt" by rocbolt is marked with CC BY-NC 2.0. Also available at https://www.flickr.com/photos/28685581@N06/48277830276)

Manhattan Project

Figure 17.12: Photograph of Trinity nuclear test. (Image credit: Kelly Michals took a photograph on November 14, 2018 of Jack Aeby's photograph of the Trinity nuclear test that occurred July 16th 1945. "Jack Aeby's Color Photo of the Trinity Nuclear Test" by rocbolt is licensed under CC BY-NC 2.0.)

The "Manhattan project" was the US government's effort to research how to build an atomic bomb and actually build it. It is named after the Army Corps of Engineers "Manhattan Engineer District" (Ref https://www.osti.gov/opennet/manhattan-project-history/Events/1942/1942.htm). The atomic explosion on July 16th of 1945, code named "Trinity," can be considered the first successful nuclear weapon (https://en.wikipedia.org/wiki/Trinity_(nuclear_test)). What led to the Manhattan project? Many events. One event was a meeting between Vannevar Bush and the US President in October 1941. The president received information that building an atomic bomb was feasible. However, at that time, the president wanted only research and development done, nothing further. (reference https://www.osti.gov/opennet/manhattan-project-history/Events/1939-1942/tentative_decision_build.htm). In December 1942, US President Roosevelt approved the building of the atomic bomb (https://www.osti.gov/opennet/manhattan-project-history/Events/1942/final_approval_build.htm).

Einstein's Treatment and Reactions

Einstein was not allowed to work on the Manhattan project (https://www.amnh.org/exhibitions/einstein/peace-and-war/the-manhattan-project). The US government considered him a security risk. He was distraught after the first atomic bomb destroyed the city of Hiroshima, Japan. He also regretted encouraging the US government into research about nuclear weapons based on hindsight. If he would have known that Nazi Germany would not succeed in building the atomic bomb, then he would not have encouraged the US government into nuclear weapons research. (https://www.amnh.org/exhibitions/einstein/peace-and-war/the-manhattan-project)

This historical experience leads to ethical questions such as:

- Should scientists get involved in politics?
- Should scientists encourage research that can be misused?
- Do scientists have individual ethical responsibility?
- Do scientists have a societal ethical responsibility?
- Should scientists do any research that is possible, or should there be limitations?
- Should society tell scientists what research is unethical and off-limits?

There is also a theater play about the ethical questions about science and atomic bomb entitled "Copenhagen: a Play about the Science, Politics, and Morality of Atomic Weapons" (Reference https://en.wikipedia.org/wiki/Copenhagen_(play) accessed 3/23/2022; https://www.armscontrol.org/blog/2017-01-24/copenhagen-play-about-science-politics-morality-atomic-weapons accessed 3/23/2022).

Website links for information:

https://www.amnh.org/exhibitions/einstein/peace-and-war/the-manhattan-project

https://www.osti.gov/opennet/manhattan-project-history/Events/1939-1942/einstein_letter.htm

https://www.osti.gov/opennet/manhattan-project-history/Events/1939-1942/tentative_decision_build.htm

https://en.wikipedia.org/wiki/Einstein%E2%80%93Szil%C3%A1rd_letter

http://www.fdrlibrary.marist.edu/archives/pdfs/docsworldwar.pdf

https://en.wikipedia.org/wiki/Copenhagen_(play)

170.

TUSKEGEE SYPHILIS EXPERIMENT

Adapted with modification from

Psychology 2e

https://openstax.org/details/books/psychology-2e?Book%20details

Published: 4/22/2020

Digital ISBN-13: 978-1-951693-23-7; retrieved 3/15/2022

Ethics and the Tuskegee Syphilis Study

Unfortunately, the ethical guidelines that exist for research today were not always applied in the past. In 1932, poor, rural black male sharecroppers from Tuskegee, Alabama, were recruited to participate in an experiment conducted by the U.S. Public Health Service, with the aim of studying syphilis in black men (Figure 2.21). In exchange for free medical care, meals, and burial insurance, 600 men agreed to participate in the study. About two thirds of the men (399) tested positive for syphilis, and they served as the experimental group (given that the researchers could not randomly assign participants to groups, this represents a quasi-experiment). The remaining syphilis-free individuals (201) served as the control group. However, those individuals that tested positive for syphilis were never informed that they had the disease. While there was no treatment for syphilis when the study began, by 1947, penicillin was recognized as an effective treatment for the disease. Despite this, no penicillin was administered to the participants in this study, and the participants were not allowed to seek treatment at any other facilities if they continued in the study. Over the course of 40 years, many of the participants unknowingly spread syphilis to their wives (and subsequently their children born from their wives) and eventually died because they never received treatment for the disease. This study was discontinued in 1972 when the experiment was discovered by the national press (https://apnews.com/article/business-science-health-race-and-ethnicity-syphilis-e9dd07eaa4e74052878a68132cd3803a). The resulting outrage over the experiment led directly to the National Research Act of 1974 and the strict ethical guidelines for research on humans described in this chapter. Throughout history, race has been divisive and, in some cases, a discouraging factor in scientific inquiry. Why is this study unethical? How were the men who participated and their families harmed as a function of this research?

Figure 17.13: A participant in the Tuskegee Syphilis Study receives an injection. (Image credit: The original image is entitled "Figure 2.21 A participant in the Tuskegee Syphilis Study receives an injection." It is from the book Psychology 2e. https://openstax.org/details/books/psychology-2e?Book%20details. Published: 4/22/2020. Digital ISBN-13: 978-1-951693-23-7; retrieved 3/15/2022. License: CC BY by OpenStax is licensed under Creative Commons Attribution License v4.0)

Link to learning:

Visit this website about the Tuskegee Syphilis Study https://www.cdc.gov/tuskegee/timeline.htm to learn more.

https://www.tuskegee.edu/about-us/centers-of-excellence/bioethics-center/about-the-usphs-syphilis-study and more about events after the study from the Legacy Committee: https://www.tuskegee.edu/Content/Uploads/Tuskegee/files/Bioethics/SyphilisStudyCommitteeReport.pdf

As a consequence, Tuskegee University created the nation's first bioethics center to study moral and ethical question in research and medical treatment of African Americans and other underserved people. The National Center for Bioethics in Research and Health Care received seed funding 2 years after the public apology of the President of the United States about the terrible, "deeply, profoundly, morally wrong" study, a study that was "clearly racist" (words in quotes are from the public apology May 16 of 1997 made by the US President).

Many very serious ethical questions arise from the Tuskegee study. Due to the egregious nature of the harm done, readers should discuss this topic and ethical questions in a larger forum and allow outmost flexibility of discussion. Listing some ethical questions might be too insensitive and might also unintentionally confine the needed space and flexibility for larger discussions.

171.

HELSINKI PROTOCOL FOR HUMAN RESEARCH

Introduction

The Helsinki Declaration was created by the World Medical Association in 1964 (ten years before the Belmont Report) and has been amended several times. The Helsinki Declaration differs from its American version in several respects, the most significant of which is that it was developed by and for physicians. The term "patient" appears in many places where we would expect to see "subject." It is stated in several places that physicians must either conduct or have supervisory control of the research. The dual role of the physician-researcher is acknowledged, but it is made clear that the role of healer takes precedence over that of scientist. In the United States, the federal government developed and enforces regulations on researcher; in the rest of the world, the profession, or a significant part of it, took the initiative in defining and promoting good research practice, and governments in many countries have worked to harmonize their standards along these lines. The Helsinki Declaration is based less on key philosophical principles and more on prescriptive statements. Although there is significant overlap between the Belmont and the Helsinki guidelines, the latter extends much further into research design and publication. Elements in a research protocol, use of placebos, and obligation to enroll trials in public registries (to ensure that negative findings are not buried), and requirements to share findings with the research and professional communities are included in the Helsinki Declaration. As a practical matter, these are often part of the work of American IRBs, but not always as a formal requirement. Reflecting the socialist nature of many European counties, there is a requirement that provision be made for patients to be made whole regardless of the outcomes of the trial or if they happened to have been randomized to a control group that did not enjoy the benefits of a successful experimental intervention. [1]

Content

It has opening statements in the **introduction** pointing out **areas of concern and importance, e.g.**:

- It states that "In medical research on human subjects, considerations related to **the well-being of the**

1. General Assembly of the World Medical Association (2014). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *The Journal of the American College of Dentists*, *81* (3), 14–18. https://pubmed.ncbi.nlm.nih.gov/25951678/

human subject should take **precedence over** the **interests of science and society**."

- It also states that "Medical research is subject to **ethical standards** that promote respect for **all human** beings and protect their health and rights. Some research **populations** are **vulnerable and need special protection**. The particular needs of the **economically and medically disadvantaged** must be recognized. Special attention is also required for those **who cannot give or refuse consent** for themselves, for those who may be subject to giving consent **under duress**, for those who will not benefit personally from the research and for those for whom the research is combined with care."

The Helsinki declaration then presents **basic principles for all medical research**. Some are listed here:

- It is the duty of the physician in medical research to **protect the life, health, privacy, and dignity of the human subject**.
- The design and performance of each experimental procedure involving human subjects should be clearly formulated in an **experimental protocol**. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed **ethical review committee**, which must be **independent** of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The **researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events**. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other **potential conflicts of interest and incentives for subjects.**
- In any research on human beings, each **potential subject** must be **adequately informed** of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the **right to abstain** from participation in the study **or to withdraw consent** to participate **at any time without reprisal**. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

Ethical questions:

- Did the Tuskegee experiment violate concerns or basic principles outlined in the Helsinki declaration?
- Which sections of the Helsinki declaration are maybe addressing problems and concerns with the Tuskegee experiment?
- What additional improvements could you suggest for the Helsinki declaration?

An interactive H5P element has been excluded from this version of the text. You can view it online here:
https://louis.pressbooks.pub/generalbiology1leclab/?p=1420#h5p-62

172.

HENRIETTA LACKS AND HELA CELLS

Henrietta Lacks, an African American mother of five children, went to Johns Hopkins hospital facilities for medical diagnosis and care, located in Baltimore, Maryland. She was diagnosed with cervical cancer, received treatment, and died less than a year after her cancer diagnosis. She succumbed to cancer in 1951. During the treatment, cancer cells were removed and used for further biomedical research without her informed consent. Some of her cells were immortal, proved very valuable for research, and were named "HeLa" cells, resembling the first two letters of her first and last name. HeLa cells were used for uncounted research projects in the past and today. Vaccines were developed with the help of the cells from Henrietta Lacks. According to scientific reporting, money was made from the HeLa cells without informing the surviving family members of Henrietta Lacks (Reference "Henrietta Lacks: science must right a historical wrong" https://www.nature.com/articles/d41586-020-02494-z). It was also reported that Henrietta Lacks genome derived from the HeLa cells was published without informing the family members. Finally, the director of the NIH met the family members, and an agreement was reached (Callaway, E. Deal done over HeLa cell line. *Nature* **500,** 132–133 (2013). https://doi.org/10.1038/500132a)

- Is it ethical to take someone's cells, tissue, organs, or fluids without their informed consent?
- Is it ethical for a scientist to use someone's cells, tissue, organs, or fluids for another type of research than the type of research that was approved by the patient?
- Is it ethical to use research data years after the patient gave approval to do some additional follow-up research, or is a second approval needed?
- Is it ethical to make monetary gains from someone's cells, tissue, organs, or fluids without the approval of the donor?

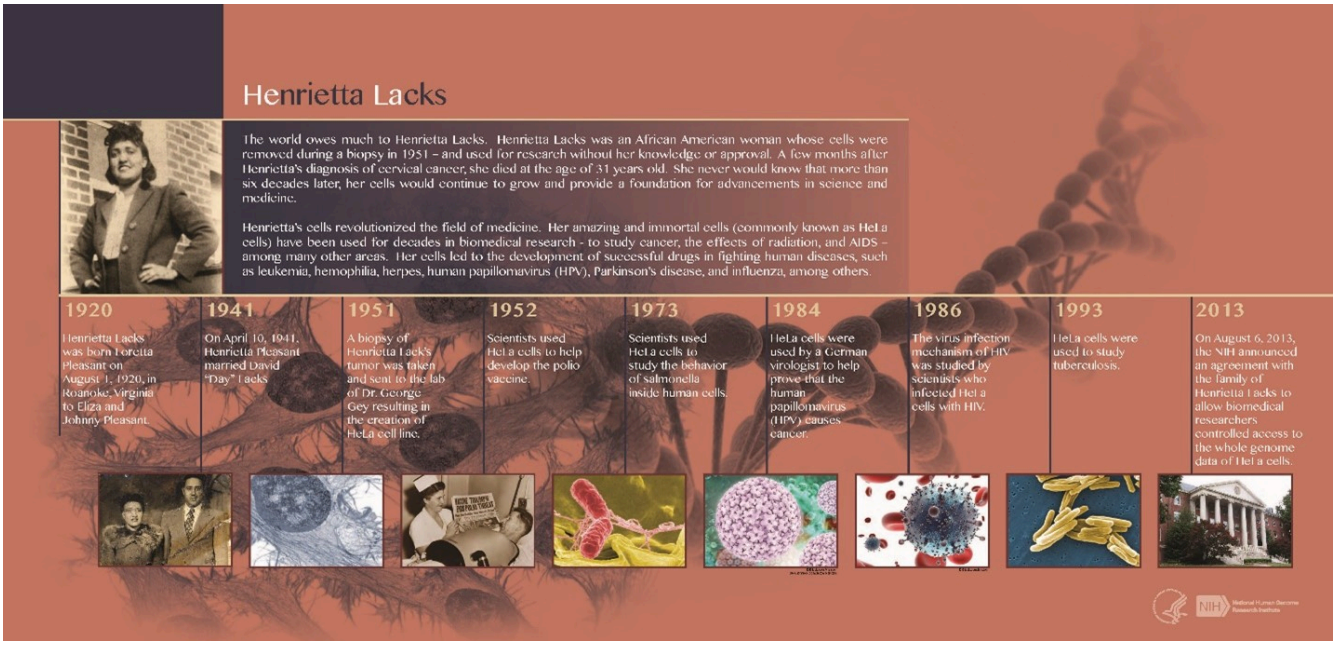

Figure 17.14 Timeline of important events of Henrietta Lacks and scientific legacies. (Image credit: "Henrietta Lacks (HeLa) Timeline" by National Institutes of Health (NIH) is marked with CC BY-NC 2.0.)

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173.

GENE THERAPY AND DEATH IN 2000

Figure 17.15 Life of The clouds....A Tear And A Smile (Image credit: Figure: "Life of The clouds....A Tear And A Smile..." by -Reji is licensed under CC BY-NC-SA 2.0.)

Some diseases are caused by inherited mutations to genes. Of these diseases, some are caused by mutations of a single gene, like sickle cell disease. Scientists developed the technology called gene therapy to treat these types of diseases for ailing animals. Gene therapy aims to replace the defective mutated gene with an unmutated version of the gene. Because of the success in animals and with individual human cells in the labs, scientists were hoping to help humans by using gene therapy for a whole living, breathing human.

In 1999, an approved clinical trial was done to test the safety of gene therapy for a disorder of nitrogen metabolism. The gene therapy was aiming to treat deficiency of the enzyme ornithine transcarbamylase. A virus containing the corrected version of the gene was used to treat 18 volunteers. 18-year-old Jesse Gelsinger suffered from a mild version of the disease and received the gene therapy. Four days after he received the gene therapy, he passed away. This tragic loss led to a lawsuit, a stop to gene therapy trials, and investigations, including one by a committee of the senate of the United States in Washington, DC. Gene therapy slowed down as a treatment option. Some ethical questions and other concerns were raised. The Food and Drug Administration created

more regulations and review processes for clinical trials using gene therapy. In 2009, scientific publications announced that gene therapy was again considered a valuable option (A Comeback for Gene Therapy. Luigi Naldini. Science. 6 Nov 2009).

Sickle cell disease (SCD) occurs in people who inherit two copies of the sickle cell gene, one from each parent. This produces abnormal hemoglobin, called *hemoglobin S*. (Hemoglobin is the protein molecule in red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the tissues to the lungs.) When an individual inherits one copy of the sickle cell gene from a parent, the person is said to have sickle cell trait (SCT). People with SCT usually do not have any of the symptoms of SCD and live a normal life.

Ethics questions

- Is it ethical that the scientist who is conducting the clinical trial also owns a company that is getting paid for participating in the clinical trial?
- Is it ethical to ask volunteers with the disease to participate in a phase 1 trial that studies only if a treatment doesn't cause unnecessary harm instead of a phase 3 trial that studies if a treatment actually improves their condition?
- What is a better ethical choice involving babies in a clinical trial phase 1 that would die from severe cases of the disease without treatment in a year? Babies cannot understand the risks of the treatment. If the treatment can cause death, but if lack of treatment also causes death within 12 months? Is it better to involve adults who can make an informed decision and understand the risk? If adults have a mild form of the disease that can survive by sticking to a harsh diet without harm to their lives, but participation in a clinical trial phase 1 has a chance of causing life-ending harm, is that a better way to conduct a phase 1 clinical trial?
- Is it ethical to withhold information from the volunteer participant that some monkeys died after receiving gene therapy?

Further reading

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174.

GENE THERAPY FOR SICKLE CELL DISEASE 2018

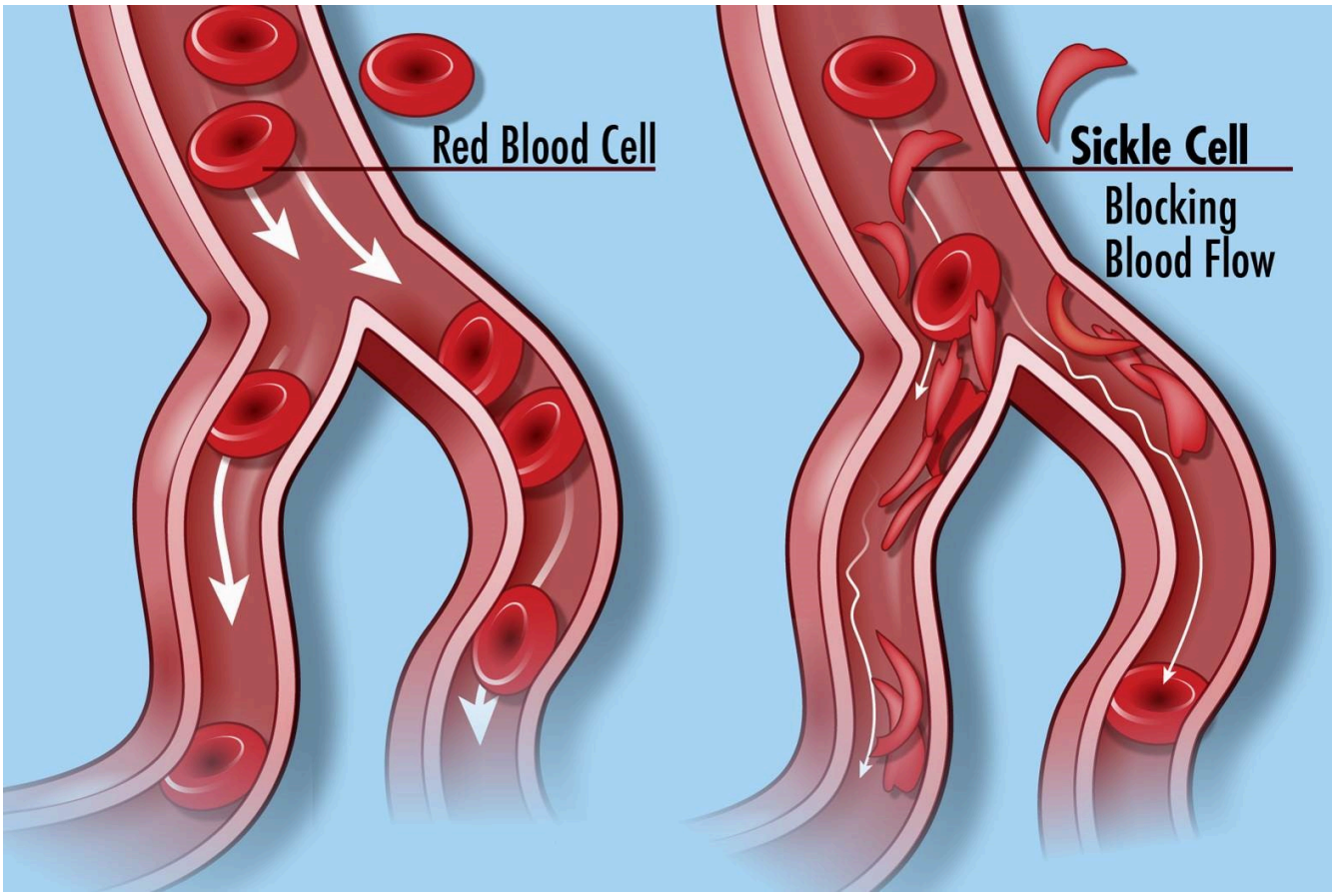

Figure 17.16 Comparison of blood flow with normal and sickle shaped red blood cells
Image credit: "Sickle Cell Disease" by National Institutes of Health (NIH) is licensed under CC BY-NC 2.0.

In sickle cell anemia, a change of one amino acid in hemoglobin molecules distorts the red blood cells and causes them to assume a crescent or "sickle" shape, which clogs blood vessels. Sickle cell anemia can lead to myriad serious health problems such as breathlessness, dizziness, headaches, and abdominal pain for those affected. A change in nucleotide sequence of the gene's coding region for the hemoglobin βchain causes the disease. (Adapted with modification from chapter 3, Biology 2e, OpenStax). This change or mutation in a single gene allows the possibility for gene therapy.

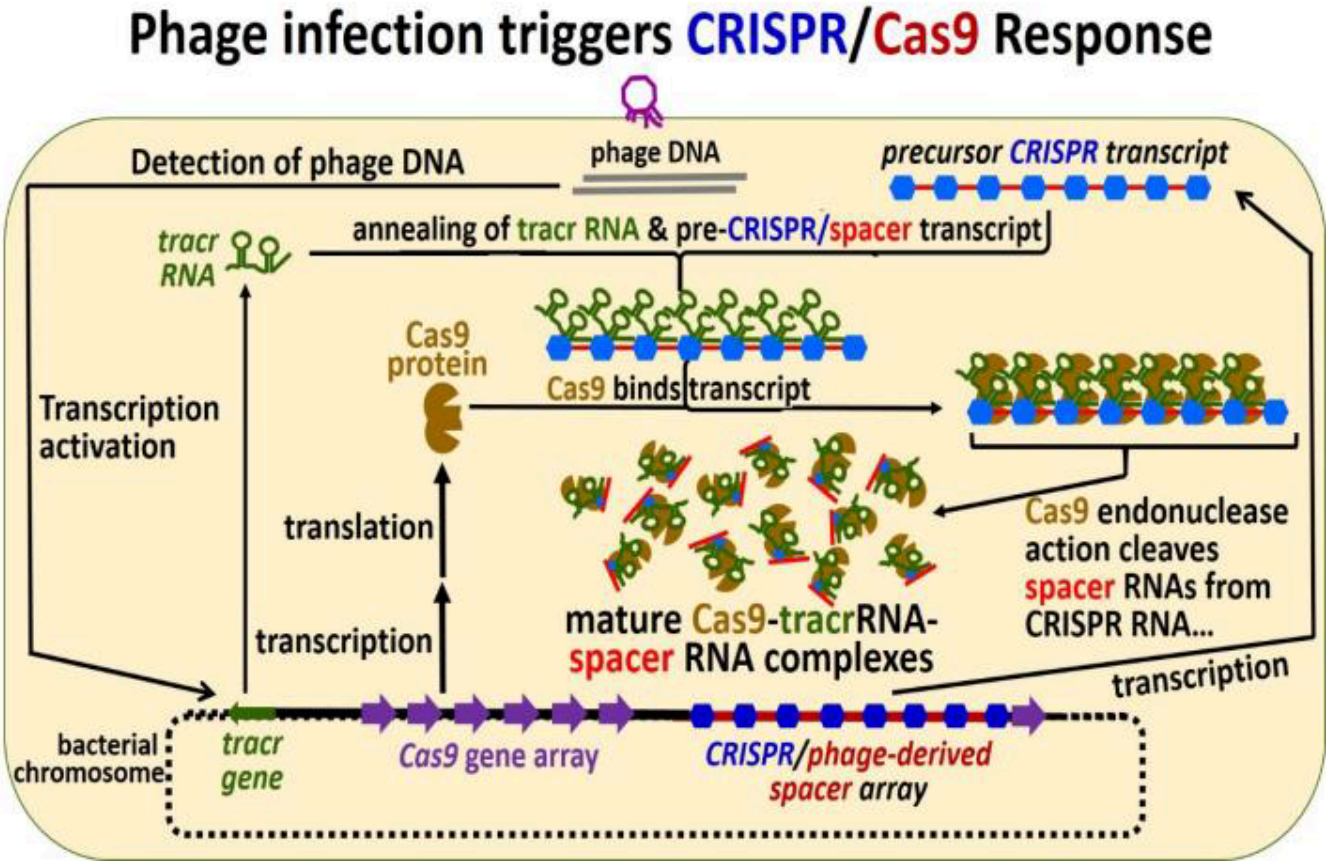

Fig. 17.27: Phage infection triggers formation of CRISPR/Cas9 array. (Image credit: Fig 13.3, page 313 of the book entitled Bergtrom, Gerald, "Annotated Cell and Molecular Biology 4e: What We Know and How We Found Out" (2020). Cell and Molecular Biology 4e: What We Know and How We Found Out – All Versions. 13. https://dc.uwm.edu/biosci_facbooks_bergtrom/13. cc-by 4.0 license)

In 2019, radio stations and newspapers reported about the experimental gene therapy of sickle cell disease patient Victoria Gray. Mrs. Victoria Gray lives in Mississippi with her husband and children. She has suffered from sickle cell disease since early childhood. It has also negatively impacted her college career and the activities she can do with her children. Mrs. Gray was offered participation in an gene therapy experiment using a new technique called CRISPR. In 2019, Mrs. Gray was the only person in the US to receive this type of gene therapy treatment. For this treatment, bone marrow cells were removed from Mrs. Gray and then genetically altered using the new CRISPR technique. The genetically changed cells have a version of hemoglobin gene that will make better-performing hemoglobin. The modified cells are then re-injected into the body of the patient. The treatment is lengthy and did also involve very unpleasant chemotherapy. The treatment showed improvement in quality of life for Mrs. Gray after some months. However, it is not clear if the improvements will last and how long. As the technique is also completely new, there are chances for unforeseen dangerous complications. A scientific study about the 15 months after treatment was published. Three severe adverse events were reported that were treated successfully. Also, 111 less severe events were recorded.

Ethics questions

- Should a scientist or a doctor offer a new experimental treatment that hasn't been tried on any human being before?
- Should a scientist or doctor include additional ethical considerations if an experimental treatment is offered to a demographic group that is exposed to disproportionally high health disparities?
- Should a scientist or doctor offer a new experimental treatment to a person that is parenting dependent children?
- Is this treatment technique a safe technique?
- Is there value in giving a potentially unsafe treatment to a volunteer with a life-shortening disease?
- Is there a value in testing new treatments for diseases that affect underserved population groups with health disparities?
- Who should be involved in discussions, planning, and decisions about research trying to overcome health disparities?

Further reading

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Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, Foell J, de la Fuente J, Grupp S, Handgretinger R, Ho TW, Kattamis A, Kernytsky A, Lekstrom-Himes J, Li AM, Locatelli F, Mapara MY, de Montalembert M, Rondelli D, Sharma A, Sheth S, Soni S, Steinberg MH, Wall D, Yen A, Corbacioglu S. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N Engl J Med. 2021 Jan 21;384(3):252-260. doi: 10.1056/NEJMoa2031054. Epub 2020 Dec 5. PMID: 33283989. https://www.nejm.org/doi/pdf/10.1056/NEJMoa2031054?articleTools=true accessed 4/6/2022; article is freely available as fulltext download;

175.

ETHICS OF GENETIC TESTING AND MEDICAL DISEASE DIAGNOSTICS

Traditionally, disease is diagnosed when a patient suffers from symptoms or measurable signs. Some diseases are caused (at least in part) by genetic changes to a single gene, some by genetic changes to multiple genes, some by changes to chromosomes or their interactions with the environment. Sickle cell anemia, cystic fibrosis, some versions of hemophilia, phenylketonuria, and Huntington's disease are caused by mutations to a specific gene or location on a chromosome. Some diseases such as a type 2 diabetes are thought to be influenced by many genes and environmental factors. Some diseases such as trisomy 21 (also known as Down syndrome) are caused when an additional third chromosome is part of someone's genetic information. Some diseases have a genetic component that increases the risk for a disease, but does not determine with 100% certainty that a person will develop that disease. An example are some mutations in BRCA1 that can increase the risk for breast cancer. Some diseases caused by genetic mutations are life-ending at an early age. Examples are mutations for the gene encoding ADA. ADA is an abbreviation for adenosine deaminase. A mutation causing lack of ADA can cause "severe combined immunodeficiency" (SCID). Persons with non-functional ADA die at a very young age (a few years old) from the lack of a functional immune system, overcome by infections turning lethal against them. Some genetic diseases such as phenylketonuria (PKU) can lead to impairing the cognitive development of a person without intervention. On the other hand, mental impairment can be avoided by giving a diet that avoids or strongly reduces the amount of phenylalanine. Some diseases with genetic components such as diabetes can be improved with improving diets, exercises, patient education, and life style changes. Other genetic diseases such as breast cancer can be monitored with routine testing. Some genetic diseases such as Huntington's disease are fatal but often start to show the signs and symptoms of illness only later in life, oftentimes when someone is in their 30s or 40s. Some genetic diseases can't be cured (as of yet but perhaps in the future). Unfortunately, Huntington's disease is one example. For some genetic diseases, a person can have a recessive disease-causing allele for a gene on one paternal chromosome and a normal allele for the same gene on the chromosome from the other parent. When individuals in this genetic situation have biological children, then there is a chance that one of the children will inherit disease-causing alleles from both parents and suffer from the genetic disease. Sickle cell disease is an example of this type of genetic situation.

In 2005, the National Society of Genetic Counselors (NSGC) defined genetic counseling as "the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease" (Waxler, JL 2012). The World Health Organization (WHO) is also developing and presenting some ethical framework for genetic diseases and genetic testing (Ethical aspects of early diagnosis of

genetic diseases. Kare Berg. World Health. No 9. 1996; Control of genetic diseases. Report by the Secretariat. World Health Organization. Executive Board. 116th Session. Provisional Agenda item 4.1. 21 April 2005). The WHO has guidelines for the "best possible treatment and prevention." The WHO promotes also the autonomy of the individual and the right to full information about the genetic disease and available options. It also promotes that genetic counseling should be educational, voluntary, and non-prescriptive. It should also be sensitive to the societal, cultural, and religious views of the individual. Besides the "right to know," the question about "the right not to know" arises. Do physicians have a duty to share the information about a risk with a patient who doesn't want to know or a family member who might be at risk and is totally unaware of the risk?

Ethics questions:

- Should parents test themselves for genetic diseases or disease conditions before they decide to have children? If parents find out that their children have a risk of getting a genetic disease, should they decide against biological children? How high does the risk have be—low, moderate, high?
- The actress Angelina Jolie wrote an open opinion letter to the *New York Times* entitled "My Medical Choice." The actress decided to test for an allele of the BRCA1 gene that can increase the breast cancer risk. The actress found out that she had the allele that causes increased risk for breast cancer. The actress then had her breast tissue removed (mastectomy) in order to reduce the risk for breast cancer. What do you think about her choice to take the test? What do you think about her choice to proactively have surgery? BRCA1 mutations can also increase risk for ovarian cancer. What do you think about proactively removing the ovaries? The government website from the national cancer institute has some medical information about the BRCA1 gene: http://www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet#q2.
- A grandparent had Huntington's disease. The parents don't want to get tested. If a child wants to know their risk of Huntington's disease, should they get tested even if the disease can't be cured? If the kid is found to have Huntington's disease, then their parent might have a high change of also having it. Can the parent forbid the child to get tested, as the parent doesn't want to know about their own risk?
- Should embryos or fetuses be tested for genetic disease? What would you do as a parent if you find out your unborn child suffers from a genetic disease? Does it make a difference if the disease is severe, mild, untreatable, treatable, life-changing in the 30s or 40s like Huntington's disease, of life-shortening like ADA SCID?

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My Medical Choice. By ANGELINA JOLIE. New York Times. MAY 14, 2013. http://www.nytimes.com/2013/05/14/opinion/my-medical-choice.html?_r=0

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Huntington Disease. Online Mendelian Inheritance in Man. Johns Hopkins University. Creation Date: Victor A. McKusick: 6/4/1986. Contributors: Ada Hamosh – updated: 12/06/2019 https://www.omim.org/entry/143100?search=huntington&highlight=huntington; accessed 4/18/2022. Note: OMIM is intended for use primarily by physicians and other professionals concerned with genetic disorders, by genetics researchers, and by advanced students in science and medicine. While the OMIM database is open to the public, users seeking information about a personal medical or genetic condition are urged to consult with a qualified physician for diagnosis and for answers to personal questions.

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176.

ETHICS OF GENE THERAPY

This topic was also discussed in earlier sections 17.7 and 17.8. Some new technology is mentioned here. There are many more technologies and examples available for discussion, but unfortunately there is not enough time and space available for this section.

This topic is also rapidly changing with the arrival of new technologies. In the past, gene therapy also aimed to treat only affected tissue cells. And the technologies were maybe more complicated and expensive. A discovery made about genes in bacteria called clustered regularly interspaced short palindromic repeats (CRISPR) technology allows scientist to change genes and thereby the genome (entire collection of the genetic information of an organism). There are some concerns about how accurate and how stable this treatment technology is. Other genes might be affected, and the treatment might not be long-lasting or strong enough or efficient enough (see also What are genome editing and CRISPR-Cas9? MedlinePlus; and the open access article by Yang Yue et al. 2021). An additional question arises about whether it safe and ethical to change genetic information of germ cells or stem cells that are passed on to children. In 2018, two twin girls were born with modified genes to make them HIV-immune. Their birth was the result of an "experiment" in which the males were HIV carriers. CRISPR technology was used to immunize the babies against HIV by disabling the CCR5 gene that enables HIV infection.

An open access article discusses the ethics of this case of gene therapy.[1] This author also criticizes that the gene therapy was not really therapeutic to treat existing HIV infection of the fertilized egg or embryo, but an experiment to test the capability of the method.

Ethics questions

- What do you think about using a new technique that has safety risks that are not fully understood?
- What do you think about using gene therapy that is not therapeutic but lets you understand if a method works? Can you use healthy organisms or humans for that?

Further reading

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177.

ETHICS OF DNA TESTING IN FORENSIC SCIENCE AND CRIMINAL JUSTICE

DNA analysis is used more and more in the legal justice system. It can be used to determine the biological father or the biological father. It can also be used to identify a deceased victim. For sexual violence, it can also be used to identify the assaulting criminal.

The modernization of DNA isolation and DNA sequencing techniques makes it affordable for the police, the civil justice system, and the criminal justice system to use DNA evidence. Questions arise, then, about when DNA evidence can be used and who can use it. A physician was convicted of attempted second-degree murder by injecting his former girlfriend with HIV from one of his patients. The unique HIV mutations of the virus sequence determined that the doctor took HIV from a patient and injected it into his girlfriend. Individuals convicted of a crime have their DNA taken and analyzed for genetic markers. The FBI collects this type of information in CODIS (Combined DNA Index System). In Louisiana, there was a case (the State of Louisiana versus Franklin) that ruled a cheek swab for DNA testing is allowed even if a person is not convicted of a crime, but only arrested (*State v. Franklin*, 76 So. 3d 423 (La. 2011)). It also referenced that on the federal level, this was allowed and established. The federal case of *U.S. v. Mitchell*, 652 F.3d 387 (3d Cir. 2011) states in the court decision, "As arrestees have a diminished expectation of privacy in their identities, and DNA collection from arrestees serves important law enforcement interests, we conclude that such collection is reasonable and does not violate the Fourth Amendment."

In another case from Louisiana, familial DNA searching was used. If the DNA found at a crime scene doesn't lead to a suspect in a DNA database, a DNA search for biological family members related to the found DNA of unknown identity can be done. This search has solved some cases, including a cold case of the "Golden State" killer. Some news reports also indicate some problems. According to a newspaper report from New Orleans, a New Orleans film maker was called via phone by police to answer questions about a hit and run. Upon return to his home in New Orleans, two law enforcement persons from Idaho and allegedly a third federal agent waited for the film maker, interviewed him, and took a cheek swab. Familial DNA search led to these events, according to the newspaper article (Jim Mustian. New Orleans filmmaker cleared in cold-case murder; false positive highlights limitations of familial DNA searching. see section further reading). DNA from a murder victim in Idaho did not turn up a suspect with matching DNA. However, familial DNA searching led to the DNA of the father of the film maker. The father's DNA had enough DNA similarities, suggesting family relationships to the unidentified murder suspect's DNA. The father's DNA was entered as part of a church initiative. The DNA information was stored by a foundation whose information was acquired

by a for-profit company specializing in genealogy. The DNA of the film maker's father was too different to be the murder suspect, but the law enforcement entities determined that the son—that is, the New Orleans film maker—could be the murder suspect, according to the newspaper article. The film maker experienced more stressful times until he was cleared. The actual murderer was finally found.

Ethics questions:

- When should DNA samples be taken? Should they be taken from convicted criminals of non-violent crimes or violent crimes? Should they be taken from suspects? Should they be taken from anyone arrested?
- How long should the genetic information be used? Only for the specific incident, arrest, law suit, civil case, criminal case or for 1 year, 10 years, a life time?
- Who should have access to the genetic information? Only the prosecutor, defendant, defense attorney, law enforcement personnel on the specific case, a special DNA unit, the police in the state of the individual, or any law enforcement person in the US?
- Should the collected DNA also be used for other purposes than originally intended? Should DNA for identification purposes also be used to identify relatives, genetic diseases, or a possible "phenotype" of a person?

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178.

USE OF ANIMALS & ENVIRONMENT AND ETHICS

Animals and Ethics

At present, the majority of human beings act as if the circle of ethical consideration stops at the border of the human species, as if no non-human animals deserve true ethical consideration. Hence, we keep certain non-human animals as pets, kill them for food and sport, and perform countless experiments on them in labs without even wondering whether this violates ethical rules that we should pay attention to.

Thoughts for consideration are:

- "Dogma of difference": This is the idea that whatever our relationship to animals may be, it is the differences between us and them that should be emphasized, not the (mostly superficial) similarities.
- Rene Descartes took the Christian idea that animals have no souls to its logical conclusion when he suggested that animals have much more in common with inanimate machines, such as clocks, than they do with human beings.
- Biology suggests that we are much more closely related to other living things than we previously suspected.
- Human needs: Until we provide some reasons why we should be permitted to use animals for food, research and entertainment without any moral consideration, we will not have a leg to stand on.
- Kant's view of morality is a relation of strict equality based on the rational recognition that another counts just as much as I do.
- Animal Welfare: As long ago as the late 18th century, the British Utilitarian philosopher Jeremy Bentham claimed that animals deserve moral consideration to the extent that they can feel pain.
- Animal rights: Utilitarian arguments alone cannot possibly be the basis for objecting to all uses of animals by humans that are currently the norm.
- Animal Welfare Act of 1966 (https://www.nal.usda.gov/animal-health-and-welfare/animal-welfare-act)

Ethics and the Environment

Philosophical Ethics. George W. Matthews, Plymouth State University. Copyright Year: 2020. Attribution CC BY-SA (https://open.umn.edu/opentextbooks/textbooks/philosophical-ethics)

A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community. It is wrong when it tends otherwise.

—Aldo Leopold, A Sand County Almanac

The assumption that there are no limits to human activity is perhaps clearest in the realm of economic activity.

Thoughts for consideration could be:

We "add value" to raw materials when we convert them into particular products, and thus we seem to get more value out of the process for free.

- Potentially negative impacts of economic activity, impacts such as pollution and resource depletion that seem to accompany many manufacturing processes as well as other human activities like transportation.
- The loss of biodiversity, or put more simply, the currently unfolding mass extinction of species.
- Our welfare depends on the stability of the climate, on the existence of biodiversity, and on the availability of energy resources.
- The interconnections between organisms, each of which depend on other organisms for their own survival. The "biotic community" is the network of interdependent organisms that make up a given ecosystem and that we ignore at our peril.

Additional Resources:

Philosophical Ethics. George W. Matthews, Plymouth State University. Copyright Year: 2020. Attribution CC BY-SA (https://open.umn.edu/opentextbooks/textbooks/philosophical-ethics)

Hubrecht, R. (2011). Guide for the Care and Use of Laboratory Animals, Eighth Edition 2011 The Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). Published by the National Research Council of the National Academies, Washington DC, USA. https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf

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KEY TERMS

ethics

study of the standards of right and wrong that inform us as to how we ought to behave

professional ethics

rules or expectations imposed on a trainee, student, employee in a company, an institution or as member of a profession

research ethics

how research is conducted and how findings from that research are used and by whom

common ethics

ethics that the majority of people agree on

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CRITICAL THINKING QUESTIONS

1. Which topic, thought, or reported fact was the most interesting for you?
2. Briefly describe something that you learned from this chapter.
3. The human genome project raised several questions:
   A. Who should have access to personal genetic information, and how will it be used?
   B. Who owns and controls genetic information?
   C. How does personal genetic information affect an individual and society's perceptions of that individual? How does genomic information affect members of minority communities?
4. Explore or discuss how question A could be a question for the field of i) research ethics, ii) professional ethics, iii) common ethics, iv) personal ethics.
5. Explore or discuss how question B could be a question for the field of i) research ethics, ii) professional ethics, iii) common ethics, iv) personal ethics.
6. Explore or discuss how question(s) C could be a question for the field of i) research ethics, ii) professional ethics, iii) common ethics, iv) personal ethics.

APPENDIX A: CHECKLIST FOR ACCESSIBILITY

This title has been reviewed to meet these accessibility practices:

Organizing Content

- Content is organized under headings and subheadings.
- Headings and subheadings are used sequentially (e.g., Heading 1, Heading 2).

Images

- Images that convey information include alternative text (alt text) descriptions of the image's content or function.
- Graphs, charts, and maps also include contextual or supporting details in the text surrounding the image.
- Images do not rely on color to convey information.
- Images that are purely decorative do not have alt text descriptions. (Descriptive text is unnecessary if the image doesn't convey contextual content information).

Links

- The link text describes the destination of the link and does not use generic text such as "click here" or "read more."
- If a link will open or download a file (like a PDF or Excel file), a textual reference is included in the link information (e.g., [PDF]).
- Links do not open in new windows or tabs.
- If a link must open in a new window or tab, a textual reference is included in the link information (e.g., [NewTab]).
- For citations and references, the title of the resource is hyperlinked, and the full URL is not hyperlinked.

Tables

- Tables are used to structure information and not for layout.
- Tables include row and column headers.
- Row and column headers have the correct scope assigned.
- Tables include a caption.
- Tables avoid merged or split cells.
- Tables have adequate cell padding.

Multimedia

- All audio content includes a transcript. The transcript includes all speech content and relevant descriptions of non-speech audio and speaker names/headings where necessary.
- Videos have captions of all speech content and relevant non-speech content that has been edited by a human for accuracy.
- All videos with contextual visuals (graphs, charts, etc.) are described audibly in the video.

Formulas

- Equations written in plain text use proper symbols (i.e., −, ×, ÷).[1]
- For complex equations, one of the following is true:
  - They were written using LaTeX and are rendered with MathJax (Pressbooks).
  - They were written using Microsoft Word's equation editor.
  - They are presented as images with alternative text descriptions.
- Written equations are properly interpreted by text-to-speech tools.[2]

Font Size

- Font size is 12 point or higher for body text in Word and PDF documents.
- Font size is 9 point for footnotes or endnotes in Word and PDF documents.

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1. For example, a hyphen (-) may look like a minus sign (−), but it will not be read out correctly by text-to-speech tools.

2. Written equations should prioritize semantic markup over visual markup so text-to-speech tools will read out an equation in a way that makes sense to auditory learners. This applies to both equations written in LaTeX and equations written in Microsoft Word's equation editor.

- Font size can be enlarged by 200 percent in webbook or ebook formats without needing to scroll side to side.

Learn more about Pressbooks' commitment to Accessibility.